Improving the gel properties of duck egg white by synergetic phosphorylation/ultrasound: Gel properties, crystalline structures, and protein structure

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ABSTRACT

To improve the gel properties of duck egg white gel and increase the industrial value of duck egg white, the mechanisms of ultrasound and synergetic phosphorylation/ultrasound treatments were examined in this study. It was found that as the ultrasound power increased, the surface hydrophobicity, hardness, and cohesiveness of the gel system increased, and the ζ-potential and water mobility decreased. Of the two treatments, phosphorylation/ultrasound had the strongest impact on the conformation and crystallinity of the gel system and promoted the formation of high molecular polymers. Both gel systems displayed enhanced compactness, stability, and gel strength because of the enhanced protein–protein interactions via hydrogen bonds and protein aggregation, and increased the content of intramolecular β-sheets following ultrasound treatment, and synergetic phosphorylation/ultrasound further improved the stability, water binding and gel properties. This experiment showed that ultrasound and, particularly, phosphorylation/ultrasound are effective methods to improve the gel properties of duck egg white. This study enhanced our understanding of the interactions of sodium pyrophosphate and egg white under ultrasound treatment, and promote the potential application of sodium pyrophosphate and ultrasound treatment of novel food products.

1. Introduction

A protein gel is a state in which a protein solution loses fluidity under certain conditions, and protein molecules aggregate to form a gel or solid. In the aggregation process, the attractive and repulsive forces between proteins reach equilibrium, thus forming a very ordered 3D gel structure which can retain lots of water [1]. Stable gel products, such as dried bean, surimi, cheese, egg sausage, and Japanese tofu, can be formed from high-protein foods, such as soybean, meat, milk, and poultry eggs, under certain conditions. These gel products provide invaluable information for the development and utilization of food. Eggs are one of the most popular foods for consumers and are excellent sources of protein, fat, calcium, phosphorus, iron, and vitamins. The edible part of an egg is mainly including egg yolk and egg white. Egg white accounts for approximately 57.0–58.5 % of the volume of whole egg, and more than 90 % of the solids in egg white are protein, which is mainly including lysozyme, ovalbumin, ovotransferrin, and ovomucin, and which provides a material basis for the gelation of egg white [2].

Substantial research has been devoted to improving and optimizing the gel characteristics of egg white gel by various physical modification (such as microwave, magnetic field, electric field, ultrasonic field, etc.) or chemical modification (such as adding chemical modifier, changing pH value, etc.) to design new types of egg white gel products. For example, the hardness, cohesiveness, and springiness of protein gel were improved to resist the extrusion of vacuum packaging (to prevent the deformation of protein gel), thus increasing the application range of protein gel and the added value of the raw material [3].

Ultrasound is the most common physical method for modifying the gelation properties of proteins, possessing the characteristics of environmental protection, efficiency, and safety [4]. In particular, low-frequency with high-intensity ultrasound (16–100 kHz) has broad prospects for improving the physicochemical properties of protein. The liquid would be vibrated sharply after ultrasonic treatment and numerous tiny bubbles would be produced. These bubbles will be...
produced in the negative pressure zone formed by longitudinal ultrasound propagation, and quickly closed in the positive pressure zone, which is called ultrasonic cavitation effect. When the ultrasound waves propagate in the protein medium, the interaction between the ultrasound waves and protein molecules produces a series of mechanical and cavitation effects capable of changing the characteristics of protein, including foamability, solubility, and gelling ability [5]. Sun, et al., [6] stated that the structure of porcine myosin was opened under ultrasound treatment, which increased the surface hydrophobicity of porcine myosin protein, and the 3D network structure of the porcine myosin protein gel became uniform with enhanced water holding capacity and hardness. Ultrasound also improved the gel characteristics of lionfish surimi patties and their sensory attributes [7].

For chemical modification, phosphorylation is a well-recognized and effective food protein modification method. Phosphate is a commonly used food additive that could not only improve the texture of a protein gel (without adverse effects on the sensory of protein gel) but also provide essential phosphorus for the human body [8]. Li et al., [9] provided evidence for enhanced gel strength and water holding capacity of whole egg gel by sodium pyrophosphate treatment due to the increased the pH value and surface hydrophobicity of the protein. Phosphate also promoted the crosslinking of myofibrillar protein (via ionic interactions) and the gel network formation [10].

In recent years, synergistic modification (physical and chemical modification) has been used to further develop the gel properties of proteins [4]. Inspired by this approach, in this study, duck egg white was treated with synergistic phosphorylation/ultrasound to provide fundamental information on improving the gelation property of the protein and offer theoretical guidance for improving traditional Asian egg product foods production technology, including egg sausage and Japanese tofu. Sodium pyrophosphate was chosen as the phosphorylating reagent in this study based on the preliminary experiment that synergistic sodium pyrophosphate/ultrasound had the best modification effect (compared with other phosphate salts tested, such as sodium diphosphate, sodium tripolyphosphate, and sodium hexametaphosphate).

2. Materials and methods

2.1. Materials and chemicals

Fresh duck eggs (60 ~ 65 g) were supplied by Jiangxi Tianyun Agricultural Development Co., ltd. (Nanchang, China). Sodium pyrophosphate (food grade) was purchased from Tianfu (Lianyungang) Food Ingredients Co., ltd. (Lianyungang, China). Other chemical agents were analytical reagents, except 2.5 % glutaraldehyde (guaranteed reagent), and were acquired from Solarbio Chemicals Co., ltd. (Beijing, China).

2.2. Gel samples preparation

>30 duck eggs were taken out, of all egg whites were placed in a beaker and stirred thoroughly by a magnetic stirrer for 300 s. Afterward, 40 mL of duck egg white was mixed thoroughly with 0.5 % sodium pyrophosphate (according to China Standard (GB 2760–2014) for Food Additives) in a vortex mixer at 2,800 rpm for 60 s and left to stand at 25 °C for 10 min to phosphorylate it. The samples were then treated in an ice bath under ultrasound power at 0, 120, 240, and 360 W for 20 min (pulse duration of off 3 s and on 3 s) using a JY92-2N ultrasound processor (NingBo Scientz Biotechnology Co., ltd., Ningbo, China). After ultrasonication, the samples were poured into casings (about 1 cm in diameter) and thermally induced at 80 °C for 300 s, and quickly cooled in ice then saved overnight in a refrigerator at 4 °C to obtain synergistic sodium pyrophosphate/ultrasound-modified duck egg white protein gel (SUDEP) systems. Duck egg white samples exposed to the same ultrasound treatment but without sodium pyrophosphate were labeled as ultrasound-modified duck egg white protein gel (UDEP) systems.

2.3. Measurement of the relaxation time (T2) of UDEP and SUDEP

Approximately 2 g of UDEP and SUDEP were separately placed in nuclear magnetic resonance (NMR) glass tubes within the radio frequency coil center of a Niumag low-field pulsed NMR analyzer (Niumag Corp., Shanghai, China) with Carr–Purcell–Meiboom–Gill (CPMG) sequences. T2 was analyzed by multi-component fitting, and made with a τ-value (time between 90 and 180 ° pulses) of 17 μs. The operating parameters were as follows: digital gain DRG1 = 3, the repeat sampling interval = 1000.00 ms, NECH = 220, scan repetitions = 4 [11].

2.4. Measurement of the surface hydrophobicity of UDEP and SUDEP

Approximately 1 g of UDEP and SUDEP, respectively, was mixed with 10 mL 0.05 M PBS buffer (pH 7.2~7.4), subsequently homogenized with a T-18 digital homogenizer (German IKA-Werke GmbH & Co., Staufen, Germany) at 12,000 rpm for 120 s, and centrifuged at 7012 g (20 min) in an Anke TGL-20B (Shanghai, China). The supernatant was measured by BCA method using a BCA assay kit, was diluted to 0.3 mg/mL by PBS. Finally, a 2.5 % 0.69 mM sodium 8-anilino-1-naphthalenesulfonate solution (dissolved in ethanol) was respectively mixed to each prepared sample and incubated in the dark for 10 min. After incubation, Fluorescence intensity was recorded at wavelength of 485 nm (emission), wavelength of 380 nm (excitation), and the emission and excitation slits were set as 5 nm using an F-7000 fluorescence spectrofluorometer (Hitachi, Tokyo, Japan) [12].

2.5. Determination of the ζ-potential of UDEP and SUDEP

Approximately 3 g of UDEP or SUDEP was added to ultrapure water (27 mL) and homogenized. After centrifugation, the supernatants were measurement of the ζ-potential using a Zetasizer Nano Z (ZEN2600; Malvern Instruments ltd., Malvern, UK) [13].

2.6. Measurement of the texture of UDEP and SUDEP

UDEP and SUDEP were cut into cylinders (5 mm in height and 10 mm in diameter) for texture determination using a Brookfield CT3 texture analyzer (Middleboro, MA, USA) with a P/50R cylindrical probe. TPA measurements were conducted at a pre-test speed of 5.00 mm/s, a test speed of 2.00 mm/s, a compression ratio of 60 %, and an auto-triggering force of 5.0 g [14].

2.7. Measurement of the SDS-PAGE of UDEP and SUDEP

UDEP and SUDEP were cut into cylinders (5 mm in height and 10 mm in diameter) for texture determination using a Brookfield CT3 texture analyzer (Middleboro, MA, USA) with a P/50R cylindrical probe. TPA measurements were conducted at a pre-test speed of 5.00 mm/s, a test speed of 2.00 mm/s, a compression ratio of 60 %, and an auto-triggering force of 5.0 g [14].

2.8. Analysis of the microstructure of UDEP and SUDEP

UDEP and SUDEP were cut into cubes (0.5 cm³) and mixed, respectively, with glutaraldehyde overnight (>12 h). After decanting the glutaraldehyde, the samples were washed with 0.05 M PBS buffer (pH 7.2) for 15 min and dehydrated in a gradient series of ethanol (70 %, 80 %, 90 %, and 100 %) for 30 min. Subsequently, the samples were pre-treatment in the –80 °C ultra-low temperature freezer and and later vacuum freeze-dried via a SCIENZT-10 N vacuum freeze dryer (Ningbo ScientzBiotechnology Co., Ltd., Ningbo, China). The freeze-dried samples were coated with gold dust and analyzed using a SU8100 cold-field-
emission scanning electron microscope (Hitachi) in low-vacuum mode at 2,500 \times \text{magnification} \ [16].

2.9. Measurement of the crystalline structures of UDEP and SUDEP

UDEP and SUDEP were directly freeze-dried and separately ground to powder. The crystalline structure of each powdered sample was analyzed using a D8 Advance X-ray diffractometer (Bruker AXS, Karlsruhe, Germany) under 40 mA/40 kV in the range of 5 to 60°. The data were smoothed and peak-divided by PeakFit 4.12 software (Seasolve Software, Inc., Framingham, MA, USA) (fitting degree $R^2 > 0.997$) \[17\].

2.10. Measurement of the Fourier transform infrared spectra (FTIR) of UDEP and SUDEP

UDEP and SUDEP were directly freeze-dried, separately ground to powder, mixed with KBr (1:100, w/w). FTIR of each sample was measured by a Thermo Nicolet iS 5 FTIR (Thermo Scientific Nicolet Corp., MA, USA). The scanning results of all samples were recorded within 4000–400 cm\(^{-1}\) at the resolution of 4 cm\(^{-1}\). To analyze the change in the secondary structure, the amide I region (ranging from 1600 to 1700 cm\(^{-1}\)) was processed by OMNIC 9.0 software \[18\].

2.11. Statistical analysis

All measurements were performed thrice, except for texture (six times). All results were analyzed using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) by a Duncan multiple-comparison test at a significance level of $p < 0.05$. Lastly, the experimental figures were drawn by Origin 8.5 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. $T_2$ analysis

Low-field NMR of a protein gel provides information about the state of water in the gel structure and the integration of hydrogen protons with the protein molecules. For this reason, it has been widely used to evaluate the stability of protein gel systems. A smaller $T_2$ value of the sample indicates a closer integration of the water molecule or hydrogen proton in the gel and a more stable protein gel system \[19\]. The $T_2$ results of UDEP and SUDEP (Fig. 1A and B) were composed of two independent peaks, labeled $T_{21}$ and $T_{22}$, respectively. $T_{21}$ represents tightly bound water (0.05–3.05 ms), and it accounted for about 5 % of the total water content. $T_{22}$ corresponds to immobilized water (21–115 ms) \[19\], and it accounted for about 95 % of the total water content. Both gel systems had a number of relatively small $T_2$ peaks in the relaxation time distribution domain, most of which were immobilized water, suggesting that the gel structures were relatively stable. The $T_{22}$ peak positions of UDEP (57.22 ms) and SUDEP (49.77 ms) remained unchanged with the increase of ultrasound power, while the $T_{21}$ peak positions decreased significantly, from 1.51 to 1.00 ms for UDEP and from 1.32 to 0.87 ms for SUDEP. These results showed that phosphorylation or ultrasound could greatly improve the stability and the water molecules-binding capacity of duck egg white protein gel. Among the two different gel systems, the structural stability of SUDEP was greater than that of UDEP. This can be attributed to the combination of the cavitation effect of ultrasound \[6\] and the enhanced integration of water molecules and protein in the gel system due to the increased ionic strength and electrostatic repulsion between protein molecules as a result of phosphorylation \[8\]. Therefore, SUDEP formed a gel system with high electrostatic repulsion and stable gel structure.

![Fig. 1. Effect of different ultrasound power on $T_2$ (A and B) and surface hydrophobicity (C and D) of UDEP and SUDEP.](image-url)
3.2. Surface hydrophobicity

For protein gel, hydrophobic interactions are crucial for maintaining the tertiary structure of a protein. In particular, the hydrophobic interactions at the protein surface play a key role in the stability, conformation, and function of the protein. Therefore, surface hydrophobicity may reflect changes in protein conformation and be used for describing the tertiary structure of a protein [20]. Surface hydrophobicity was measured in the present study through fluorescence spectrometry using the ANS probe. As shown in Fig. 1 C and D, the peak positions of UDEP and SUDEP remained unchanged after ultrasound treatment or phosphorylation, which indicated that the environments of the hydrophobic groups (such as tryptophan) of the protein had not changed. The surface hydrophobicity of UDEP and SUDEP increased significantly because the shear forces generated in the sample interior by the cavitation effect of ultrasound would unfold the protein structure, exposing the hydrophobic amino acid residues of protein [6]. In addition, Ai et al., [17] reported that phosphate ions and sodium ions (generated by hydrolysis of sodium pyrophosphate) easily bind to protein, resulting in conformational changes in the protein and increased surface hydrophobicity. Furthermore, ultrasound treatment would decrease the size of the protein particles (specific surface area decreased) by breaking peptide bonds [21], which may also promote the interaction of phosphate ions and sodium ions with protein, which is consistent with the higher surface hydrophobicity of SUDEP compared to UDEP.

3.3. \( \zeta \)-potential analysis

The \( \zeta \)-potential can be used to characterize the quantity and properties of charges on the surface of protein. The over-representation of negative-charged amino acids (such as glutamic acid and aspartic acid) on the protein surface results in a negative \( \zeta \)-potential and vice versa [22]. Moreover, the \( \zeta \)-potential may influence the physical stability of protein system; the higher the absolute value of the \( \zeta \)-potential, the more stable the protein system. In this study, the \( \zeta \)-potential values are the absolute \( \zeta \)-potential values.

As shown in Fig. 2A, the \( \zeta \)-potential decreased (\( p < 0.05 \)) from 44.5 to 42.6 mV (UDEP) and from 48.2 to 45.4 mV (SUDEP) with the increase in ultrasound power. It indicated that ultrasound treatment had slightly reduced the physical stability of the protein gel system. However, because the \( \zeta \)-potentials were still relatively high (>30 mV) [23], it suggested that the gel systems of UDEP and SUDEP were still relatively stable. The decrease in the \( \zeta \)-potential may be due to the unfolding of the protein molecules under the action of ultrasound, exposing more lysine and histidine on the protein surface, resulting in the neutralization of some of the negatively charged amino acids on the surface of protein [24]. Therefore, the \( \zeta \)-potential decreased significantly for both UDEP and SUDEP. In addition, the phosphate ions released upon hydrolysis of sodium pyrophosphate would easily attach to the protein, thereby increasing the ionic strength and electrostatic repulsion between protein–protein [8,10], which is consistent with the higher \( \zeta \)-potential of SUDEP compared to UDEP. These results were consistent with that reported by Mao et al. [25], who found that sulphate group (\(-\text{OSO}_3^-\)) released upon hydrolysis of kappa-carrageenan would interact with the amino group (\(-\text{NH}_3^+\)) of ovalbumin through electrostatic interaction, thus affecting the \( \zeta \)-potential and structure of ovalbumin.

3.4. Texture profile analysis

Food texture is a physical property determined by the composition and structure of the sample. Hardness, cohesiveness, and springiness are considered to be important textural parameters of gel systems [9]. Hardness is the force exerted when the gel sample is forced to reach a certain deformation by external pressure. It is usually related to the gel structure of the protein and the forces between the constituent protein components. As shown in Fig. 2B, the hardness firstly increased (\( p < 0.05 \)) from 890.84 to 1,272.67 g for UDEP and from 1,132.04 to 1,258.07 g for SUDEP with the increase in ultrasound power, then

![Fig. 2. Effect of different ultrasound power on \( \zeta \)-potential (A), hardness (B), cohesiveness (C), and springiness (D) of UDEP and SUDEP.](image-url)
remained essentially unchanged at the ultrasound power applied (120 W). However, except for 0 W, the hardness between UDEP and SUDEP have no significant difference \( (p > 0.05) \). Compared with ultrasound, synergetic phosphorylation/ultrasound had no obvious effect. Therefore, ultrasound is the main factor that affects the hardness of duck egg white gel. This was probably because the gel structures of UDEP and SUDEP were relatively stable after the ultrasound power exceeded 120 W, and it was difficult to increase the strength of the gel systems further.

After the first compression deformation, the relative resistance of the sample to the second compression is defined as cohesiveness, and cohesiveness is related to the shrinkage force and degree of protein crosslinking; the greater the shrinkage force and crosslinking degree of the protein gel, the stronger the cohesiveness. As presented in Fig. 2C, with the increase in ultrasound power, the cohesiveness firstly increased \( (p < 0.05) \) from 0.702 to 0.740 for UDEP and from 0.748 to 0.776 for SUDEP, then remained essentially unchanged at 120 W, which was similar to the trend of hardness (Fig. 2B). However, unlike the hardness, the cohesiveness of SUDEP was greater than that of UDEP regardless of the ultrasound power \( (p < 0.05) \). This indicated that the degree of crosslinking of duck egg white protein can be improved by ultrasound and further intensified by synergetic sodium pyrophosphate/ultrasound treatment, which corresponded with the surface hydrophobicity observations (Fig. 1C and D). It may be that the ultrasound-induced decrease in the particle size of the samples increased the interaction and aggregation rate of protein molecules \( (5,26) \), facilitating chemical crosslinks and thus improving the cohesiveness of UDEP and SUDEP. This agrees with a previous observation that ultrasound enhanced the non-covalent and covalent interactions between soy protein isolates, which was conducive to the crosslinking of protein \( (27) \). Moreover, sodium pyrophosphate may also promote the oxidation of sulfhydryl and crosslinking of proteins via disulfide bonds \( (8) \), which is consistent with the higher cohesiveness of SUDEP compared to UDEP.

Springiness is the ratio of the recovery altitude after the second compression to the amount of the first compression deformation. As shown in Fig. 1D, the springiness of UDEP have no significant difference \( (0.960, p > 0.05) \) with the increase of ultrasound power, but increased from 0.972 to 0.986 in SUDEP \( (p < 0.05) \). It suggested that ultrasound alone can not improve the recovery degree of the protein gel, which is in agreement with a previous study \( (4) \). With synergetic sodium pyrophosphate/ultrasound treatment, the recovery degree of the egg white gel was significantly improved, perhaps because the intermolecular forces of the egg white gel could be regulated by sodium pyrophosphate \( (9) \). In addition, the change of springiness may be related to the protein gel structure \( (28) \). That is to say, SUDEP might form a gel structure with high recovery ability. In any case, it can be emphasized that ultrasound and synergetic phosphorylation/ultrasound significantly improved the gel properties of duck egg white protein.

### 3.5 SDS-PAGE analysis

SDS-PAGE is an effective method for detecting and discriminating between protein components based on their molecular weight. The electrophoretic patterns of UDEP and SUDEP are shown in Fig. 3. Three main bands corresponding to ovalbumin \( (35 \text{ kDa}) \), ovoglobulin \( (49 \text{ kDa}) \), and ovotransferrin \( (60 \text{ kDa}) \) could be detected \( (12,29) \). These three bands were all lower than that the natural weights proteins (ovoalbumin was 45 kDa, ovoglobulin was 55 kDa, and ovotransferrin was 76 kDa), which could be attributed to the protein molecule of egg white protein would be partially degraded under high temperature treatment \( (12) \). After ultrasound treatment, the positions of the UDEP and SUDEP electrophoretic bands were fundamentally unchanged (none disappeared, and no new bands were generated), and the intensity of the bands was not changed, suggesting that ultrasound had not affected the molecular weight of the proteins. A previous study reached the same conclusion for ultrasound-modified egg yolk \( (30) \). Moreover, compared with UDEP, the crosslinked protein at the top of the gel in SUDEP was more intense, which indicated that sodium pyrophosphate might promote crosslinking between proteins, and this higher degree of crosslinking degree in SUDEP compared to UDEP was consistent with the results of cohesiveness (Fig. 2C).

### 3.6 Microstructure analysis

Scanning electron microscopy is an effective means to characterize the microstructure and state of a protein gel. The scanning electron micrographs of UDEP and SUDEP presented a laminated and dense granular gel structure (Fig. 4). This could be attributed to the fact that UDEP and SUDEP were thermally induced gels, their aggregation rates were much higher than their denaturation rates, and the gels were 3D structures with a high degree of protein polymerization \( (31) \). These results were similar to the results of a previously reported thermally induced egg white gel \( (32) \) and were consistent with the results of \( T_2 \)

![Fig. 3. Effect of different ultrasound power on SDS-PAGE of UDEP and SUDEP.](image)

![Fig. 4. Effect of different ultrasound power on microstructure of UDEP and SUDEP.](image)
The protein gel structure would affect its gel strength, and a dense and ordered network gel structure could enhance the protein gel strength [33,34]. In addition, the degree of crosslinking and the polymerization between SUDEP proteins were higher than that of UDEP, which was also consistent with the results of cohesiveness (Fig. 2C) and SDS-PAGE (Fig. 3). In summary, after ultrasound treatment, the gel structures of UDEP and SUDEP became denser and more stable, and the crosslinking between protein molecules may also be promoted by sodium pyrophosphate, thus further improving the gel properties of SUDEP.

3.7. Crystalline structures

The crystalline characteristics of protein molecules influence the physicochemical properties of the protein, including solubility, stability, and gelation, thereby affecting the storage, processing, and digestion stability of food in which it is incorporated. The crystalline structure changes of UDEP and SUDEP were presented in Fig. 5. The absorption peaks appeared at 9.3° and 20.1° were corresponded to the absorption peaks of ovalbumin [35]. Compared with UDEP, the absorption peak at 20.1° of SUDEP was significantly lower and wider, suggesting that sodium pyrophosphate may reduce the crystallinity of the egg white protein. Ultrasound treatment significantly changed the intensity of the peaks of UDEP and SUDEP but not the peak positions. In order to further analyze the changed in the crystalline structures of UDEP and SUDEP, the peaks were fitted and analyzed by PeakFit software. UDEP and SUDEP were composed of four characteristic diffraction peaks (I – IV) at 9.3°, 20.1°, 28.0°, and 38.5°, and peak II was the main peak. For UDEP, peak I decreased from 14.4 % to 12.2 %, peak II increased from 48.6 % to 48.9 %, peak III increased from 19.7 % to 23.1 %, and peak IV decreased from 17.3 % to 15.8 %. For SUDEP, peak I increased from 11.7 % to 14.9 %, peak II decreased from 45.3 % to 46.1 %, peak III decreased from 18.0 % to 11.5 %, and peak IV increased from 25.0 % to 27.5 %. These results indicated that ultrasound affected the conformation and crystalline structures of the protein. In addition, the crystalline structures of the protein could be related to the degree and type of aggregation and crosslinks [36], while the proportion of peak IV of SUDEP increased significantly, suggesting that a low proportion of peak II and a high proportion of peak IV in egg white protein might promote protein aggregation. Therefore, SUDEP formed a denser (Fig. 4) and more stable gel structure (Fig. 2B-D).

Fig. 5. Effect of different ultrasound power on crystalline structure of UDEP and SUDEP.
3.8. FTIR analysis

FTIR is a powerful and reliable analytical tool that can analyze a series of functional groups and is very sensitive to changes in functional groups structure, making it a popular method for characterizing the structure and conformation of proteins. The FTIR data (Fig. 6A and B) of UDEP and SUDEP revealed that regardless of the ultrasound power or the addition of sodium pyrophosphate, the peak positions did not change significantly, no peaks disappeared, and no new peaks appeared, indicating that protein functional groups had not been entirely destroyed or generated under ultrasound or synergetic sodium pyrophosphate/ultrasound treatment. Among these peaks, with the increase of ultrasound power, the intensity of the hydroperoxide group (∼OO-H) absorption peak (3442 cm⁻¹) [37] of UDEP and SUDEP increased significantly, indicating that egg white protein might produce peroxide groups under ultrasonic treatment. These peroxide groups have the potential to oxidize sulfhydryl groups (to form disulfide bonds) and other groups to promote the crosslinking of protein. The absorption peaks at 1637 and 1508 cm⁻¹ represented the amide I and II bands of proteins, respectively. These can be used to characterize the strength and formation of hydrogen bonds and the types of secondary structure in a protein [38]. The intensities of the amide I and II bands of UDEP and SUDEP were enhanced by ultrasound treatment, suggesting that ultrasound promoted hydrogen bonds between neighboring proteins, thus improving the degree of protein crosslinking.

To gain further insight into the effects of ultrasound and synergetic sodium pyrophosphate/ultrasound on the secondary structure of the protein, Fig. 6 (C, D) is the second derivative spectra of UDEP and SUDEP in the region of 1700 to 1600 cm⁻¹ (amide I band). Several characteristic absorption peaks could be observed, namely, intermolecular antiparallel β-sheets at 1700 to 1690 cm⁻¹, β-turns at 1690 to 1661 cm⁻¹, α-helices at 1660 to 1651 cm⁻¹, random coils at 1650 to 1640 cm⁻¹, intramolecular β-sheets at 1642 to 1612 cm⁻¹, and intermolecular β-sheets at 1625 to 1615 cm⁻¹ [32,38]. The intermolecular β-sheet content of UDEP and SUDEP gradually decreased, and the intramolecular β-sheet content gradually increased with the increase of ultrasound power, suggesting that ultrasound treatment could promote the aggregation of protein molecules. This is because the intermolecular β-sheets are converted into the more stable intramolecular β-sheets during the aggregation of protein [39]. For UDEP and SUDEP, intramolecular β-sheets were the major type of secondary structure of protein, that indicated that UDEP and SUDEP had relatively stable gel structures. In addition, the content of intramolecular β-sheets was much higher in SUDEP than in UDEP, indicating that the gel structure of SUDEP was more stable than that of UDEP, which were consistent with the results of T2 (Fig. 1A and B) and TPA (Fig. 2B-D). The other types of secondary structure of SUDEP were essentially unchanged with the increase in ultrasound power. This may be because SUDEP was mainly composed of intramolecular β-sheets, and its protein secondary structure was very stable; therefore, the effect of ultrasound treatment was slight. For UDEP, the content of α-helices increased significantly, indicating that the gel structure of UDEP might be more compact compared to SUDEP [32]. Overall, the gel conformation of UDEP and SUDEP became more stable after ultrasound treatment.

4. Conclusion

This paper proved that ultrasound and synergetic sodium pyrophosphate/ultrasound are effective and practical methods to enhance the gel properties of egg white protein. Ultrasound treatment, on the one hand, unfolded the protein structures of UDEP and SUDEP in the region of 1700 to 1600 cm⁻¹ (amide I band). Several characteristic absorption peaks could be observed, namely, intermolecular antiparallel β-sheets at 1700 to 1690 cm⁻¹, β-turns at 1690 to 1661 cm⁻¹, α-helices at 1660 to 1651 cm⁻¹, random coils at 1650 to 1640 cm⁻¹, intramolecular β-sheets at 1642 to 1612 cm⁻¹, and intermolecular β-sheets at 1625 to 1615 cm⁻¹ [32,38]. The intermolecular β-sheet content of UDEP and SUDEP gradually decreased, and the intramolecular β-sheet content gradually increased with the increase of ultrasound power, suggesting that ultrasound treatment could promote the aggregation of protein molecules. This is because the intermolecular β-sheets are converted into the more stable intramolecular β-sheets during the aggregation of protein [39]. For UDEP and SUDEP, intramolecular β-sheets were the major type of secondary structure of protein, that indicated that UDEP and SUDEP had relatively stable gel structures. In addition, the content of intramolecular β-sheets was much higher in SUDEP than in UDEP, indicating that the gel structure of SUDEP was more stable than that of UDEP, which were consistent with the results of T2 (Fig. 1A and B) and TPA (Fig. 2B-D). The other types of secondary structure of SUDEP were essentially unchanged with the increase in ultrasound power. This may be because SUDEP was mainly composed of intramolecular β-sheets, and its protein secondary structure was very stable; therefore, the effect of ultrasound treatment was slight. For UDEP, the content of α-helices increased significantly, indicating that the gel structure of UDEP might be more compact compared to SUDEP [32]. Overall, the gel conformation of UDEP and SUDEP became more stable after ultrasound treatment.

Fig. 6. Effect of different ultrasound power on FTIR of original spectrum (A, B) and second derivative spectrum(C, D) of UDEP and SUDEP.
hydrophobic and hydrogen bonds. On the other hand, although the ultrasonic treatment slightly decreased the net charge on the surface of the protein and decreased the electrostatic repulsion between protein–protein molecules, it promoted protein–protein interactions and protein aggregation, resulting in a dense and stable protein gel structure, thus increasing the hardness, cohesiveness, and water binding capacity of UDEP and SUDEP. In particular, synergetic sodium pyrophosphate/ultrasound greatly enhanced the crosslinking between proteins in SUDEP and the stability of the secondary structure and gel structure of the protein. Hence, the gel properties of SUDEP were superior to those of UDEP. These findings could provide theoretical guidance for improving traditional Asian egg product foods production technology, including egg sausage and Japanese tofu.

CRediT authorship contribution statement

Hui Xue: Project administration, Investigation, Writing – original draft, Formal analysis, Data curation. Huiyan Liu: Project administration, Formal analysis. Na Wu: Writing – review & editing. Guowen Zhang: Writing – review & editing. Yongzhe Tu: Conceptualization, Funding acquisition, Project administration, Validation, Writing – review & editing. Yan Zhao: Conceptualization, Funding acquisition, Project administration, Validation – Writing & 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.

Data availability

No data was used for the research described in the article.

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