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Effects of ultrasound-assisted glycosylation on the interface and foaming characteristics of ovotransferrin

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ABSTRACT

Ovotransferrin (OVT) is one of the major functional proteins in egg white protein. Most of the industry only paid attention the biological activity of OVT in iron supplement, antibacterial and other aspects, few reports were carried out on its processing characteristics such as foaming, interfacial behavior such as emulsification and foaming, which was an important processing functional attribute affecting its application scenario. In this study, the effects of ultrasound-assisted glycosylation on the interface and foaming characteristics of OVT were investigated. The results showed that proper ultrasonic treatment had a significant effect on the structure and physicochemical properties of OVT glycosylation products. When ultrasonic treatment lasted for 20 min, the grafting degree of OVT was 20.98%, the particle size decreased and the absolute value of potential increased. The foaming ability of OVT increased first and then decreased after ultrasonic-assisted glycosylation treatment. The foaming ability of OVT increased from 43.54% to 96.73% and the foaming stability increased from 68.92% to 89.19% after ultrasonic-assisted glycosylation treatment for 20 min. The experimental study effectively discovered the effect of ultrasound-assisted glycosylation on the foaming property of OVT, and would provide important technical references for expanding its application in food, biology, medicine and other fields.

1. Introduction

Ovotransferrin (OVT) is a natural protein with multiple functions and is widely used in various food systems. OVT in egg white is a glycoprotein composed of single chain polypeptide, accounting for about 12% of the total protein in egg white, and it is the main protein in egg white [1]. As the second highest abundant protein in egg white, the physicochemical properties of OVT (such as solubility and foaming properties) undoubtedly greatly affect the application potential of egg white in food [2]. In previous studies, it was rarely seen to combine ultrasound and polysaccharides and apply in OVT to improve its foaming property. In this experiment, the OVT-carbohydrate complex prepared by hydrothermal method was ultrasonically pretreated, the hydrophobicity, particle size, foaming ability and foaming stability of the complex were analyzed. The effect of ultrasound-assisted glycosylation on the foaming property of OVT was explored. It provided a theoretical basis for further understanding the mechanism of OVT glycoconjugates under ultrasound.

Foaming property of food dispersions frequently played an important role in determining the quality of various foods (milk, meat, mayonnaise, ice cream, cake, bread, etc.) [3]. The structure of these foods often depended on the formation of foam and the stability of foam [4], but the foam was thermodynamically unstable, and its relative stability depended on the properties of the active surface components in the system. The foaming performance of egg white protein was prone to be affected by many factors, such as protein adsorption at the air–water interface, content of adsorbed protein, conformational rearrangement at the interface, etc [5,6]. In addition, the poor thermal stability of OVT also increased the risk of application of its foaming properties. Wu et al. found that, OVT was prone to denature and aggregate at 53 ℃ ~ 65 ℃,

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causing the change of its foaming property at the base interface [7]. Considering that the foaming characteristics of OVT played an important role in its application in food, medicine and other fields. Based on this, it was of great theoretical and practical significance to explore how to improve and enhance the foaming property of OVT.

As a safe, non-toxic and environmentally friendly means of physical modification, ultrasonic technology was increasingly used in food science, such as emulsification, solid dispersion, crystallization, degassing and extraction [8]. Ultrasonic treatment could change the physicochemical properties of proteins through continuous ultrasonic cycles in viscous media, resulting in the collapse of cavities [9], so it could improve the functional properties of food by changing the molecular characteristics of food proteins. In recent years, ultrasound has been widely applied to enhance the foaming ability of various proteins, such as egg white protein, soy protein isolate, wheat protein, meat protein, etc. [10,11]. The protein conformational change induced by ultrasound, which lead to protein folding and then exposed hydrophilic region to water phase, while hydrophobic region to gas phase, which enhanced the foaming ability of protein to a certain extent [12]. Xanthan gum (XG), with double helix conformation in water, was a kind of anionic polysaccharide with considerable practical value. The existence of its molecular conformation and charged functional groups made Xanthan gum display such “weak gel” characteristics and high shear thinning behavior (easy to flow). The property provided a long-term stable environment for colloidal systems [13]. After Xanthan gum and protein mixed, the viscosity of the liquid phase increased, resulting in the decrease of the liquid discharge rate of the foam film, thus enhancing the foam stability of protein.

In previous studies, it was rarely seen to combine ultrasound and polysaccharides and apply in OVT to improve its foaming property. In this experiment, the OVT-carbohydrate complex prepared by hydrothermal method was ultrasonically pretreated, the hydrophobicity, particle size, foaming ability and foaming stability of the complex were analyzed. The effect of ultrasound-assisted glycosylation on the foaming property of OVT was explored. It provided a theoretical basis for further understanding the mechanism of OVT glycoconjugates under ultrasound.

2. Experimental materials and equipment

2.1. Materials and reagents

The OVT was prepared in our own laboratory (with the reagent purity over 80%). The Xanthan gum, Potassium bromide, Sodium tetraborate, β- Mercaptoethanol and SDS were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). O-phthalaldehyde (OPA) was purchased from Sigma company (USA). 8-aniline-1-naphthalenesulfonic acid (ANS) was purchased from Shanghai McLean Biochemical Technology Co., Ltd (Shanghai, China). Nile blue was purchased from Sigma company (USA). All the reagents used in the experiments were analytical reagents with high purity.

2.2. Instruments and equipment

The required instruments and equipment used in the following experiments were listed as the following: Multifuge high speed freezing centrifuge (Thermo Fisher Technology (China) Co., Ltd), DF-101S collector constant temperature heating magnetic stirring water bath (Gongyi Yuhua Instrument Co., Ltd), TRACKER interface rheometer (TECLIS Interface Technology Co., Ltd) and DFA100 dynamic foam analyzer (Kruss, Germany).

3. Experimental methods

3.1. Ultrasound-assisted preparation of glycosylated ovotransferrin samples

OVT (1 mg / ml) was dissolved in phosphate buffer (0.1 mol / L, pH 7.4), and then half of the mass of XG of OVT was added to the solution and mixed evenly to prepare OVT / XG mixture and stored at 4 ℃ and hydrate overnight after mixing. Then, the ultrasonic treatment was carried out via ultrasonic cell pulverizer. The ultrasonic conditions were set as pulse mode (on for 2 s and off for 4 s), ultrasonic power (300 W) and 40% amplitude at 25 ℃. After ultrasonic treatment for 5, 10, 20 and 40 min respectively, the mixture was put into 95 ℃ water bath for stirring and heating for 1 h, and then quickly cooled to room temperature to obtain ultrasonic OVT / XG complex solution. After freeze-drying, the ultrasonic mixture was obtained and recorded as U-OVT/XG-5MIN, U-OVT/XG-10MIN, U-OVT/XG-20MIN and U-OVT/XG-40MIN, respectively. The same mixture was directly treated in 95 ℃ water bath without ultrasonic pretreatment, which was recorded as U-OVT/XG-0MIN. OVT samples without adding sugar were marked as the control group, and the obtained samples were recorded as N-OVT.

3.2. Determination of grafting degree

Referring to the method of Zhao Chengbin et al, the degree of grafting was determined by OPA reagent method [14].

3.3. Polyacrylamide gel (SDS-PAGE) electrophoresis

Referring to Las é’s method, SDS-PAGE was used to analyze the changes of protein molecular weight [15]. After electrophoresis, the cells were stained with Coomassie brilliant blue R-250 for 4 h. Then the mixed solution of methanol, glacial acetic acid and double distilled water was handled for decolorization for 12 h, and the decolorization solution was changed every 3 h.

3.4. Determination of particle size and potential

OVT and PPP-OVT were dissolved in distilled water and PBS buffer (50 mmol / L, pH = 7.4) to form 0.1 mg / ml protein solution, after fully dissolved and then hydrated overnight. The size and potential of Zetasizer Nano-ZS nanoparticles were measured at 25 ℃ for 1 min.

3.5. Determination of ultraviolet spectrophotometry

The lyophilized protein-carbohydrate complex was dissolved in phosphate buffer solution (pH = 7.4) at a concentration of 5 mg / ml. The absorbance value was determined at 420 nm wavelength, which could be regarded as the final stage of glycosylation reaction [16]. Then the concentration of the solution sample was diluted to 0.5 mg / ml, and the absorbance value was determined at 295 nm wavelength, which could be regarded as the intermediate stage of glycosylation reaction. At the same time, the above method was used to prepare the protein sugar complex solution with the concentration of 1 mg / ml, and the UV spectrum was scanned in the wavelength range of 200 nm ~ 800 nm.

3.6. Scanning of endogenous fluorescence spectrum

Fluorescence spectrometry was used to measure the fluorescence of solid colored ammonia [17]. The protein sample was dissolved in phosphate buffer (pH = 7.4) and diluted to the final concentration of 0.1 mg / ml. The excitation wavelength was set at 295 nm, the emission wavelength range was 300 nm ~ 500 nm, the slit width was 5 nm, and the voltage was 400 V.

3.7. Fourier transform infrared (FTIR) spectroscopy

KBr compression method was used for infrared spectrum scanning analysis [18]. The background (air) of each sample was deducted before scanning, and 3 parallel experiments were made for each sample.

3.8. Differential scanning calorimetry (DSC)

The thermal denaturation temperatures of OVT and PP-OVT were determined by differential scanning calorimetry (DSC). Weighed 5 mg of OVT and PP-OVT respectively, put them in an aluminum crucible, and pressed them to seal. The aluminum crucible with the sample was put into the heating furnace, with the empty aluminum crucible as the reference, nitrogen as the protective gas, the temperature was raised from 25 ℃ to 120 ℃, the heating rate was controlled at 5 K / min, and the equilibrium time was 5 min at 120 ℃.

3.9. Determination of solubility

The protein solubility was determined by Coomassie brilliant blue method [19]. BSA was used to draw the standard curve. Weighed egg white powder with different ultrasonic power and dissolved in distilled water. After centrifugation at 4 ℃ and 15,000 R / min for 15 min, the supernatant was fully mixed with Coomassie brilliant blue G-250 protein reagent (ratio 1:4), and the absorbance was determined at 595 nm. The mixture of Coomassie brilliant blue and up water was used as blank control. The absorbance was substituted into the standard curve to calculate the soluble protein content. The solubility formula was as follows:

$$NSI = soluble\ protein(mg)/total\ protein(mg) \times 100\% \quad (2\text{-}1)$$

3.10. Determination of foaming properties

Referring to the method of Sheng Long et al. [20], the foaming properties of the original white and modified protein were determined. Weighed 0.1 g lyophilized protein powder and dispersed it into 20 ml up water (5 mg / ml), then transferred the solution to a 100 ml cylinder, and sheared it for 1 min with a high-speed shear at 8000 R / min at room temperature. The volume of the sample (V_1) after 30 s stirring and the volume of the sample (V_{30}) after storing 30 min at room temperature were read respectively. The foaming ability and foaming stability were calculated according to the following equation:

$$FA(\%) = V_1/20 \times 100 \quad (2\text{-}2)$$

$$FS(\%) = V_{30}/V_1 \times 100 \quad (2\text{-}3)$$

3.11. Determination of interfacial properties

The surface tension and expansion modulus of air–water interface were measured by using tracker interface rheometer in oscillating mode. At room temperature, a thoroughly cleaned syringe with a U-shaped needle was immersed in a quartz sample cell containing protoprotein or ultrasound-assisted glycosylated protein solution (0.1% w / W). The sample cell was located between the light source and the high-speed CCD camera. About 0.5 μL bubble was injected into the liquid solution through the needle, the surface area of the bubble fluctuated sinusoidally with time at the condition of 0.05 Hz oscillation frequency and the 5% relative amplitude, and the measurement time was set for 12000 s.

3.12. Foam interface structure observation

Olympus laser confocal microscopy was used to observe the micromorphology of the stabilized foam of proalbumin and ultrasound assisted glycosylation. The prepared sample solution was added with Nile blue dye solution (1 mg/mL), cut at the speed of 8000 r/min by a high-speed shearing machine for 1 min at room temperature. The micromorphology of the foam after 30 min was observed by the same production method. The excitation wavelength of the laser scanning confocal microscope was 633 nm (Nile blue).

3.13. Determination of foam properties

Foam properties (foaming and foaming stability) were measured by DFA100 dynamic foam analyzer, and the height of foam and the change of foam structure with time were measured at the same time. In the experiment, 3 mg / ml sample solution was prepared, took 50 ml solution and added into the measuring container, the air flow rate was set at 0.3 L / min, the foaming procedure was set to stop foaming when the total height reached 180 mm, and the measurement time was 7200 s.

4. Results and discussion

4.1. Ultrasound-assisted modification effects

Grafting degree and browning degree were two factors used to evaluate the glycosylation effect. Maillard reaction was a complex process, with many intermediates and complex final products, which were affected by many factors [21]. The content of active amino acids could be used to judge the degree of Maillard reaction and expressed in the form of grafting degree [22]. As shown in Fig. 1A, the grafting degree (DG) increased significantly with the ultrasonic assisted glycosylation treatment time, and reached the maximum value of 20.98% at 20 min after ultrasonic treatment. The possible reason was that appropriate ultrasound treatment changed the spatial conformation of proteins and exposed more reactive groups (ε amino acid lysine residue group), forming better covalent bond between OVT and xanthan gum, thus leading to the increase of grafting degree. The result could also be confirmed in the following infrared spectrum.

SDS-PAGE was usually used to analyze the molecular weight composition of protein subunits. From the aspect of reaction, it was considered that the higher molecular weight of protein was an important indicator for the formation of conjugates [23]. In order to further confirm whether OVT and XG were successfully combined, SDS-PAGE analysis was carried out under non-reducing conditions. As shown in Fig. 1B, the molecular weight of the product was concentrated between 55 kDa ~ 70 kDa, and the color of the band gradually deepened and shifted upward with the increase of ultrasonic assisted glycosylation time. The reason for this phenomenon might be that the reaction between subunits of OVT and xanthan caused to form macromolecular polymer, which indicated that changes of the structure of protein occurred during Maillard reaction, further verifying the successful combination of OVT and xanthan to a certain extent.

4.2. Particle size, zeta potential, and solubility of OVT-XG

The particle size of the solution was largely affected by ultrasonic treatment time and glycosylation. After ultrasonic assisted glycosylation, the protein particle size was significantly reduced (Fig. 2A), which might be caused by the destruction of non-covalent bonds between OVT molecules by ultrasonic induced cavitation and microbeam interaction, or the protein denaturation caused by glycosylation hindering the thermal effect of ultrasonic treatment, thus reducing the aggregation of protein molecules, and then resulting in the particle size reduction [24]. Zeta potential was an important parameter that affected the stability and foaming properties of protein. As shown in Fig. 2B, the absolute value of protein potential increased from 4.14 mV to 16.07 mV after modification, which was probably due to the introduction of negatively charged xanthan gum, covalently combined with OVT and increased the surface electronegativity of protein, thus increased the absolute value of potential.

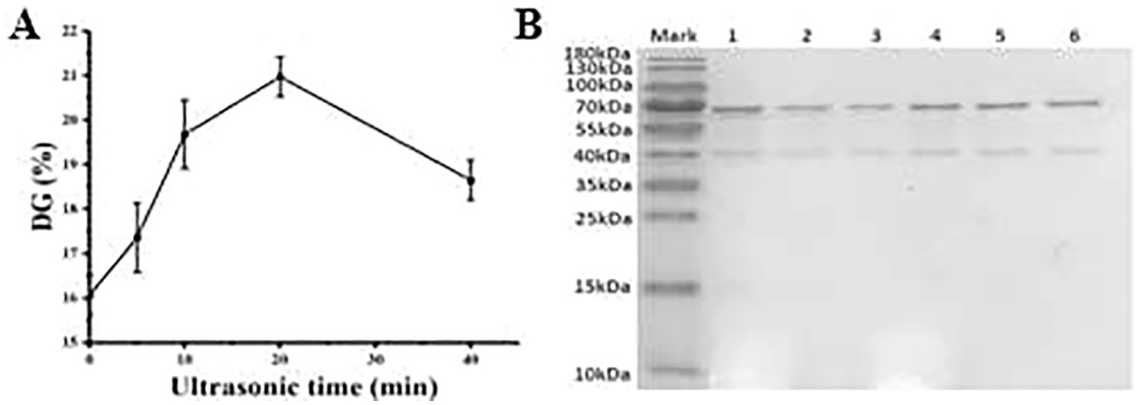

Fig. 1. Grafting degree (A) and electrophoresis diagramsunder (B, lane 1–6 represented N-OVT, U-OVT/XG-0MIN, U-OVT/XG-5MIN, U-OVT/XG-10MIN, U-OVT/XG-20MIN, U-OVT/XG-40MIN, respectively) of OVT with different ultrasound time.

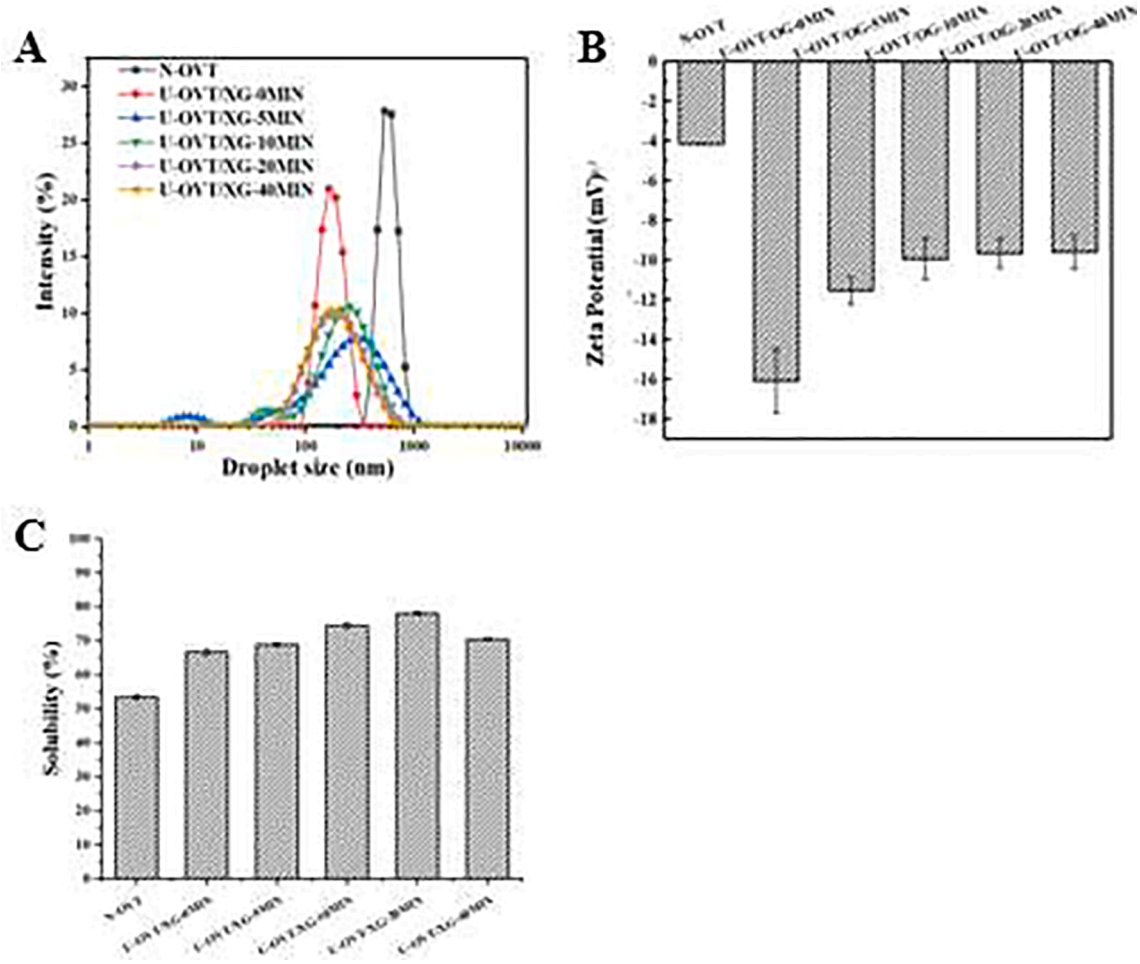

Fig. 2. Particle size distribution (A), zeta potential (B) and solubility (C) of OVT with different ultrasound time.

Solubility is one of the key factors affecting the foaming properties of protein, which has been widely studied. Maillard reaction can also increase protein solubility to a certain extent [25]. Fig. 2C showed the solubility of OVT, OVT-XG and U-OVT / XG at room temperature. Ultrasound assisted glycosylation could effectively improve the solubility of OVT, and the maximum solubility was 78.18% at 20 min, which was consistent with the change of glycosylation degree. Wang et al. found that the Maillard reaction of mung bean protein isolates with glucose had a similar effect under ultrasound assisted conditions [26]. Therefore, the combination of hydrophilic polysaccharide and protein would increase the hydrogen bonding ability of protein, thus changing the surface hydrophobicity of protein. Similar results were also found by Zhang et al., saccharification of OVT with glucose resulted in a decrease in surface hydrophobicity, and sonication-assisted saccharification resulted in a greater decrease in surface hydrophobicity [27]. These results suggested that ultrasound-assisted glycation was an effective way to reduce the surface hydrophobicity of OVT and improve the solubility of proteins.

4.3. Structural characterization and thermal stability of OVT-XG

Previous studies have shown that the formation of brown pigment (melanoid) occurred at the late stage of Maillard reaction, which could be detected at 420 nm of ultraviolet (UV) [28]. As shown in Fig. 3A, the absorbance of OVT at 420 nm increased from 0.11 to 0.24 after glycosylation, and further increased after ultrasonic assisted glycosylation. The results showed that ultrasound-assisted glycosylation could induce the browning reaction of OVT, but the absorbance value decreased significantly when the ultrasound time was 40 min, which indicated that excessive ultrasound treatment might interfere with the formation of melanoids by inhibiting the polymerization of primary or intermediate products, resulting in the reduction of browning degree. The absorbance value at 294 nm of the solution modified by ultrasonic assisted glycosylation reflected the rate change in speed of the intermediate reaction stage of glycosylation. It was seen from the figure that the absorbance increased from 0.13 to 0.50 when the solution was treated with ultrasonic for 20 min, which indicated that ultrasonic could promote the glycosylation reaction and that OVT and XG were combined successfully.

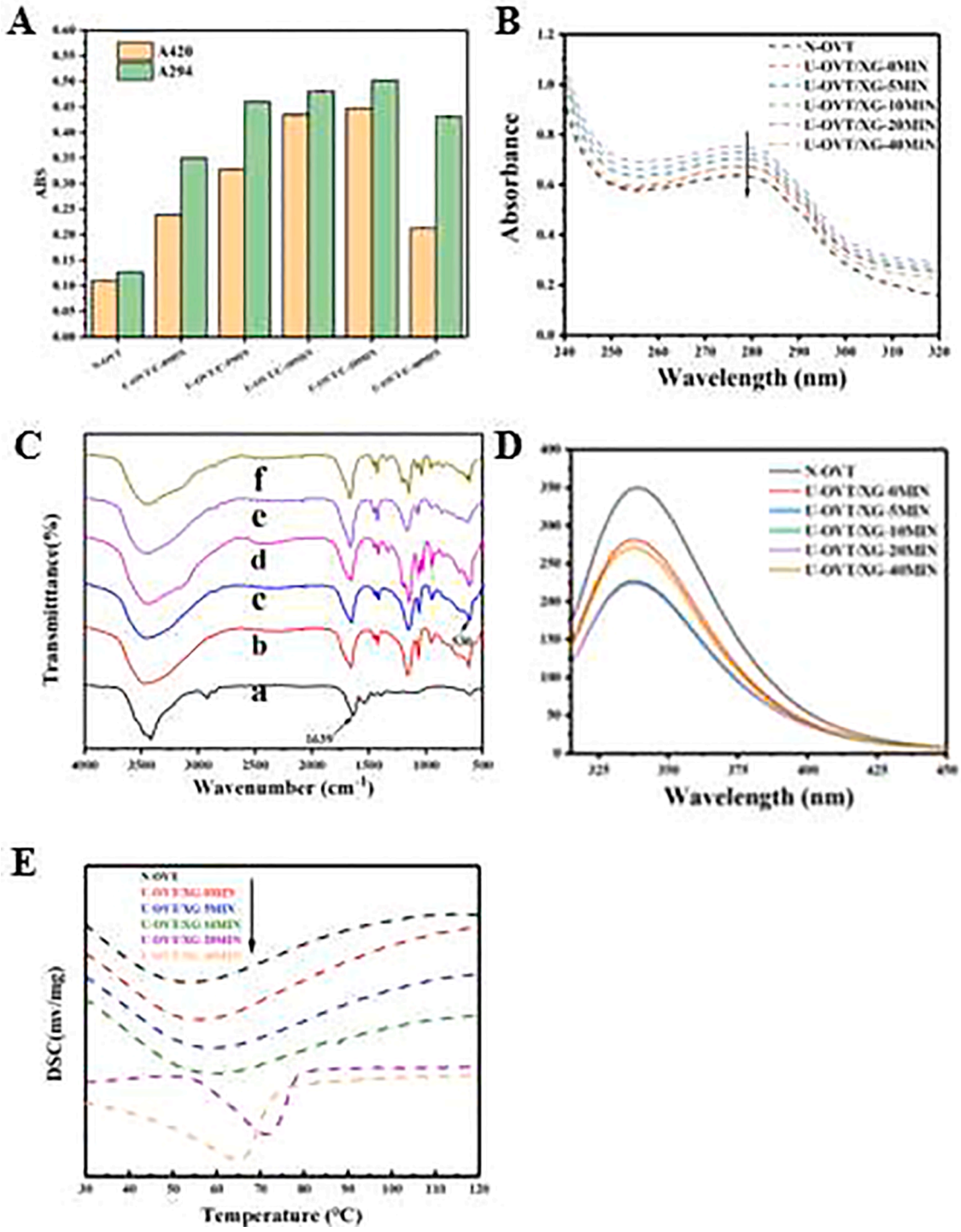

Fig. 3. The ultraviolet of A420 and A294 (A), near ultraviolet spectrum (B), infrared spectra(C), emission fluorescence spectrum(D) and DSC curve (E) of OVT with different ultrasound time.

In order to further verify the covalent binding between OVT and XG, the UV spectra were obtained in the range of 200 nm ~ 800 nm. Fig. 3B clearly distinguished the glycosylation of conjugates formed by N-OVT, OVT/XG and U-OVT/XG. Compared with N-OVT, the absorption strength of the treated protein increased at 280 nm, which was attributed to the participation of amino acids such as tyrosine, histidine, phenylalanine and glutamine in Maillard reaction [29]. Ultrasonic assisted glycosylation expanded the structure of OVT, which made the Maillard reaction much smoother between OVT and XG. The result was consistent with the reports of Yang et al, who found that the UV absorption intensity of ovalbumin increased with the increase of ultrasonic power after ultrasonic assisted glycosylation treatment [30].

The infrared structure of the glycosylated protein modified by ultrasound was determined, which could be used to observe the changes of the secondary structure of the protein. As shown in Fig. 3C, in the wavelength range of 3700 cm^{-1} ~ 3200 cm^{-1}, the peak shape of U-OVT / XG was significantly stronger than that of N-OVT, which might be caused by the increase of stretching vibration intensity of –OH bond after Maillard reaction. Maillard reaction led to the introduction of polysaccharide molecules into the protein side chain, resulting in the enhancement of hydroxyl vibration peak. The absorption peaks of OVT / XG complex changed in varying degrees at 1639 cm^{-1} (amide I band), 1542 cm^{-1} (amide II band) and 1405 cm^{-1} (amide III band), which were attributed to C = O stretching vibration and N–H bending vibration, which also indicated the change of protein structure, thus explained the glycosylation reaction from another side. In the wave number range of 1260 cm^{-1} ~ 1000 cm^{-1}, there was an obvious absorption peak, stronger than that of N-OVT, which was a typical feature of sugar molecules forming complex with protein by covalent bond [24], Such findings indicated that OVT binds XG in the form of covalent bond before and after Maillard reaction.

Fluorescence spectroscopy was used to evaluate the conformational changes of tryptophan residues and characterize the tertiary structure of proteins [31]. The typical absorption peak appeared at 338 nm (Fig. 3D), and the fluorescence intensity of OVT was slightly blue shifted compared with that of N-OVT due to ultrasound-assisted glycosylation. This might be due to the slight aggregation of protein that led to the occurrence of fluorescence quenching caused by the explosion of chromogenic groups to solvent and the reduction of fluorescence intensity. Combined with the previous experimental results, the higher the degree of glycosylation was, the stronger the screening effect of ultrasound-assisted glycosylation on tryptophan residues would be, so the greater change in protein conformation would occur.

DSC was used to investigate the effect of ultrasound-assisted glycosylation on the thermal properties of OVT. As shown in Fig. 3E, the denaturation temperature of N-OVT was about 54.9 ℃. With the increase of ultrasonic assisted glycosylation time, the peak denaturation temperature of protein gradually shifted to the right, and reached the maximum denaturation temperature of 70.1 ℃ in 20 min. This might be caused by the proper ultrasonic treatment promoting glycosylation reaction, inhibiting the cross-linking reaction between proteins and forming more polymers, The results showed that ultrasound assisted glycosylation could improve the denaturation temperature and thermal stability of OVT. However, when the ultrasonic time was 40 ℃, the peak value of denaturation temperature shifted to the left, and the denaturation temperature decreased to 65.8 ℃, indicating that excessive ultrasound would cause protein folding to produce aggregation structure, which was consistent with the previous research results.

The above research results showed that ultrasonic treatment promoted the combination of OVT and XG, changed the secondary structure and tertiary conformation of OVT to a certain extent, and enhanced the thermal stability of OVT, which may be beneficial to foam formation and stability.

4.4. Evaluation of foam properties of OVT-XG

Foaming occurs at the gas–water interface, which is related to the surface hydrophobicity and molecular flexibility of proteins [32,33]. Fig. 4A and 4B showed the foaming properties of N-OVT and ultrasound-assisted glycosylated OVT at the gas–water interface. Fig. 4A showed that the foaming ability of ultrasound-assisted glycosylated OVT increased from 43.54% to 96.73%, and then decreased, reaching the maximum after 20 min of ultrasound-assisted glycosylation. It could be seen from Fig. 4B that the foaming stability increased from 68.92% to 89.19%. The reason for the improvement of foaming ability might be that the structure of protein unfolded, hydrophobic groups were exposed and flexibility increased during Maillard reaction. The expansion of peptide chain made the interaction between hydrophobic residues stronger than the natural state, so as to improve the foaming stability.

DFA 100 dynamic foam analyzer was used to measure foaming capacity, half-life of foam (i.e. time lasts from the half period of foam height to its initial value), dynamic change of foam and variation rule of foam height with time. Foaming ability was evaluated by the ratio of the maximum foam volume to the volume of gas consumed by foaming [34]. As shown in Fig. 4C, compared with the foaming ability of N-OVT (0.3), the foaming ability of the modified protein (1.2) was about 4 times that of N-OVT, which indicated that the ultrasound-assisted glycosylation modified OVT had good foaming properties. This might be due to the more loose and elastic protein polymer formed by covalent bond under ultrasound-assisted conditions, which could be quickly adsorbed on the gas–water interface to form an adsorption layer, so as to improve its foaming ability. The stability of foam was evaluated by measuring the decay of foam height with time, and the change was observed through the half-life of foam. It could be seen from the diagram that the foaming and foaming stability of OVT were all bad. The half-life of the foam was only 456.9 s, and it had completely defoamed at less than 1000 s. The half-life of the foam via the ultrasound assisted glycosylation modified transferrin was significantly higher than that of the original single white. With the increase of ultrasonic time, the half-life of the foam increased first and then decreased, reaching the maximum at 5314.9 min at 20 min. It could be seen that the foaming ability and foaming stability of OVT after ultrasonic assisted glycosylation treatment have been significantly improved. Fig. 4D was the attenuation curve of foam height with time under different ultrasonic treatment time. Because of the larger gravity of water, the liquid drainage would start immediately after the formation of foam. The polymerization and disproportionation of foam would also cause instability of foam. With the extension of time, the volume of liquid increased, the polymerization and defoaming of foam increased, and the height of foam gradually decreased [35]. But at 10 min and 20 min, the height of foam dropped relatively slowly. This might be due to the large viscosity of the mobile phase and the rapid decrease of interfacial tension. The polymerization between the foam and the foam was not easy to occur, and the drainage velocity was weakened, thus improving the stability of the foam.

The dynamic changes of the number and size of bubbles were monitored in real time to further reflect the foaming characteristics before and after protein modification, as shown in Fig. 4E. After horizontal comparison, it was found that with the extension of time, the number of bubbles decreased and the size increased, which was caused by the diffusion of air, leaching, agglutination, disproportionation and other reactions. Longitudinal contrast showed that with the increase of ultrasonic time, the performance of foam increased first and then decreased.

4.5. Mechanism of foam properties improvement

4.5.1. Interfacial adsorption behavior and surface pressure

The foaming properties of proteins are related to their adsorption behavior at the gas–water interface [29]. Therefore, the gas–water interface behaviors of OVT and U-OVT / XG were analyzed to

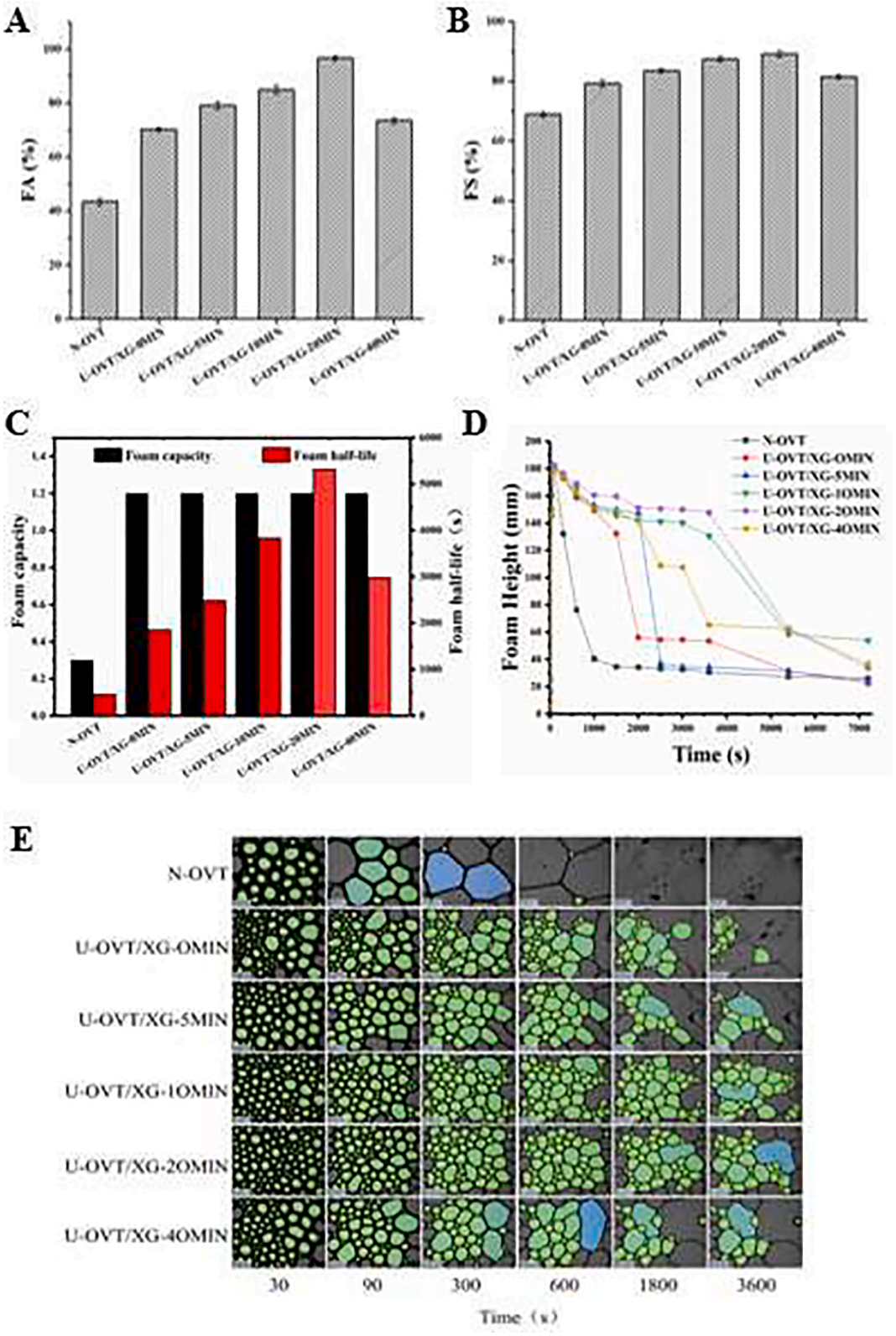

Fig. 4. Foaming property (A), foaming stability (B),foaming ability and half-life of foam (C), change curve of foam height (D) and change graph of bubble size with time (E) of OVT with different ultrasound time.

understand their foaming properties. Fig. 5A showed the change of the surface tension of the original protein and the modified protein on the air–water interface with the adsorption time (T). It could be seen that in the first 500 s, the protein polysaccharide complex quickly adsorbed on the air–water interface, resulting in the rapid decrease of the surface tension. From 500 s to 1000 s, the surface tension decreased slowly until constant. However, the surface tension decay rates of different samples were different, which indicated that the diffusion and adsorption rates of the samples were different. The surface tension decay rate of N-OVT was the slowest, and the surface tension equilibrium value was the highest,

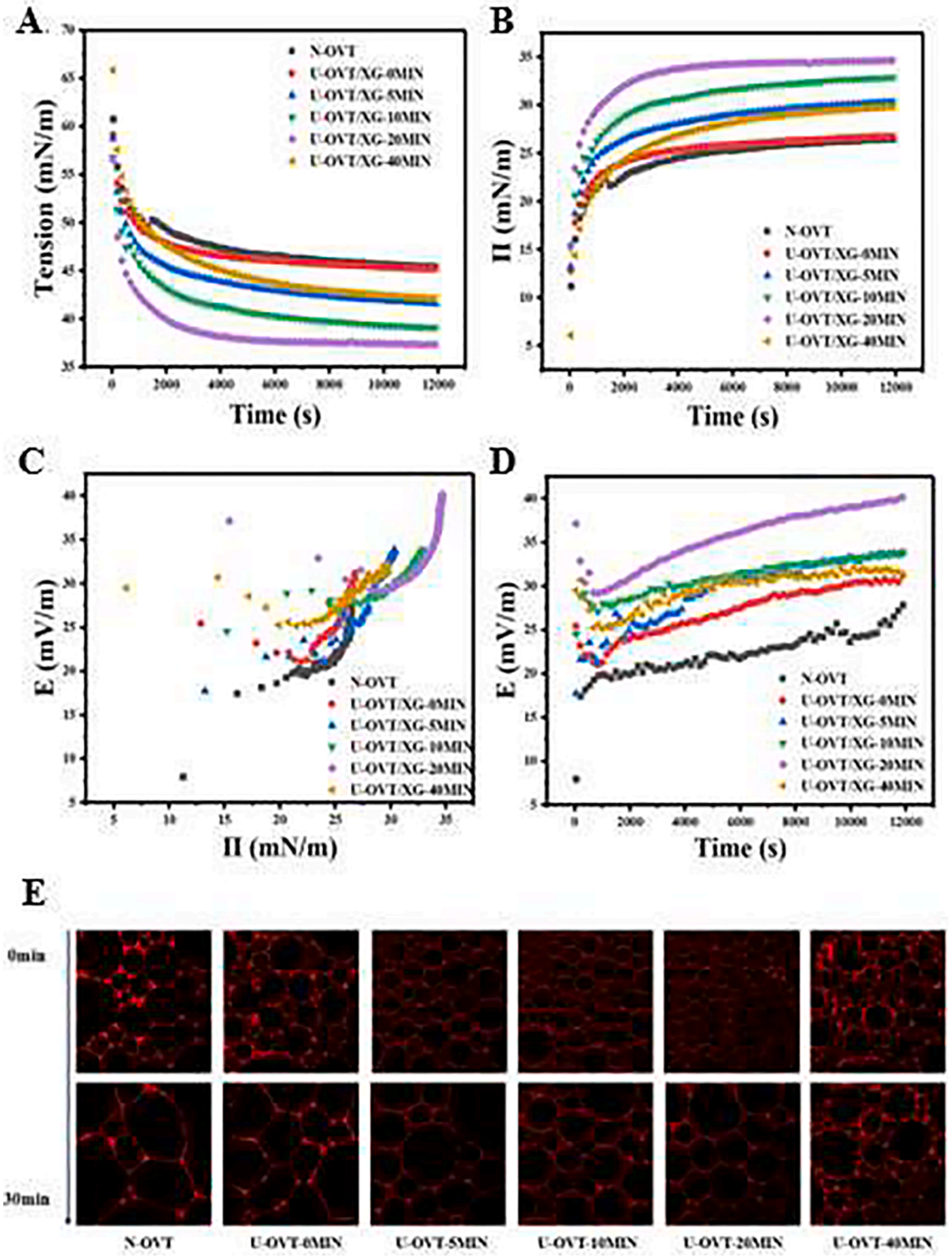

Fig. 5. Surface tension (A), surface pressure (B), E-π curve (C), viscoelastic modulus (D) and foam interface structure (E) of OVT with different ultrasonic time.

which indicated that the surfactant was poor and the adsorption kinetics was slow. On the contrary, after ultrasonic assisted glycosylation treatment, the surface tension decay rate was greatly improved, which was most obvious at 20 min of ultrasonic assisted treatment, so it had faster adsorption rate and better foaming performance. This result corresponded to the best foaming performance of OVT/XG at 20 min of ultrasonic treatment (Fig. 4A). Fig. 5B showed the surface pressure of OVT at the gas–water interface for different ultrasonic assisted glycosylation time (π)with the change of adsorption time (T). The surface pressure increased rapidly in the first 1 000 s, but it increased slowly when the adsorption time was between 1 000 s and 12 000 s. The results of interfacial tension and surface pressure echo that the rapid adsorption of ultrasound assisted glycosylation treatment on the interface facilitates rapid formation of elastic interfacial film, thereby improving foam stability.

4.5.2. Surface film rheology

The E- π curve and its slope reflected the equilibrium state of adsorbed substances on the interface [36]. Fig. 5C showed the E- π Curve of OVT at the gas–water interface under different ultrasonic assisted glycosylation time after ultrasonic glycosylation. When π value was higher than 17 mN/m, the E of OVT forms at the interface greatly increased with the increase of π. At this moment, the slopes of the E- π curves were significantly greater than 1 (The ideal gas slope of E- π curve), which indicated that there was a stronger interaction between protein residues adsorbed on the interface [37]. However, the electrostatic repulsion between particles might be enhanced and the interaction between particles might be inhibited when the ultrasound was applied for 40 min, resulting in the decrease of the slope. Fig. 5D showed that the viscoelastic modulus (E) of OVT adsorbed on the gas water interface varied with the adsorption time (T) at different ultrasonic assisted glycosylation time. The maximum E value of OVT/XG was 20 min under ultrasonic treatment, showing that the adsorption layer of protein polysaccharide at the interface was the most elastic and rigid, which was conducive to the stability of foam properties. The result of the study was consistent with the findings of foaming stability.

4.5.3. Foam interface structure

The morphological changes of the bubbles observed by laser confocal microscope could reflect the bubble characteristics of ultrasound-assisted glycosylation modified OVT from a microscopic point of view, thus revealing its interface structure. Results were shown in Fig. 5E that the freshly prepared foam was evenly stained by Nile blue, and the fluorescent protein (marked red) was located around the foam. It could be seen that the shape of N-OVT foam was irregular and large, and the size was not uniform. With the increase of ultrasonic assisted glycosylation time, the number of foams gradually increased, and the size and distribution were more uniform. A more uniform and more complete interfacial film tends to form at the gas water interface. This result confirmed the promotion effect of ultrasound assisted glycosylation on OVT foaming. Due to the phenomenon of drainage, polymerization, disproportionation and so on in the process of foam placement can be observed from the diagram [37]. The amount of shear prepared foam reduced to varying degrees after placing 30 min, and the diameter increased. However, compared with N-OVT, the modified protein foam had a relatively large number and relatively regular shape, so ultrasound assisted glycosylation improved the foam stability of OVT. This was consistent with the results of bubble size changing with time (Fig. 4E)

5. Conclusion

In this study, the effects of ultrasound-assisted glycosylation on the physicochemical and foaming properties of OVT were compared and analyzed. The results showed that ultrasound-assisted glycosylation improved the dispersion of OVT in solvent, reduced the particle size, increased the electronegativity and made the solution more stable. Ultrasonic treatment could significantly improve the degree of glycosylation of OVT, and the highest degree of grafting of OVT was 20.98% when ultrasonic assisted glycosylation for 20 min. Meanwhile, the denaturation temperature of OVT increased from 54.9 ℃ to 70.1 ℃, and its thermal stability increased. Ultrasound assisted glycosylation could effectively improve the solubility of OVT, and the maximum solubility was 78.18% at 20 min, which was consistent with the change of glycosylation degree. The foaming ability of OVT increased from 43.54% to 96.73%, and the foaming stability also increased significantly. Ultrasound assisted glycosylation made the surface tension of OVT decrease rapidly and the surface pressure increase, which made the protein particles a faster adsorption rate, which was conducive to the formation of viscoelastic film, so it had high foaming property and foaming stability.

The experimental results showed that the modified OVT was suitable as a new foaming agent and widely used in food, cosmetics and pharmaceutical industries. However, there was still a lack of systematic research on its specific application fields and product situation, and the influence mechanism of ultrasound-assisted glycosylation modification of OVT to improve its foaming ability is not deep enough, which needs to be further strengthened.

CRediT authorship contribution statement

Shugang LI: Project administration, Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Shan ZHANG:** Investigation, Data curation, Writing – original draft. **Ying LIU:** Investigation, Formal analysis, Writing – review & editing. **Xing FU:** Resources, Investigation, Writing – review & editing. **Xiaole XIANG:** Methodology, Data curation, Writing – review & editing. **Sihai GAO:** Resources, Conceptualization, Supervision, 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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