



Effects of high-intensity ultrasound processing on the physiochemical and allergenic properties of shrimp

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ABSTRACT

Shrimp is a common seafood with high nutritional value and delicious flavor. However, many individuals are allergic to shrimp, but no therapies have been provided until now. The aim of this study was to evaluate the impacts of the high-intensity ultrasound processing (0, 5, 10, 15, 20 min, at room temperature) on the physiochemical and allergenic properties of shrimp samples. The results indicated allergenicity decreased with increasing treatment time, and the best hypoallergenic effect showed at 20 min with a 76% reduction of tropomyosin. At 20 min, the total soluble protein content decreased by 28.26%, while the *in vitro* digestibility, peptide content, total antioxidant capacity strengthened by 7.53%, 0.81%, and 71.29%, respectively. β -Sheets and α -helices increased with processing time, accompanied by the reduction of turns and unordered conformations determining by Fourier-transform infrared spectroscopy. More fragments, strips, and holes were observed after 20 min, which indicated the physical essence of ultrasound treatment in improving the extraction of bioactive molecules.

Industrial relevance: The high-intensity ultrasound as a novel non-thermal processing technique exhibits great potential in reducing the allergenicity of food products. In comparison to thermal processing techniques, it consumes less energy and has a higher mass transfer rate without altering the fundamental properties of foods. Shrimp allergens can be efficiently modified or even eliminated after the optimal condition of ultrasound treatment. The sensory and nutritional properties of shrimp can be retained to meet the marketing requirements for consumers. Therefore, the manufacture of hypoallergenic shrimps with high quality will be one of the most popular products in the future. The findings in this study provide a better understanding of ultrasound application in both lowering allergenicity and retaining the nutritional characteristics of shrimp.

1. Introduction

Shrimp, belonging to crustacean species in seafood, is an excellent source for human health. Shrimps are rich in proteins (up to 20%), well-balanced amino acids, a large number of minerals (e.g., calcium and selenium) and highly unsaturated fatty acids (HUFA) such as eicosapentaenoic (20:5n3, EPA) and docosahexaenoic (22:6n3, DHA) acids (Akonor et al., 2016; Sriket et al., 2007). Shrimp is also abundant in antioxidants, including vitamin B12, phosphorus, astaxanthin, and a fat-soluble carotenoid (Venugopal, 2008). These bioactive compounds can boost the immune system and reduce the risk of cardiovascular disease, stroke, and diabetes (Smith & Guentzel, 2010).

However, shrimp belong to the shellfish family, which is one of the “big eight” allergenic foods, has become a significant public health concern leading to a series of allergic reactions from the mild symptoms to life-threatening anaphylaxis (Hoffmann-Sommergruber, 2000; Silke,

2017). Patients suffering with a shrimp allergy are cross-reactive with other seafood such as crabs and clams. An investigation was conducted among 33 individuals with a shrimp allergy history and the main symptoms including urticarial/angioedema (28%), gastrointestinal (13%), pulmonary (9%), systemic anaphylaxis (7%), generalized pruritus (2%), and other symptoms (2%) were found (Daul et al., 1987). Shrimp is the most frequent offender in children and adults (Sicherer et al., 2004). In North America, the prevalence of shellfish allergy is approximately 1.3%, in which shrimp is the most commonly reported allergen (Chokshi et al., 2015). In Europe, a study found that shrimp is the most common allergenic food with 48 patients suffering from 142 food-sensitized patients surveyed in Spain (Castillo et al., 1996). In Asia, a questionnaire survey also reported that shrimp is the most common food causing allergic reactions (33.3%) in children aged 3–7 years in Thailand (Lao-araya & Trakultivakorn, 2012). Thus, shrimp allergy has become a challenge causing health-related issues all

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over the world.

The major shrimp allergen is tropomyosin (TM), which was first described as a crustacean allergen in 1981 (Ahmed et al., 2018; Hoffman et al., 1981). TM is a heat-stable protein that can maintain its activity even after being boiled in water (Besler et al., 2001). It is estimated that at least 80% of individuals with shrimp allergy history showed allergic reactions to this allergen-producing shrimp-specific IgE antibody. TM has also been described as a primary allergen in other crustaceans such as lobsters, crabs, and mollusks, as well as other arthropods (house dust mites and cockroaches) (Ayuso, Lehrer, Reese, 2002). In addition, studies have reported that people with shrimp allergy history showed cross-reactivity to the animals mentioned above due to their sharing similar amino acid sequences (Ayuso, Reese, et al., 2002).

Novel techniques have been applied to the modification of shrimp allergens by denaturation and interaction reactions (Ekezie et al., 2018; Rahaman et al., 2016). To reduce the content of shrimp allergen, many methods have been used such as ultrasound processing (Li et al., 2011; Li, Linhong, Khalid, 2006; Zhang et al., 2018), thermal treatment (Wal, 2003), pulsed UV light (Shriver et al., 2011), gamma irradiation (Li et al., 2007), cold plasma (Shriver, 2011), fermentation (Kim et al., 2008), etc. Among them, ultrasound processing showed the most efficient impact in reducing the allergenicity of shrimp. Li, Lin, et al. (2006) treated shrimp with ultrasound treatment (30 Hz, 800 W) for 30–180 min. The results demonstrated that the allergenicity of shrimp allergen showed a decreasing trend with the rise of ultrasound processing duration. The maximum reduction was up to 75% analyzed by the ELISA test (Li, Lin, et al., 2006). In another study, Zhang et al. (2018) treated tropomyosin with 100–800 W ultrasound for 15 min. They found the allergenicity of tropomyosin significantly decreased using immunoblotting and ELISA test, which was associated with the degradation of TM under a high-intensity ultrasound (Zhang et al., 2018).

However, most ultrasound treatments were conducted under a long-time duration accompanying high energy consumption. The objective of this study was to evaluate the effect of high-intensity ultrasound processing (0, 5, 10, 15, and 20 min at 400 W) on the allergenicity, total soluble protein content, secondary structure, and microstructure of shrimp. The total antioxidant capacity and *in vitro* protein digestibility of shrimp samples are also measured. The experimental results may further provide strategies for better management and development of shrimp hypoallergenic and positive health attributes to the population.

2. Materials and methods

2.1. Sample preparation

Frozen shrimps (*Litopenaeus vannamei*, whiteleg) purchased from a local supermarket (Liantai Chinese Market, Montreal, Canada) were stored at -20°C until their further analysis. The average length and weight of shrimps were around 8.79 ± 0.42 cm and 32.54 ± 0.85 g, respectively. As shown in Fig. 1, the shrimps were thawed, beheaded, and deshelled. The experimental samples were prepared by mincing the edible portion with double distilled water by a ratio of 1:3 (per gram/per milliliter) using a juicer.

2.2. Ultrasound treatment

Ultrasound processing was conducted using a Branson Sonifier 450 (Branson Ultrasonic Corp., Danbury, CT, USA) with a probe at a 20-kHz frequency, 400 W. The sonication probe was immersed into the liquid samples maintained in a glass jar. As shown in Table 1, 50 mL of samples were treated by ultrasonic processor with the duty cycle at 50% for 0 min (US0), 5 min (US5), 10 min (US10), 15 min (US15), and 20 min (US20), respectively. After the treatments, some samples were stored at 4°C for microstructure analysis. The leftover processed

samples were dried by a freeze dryer (7420020, Labconco Corporation, Kansas City, USA) for 48 h and then were stored at -20°C until further utilities (Fig. 1). All treatments and analyses were performed in triplicates.

2.3. Total soluble protein content

The shrimp sample (0.5 g) was mixed with PBS buffer (0.1 M, pH = 7.0). After incubation at room temperature for 30 min, the mixture was centrifuged at $4000 \times g$ for 10 min. The supernatant was collected for further measurement. In the present study, a Pierce BCA protein assay kit (purchased from Thermo Fisher Scientific, Canada) was used to determine the total soluble protein of shrimp samples according to the provided protocol (Wang, Vanga, Raghavan, 2019).

2.4. SDS-PAGE measurement

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Zhang et al. (2018) with some modifications. The extracted protein solution was mixed with sample buffer (10 μL β -mercaptoethanol, 90 μL $4\times$ Laemmli sample buffer) at the ratio of 1:1 (v/v). After heating at 95°C for 5 min in a water bath, the mixture was cooled down at room temperature. Then, 10 μL of denatured protein samples and 5 μL of the molecular marker (10–250 kDa, Bio-Rad, Philadelphia, PA, USA) were loaded in the gel. Electrophoresis was performed in a vertical unit (Mini-PROTEAN[®] Tetra System, BIO-RAD, Philadelphia, PA, USA) at 80 V. After electrophoresis, the protein gel was stained with 0.1% Coomassie Brilliant Blue R-250 (Sigma-Aldrich Chemical, St. Louis, MO, USA) for 10 min, then destained with a solution containing 90 mL of 50% methanol and 10 mL of acetic acid. The related figure was obtained using a digital camera (Canon, EOS Rebel SL2 DSLR Camera, America).

2.5. Sandwich ELISA

In the present study, a Sandwich ELISA kit (Shrimp Tropomyosin ELISA 2.0, Indoor Biotechnologies, USA) was used to determine the tropomyosin content of shrimp samples. The ELISA kit contains a microtiter plate coated with an anti-tropomyosin monoclonal antibody, and reagents including tropomyosin allergen standard (500 ng/mL), rabbit anti shrimp tropomyosin antiserum, peroxidase-conjugated goat anti-rabbit IgG, wash buffer ($10\times$), assay buffer ($10\times$), TMB developing substrate, and stop solution. According to the protocol provided by the kit, the final absorbance of tropomyosin in each sample was recorded at 450 nm. The concentration of tropomyosin was calculated according to the standard curve obtained by the standard.

2.6. Secondary structure

Fourier transform infrared spectroscopy (FTIR) was used to study the changes in secondary structures present in shrimp proteins. The powder sample (0.1 g) was transferred to the diamond crystal at room temperature. The data was analyzed by Windows-based OMNIC software (Version 8, Thermo Nicolet Co., Madison, WI) connected to the FTIR spectrometer (Nicolet Magna 158,750 FTIR, Nicolet Instrument Corp., Madison, WI). Thirty-two scan spectra at a spectral resolution of 4 cm^{-1} will be recorded and averaged in the mid-infrared region ($4000\text{--}500\text{ cm}^{-1}$). A background spectrum without the sample was collected before each determination to avoid the influence of air.

A curve-fitting procedure was applied using OriginPro (Version 9, Origin Lab Corporation, Northampton, MA, USA) to quantify the conformational changes of shrimp proteins in the amide I band. The sum of squares of the differences was minimized between experimental spectra and the computed spectra developed by the summation of the component curves. The component bands were determined using the second

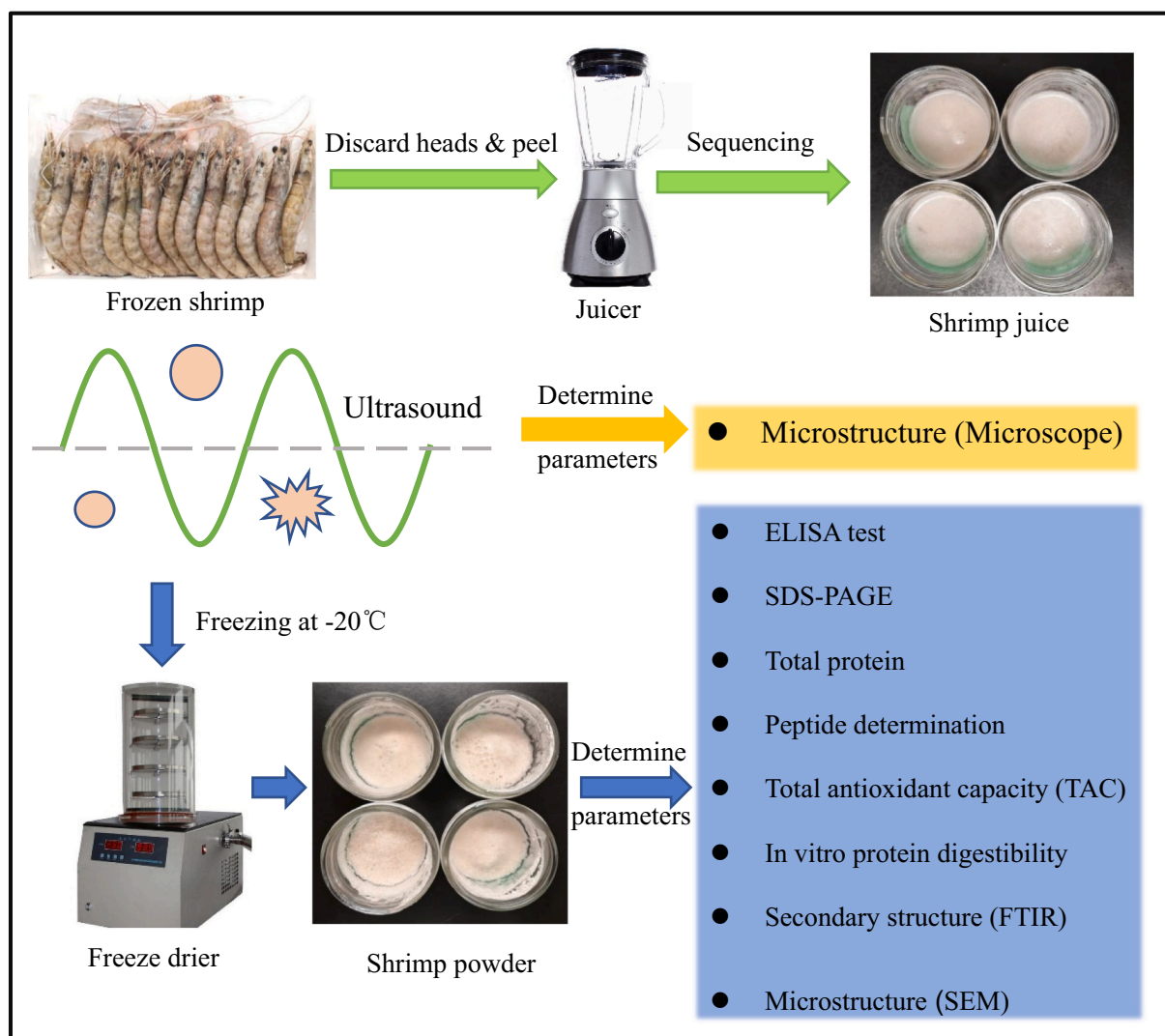


Fig. 1. Schematic overview of experimental setup.

Table 1
Ultrasound treatment index applied to shrimp samples.

Treatment	Amount/mL	Time duration/ min	Duty cycle/ %	Frequency and power
US0	50	0	50%	20 kHz, 400w
US5	50	5	50%	20 kHz, 400w
US10	50	10	50%	20 kHz, 400w
US15	50	15	50%	20 kHz, 400w
US20	50	20	50%	20 kHz, 400w

derivative spectrum generated by the software, and the results were given in terms of the percentage of secondary structures at corresponding wavelengths.

2.7. Analysis of total antioxidant capacity

As described by Wang, Wang, et al. (2019), a ferric-reducing/antioxidant power (FRAP) assay was performed to test the total antioxidant activity of shrimp samples. Briefly, the FRAP working solution was prepared by mixing ferric chloride solution (20 mM), acetic acid buffer (300 mM), and 2,4,6-tripyridyl-S-triazine (TPTZ) (10 mM) in the ratio of 1:1:10 (v/v/v), respectively at room temperature. The extraction of antioxidants from shrimp samples was done by mixing samples with

methanol. FRAP solution (200 mL) and the extract (67 μ L) were added to the microwells of plates, respectively. After a 5-min incubation with slight shaking at 37 °C in the dark, the color change was measured at a wavelength of 593 nm by a spectrophotometer. Standard solutions were prepared from a 1000 μ M ferrous sulfate solution to make a standard curve, and the antioxidant activity of shrimp samples was presented as μ mol Fe (II)/100 mg of samples.

2.8. Microstructure

According to the method described by Stratakis, Delgado-Pando, Linton, Patterson, and Koidis (2016), 20 μ L of diluted shrimp samples were transferred to the glass slide and then were stained using 0.1% of toluidine blue (obtained from Sigma-Aldrich (Quebec, Canada)) solution for 2 min. The mixture was observed under an optical microscope equipped with a digital camera (Leica DM500, Leica Microsystems Inc., Canada). The images were captured using imaging software (Leica LAS EZ, Leica Microsystems Inc., Canada) at a 10 $\times$ objective.

A Scanning Electron Microscope (SEM) (TM3000, Hitachi High-Technologies Corporation, Tokyo, Japan) was applied to observe the microstructural alteration of each sample (Wang, Vanga, Raghavan, 2019). The freeze-dried shrimp samples were transferred to the measuring platform. The level of magnification was set at $\times$ 500, and the related figures were captured using the software equipped with SEM.

2.9. *In vitro* protein digestibility

The *in vitro* protein digestibility of shrimp proteins was conducted following a two-stage procedure suggested by Hejazi and Orsat (2016) and Vilela et al. (2006) with slight modifications. In the first stage of the digestion, shrimp powder was dissolved in the double distilled water to a final protein concentration of 3 mg/mL, and the pH of the sample solution was adjusted to 1.5 using 0.1 N HCl. The flasks were put in a water bath at 37 °C, and the digestion was started by adding freshly prepared pepsin solution (5 mg pepsin/mL in 0.01 M HCl) to reach an enzyme to substrate ratio of 1: 100. After 30 min, the digestion was stopped by adding 1.0 M NaOH solution moderately. The pH of the solution was adjusted to 7.8, using 1.0 M NaOH to start the second stage of the digestion. Digestion was started by adding prepared pancreatin stock solution (5 mg/mL in sodium phosphate buffer, pH 7.0) to reach an enzyme to substrate ratio of 1: 30 and incubated at 40 °C. A 150 mM Na₂CO₃ solution was used to stop the reaction after 60 min of digestion. The total protein contents of shrimp samples after the first-stage and second-stage digestion were determined using the BCA method (Beazley, 2017). The *in vitro* protein digestibility was calculated using the following equation:

$$\text{IVPD\%} = \frac{\text{initial protein} - \text{final undigested protein}}{\text{initial protein}} \times 100$$

2.10. Determination of peptide content

The α-amino group content was determined using the OPA reagent (Church, Porter, Catignani, & Swaisgood, 1985). OPA reagent (50 mL) was obtained by mixing 25 mL of sodium tetraborate (100 mM) with 2.5 mL of sodium dodecyl sulfate buffer (20%, wt/wt), OPA solution (40 mg dissolved in 1 mL of methanol), and 100 μL of β-mercaptoethanol. The digested samples at different stages (50 μL) were incubated with 950 μL OPA reagent solution for 2 min at room temperature. The absorbances were recorded at 340 nm using a plate reader (Thermo Fisher Scientific, Canada). A standard curve was constructed using leucine-glycine as a standard peptide.

2.11. Statistical analysis

The experimental data were analyzed using the analysis of variance (ANOVA) of the SPSS software (SPSS Inc., Ver. 18, Chicago, IL, USA). The Duncan, multiple range test, was applied to separate the means and to establish significance, which was accepted at $p < 0.05$.

3. Results and discussion

3.1. Total antioxidant capacity (TAC)

As shown in Table 2, the influences of ultrasound treatment on the antioxidant capacity of shrimp samples were outlined. The total antioxidant capacity of shrimp samples gradually increased with the rise of processing duration from 5 min to 20 min. There is no significant difference between the treatment US0 US5, US10, and US15. The highest

Table 2
Changes of bioactive compounds in ultrasonic-treated shrimp samples.

Treatment	Total protein (mg/g)	TAC (μmol/100 mg)	Tropomyosin (ng/mg)
US0	255.16 ± 8.68 ^a	29.50 ± 1.61 ^b	1.50 ± 0.13 ^a
US5	228.17 ± 1.49 ^{ab}	35.03 ± 2.86 ^b	1.24 ± 0.14 ^{ab}
US10	200.25 ± 11.43 ^{bc}	42.70 ± 8.41 ^{ab}	0.88 ± 0.11 ^b
US15	186.34 ± 33.06 ^c	46.28 ± 21.25 ^{ab}	0.38 ± 0.087 ^c
US20	183.04 ± 5.67 ^c	50.53 ± 3.71 ^a	0.36 ± 0.065 ^c

Note: Values with different letters in the same column are significantly different ($p < 0.05$) from each other.

total antioxidant capacity of shrimp was observed in US20 treated samples (50.53 μmol/100 mL) with a significant difference, followed by US15 (46.28 μmol/100 mL), US10 (42.70 μmol/100 mL), US5 (35.03 μmol/100 mL), and US0 (29.50 μmol/100 mL).

These results indicate that ultrasound processing with a long time can contribute to the increase in total antioxidant capacity. In rainbow trout (*Oncorhynchus mykiss*), samples after ultrasound treatment showed higher antioxidant capacities by FRAP values due to the alterations of fraction structures affected by ultrasound (Misir & Koral, 2018). Similarly, in red raspberry puree, the total antioxidant capacity increased by 17.3% when a 20-kHz ultrasound was applied for 10 min (Golmohamadi et al., 2013). Moreover, the enhancement of total antioxidant capacity was also reported in carrot-grape juice treated by ultrasonication (20 kHz, 525 W) for 2– 6 min (Nadeem et al., 2018). These increases mainly resulted from the rise of antioxidants (e.g., phenolics, ascorbic acids) after ultrasound processing (Tang et al., 2015). Moreover, inactivation reactions of some oxidation related enzymes resulted from the sheer force, such as polyphenol oxidases, occurred during the processing period, which would also increase total antioxidant capacity in food products (Cheng et al., 2007).

3.2. Total soluble protein determination

The total soluble protein content of shrimp samples was significantly affected by ultrasound processing (Table 2). The results showed that the total protein content of shrimp samples significantly decreased after treatment ($p < 0.05$). Specifically, the total protein content at US20 treated samples was reduced by 28.26% compared to the untreated sample, followed by US15 (26.97%), US10 (21.52%), and US5 (10.58%).

Similarly, the total protein of kiwifruit significantly decreased ($p < 0.05$) after the same ultrasound treatment for 4– 16 min (Wang, Vanga, Raghavan, 2019). Kang et al. (2016) also found a decrease in beef proteins by 20-kHz ultrasound treatment for 30– 120 min. These decreases might be associated with the breakdown of hydrogen bonds and peptide chains represented in the proteins under ultrasound treatment, which in turn could alter the protein structure (e.g., secondary or tertiary structure) (de São José et al., 2014). Thus, ultrasound treatment has a potential application to modify the structure of the protein or rupture their peptide chains, which may further affect the allergenicity of related allergens.

3.3. *In vitro* protein digestibility and peptide determination

As shown in Fig. 2, the *in vitro* digestibility of shrimp proteins increased with the rise of processing time. During the first 15 min ultrasound processing, a slight increase, but no significant difference was observed in the *in vitro* digestibility of shrimp proteins (79.61%) when compared with the initial level (76.42%). Whereas the *in vitro* digestibility of shrimp proteins significantly increased to 83.95% after 20-min ultrasound processing.

During the determination of *in vitro* protein digestibility, two-stage digestion, including pepsin digestion and pancreatic digestion, was conducted in the present study. Pepsin tended to cleave peptide bonds next to phenylalanine or tyrosine residues or leucine bonds. At the same time, pancreatin was more likely to cleave tryptophan, arginine, tyrosine, leucine, phenylalanine, and lysine bonds (Tapal & Tikku, 2019). During such two-stage enzymatic hydrolysis, an ample variety of smaller peptide and free amino acids were generated. The size, level, and compositional changes of these small peptides and amino acids can affect the antioxidant activity of samples (Kou et al., 2013). Peptide content was measured to assess the generated or hydrolyzed peptide during the ultrasonic process. Different from the IVPD of samples, there was no significant change of peptide content in treated samples compared with the untreated sample after two-stage hydrolysis (Table 3). However, a sharp increase of peptide amount was observed after partial

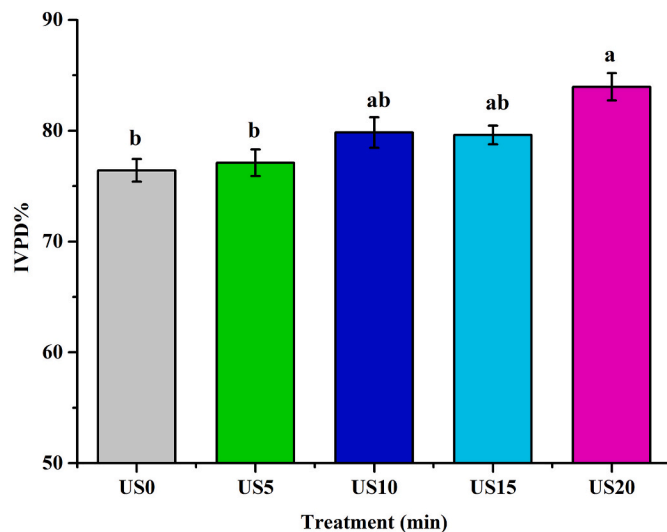


Fig. 2. Effect of ultrasonic processing on protein digestibility (IVPD %) of shrimp meat protein.

Table 3
Effect of ultrasonic processing on peptide generation of shrimp proteins.

Treatment	Initial peptide (mM)	After pepsin (mM)	After pancreatin (mM)
US0	10.09 ± 0.06 ^b	15.64 ± 0.24 ^b	19.65 ± 0.22 ^b
US5	10.02 ± 0.01 ^b	18.02 ± 2.04 ^a	20.05 ± 0.12 ^a
US10	9.97 ± 0.09 ^b	16.92 ± 1.03 ^{ab}	19.79 ± 0.07 ^{ab}
US15	10.22 ± 0.06 ^a	16.91 ± 0.26 ^{ab}	19.71 ± 0.23 ^b
US20	10.04 ± 0.09 ^b	16.88 ± 0.55 ^{ab}	19.81 ± 0.18 ^{ab}

Note: Values with different letters in the same column are significantly different ($p < 0.05$) from each other.

hydrolysis of proteins by pepsin, further accompanied by a continuing generation of peptides after pancreatin hydrolysis. In the first stage, the peptide contents rapidly increased from 9.97–10.22 mM to 15.64–18.02 mM. In comparison, the increasing degree of peptides was slowed down in the stage of pancreatic digestion, whereas a light enhancement in the peptides in the shrimp samples was observed. The final peptide amount in each treatment was approximately two times the initial peptides.

In egg white protein, similar results were described by Zhu et al. (2018). The study reported that the *in vitro* digestibility of egg white proteins significantly increased from 73% to 79% after 16-min ultrasound processing at the frequency of 20 kHz, 400 W. Su et al. (2006) also reported the digestibility of egg white proteins could be improved by 13–35% under 40-kHz ultrasound treatment for 2 h. In shrimp (*Exopalaemon modestus*) tropomyosin, the significant enhancement of digestibility was observed in SDS-PAGE when treated by ultrasound treatment (100–800 W, 15 min) (Zhang et al., 2018). The reason was probably that high-intensity ultrasound-induced conformational changes of proteins and protein aggregates, leading to the exposure of enzyme cleavage sites, further resulting in enhanced digestibility (Zhang et al., 2018). However, an opposite result was obtained in faba bean protein, as described by Martínez-Velasco et al. (2018). The *in vitro* digestibility decreased by 3.6% due to the structural changes caused by 17.92-min ultrasonication (Martínez-Velasco et al., 2018). Such an opposite result might be due to the different structures of food proteins.

With ultrasound treatment, the increased peptide yield was also observed by Wang et al. (2016). The study reported the peptides in rapeseeds protein increased by 40.88% after dual-frequency ultrasound treatment. Jia et al. (2010) revealed the release of peptides from defatted wheat germ protein during enzymatic hydrolysis was promoted

up to 42.9% by ultrasonic treatment at 20 kHz, 1500 W for 20 min. Therefore, the ultrasonication could greatly contribute to the reaction of enzymolysis and result in the significant increase of the peptides from rapeseed proteins. This was due to the intense energy generated by ultrasound led to the alteration of the protein structure. As known, general proteins were curled and folded tightly, so enzymes have no access to react with them in such circumstances adequately. Whereas ultrasonication could unfold and uncurl to the secondary and tertiary structure to the active sites in the protein, which further caused the rise of infinities between proteins and enzymes (Wang et al., 2016). In the study, the secondary conformation changes of the shrimp protein were investigated by FTIR analysis.

3.4. FTIR analysis of secondary structural changes in shrimp protein

FTIR analysis was utilized to investigate the effects of ultrasound processing on the secondary structures of shrimp proteins. In FTIR spectra, is one of the most valuable and sensitive areas, which is primarily used to study the secondary structure deviations in proteins (Vanga et al., 2016). It includes overlapping bands of various secondary structures, including β -sheets, α -helices, turns, and randomly coiled conformations (Wang et al., 2018). The absorbances of each sample in the amide I region of the typical FTIR spectra were observed (Fig. 3). For the amide I region, different frequency ranges (cm^{-1}) correspond to different secondary structures, including β -sheets (1613–1637; 1682–1696), α -helices (1645–1662), turns (1662–1682; 1630), and unordered (1637–1645).

The changes of secondary structures in shrimp proteins under ultrasound processing are represented in Fig. 4. It was noticed that β -sheets were the main secondary structure present in shrimp, representing about 46.48–63.28% of the total secondary structures. Unordered structures accounted for 8.22–12.42% of the total protein secondary structures, which were converted from the helices present in the untreated sample. In comparison to the untreated sample, a noticeable increase in the α -helices was observed after ultrasound processing, while a remarkable decline in the turn structures was presented in proteins. These results suggested ultrasound treatment led to the unfolding of the turns, simultaneously promoting the formation of β -sheets, α -helices, and decreasing interaction between amino acids that were in the random coiled region.

These findings agree with the results obtained by Yang et al. (2017). They treated rice proteins with 300-W ultrasound at various frequencies (20/35/50 kHz). The FTIR analysis results showed that all the ultrasound treatments caused a reduction in the turns and increased β -sheet structures present in rice proteins. Besides, Jin et al. (2016) investigated the effect of dual-frequency power ultrasound pretreatment on corn gluten meal hydrolysates. The results found that ultrasound processing significantly decreased the β -turn structure by 27.2%, while the α -helices and β -sheets were increased by 3.8% and 16.5%, respectively. The difference in secondary structure content might be due to the shear forces of ultrasound mechanical action, which disrupted the interactions between the protein molecules and influenced the protein molecule internal structures (Yang et al., 2017). Baltacıoğlu, Bayındırlı, and Severcan (2017) treated mushroom polyphenol oxidase with thermosonication, and the results showed a loss in the α -helix and β -sheet structures, but an increase in the turns and random coil structures after processing. Thus, the structural changes under ultrasound processing are different in various proteins, and further studies are still in need in the future.

3.5. Allergenicity

In the present study, a sandwich ELISA test was used to determine the changes of shrimp allergen under ultrasound processing. As shown in Fig. 5, the tropomyosin decreased gradually with the rise of processing time. In comparison to US0 (1.50 ng/mg), the maximum

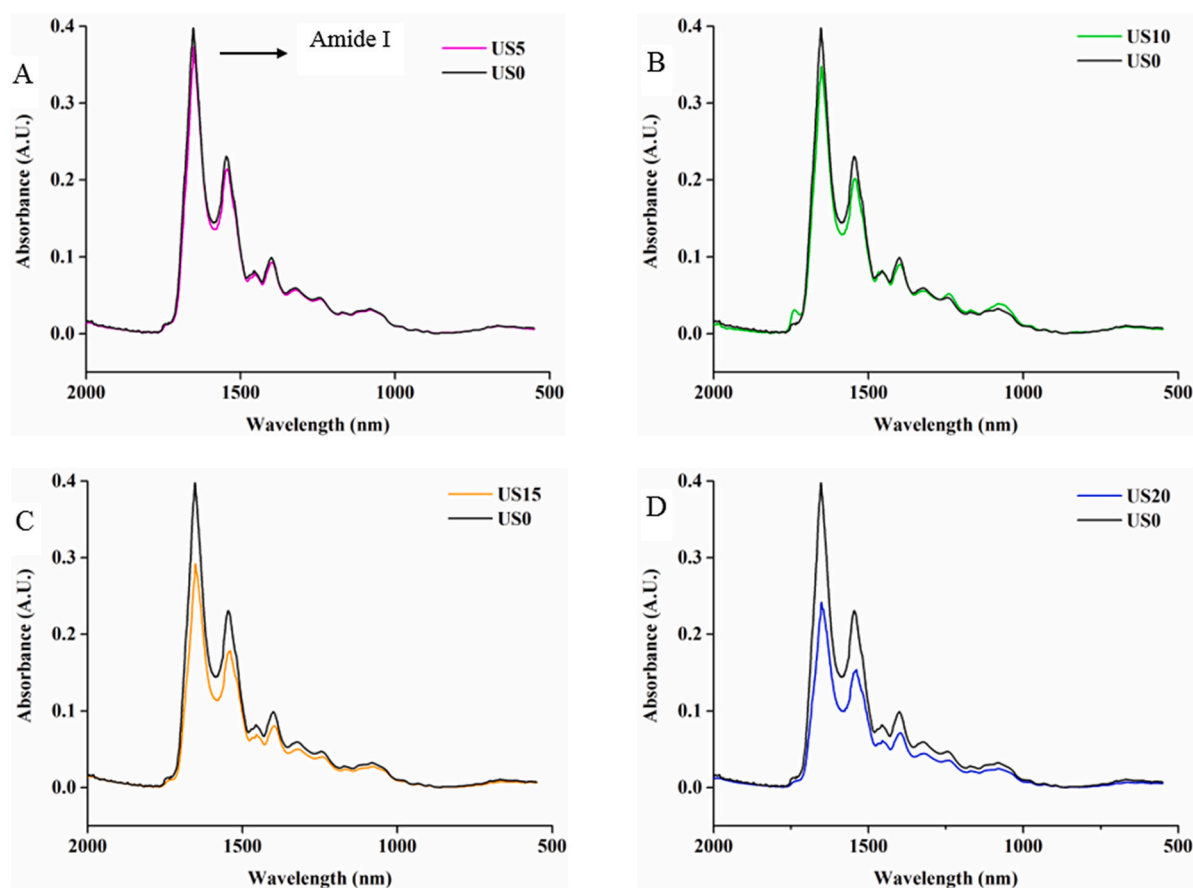


Fig. 3. The FTIR spectra of shrimp samples in the region of amide I.

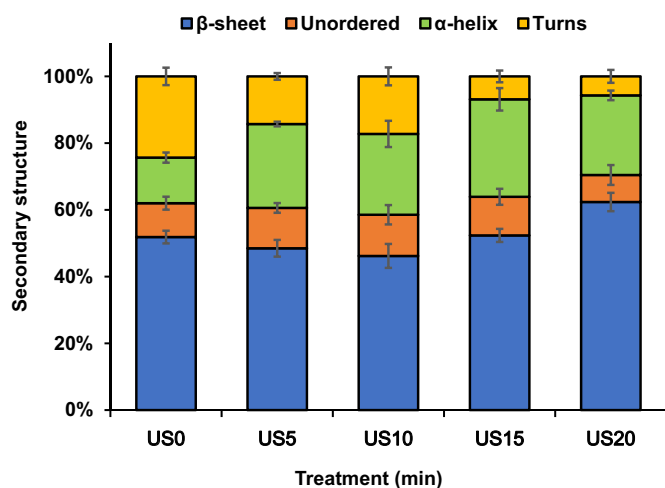


Fig. 4. Secondary structures in shrimp samples with different ultrasonic processing time.

decrease of 76% was observed in US20 (0.36 ng/mg), followed by US15 (0.38 ng/mg), US10 (0.88 ng/mg), and US5 (1.24 ng/mg). The tropomyosin content in US10 had a significant decrease ($p < 0.05$) in comparison to that in control. Similarly, tropomyosin contents in US15 and US20 were also significantly lower ($p < 0.05$) than the tropomyosin content in US10, whereas no significant decrease was observed between US15 and US20.

As shown in SDS-PAGE (Fig. 6), the intensity of the TM protein band at 36-kDa tropomyosin showed a decreasing trend with the increase of processing time under ultrasound treatment. The lightest TM protein

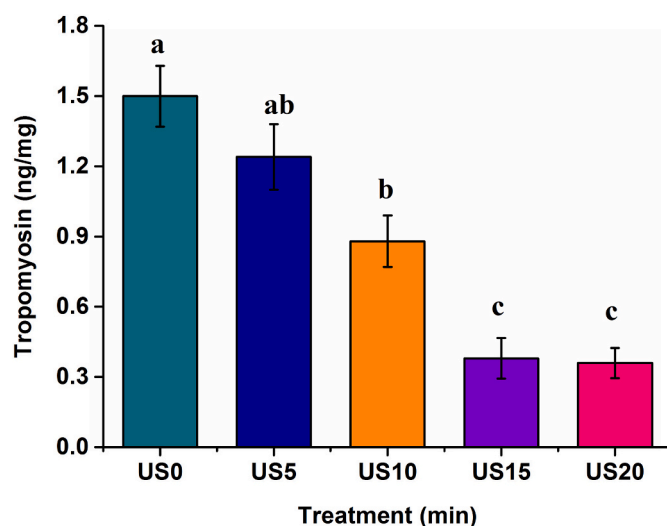


Fig. 5. Tropomyosin contents of shrimp samples with and without ultrasound treatment.

band was observed in US20, which was consistent with the results in the ELISA test.

In milk, Liu et al. (2018) reported a 61% reduction in the allergenicity of β -lactoglobulin after a 400-W ultrasound treatment resulting from the improvement of the glycation extent of some glycation sites under ultrasound. The allergenicity of shrimp tropomyosin from *Exopalaemon modestus* treated by ultrasound (100–800 W, 15 min) reached the lowest OD₄₅₀ value of 0.95 at 800 W according to the results of ELISA test and SDS-PAGE (Zhang et al., 2018). The degradation of

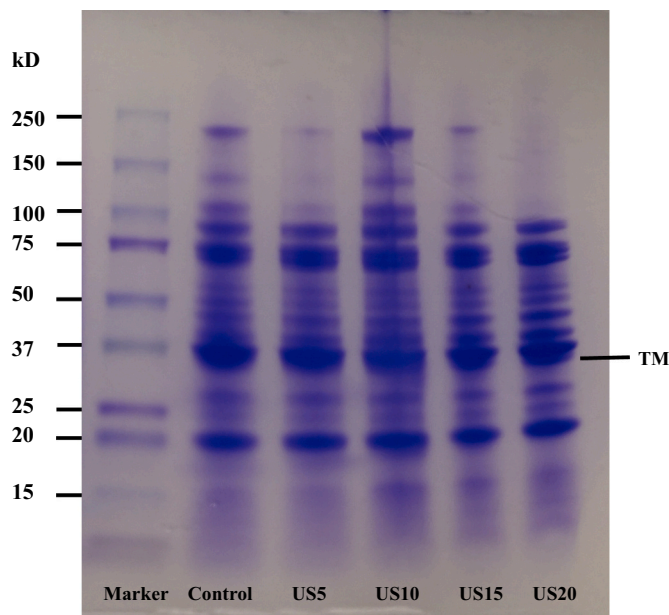


Fig. 6. SDS-PAGE of ultrasound-treated and untreated proteins in shrimp samples.

tropomyosin might be due to the cleavage of bonds in conformational structures by ultrasound-induced free radicals attack (Zhang et al., 2018). In detail, hydroperoxyl radical (HO_2) is the main species of free radicals with highly-reactive and non-selective characteristics. It generally reacts with molecules in atom abstraction or additional reactions driven by bond strength energetics and further disrupts or cleavages the primary structure of tropomyosin molecules (Pera-Titus et al., 2004). Thus, ultrasonic processing shows a potential application in reducing the immunology of shrimp.

3.6. Microstructure observation

Food microstructure is the arrangement of structural elements within the food and the forces that bind them together (Aguilera et al., 2000). Various food components are assembled into structures determining the overall properties of foods (Morris & Groves, 2013). Microstructure and its degradation in food are generally used to assess food quality intuitively. The microstructure of shrimp samples was observed by a scanning electron microscopy (SEM) and an optical microscope.

The results showed that the degradation of microstructures in shrimp samples significantly increased with the rise of processing time (Fig. 7). As the figures obtained by SEM shown, the cells in US0 exhibited smooth and intact edges, whereas large cell fragments started to appear at 5 min compared with the untreated sample. After 10-min ultrasound treatment, there were more flaky fragments generated in the shrimp samples. In US15, part of flaky fragments converted into strips. Shrimp samples showed the most significant degradation in cell structures when the samples were treated for 20 min. In comparison to the control and other treatments, cells were disrupted to smaller pieces and irregular holes accrued on the cell surface after 20 min treatment. The changes in shrimp cell status obtained by an optical microscope are shown in Fig. 8. In US0, an intact cell was observed at the center of the view. Afterward, the edge of the cell started to be indistinct and destroyed in US5, which was followed by the cell to be out of shape in US10 and especially in US15. Eventually, no cell could be observed in US20, which indicated ultrasound treatment had a significant effect on the structural degradation of shrimp samples.

Similar observations regarding microstructure changes have been reported in kiwifruit juice (Wang, Wang, et al., 2019), apple and onion (Rajewska & Mierzwa, 2017), carrot (Nowacka & Wedzik, 2016), peach juice (Rojas et al., 2016). They found ultrasound enhanced the irreversible changes in structure (growth of porosity, loss of tissue coherence) and cell composition (destruction of cell components) with the further rise of processing time. These phenomena result from shear stresses, cavitation, removal of the internal cell components, and further leading to biochemical reactions (Rajewska & Mierzwa, 2017).

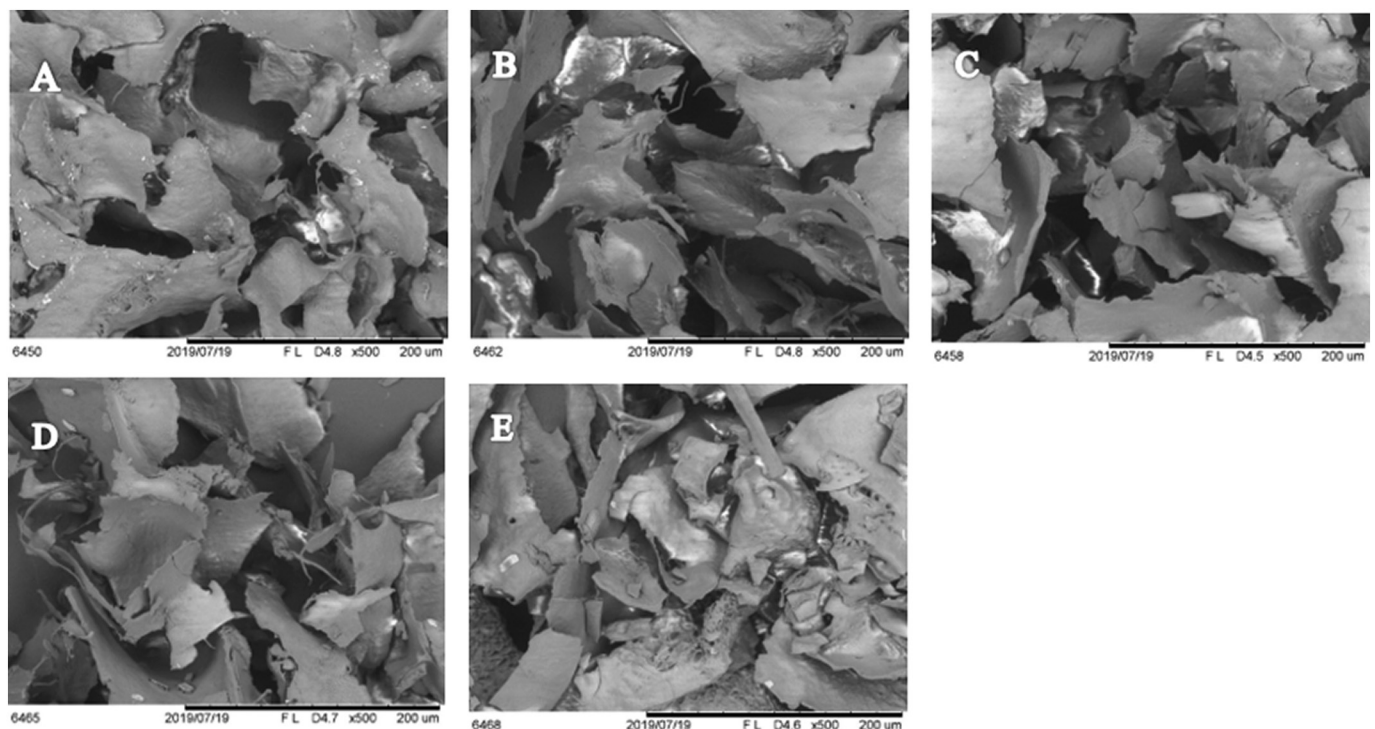


Fig. 7. SEM photomicrographs of freeze-dried shrimps (A) US0 (untreated sample), (B) US5, (C) US10, (D) US15, and (E) US20 (magnification at 500 $\times$).

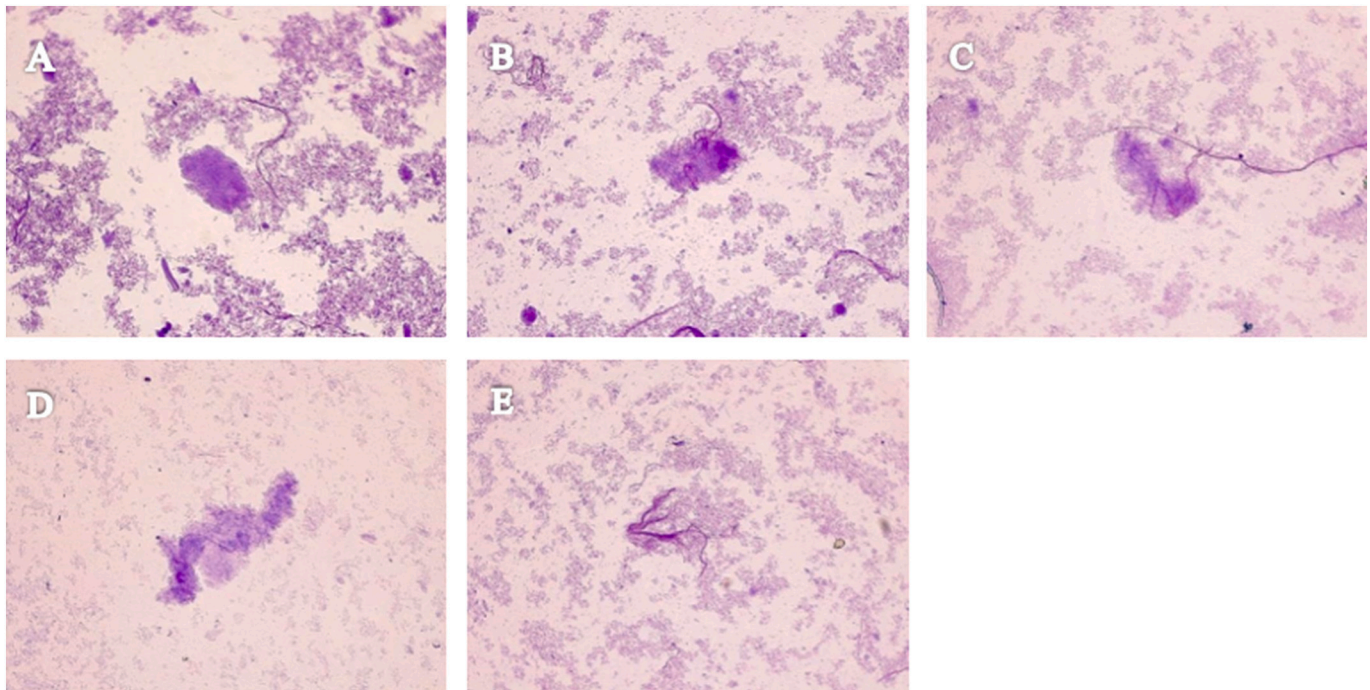


Fig. 8. Photographs by microscopy of shrimp samples before freeze-dried (A) US0 (untreated sample), (B) US5, (C) US10, (D) US15, and (E) US20 (magnification at $10\times$).

Therefore, the modified conformations of ultrasound-processed foods can induce the decrease of allergenicity by unfolding and aggregation of allergenic proteins, which contribute to the descend of the total soluble protein, enhancement of peptides and digestibility.

4. Conclusion

In our study, we evaluated the influence of high-intensity ultrasound treatment on the allergenicity, antioxidant capability, microstructures, secondary structures, *in vitro* protein digestibility of shrimp. Total soluble protein content decreased gradually with the increase of time and reached the lowest value at 20 min, simultaneously accompanying the production of peptides, which also related to the enhancement of total antioxidant capacity compared to the untreated sample. The *in vitro* protein digestibility and peptide production showed an increment with the increase of time. More and more fragments and strips were observed by microscopy. Four kinds of secondary structures transfer with the increasing time, explicitly showing the upward trend of β -sheets and α -helices, and the downward trend of turns and unordered. These results demonstrated ultrasound treatment has the potential to promote the elimination process of shrimp allergenicity with minimum destruction of shrimp original physiochemical properties. However, longer ultrasound processing time still needs to be studied in further work.

Credit author statement

Xin Dong: Conceptualization, Methodology, Software, Writing - Original draft preparation.

Jin Wang: Data curation, Visualization, Investigation, Writing - Reviewing and Editing.

Vijaya Raghavan: Supervision, Writing - Reviewing and Editing.

Declaration of competing interest

The authors declare no conflict of interest reported in this paper.

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References

- Aguilera, J. M., Stanley, D. W., & Baker, K. W. (2000). New dimensions in microstructure of food products. *Trends in Food Science & Technology*, 11(1), 3–9.
- Ahmed, I., Lv, L., Lin, H., Li, Z., Ma, J., Guanzhi, C., ... Xu, L. (2018). Effect of tyrosinase-aided crosslinking on the IgE binding potential and conformational structure of shrimp (*Metapenaeus ensis*) tropomyosin. *Food Chemistry*, 248, 287–295.
- Akonor, P., Ofori, H., Dziedzoave, N., & Kortei, N. (2016). Drying characteristics and physical and nutritional properties of shrimp meat as affected by different traditional drying techniques. *International Journal of Food Science*, 2016.
- Ayuso, R., Lehrer, S., & Reese, G. (2002). Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). *International Archives of Allergy and Immunology*, 127(1), 27–37.
- Ayuso, R., Reese, G., Leong-Kee, S., Plante, M., & Lehrer, S. B. (2002). Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *International Archives of Allergy and Immunology*, 129(1), 38–48.
- Baltacıoğlu, H., Bayındır, A., & Severcan, F. (2017). Secondary structure and conformational change of mushroom polyphenol oxidase during thermosonication treatment by using FTIR spectroscopy. *Food Chemistry*, 214, 507–514.
- Beazley, S. F. (2017). *Effects of ultra-sonication process on digestibility of kafirin in sorghum*. University of Tennessee Honors Thesis Projects.
- Besler, M., Steinhart, H., & Paschke, A. (2001). Stability of food allergens and allergenicity of processed foods. *Journal of Chromatography. B, Biomedical Sciences and Applications*, 756(1–2), 207–228.
- Castillo, R., Delgado, J., Quiralte, J., Blanco, C., & Carrillo, T. J. A.e.i. (1996). *Food hypersensitivity among adult patients: Epidemiological and clinical aspects*. 24(3), 93–97.
- Cheng, L., Soh, C., Liew, S., & Teh, F. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry*, 104(4), 1396–1401.
- Chokshi, N. Y., Maskatia, Z., Miller, S., Guffey, D., Minard, C. G., & Davis, C. M. (2015). Risk factors in pediatric shrimp allergy. *Allergy and Asthma Proceedings*, 36(4), 65–71.
- Church, F. C., Porter, D. H., Catignani, G. L., & Swaisgood, H. E. (1985). An o-phthalaldehyde spectrophotometric assay for proteinases. *Analytical Biochemistry*, 146(2), 343–348.
- Daul, C. B., Morgan, J. E., Waring, N. P., McCants, M. L., Hughes, J., & Lehrer, S. B. (1987). Immunologic evaluation of shrimp-allergic individuals. *Journal of Allergy and Clinical Immunology*, 80(5), 716–722.
- de São José, J. F. B., de Andrade, N. J., Ramos, A. M., Vanetti, M. C. D., Stringheta, P. C., & Chaves, J. B. P. (2014). Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control*, 45, 36–50.
- Ekezie, F. G. C., Cheng, J. H., & Sun, D. W. (2018). Effects of nonthermal food processing

- technologies on food allergens: A review of recent research advances. *Trends in Food Science and Technology*, 74, 12–25.
- Golmohamadi, A., Möller, G., Powers, J., & Nindo, C. (2013). Effect of ultrasound frequency on antioxidant activity, total phenolic and anthocyanin content of red raspberry puree. *Ultrasonics Sonochemistry*, 20(5), 1316–1323.
- Hejazi, S. N., & Orsat, V. (2016). Malting process optimization for protein digestibility enhancement in finger millet grain. *Journal of Food Science and Technology*, 53(4), 1929–1938.
- Hoffman, D., Day, J. E., & Miller, J. (1981). The major heat stable allergen of shrimp. *Annals of Allergy*, 47(1), 17–22.
- Hoffmann-Sommergruber, K. (2000). Plant allergens and pathogenesis-related proteins. *International Archives of Allergy and Immunology*, 122(3), 155–166.
- Jia, J., Ma, H., Zhao, W., Wang, Z., Tian, W., Luo, L., & He, R. (2010). The use of ultrasound for enzymatic preparation of ACE-inhibitory peptides from wheat germ protein. *Food Chemistry*, 119(1), 336–342.
- Jin, J., Ma, H., Wang, B., Yagoub, A. E.-G. A., Wang, K., He, R., & Zhou, C. (2016). Effects and mechanism of dual-frequency power ultrasound on the molecular weight distribution of corn gluten meal hydrolysates. *Ultrasonics Sonochemistry*, 30, 44–51.
- Kang, D.-c., Zou, Y.-h., Cheng, Y.-p., Xing, L.-j., Zhou, G.-h., & Zhang, W.-g. (2016). Effects of power ultrasound on oxidation and structure of beef proteins during curing processing. *Ultrasonics Sonochemistry*, 33, 47–53.
- Kim, S. M., Park, J. G., Saeki, H., Nakamura, A., Lee, J. W., Byun, M. W., & Ahn, D. H. (2008). Changes in the allergenicity of saeujeot by fermentation. *Food Science and Biotechnology*, 17(5), 919–924.
- Kou, X., Gao, J., Xue, Z., Zhang, Z., Wang, H., & Wang, X. (2013). Purification and identification of antioxidant peptides from chickpea (*Cicer arietinum* L.) albumin hydrolysates. *LWT- Food Science and Technology*, 50(2), 591–598.
- Lao-araya, M., & Trakultivakorn, M. (2012). Prevalence of food allergy among preschool children in northern Thailand. *Pediatrics International*, 54(2), 238–243.
- Li, X., Li, Z., Lin, H., & Samee, H. (2011). Effect of power ultrasound on the immunoactivity and texture changes of shrimp (*Penaeus vannamei*). *Czech Journal of Food Sciences*, 29(5), 508–514.
- Li, Z., Lin, H., Cao, L., & Jameel, K. (2006). Effect of high intensity ultrasound on the allergenicity of shrimp. *Journal of Zhejiang University. Science. B*, 7(4), 251–256.
- Li, Z., Lin, H., Cao, L., & Khalid, J. (2007). The influence of gamma irradiation on the allergenicity of shrimp (*Penaeus vannamei*). *Journal of Food Engineering*, 79(3), 945–949.
- Li, Z., Linhong, C., & Khalid, J. (2006). Reduction of allergenic properties of shrimp (*Penaeus Vannamei*) allergens by high intensity ultrasound. *European Food Research and Technology*, 223(5), 639.
- Liu, G.-x., Tu, Z.-c., Yang, W., Wang, H., Zhang, L., Ma, D., ... Li, X. (2018). Investigation into allergenicity reduction and glycation sites of glycated β -lactoglobulin with ultrasound pretreatment by high-resolution mass spectrometry. *Food Chemistry*, 252, 99–107.
- Martínez-Velasco, A., Lobato-Calleros, C., Hernández-Rodríguez, B. E., Román-Guerrero, A., Álvarez-Ramírez, J., & Vernon-Carter, E. J. (2018). High intensity ultrasound treatment of faba bean (*Vicia faba* L.) protein: Effect on surface properties, foaming ability and structural changes. *Ultrasonics Sonochemistry*, 44, 97–105.
- Misir, G., & Koral, S. (2018). Effects of ultrasound treatment on biochemical, structural, functional properties and antioxidant activity of protein hydrolysate of rainbow trout (*onchorhynchus mykiss*) by-products. *Italian Journal of Food Science*, 31(2).
- Morris, V., & Groves, K. (2013). *Food microstructures: Microscopy, measurement and modelling*. Oxford: Woodhead.
- Nadeem, M., Ubaid, N., Qureshi, T. M., Munir, M., & Mehmood, A. (2018). Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrasonics Sonochemistry*, 45, 1–6.
- Nowacka, M., & Wedzik, M. (2016). Effect of ultrasound treatment on microstructure, colour and carotenoid content in fresh and dried carrot tissue. *Applied Acoustics*, 103, 163–171.
- Pera-Titus, M., García-Molina, V., Baños, M. A., Giménez, J., & Esplugas, S. (2004). Degradation of chlorophenols by means of advanced oxidation processes: A general review. *Applied Catalysis B: Environmental*, 47(4), 219–256.
- Rahaman, T., Vasiljevic, T., & Ramchandran, L. (2016). Effect of processing on conformational changes of food proteins related to allergenicity. *Trends in Food Science and Technology*, 49, 24–34.
- Rajewska, K., & Mierzwa, D. (2017). Influence of ultrasound on the microstructure of plant tissue. *Innovative Food Science & Emerging Technologies*, 43, 117–129.
- Rojas, M. L., Leite, T. S., Cristianini, M., Alvim, I. D., & Augusto, P. E. (2016). Peach juice processed by the ultrasound technology: Changes in its microstructure improve its physical properties and stability. *Food Research International*, 82, 22–33.
- Shriver, S., Yang, W., Chung, S. Y., & Percival, S. (2011). Pulsed ultraviolet light reduces immunoglobulin E binding to Atlantic white shrimp (*Litopenaeus setiferus*) extract. *International Journal of Environmental Research and Public Health*, 8(7), 2569–2583.
- Shriver, S. K. (2011). *Effect of selected nonthermal processing methods on the allergen reactivity of Atlantic white shrimp (Litopenaeus setiferus)*. University of Florida.
- Sicherer, S. H., Muñoz-Furlong, A., & Sampson, H. A. (2004). Prevalence of seafood allergy in the United States determined by a random telephone survey. *The Journal of Allergy and Clinical Immunology*, 114(1), 159–165.
- Silke, A. (2017). Effect of enzymatic hydrolysis on the allergenic capacity of shrimp tropomyosin. *Honors College*, 263.
- Smith, K. L., & Guentzel, J. L. (2010). Mercury concentrations and omega-3 fatty acids in fish and shrimp: Preferential consumption for maximum health benefits. *Marine Pollution Bulletin*, 60(9), 1615–1618.
- Sriket, P., Benjakul, S., Visessanguan, W., & Kijroongrojana, K. (2007). Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. *Food Chemistry*, 103(4), 1199–1207.
- Stratakou, A. C., Delgado-Pando, G., Linton, M., Patterson, M. F., & Koidis, A. (2016). Industrial scale microwave processing of tomato juice using a novel continuous microwave system. *Food Chemistry*, 190, 622–628.
- Su, Z., Li, X., & Jin, L. (2006). Comparison between enzymatic hydrolysis of egg white proteins with ultrasound and without ultrasound. *Food Science and Technology (in Chinese)*, 31(12), 74–76.
- Tang, Y., Li, X., Zhang, B., Chen, P. X., Liu, R., & Tsao, R. (2015). Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. *Food Chemistry*, 166, 380–388.
- Tapal, A., & Tikku, P. K. (2019). Nutritional and nutraceutical improvement by enzymatic modification of food proteins. *Enzymes in Food Biotechnology*, 471–481.
- Vanga, S. K., Singh, A., Kalkan, F., Gariepy, Y., Orsat, V., & Raghavan, V. (2016). Effect of thermal and high electric fields on secondary structure of peanut protein. *International Journal of Food Properties*, 19(6), 1259–1271.
- Venugopal, V. (2008). *Marine products for healthcare: Functional and bioactive nutraceutical compounds from the ocean*. CRC press.
- Vilela, R. M., Lands, L. C., Chan, H. M., Azadi, B., & Kubow, S. (2006). High hydrostatic pressure enhances whey protein digestibility to generate whey peptides that improve glutathione status in CFTR-deficient lung epithelial cells. *Molecular Nutrition Food Research International*, 50(11), 1013–1029.
- Wal, J. M. (2003). Thermal processing and allergenicity of foods. *Allergy*, 58(8), 727–729.
- Wang, B., Meng, T., Ma, H., Zhang, Y., Li, Y., Jin, J., & Ye, X. (2016). Mechanism study of dual-frequency ultrasound assisted enzymolysis on rapeseed protein by immobilized Alcalase. *Ultrasonics Sonochemistry*, 32, 307–313.
- Wang, J., Vanga, S. K., & Raghavan, V. (2019). High-intensity ultrasound processing of kiwifruit juice: Effects on the ascorbic acid, total phenolics, flavonoids and antioxidant capacity. *LWT*, 107, 299–307.
- Wang, J., Wang, J., Ye, J., Vanga, S. K., & Raghavan, V. (2019). Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control*, 96, 128–136.
- Wang, Y., Yang, R., Ding, L., Xie, X., & Liu, H. (2018). FTIR and proteome analysis of sunflower seedling (*Helianthus annuus* L.) under lead stress. *IOP Conference Series: Earth and Environmental Science*, 199, Article 032064.
- Yang, X., Li, Y., Li, S., Oladejo, A. O., Ruan, S., Wang, Y., ... Ma, H. (2017). Effects of ultrasound pretreatment with different frequencies and working modes on the enzymolysis and the structure characterization of rice protein. *Ultrasonics Sonochemistry*, 38, 19–28.
- Zhang, Z., Zhang, X., Chen, W., & Zhou, P. (2018). Conformation stability, in vitro digestibility and allergenicity of tropomyosin from shrimp (*Exopalaemon modestus*) as affected by high intensity ultrasound. *Food Chemistry*, 245, 997–1009.
- Zhu, Y., Vanga, S. K., Wang, J., & Raghavan, V. (2018). Effects of ultrasonic and microwave processing on avidin assay and secondary structures of egg white protein. *Food and Bioprocess Technology*, 11(11), 1974–1984.