

Impact of microwave processing on the secondary structure, *in-vitro* protein digestibility and allergenicity of shrimp (*Litopenaeus vannamei*) proteins

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ABSTRACT

This study aimed to evaluate the effects of microwave processing (2.45 GHz, 1000 W, 75–125 °C, and 5–15 min) on the secondary structures, *in-vitro* protein digestibility, microstructural characteristics, and allergenicity of shrimp. SDS-PAGE analysis showed that the band intensity of tropomyosin reduced with the increase of processing temperatures and durations. The significant reduction in the allergenicity of tropomyosin was up to 75% when treated with microwave at 125 °C for 15 min. A significant reduction by 30–75% in the total soluble protein content, peptide content, and *in-vitro* protein digestibility of shrimp protein was observed. These changes mentioned above were strongly associated with the modification of the secondary structure of shrimp proteins, including the increase in β -sheets, and the loss in turns. Also, more microscopic holes, fragments, strips in treated samples were observed by scanning electron microscopy. Therefore, high-intensity microwave treatment showed great potential in reducing the allergenicity of shrimp.

1. Introduction

Shrimp is considered as one of the most popular seafood worldwide due to its nutritional values and health benefits. Shrimp contains various essential nutrients such as vitamins, minerals, omega-3 fatty acids, and astaxanthin antioxidants (Bowen, Harris, & Kris-Etherton, 2016; Fasset & Coombes, 2011; Van Blarigan et al., 2017). Among them, abundant selenium presenting in shrimp is helpful in maintaining or boosting the immune system. Astaxanthin not only can protect human brains and hearts to reduce the risks of mental fatigue and heart attack but also is beneficial to male fertility (Capelli & Ding, 2019). Besides, studies confirmed that omega-3 fatty acids present in shrimp are playing an important role in improving the cardiovascular system (Bäck & Hansson, 2019).

However, many clinical studies have reported that shrimps can trigger a series of allergenic reactions ranging from mild to life-threatening, and also the cases of shrimp allergy are increasing around the world every year (Lee, Thalayasingam, & Lee, 2013). The mild allergic reactions commonly include skin symptoms (urticaria or angioedema), respiratory symptoms (rhinitis or wheezing), or gastroenterological symptoms (abdominal pain, nausea, emesis, diarrhea), whereas the life-threatening situation may even lead to death. Tropomyosin has been confirmed as the major allergen in shellfish, which has been included in the “big eight” allergenic foods. In 1981, tropomyosin was first

identified in shrimp (Hoffman, Day, & Miller, 1981). In 1994, tropomyosin was detected in brown shrimp (*Penaeus aztecus*), reacting with 82% of shrimp-allergic patients (Daul, Slattery, Reese, & Lehrer, 1994). In recent years, studies reported that tropomyosin is a heat-stable and water-soluble allergen with a 35–38 kDa molecular mass, consisting of two identically coiled subunits (Rolland et al., 2018). Besides, minor allergens in shrimp were also identified, such as arginine kinase (AK), myosin light chain (MLC), and sarcoplasmic calcium-binding protein (SCP) (Lee et al., 2018).

Microwave is an electromagnetic wave with a frequency range of 0.3–300 GHz. Microwave heating (2450 and 915 MHz) belongs to the dielectric treatment and has been applied to the food industry for many years. The Institute of Electrical and Electronics Engineers (IEEE) reported that proteins and peptides have higher dielectric constant, which indicates microwaves may have a significant impact on their activity and structural properties (Plagemann, von Langermann, & Kragl, 2014). In recent years, microwave treatment as a novel thermal processing has been used to reduce the allergenicity of eggs (Zhu, Vanga, Wang, & Raghavan, 2018), almonds (Su, Venkatachalam, Teuber, Roux, & Sathe, 2004), and fishes (Ketnawa & Liceaga, 2017). However, few studies on microwave treatment were performed to evaluate its effects on the modification and elimination of shrimp allergens. In this study, high-temperature microwave treatment (75–125 °C for 5–15 min) was performed to evaluate its impact on the allergenicity of shrimp.

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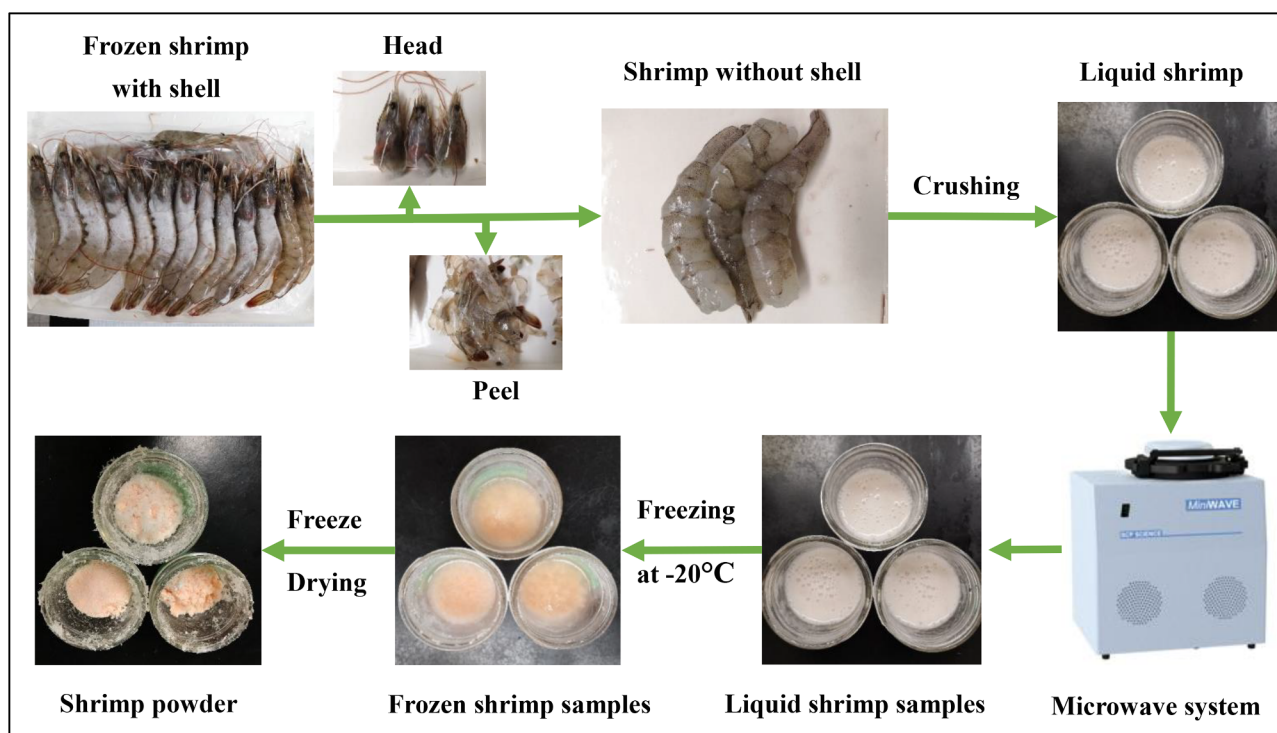


Fig. 1. Schematic overview of the pretreatment of shrimp samples.

Meanwhile, the physicochemical characteristics of shrimp samples such as secondary structure, microstructure, antioxidant capacity, *in-vitro* protein digestibility were also investigated.

2. Materials and methods

2.1. Sample preparation and microwave treatment

Frozen shrimps (*Litopenaeus vannamei*, whiteleg) purchased from a local supermarket (Liantai Chinese Market, Montreal, Canada) were stored at -20°C before the experiments. The mean length and weight of the selected shrimps were 8.51 ± 1.11 cm and 31.87 ± 1.35 g, respectively. As shown in Fig. 1, the edible part of shrimps was obtained after peeling, and then was minced and homogenized with the water by the ratio of 1:2 using a juicer (Wang, Vanga, & Raghavan, 2019).

Microwave processing was conducted using a Mini WAVE Digestion Module (SCP Science, 115 V, 60 Hz, 15 A, 1000 W, Canada) at a frequency of 2.45 GHz. The relevant treatments were set as follows: (1) 75°C for 5 min (75–5); (2) 75°C for 10 min (75–10); (3) 75°C for 15 min (75–15); (4) 100°C for 5 min (100–5); (5) 100°C for 10 min (100–10); (6) 100°C for 15 min (100–15); (7) 125°C for 5 min (125–5); (8) 125°C for 10 min (125–10); (9) 125°C for 15 min (125–15); (10) the untreated sample was set as control group. The temperatures inside of the oven were monitored through infrared sensors located on the sidewalls as well as a single magnetron located at the bottom of the mini-wave reaction chamber. After processing, the samples were immediately taken out and were cooled down to the room temperature (after 2.5 h). And then, 5 mL of the sample from each treatment were stored at 4°C for microstructure analysis, the leftover samples were dried using a freeze dryer (7420020, Labconco Corporation, Kansas City, USA) for 48 h. The dried shrimp powder was stored at -20°C for further analysis. All treatments and analyses were performed in triplicates.

2.2. Total soluble protein content and SDS-PAGE analysis

Freeze-dried shrimp powder (0.4 g) was extracted with phosphate-

buffered saline (PBS) solution (0.1 M, pH = 7.0). After 30-min incubation at room temperature, the mixture was centrifuged at $4,000 \times g$, 4°C for 10 min. The supernatant was collected for further analysis. The total soluble protein content in shrimp samples was determined by a Pierce™ BCA protein assay kit (Thermo Fisher Scientific, Canada) according to the provided protocol (Wang, Wang, Ye, Vanga, & Raghavan, 2019).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the provided protocol. The shrimp protein extract was diluted at the ratio of 1:1 (v/v) using a sample buffer (10 μL β -mercaptoethanol, 90 μL 4x Laemmli sample buffer). The mixture was heated at 95°C for 5 min, and then was cooled down at room temperature. Running buffer was prepared by mixing 100 mL of 10x Tris/Glycine/SDS with 900 mL of distilled water. Each sample was loaded for 10 μL per lane, and a molecular weight marker (10–250 kDa) (Bio-Rad, Philadelphia, PA, USA) was loaded for 5 μL . Electrophoresis was performed in a vertical unit (Mini-PROTEAN® Tetra System, BIO-RAD, Philadelphia, PA, USA) at 80 V. After electrophoresis, gels were stained by 0.1% Coomassie Brilliant Blue R-250 (Sigma-Aldrich Chemical, St. Louis, MO, USA) for 10 min, then destained using a de-staining solution (mix of 90 mL of 50% methanol and 10 mL of acetic acid). The relevant figures were obtained using a digital camera (Canon, EOS Rebel SL2 DSLR Camera, America).

2.3. Tropomyosin allergen content –using enzyme-linked immunosorbent assay (ELISA)

A sandwich ELISA (Shrimp Tropomyosin ELISA 2.0, Indoor Biotechnologies, USA) was utilized to quantify the tropomyosin content in the shrimp samples. The ELISA kit consists of 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 (10x), assay buffer (10x), TMB developing substrate, and stop solution. All the steps were performed according to the protocol provided by the kit. The absorbance of the tropomyosin was recorded at 450 nm, and the concentration of tropomyosin was calculated

based on the standard curve obtained by the standard.

2.4. Secondary structure of shrimp protein

Fourier transform infrared spectroscopy (FTIR) was applied to investigate the secondary structures of shrimp proteins. Each sample (0.1 g) was loaded on the diamond crystal of the FTIR spectrometer (Nicolet Magna 158,750 FTIR, Nicolet Instrument Corp., Madison, WI). The scan spectra at 4 cm^{-1} resolution were averaged in the mid-infrared region ($1000\text{--}2000\text{ cm}^{-1}$). The spectra obtained were analyzed by OMNIC software (Version 8, Thermo Nicolet Co., Madison, WI). To avoid the influence of air, the spectrum of an empty diamond was regarded as the background reference.

A curve-fitting procedure was performed using the OriginPro software (Version 9, Origin Lab Corporation, Northampton, MA, USA) to quantify the conformational changes of shrimp proteins in the amide I band ($1700\text{--}1600\text{ cm}^{-1}$). 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 derivative spectrum generated by the software, and the results were presented in terms of the peak areas at corresponding wavelengths.

2.5. Total antioxidant capacity measurement

As described by Wang et al. (2019), the ferric-reducing/antioxidant power (FRAP) assay was used to determine 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 was obtained by mixing shrimp samples (0.1 g) with methanol (5 mL). FRAP solution (200 mL) and the extract (67 μL) were added to the microwells of plates, respectively. After a 5-min incubation at $37\text{ }^{\circ}\text{C}$ in the dark, the color changes were measured by a spectrophotometer at 593 nm. Standard solutions were prepared with a range of $0\text{--}1000\text{ }\mu\text{M}$ ferrous sulfate solution to make a standard curve, and the antioxidant activity of the shrimp sample was presented as $\mu\text{mol Fe (II)}/100\text{ mL}$ of samples.

2.6. Microstructure

A Scanning Electron Microscope (SEM) (TM3000, Hitachi High-Technologies Corporation., Tokyo, Japan) was used to investigate the microstructural changes of shrimp samples. The freeze-dried shrimp sample was transferred to the measuring platform. The relevant photos were captured at a $500\times$ magnification.

2.7. In-vitro protein digestibility

The *in-vitro* digestibility of shrimp proteins was performed in two stages following the procedure suggested by Hejazi and Orsat (2016). Freeze-dried shrimp powder (0.5 g) was mixed with a PBS buffer (0.1 M, 20 mL, pH = 7.0) to a final mixture concentration of 25 mg/mL. The pH of the mixture was adjusted to 1.5 using 0.1 N HCl solution. The first-stage digestion was started by adding pepsin solution (10 mg/mL, 50 μL) at $37\text{ }^{\circ}\text{C}$. After 30 mins, NaOH solution (1.0 M) was added to stop the first-stage digestion and adjusted the pH to 7.8. The second-stage digestion was started by adding pancreatin (10 mg/mL, 200 μL) at $40\text{ }^{\circ}\text{C}$. After 60 mins, Na_2CO_3 solution (150 mM, 50 μL) was used to stop the reaction. The total protein contents of shrimp samples before and after two-stage digestion were determined by the BCA method. The *in-vitro* protein digestibility was calculated using the following equation:

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

2.8. Determination of peptide content

The peptide content was determined using the o-Phthaldialdehyde (OPA) Reagent. The OPA reagent was prepared by adding 25 mL of 100 mM sodium tetraborate in water, 2.5 mL of 20% (w/v) sodium dodecyl sulfate, 40 mg of OPA dissolved in 1 mL of methanol, 100 μL of β -mercaptoethanol, and ddH_2O to a final volume of 50 mL. A 50 μL of digestion mixture was incubated for 2 min with a 950 μL OPA reagent solution. The absorbances were read at 340 nm using a plate reader (Spectra Max M2, Molecular Devices, QC, Canada). Leucine-glycine was used to obtain the standard curve.

2.9. 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 $p \leq 0.05$.

3. Results and discussion

3.1. Total soluble protein and total antioxidant capacity (TAC)

As shown in Table 1, the results of total soluble protein and total antioxidant capacity (TAC) of shrimp samples are summarized. A decreasing trend in the protein solubility was investigated with the increase of processing duration and temperature. It was noticed that the total soluble protein content of microwave-treated samples significantly decreased by 50–75% compared with the control group. Among all the treatments, microwave processing at $75\text{ }^{\circ}\text{C}$ for 10 min resulted in the lowest concentration of total soluble protein (49.57 mg/g) present in shrimps. Then, further processing duration and temperature increased the total soluble protein content to 97.41 mg/g after 15-min microwave treatment at $125\text{ }^{\circ}\text{C}$. The reduction in the total soluble protein content resulted from the degradation and hydrolysis of proteins treated with microwave, where generated a consequence of the frictional force in the electromagnetic field (Iuliana, Rodica, Sorina, & Oana, 2015). Amponsah and Nayak (2016) reported the total protein concentration in soy flour decreased by 3 mg/ml with the increasing temperature of the microwave treatments (60, 70, $100\text{ }^{\circ}\text{C}$ for 5 and 10 min). The reduction could be attributed to the proteins in raw soy flour gradually denatured under the microwave heat with increasing temperature. Heat treatment can cause protein denaturation and unfolding of proteins leading to surface exposure of hydrophobic and sulfhydryl groups located in the interior of the molecule. Further, the irreversible protein aggregates and covalent complexes form to result in the reduction of protein solubility, which eventually showing lower total soluble protein detected (Renkema, Lakemond, Jongh, Gruppen, & Vliet, 2000). However, no changes in total soluble proteins were observed by SDS-PAGE and SE-HPLC in wheat kernels treated with microwave at 1000 W for

Table 1

Changes of total protein and antioxidant activity in microwave treated shrimp samples. Note: values with different letters in the same column are significantly different ($p < 0.05$) from each other.

Treatment	Total protein (mg/g)	TAC ($\mu\text{mol}/100\text{ mL}$)
Control	193.88 ± 19.18^a	31.78 ± 3.22^f
75–5	56.40 ± 10.52^{fg}	24.20 ± 6.79^g
75–10	49.57 ± 3.67^h	46.93 ± 4.28^e
75–15	63.38 ± 2.46^d	59.50 ± 16.55^c
100–5	62.24 ± 1.22^{de}	47.13 ± 3.43^e
100–10	53.42 ± 8.85^g	50.03 ± 13.4^e
100–15	59.45 ± 1.81^{ef}	71.13 ± 5.13^a
125–5	58.67 ± 1.55^f	59.55 ± 1.06^c
125–10	76.75 ± 1.05^c	55.20 ± 18.88^d
125–15	97.41 ± 1.15^b	65.78 ± 6.54^b

2 min, when compared with the untreated samples (Lamacchia, Landriscina, & D'Agello, 2016). Similarly, Walde et al. (2002) found the total soluble protein content of wheat samples dried by microwave (700 W, up to 150 s) remained the same as that of the control (9.9%). Thus, the changes in the total soluble proteins are different among various food sources and processing conditions.

As outlined in Table 1, an increasing trend in the total antioxidant capacity (TAC) of shrimp samples were observed with the rise of microwave processing duration and temperatures. Specifically, 75–5 treatment caused a 24% of reduction in the TAC, and then increased to 46.93 $\mu\text{mol}/100\text{ mL}$ after 15-min microwave treatment at the same temperature, when compared with the initial level (31.78 $\mu\text{mol}/100\text{ mL}$). The highest TAC of 71.13 $\mu\text{mol}/100\text{ mL}$ was observed in 110–15 treated samples. Similarly, an increase of antioxidant activity was observed in *Scomberomorus niphonius* under microwave treatment (400 W, 40 °C) for 5 min by Huang et al. (2017). In comparison, the total antioxidant capacity of broccoli florets and stems declined from 60.5% to 21.0% and 62.8% to 21.7%, respectively, with the time increase (0–300 s) of microwave processing due to a large number of antioxidants being dissolved in the solvent. Ketnawa et al. (2018) found the peptides hydrolyzed from protein can be a rich source of natural antioxidants. It was reported that the unfolding protein and increasing peptide bonds might contribute to bioactivities, such as the increase of TAC (Ketnawa & Liceaga, 2017). It is generally agreed upon that peptides possess substantially better antioxidant activities than their parent proteins; this is possibly due to increased accessibility of the functional side of the chain to the reactive species and the electron-dense peptide bonds generated by enzymatic hydrolysis (Udenigwe & Aluko, 2011). Further, an increase in antioxidant activity is probably related to the molecular weight of peptide. Specifically, the higher antioxidant activity might be associated with the lower molecular-weight peptides (Sae-Leaw & Benjakul, 2018). However, in the present study, the peptides content significantly decreased in the samples when treated with microwave processing. Thus, further studies regarding the relationship between the characteristics of peptides and the antioxidant activity of shrimp proteins are still in need.

3.2. *In-vitro* protein digestibility and peptide content

The effect of microwave processing on the *in-vitro* protein digestibility (IVPD) of shrimp samples is demonstrated in Fig. 2a. The digestibility of the untreated sample was 80.46%, while the digestibility of the treated samples was significantly decreased, with the lowest IVPD at 64.19% in 75–10. In a similar study, durum wheat semolina was heated in a microwave oven set at 900 W for 1–2 min. The results found that a 2-min microwave significantly decreased the *in-vitro* protein digestibility of wheat to 53.64% from the initial level of 71.35% (Kamble, Singh, Kaur, Rani, & Upadhyay, 2020). The reduction in the protein digestibility may be attributed to the alteration of protein molecules, such as the active amino acid side blocking and crosslink formation. Also, some food compounds may produce derivatives through Maillard reactions to inhibit enzymes or aminopeptidase (Mauron, 1990).

However, other studies have reported that microwave processing potentially increased the *in-vitro* protein digestibility in foods. Vagadia et al. (2018) treated soymilk with microwave (2450 MHz, 1000 W) at 70–100 °C for 2–8 min, where they found IVPD increased by around 4% after 5 min. In egg white protein, Zhu et al. (2018) reported that the microwave treatment at 60–80 °C for 1–5 min significantly increased the IVPD by up to 15.82% compared with the untreated samples. Alajaji and El-Adawy (2006) reported that the proteins in chickpea (*Cicer arietinum* L.) seeds treated with microwave cooking for 15 min resulted in a 5.79% increase of IVPD compared with the raw sample. The increase of digestibility might be due to the reduction of the enzymatic inhibitor activity, which was caused by the protein denaturation and the proceeding digestion as the increasing time and temperatures (Vagadia

et al., 2018).

Microwave treatment showed a negative effect on the peptides content of shrimp when compared with untreated samples (Fig. 2b). The peptide contents before digestion in each treatment were at the similar level. After pepsin digestion, significant differences in the peptides content were observed in microwave treated samples, compared with the untreated sample. In treated samples, the peptide content showed an increasing trend after pancreatin digestion, and the highest peptide content (15.42 mM) was observed in the treated sample (125–15), whereas it was still lower than that of the control (19.13 mM). This decrease of peptides in treated samples might be associated with the protein denaturation and polymerization during microwave treatment. It has been reported that proteins often aggregate under high temperatures, before they open by pulling out hydrophobic groups, and then they interact between them through covalent bonds, which may lead to a low *in-vitro* digestibility and peptide content in the microwave treated shrimp samples (Bogahawaththa, Chau, Trivedi, Dissanayake, & Vasiljevic, 2019).

However, Nguyen et al. (2017) found that the hydrolyzed peptides in fish samples increased by 7% when treated by microwave (1200 W, 20% power, 50–55 °C, 10 min). Similarly, Uluko et al. (2013) reported the large amounts of hydrolyzed peptides generated in milk proteins treated by microwave at 800 W up to 8 min, and the increasing time duration contributed to the degree of hydrolysis. These increasing peptides were probably because the protein bonds were susceptible to enzymatic hydrolysis when microwave was applied (Uluko et al., 2013). Thus, the effects of microwave processing on the peptide content of various proteins are different, and further studies are still needed in the future.

3.3. Changes of secondary structure

In the present study, Fourier transform infrared spectroscopy (FTIR) was used to analyze the changes in the secondary structure of shrimp proteins (Fig. 3a, 3b, and 3c). The amide I frequency bands (1600–1700 cm^{-1}) of protein secondary structures include β -sheet (1613–1637; 1682–1696), α -helix (1645–1662), turns (1662–1682; 1630), and unordered structures (1637–1645) (Vanga et al., 2016). As shown in Fig. 3d, the results found that the β -sheet structure accounted for the highest percentage (53.19%) in the raw shrimp proteins, followed by the α -helix structure (20.83%), turns (18.88%), and unordered structure (7.2%). After microwave treatment at 75–125 °C for 5–15 min, the results showed an increase in the β -sheets, while a loss in the α -helix structure and turns, with a fluctuation of the unordered structure, compared with the control group. Among all the treatments, the highest percentage of β -sheets (67.8%) and the lowest percentage of turns (5.67%) were observed in the shrimp proteins when treated with microwave processing at 75 °C for 10 min. The lowest percentage of the α -helix structure (15.56%) was observed in the treatment at 75 °C for 15 min. In addition, 10-min microwave treatment at 100 °C contributed to the highest percentage of unordered structures (14.9%) compared with other treatments and control.

Similarly, Gomaa et al. (2016) found that α -helical structures in bovine β -lactoglobulin were significantly decreased by up to 45% after microwave treatment at 40–90 °C compared with the untreated samples, which was because microwave might enhance unfolding pathways in protein structure due to its non-thermal effects. Zhu et al. (2018) reported that β -sheets in egg white increased up to 2 times when treated with microwave at 60–80 °C for 1–5 min, whereas, α -helices decreased with the rise of processing time at each temperature, with turns and unordered structures stable. Vanga et al. (2016) found that α -helices and unordered structures in peanut samples were increased with the rise of processing time and temperature when processed by microwave at 50–100 °C for 5–20 min. In all the percentage changes of each secondary structure might be attributed to the relocations from each other (Vanga et al., 2016). The enhancement of β -sheets and the decline of

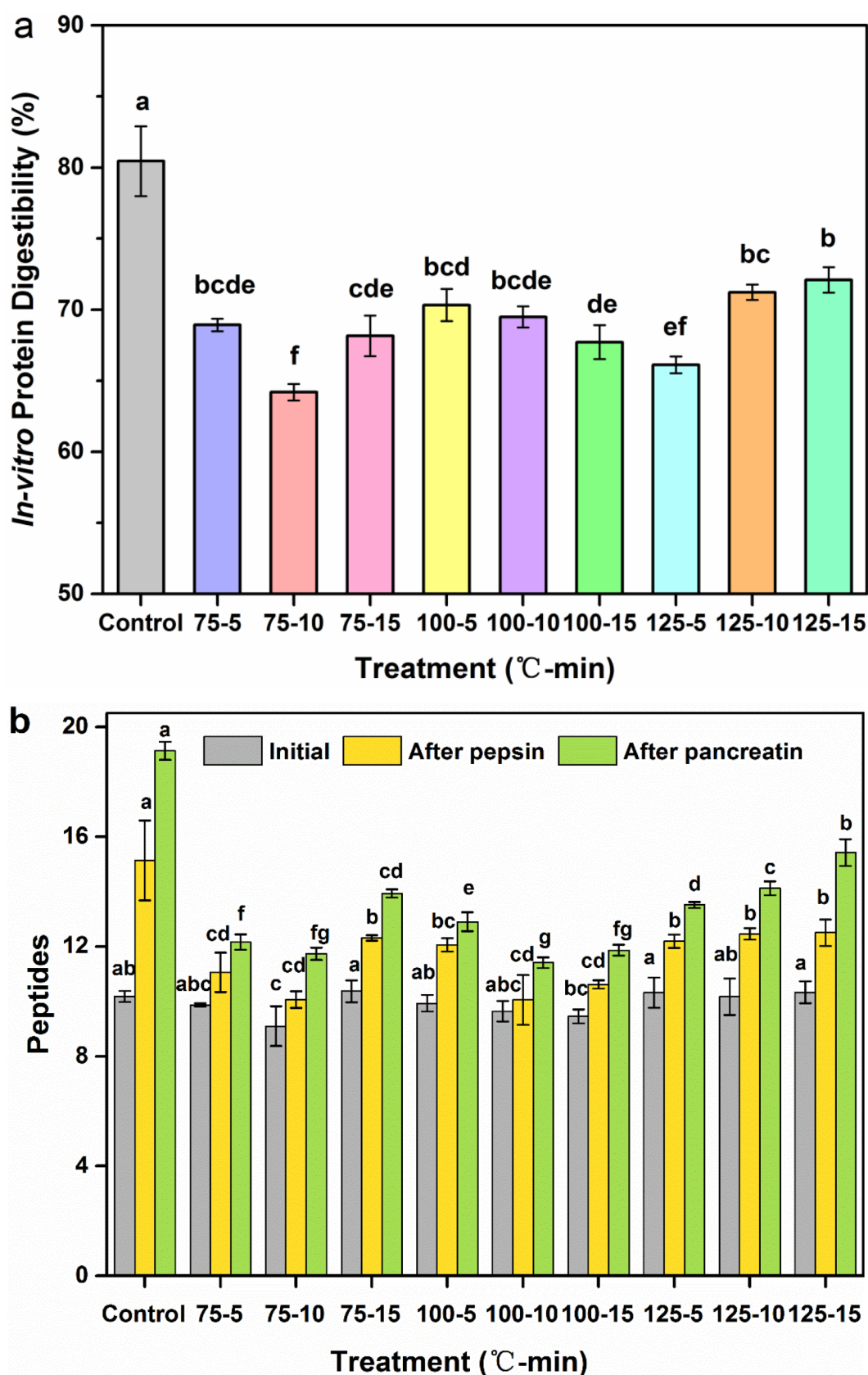


Fig. 2. Changes of *in-vitro* protein digestibility (a) and hydrolyzed peptides content (b) of shrimp samples under different microwave treatments.

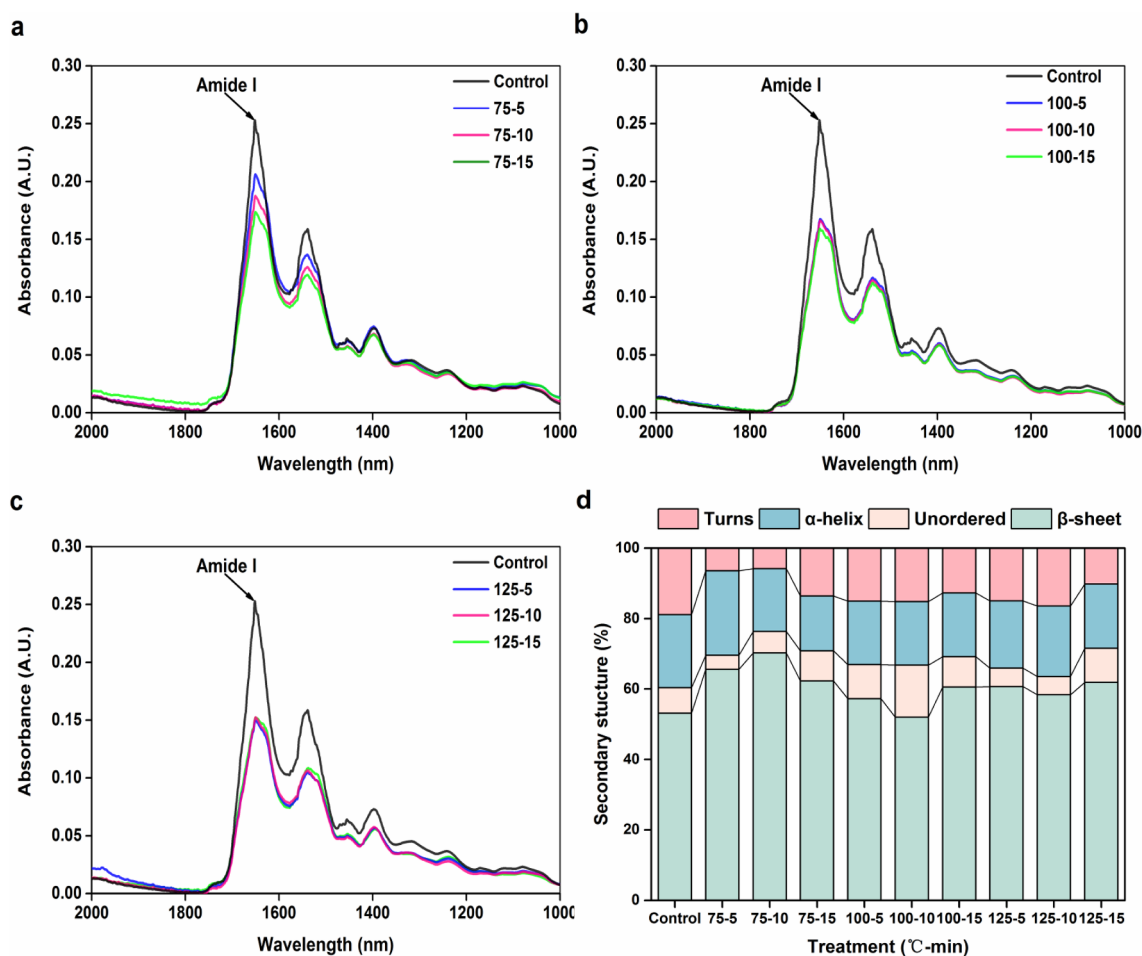


Fig. 3. FTIR spectrum of shrimp samples under different microwave treatments (a–c) and variations in relative areas of the bands fitted to the normalized FTIR spectra of the Amide I region (1700–1600 cm⁻¹) of shrimp samples (d).

the α -helices may be due to the exposure of hydrophobic regions and the disruption of the hydrogen bonds when external stresses generated from the microwave processing (Zhu et al., 2018). Thus, microwave processing could speed up the rates of the protein denaturation and folding process by loosening protein structures. Besides, long-time and high-temperature microwave effectively increase the kinetic energy of protein molecules and lead to the vibrations of polar groups, and further affect the changes of secondary structures (Wang & Chi, 2012).

3.4. Allergenicity

In this study, an SDS-PAGE measurement was used as a qualifying method to analyze the changes in the shrimp allergen after microwave treatment. As represented in Fig. 4a, the results showed that microwave treatment caused a decrease in the intensity of shrimp protein bands. In addition, the protein band intensity located at 36 kD in the gel corresponding to tropomyosin (TM, a major shrimp allergen) showed a reducing trend with the rise of processing duration and temperature. Among all the microwave treatments, the lowest intensity of TM band was observed in the samples when treated at 125 °C for 10 min. It indicates that microwave as a thermal processing showed a potential application in reducing the allergenicity of shrimp.

Furthermore, a quantified method, sandwich enzyme-linked immunosorbent assay (ELISA), was used to evaluate the IgE binding capacity of shrimp allergen. As shown in Fig. 4b, tropomyosin contents in microwave-treated samples exhibited a significant decline in the immune response as the increasing temperatures and time. During the microwave treatment at 75 °C for 5–15 min, a 48–58% reduction in the

tropomyosin content was observed. Whereas, a significant increase in the tropomyosin content when increased the temperature to 100 °C for 5 min. And then, the allergen content was dramatically decreased to a stable level. The microwave treatment at 125 °C for 5–15 min showed the highest reduction (75%) in the tropomyosin content compared with the initial level (125 ng/100 mg). The decrease in the tropomyosin content might be associated with the conformational changes or destructions in the linear epitopes of tropomyosin molecules caused by microwave (Venkatachalam et al., 2008). Further, secondary structure changes strongly associated with the allergenicity of relevant allergens (Dong, Wang, & Raghavan, 2020; Wang, Vanga, McCusker, & Raghavan, 2019; Wang, Vanga, & Raghavan, 2020). In this study, the results revealed that microwave caused a loss in the turn structures, and an increase of β -sheet and unordered structures with the rise of processing temperature and duration (Fig. 3d).

In wheat flour, Leszczynska et al. (2003) treated gliadins with microwave powers at 500 W for 1–5 min, resulting a significant decrease in the immunoreactivity of samples. In wheat kernels, Lamacchia et al. (2016) also reported the gliadin antigenicity reduced significantly (99%) when treated by 1000-W microwave for 2 min. Similarly, Zellal et al. (2011) found that microwave irradiation (300 or 700 W) markedly reduced the allergenicity of β -lactoglobulin of whey proteins tested by ELISA. Microwave processing showed potential in altering the native structure of proteins, and therefore it might potentially modify the relevant protein structure causing a less opportunity to be recognized by IgE of sensitized subjects (Jiménez-Saiz, Benedé, Molina, & López-Expósito, 2015).

However, different effects were observed in other foods. Cabanillas

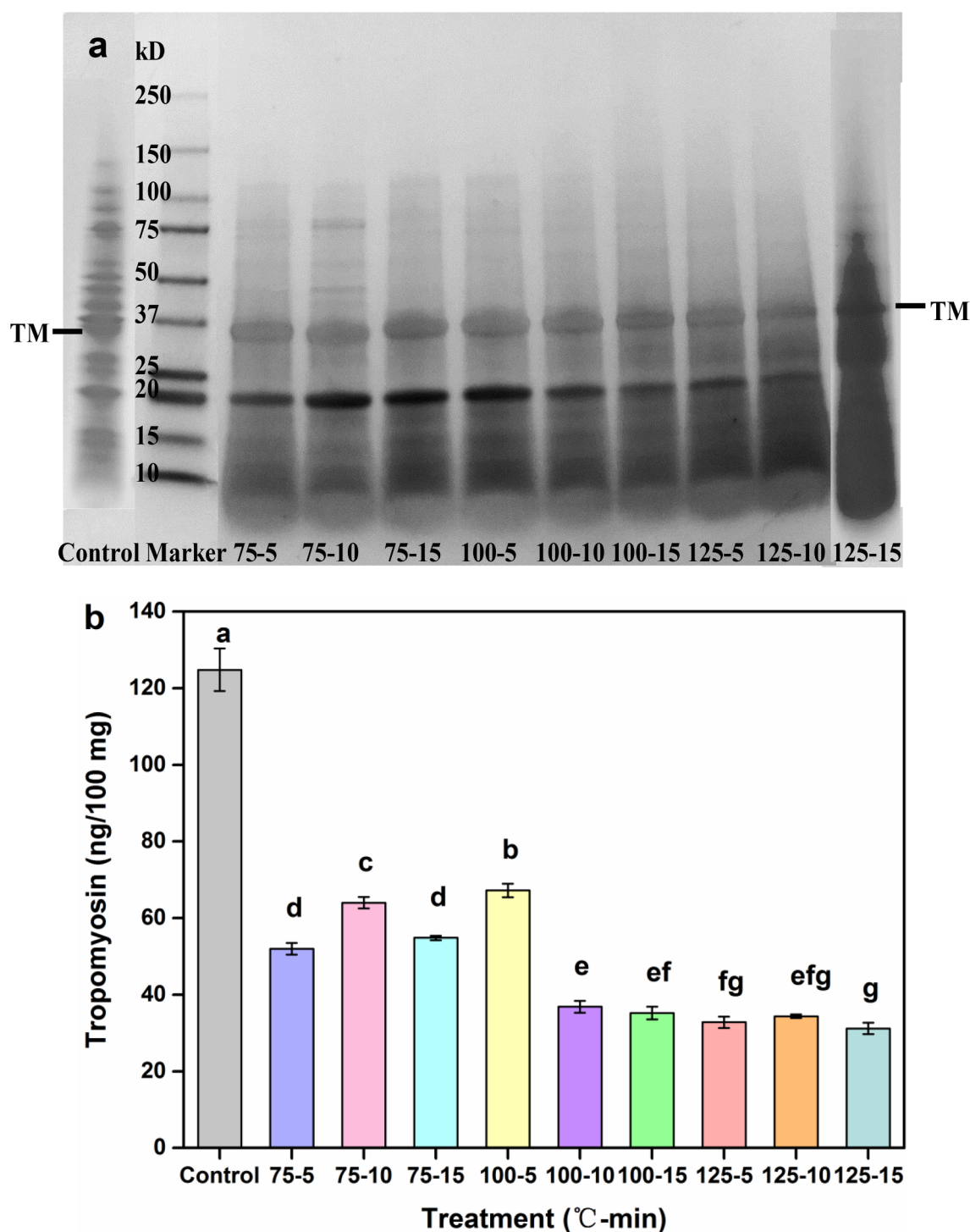


Fig. 4. SDS-PAGE (a) and tropomyosin contents (b) of microwave treated and untreated shrimp samples.

and Novak (2019) reported microwave processing showed a very limited effect on the allergenicity of many foods. Similarly, no significant difference in the three allergens (Ana o 1, Ana o 2, and Ana o 3) content in cashew nut were observed when treated with microwave heating (1–2 min, 500–1000 W) (Venkatachalam et al., 2008). Su et al. (2004) found the allergenicity of the proteins in tree nuts, including almond, cashew or walnut, remained stable after microwave heating at 500 W for 1–3 min. The unchanged allergenicity was because the related epitopes in allergenic proteins were not susceptible, even treated by long-time or high-temperature microwave heating (Venkatachalam et al., 2008). In this study, our results showed that microwave

treatment shows an obvious potential to eliminate the allergenicity of tropomyosin in shrimp.

3.5. Microstructure observation

Fig. 5 shows the SEM images of the freeze-dried shrimp samples after different microwave treatments. The samples treated at 75 °C showed destroyed structures with rough surfaces and microstructural holes compared with the control. The damage in the structure of shrimp tissues was enhanced as an increase in processing time and temperature. When the temperature increased to 100 °C, more alveolate holes

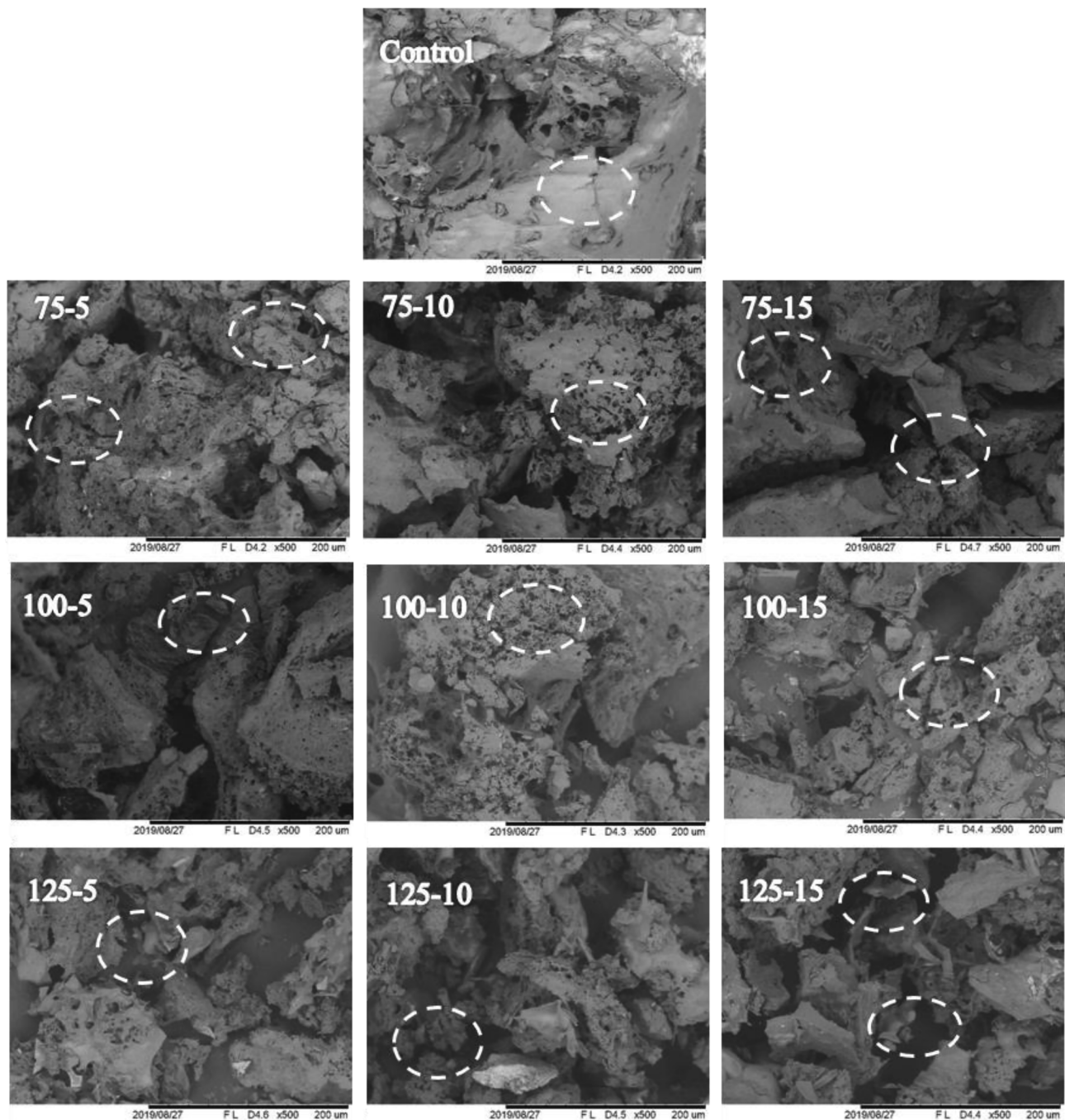


Fig. 5. SEM photomicrographs of freeze-dried shrimp samples treated with different microwave conditions (magnification at 500 $\times$).

and fragments were observed in the samples, especially in the treatment of 100–15. At 125 °C, more fragments and strips were generated, leading to the extremely degraded and collapsed structures. Similarly, [Izli and Isik \(2015\)](#) treated tomato samples with microwave (90, 160, 350, and 500 W), where they found a higher temperature and microwave power caused more considerable damage in the microstructure of the tomato samples. [Yarmand et al. \(2013\)](#) reported that the microwave treatment (2450 MHz, 600 W for 3 min) can affect the microstructure of camel longissimus dorsi muscle, which could be attributed to the high loss of water and fat caused by protein denaturation and disintegration of the texture matrix with the microwave treatment. In beef semitendinosus muscle treated with microwave heating (50, 70, 90 °C); the higher temperature, along with the longer heating time, led to higher insoluble collagen content viewed by SEM due to the thermal

denaturation and shrinkage of collagen ([Chang et al., 2011](#)). These changes were due to its structural damage, destroying the external surfaces of the samples during the high temperature and long-time processing ([Chang et al., 2011](#); [Izli & Isik, 2015](#)). Therefore, microwave treatment can destruct the shrimp structure considerably. The increasing temperatures and time of microwave heating effectively boost protein degradation and cell distortions. The intact cells completely changed into strips and fragments in the treatments at 125 °C, whereas no obvious differences were observed in the treatments at the same temperatures for different durations ([Fig. 5](#)). It indicates that high temperatures of microwave showed potential in modifying the shrimp protein structures, which may explain why high-temperature and long-time microwave processing could contribute to the reduction in the allergenicity of shrimp.

4. Conclusion

In the present study, microwave processing showed a potential application in reducing the allergenicity of shrimp. The percentage of reduction in the allergenicity was positively related to the processing temperatures and time. The highest reduction (up to 75%) in allergenicity was observed in 125–15 treated sample. The total antioxidant capacities significantly increased by two-fold after microwave processing. However, the total soluble protein content decreased by 50–75%, as well as a reduction in the *in-vitro* protein digestibility (64%–80%), which caused a decrease of peptides content. These changes obtained from microwave processing, were strongly attributed to an increase in β -sheets and a loss in turns of the secondary structure of shrimp proteins. Further, numerous microstructural holes and fragments of shrimp were observed after microwave treatments, especially in treatments at 125 °C. However, the mechanisms of microwave processing caused a reduction in the allergenicity of shrimp are still not clear. Further studies regarding the microwave parameters and relevant medical research need to be performed in the future.

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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