Effect of ultrasound on the glycosylation reaction of pea protein isolate–arabinose: Structure and emulsifying properties

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A B S T R A C T
This study investigated the effects of different ultrasonic power and ultrasonic time on the structure and emulsifying properties of pea protein isolate (PPI)–arabinose conjugates. An examination of the absorbance and color development of PPI-arabinose (Ara) conjugates found that compared with traditional heating, the degree of glycosylation of protein reached the maximum when the ultrasonic treatment power was 150 and the treatment time was 30 min. Structural analysis revealed that the content of disordered structures (β-turn and random coil) of the protein conjugates increased, the maximum emission wavelength of the fluorescence spectrum was red-shifted, and the UV second-order derivative values decreased. The protein structure unfolded, exposing more hydrophobic groups on the molecular surface. Ultrasonic treatment improved the emulsification of protein conjugates. The emulsifying activity index (EAI) increased to 19.7 and 19.3 m²/g, and the emulsifying stability index (ESI) also increased. The contact angle and zeta potential also demonstrate that ultrasonic power has a positive effect on emulsion stability. Based on examining the thermal stability of the emulsion, the ultrasonic treatment increased the thermal denaturation resistance of the protein. This result confirms that mild sonication can increase the degree of glycosylation reaction and improve the emulsification properties of protein–Ara conjugates, providing a theoretical basis for developing foods with excellent emulsification properties.

1. Introduction

Compared with animal proteins, plant-derived proteins have a wide variety and more abundant sources, and the nutritional value of plant proteins is not even lower than that of animal proteins [1,2]. Pea protein isolate (PPI), a potential animal protein substitute, has always been the focus of food industry research because of its high nutritional value and beneficial effects on human health [3–5]. However, the solubility of PPI is weak, which limits the functional properties of the protein, such as emulsification, foaming, and gelation [6,7]. Eliana et al. [8] prepared a PPI-polysaccharide high internal phase emulsion system. Because of the instability of the composite system, it exhibited an oil release of 11% after four weeks of storage at 5°C. Some studies improved the solubility of protein solutions by adjusting the pH of the protein solution [9]. The isoelectric point of PPI is pH 4.0–5.0, and its solubility reaches 65–95% when the pH of the protein solution is 7.0–9.0 [10]. However, the pH value of the solution is too high, producing a condensation reaction of serine or cystine residues in the protein with the ε-amino group of lysine to generate toxic lysine amide.

The protein is sensitive to environmental factors such as temperature and acid, and many studies have focused on the group modification of PPI Glycosylation (Maillard reaction), a naturally occurring reaction of proteins and reducing sugars under heating conditions, has been explored to improve functional properties, especially solubility and the surface properties of proteins [11,12]. Dickinson et al. [13] found that during glycosylation, reducing sugars are covalently bound to proteins so that proteins are protected under low pH and high ionic strength solution, improving protein solubility and stability [14]. Zha et al. [15] studied the effect of gum arabic (GA) on the functional properties and flavor characteristics of pea protein concentrate (PPC). They found that the solubility and emulsifying properties of the PPC-GA composites were significantly improved within an appropriate processing time, demonstrating high physical stability.

The glycosylation reaction is time-consuming, and the complex structure inside the protein may reduce its reaction rate [16,17]. The cavitation effect produced by ultrasound has significant potential to accelerate the Maillard reaction [18]. Li et al. [19] induced the Maillard reaction of myofibrillar proteins with different molecular weights (40, 70 and 150 kDa) by ultrasound, and found that the emulsification properties of myofibrillar protein structures were improved. The particle
size of the emulsion prepared by the conjugate of 70 kDa DX induced by ultrasound was 30.12 μm. Cucheval et al. [20] prepared oil/water (O/ W) emulsions using ultrasonic-assisted emulsification and found that the emulsions had uniform droplet distribution and a droplet size of approximately 0.7 μm. However, few studies have investigated ultrasound–induced glycosylation of PPI.

Arabinose is widely used in the biological, pharmaceutical, and food industries, and is considered an aldopentose with a high market value for blood sugar control [21,22]. It is always combined with other monosaccharides and exists in plants, colloids, hemicelluloses, bacterial polysaccharides, and some glycosides in the form of heteropoly saccharides. α-arabinose (Ara) can be obtained by degrading d-glucose and exists in a free state. It can be absorbed by the human body and has high stability. Therefore, different ultrasonic power (0–300 W) and ultrasonic time (0–30 min) were used to treat PPI–Ara conjugates. The degree of reaction was evaluated by the absorbance value and color change in glycosylation products. The structural changes were determined by Fourier transform infrared spectroscopy (FTIR), fluorescence spectroscopy and ultraviolet spectroscopy. The emulsifying properties of PPI–Ara conjugates were also investigated based on emulsifying activity, emulsifying stability, contact angle and thermal stability.

2. Materials and methods

2.1. Materials

The PPI (protein ≥80 %, moisture ≤10 %, ash ≤8 %) was acquired from Yantai Oriental Protein Technology Co., Ltd. (Shandong, China), Ara (98 %, molecular weight 150.13) was acquired from Shanghai Yuanue Biotechnology Co., Ltd. (Shanghai, China), and soybean oil (Nine San Brand Grade III Soybean Oil) was acquired from Beidahuang Group (Harbin, China). All chemicals and reagents in this study were analytical grade.

2.2. Preparation of the PPI–Ara conjugates

The PPI–Ara conjugate was prepared based on the method of Li et al. [19] with some modifications. PPI (10 mg/mL) and Ara (5 mg/mL) were completely dispersed in a phosphate buffer (0.1 M pH 7.0) and hydrated overnight at 4 °C. The pH of the solution was adjusted to 7.0 with a 1 mol/L HCl solution. Then, the PPI–Ara solution was transferred into a beaker and placed in a temperature-controlled shaker at 80 °C, followed by ultrasonic processing (Scientz Biotechnology Co., Ltd., Ningbo, China) with a 1.5-cm diameter titanium probe. During this time, the temperature of the solution was adjusted with ice cubes. Samples were run at different power output levels (100, 150, 200, and 300 W) and processing times (10, 20, and 30 min). Finally, the solution was cooled in an ice bath, dialyzed at 4 °C for 24 h, and freeze-dried.

2.3. Degree of Maillard reaction

2.3.1. Degree of browning

Consistent with the method of Maria et al. [23], the sample was dissolved in deionized water (1 mg/mL), and the absorbance values were measured at 294 and 420 nm using a UV–vis spectrophotometer (UV2600, Shimadzu Instrument Co., Ltd., Japan).

2.3.2. Color change

The color of samples was measured using a Chroma Meter (ZE6000, Japan) with a diffuse illumination/0-degree viewing geometry to obtain CIE L* a* b* values [24]. The chromameter was calibrated with a standard white tile (Y = 92.2, x = 0.3162, and y = 0.3324). The color was calculated as follows:

\[
\begin{align*}
\Delta E' &= \sqrt{(\Delta L')^2 + (\Delta a')^2 + (\Delta b')^2}
\end{align*}
\]

2.4. Structure determination

2.4.1. Fourier transform infrared spectroscopy (FTIR)

The PPI–Ara conjugate powder and KBr were thoroughly mixed in a mass ratio of 1:100–1:200, and compressed into transparent tablets under dry conditions. FTIR spectra were scanned using a Nicolet iS50 (Thermo Fisher Scientific GmbH, Germany). The scanning wavenumber range was 4000–400 cm⁻¹, the resolution was 4 cm⁻¹, and the average number of scans was 32.

2.4.2. Fluorescence spectrometry

The samples were dissolved in (pH 7.0, 0.01 M) phosphate buffer and measured using a spectrofluorimeter (F-7100, Hitachi, Japan) [25]. The measurement conditions were set as follows: an excitation wavelength of 347 nm, an emission wavelength range of 300–500 nm, an excitation slit and emission slit width of 5 nm, and a medium scanning speed. The phosphate buffer solution was the control.

2.4.3. UV spectrum

Consistent with the experimental method of Dong [26] et al., with slight modifications, the sample concentration was diluted to 0.025 mg/ mL with deionized water. An unsaponified PPI–Ara conjugate was used as a blank control and scanned in the 190–300 nm range using a UV–vis spectrophotometer.

2.4.4. Surface hydrophobicity

The protein solutions before and after sonication were centrifuged, and the supernatant was used to dilute the sample with phosphate buffer (0.01 M, pH 7.0) to a concentration range of 0.05–0.4 mg/mL. The sample solution (4 mL) and 8-anilino-1-naphthalenesulfonic acid (ANS) solution (20 μL at 8 mmol/L) were mixed by vortexing to measure the fluorescence intensity. The excitation wavelength was set to 390 nm, the emission wavelength to 468 nm, and the scan speed to 10 nm/s. A linear fit was performed between the fluorescence intensity and the protein concentration in the complex, and the slope was the value of the surface hydrophobicity (Hₒ).

2.4.5. Determination of sulphhydril groups (SH)

The sonicated PPI conjugate solution was mixed with the prepared Tris-Gly buffer (0.086 mol/L Tris-0.09 mol/L Glycine-4 mmol/L Na₂EDTA, pH 8.0), followed by the addition of Ellman’s reagent (0.01 mol/L) [27], and centrifuged at 6000g for 10 min after a sufficient reaction. The supernatant was used to measure the absorbance at 412 nm with a UV spectrophotometer.

2.4.6. Protein solubility

Consistent with Chen et al. [28], the 1 % protein solution was reacted with a biuret reagent, and colorimetry was performed at 540 nm. The measured protein absorbance value was substituted into the standard curve to obtain the measured protein solubility.

2.4.7. The particle size

The particle size distribution and zeta-potential of the samples were measured using a Malvern Zetasizer Nano ZS potential and particle size distribution analyzer (NANO ZS90 Malvern Instruments Ltd. UK). The sample solution was diluted with a 0.01-M phosphate buffer (pH 7.0) dispersant. The particle refractive index was set to 1.46, the refractive index in the dispersant was set to 1.33, and the particle size distribution was measured at room temperature.

2.5. Preparation of emulsions

The sample solution (6.25 wt%) was mixed with soybean oil to a final concentration of 5 wt% of the solution. The solution was mixed for 2 min using an Ultra Turrax T25 homogenizer at a speed of 13,500 rpm, and...
the pH of the emulsion was adjusted to 7.0 with 0.1 M of NaOH to be tested.

2.5.1. Emulsifying activity index (EAI) and emulsifying stability index (ESI)

A 50 μl pipette of the emulsion was dispersed in sodium dodecyl sulfate (SDS) solution. After vortexing, the absorbance value of the sample at 500 nm was measured with a UV spectrophotometer, and the SDS solution with the same concentration was used as a blank control. After waiting for 10 min, the absorbance value of the composite emulsion was measured again.

2.5.2. Contact angle

The contact angle of the samples was determined by the sitting drop method using a contact angle analyzer (SL200KS Shanghai Solon Information Technology Co., Ltd.) at room temperature. A small amount of the complex emulsion was pipetted onto a glass slide to make a film of approximately 10 mm in diameter. A high-precision syringe was used to drop a drop of water on the surface of the film formed by the composite emulsion, and the image of the droplet was recorded immediately after falling from the syringe through a camera.

2.5.3. Zeta-potential

The zeta-potential of the emulsion was measured using a Malvern Zetasizer Nano ZS.

2.5.4. Differential scanning calorimetry (DSC)

The thermal stability of the emulsions was measured by differential scanning calorimetry (DSC). Thermal testing was performed using a DSC 250. A sample of approximately 2 mg of the emulsion was first placed in an aluminum pan and sealed with an aluminum lid. The sample was heated from 20 to 160 °C at a heating rate of 5 °C/min, and the DSC curve was recorded in a nitrogen atmosphere of 20 mL/min.

2.6. Data analysis

This study established three parallel experiments, and the experimental data were processed and analyzed using SPSS 16.0 and Origin 2021. The values marked with “±” represent the standard deviation, and p < 0.05 indicates a significant difference between the data.

3. Results and discussion

3.1. Absorbance of PPI-Ara conjugates

The glycosylation reaction refers primarily to the condensation reaction between the carbonyl group of reducing sugar and the unmodified amine group. The UV–vis absorbance at 294 and 420 nm was used to demonstrate the formation of Amadori compounds and the final stage of advanced glycation end-products (AGEs) [29]. Fig. 1 illustrates the changes in the degree of glycosylation of PPI under different sonication power levels and treatment times. Compared with the unsonicated PPI–Ara conjugate, A294 and A420 changed significantly. Moreover, A294 > A420 indicates that the early reaction of glycosylation was dominant [30], consistent with the report of Delgado Andrade et al. [24].

The degree of glycosylation of the protein increased with an increase in sonication treatment power/time and reached the maximum at 150 W/30 min. This result indicates that ultrasonication promotes the rate of protein glycosylation reaction, which may be caused by the local translational motion induced by the ultrasonic field, which increases the proximity of protein molecules to sugars. Furthermore, ultrasonic cavitation can increase the excluded volume and flexibility of proteins, accelerating the insertion of hydrophilic groups into protein structures [31,32]. This result also confirms that A294 and A420 increased significantly when the ultrasonic treatment time was prolonged to 30 min. With the prolongation of sonication time, more of the internal structure of the protein was expanded outward, and reducing sugars were then bound to the protein’s interior, significantly increasing the reaction rate of glycosylation. However, when the ultrasonic power was increased to 300 W, A294 decreased, indicating that too high ultrasonic power disrupted the covalent bonding between proteins and sugars.

3.2. Color development

As presented in Table 1, compared with the control group (group 0),

Table 1

|       | L*   | a*   | b*   | dE   |
|-------|------|------|------|------|
| 0     | 30.65 ± 0.24a       | 0.48 ± 0.06b       | 2.27 ± 0.11a       | 14.39 ± 0.15b       |
| 10 min| 28.38 ± 0.19a       | 0.75 ± 0.12a       | 1.53 ± 0.18a       | 18.21 ± 0.14a       |
| 20 min| 40.22 ± 3.06b       | 0.38 ± 0.02a       | 1.48 ± 0.20a       | 20.86 ± 1.13a       |
| 30 min| 46.68 ± 1.14a       | 0.38 ± 0.03a       | 0.75 ± 0.14a       | 19.44 ± 0.09a       |
| 60 min| 30.65 ± 0.24a       | 0.48 ± 0.06a       | 0.27 ± 0.11a       | 14.39 ± 0.15a       |
| 100 W | 44.95 ± 2.30b       | 0.38 ± 0.04a       | 0.75 ± 0.14a       | 19.44 ± 0.09a       |
| 150 W | 46.70 ± 1.04b       | 0.13 ± 0.04a       | 0.15 ± 0.03a       | 23.42 ± 0.86a       |
| 200 W | 46.04 ± 0.23c       | 0.39 ± 0.17c       | 0.23 ± 0.05c       | 24.75 ± 0.90c       |
| 300 W | 45.70 ± 1.01c       | 0.35 ± 0.06c       | 0.18 ± 0.09c       | 23.70 ± 0.67c       |

Note: the colour of each dispersion was measured and expressed as L* values (lightness), a* values (redness (+)–greenness (−)), and b* values (yellowness (+)–blueness (−)). dE (color difference) represents the total color change with the control group; different letters in the same column indicate significant difference (p < 0.05).
glycosylation reaction. This finding is also consistent with the trend in
the degree of browning of proteins in Fig. 1. During the reaction, the
dE of the different treatment groups increased significantly (p < 0.05), indicating that the ultrasonic treatment promoted the protein
amino group of the protein is deprotonated (RNH₂ → RNH₂⁺), and the nucleophilicity is increased. The carbonyl group of Ara interacts with
the affinity amino group of amino acids under heat treatment condi-
tions, which is an endothermic reaction system [33]. The energy
released by the cavitation effect generated by ultrasound induces effi-
cient binding of PPI–Ara conjugates. Moreover, the gain effect of the
ultrasonic treatment power is higher than that of treatment time. During
the ultrasonic modification of proteins, excessive power or long treat-
ment time may cause excessive protein aggregation.

Table 2
Changes in secondary structure of various PPI–Ara conjugates.

|                | α-helix | β-sheet | β-turn | random coil |
|----------------|---------|---------|--------|-------------|
| 0              | 12.92 ± 0.68a | 38.57 ± 0.28a | 9.58 ± 0.51c | 14.1 ± 0.43d |
| 10 min         | 15.67 ± 0.28a | 40.08 ± 0.23b | 16.08 ± 0.44a | 21.04 ± 0.37b |
| 20 min         | 11.54 ± 0.74a | 40.06 ± 0.19b | 12.69 ± 0.56a | 18.20 ± 0.47b |
| 30 min         | 11.36 ± 0.42a | 42.69 ± 0.27b | 16.84 ± 0.23a | 22.91 ± 0.35a |
| 0              | 12.92 ± 0.68a | 38.57 ± 0.28a | 9.58 ± 0.51c | 14.10 ± 0.43c |
| 100 W          | 15.30 ± 0.26a | 40.70 ± 0.15b | 15.54 ± 0.40b | 19.66 ± 0.52b |
| 150 W          | 12.46 ± 0.28a | 41.92 ± 0.13b | 20.30 ± 0.66b | 21.96 ± 0.41b |
| 200 W          | 10.58 ± 0.30b | 37.42 ± 0.28b | 10.43 ± 0.37b | 16.95 ± 0.41b |
| 300 W          | 11.04 ± 0.19b | 38.09 ± 0.17b | 9.57 ± 0.78c | 25.70 ± 0.34c |

Note: different letters (a–e) in the same column indicate significant difference (p < 0.05).

The dE of the different treatment groups increased significantly (p < 0.05), indicating that the ultrasonic treatment promoted the protein
glycosylation reaction. This finding is also consistent with the trend in
the degree of browning of proteins in Fig. 1. During the reaction, the
appearance of peaks related to reducing sugars are located at 3500–3000 cm⁻¹ and 1200–900 cm⁻¹, and the hydroxyl stretching band (1080 cm⁻¹) of the PPI–Ara sample after sonication has a higher absorption peak, indicating that more –OH groups were formed in the protein secondary structure after sonication
[34]. Moreover, there are the highest absorption peaks at 150 and 30
min, consistent with the degree of protein browning. A new absorption
band was generated at 3315 cm⁻¹, confirming the generation of Schiff
bases and the progress of the conjugation reaction between PPI and Ara
[35]. Table 2 presents the change analysis results of the secondary
structure of PPI–Ara conjugates under different sonication power levels
and times.

During sonication of PPI–Ara conjugates, more sugar residues are
promoted to attach to the protein surface, changing the protein surface
charge and conformation and affecting its functional properties [36].
Compared with the untreated PPI–Ara complex, the content of β-turn
and random coil of the protein increased significantly (p < 0.05) after
different ultrasonic power/time treatments, reaching the maximum at
150 and 30 min, and the protein structure shifted to a disordered
arrangement. Stanic et al. [30] found that when proteins are covalently
bound to polysaccharides, the intramolecular and intermolecular
hydrogen bond structures are destroyed, causing protein denaturation
and unfolding—manifested in the changes in the content of α-helix and
β-sheet.

Ultrasound-assisted treatment accelerated the rate of protein-
disordered structural transformation, consistent with the decreasing
α-helix content. Moreover, the local high temperature, shear force, and

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and after sonication. The regions dominated by peaks related to
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Fig. 2. The FTIR spectra of various PPI–Ara conjugates.

Fig. 3. Fluorescence spectra of various PPI–Ara conjugates.
shock waves caused by cavitation bubbles disrupted the protein–protein interactions within the complex PPI structure, dissociating more hydrogen bond structures. Furthermore, when the ultrasonic power was increased to 300 W, the content of random coils increased significantly (p < 0.05), likely caused by the formation of more free radicals [37] in the protein, causing excessive oxidation and protein aggregation.

3.4. Fluorescence spectrogram analysis

Compared with the untreated PPI–Ara conjugate, its fluorescence intensity decreased after sonication (Fig. 3), indicating that the sonication–assisted modification improved the hydrophilicity of the protein, consistent with Wang et al. [38]. During glycosylation modification, the protein unfolds to form a more flexible conformation, which also promotes the movement of amino acids to a hydrophilic environment [39]. The structure of the protein molecule becomes looser during the sonication-assisted treatment, increasing the degree of browning of the protein.

During the covalent binding of PPI to Ara, arabinose functions as a ligand to bind the tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) residues of the protein, which are located in the hydrophobic part of the protein structure. Trp has the maximum emission intensity (\( \lambda_{\text{max}} \)) at 348 nm. With the increase in ultrasonic power (longer processing time), the \( \lambda_{\text{max}} \) of PPI–Ara shifted to the farthest red to 352 nm, indicating that the surface microenvironment of Trp molecules shifted to hydrophilic. Collapse pressure, turbulence, and shear stress may be generated by the cavitation effect produced by ultrasound, which affects the local sequence of amino acids and the interactions between molecules. When the ultrasonic power was increased to 300 W, \( \lambda_{\text{max}} \) was blue-shifted, and the amino acid environment was shifted to the hydrophilic direction [40]. Under strong ultrasound fields, hydrophobic interactions and protein–protein hydrogen bonding dominate, rather than protein–polysaccharide interactions.

3.5. UV spectrogram analysis

Trp, Tyr, and Phe residues produce different UV absorption peaks, and the relative movement of amino acid residues can reflect the changes in tertiary protein conformation in protein side chains [41]. The UV absorption peak is the characteristic absorption peak of protein aromatic amino acid residues at 280 nm, which masked the absorption peak of tyrosine because of the broad UV absorption peak of tryptophan. The signal superposition of spectral peaks is challenging to distinguish the specific characteristics of the peaks. Therefore, the ultraviolet absorption spectrum is derived to obtain its second derivative spectrum, which is used to analyze the complex protein spectrum migration information in the near-ultraviolet region.

As depicted in Fig. 4, the second derivative value of the UV spectrum of the PPI–Ara conjugate increases with the prolongation of the ultrasonic time, and reaches the maximum value at 30 min—confirming that the sonication loosened the protein structure and exposed more hydrophobic groups on the surface of the protein molecule. With the increase in ultrasonic power, the second derivative of the composite solution decreased, likely caused by the cavitation and microbeam effects of ultrasonic waves that promoted the exposure of the hydrophobic groups of the composites. The steric hindrance of the Ara molecule produced a shielding effect on the three aromatic amino acids [42]. When the ultrasonic power was further increased to 300 W, the value of the second derivative increased significantly, likely because the excess energy generated by the ultrasonic reformed the protein molecules into insoluble aggregates, and the hydrophobic groups were re-embedded.

3.6. \( H_\text{o} \)

Hydrophobic groups and SH are usually packed inside compact protein structures [43]; therefore, changes in their content can reflect the degree of tertiary structure changes. Table 3 presents the \( H_\text{o} \) changes of PPI–Ara conjugates under different sonication power levels and times. Compared with the control group, \( H_\text{o} \) decreased significantly after ultrasonication (p < 0.05), consistent with the change in the UV second derivative value, indicating that the cavitation effect produced by ultrasonication contributed to the extension of the protein molecular structure and promoted the covalent binding of protein molecules to Ara.

During protein glycosylation, protein molecules generate more –OH groups, indicating that more hydrophilic groups are coupled to the protein surface, causing a decrease in surface hydrophobicity. When the ultrasonic power was increased to 200 W (the ultrasonic treatment time was extended to 30 min), the high physical and mechanical force induced by the ultrasonic field severely denatured the protein. A large

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**Table 3**

| \( H_\text{o} \) | Solubility | Particle size (nm) | PDI |
|-------------|---------------|----------------|---------|
| 0           | 2575.5 ± 100.3\(^b\) | 0.36 ± 0.02\(^d\) | 188.2 ± 15.1\(^e\) | 0.42 ± 0.03\(^d\) |
| 10 min      | 1585.1 ± 99.7\(^b\) | 0.52 ± 0.08\(^d\) | 265.9 ± 23.1\(^f\) | 0.63 ± 0.03\(^e\) |
| 20 min      | 1973.1 ± 63.5\(^b\) | 0.67 ± 0.03\(^d\) | 301.2 ± 17.0\(^f\) | 0.50 ± 0.04\(^f\) |
| 30 min      | 1051.7 ± 101.4\(^b\) | 0.51 ± 0.05\(^d\) | 303.9 ± 15.4\(^f\) | 0.54 ± 0.05\(^f\) |
| 0           | 2575.5 ± 100.3\(^b\) | 0.36 ± 0.02\(^d\) | 188.2 ± 15.1\(^e\) | 0.42 ± 0.03\(^d\) |
| 100 W       | 1901.3 ± 41.3\(^b\) | 0.44 ± 0.04\(^d\) | 265.9 ± 9.10\(^f\) | 0.66 ± 0.06\(^g\) |
| 150 W       | 1001.3 ± 41.3\(^b\) | 0.70 ± 0.03\(^d\) | 237.6 ± 10.31\(^f\) | 0.73 ± 0.05\(^g\) |
| 200 W       | 1546.3 ± 43.7\(^b\) | 0.61 ± 0.03\(^d\) | 275.7 ± 7.31\(^f\) | 0.55 ± 0.04\(^h\) |
| 300 W       | 1768.7 ± 57.0\(^b\) | 0.54 ± 0.05\(^d\) | 295.1 ± 11.03\(^f\) | 0.50 ± 0.07\(^i\) |

Note: All the data are expressed as mean ± SD. Means with the different superscript letters within the same column are significantly different (p < 0.05).

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**Fig. 4.** Effects of different sonication power/time on the second derivative of PPI–Ara conjugates.
number of hydrophobic groups were linked to each other to form a hydrophobic interaction, forming polymers such as dimers and trimers, which made the H₀ of the protein conjugate continue to decrease.

3.7. Sh

The content of SH can reflect the degree of protein denaturation and are essential to the functional properties of proteins [44]. The content of free SH increased after sonication, reaching maximum values of 7.1 and 5.1 μmol/g when the ultrasonic power was 150 and the treatment time was 30 min (Fig. 5), indicating that ultrasonication destroyed the cross-linking of the protein and made it more dispersible. When the ultrasonic power was applied at 200–300 W, the content of free SH decreased, likely caused by the intense cavitation phenomenon formed by the increase in ultrasonic intensity, stretching of protein molecules, and increase in the degree of the Maillard reaction. However, with the rapid increase in temperature and the formation of a large number of free radicals to make the protein more reactive [45], the SH in cysteine and methionine are reactively oxidized to form disulfide bonds.

3.8. The protein solubility and particle size

The solubility of untreated PPI was 0.32, and it is easy to form aggregates when forming a solution. The steric effect of sugar molecules after glycosylation of proteins hinders protein–protein aggregation, improving solubility [46]. The solubility of the protein complexes increased to 0.70 and 0.67 at 150 W/20 min, with increasing ultrasonic power (longer treatment time). During sonication, the chemical bonds between proteins were destroyed, destroying the close relationship between molecules and promoting the loosening of protein molecular structures. When the ultrasonic power was increased to 300 W (the time was extended to 30 min), the protein molecules were over-oxidized to form dimers, trimers, or precipitates [47]. Moreover, the violent cavitation effect destroyed the covalent bonding of PPI–Ara, decreasing its solubility.

The average particle size and polydispersity index (PDI) were determined to study the aggregation behavior of protein molecules. The average particle size of the glycosylated protein was 488.2 nm and the PDI value was 0.42, confirming that the Maillard reaction would cause protein aggregates to dissociate to a certain extent and prevent the formation of aggregates. For the conjugates treated with different ultrasonic power, the average particle size decreased to 265.9 nm (100 W), 237.6 nm (150 W), 275.7 nm (200 W), and 295.1 nm (300 W), and the PDI decreased significantly (p < 0.05). The particle size of the protein complexes decreased to 265.9, 301.2, and 303.9 nm after different sonication times. Consequently, ultrasonic treatment inhibits the aggregation of proteins, improves the dispersion of the solution, and more closely combines the PPI and Ara.

3.9. EAI/ESI

The ability of proteins to form and stabilize emulsions can be determined by their emulsifying properties [48]. As depicted in Fig. 6, the EAI of PPI–Ara conjugates increased significantly from 15.1 to 19.7 and 19.3 m²/g with increasing ultrasonic power (longer treatment time). The hydrophobicity of the protein surface decreases when the degree of glycosylation is induced by ultrasound, and the hydrophilic and hydrophobic ratios on the molecular surface reach equilibrium, promoting the rapid adsorption of proteins at the oil–water interface, reducing the interfacial tension [49].

Fig. 5. The SH content of PPI–Ara conjugates under different sonication power/time; Different capital letters (a-e) indicate significant differences among various treated PPI (p < 0.05).

Fig. 6. The EAI/ESI of various PPI–Ara emulsions.
Furthermore, the increased protein solubility favored its diffusion rate at the oil–water interface, increasing the EAI. After ultrasonic treatment, ESI increased and then decreased, reaching the maximum value at 150 and 20 min. The change in ultrasonic power has a significant effect on EAI ($p < 0.05$), which may be because the temperature and shear stress generated by the increase in ultrasonic power make the protein conformation change rapidly, promoting the stability of proteins in the emulsion system.

When the ultrasonic power was increased to 300 W, the EAI and ESI of the emulsion decreased, indicating that the ultrasonic cavitation effect destroyed the non-covalent bonds that maintained the spatial protein structure and formed protein aggregates, reducing the emulsifying performance. When the sonication time was prolonged to 30 min, the EAI of the protein did not increase significantly, likely caused by the severe denaturation of the protein by excessive sonication, causing oxidative aggregation and the formation of multimers, which was not conducive to the stability of the emulsion.

3.10. Analysis of contact angle

Contact angle ($\theta$) is an important indicator that can reflect the wettability of proteins at the emulsion interface; its value has an impact on the stability of the emulsion [50]. The contact angles of the emulsions depicted in Fig. 7 are all acute, indicating that they were hydrophilic. Compared with the 0 W sample (82.62$^\circ$), the $\theta$ of the sample after sonication decreased significantly, reaching a minimum value of 66.88$^\circ$ and 70.37$^\circ$ at 200 and 30 min, which indicates that ultrasonic treatment improves the hydrophilicity of protein particles and is more conducive to the formation of oil-in-water emulsions, consistent with the research results of Wang et al. [51]. The contact angle of the sample increased (72.06$^\circ$) when the ultrasonic power was 300 W because the cavitation increased the wettability of the composite emulsion. In contrast, excessive ultrasonic treatment was not conducive to the stability of the emulsion, and agglomeration tends to occur in aqueous solutions.

3.11. Zeta potential

The presence of protein charge and zeta potential in the emulsion also affects the emulsion’s stability. The absolute value of the emulsion zeta potential increased significantly ($p < 0.05$) with increasing ultrasonic power (prolongation of treatment time) in Fig. 8, reaching maximums at 150 and 30 min. This phenomenon is caused by the fact that ultrasonic treatment loosens the compact structure of the protein, which breaks the hydrogen bonds, hydrophobic interactions, and disulfide bonds between protein molecules—improving the electrostatic repulsion between particles, inhibiting the aggregation of proteins, and increasing the stability of the emulsion [52]. With the increase in ultrasonic power, the zeta potential decreased, likely because the excessive ultrasonic treatment destroyed the balance of electrostatic repulsion and electrostatic attraction, causing the proteins to re-aggregate, and reducing the stability of the emulsion.

Fig. 7. Contact angle of PPI-Ara emulsions under different ultrasonic power/treatment time.

Fig. 8. Zeta-potential of sonicated PPI-Ara conjugated emulsions. Error bars represent standard deviation obtained from three determinations. Different letters represent significant differences ($p < 0.05$).
samples after sonication increased, indicating that sonication increased the thermal denaturation resistance of the protein. Furthermore, ultrasound-assisted treatment reduces the interfacial tension of proteins, increasing the stability of the emulsion. The two endothermic peaks present in the treated samples are caused by the thermal denaturation of the protein and the glycosylation reaction facilitated the interaction between protein molecules to improve their solubility. Moreover, the emulsion stability was significantly improved compared to the untreated group under different ultrasonic treatment times. The EAI of the emulsion prepared by different ultrasonic powers improved significantly, and the thermal denaturation resistance increased.

CRediT authorship contribution statement

Xing Chen: Methodology, Writing – original draft, Writing – review & editing. Yajie Dai: Investigation, Validation. Zhe Huang: Formal analysis, Visualization. Linwei Zhao: Validation, Visualization. Jing Du: Conceptualization, Software. Wei Li: Writing – review & editing. Dianyu Yu: Writing – review & editing, Project administration, Funding acquisition.

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

The data that has been used is confidential.

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Table 4

| Td and ΔH of PPI–Ara conjugated emulsions under ultrasonication. | Td (°C) | ΔH (J/g) | Td (°C) | ΔH (J/g) |
|---|---|---|---|---|
| 0 | 68.68 ± 2.17 | 51.29 ± 0.17 | 110.27 ± 1.67 | 4.14 ± 0.11 |
| 10 min | 80.07 ± 1.69 | 88.59 ± 0.99 | 135.23 ± 2.04 | 11.28 ± 0.20 |
| 20 min | 80.17 ± 1.13 | 85.90 ± 0.97 | 135.49 ± 1.12 | 11.43 ± 0.09 |
| 30 min | 80.34 ± 1.53 | 83.00 ± 0.13 | 136.19 ± 2.17 | 12.58 ± 0.18 |
| 0 | 68.68 ± 2.17 | 51.29 ± 0.17 | 110.27 ± 1.67 | 4.14 ± 0.11 |
| 100 W | 80.21 ± 2.21 | 86.87 ± 0.12 | 135.71 ± 2.17 | 11.53 ± 0.19 |
| 150 W | 80.88 ± 1.34 | 101.5 ± 0.08 | 134.69 ± 1.33 | 14.64 ± 0.13 |
| 200 W | 80.34 ± 1.16 | 93.18 ± 0.10 | 136.19 ± 2.01 | 12.68 ± 0.13 |
| 300 W | 83.48 ± 2.07 | 66.87 ± 0.07 | 133.78 ± 1.10 | 6.02 ± 0.11 |

Note: different letters (a-e) in the same column indicate significant difference (p < 0.05).

Fig. 9. Thermal Characteristics of PPI–Ara Conjugated Emulsion.

3.12. DSC

The thermal stability of the emulsions was investigated by DSC (Fig. 9). Two endothermic peaks were identified based on the thermal characteristic curve of the emulsion. The first peak appeared in the temperature range of 80–100 °C and the second at 130–150 °C. The two endothermic peaks present in the treated samples are caused by the altered structural features of the protein after glycosylation without disturbing the structure of its peptide chain, consistent with the thermal behavior of the protein reported by Bülent et al. [53].

Compared with the control group (Table 4), the Td and ΔH of the samples after sonication increased, indicating that sonication increased the thermal denaturation resistance of the protein. Furthermore, ultrasonic treatment reduces the interfacial tension of proteins, increasing the protein adsorbed on the interface layer, and the proteins are arranged in an orderly manner at the oil–water interface, increasing the thermal stability of the emulsion system. When the ultrasonic power was further increased, the physical and thermal effects caused by ultrasonic treatment disturbed the internal structure of the emulsion, negatively affecting its thermal stability.

4. Conclusion

This study demonstrated that the structure, solubility, and emulsifying properties of PPI changed significantly under ultrasound-assisted glycosylation. Under the cavitation effect generated by ultrasonic waves, the glycosylation reaction of protein and sugar was promoted, and the reaction rate of glycosylation improved. Under ultrasonic treatment, the protein structure changed to disorder, more hydrophobic groups were exposed on the molecular surface, and the content of free SH increased. At this time, the protein structure became loose, which was conducive to the glycosylation reaction of protein sugar. Moreover, the glycosylation reaction facilitated the interaction between protein and water, and more hydrophilic groups were formed on the surface of protein molecules to improve their solubility. Moreover, the emulsion stability was significantly improved compared to the untreated group after different ultrasonic treatment times. The EAI of the emulsion prepared by different ultrasonic powers improved significantly, and the thermal denaturation resistance increased.
