Mechanism of ultrasound and tea polyphenol assisted ultrasound modification of egg white protein gel

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In order to improve the characteristics of egg white protein gel and the stability of egg white protein gel products. In this work, the changes of the texture, physicochemical properties, secondary structure and microstructure of ultrasound modified egg white protein gel (UEP) and tea polyphenols (TP) assisted ultrasound modification of egg white protein gel (TUEP) with different ultrasonic power (0–360 W) were studied. With the increase of ultrasonic power, soluble protein, surface hydrophobicity and disulfide bonds of TUEP and UEP showed an increasing trend. In particular, with the increase of ultrasonic power, the content of intramolecular \(\beta\)-sheets and \(\alpha\)-helices of TUEP showed an increasing trend, and significantly improved the relaxation time and microstructure, thus enhancing TUEP gel stability. In addition, the hardness and water holding capacity (WHC) of TUEP and UEP can be increased by ultrasonic treatment, and when the ultrasonic power reached 120 W, the hardness and WHC reached the maximum. The hardness and WHC of TUEP were significantly better than that of UEP. SDS-PAGE results showed that the peptide chain of protein without being broken under ultrasonic treatment. Ultrasonic treatment can improve the gel strength of egg white protein by promoting the cross-linking of proteins to form a dense gel structure, and egg white protein gel form more disulfide bonds and a more stable gel conformation under TP assisted ultrasound. In conclusion, ultrasound and TP assisted ultrasound modification of egg white protein gel is a reliable technique, which can improve the value of egg white protein in food processing.

1. Introduction

Egg white protein is mainly composed of ovalbumin, lysozyme and ovomucin, which provide a material basis for the formation of egg white gel. In the early formation of egg white gel, ovotransferrin, lysozyme, ovomucin are involved in the formation of the protein gel skeleton \cite{1}, and after the egg white gel is completely formed, ovalbumin is the major protein that formed the structure of gelatin \cite{2}. In addition, after the egg white protein is divided into thick egg white and thin egg white gel, the heat induced thick egg white gel appears tough soft (high cohesiveness and low hardness), while thin egg white gel appear brittle hard (low cohesiveness and high hardness) \cite{3}. Due to the excellent gel properties of egg white, it could be used as a gel filler and cross-linking agent in the processing of surimi and sausage gel to increase gel properties \cite{4}. Furthermore, in order to improve the added value of egg white gel, many scholars \cite{2,5} have increased the application scope of egg white protein gel through modification (mainly through chemical modification and physical modification). Liu, et al \cite{5} found that, egg white protein would form a blocklike gel structure with high water holding capacity after pasteurization, while egg white protein would form a mesh-like gel structure with high cohesiveness after spray-drying. In addition, there are small molecules (such as polyphenols, polysaccharides, and phosphates) could also improve the gel properties of egg white protein to a certain extent \cite{2}.

Tea polyphenols (TP) are the main active components in tea, accounting for 30% of the dry weight of tea, which are composed of tea catechins, flavonoids, anthocyanins and phenolic acids. Due to TP have strong antioxidant properties, there are widely used in food processing, such as milk, chewing gum, biscuits, etc. \cite{6}. In addition, TP has a significant affinity for proteins, and the hydroxyl groups in TP could...
enhance the functional properties of protein through hydrophobic interactions, hydrogen bonds, and other interactions with proteins [7]. TP could be combined with egg white protein, thus improving the antioxidant and digestibility of egg white protein [8]. Furthermore, there have been a number of researches on TP modified protein gel [9,10]. However, because TP is extremely sensitive to proteins, it tends to lead to the formation of soluble complexes, and these complexes would gradually polymerize and even form deposits [9], which would affect the taste and sensory of food to a large extent, reduce the quality of food.

Ultrasound is a kind of physical technique, which has the characteristics of safety, high efficiency, and environmental protection. It could be divided into low-intensity high-frequency (0.1–1 MHz) ultrasound and high-intensity low-frequency (16–100 kHz) ultrasound [11]. Low-intensity high-frequency ultrasound is normally designed for the analysis and detection of samples. It could determine the structure and physical state of the sample without destroying the sample [12]. High-intensity low-frequency ultrasound could improve the properties of the samples, in liquid medium, the physicochemical characterization of the samples are changed by collapse of cavities and cyclic generation. In particular, high-intensity low-frequency ultrasound has a broad prospect in the improvement of proteins functional properties. The solubility and foaming ability of egg white protein were greatly improved under 20/40 kHz dual-frequency ultrasound [13]. Fu et al. [14] found that ultrasound could promote the glycation of xylose and egg white protein and improve the solubility of egg white protein. Considering that ultrasound could improve the solubility of protein, through the preliminary experiments, it was found the TP-modified egg white protein would generate partial insoluble matter. And the insoluble matter almost disappeared after ultrasonic treatment, and the gel strength was greatly enhanced after thermal induction (compared with non-ultrasound and no-TP). Therefore, in this study, the mechanism of ultrasound assisted TP-modified egg white protein gel was explored by studying the changes of gel strength, physicochemical properties and microstructure. So as to provide a theoretical basis for the modification research and processing of egg white protein.

2. Materials and methods

2.1. Materials

Fresh chicken eggs (54.2 ± 3.5 g) were supplied by a supermarket (Wangzhongwang Supermarket) in Qingshanhu district, Nanchang, China. TP (purity ≥ 98%, derived from green tea) and spectroscopic grade potassium bromide (KBr) were bought from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). All other chemical reagents were analytical grade.

2.2. Sample preparation

Separate the egg white from the yolk, the egg white was stirred in a magnetic stirrer for 5 min, and was mixed with 0.05% of TP and homogenized by using a T-18 Digital homogenizer (German IKA Company, Staufen, Germany) at 12 k r/min for 2 min. Afterward, the mixtures were stirred in a magnetic stirrer for 10 min and stored at 4 °C for 3 h, and the sample was treated using a JY92-1IN ultrasonic processor (NingBo Scientz Biotechnology Co. Ltd., Ningbo, China) with a 6 mm high-grade titanium tip in the ice bath (the temperature of the sample was controlled below 25 °C). The ultrasound condition at an ultrasonic power of 0 W, 30 W, 60 W, 120 W, 240 W, and 360 W with pulse mode (3 s on and 3 s off) for 15 min. After that, the sample was added to a 10 mm casings and thermal treatment at 85 °C for 20 min. At last, the sample was rapid cooling in the ice bath and overnight at 4 °C. Mark the above heat induced samples as tea polyphenol assisted ultrasound modified egg white protein gel (TUEP). A control trial without TP was used as a blank sample and marked as ultrasound modified egg white protein gel (UEP).

2.3. Determination of ζ-potential

The ζ-potential of TUEP and UEP were measured with Zetasizer Nano Z ZEN2600 instrument (Malvern Instruments Ltd., Malvern, UK). TUEP and UEP were diluted 10 times with deionization water and homogenized at 12 k r/min for 2 min, after centrifugation at 8 k r/min for 10 min (Model TGL-20B, Anke, Shanghai, China), and the supernatant was diluted 10 times to measure.

2.4. Determination of soluble protein

Accurately weigh 1 g of TUEP and UEP, mixed with 9 mL PBS buffer (0.05 M, pH = 7.2). Then, the mixture was homogenized at 12 k r/min for 2 min and centrifuged at 8 k r/min for 10 min, and the supernatant of sample was measured by BCA methods [15].

2.5. Determination of free sulphydryl

TUEP and UEP were diluted 10 times with PBS buffer, then the mixture was homogenized and centrifuged, and the protein concentration of supernatant was measured by BCA methods. Subsequently, the supernatant was diluted 14 times with Tris-Gly buffer (pH = 8, 100 mmol/L Gly, 4 mmol/L EDTA, 100 mmol/L Tris) and 0.1 times Ellman’s reagent (4 mg/mL 5,5-dithiobis-(2-nitrobenzoic acid) dissolved in Tris-Gly buffer), and heat at 40 °C for 40 min to complete reaction of the mixture. Finally, the sample was analyzed for free sulphydryl content using a Hitachi F-7000 Fluorescence Spectrofluorometer (Hitachi, Tokyo, Japan) at the emission wavelength and excitation wavelength of 485 nm and 380 nm [17].

2.6. Determination of surface hydrophobicity

TUEP and UEP were diluted 10 times with PBS buffer, then the mixture was homogenized and centrifuged, and the protein concentration of supernatant was diluted to 0.3 mg/mL. Then, 2 mL of diluted sample was mixed with 40 μL ANS solution (sodium 8-anilino-1-naphthalenesulfonate dissolved in ethanol, 1 mmol/L) and store in the dark for 10 min. Finally, the sample was analyzed for surface hydrophobicity using a Hitachi F-7000 Fluorescence Spectrofluorometer (Hitachi, Tokyo, Japan) at the emission wavelength and excitation wavelength of 485 nm and 380 nm [17].

2.7. Microstructure analysis

The microstructure of TUEP and UEP were measured with SU8100 Cold-Field-Emission Scanning Electron Microscope (CFESEM, Hitachi, Tokyo, Japan). TUEP and UEP were immobilized overnight in 2.5% glutaraldehyde, and rinsed with the PBS buffer. Then, the sample was dehydrated with ethanol (60–100% (v/v)) and freeze dried with Scientz-10 N vacuum freeze dryer (NingBo Scientz Biotechnology Co. Ltd., Ningbo, China). Finally, the sample was sprayed with gold and observed under 5 k V accelerating voltage at 5 k magnification [18].

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

Determination of relaxation time (T2) was using a Bruker low-field pulsed NMR Analyzer (Bruker Co., Ltd., China) by multi-component fitting analysis at repeat sampling interval was 800.000 ms, scan repetitions was 4, and echo number was 240 [19].
2.9. Determination of water holding capacity (WHC)

The WHC of TUEP and UEP were measured with the methods of direct centrifugation [20]. About 3 g of TUEP and UEP (mark as M) was placed in a 50 mL centrifuge tube (with a MWCO = 100 KDa ultrafiltration tube) and centrifuged at 8 k r/min for 30 min. The mass of TUEP and UEP were recorded after centrifugation and mark as m. The calculation of WHC as follows: WHC (%) = 100%*m/M.

2.10. Texture profile analysis (TPA)

The TPA of TUEP and UEP were measured with BROOKFIELD CT3 instrument (Brookfield, Middleboro, MA, USA). The sample was cut into 1 cm high cylinder, and measured at a compression deformation of 60% and crosshead speed of 2 mm/s with a P/36R metal plate probe. The hardness, and springiness of sample were recorded and analyzed by using Texture Expert version 1.22 (Brookfield, Middleboro, MA, USA).

2.11. SDS-PAGE analysis

The protein composition of TUEP and UEP were measured with a Mini Bio-Rad vertical electrophoresis unit (Bio-Rad Co., Ltd., Hercules, CA, USA). About 3 g of TUEP and UEP were mixed with 27 mL of Tris-HCl (pH = 8.8, 10%), after homogenization and centrifugation, the sample was mixed with loading buffer and boiled for 4 min. Then, the sample was added to the electrophoresis (separating and stacking gels were 10% and 5%) [21].

2.12. Fourier transformed infrared (FTIR) analysis

The FTIR of TUEP and UEP were measured with a Thermo Nicolet iS 5 FTIR (Thermo Fisher Scientific, Waltham, MA, USA). The freeze-dried of TUEP and UEP was grinded with KBr (1:100) and pressed into thin slices. The measurement of FTIR was carried out at room temperature from 400 to 4000 cm\(^{-1}\) for 32 scans. In addition, the amide I band (1600–1700 cm\(^{-1}\)) of the original data was derived by OMNIC 6.0 Software to observe the protein secondary structure [22].

2.13. Statistical analysis

Except for TPA (six times), all experiments were carried out three times. These results were analysis using SPSS version SPSS19.0 (SPSS Inc., Chicago, IL, USA) by variance at a level of p < 0.05. The research results presented as means ± SD using Origin 8.5 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Changes in physicochemical properties of TUEP and UEP

3.1.1. Changes in \(\zeta\)-potential of TUEP and UEP

The \(\zeta\)-potential could reflect the net charge of the protein surface, the \(\zeta\)-potential of the protein is positive, indicated that there are more positively charged amino acids on the protein surface than negatively charged amino acids, and vice versa [23]. The decrease of the absolute value of \(\zeta\)-potential suggested that the stability of protein dispersive system becomes worse or aggregation gradually. As shown in Fig. 1A, the \(\zeta\)-potential of both TUEP and UEP were negative means that the surface of TUEP and UEP were mainly covered by negative charge. With the increase of ultrasonic power, the absolute values of TUEP and UEP \(\zeta\)-potential showed a decreasing trend (p < 0.05), from 32.7 to 28.7 mV and 35.4 to 31.9 mV, respectively, and the absolute values of UEP \(\zeta\)-potential were higher than that of TUEP. It is generally believed that the protein sample would be broken and the protein particles would become smaller after ultrasonic treatment. Therefore, this suggested that TUEP and UEP particles became smaller under ultrasonic effect, and alter the charge distribution of protein molecules, the positively charged amino acids (such as lysine and arginine) within a protein were exposed from 400 to 4000 cm\(^{-1}\) for 32 scans. In addition, the amide I band (1600–1700 cm\(^{-1}\)) of the original data was derived by OMNIC 6.0 Software to observe the protein secondary structure [22].

3.1.2. Changes in soluble protein of TUEP and UEP

The soluble protein of TUEP and UEP were measured with a BIO-RAD protein assay unit (Bio-Rad Co., Ltd., Hercules, CA, USA). The soluble protein of TUEP and UEP were increased with the increase of ultrasonic power (p < 0.05), from 0.8 to 1.2 mg/mL and 0.9 to 1.3 mg/mL, respectively.

3.1.3. Changes in free sulfhydryl of TUEP and UEP

The free sulfhydryl of TUEP and UEP were measured with a SNOBU-100 spectrophotometer (Shanghai, China). The free sulfhydryl of TUEP and UEP were increased with the increase of ultrasonic power (p < 0.05), from 0.2 to 0.6 mmol/g protein and 0.3 to 0.7 mmol/g protein, respectively.

3.1.4. Changes in surface hydrophobicity of TUEP and UEP

The surface hydrophobicity of TUEP and UEP were measured with a HPA-2000 spectrophotometer (Haiyi, China). The surface hydrophobicity of TUEP and UEP were increased with the increase of ultrasonic power (p < 0.05), from 1000 to 2500 AU and 1500 to 3000 AU, respectively.

Fig. 1. Effect of different ultrasonic power on \(\zeta\)-potential (A), soluble protein (B), free sulfhydryl (C), and surface hydrophobicity (D) of TUEP and UEP.
[24] and neutralize the negative charge on the protein surface. Therefore, the absolute values of TUEP and UEP ζ-potential decreased with the increase of ultrasonic power. TP contains a large number of carboxylic acid groups (–COO⁻), which may bind to the nitrogen groups (–NH₃⁺) of aspartic acid and glutamic acid through electrostatic reaction [25], resulting in a lower absolute value of TUEP ζ-potential than that of UEP.

3.1.2. Changes in soluble protein content of TUEP and UEP

Protein solubility is a key indicator to reflect protein aggregation, degradation and conformational [26], which will affect other functional properties of protein, such as foaming and gelling. As shown in Fig. 1B, with the increase of ultrasonic power, the soluble protein content of TUEP and UEP gradually increased (p < 0.05), and the soluble protein content of TUEP was significantly higher than that of UEP (p < 0.05). The increase of soluble protein content may be attributed to two reasons. First, the triggering of physical effects (through ultrasonic treatment) alter the protein structure, which was consistent with the research results of Sun, et al. [13], they found that appropriate ultrasonic treatment would change the molecular structure of protein, thus increasing the solubility of protein. In particular, the polarity of the egg white protein was enhanced after combined with polyphenols, exposing the hydrophilic region of the protein to water and the interaction between water and protein were enhanced. Therefore, the soluble protein content of TUEP was higher than that of UEP. Second, non-covalent interaction bonds in proteins (such as hydrophobic interaction, electrostatic interaction and hydrogen bond) were altered by mechanical forces such as shear and microflow (generated by ultrasonic cavitation) [27], resulting in the original protein gel aggregates were destroyed and the particle size of protein gel became smaller, which increased the solubility of TUEP and UEP.

3.1.3. Changes in free sulfhydryl groups of TUEP and UEP

The content of free sulfhydryl groups and disulfide bonds play a very important role in the stability of protein structure. Disulfide bonds were easily formed by free sulfhydryl groups through peroxidation, thus improving the gel network structure of protein [28]. The more disulfide bonds in protein gel, the more stable the protein gel structure is. As shown in Fig. 1C, with the increase of ultrasonic power, the content of free sulfhydryl groups in TUEP and UEP first increased (p < 0.05) and then decreased (p < 0.05), and subsequently remain essentially unchanged after 60 W ultrasound power (p > 0.05), and the content of free sulfhydryl groups in TUEP was significantly lower than that of UEP (p < 0.05). Theoretically, the aggregate degree of protein gel would be reduced after ultrasonic treatment, thus exposing the free sulfhydryl groups inside the protein. This was consistent with the result that the content of free sulfhydryl groups increased in egg yolk after ultrasonic treatment [29]. But on the other hand, high-intensity ultrasonic treatment may generate free radicals through cavitation effect, and the exposed free sulfhydryl groups were oxidized to disulfide bonds, thus reducing the TUEP and UEP free sulfhydryl groups. In particular, TP in TUEP was easy to be oxidated into quinones by free radicals (from cavitation effect), while a large number of active groups on quinones were easy to oxidize free sulfhydryl groups to disulfide bonds in TUEP, thus reducing the content of free sulfhydryl groups [30,31]. Therefore, the content of free sulfhydryl groups in TUEP was lower than that of UEP. In addition, it also indicated that the content of disulfide bond in TUEP was higher than that in UEP and polyphenols can improve the stability of protein gel. The total amount of sulfhydryl groups in protein were fixed, when a large number of free sulfhydryl groups were oxidized into disulfide bonds, the content of free sulfhydryl groups on the surface of protein was low, and free sulfhydryl groups were difficult to contact each other and form disulfide bonds through oxidation. Therefore, the content of free sulfhydryl groups remains unchanged after 60 W ultrasound power.

3.1.4. Changes in surface hydrophobicity of TUEP and UEP

Surface hydrophobicity of proteins could reflect the distribution of hydrophobic amino acid residues (such as tryptophan residues and tyrosine residues) on the surface of proteins, and can also indirectly reflect the changes of protein conformation, which is an important factor to determine the functional properties of proteins. The changed in surface hydrophobicity of TUEP and UEP were shown Fig. 1D. With the increase of ultrasonic power, the surface hydrophobicity of TUEP and UEP showed an increasing trend, and the surface hydrophobicity of TUEP was lower than that of UEP. Surface hydrophobicity gradually increased with the increase of ultrasonic power, which indicated that the hydrophobic amino acid residues of protein were exposed to water environment by ultrasonic treatment. It may be because ultrasonic cavitation may generate strong shear force in the local protein sample, which would unfold of TUEP and UEP structure and expose the hydrophobic amino acid residues on protein surface [32]. The combination of polyphenols with protein (hydrophilic hydroxyl groups of polyphenols bind to lipophilic groups of protein) may promote protein aggregation, leading to the decrease of protein surface hydrophobicity. Therefore, the surface hydrophobicity of TUEP was smaller than that of UEP. The increased surface hydrophobicity of TUEP and UEP can promote the formation of hydrophobic aggregates, and the network structure became more compact, which was conducive to the formation of a more stable protein gel structure.

From the results of physicochemical properties, the protein particles of TUEP and UEP became smaller and the protein structure was destroyed under ultrasonic treatment. In this process, positively charged amino acids, hydrophobic amino acid residues and free sulfhydryl groups were exposed on the protein surface, which promoted interaction between proteins of TUEP and UEP (through the formation of disulfide bonds and hydrophobic aggregates). Moreover, in TUEP, after polyphenols were combined with protein, due to the strong polarity of phenolic acid, on the one hand, the protein solubility of TUEP was higher than that of UEP, on the other hand, the surface hydrophobicity of TUEP was lower than that of UEP.

3.2. Changes in microstructure and macro-characterisation of TUEP and UEP

3.2.1. Changes in microstructure of TUEP and UEP

Scanning electron microscopy plays an important role in scientific research, which can characterize the shape and size of protein gels at the microscopic level. The microstructure of TUEP and UEP were shown in Fig. 2. Without ultrasound (0 W), the gel structures of TUEP and UEP were relatively complete, but the distribution of protein was uneven, with larger protein particles and higher protein aggregation degree. With the increase of ultrasonic power, the protein gel particles significantly decreased (from about 5 μm to 2 μm). It means that ultrasound increased the surface area and the opportunity for proteins to contact water of TUEP and UEP molecules, resulting in increased soluble protein content (Fig. 1B) and surface hydrophobicity (Fig. 1D) of UTEP and UEP. In addition, the distribution of TUEP and UEP particles were more well-proportioned and formed a dense, smooth, and ordered network structure. Generally speaking, the formation of protein gel structure was determined by the relative rate of protein expansion and aggregation. When the protein aggregation rate was greater than the expansion rate, the protein would over-aggregate (such as the gel structure of the sample without ultrasound), and resulting in uneven distribution of protein aggregates and large protein particles [33]. The protein structure (tertiary structure and secondary structure) could be expanded by appropriate ultrasonic treatment, thus reducing the relative rate of protein aggregation [32]. Therefore, ultrasonic treatment improved the gel structure of TUEP and UEP.

3.2.2. Changes in T2 of TUEP and UEP

Low field NMR technology can analyze samples without destroying.
the TUEP and UEP gel systems were non-flowing multi-molecular layer bound water, and this gel system has a relatively stable gel structure. With the increase of ultrasonic power, the \( T_2 \) value of TUEP showed a decreasing trend \((p < 0.05)\), from 52.18 to 47.79 ms, while the \( T_2 \) value of TUEP remained essentially unchanged \((p > 0.05)\), and remained between 53.58 and 54.84 ms. This indicated that separate ultrasonic treatment could not enhance the water-binding ability of protein gel. However, under TP assisted ultrasound, the distribution of water molecules in TUEP gel was improved, and water molecules were more tightly bound by proteins. This result may be attributed to the decreased of TUEP particles (Fig. 2), the specific surface area and the contact area between polyphenols-proteins and water have increased, thus improving the ability of polyphenols-proteins to attract water molecules (through hydrogen bond) and reducing the \( T_2 \) value of TUEP. With the increase of ultrasonic power, although the decreased of UEP particles, the ability of individual protein to attract water molecules through hydrogen bonds was weak (lack of a large number of hydroxyl groups), therefore the \( T_2 \) value of UEP remained essentially unchanged.

3.2.3. Changes in WHC of TUEP and UEP

WHC can characterize the retaining water ability of protein gel, which was one of the most important indicators of heat-induced protein gel. Namely, WHC is related to the gel structure and water-protein interaction state of the protein. A dense and stable gel structure and strong water-protein interaction could improve the WHC of the gel sample. As shown in Fig. 3B, with the increase of ultrasonic power, WHC of TUEP first increased from 74.2\% (0 W) to 82.8\% (120 W) \((p < 0.05)\), and then decreased \((p < 0.05)\), while WHC of UEP increased from 70.16\% (0 W) to 77.53\% (360 W) \((p < 0.05)\). These results indicated that ultrasonic treatment may be an effective method to improved the water retention of protein gel. The enhancement of WHC may be due to the protein structures of TUEP and UEP to be expanded and folded through the hole effect produced by ultrasonic treatment, and formed a more dense and stable gel structure (Fig. 2), thus improving the WHC of TUEP and UEP. In particular, after ultrasonic treatment, polyphenols-proteins molecules were easy to interact with water molecules, and water molecules were tightly wrapped by the protein network structure, thus increasing the WHC of TUEP. Therefore, the WHC of TUEP was higher than that of UEP. This was consistent with the previous conclusion [34], which hypothesized that ultrasonic treatment can promote the interaction of water-protein, thus increasing the WHC of gluten protein.

3.2.4. Changes in TPA of TUEP and UEP

TPA can directly characterize the strength of protein gel, which is a comprehensive expression of protein properties (including the gel structure of protein, the interaction forces that the constitute of protein, and the secondary and tertiary structures, etc.). The springiness of TUEP and UEP were shown in Fig. 3C. With the increase of ultrasonic power, the springiness of TUEP and UEP remained essentially unchanged \((p > 0.05)\), which was close to 1, and the springiness of TUEP \((0.974\text{-}0.982)\) was higher than that of UEP \((0.951\text{-}0.956)\). These results indicated the effect of the ultrasonic treatment on the springiness of TUEP and UEP was very feeble, and both TUEP and UEP could maintain a highly elastic gel structure, while the addition of polyphenols could slightly improve the springiness of protein gel. This result may be attributed to polyphenols could improve the toughness of protein gel by connecting the three-dimensional network structure, thus improving TUEP springiness [22]. The hardness results were shown in Fig. 3D. With the increase of ultrasonic power, the hardness of TUEP first increased \((p < 0.05)\), from 1515.8 g (0 W) to 1919.1 g (120 W), increased by 26.6\%, and then remained essentially unchanged \((p > 0.05)\). The hardness of UEP increased from 1384.4 g (0 W) to 1650.0 g (360 W) \((p < 0.05)\), increasing by 19.2\%. The results were consistent with WHC change trend (Fig. 3B). These results may be due to the ultrasonic treatment could improve the compactness and structural integrity (Fig. 2) of the

Fig. 2. Effect of different ultrasonic power on microstructure of TUEP (A) and UEP (B).
protein gel, and this stable gel structure can resist external extrusion. In addition, from the results of free sulfhydryl groups (Fig. 1C) and surface hydrophobicity (Fig. 1D), ultrasonic treatment would promote the interaction and cross-linking between proteins through hydrophobic interactions and disulfide bonds of TUEP and UEP [35]. In particular, from the results of free sulfhydryl groups, TUEP may promote the formation of more disulfide bonds, so that the hardness of TUEP was higher than that of UEP. Interestingly, from the results of WHC and TPA, at 120 W ultrasound power, ultrasonic and TP assisted ultrasonic modified egg white protein gel strength was a appropriate power. The enhancement of egg white protein gel hardness was very beneficial to the processing of egg products. At present, most egg products (such as Japanese tofu and egg sausage, etc.) were vacuum packaging, egg products would deform (compressed by atmospheric pressure) if the protein gel was weak, thus affecting the senses of egg product. These results have proved that ultrasonic and TP assisted ultrasonic modification of egg white protein may be a very effective method to improve the quality of egg products in the future.

According to the results of microstructure and macro-characterization, after ultrasonic treatment, the gel structure of TUEP and UEP became uniform, dense and complete. This intact network structure could retard water molecules loss to a certain extent, and provide a better rigidity to resist external force of extrusion, therefore the WHC and hardness of TUEP and UEP were improved after ultrasonic treatment. Besides, after polyphenols interact with protein, the ability of polyphenols-proteins in TUEP to attract water molecules was improved, thus increasing the ability of bound water molecules and water holding capacity, as well as the stability of the gel structure. Therefore, the T2, WHC, and hardness of TUEP were higher than UEP.

3.3. Changes in protein structure of TUEP and UEP

3.3.1. Changes in SDS-PAGE of TUEP and UEP

SDS-PAGE electrophoresis is a very effective method to analyze the molecular weight and content distribution of proteins. As shown in Fig. 4, the location and depth of the main protein bands of TUEP and UEP were not significantly different, including ovotransferrin (70 kDa), ovoglobulin (48 kDa), and ovalbumin (38 kDa), respectively [19]. Among them, ovalbumin was the main band, because ovalbumin was the main protein in egg white (accounting for 54% of all proteins), which was consistent with previous studies [36]. In addition, the molecular weights of ovotransferrin (76 kDa) and ovalbumin (45 kDa) at the natural state were both higher than that of TUEP and UEP, which was probably because egg white protein molecules may be partially degraded under high temperature treatment [22]. In addition, high molecular weight proteins were appeared on the electrophoretic bands top, that maybe the result of heat-induced protein aggregation. With the increase of ultrasonic power, the electrophoretic bands of TUEP and UEP did not change significantly, which indicated that ultrasonic treatment on the protein molecular weight was very feeble, and ultrasonic treatment would not lead to the cleavage and formation of protein covalent bonds. This was consistent with the results of thermosonication-induced modification of mung bean protein [37].

3.3.2. Changes in FTIR of TUEP and UEP

The variation of protein secondary structure and function group of TUEP and UEP were measured by FTIR spectroscopy, as shown in Fig. 5. The result of Fig. 5A and B revealed that the shape and position of the peak pattern in the infrared spectrum did not change significantly, indicated that protein functional groups would not be completely destroyed or formed under ultrasonic treatment. Among these peaks, at 3291 cm\(^{-1}\) corresponded to N–H hydrogen bonding and stretching coupling, which depended on the strength of hydrogen bond in protein [38]. With the increase of ultrasonic power, the intensity of the two absorption peaks of TUEP significantly increased, while UEP subsequently remained essentially unchanged, and was much smaller than that of TUEP. This indicated that the synergistic action of TP and ultrasonic treatment could enhanced the hydrogen bond interaction of...
TUEP. This was probably because the gel particles of the polyphenols modified protein decreased and the specific surface area of the protein increased under ultrasonic treatment, which promoted the close bonding of the hydroxyl groups in the polyphenols-proteins through hydrogen bonds, thus enhancing the hydrogen bond strength of TUEP.

Two absorption peaks were observed at 2962 cm\(^{-1}\) and 2935 cm\(^{-1}\), corresponded to C–H stretching vibrations of methyl and methylene groups in proteins and lipids [39], the intensity of these two absorption peaks significantly increased in TUEP under ultrasonic treatment, which showed that the contents of methyl and methylene groups increased under TP assisted ultrasound. Moreover, the absorbance of protein amide I (1654 cm\(^{-1}\), represented COO– hydrogen bond coupling and C = O stretching vibration), amide II (1541 cm\(^{-1}\), represented N–H stretching vibration and C–N bending vibration), and amide III (1237 cm\(^{-1}\), represented N–H bend) [40] significantly changed, which indicated that TP might change the protein structure through the reaction with the C-N, N–H, and COO– groups of protein under ultrasound treatment.

The amide I band (1600–1700 cm\(^{-1}\)) mainly represented the extension of the amide group (C = O), and the shape and position of amide I band could reflect the conformation of the protein and characterize the secondary structure of the protein [41]. As shown in Fig. 5C and Fig. 5D, there were several keys absorption peaks were observed in the second derivative of TUEP and UEP. They were intramolecular β-sheets (1612–1642 cm\(^{-1}\)), intermolecular β-sheets (1615–1625 cm\(^{-1}\)), random coils (1640–1650 cm\(^{-1}\)), α-helices (1650–1660 cm\(^{-1}\)), β-turn (1661–1690 cm\(^{-1}\)) and intermolecular antiparallel β-sheets (1690–1700 cm\(^{-1}\)) [19]. Among them, intramolecular β-sheets was the
main secondary structure to maintain TUEP and UEP structure, indicated that TUEP and UEP had a ordered secondary structure (β-sheets was an ordered and stable secondary structure). The contents of intramolecular β-sheets and intermolecular antiparallel β-sheets of TUEP were much higher than that of UEP, suggested that polyphenols could promote the formation of stable secondary structure. In addition, the hardness of TUEP was much higher than that of UEP may due to TUEP has stable secondary structure. With the increase of ultrasonic power, the secondary structure of UEP does not vary significantly, indicated the effect of the separate ultrasonic treatment on the secondary structure of protein was very feeble. However, with the increase of ultrasonic power, the content of intramolecular β-sheets of TUEP increased significantly with a decrease in the content of intermolecular β-sheets. It was probably because ultrasonic treatment promoted the cross-linking between polyphenols-proteins, resulting in intermolecular β-sheets transformed into intramolecular β-sheets, which corresponded to the results of free sulfhydryl groups (Fig. 1C) and surface hydrophobicity (Fig. 1D). The contents of random coils and β-turn in TUEP remain essentially unchanged, which might be because the stable gel structure of TUEP could inhibit the changed of unstable secondary structure. With the increase of ultrasonic power, the α-helices of TUEP showed an increasing trend, which may be because the α-helices were maintained by the hydrogen bonds (formed by the carbonyl group and amino group of polypeptide chain) [42], and the increased polarity of protein may affect the hydrogen bond of α-helices, thus increasing the content of α-helices. Interestingly, the results of the α-helices in this study were contrary to those of porcine myosin gel under ultrasonic treatment [32], suggested that the effects of ultrasonic treatment may be different for different proteins.

From the protein structure results, in UTEP and UEP, ultrasonic treatment would not lead to the formation and cleavage of protein covalent bonds, nor completely destroy or generate the functional groups of proteins. For UEP, ultrasonic treatment on the protein structure was very feeble. However, for TUEP, ultrasound treatment not only would enhance the hydrogen bond between proteins and promote the interaction of proteins, but could also convert the secondary structure of TUEP into a more stable structure, resulting in formed a more stable gel structure.

3.4. Schematic mechanism

Combined with the above research results, the schematic diagram of ultrasonic and ultrasonic-assisted TP modification of egg white protein gel was simulated and shown in Fig. 6. The gel particles of TUEP and UEP are shown in the figure. The protein aggregates (hydrophobic groups in protein inside) and protein aggregates (hydrophobic groups on protein surface) are represented by different symbols, and the tea polyphenols, cysteine, lysine or arginine, hydrogen bond, disulfide bond, water molecule, and negative charge are represented by different symbols as well. The effect of ultrasound on gel structure of egg white protein is shown in the figure.

**Fig. 6.** Schematic diagram of influence mechanism of ultrasound assisted TP on gel structure of egg white protein.
UEP decreased under ultrasonic treatment, the hydrophobic amino acids and positively charged amino acids were exposed to the surface of the protein, and hydrophilic amino acids were wrapped inside the protein, thus enhancing the interaction between the proteins and decreasing the net charge on the protein surface. Under ultrasonic treatment, free sulfhydryl groups were oxidized to disulfide bonds, thus promoted the cross-linking between proteins. The gel structure of TUEP and UEP net charge on the protein surface. Under ultrasonic treatment, free sulfhydryl groups were oxidized to disulfide bonds, thus promoted the cross-linking between proteins. The gel structure of TUEP and UEP became more stable and dense, which can bind more water molecules and enhance the retaining water ability protein gel. In addition, for TUEP, the polarity of the protein and the content of intramolecular β-sheets and α-helices increased under ultrasonic treatment, leading to the transformation of the protein secondary structure into a more stable conformation. These results showed that ultrasonic treatment was a very effective method to improve egg white protein gel, while TP assisted ultrasound has a better effect.

4. Conclusions

In this study, it was found that the gel properties of egg white protein modified by ultrasound and TP assisted ultrasound were greatly improved after heat induction. These results indicated that ultrasound and TP assisted ultrasound were useful, energy-saving and safe methods to modify the gel strength of egg white protein. With the increase of ultrasonic power, the gel strength and WHC of TUEP and UEP showed an increasing trend, and reached their maximum at 120 W, indicating that 120 W ultrasound power modification of egg white protein may be an effective method to improve egg white protein gel structure. This study could provide a theoretical and technological basis for the processing and utilization of ultrasonic treatment in egg products, and also provide a new idea for the study of other protein gel modification.

CRediT authorship contribution statement

Hui Xue: Investigation, Data curation, Formal analysis, Writing – original draft. Yonggang Tu: Funding acquisition, Conceptualization, Validation, Project administration, Writing – review & editing. Guowen Zhang: Writing – review & editing. Xiaojun Xin: Writing – review & editing. Hui Hu: Writing – review & editing. Wei Qiu: –. Dandan Ruan: Writing – review & editing. Yan Zhao: Funding acquisition, Conceptualization, Validation, Project administration, Writing – review & editing.

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.

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