



Positive effects and mechanism of ultrasound on chitin preparation from shrimp shells by co-fermentation

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ABSTRACT

The objective of this study is to explore the effect and mechanism of ultrasound on chitin extraction from shrimp shells powder (SSP) by the co-fermentation of *Bacillus subtilis* and *Acetobacter pasteurianus*. After pre-treating the SSP with high-intensity ultrasound (HIU) at 800 W, the protease activity in the fermentation solution reached 96.9 U/mL on day 3, which was significantly higher than for SSP that had not been pre-treated with ultrasound (81.8 U/mL). The fermentation time of the chitin extraction process was 5.0 d without ultrasound pre-treatment, while it was shortened to 4.5 d when using ultrasound at 800 W to treat SSP. However, there were no obvious differences when we applied ultrasound at low power (200 W, 400 W). Furthermore, chitin purified from shrimp shells pre-treated with HIU at 800 W exhibited lower molecular weight (11.2 kDa), higher chitin purity (89.8%), and a higher degree of deacetylation (21.1%) compared to SSP with no ultrasound pre-treatment (13.5 kDa, 86.6%, 18.5%). Results indicate that HIU peels off the protein/CaCO₃ matrix that covers the SSP surface. About 9.1% of protein and 4.7% of Ca²⁺ were released from SSP pre-treated with HIU at 800 W. These figures were both higher than with no ultrasound pre-treatment (4.5%, 3.2%). Additionally, the amount of soluble protein extracted from SSP through HIU at 800 W was 50% higher than for the control sample. SDS-PAGE analysis indicated that the soluble protein was degraded to the micromolecule. It also revealed that HIU (600, 800 W) induced the secondary and tertiary structure destruction of protein extracted from SSP. In conclusion, HIU-induced degradation and structural damage of protein enhances the protein/CaCO₃ matrix to be peeled off from SSP. Also, in the co-fermentation process, an increase of protease activity further accelerates deproteinization.

1. Introduction

Chitin, a biopolymer of 2-acetamide-2-deoxy-D-glucopyranose (GlcNAc) units linked by β-1, 4 glycosidic bonds, is widely distributed in the exoskeleton of shrimp, crab, and the cell wall of fungi [1]. There is about 20% of chitin in the dried shrimp shells. Chitin extraction from shrimp shells is an excellent strategy for environmental protection and high efficient utilization of natural resources [2]. Chitosan and chitooligosaccharides are derived from deacetylation and depolymerization of chitin, respectively. Generally, it is considered as chitosan when the deacetylation degree (DD) of chitin is more than 55% [3]. Because the two chitin derivatives possess good biodegradability, biocompatibility, antimicrobial durability, they are excellent functional biomaterial for

potential application in the food industry, environmental and medicinal [4]. Accordingly, molecular weight (M_w) and DD are the key parameters of chitin quality, and have a great impact on the physicochemical and biological properties of chitin [5].

Among various measures to extract chitin from shrimp shells, conventional chemical deproteinization (DP) and demineralization (DM) usually produce large amounts of wastewater containing strong acid or alkaline. In contrast, biological extraction methods like protease catalysis and microbial fermentation are getting increasing attention due to no pollution for the environment and mild conditions [6]. Protein from shrimp shells is generally removed by some *Bacillus*, e.g., *B. subtilis*, *B. licheniformis*, and *B. cereus*, while minerals are removed using lactic acid bacteria or acetic acid bacteria [7]. Physical means including

Abbreviations: SSP, shrimp shells powder; HIU, high-intensity ultrasound; DD, deacetylation degree; DP, deproteinization; DM, demineralization; CD, Circular dichroism.

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ultrasound, microwave and grinding can accelerate the separation of chitin from shrimp shells [8–10]. These physical technologies for chitin extraction have the advantages of improving overall process efficiency and reducing operation cost.

At present, a lot of reports deal with ultrasound to extract chitin and astaxanthin from shrimp waste [11], to enhance the shrimp shells loosening [12], to assist the hydrolysis of chitin [13], as well as to prepare chitin nanomaterials [14] and highly deacetylated chitosan [15]. Besides, ultrasound has been proved to make enzymes activate or deactivate. For example, the binding ability of chitinase onto the substrate was strengthened through ultrasound, which helped to accelerate the enzyme reaction [16]. However, the excessive ultrasound led to protein denaturation and decrease of enzyme activity [17,18]. Indeed, ultrasound is an effective way to assist extraction of active components from some plants or animals, and has been widely used in different fields. The extraction process was enhanced through ultrasound, which was attributed to localized pressure generated by bubbles explosively collapse, and causing the release of intracellular substances from the tissue [19]. So far, some work has been carried out about ultrasound-assisted chemical extraction of chitin or chitosan from shrimp waste [8,20], but the assistant effects of ultrasound on fermentation extraction of chitin has not been published in the literature.

Ultrasound with intensity greater than 1 W/cm² and frequency ranging from 18 to 100 kHz was considered as high-intensity ultrasound (HIU) [21]. Several studies have demonstrated that HIU caused changes in the spatial structure of protein. After treating with HIU, the structure of tropomyosin from shrimp could be loosened, with the decreased α -helix and increased β -turn [22]. Shi et al. [23] indicated that HIU combined with potassium alginate treated on chicken breast meat induced the change in polar microenvironment through fluorescence spectroscopy. In this work, based on our previous study of chitin extraction by a successive co-fermentation process [24], we designed to pre-treat shrimp shells powder (SSP) with HIU before chitin extraction. We aim to improve the efficiency of chitin extraction by ultrasound-assisted co-fermentation process and understand the mechanism of ultrasound effects on chitin extracted by co-fermentation.

2. Materials and methods

2.1. Materials

SSP was obtained by crushing dry *Penaeus vannamei* shells into particles of 0.25–0.425 mm. *Bacillus subtilis* was isolated from an aquatic product factory, and *Acetobacter pasteurianus* was purchased from Guangdong Microbial Culture Collection Centre, China.

2.2. Ultrasound-assisted co-fermentation for extracting chitin from shrimp shells

The ultrasound treatment of SSP was performed according to Liu et al. [25] with slight modifications. In 50 mL centrifuge tube, 2.5 g of SSP was dispersed uniformly in 20 mL of distilled water. Then the samples were pre-treated with ultrasound at 20 kHz at different power settings (0, 200 W, 400 W, 600 W, 800 W) for 30 min (duration of 5 s on and 5 s off) using an ultrasonic cell disruptor (JYD-650 L, Ningbo Scientz Biotechnology Co., Ltd).

After ultrasound treatment, SSP was transferred to a 250 mL of flask. The fermentation media of 50 mL contained 1 g/L of yeast extracts, 50 g/L of glucose and 50 g/L of SSP. According to the optimized conditions shown in the previous study [24], the chitin extraction process consisted of DP fermentation with *B. subtilis* for 3 d and DM fermentation with *A. pasteurianus* for 2 d.

2.3. Assay of protease, soluble protein, Ca²⁺, DP and DM efficiency

The determination of neutral protease activity during fermentation

was performed by Folin-phenol reagent method according to the study of Chatterjee et al. [26].

The soluble calcium in fermentation liquid was measured using an atomic absorption spectrometer (TAS-990 SUPER AFG, Beijing Purkinje General Instrument Co., Ltd, China). The dissolution percentage of Ca²⁺ was calculated to be the mass ratio of Ca²⁺ in the supernatant and Ca²⁺ in SSP.

The soluble protein was quantified by the colorimetric method of Foline-phenol reagent. The dissolution percentage of protein was calculated to be the mass ratio of protein in the supernatant and protein in SSP.

The ash in SSP and chitin samples was analyzed using a muffle furnace (MF-1200C, BEQ, China), and protein content in SSP and chitin samples was determined by an automated Kjeldahl apparatus (K9840, Hanon, China). Based on the values of residual ash and protein in samples, the DP and DM efficiency could be calculated by referring to the equations listed in the study of Zhang et al. [24].

2.4. Molecular weight and deacetylation degree of chitin

To measure the M_w of chitin, chitin samples were dissolved in the solution of 5% LiCl/ N, N-dimethylacetamide (DMAC). Viscometric analysis and Mark-Houwink mathematical equation were used to determine the value of M_w , which was described in the study of Setoguchi et al. [27].

The deacetylation degree (DD) of chitin samples was derived from the carbon/ nitrogen ratio (C/N) using a stable isotope ratio mass spectrometer (isoprime precisiON, Elementar Analysensysteme GmbH, Germany). The value of DD could be calculated according to Eq. (1) [28].

$$DD(\%) = [1 - (C/N - 5.14)/1.72] \times 100 \quad (1)$$

2.5. Surface morphology of shrimp shells

After being treated with HIU at different power as described in Section 2.2, SSP were dried at 60 °C for 24 h. Before observation, samples were covered by a thin layer of gold to obtain conductivity. A field emission scanning electronic microscopy (Verios G4 UC, Thermo Scientific, USA) was used to observe the surface morphology of shrimp shells.

2.6. Extraction of water-soluble proteins derived from shrimp shells

The extraction of water-soluble protein from SSP was carried out based on the method described by Pan et al. [29]. SSP of 2.5 g was suspended in 20 mL of boric acid buffer (0.3 M, pH6.8). The mixture above was treated with HIU at different power (0, 200 W, 400 W, 600 W, 800 W) for 30 min (duration of 5 s on and 5 s off), then stirred for 18 h, followed by adding 75 mL pre-cooled ethylenediamine tetraacetic acid (EDTA) solution (pH7.0) to a final concentration of 10% (w/v), and next continued to stir for 12 h. The mixture was centrifuged at 8000 g for 15 min to remove the shrimp shells and save the supernatant. The soluble protein in the supernatant was precipitated by ammonium sulfate with 60% saturation. After keeping at 4 °C for 4 h and centrifuging at 8000 g for 15 min, the precipitate was re-dissolved in 5 mL of phosphate buffer (20 mM, pH7.0). Finally, the soluble protein was dialyzed for overnight to remove the residual ammonium sulfate.

2.7. Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE)

The soluble protein extracted from SSP treated with ultrasound was analyzed by SDS-PAGE, as described by Pan et al. [29]. The protein samples were mixed with the protein loading buffer (P0015, Beyotime, China) in proper proportion, and boiled for 5 min. The electrophoresis

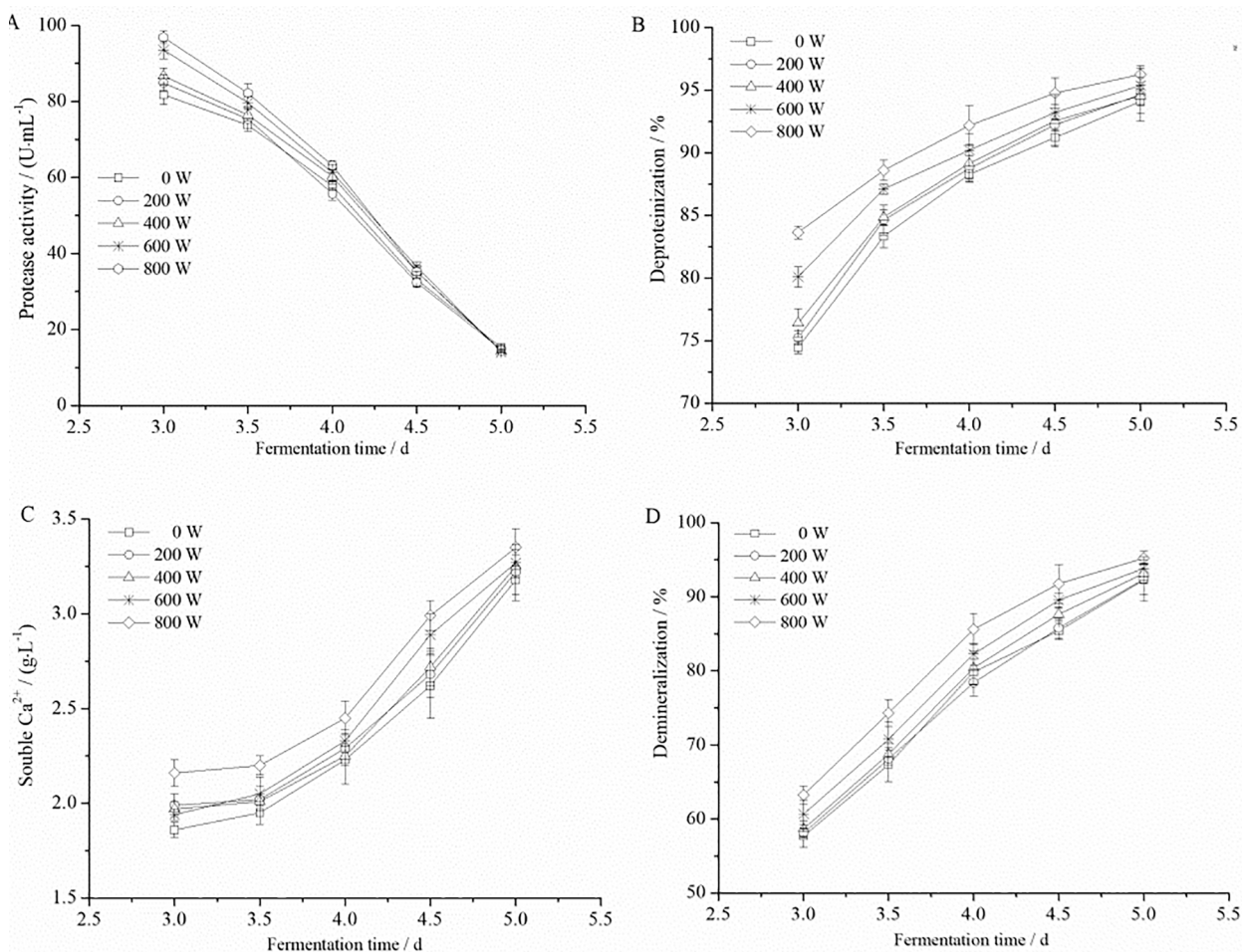


Fig. 1. Effects of ultrasound power on the protease (A), deproteinization (B), soluble Ca²⁺ (C) and demineralization (D) during chitin extraction by ultrasound-assisted co-fermentation.

was performed on a 4–20% precast gel (P0523S, Beyotime, China), and stained with FastBlue protein stain solution (BL607A, Biosharp, China). The Prestained Color Protein Marker (10–170 kDa, 26616, Thermo Fisher Scientific.) was used as the reference.

2.8. Protein conformation analysis

2.8.1. Circular dichroism (CD) spectra

The secondary structure of the soluble protein extracted from SSP was characterized by the far-UV CD spectra, as described by Zhang et al. [30]. Protein samples were diluted to 0.1 mg/mL, and scanned in the region of 200–250 nm at a rate of 5 nm/s by a MOS-450 spectrometer (Biologic, Claix, France).

2.8.2. Fluorescence spectra

The intrinsic fluorescence spectra of proteins samples were recorded by an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan), as described by Zhang et al. [30]. Protein solution (0.1 mg/mL) was scanned with the excitation wavelength 295 nm, and the emission wavelength was varying from 300 nm to 450 nm at a scanning speed of 12000 nm/min.

2.8.3. Surface hydrophobicity

The surface hydrophobicity of protein extracted from shrimp shells was measured by a fluorescence probe, as described by Zhang et al. [22]. In 4 mL of protein solution diluted to 0.1–0.5 mg/mL, 20 μ L of 8-anilino-1-naphthalenesulfonic acid (ANS) solution (8 mM ANS dissolved in 0.1

M of potassium phosphate buffer, pH7.0) was added and mixed well. The fluorescence intensity at 470 nm was recorded by a fluorescence spectrophotometer. The surface hydrophobicity of protein samples was determined by the initial slope of fluorescence intensity versus proteins concentration plot.

2.9. Statistical analysis

All data in tables or figures were the mean of three experiments, and were presented as mean \pm standard deviation (SD). The software of SPSS version 26.0 (IBM, USA) was used to perform the statistical analysis, and a significant difference was determined by Duncan's multiple range test ($p < 0.05$).

3. Results and discussions

3.1. Ultrasound-assisted co-fermentation for extracting chitin

In this work, we used HIU (200–800 W) to assist co-fermentation for the extraction of chitin from SSP. The associated parameters, including protease activity, soluble Ca²⁺ concentration, DP, and DM efficiency were determined during co-fermentation and are presented in Fig. 1. When SSP was pre-treated with HIU at 600 W and 800 W, the protease activity in the fermentation solution on day 3 reached 93.5 U/mL and 96.9 U/mL, respectively. These numbers are both considerably higher than the protease activity with no ultrasound treatment (81.8 U/mL). On day 5, the protease activity for all test samples decreased rapidly to

Table 1

Physicochemical parameters of chitin prepared by ultrasound-assisted co-fermentation.

| Chitin Power | Ash/% | Protein/% | Chitin/% | M_w /kDa | DD/% |
|--------------|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
| Control | 4.5 ± 0.2 ^a | 8.3 ± 0.3 ^a | 86.6 ± 1.5 ^a | 13.5 ± 1.1 ^a | 18.5 ± 0.4 ^c |
| 200 W | 4.4 ± 0.3 ^a | 7.8 ± 0.4 ^{ab} | 87.3 ± 0.9 ^{ab} | 13.9 ± 0.6 ^a | 19.3 ± 0.8 ^{bc} |
| 400 W | 4.2 ± 0.1 ^{ab} | 7.9 ± 0.4 ^{ab} | 87.5 ± 1.1 ^{ab} | 13.2 ± 0.4 ^a | 19.5 ± 0.4 ^{bc} |
| 600 W | 3.8 ± 0.3 ^b | 7.6 ± 0.2 ^b | 88.2 ± 2.0 ^{ab} | 12.6 ± 0.3 ^a | 20.2 ± 0.5 ^{ab} |
| 800 W | 2.9 ± 0.3 ^c | 6.8 ± 0.2 ^c | 89.8 ± 0.6 ^b | 11.2 ± 0.8 ^b | 21.1 ± 0.6 ^a |

M_w : molecular weight; DD: deacetylation degree. All values were calculated by averaging the data of three parallel experiments (n = 3). Superscripts containing the same letter in each column mean no significant difference (p > 0.05).

about 15 U/mL, which can be attributed to the inhibitive effects of the acetic acid produced by *A. pasteurianus*. Fig. 1B shows that the DP efficiency was enhanced by HIU at 800 W, and the fermentation time was reduced from 5 d to 4.5 d. This is because the DP efficiency on day 4.5 (94.8%) with ultrasound at 800 W was similar to that of no ultrasound treatment on day 5 (94.1%). According to Fig. 1C, HIU at 800 W also facilitates the dissolution of Ca^{2+} from shrimp shells during fermentation. The soluble Ca^{2+} concentration on day 5 was 3.35 g·L⁻¹, compared to 3.18 g·L⁻¹ with no ultrasound treatment. However, the soluble Ca^{2+} concentration didn't increase noticeably with ultrasound at low power (200 W and 400 W), whether on day 3 or day 5. In Fig. 1D we can see that the DM efficiency on day 4.5 (91.8%) of chitin extracted from SSP pre-treated by HIU at 800 W was comparable to that on day 5 for the sample with no ultrasound (92.4%). This enhanced DM efficiency can be ascribed to the fact that perhaps the protease-induced protein removal from the protein/ CaCO_3 matrix simultaneously leads to partial CaCO_3 removal except for the effect of ultrasound. We conclude that the DP and DM processes are both promoted by HIU at higher power (800 W), while HIU below 600 W of power produces a negligible difference compared to no ultrasound treatment. In shrimp shells in their natural condition, chitin molecules are arranged as microfibrils embedded in the protein/ CaCO_3 matrix. Due to the loosening effect of ultrasound, the protein/ CaCO_3 matrix peels off from the shrimp shells into the liquid medium [12]. The protease produced by *B. subtilis* is an inducible enzyme, and the peeled protein induces more protease production, thus resulting in improved DP. Ultrasound has already been proven as an excellent auxiliary approach for chitin preparation. For instance, Singh et al. [20] discovered that ultrasound effectively reduced the DP time in the chitin extraction from squid pens. This is probably because ultrasound produced cavitation in samples, which induced the breakage of nitrogen bonds and other intramolecular bonds, thus enhancing the DP process.

Table 1 displays the physicochemical parameters of chitin prepared by successive co-fermentation after pre-treatment of shrimp shells with ultrasound at different power (200–800 W). The purity of chitin increases along with ultrasound power and corresponds to the results in Fig. 1, which presents the effects of ultrasound power on DP and DM efficiency. Chitin extracted from SSP pre-treated with HIU at 800 W has a purity level of 89.8%, which is higher than chitin with no ultrasound treatment (86.6%). Meanwhile, Table 1 also indicates that ultrasound at 800 W reduces the chitin M_w (11.2 kDa) and increases the DD value (21.1%), compared with $M_w = 13.5$ kDa and DD = 18.5% for no ultrasound treatment. A previous report suggested that ultrasound was an effective technique for reducing the M_w value of polysaccharides without changing the chemical structure. The reduction in M_w was attributed to the breakage of covalent bonds in the backbone of amylopectin, chitosan and pullulan [31]. Additionally, ultrasound was used for chitosan preparation, and the DD was enhanced with an increase in ultrasound power [8]. Thus, chitin with lower M_w and higher

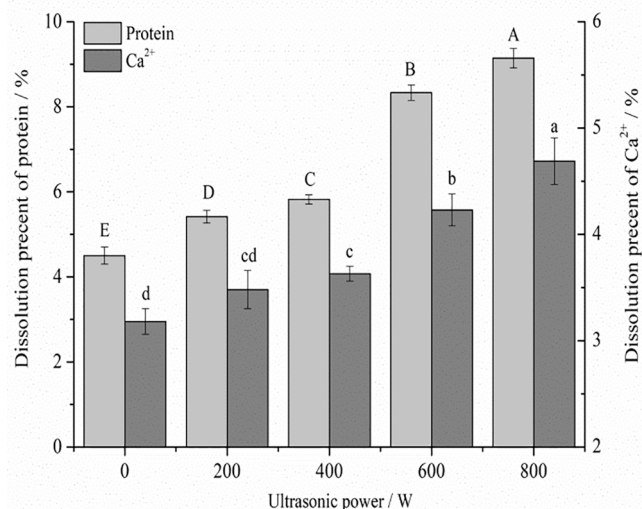


Fig. 2. Effects of ultrasound power on the dissolution percentage of protein and Ca^{2+} from shrimp shells.

DD values, extracted by ultrasound-assisted co-fermentation, has potential applications in functionalized nanomaterials and chitosan preparation.

3.2. Effect of ultrasound power on the dissolution of protein and Ca^{2+} from shrimp shells

To confirm the influence of ultrasound on the removal efficiency of protein and minerals from SSP, 2.5 g SSP was dispersed uniformly in 20 mL of distilled water, then treated with HIU (200–800 W) for 30 min. Ultrasound effects on shrimp shells, such as loosening, altering protein spatial structure, and heating, are closely related to ultrasound strength [12]. The dissolution percentage of protein and Ca^{2+} from SSP treated with ultrasound is displayed in Fig. 2. More protein and Ca^{2+} were released from SSP with an increase in ultrasound power. When SSP was treated with ultrasound at 800 W, the dissolution percentage of protein and Ca^{2+} were 9.1% and 4.7%, respectively. These values are both higher than with no ultrasound (4.5%, 3.2%). It was important to note that deproteinization efficiency on day 3 was 9.2% higher when SSP was pre-treated with HIU at 800 W than with no ultrasound (Fig. 1B), but the increment value in dissolution protein was only 3.6%. This indicates that the higher deproteinization obtained with HIU at 800 W is due to the combined effects of higher protein solubility and enhanced protease activity.

3.3. Changes in surface morphology of shrimp shells treated by ultrasound

The surface morphology of shrimp shells treated with HIU (200–800 W) is presented in Fig. 3. The surface of shrimp shells without ultrasound treatment was tight and rough (Fig. 3A, B), and significantly different from the surface of shrimp shells treated with HIU (Fig. 3C, D, E, F). Particles were produced on the surface of SSP treated with ultrasound, but the amount decreased with increasing ultrasound power. It can be postulated that these particles are the protein/ CaCO_3 matrix being stripped from SSP by HIU. Fig. 3C and Fig. 3D indicate that there were more microfibrillar deposits observed on the surface of SSP treated with ultrasound at 400 W than on the SSP treated with 200 W. When the ultrasound power was increased to 800 W, both microfibrillar and a noticeable porous structure were observed on the surface of SSP (Fig. 3F). According to previous reports, the microfibrillar and porosity were the characteristic surface structure of shrimp shell chitin [24,32].

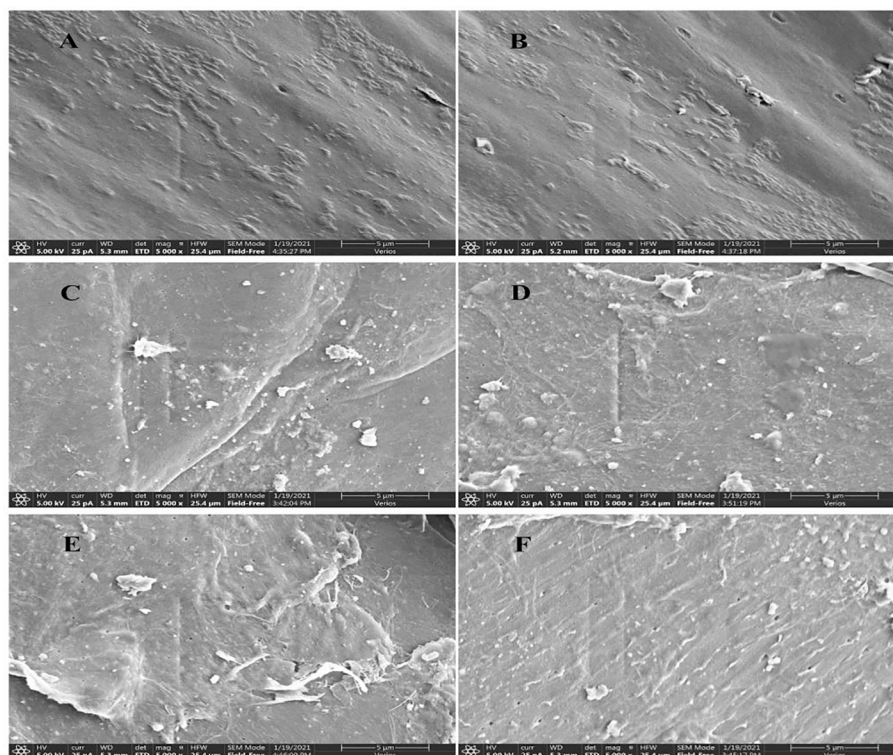


Fig. 3. The surface of shrimp shells treated with different ultrasound power. A, B: shrimp shells treated with no ultrasound; C, D, E, F represent shrimp shells treated with ultrasound at 200, 400, 600, 800 W, respectively.

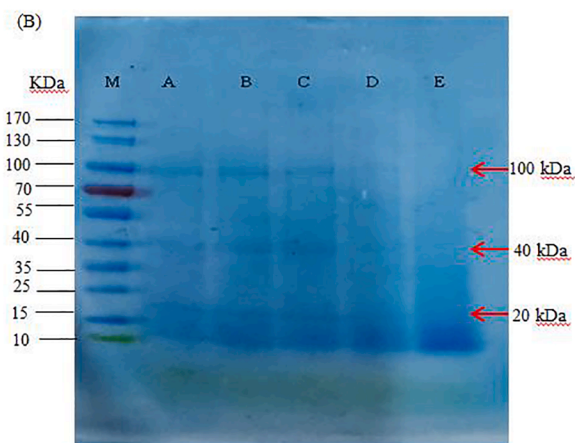
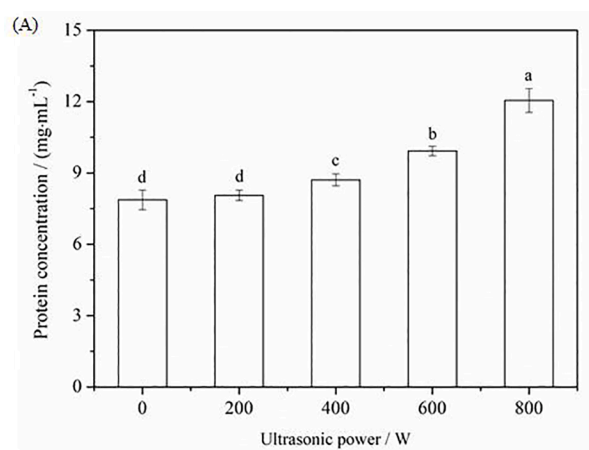


Fig. 4. Concentration and SDS-PAGE of soluble protein extracted from SSP treated with ultrasound at different power. M: Protein Marker; Lane from A to E represent the soluble protein extracted from shrimp shells treated with ultrasound at 0, 200, 400, 600, and 800 W.

3.4. Effects of ultrasound on the soluble protein extracted from SSP

Soluble protein was extracted from ultrasound-treated SSP using the boric acid buffer and EDTA, as described in Section 2.6. We noticed that the soluble protein concentration increased with rising ultrasound power (Fig. 4A). This result is due to the dissolution of more protein from SSP treated by HIU, which is consistent with the results displayed in Fig. 2. Besides, the soluble protein extracted from SSP was analyzed by SDS-PAGE, and the results are displayed in Fig. 4B. The lanes of A, B, and C all exhibited 20 kDa, 40 kDa, and 100 kDa protein bands. However, these protein bands disappeared when the ultrasound power increased to 600 W or 800 W. This indicates that ultrasound at high power (600, 800 W) induces the degradation of protein, which may promote the dispersion and solubilization of shrimp shell protein [33].

There have been very few particular proteins isolated from shrimp shells until now. The protein components are different depending on the shrimp species. A novel lipophilic protein abundantly containing hydrophobic amino acids was isolated from the shells of *Pandalus borealis* [34]. Pan et al. [29,35] isolated red color-related proteins from the shells of *Litopenaeus vannamei* and *Procambarus clarkia*, with the monomer molecular mass of 75 and 24 kDa, respectively. The isolation, identification and characterization of particular proteins from shrimp shells deserve further studies.

3.5. Conformation of shrimp shells protein affected by ultrasound

To further determine the effects of ultrasound on the secondary structure of the soluble protein, we recorded the spectra of far-UV CD,

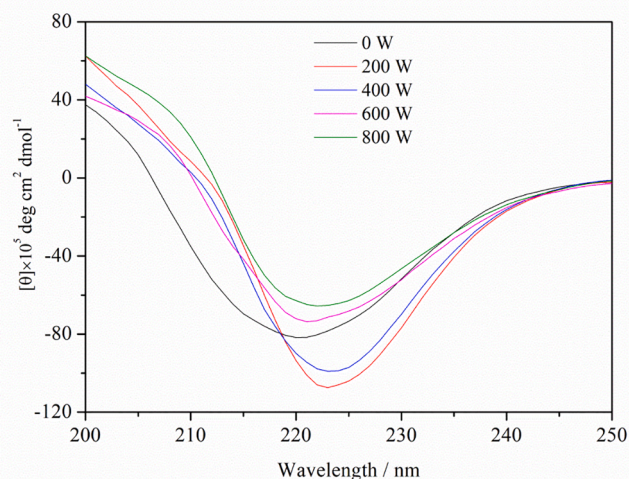


Fig. 5. Far-UV CD Spectra of soluble protein extracted from shrimp shells treated with ultrasound at different power.

and the results are shown in Fig. 5. The characteristic negative peak (220 nm) for a β -sheet was observed in the CD spectrum of protein from the untreated SSP. The soluble protein extracted from shrimp shells treated with ultrasound at low power (200, 400 W) exhibited enhancement in the negative peak around 220 nm compared to no ultrasound treatment. This is probably because ultrasound at 200 W or 400 W hardly caused the damage to protein structure, but promoted more soluble protein to be dissolved out from SSP than no ultrasound treatment (Fig. 4A). However, at higher ultrasound power (600, 800 W), the secondary structure of the protein was severely damaged, which resulted in the higher values of $[\theta]$ round 220 nm than with no ultrasound treatment. Changes in the secondary structure are a result of disruption to some interactions between different parts of molecules. In the study of Zhang et al. [22], after HIU treatment, there were fewer α -helix and more β -sheet structures in shrimp tropomyosin. Differences in modifications to the secondary structure of a protein may be due to the protein species and ultrasound power.

The polarity of the microenvironment surrounding tryptophan residue is a decisive factor for the intrinsic fluorescence spectra, which are used to monitor tertiary structural changes in protein [36]. The fluorescence spectra of SSP protein after HIU treatment are presented in Fig. 6A. Ultrasound treatment causes changes to occur in the

fluorescence spectra, such as maximum wavelength and fluorescence intensity. We observed that the maximum fluorescence intensity increased in SSP protein after ultrasound at 200 W. However, the maximum fluorescence intensity decreased as the ultrasound power increased from 400 to 800 W. Reduced fluorescence intensity is an indication of protein unfolding and more chromophores exposure [37]. Besides, HIU at 600 or 800 W also causes a redshift in the maximum wavelength, which leads to an increase in the microenvironment polarity of tryptophan residue [38]. The shift toward a longer wavelength suggests that the hydrophobic interaction is destroyed by ultrasound, and the hydrophobic groups are exposed to the surface of molecules [39]. As indicated in Fig. 6B, the surface hydrophobicity (S_0 -ANS) of protein with HIU increases, compared to no ultrasound treatment, which supports the idea of the hydrophobic interaction destruction by ultrasound. However, the surface hydrophobicity of protein from SSP treated with HIU at 800 W was slightly lower than that of 600 W, suggesting aggregation or partial denaturation of protein owing to HIU (800 W) [40].

In summary, HIU at high power (600, 800 W) contributes somewhat to the destruction of the secondary and tertiary structure of protein extracted from SSP, which might further promote the dissolution of protein from shrimp shells. Liu et al. [25] reported that HIU treatment effectively improves the solubility and dispersion stability of myofibrillar protein in water. The main mechanism of solubilization is due to the disruption of the intact structure and changes in the filamentous myosin spatial structure.

4. Conclusion

HIU pre-treatment of SSP at 800 W before co-fermentation facilitates higher DP and DM efficiency of chitin extraction process. It also improves the quality of application for chitin, such as a higher degree of deacetylation and lower molecular weight. The mechanism of positive effect on chitin extraction is that more protein stripped by HIU at 800 W promotes the production of protease by *B. subtilis* and further hydrolyzed the protein covering the SSP surface. Additionally, HIU-induced degradation and structural destruction of protein enhances the dissolution of protein from shrimp shells. In brief, ultrasound is an effective method for promoting chitin extraction by microbial fermentation.

CRediT authorship contribution statement

Qiao Zhang: Conceptualization, Methodology, Writing – original

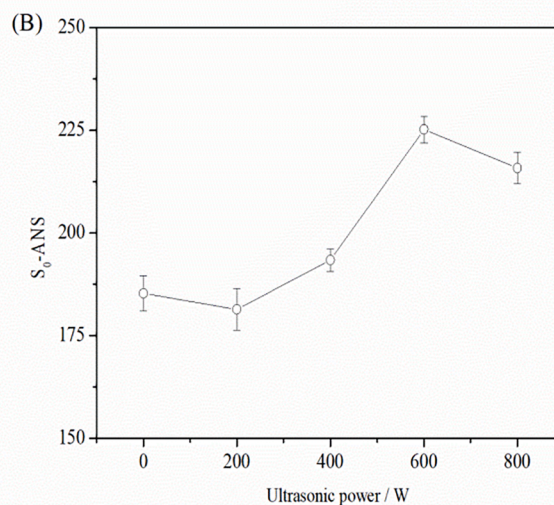
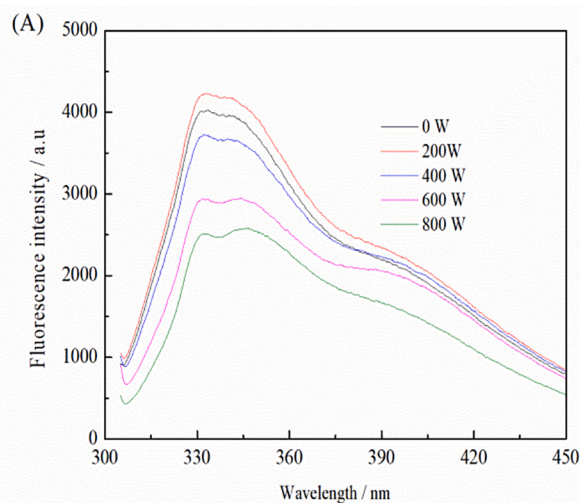


Fig. 6. Intrinsic fluorescence spectra (A) and surface hydrophobicity (B) of the soluble protein extracted from shrimp shells treated with ultrasound at different power.

draft. **Lirui Duan**: Software, Supervision, Visualization. **Yongcheng Li**: Conceptualization, Writing – review & editing, Project administration.

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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