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Functional and structural properties of glycosylation ovalbumin with pectin through wet-heating and ultrasound method

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Abstract

Due to the good functional characteristics and nutritional value, ovalbumin (OVA) has a widely ranges of applications in food industry. But the characteristics of being susceptible to external conditions, such as processing, storage conditions and food ingredients, makes it display a relative lower functional property and limits its applications. Glycosylation is a natural, green approach of protein modifications that has an impact on its functional properties. In this work, we prepared the OVA-pectin conjugates through wet-heating and ultrasound glycosylation to investigate the effects on the functional and structural properties of OVA. Both wet-heating and ultrasound treated OVA at present of pectin showed higher emulsify and foam properties than untreated OVA. The results of particle size, SDS-PAGE, FT-IR, and SEM indicated that the covalent binding of pectin with OVA molecule. This study suggests that wet-heating and ultrasound glycosylation with pectin are promising method to improve the functional properties of OVA.

Keywords: ovalbumin; glycosylation modification; pectin; wet heating method; ultrasound method.

Practical Application: Ovalbumin is an excellent source of animal protein with great nutrition. However, it appears a highly aggregated conformation in aqueous solution and has low solubility limiting its application. This study investigated the effects of glycosylation through wet-heating and ultrasound method with pectin on the functional and structural properties of ovalbumin. Both glycosylation modification methods significantly improved the functional properties of ovalbumin. Glycosylation of ovalbumin with pectin through wet-heating and ultrasound method can be recognized as effective methods to improve the functional properties of ovalbumin and applied in food industry.

1 Introduction

Ovalbumin (OVA) is the most abundant protein of egg white, accounting for about 54% of the egg white protein (Zhang et al., 2020). It is consisted of 17 kinds of amino acids, about 385 residues, of which the content of hydrophobic amino acids is more than a half (Razzak & Choi, 2021; Sang et al., 2018). And the structural feather of number of hydrophobic residues on the surface makes it widely used as emulsifiers and foaming agents to enhance food stability and improve sensory and texture (Sheng et al., 2019). But it is susceptible to the processing and storage conditions such as solution pH, ambient temperature, ionic strength and so on, makes it display a relative lower functional property and thus limits its applications. Consequently, it is necessary for the OVA to be modified for improving its functional properties.

Protein modification methods include physics, chemicals and enzymatic (Abedi & Pourmohammadi, 2021; Lee & Kim, 2005; Li et al., 2019). Among these methods, glycation is a green, safety and economical modification method to enhance the functional characteristics of protein (Zhang et al., 2019). The glycation process is the primary stage of Maillard reaction. It is covalent reactions that occurs between the free amino group of the protein and the carbonyl group of saccharides (Zhao et al., 2017). The reaction process is simple, no chemical substances or enzymes added, and the reaction conditions are easy to control. Many studies have also verified that glycosylation modification was an effective approach to enhance the solubility, emulsification, foaming and gelation of the proteins (He et al., 2021; Wei & Huang, 2019; Zheng et al., 2020; Ai & Jiang, 2021). And the key of the glycosylation modification is to choose the suitable saccharides. Owing to the superior surface activity and solubility, monosaccharides such as glucose (Yan et al., 2022), galactose (Li et al., 2022) and ribose (Wang et al., 2022a) had been successfully used in the protein glycosylation. However, the research on the applications of polysaccharides in the protein glycosylation is still limited (Ai et al., 2021). Pectin is a class of acidic heteropolysaccharides consisting of D-galacturonic acid linked by α-1,4-glycosidic bonds, in addition to neutral sugars such as arabinose, galactose, rhamnose, and xylose. It contains a variety of carbonyl groups, which can react with amino groups of proteins through Maillard reaction. Therefore, since the good emulsifying and thickening ability, easily obtain and cheap price of pectin, it is an interesting concept to bond the pectin with OVA by glycosylated reaction and make the improvement of functional properties.

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Protein is modified through the Maillard reaction by traditional dry heat and moist heat method. Compared with the dry heat method, moist heat method conducts the reaction in solution, which greatly shortens the reaction time. Ultrasound offers significant advantages over traditional heat treatment, such as low cost, highly efficient, preservation of food nutrients and environmentally (Scudino et al., 2022). Moreover, ultrasound has been demonstrated to improve the functional properties of protein by reducing the size of the aggregates and increasing solubility (Glover et al., 2022). Ultrasound-assisted glycosylation can make up for the disadvantage of high equipment requirements, large energy consumption, and difficulty in large-scale production. In protein research, it has been applied to modify the structure of protein, such as alpha-lactalbumin (Wang et al., 2022b), bovine serum albumin (Xu et al., 2020), fava bean protein (Martínez-Velasco et al., 2018), and pea protein (Chen et al., 2022). It had been investigated that ultrasound can modify the glycation of ovalbumin with xylose thereby improving the foaming properties (Fu et al., 2019). In addition, as a new technology, the sensory acceptability of samples after ultrasonic treatment is good. There are studies have shown that ultrasound treatment can improve the odor, appearance and cloudiness of food products, which are more conducive to the application of ultrasound in the food industry (Ribeiro et al., 2022). To our knowledge, there are no detailed studies so far on ultrasound glycosylation modification of ovalbumin with pectin to make it suitable for processing.

In this study, the effects of glycosylation induced by wet-heating and ultrasound-assisted on the changes of emulsify ability, emulsion stability, foaming ability and foam stability of OVA were evaluated respectively. And structural properties of the OVA-pectin, such as secondary structure, particle size, zeta potential, morphology and molecular weight distribution, were also examined.

2 Materials and methods

2.1 Materials

Ovalbumin (food grade, 90%) and pectin (food grade, 45 kDa) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd (Shanghai, China). Soybean oil was obtained from a Supermarket in Jilin, China. Hepes electrophoresis solution, SDS-PAGE gel preparation kit Coomassie brilliant blue rapid staining solution were supplied from Biyuntian Biotechnology (Shanghai, China). Potassium bromide and sodium lauryl sulfate were provided by Sigma (St. Louis, MO, USA). All other chemical and reagents were all analytic grade.

2.2 Sample preparation

Ovalbumin-pectin glycosylated products were prepared by wet-heating and ultrasound through Maillard reaction, as reported previously (Sheng et al., 2022). Ovalbumin and pectin were dissolved (1:1, w/w) in 20 mM sodium phosphate buffer solution (pH 7.5). The ionic strength was adjusted to 400 mol/L with sodium chloride, the pH of the solution was adjusted to 6 with 1 M HCl. In order to completely dissolve the protein and pectin, samples were stirred on a magnet stirrer (HJ-4, Jintan Liangyou Instrument Co. Ltd., Changzhou, China) at room temperature for 10 min. Other 50 mL same protein and pectin mixtures were heated at 90 °C concurrently for 50 min to carry out glycation, which was named as HG-OVA. An ultrasound cell crusher (JY92-2D, Xinzhi Biotechnology Co. Ltd., Ningbo, China) was used to sonicate 50 mL of mixture solution at 400 W for 70 min at 60 °C, which was designated as UG-OVA. Individual OVA with the same conditions of ultrasound and bath heating respectively were used as controls, which define as U-OVA and H-OVA. Finally, the samples were freeze-dried into powder and stored in a sealed bag for later use.

The grafting degrees of UG-OVA and HG-OVA were 38.94% and 40.56%, and the degree of browning were 1.24 and 0.88, respectively.

2.3 Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were measured using the method described by (Xie et al., 2020) with minor modifications. In brief, 20 μL of reaction samples were mixed with 5 mL of sodium phosphate buffer solution (20 M, pH7.2) and 5 mL of soybean oil were slowly added while stirring in a 50 mL beaker. The mixtures were homogenized at 2000 r/min for 2 min at room temperature. The prepared emulsion was sampled 1 mL from the bottom of the solution at 0 min and 10 min, respectively, and added to the 20 mL of 0.1% (w/v) SDS solution. The absorbance of the solutions was determined using an UV spectrophotometer (UV-2550, Beijing Huaerda Technology and Trade Co., Ltd., Being, China) at 500 nm and the SDS solution (0.1% (w/v)) was used as blank. The EAI and the ESI were determined according to following formula (Equations 1-2):

$$EAI\left(m^2/g\right) = \left(A_0 \times 2.303\right) \times 2 \times N \times 10^{-4} / \varphi LC \quad (1)$$

$$ESI = A_0 \times \Delta T / \Delta A = A_0 \times \Delta T / \left(A_0 - A_{10}\right) \quad (2)$$

Where A_0 and A_{10} represent the absorbance values at 0 and 10 min at 500 nm wavelength, respectively, ΔT = 10 min. N means the dilution ratio; φ represents the fraction of oil phase in the system volume (φ = 0.5); C represents the protein concentration (g/mL); L represents the light path of the cuvette (1 cm).

2.4 Foaming Ability (FA) and Foam Stability (FS)

Foaming ability (FA) and foam stability (FS) were analyzed by the way described by Kuan et al. (2011). Dissolve the samples with distilled water to 5% (w/v) in 100 mL plastic measuring cylinders. The foams of solutions were formed using high-speed homogenizer (Vortex-Genie 2, Scientific Industries, US) for 1 min at 12000 rpm. Recording the foam volume at 0 min after homogenization and after 30 min. The FA and FS were accounted by Equations 3-4:

$$FA = V_1 / V_0 \quad (3)$$

$$FS = V_2 / V_1 \quad (4)$$

Where V_0 represents liquid volume at the initial stage, V_1 represents the total volume at 0 min after homogenization, and V_2 represents the total volume at 30 min after homogenization.

2.5 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was followed as the way represented by Vinayashree with slight changes (Vinayashree & Vasu, 2021). The reduced SDS-PAGE was performed using the 12% running gel and 5% stacking gel. The samples were thoroughly solubilized in Tris-HCl buffer (2 mg/mL) which contains 0.1% bromophenol blue, 10% (w/v) glycerol, 5% (w/v) mercaptoethanol, and 2% (w/v) SDS. Boiling the sample solution in water bath for 5 min before loading. The samples were cooled to ambient temperature in an ice bath subsequently and 15 μL of each sample (6 mg/mL) was loaded into gel lanes mounted in an electrophoresis equipment (Bio-Rad Co., Ltd, America). A constant voltage of 120 V for 1 h was set as the condition for electrophoresis. After electrophoresis, gels were stained with 0.25% Coomassie Brilliant Blue R250 for 1 h and then decolorize in the solution containing 10% methanol and 7% acetic acid.

2.6 Fourier Transform Infrared Spectroscopy (FT-IR)

Analysis of secondary structure changes of OVA after glycosylation by FTIR spectroscopy (IRPRESTIGE-21, Honshimazu, Co, Ltd., Japan). The freeze-dried samples (2 mg) were mixed with 198 mg KBr and ground to a homogeneous powder using a mortar then pressed to pellets. The FTIR spectrum of samples were scanned 32 times in the wavenumber range of 500 to 4000 cm^{-1} (Sheng et al., 2020).

2.7 Scanning Electron Microscopy (SEM)

The morphology of OVA before and after glycosylation was observed with SEM (Phenom-Pro-X, Phenom-World Co., Ltd., Netherlands). Before imaging, all freeze-dried powders were adhered to the black adhesive strip and sputtered with a thin coating gold. The micro appearances of the samples were observed through SEM under different magnifications.

2.8 Measurement of zeta potential and particle size

The zeta-potential and average particle size of samples were measured on a Laser particle size analyzer (Nano-ZS 90 instrument, Malvern, UK) at room temperature in accordance with the previous method (Zhou et al., 2021). Samples were diluted with distilled water at the ratio of 1:200. Prior to determining, the dispersions were centrifugated at 10000 × g for 15 min to detach any insoluble material to reduce multiply scattering effects.

2.9 Statistical analyses

According to the above methods, all experiments were repeated three times. The dates were presented as mean value ± standard. Statistical analyses were performed through 24.0 version SPSS software (IBM Corporation, Chicago, IL, USA).

3 Results and discussion

3.1 Emulsifying properties

Emulsification is one of important functional properties of protein, which were examined by measuring EAI and ESI. The EAI and ESI of native and glycated OVA were described in Figure 1. Both EAI of U-OVA and H-OVA were significantly lower ($p < 0.05$) than that of the native OVA, indicating that heating and ultrasonication declined emulsion formation of OVA. The reason was that the extensive protein unfolding caused by over-heating and over-ultrasonic processing, could lead to the aggregation of OVA, producing water-insoluble OVA molecules, at the oil/water interface preventing OVA molecules from being adsorbed (Dev et al., 2021; Zhong et al., 2019). However, the glycosylated group, HG-OVA and UG-OVA samples, exhibited stronger emulsifying characteristics compared to the native OVA, which demonstrated that the glycosylation treated by heating and ultrasonic really significantly improved the EAI of the OVA ($P < 0.05$). The improvement of OVA emulsification ability after glycated was mainly attributed to the tighter interfacial bonding of covalent glycoconjugates than that of the corresponding non-covalent ones, which could help to assemble the glycoconjugates into the viscoelastic and strong multilayer membranes around the oil drops to avoid coalescence, flocculation and creaming (Zheng et al., 2022). The graft can be quickly and tightly adsorbed on the water/oil interface, thus enhancing the emulsification of OVA (Chen et al., 2018). Data also showed that the EAI of the UG-OVA was significantly higher ($p < 0.05$) than that of HG-OVA, which indicated that glycosylation OVA with pectin through ultrasonic treatment was an efficient way to enhance the EAI of OVA. In addition, the ESI of HG-OVA and UG-OVA showed no significant changes compared to the native OVA.

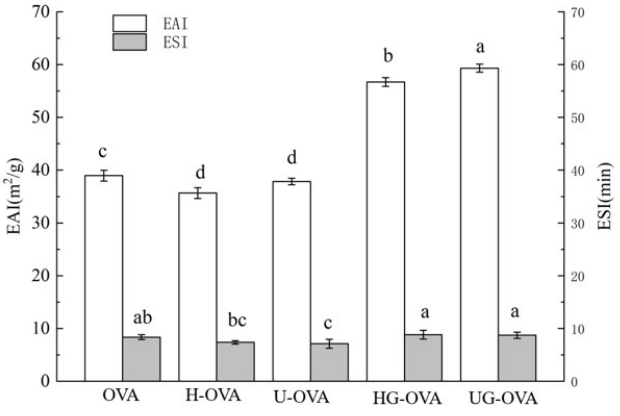

Figure 1. Changes in emulsifying (EAI) and emulsification stability (ESI) of OVA after glycated with pectin through Wet-Heating and Ultrasound Method. OVA, native ovalbumin; H-OVA, ovalbumin solution treated by heating (90 °C for 50 min) in water bath; U-OVA, ovalbumin solution treated by ultrasound (500 W for 70 min at 60 °C); HG-OVA, the mixture of ovalbumin and pectin treated by heating at 90 °C for 50 min; UG-OVA, the mixture of ovalbumin and pectin treated by the high-intensity ultrasound at the power of 500 W for 70 min at 60 °C. Different superscript letters in the figure denote significant differences ($p < 0.05$).

3.2 Foaming properties

Figure 2 shows the FA and FS of various OVA samples. For H-OVA, FA shows an increasing trend while a down trending in FS. In comparison to OVA, both FA and FS of U-OVA demonstrated comparably big differences ($P < 0.05$). The reasons might be as follows. The structure of OVA was unfolded and assembled after heating treatment, increasing the molecular force between the proteins, which makes it easy to form foam with the surrounding air (Zhang et al., 2022); In addition, the molecular conformation, particle size and surface charge of protein had been changed after ultrasound treatment. After treated by ultrasound, the structure of the OVA was unfolded and the internal groups of OVA were exposed, and thus led to an increasing of foam properties (Dev et al., 2021). However, in the case of low foam strength, high temperature could lead to the destruction of the foam, which might be the reason for the decreased FS of H-OVA (Alavi et al., 2021). Both FA and FS indices of OVA-pectin conjugates were significantly enhanced ($P < 0.05$) in comparison with native OVA, H-OVA and U-OVA, which suggested that synergistic modification could improve the foaming ability of OVA. The FA and FS of HG-OVA increased by 30.6%, 19.7%, respectively. The FA and FS of UG-OVA increased by 28.9%, 22.4%, respectively. The electrostatic attraction and interfacial tension between pectin molecules were increased because of a high amount of hydroxyl groups were introduced (Du et al., 2021). The strength of the film formed at the water and air interface was improved, and it was not easy to break or collapse. Moreover, the steric repulsion between the two adjacent interfaces could be inhibited by covalently bound pectin protruding into the aqueous phase. Therefore, conjugated polysaccharide prevented surface bubbles from approaching, delaying the aggregation and coalescence among bubbles (Fu et al., 2019). Hence, Glycosylation OVA with pectin through wet-heating and the ultrasound methods are recognized as effective techniques of the improvement of OVA solution foaming properties.

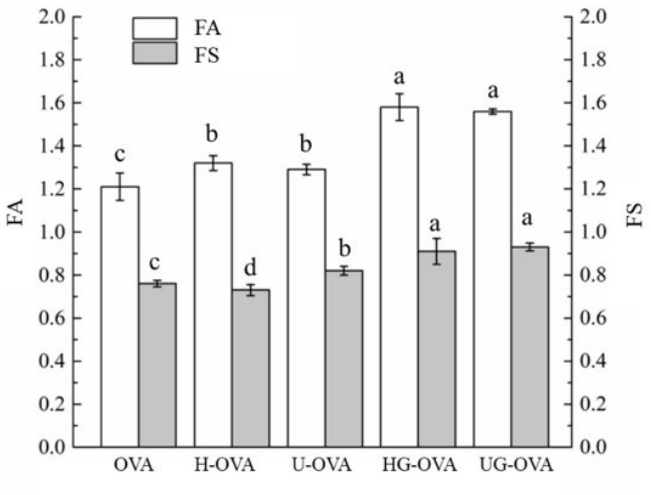

Figure 2. Changes in foaming ability (FA) and foam stability (FS) of ovalbumin (OVA) after glycated with pectin through Wet-Heating and Ultrasound Method. OVA, native ovalbumin; H-OVA, ovalbumin solution treated by heating (90 °C for 50 min) in water bath; U-OVA, ovalbumin solution treated by ultrasound (500 W for 70 min at 60 °C); HG-OVA, the mixture of ovalbumin and pectin treated by heating at 90 °C for 50 min; UG-OVA, the mixture of ovalbumin and pectin treated by the high-intensity ultrasound at the power of 500 W for 70 min at 60 °C. Different superscript letters in the figure denote significant differences ($p < 0.05$).

3.3 SDS-PAGE analysis

SDS-PAGE was used to further confirm whether the protein and pectin formed a covalent linkage and to detect molecular weight changes in the samples after the glycosylation reaction. As shown in Figure 3, the untreated OVA displayed two bands with a primary molecular weight of approximately 75 kDa and 45 kDa, representing lecithin and ovalbumin (Geng et al., 2019). Compared to the native OVA, there was no dramatic difference in the electrophoretic patterns for the H-OVA and U-OVA protein samples. This finding suggests that the molecular weight of OVA was not altered by heating and ultrasonication treatment. It had also been noted that a weak band (between 45 and 65 kDa, lane HG-OVA and UG-OVA) emerged in the higher molecular weight region after treatment by heating and ultrasonication respectively. This phenomenon suggested that treatment of heating and ultrasonication bought mild protein aggregation, which often occurred in the advanced stages of Maillard reaction (Hu et al., 2019). In addition, a new high-molecular-weight band appeared at the top of gels for two glycated samples, HG-OVA and UG-OVA, which indicated OVA and pectin form larger molecules

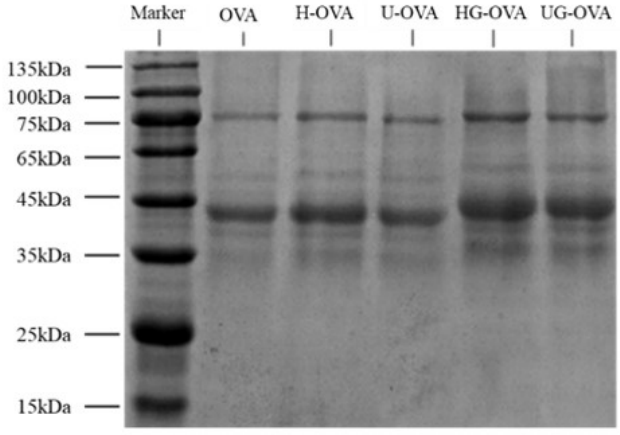

Figure 3. SDS-PAGE profiles for OVA, H-OVA, U-OVA, HG-OVA and UG-OVA. OVA, native ovalbumin; H-OVA, ovalbumin solution treated by heating (90 °C for 50 min) in water bath; U-OVA, ovalbumin solution treated by ultrasound (500 W for 70 min at 60 °C); HG-OVA, the mixture of ovalbumin and pectin treated by heating at 90 °C for 50 min; UG-OVA, the mixture of ovalbumin and pectin treated by the high-intensity ultrasound at the power of 500 W for 70 min at 60 °C.

by covalent bonds to. A new diffused bands appeared which can be visualized around the loaded edge of the gel. It demonstrates the production of a large number of large molecular masses ovalbumin-pectin conjugates were generated (Wu et al., 2022). Furthermore, one possible explanation for the highly diffuse

bands of OVA-pectin conjugates was protein molecules were grafted onto one or more polysaccharides.

3.4 FT-IR analysis

FTIR spectroscopy was performed to observe the intramolecular interactions of the protein and pectin and investigate the changes in hydrogen bonding and secondary structure of OVA treated by glycation, ultrasonication and heating. The influence of heating, ultrasonication and glycosylation on the FT-IR spectra intensity of OVA were shown in Figure 4. The spectra of H-OVA and U-OVA showed similar characteristics to untreated ovalbumin, and C-O stretching makes the appearance of a sharp band at about 1020 cm^{-1}. This phenomenon suggested that the heating and ultrasonication process may lead to changes in the secondary structure of ovalbumin. The spectra of the ovalbumin-pectin conjugates revealed noticeable differences compared to the untreated OVA. The typically characterized of glycosylation modification of ovalbumin was an increasing content of the hydroxyl groups in protein molecule, which appeared as characteristic absorption enhancement of the hydroxyl group in the infrared spectrum. The reason for the absorption peaks in the range of 3700~3200cm^{-1} after the graft modification treatment was the -OH stretching vibration of the grafted modified product, which proved that the ovalbumin glycosylated grafted pectin increases the amount of -OH (Chen et al., 2022). Meanwhile, C-O stretching caused the broad bands in the range of 3700~3200 cm^{-1} and 1260~1000 cm^{-1} and a sharp band appeared in the range of 1640~1550 cm^{-1} was induced by decrease of the bending vibration of -NH_2 (Sheng et al., 2016).

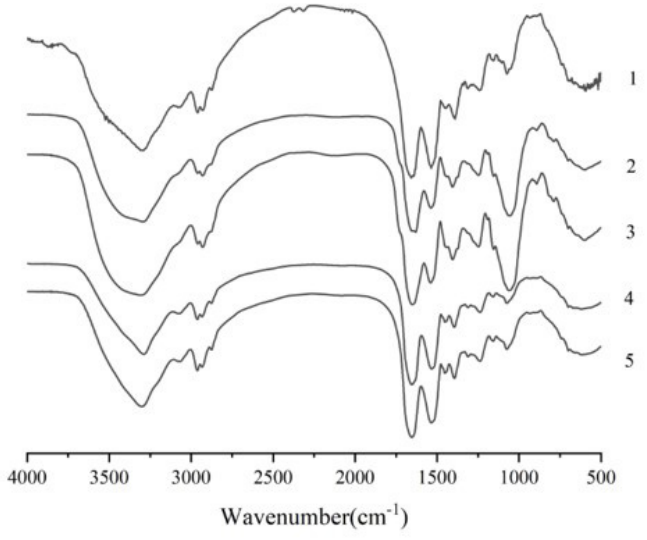

Figure 4. FT-IR spectrum characterization of OVA, H-OVA, U-OVA, HG-OVA and UG-OVA. 1: OVA, native ovalbumin; 2: H-OVA, ovalbumin solution treated by heating (90 °C for 50 min) in water bath; 3: U-OVA, ovalbumin solution treated by ultrasound (500 W for 70 min at 60 °C); 4: HG-OVA, the mixture of ovalbumin and pectin treated by heating at 90 °C for 50 min; 5: UG-OVA, the mixture of ovalbumin and pectin treated by the high-intensity ultrasound treatment at the power of 500 W for 70 min at 60 °C.

Peak at 1715 cm^{-1} corresponded to the stretching vibrations of the -C=O ester bond. When the polysaccharide was attached to ovalbumin successfully, conjugation of carbonyl and ethylenic bond reduced absorption frequency (Liu et al., 2022). The new peak in the range of 1450~1350 cm^{-1} appeared in the spectra of ovalbumin-pectin conjugates, which indicates that the absorption strength of the newly created C-N covalent bonds in the molecule increased. It can be seen that modification of glycosylation OVA with pectin through wet heating and ultrasound method respectively, caused similar glycosylation, covalent binding of pectin induced a high amount of sugar molecules, which was in accordance with the graft rate results.

3.5 Scanning Electron Microscope (SEM)

The SEM images displayed the differences in protein shape of OVA, H-OVA, U-OVA, HG-OVA, and UG-OVA (Figure 5). As for untreated ovalbumin, uniform spherical structure was described and the sphere was smooth and concave (Ma et al., 2019). In addition, H-OVA displayed large block structure with slender branches and obvious edges compared with OVA, and a small amount of incompletely expanded ovalbumin was attached to the block structure. There are a lot of slender branches and dendritic structures in U-OVA and all ovalbumin sphere structures were destroyed under ultrasonic treatment. The U-OVA had less block structure and more broken structure than H-OVA, indicating that ultrasound can destroy protein structure more completely (Zhu et al., 2018). Compared with H-OVA, the block structure of GH-OVA was reduced, presenting a dendritic and tortuous structure. The reason for the phenomenon was that the amino groups of ovalbumin and the carbonyl groups of pectin were grafted to unfold and separate the structure of ovalbumin (He et al., 2021). The structure of UG-OVA was filamentous and dendritic, and the ovalbumin was fully expended and covalently grafted with pectin molecules. Contrast with U-OVA, the molecular structure become larger and entangled with each other after glycosylation modification. Compared with HG-OVA, the dendritic structures were increased significantly after ultrasonic treatment. Therefore, glycosylation modification changed the structure of ovalbumin, making it more stretched and having a larger contact area (Zha et al., 2019). The heating treatment caused the structure to be destroyed, and the glycosylation reaction with pectin reduces the block structure (Ma et al., 2019). The ultrasonic glycosylation modification damaged ovalbumin in a large extent, and the structure was messy and winding. The changes of structure affected the emulsification and foaming functional properties of protein.

3.6 Zeta-potential and particle size analysis

Zeta-potential and particle size are essential ingredients that affect the stability and adsorption of OVA in the water/oil interface (Yu et al., 2021). The zeta-potential value has a tight relationship with the stability of colloidal dispersion. It indicates the repulsion degree among similarly charging particle (Li et al., 2018). The smaller the combined particles or individual molecules, the higher the potential difference, and the more stable the system (Duan et al., 2018). The zeta-potentials of OVA, H-OVA, U-OVA, HG-OVA, and UG-OVA are shown in

Figure. 6A. Compared with untreated OVA, the absolute value of zeta-potential of proteins significantly ($P < 0.05$) increased after different treatments and that of HG-OVA was highest. A larger number of negatively charged amino acids were exposed to the protein surface by glycosylation. A higher negative potential demonstrated a strong surface electrostatic repulsion between the molecules, which inhibited the aggregation of molecules and formed a stabilized system (Yan et al., 2022). Wang et al. (2019) discovered that the absolute value of zeta-potential of the egg white protein and isomalto-oligosaccharide conjugates by Maillard reaction significantly increased, which was in accordance with our finding. The zeta potential of H-OVA was lower than U-OVA, this may be due to the unfolded protein structure by cavitation of ultrasonic while protein molecules aggregate at high temperature resulting in some charged groups being buried inside the protein. Zeta-potential was essential for the stabilization of colloids, the large absolute values of zeta-potential allowed colloidal particulates (such as polysaccharide and protein) had a strong electrical repulsive force, which could partly explained the better foaming and emulsification of HG-OVA and UG-OVA (Ai et al., 2021). As shown in Figure 6B, the treatment of ultrasound resulted in a more dramatic reduction

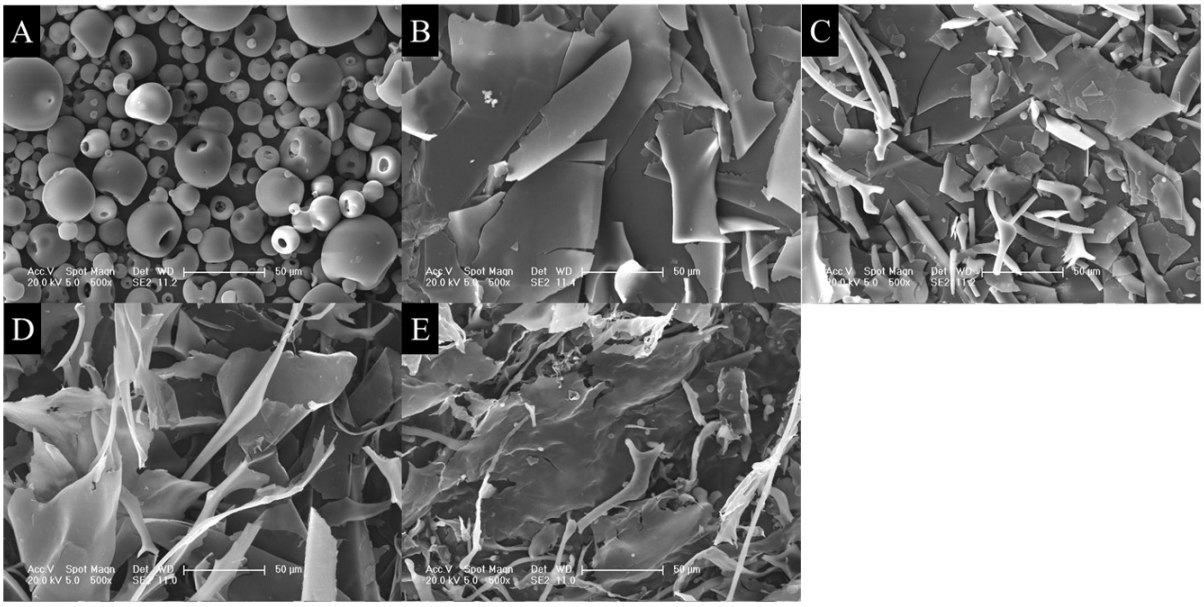

Figure 5. SEM images of OVA (A), H-OVA (B), U-OVA (C), HG-OVA (D), UG-OVA (E) (Magnification: 500×). OVA, native ovalbumin; H-OVA, ovalbumin solution treated by heating (90 °C for 50 min) in water bath; U-OVA, ovalbumin solution treated by ultrasound (500 W for 70 min at 60 °C); HG-OVA, the mixture of ovalbumin and pectin treated by heating at 90 °C for 50 min; UG-OVA, the mixture of ovalbumin and pectin treated by the high-intensity ultrasound at the power of 500 W for 70 min at 60 °C.

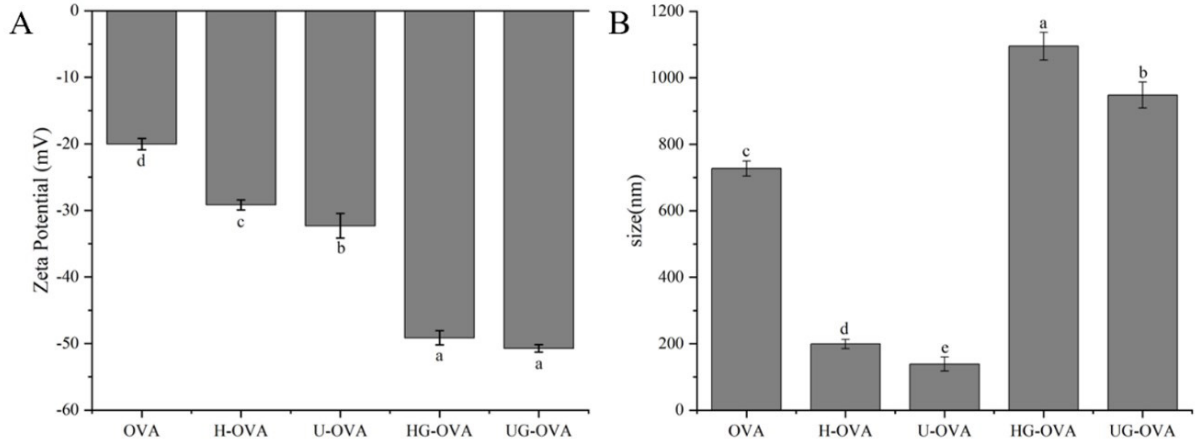

Figure 6. The zeta potential (A) and particle size (B) of OVA, H-OVA, U-OVA, HG-OVA and UG-OVA. OVA, native ovalbumin; H-OVA, ovalbumin solution treated by heating (90 °C for 50 min) in water bath; U-OVA, ovalbumin solution treated by ultrasound (500 W for 70 min at 60 °C); HG-OVA, the mixture of ovalbumin and pectin treated by heating at 90 °C for 50 min; UG-OVA, the mixture of ovalbumin and pectin treated by the high-intensity ultrasound at the power of 500 W for 70 min at 60 °C. Values are given as means ± standard deviation (n = 3). Different letters indicate significant differences between groups ($P < 0.05$).

of the OVA particle size from 727 to 139 nm. This phenomenon was contributed to the strong mechanic destructive forces produced by ultrasonic process. The particle size significantly increased ($P < 0.05$) when OVA was glycosylated by pectin. The increase of particle size may be attributed to two reasons, one part was the formation of glycosylation products, the other was the ovalbumin-pectin polymer, which was not fully grafted and glycosylated. Moreover, the particle size of HG-OVA was larger than UG-OVA, which was due to a higher grafting rate of HG-OVA (44.89%) than UG-OVA (40.17%) (Xie et al., 2020).

4 Conclusion

In this work, the OVA-pectin conjugates were acquired through glycosylation by wet heating method and ultrasound method. The grafted OVA displayed distinctly high levels of emulsification and foaming properties when compared to native OVA. Hence, the glycosylation reaction of OVA and pectin is an efficient approach to improve the properties of ovalbumin to make it more suitable for industrial production. There are in apparent differences in the extent of glycation between the wet heating method and ultrasound method. The glycosylation rate by ultrasound treatment were higher than that by wet heating treatment. And the improvements in emulsifying activity were more pronounced under ultrasound treatment than under wet heating treatment. The investigation of SDS-PAGE, FT-IR, SEM, zeta-potential and particle size indicated the improvement of the emulsification and foaming properties of OVA-pectin conjugates was attributed to the dynamic quenching of OVA caused by the successful glycation reaction with pectin and altered the polarity of the hydrophobic microenvironment of OVA to unfold conformational changes. These results indicated that ultrasound treatment could be used as an effective technique to promote the protein glycation reactions, altering conformational structure and remarkably improving the functional properties of OVA. It also had the advantages of short time-consuming, high processing efficiency and environmental protection as compared to the wet heating method.

Acknowledgements

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