Effect of ultrasonic treatment on the structure and functional properties of mantle proteins from scallops (*Patinopecten yessoensis*)

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**ABSTRACT**

In this study, scallop mantle protein was treated by ultrasound at different powers, and then analyzed by ANS fluorescent probes, circular dichroism spectroscopy, endogenous fluorescence spectrum, DNTB colorimetry and in-vitro digestion model to elucidate the structure-function relationship. The results indicated that ultrasound can significantly affect the secondary structure of scallop mantle protein like enhancing hydrophobicity, lowering the particle size, increasing the relative contents of α-helix and decreasing contents of β-sheet, β-turn and random coil, as well as altering intrinsic fluorescence intensity with blue shift of maximum fluorescence peak. But ultrasound had no effect on its primary structure. Moreover, the functions of scallop mantle protein were regulated by modifying its structures by ultrasound. Specifically, the protein had the highest performance in foaming property and in-vitro digestibility under ultrasonic power of 100 W, oil binding capacity under 100 W, water binding capacity under 300 W, solubility and emulsification capacity under 400 W, and emulsion stability under 600 W. These results prove ultrasonic treatment has the potential to effectively improve functional properties and quality of scallop mantle protein, benefiting in comprehensive utilization of scallop mantles.

1. Introduction

As high economic shellfish, scallop (*Patinopecten yessoensis*) is commonly cultured in East Asian coast [1]. With annual aquaculture production of nearly 2.0 million tons in China, the production of scallop processing byproduct is on the increase yearly. Yesso scallop mantle is the main byproduct during processing and regarded as good resource for anti-virus, anti-tumor and anti-aging due to their abundant nutrients like protein, fat, folic acid, taurine and trace elements [2–5]. Growing attention is focused on marine proteins for their high nutritional value and good functional characteristics. However, most of yesso scallop mantle are directly discarded and wasted while only small portion are processed to low-value feed [6]. Therefore, it is particularly necessary to improve physicochemical and functional characteristics to enhance their utilization rate.

As an effective non-thermal food processing, ultrasonic treatment is widely used in the food industry to change protein structure and regulate its aggregation by inducing interactions between ultrasound and media. Firstly, protein aggregation was dismantled under the shearing force, high temperature and high pressure released by the crack of the cavitation bubbles at the compression period. Secondly, the mass transfer of protein solution was accelerated by micro jets and micro turbulence due to cavitation bubbles explosion. Finally, intermolecular forces of protein, such as hydrophobic forces and hydrogen bonds, were broken up by peroxides or induced to form cross-linking, which were formed through active free radicals from water molecules [7].

Ultrasonic treatment is an effective method to improve food functionality of protein by changing the internal structure of protein molecules and intermolecular forces [8]. Arredondo-Parada et al. [9] stated that foaming properties of giant squid protein were improved after ultrasonic treatment. The sea cucumber gonadal protein with tight and even structure had good stability due to the facts that hydrogen bond was broken and the side chains of sea cucumber gonadal protein were unfolded at 200 W [10]. Zou et al. [11] reported that high-intensity ultrasonic treatment could improve solubility, foaming, foaming stability, emulsification activity and emulsification stability of mussel sarcoplasmic protein. Sha et al. [12] indicated that ultrasonic treatment could significantly increase the solubility, hydrophobicity, and surface activity of pea protein isolate. Zhang et al. [13] also found that ultrasound treatment could promote the physical stability of the emulsions.

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by modifying almond protein isolate structure. Although most studies have focused on the change of protein structure and function under ultrasonic treatment, knowledge of the effect of ultrasound on scallop mantle protein is limited, especially comprehensive and systemic study on structure and function properties. Function-oriented scallop mantle proteins are urgent to explore based on ultrasonic treatment.

The aim of the study was to comprehensively assess the effect of ultrasound on the structure and different functions and clarify the relationship between structure and function of scallop mantle protein so as to explore function-oriented scallop mantle protein. After scallop mantle protein was processed by ultrasound at the power of 100 W, 200 W, 300 W, 400 W, 500 W and 600 W respectively, SDS-PAGE, circular dichroism spectroscopy and endogenous fluorescence spectrum were applied to analyze its primary, secondary and tertiary structures respectively. Moreover, particle size, microstructure, surface hydrophobicity and sulfhydryl content of scallop mantle were detected. Importantly, the relationship between the structure and function of scallop mantle protein including solubility, foaming property, emulsification and in vitro digestibility coefficient was demonstrated under ultrasonic treatment. The results would broaden the application of scallop mantle protein and indeed provide a scientific reference for the comprehensive utilization of scallops.

2. Materials and methods

2.1. Materials

Frozen Yesso scallop (Patinopecten yessoensis) mantle was purchased from Gyrifalcon Co., Ltd. (Qinghuangdao, China). Ellman’s reagent (5,5′-dithiobis (2-nitrobenzonic acid) (DTNB), 8-Anilino-1-naphthalensulfonic acid (ANS), pancreatic from porcine pancreas (8 × USP specifications), Pepsin (400 units/mg, P7125) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo., U.S.A.). Ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), o-phthalaldialdehyde (OPA) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). All chemicals and reagents were analytical grade. All solutions were prepared by Milli-Q water.

2.2. Methods

2.2.1. Hydrophilic scallop mantle protein preparation

Scallop mantle was mixed with 0.05 mol/L phosphate buffer saline (PBS) at a protein proportion of 1:2 (w/v). The suspension was blended using high-speed grinder at 5000 r/min for 2 min and then using electric blender at 800 r/min for 1.5 h. The supernatant was collected using centrifuge at 4000 r/min for 20 min. The scallop mantle protein was extracted three times. And all extracts were combined [14].

2.2.2. Ultrasonic treatment

60 mL of scallop mantle protein solution (1%, w/v) was put in the 100 mL beaker at ice bath, and treated with ultrasonic power of 100 W, 200 W, 300 W, 400 W, 500 W and 600 W (JY92-IIDN, Ningbo Scientz Biotechnology Co. Ltd, Ningbo, Zhejiang, China), for 15 min. The mixture was measured by the fluorescence spectrometer at 390 nm excitation wavelength and 470 nm emission wavelength, respectively. The surface hydrophobicity was indicated by the slope of the initial segment in the line graph with protein concentration as the horizontal axis and the fluorescence intensity as the vertical axis.

2.2.3. Molecular mass analysis

The molecular mass of scallop mantle protein was measured following the method in Laemmli [15] with minor modifications. The concentration of separation gel and stacking gel was 12% and 5%, respectively. With different molecular weight marker (10 kDa-250 kDa) for size estimation, SDS-PAGE was run under 80 V and 120 V voltage for the separation gel and stacking gel, respectively.

2.2.4. Secondary structure analysis

The Circular Dichroism Spectropolarimeter (Jasco-815, Jasco Corp., Tokyo, Japan) was used to determine the secondary structure of scallop mantle protein solution (0.05%, w/v). With deionized water as the blank control group, α-helix, β-sheet, β-turn and random coil were conducted at 60 nm/min configured with 0.5 mm optical path, scanned area of 190–250 nm and 1 nm bandwidth [16,17].

2.2.5. Tertiary structure analysis

To determine the tertiary structure, the endogenous fluorescence spectrum of 0.2 mg/mL samples prepared with 0.05 mol/L PBS (pH 7.0) was analyzed by the fluorescence spectrometer (F-320, Tianjin Gang-Dong Scientific Co. Ltd., Tianjin, China) with the excitation wavelength of 290 nm, the emission wavelength of 300–400 nm, and 5.0 nm constant crack width between the excitation wavelength and the emission wavelength [18].

2.2.6. Surface hydrophobicity measurement

The surface hydrophobicity (Hs) of scallop mantle protein was measured using modified ANS fluorescent probes [19]. The scallop mantle protein solution with different concentrations of 0.04-0.2 mg/mL was mixed evenly with 50 μL ANS solution (8 mmol/L) to react for 15 min. The mixture was measured by the fluorescence spectrometer at 390 nm excitation wavelength and 470 nm emission wavelength, respectively.

2.2.7. Particle size and polydispersity index measurement

The particle size and polydispersity index of the prepared scallop mantle protein were detected by Nano ZS90 and all analyses were performed in triplicate [20].

2.2.8. Total sulfhydryl (SH) and free sulfhydryl determination

Total SH and free SH were obtained with DTNB rate colorimetry [21]. For the total SH, scallop mantle protein solution (1 mg/mL, 0.5 mL) was mixed with 5 mL of buffer solution (10 mM EDTA, 8 M urea, 20 mM Tris–HCl, pH 6.0). Then the mixture was incubated at room temperature for 30 min after adding 100 μL of 10 mmol/L DTNB, while the scallop mantle protein solution (1 mg/mL, 5.5 mL) was mixed with 100 μL of 10 mmol/L DTNB at 4 °C for 1 h for free SH detection. The absorbance values were detected at 412 nm. The SH contents were calculated with the molar extinction coefficient (13,600 L/mmol • cm).

2.2.9. Protein microstructure observation

According to the method [22] with a few modifications, the scanning electron microscope (JSM7800, JEOL, Japan) was used to observe the morphology of the scallop mantle protein powder which was coated with gold film at 10 mA for 60 s.

2.2.10. Solubility measurement

The sample (0.3 g) was dissolved in 30 mL of PBS (50 mM, pH 7) and centrifuged at 3500 r/min for 10 min at 4 °C. The supernatant was collected and diluted 60 times using PBS (50 mM, pH 7). The diluted protein solution (1 mL) mixed with 5 mL of 1% CBB G-250 reagent and reacted for 2 min. The absorbance was detected at 595 nm. The protein solubility was calculated using the following formula (1).

\[
\text{Solubility (％) = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100}
\]  

2.2.11. Water holding capacity (WHC) and oil holding capacity (OHC) measurement

The freeze-dried scallop mantle protein powder (0.5 g) was fully mixed with 5 mL of deionized water or soybean oil in a 10 mL centrifugal tube and standstill for 30 min at room temperature. And then it was centrifuged at 5000 r/min for 15 min to remove the supernatant. The WHC and OHC were calculated using Formula (2) and Formula (3) as follows.


\[
\text{WHC (g/g)} = \frac{M_2 - M_1}{M_0}
\]  

(2)

\[
\text{OHC (g/g)} = \frac{M_3 - M_1}{M_0}
\]  

(3)

Where \(M_0\) is the mass of powder sample (g); \(M_1\) is the total mass of the centrifugal tube and the sample (g); \(M_2\) is the total mass of the centrifugal tube and the deposit with water (g); and \(M_3\) is the total mass of the centrifugal tube and the deposit with oil (g).

2.2.12. Emulsification capacity and emulsification stability measurement

Based on the method of Kevin et al. [23] with some modification, 15 mL protein solution (10 mg/mL) was high-speed homogenized with 5 mL soybean oil at 10,000 r/min for 1 min at 25 °C. Protein emulsion (50 μL) taken from the bottom at 0 min and 10 min respectively was combined with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance value was measured at 500 nm by spectrophotometer with the SDS solution as the blank control group. The calculation formula (4) of emulsification capacity index (EAI) and the formula (5) of emulsification stability index (ESI) are shown as follows:

\[
\text{EAI(m²/g)} = \frac{2 \times 2.303 \times A_0 \times N}{\varphi \times \text{Protein weight(g)}}
\]  

(4)

\[
\text{ESI(min)} = \frac{A_0 \times \Delta t}{A_0 - A_10}
\]  

(5)

Where \(A_0\) and \(A_10\) are the absorbance values at 0 min and at 10 min respectively, while \(N\) is the dilution factor of the emulsion, \(\varphi\) is the volume fraction of the oil phase in the solution (\(\varphi = 0.25\)) and \(\Delta t\) is 10 min.

2.2.13. Foaming capacity (FC) and stability (FS) test

After homogenized at 10,000 r/min for 1 min in centrifuge, 20 mL of 10 mg/mL scallop mantle protein solution was placed at room temperature for 30 min. Then the volume of solution was measured at 0 min and 30 min, respectively [24]. The calculation formula (6) of foaming capacity and the formula (7) of foaming stability are shown as follows:

\[
\text{FC(%) = } \frac{V_1 - V_0}{V_0} \times 100
\]  

(6)

\[
\text{FS(%) = } \frac{V_2 - V_0}{V_0} \times 100
\]  

(7)

where \(V_0\) is the volume of solution before homogenization; \(V_1\) is the volume at 0 min after homogenization; and \(V_2\) is the volume at 30 min after homogenization.

2.2.14. In vitro digestibility coefficient and the degree of hydrolysis (DH) measurement

According to the reference with some modifications, the in vitro digestibility was performed [25]. Scallop mantle protein powder (0.3 g) was dissolved into distilled water (30 mL). Then the pH value of the protein solution was adjusted to 3.0 with 1 mol/L HCl at 37 °C. The simulative gastric digestion was conducted for 2 h at 37 °C with pepsin added at 3:100 (w/w), which is the mass ratio of pepsin to protein. Then, the intestinal digestion was simulated for 2 h at 37 °C after adjustment of the pH value to 7.0 using 1 mol/L NaOH and trypsin addition at 3:100 (ES). The reaction was ended by heating up the mixture at 100 °C for 5 min to eliminate enzymatic activity.

The supernatant was collected for DH measurement after the reactive mixture was centrifuged at 10,000 r/min for 5 min at 4 °C. After diluted by 10 times, 400 μL supernatant was mixed well with 3 mL OPA and placed in dark for 2 min. The absorbance was measured at 340 nm by spectrophotometer [26]. The calculation formula (8) and (9) of DH is shown as follows:

\[
\text{DH(%) = } \frac{h}{h_{\text{tot}}} \times 100
\]  

(8)

\[
\text{h(meqV/g protein)} = \frac{\text{Serine} - \text{NH}_2 - \beta}{\alpha}
\]  

(9)

where \(\alpha\) is 1 and \(\beta\) is 0.4. The \(h_{\text{tot}}\) is 7.71.

2.3. Statistical analysis

All the experiments were conducted in triplicate. All data were reported as mean ± standard deviation (n = 3). One-way ANOVA was applied to compare multiple treatments with Duncan’s test by using SPSS 25.0 at the significance level of \(P < 0.05\). The results were plotted using Origin 2019.

3. Results and discussion

3.1. The impacts of ultrasound on structure of scallop mantle protein

3.1.1. Primary structure

SDS-PAGE was applied to visualize the effect of ultrasound on primary structure of scallop mantle protein. As shown in Fig. 1, hydrophilic scallop mantle protein, with the wide molecular weight of 14–115 kDa, is mainly composed of actin, tropomyosin and myosin light chain weighing 43 kDa, 36 kDa and 27 kDa, respectively. There was not statistically significant difference between different samples, suggesting that ultrasound didn’t supply enough energy to break chemical bonds and modify the primary structure of scallop mantle protein [27].

3.1.2. Secondary structure

CDS was carried out to estimate the relative contents of α-helix, β-sheet, β-turn, and random coil of treated and untreated protein samples. As shown in Table 1, the α-helix content increased significantly from 29.70 ± 0.08% (0 W) to 38.70 ± 0.08% (600 W) while the β-sheet proportion decreased from 17.56 ± 0.12% (0 W) to 12.07 ± 0.17% (600 W), the β-turn proportion decreased from 21.20 ± 0.08% (0 W) to 19.23 ± 0.05% (600 W), the random coil proportion decreased from 31.50 ± 0.08% (0 W) to 28.60 ± 0.08% (600 W). In general, ultrasonic treatment significantly increased the relative content of α-helix but significantly decreased the relative content of β-sheet, β-turn and random coil. Ultrasound instead of different ultrasonic power reduced the relative content of random coil. That’s probably because ultrasound could...
convert β-sheet, β-turn and random coil into stable and orderly α-Helix stabilized by hydrogen bonds, but their conversion rates are different [28,29]. Since α-helix was stabilized by intra hydrogen bonds of peptide chains and β-sheet depended on the hydrogen bonds between the peptide chains, ultrasonic treatment could promote the reconstruction of the internal hydrogen bonds of the scallop mantle protein. Therefore, the secondary structure of scallop mantle protein was changed by physical forces and free radicals induced by ultrasonic cavitation, which proved that ultrasonic treatment was an effective method to significantly increase the content of orderly structures and stable conformation in scallop mantle protein [22,30].

### 3.1.5. Particle size and PDI

Particle size and PDI were key parameters that affected the protein functions like solubility, emulsification and foamy. Particle size distribution was used to characterize the degree of protein aggregation [39]. PDI had negative correlation with the homogeneous protein due to the non-covalent bonds, unfold the molecular structure of scallop mantle protein and expose hydrophobic amino acid residues like Phe, Trp and Tyr. It could be explained that the intermolecular interactions of scallop mantle proteins were weaken because the solubility was increased after ultrasound [36]. Previous studies had similar results that the exposure rate of hydrophobic amino acid residues in protein was increased under ultrasonic treatment, which enhanced the surface hydrophobicity of proteins, such as faba bean protein [37], oat protein [38] and protein from sea cucumber gonad [10]. It could be concluded that appropriate ultrasound increased the hydrophobicity of protein surface by folding the protein to increase the exposure of hydrophobic regions.

### 3.1.4. Surface hydrophobicity

Surface hydrophobicity is an important factor for the conformation, stability and functional properties of protein by hydrophobic interactions. The surface hydrophobicity of scallop mantle protein raised from 417.87 ± 14.50 to 652.83 ± 2.70 with the increase of ultrasonic power (p < 0.05) (Fig. 3). Higher ultrasonic power, stronger effects of cavitation with more tiny bubbles and microbeams, which was conducive to break the non-covalent bonds, unfold the molecular structure of scallop mantle protein and expose hydrophobic amino acid residues like Phe, Trp and Tyr. It could be concluded that appropriate ultrasound increased the hydrophobicity of protein surface by folding the protein to increase the exposure of hydrophobic regions.

### Table 1

| Ultrasound power (W) | α-Helix (%) | β-Sheet (%) | β-Turn (%) | Random coil (%) |
|---------------------|------------|-------------|------------|-----------------|
| 0                   | 29.70 ± 0.08b | 17.56 ± 0.12d | 21.20 ± 0.06c | 31.50 ± 0.08c |
| 100                 | 36.56 ± 0.17c | 13.63 ± 0.05d | 19.77 ± 0.11e | 30.43 ± 0.05c |
| 200                 | 37.46 ± 0.05c | 13.50 ± 0.05c | 19.87 ± 0.19c | 28.5 ± 0.05c   |
| 300                 | 37.43 ± 0.05c | 13.77 ± 0.12e | 19.53 ± 0.14c | 28.53 ± 0.12c  |
| 400                 | 37.63 ± 0.05c | 12.93 ± 0.12c | 19.03 ± 0.05c | 28.53 ± 0.05c  |
| 500                 | 38.43 ± 0.05c | 11.97 ± 0.12c | 19.13 ± 0.14c | 28.67 ± 0.12c  |
| 600                 | 38.70 ± 0.05c | 12.07 ± 0.12c | 19.23 ± 0.14c | 28.60 ± 0.08c  |

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

Fig. 2. The intrinsic fluorescence spectrum of scallop mantle protein treated by ultrasound.

Fig. 3. Surface hydrophobicity of scallop mantle protein under different ultrasonic treatments.
and shear forces of ultrasound treatments, the structure of protein increased, which improved its solubility and surface hydrophobicity. Observed by SEM (Fig. 5). When the ultrasonic power was lower than became loose, resulting some of the exposed free sulfhydryl groups are produced by the ultrasound treatment contributed to the loosen protein mantle protein was improved [41]. Meanwhile, it may be the main reason of free sulfhydryl groups reduction that free sulfhydryl but didn’t affect significantly total sulfhydryl. Due to cavitation and shear forces of ultrasound treatments, the structure of protein became loose, resulting some of the exposed free sulfhydryl groups are easily to form disulfide bonds. Thus, the structural stability of scallop mantle protein was improved [41]. Meanwhile, it may be the main reason of free sulfhydryl groups reduction that free sulfhydryl groups were oxidized by hydrogen peroxide produced by ultrasound [42].

3.1.7. Protein microstructure

The microstructures of untreated and treated protein samples were observed by SEM (Fig. 5). When the ultrasonic power was lower than 500 W, longer sonication duration led to the smaller particle size of protein samples and surface was rougher compared with those of untreated ones because the shear force, impact force and cavity effect produced by the ultrasound treatment contributed to the loosen protein structure and had a certain dispersive effect on the protein structure as shown in the white dotted circle. In other words, smaller dispersion systems may lead to higher solubility. Due to the exposure of hydrophobic and polar groups, the contact area of protein and water was increased, which improved its solubility and surface hydrophobicity. However, aggregate occurred among dissociated subunits when the ultrasonic power was over 500 W, which increased the protein particle size, as shown in the red dotted circle [43].

### 3.2. Effect of ultrasound on protein solubility

As the indicator of protein aggregation and denaturation, solubility could significantly affect its many functions like emulsification and foaming properties [44].

As presented in Fig. 6, with the increase of ultrasonic power, the solubility increased gradually and reached maximum at 400 W. It was the results from the balance between attractive and repulsive forces of protein molecules, which depends on the intermolecular conformational changes caused by ultrasound. The molecular structure of insoluble protein aggregates turned loose in the appropriate range of ultrasonic power, resulting that soluble protein aggregated and its formation was promoted due to the exposure of internal hydrophilic groups. Similar results were reported in previous study [35]. In addition, ultrasonic treatment was benefitted to reduce the particle size, expand the contact area and increase the interaction between protein and water, which increased the protein solubility [45]. It may be explained that excessive protein denaturation and insoluble aggregates formation decreased the protein solubility when the ultrasonic power was over 400 W, in agreement with the results of protein microstructure.

### 3.3. Effect of ultrasound on the water holding capacity (WHC) and oil holding capacity (OHC)

WHC was a key factor for diet freshness, taste and texture, while OHC affected the taste, flavor and the longer shelf life of foods [46]. WHC and OHC tended to increase at first and decrease later with the increase of ultrasonic power (Fig. 7). 300 W was the optimal ultrasonic power for WHC as well as 200 W for OHC. As to WHC, the improvement of protein solubility and the reduction of particle size facilitated combination between hydrophilic groups of proteins and water molecules. If the ultrasonic power increased after the optimal, disulfide bonds were formed and free sulfhydryl groups decreased as described in the section of 3.1.6, which boosted protein aggregation and was detrimental to absorb water [47]. As to OHC, non-polar groups were exposed and protein hydrophobicity was improved due to conformation changes of proteins under proper ultrasonic treatment as discussed earlier, which enhanced the absorbed oil content. But overhigh ultrasonic power would lead to reaggregation of proteins and attenuation of hydrophobic interactions, reducing the oil holding capacity [48].

### 3.4. Effect of ultrasound on the emulsification property

Good emulsification property could broaden the protein application in foods like beverages, seasoners, baking ingredients, and dairy as stabilizer, surfactant, humectant, softener and plasticizer [49]. EAI and ESI were important indexes to evaluate the emulsifying property of the scallop mantle protein.

The results in Fig. 8 indicated that appropriate ultrasonic treatment could significantly improve EAI and ESI of scallop mantle protein. EAI got its max at 400 W (85.28 ± 1.35 m²/g) while ESI peaked at 600 W (17.25 ± 0.72%). The emulsification property was bound up with solubility and surface hydrophobicity of protein [35]. The affinity of the proteins was optimal to the oil–water interface during emulsification when the protein solubility reached its peak at 400 W, as described above. In addition, high solubility can promote absorbed protein content to the oil–water interface and the oil–water interface surface activity during emulsification [50]. Correspondingly, when ultrasound was carried out at 600 W, the protein density on the oil–water interface was in the upmost level. That was is consistent with the tendency of surface hydrophobicity, which accelerated protein molecules toward the oil–water surface.

### 3.5. Effect of ultrasound on the foaming properties

The foaming properties of protein including foaming capacity (FC)
and stability (FS) were crucial to puff food and foamed drinks as it related to food mellowness, softness, smoothness, and brightness. Both FC and FS largely depended on structural properties of protein such as the particle size (diffusion rate), surface hydrophobicity (affinity to the air–water interface) and structural flexibility (unfolding ability on the interface) [51].

As shown in Fig. 9, FC and FS were significantly improved when the ultrasonic power was between 0 and 400 W. And 100 W was the best power for FC and FS. After ultrasonic treatment, the reduced particle size could accelerate the diffusion of protein toward the air–water and oil–water interfaces. Moreover, the improvement of affinity to the interface also contributed to the enhancement of foaming properties, resulting from the exposure of hydrophobic groups and partially unfolding of proteins [9]. However, the solubility reduction and the formation of aggregates induction were disadvantaged to form the foam of scallop mantle protein at overhigh ultrasonic power because of the damage of molecular structure and boundary film [52].

3.6. Effect of ultrasound on the in vitro digestibility

Digestibility was indexed to the nutritional quality of protein and the bioavailability of amino acids, which may be affected by protein structure. Degree of hydrolysis (DH) is a major parameter to evaluate protein digestibility [53].
Ultrasonic treatment could enhance the DH (Fig. 10). The DH of scallop mantle protein was significantly enhanced to 86.30% from 70.30% after ultrasonic treatment at 100 W. It could be explained that hydrolysis reaction rate was improved due to the increase of binding site with enzyme hidden in protein after ultrasonic shearing force and cavitation damage [54]. In addition, the reduction of the particle size of the protein accelerated the penetration of the enzyme into the protein matrix and improved the efficiency of enzymatic hydrolysis [55]. The results was consistent with previous study that proper ultrasonic treatment increased the DH of buckwheat protein isolates [22]. Noteworthily, the influence of ultrasonic power had not greatly difference on protein digestibility.

4. Conclusion

This study systematically demonstrated the effect of ultrasonic treatment on the structure and functional properties of scallop mantle protein. Ultrasonic treatment was an effective method to modify the protein structure except the primary structure. The stability and amphiphilicity of the protein structure could be regulated with appropriate ultrasonic power. Differences in protein structure led to various functional properties. Except for the stability caused by the changes of secondary structure, the particle size and the exposure rate of hydrophobic groups were related to the hydrophobicity and solubility of scallop mantle protein, which affected the water binding capacity, oil binding capacity, foaming property and emulsification property and in vitro digestibility of protein. Therefore, this study can provide...
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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