Effect of Power Ultrasound on the Immunoactivity and Texture Changes of Shrimp (*Penaeus vannamei*)

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Abstract

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The aim of this study was to examine the effect of power ultrasound on the allergenicity and texture properties of shrimp (*Penaeus vannamei*). For this purpose, raw and boiled shrimps were treated with power ultrasound (30 kHz, 800 W) at 0°C and 50°C for 0, 2, 8, 10, and 30 minutes. The results showed that the ultrasound treatment had a greater effect on the allergenicity of the boiled shrimps than of the raw ones, while with hardness it was *vice versa*. The allergenicity of the boiled shrimps treated at 0°C (treatment 3) and 50°C (treatment 4) decreased by nearly 50% and 40%, respectively, with 10 min of the treatment duration. As for the raw shrimps, with the treatment at 0°C (treatment 1) their allergenicity increased in the first 10 min and then decreased, while at 50°C (treatment 2), a slight reduction of 8% in allergenicity occurred. After treating with ultrasound for 30 min the hardness in treatment 1 increased to a peak-1.5-fold higher than the control, compared with 27% increase in treatment 2 and 15% increase in treatments 3 and 4. The results suggest that allergenicity can be reduced by power ultrasound with no change in the texture.

Keywords: shrimp; power ultrasound; allergenicity; texture

Food allergy is recognised as a safety problem worldwide and has become more serious in recent years. Recent epidemiologic studies showed that nearly 3.5–4% of Americans are affected by food allergies (Furlong *et al*. 2004), and shellfish is one of the most important causes of food allergy in adults in the United States, being responsible for the majority of emergency department visits due to food allergy (Sicherer *et al*. 2004). Shellfish allergy is typically lifelong, often severe, and potentially fatal (Wuthrich & Weber 2001). Shrimp is commonly identified as a major cause of shellfish hypersensitivity (Jeounga *et al*. 1997), with some heat-stable proteins as allergens (Daul *et al*. 1988; Naqpal *et al*. 1989). To ensure the shrimp safety for some allergic people, certain measures have been reported to decrease the allergenicity of shrimp. Among these measures, food processing has been recognised as a potentially effective way.

In previous studies, some kinds of food processing were reported to have a great influence on the activity of food allergens. Irradiation was confirmed to diminish the antigenic properties of proteins in eggs, milk, peanut, and shrimp by altering the structure of epitopes (Kume & Matsuda 1995; Byun *et al*. 2000, 2002; Li *et al*. 2007; Oh *et al*. 2009), the amounts of intact allergens having been reduced depending upon the irradiation dose. By disrupting the sequential and conformational epitopes, enzymatic hydrolysis may also help eliminate certain epitopes. Burks

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et al. (1992) demonstrated that the IgE-binding capacity of peanut and soybean proteins extracts can be reduced 100-fold and 10-fold, respectively, when treated with enzymes in the immobilised digestive enzyme assay system, and Shimakura *et al*. (2003) stated that the allergenicity of the crustacean extractives was almost completely lost on digestion with proteases. However, irradiation needs expensive instruments and the security of the irradiated food is under suspicion. On the other hand, protein hydrolysis may result in undesirable and unacceptable changes in the food texture and sensory attributes (Sathe *et al*. 2005; Thomas *et al.* 2007), thus such application is limited. It is urgent to find some non-vemethods to decrease the allergenicity of foods.

Since recently, power ultrasound has become an important processing means which is widely used in food processing, The power ultrasound waves can be absorbed by food and cause various changes of the food characteristics, especcially as concerns the protein component (Choa *et al.* 1985; Jambrak *et al.* 2008). Various effects of ultrasound arise from acoustic cavitation: the formation, growth, and implosive collapse of bubbles in the liquid. Cavitational collapse produces intense local heating and high pressure (Suslick *et al*. 1999). These effects result in changes of native protein structure, such as the alteration of conformation, loss of the secondary structure, formation of new intra/inter molecular interactions, and rearrangements of disulfide bond (Owen & Simons 1957; Pavlovskaya *et al.* 1992). Alteration of the protein structure may affect the allergenicityand texture of food. The results of our previous studies showed that the allergenicity of raw shrimp profoundly reduced after the treatment with power ultrasound for 1.5 h at 50°C (Li *et al*. 2006), indicating that ultrasound can affect immunogenicity of the shrimp allergen; however, no data on the texture of the shrimp treated with ultrasound have been reported.

Texture is one of the most important indexes of food quality. Texture profile analysis (TPA) is conducted to assess the textural properties of food; many researches have demonstrated it as a useful method for the prediction of the sensory texture (Tabilo *et al*. 1999; Huidobro *et al.* 2005). In order to evaluate the changes of shrimp after power ultrasound treatment, texture properties have been determined. Some previous reports demonstrated that the ultrasound treatment accelerated the ageing process of beef (Stadnik *et al*. 2008), and Yang *et al*. (2006) reported that the combined application of ultrasound and papain help to tenderise the shrimp by reducing the shear force. Therefore, in the present research, power ultrasound was used to treat shrimps for different periods of time, and the allergenicity and texture properties were subsequently analysed.

MATERIAL AND METHODS

Reagents and materials. Bovine serum albumin (BSA) was purchased from Solarbio (Beijing, China). HRP-goat-anti-human IgE was obtained from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, USA). 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich (St. Louis, USA).

Sera from patients with shrimp allergy were collected in Qingdao Municipal Hospital (Qingdao, China) and stored in aliquots at –80°C. The allergic response had been confirmed by the clinical history and diagnosis, skin prick testing, and objective manifestations observed after shrimp ingestion. Sera from healthly people without shrimp-allergy were employed as negative controls.

Live farmed shrimp (*Penaeus vannamei*) were purchased from Nanshan fishery market (Qingdao, China).

Shrimp muscle treatment. Shrimp were deshelled, then the muscle was sealed in plastic bags and subjected to different treatments: treatment 1: the treatment with power ultrasound (30 kHz, 800 W) at 0°C for 0, 2, 8, 10, and 30 min; treatment 2: the treatment with power ultrasound (30 kHz, 800 W) at 50°C for 0, 2, 8, 10, and 30 min; treatment 3: heating in boiling water for 15 min, then cooling at room temperature; after that, shrimp muscle was treated with power ultrasound (30 kHz, 800 W) at 0°C for 0, 2, 8, 10, and 30 min; treatment 4: the same operation as in treatment 3 was done with shrimp muscle which was after wards treated with power ultrasound (30 kHz, 800 W) at 50°C for 0, 2, 8, 10, and 30 minutes. In order to clarify the alteration during the ultrasound treatment, a control sample was set in each group: ultrasound treatment for 0 min at corresponding temperature.

The preparation of shrimp protein extracts (PE). Peeled shrimp after the treatment with ultrasound were quick-frozen in an ultra-low temperature refrigerator and kept at –80°C until used.

Shrimp acetone powder was prepared according to Smillie (1982). Shrimp extracts were prepared as described previously (Yu *et al*. 2003) with few modifications. Five grams of shrimp acetone powder was immersed in 50 ml of extraction buffer (1M KCl, containing 0.2mM 1,4-dithiothreitol and 1mM phenyl methyl sulfonyl fluoride) at 4°C for 24 h at constant stirring. After centrifugation at 12 000 g at 4°C for 15 min, the supernatant was dialysed at 4°C for 72 h against 0.01M PBS, pH 7.4, was replaced every 12 h resultant solution was freeze-dried and stored at –80°C until used. The concentration of protein in PE was determined using Bradford's method (Bradford 1976).

SDS-PAGE. SDS-PAGE was performed in a discontinuous buffer system on 5–12% gradient gels using the principles described by Laemmli (1970). Proteins were stained with Coomassie Brilliant Blue R250, the molecular weights were estimated by comparison with pre-stained marker proteins.

Indirect ELISA. For indirect ELISA, the microplates were coated with 100 µl of allergen (40 ng/ml) antigens in carbonate buffer (0.05M, pH 9.6, CBS) at 4°C overnight. The wells were washed three times with PBS containing 0.1% Tween 20 (PBST) and then blocked with 1% BSA (in PBST) at 37°C for 1.5 hour. The microplates were washed three times, and then 100 µl of positive serum diluted with 1% BSA (in PBST) was added to each well followed by incubation for 1.5 h at 37°C, and further by the addition of 100 µl HRP-labelled goat anti-human IgE. The microplates were then incubated at 37°C for 1.5 h and washed three times with PBST, and then 100 µl substrate solution (100 µg/ml TMB in 0.05M phosphate citrate buffer (pH 5.0) with 0.04% H_2O_2) was added. After incubation at 37°C for 20 min in the dark, the reaction was stopped by the addition of 50 µl H_2SO_4 (2M) to each well. The absorbance value of each well was read at 450 nm with a plate reader (Multiskan MK3, Thermo Labsystems, Vantaa, Finland). The negative control wells were treated in the same way as the test wells except that the sera collected from healthy people replaced the positive sera.

Texture profile analysis. Smooth abdominal muscle (the front 3 sections of abdominal) was selected for the texture profile analysis. Five parallel samples were taken from different treatments. The texture measurements in the form of texture profile analysis (Bourne 1978) were performed at room temperature with a Texture Analyser (TMS-PRO, Food Technology Corporation, Sterling, USA).

A double compression cycle test was performed at up to 30% compression of the original sample height with a cylindrical probe of 3.6 mm diameter. The cross-head moved at a constant speed of 1 mm/s.

The following parameters were quantified (Bourne 1978): hardness (N) – maximum force required to compress the sample during the first compression, springiness (m) – the ability of the sample to recover its original form after deforming force was removed, adhesiveness (N·s) – the area under the abscissa after the first compression; cohesiveness – the extent to which the sample could be deformed prior to rupture; and chewiness (J) – the work required to masticate the sample before swallowing, which is defined as the product of hardness, springiness, and cohesiveness.

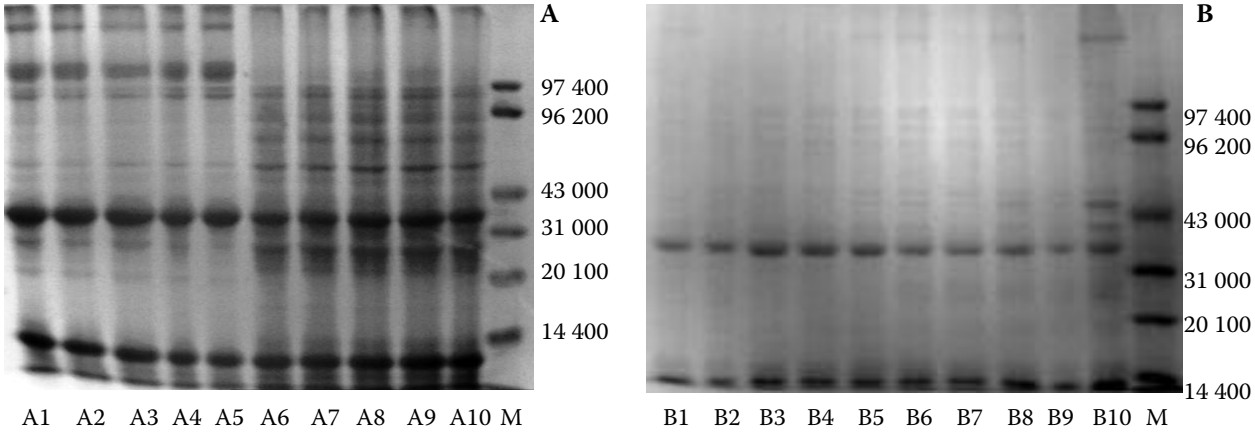
RESULTS AND DISCUSSION

Composition of shrimp protein extracts

The allergen extracts of shrimp exposed to different treatments were analysed using SDS-PAGE. The treatment profiles related to various time intervals are shown in Figure 1. Significant changes were observed in the protein profiles of shrimp allergen extracts when the shrimp were heat-treated, however, one of the bands with the molecular mass of approximately 36 kDa, corresponding to what is known as the major shrimp allergen, namely Pen a 1 (Hamada *et al*. 2003), was present even if the shrimp was treated in boiling water. At the same temperature, there were no noticeable changes in the composition of protein extracts after the treatment with power ultrasound for different periods of time.

Allergenicity

Allergenicity of PE from differently treated shrimps was analysed by indirect ELISA and was measured at OD_{450}. As shown in Figure 2, there were no significant changes in the allergenicity of PE following treatment 2. The allergenicity of PE from treatment 1 increased slightly during the first ten minutes and then decreased. After 10 min treatment, significant reduction was observed in the allergic activity of PE with treatment 3 and treatment 4, i.e. 50% and 40%, respectively.



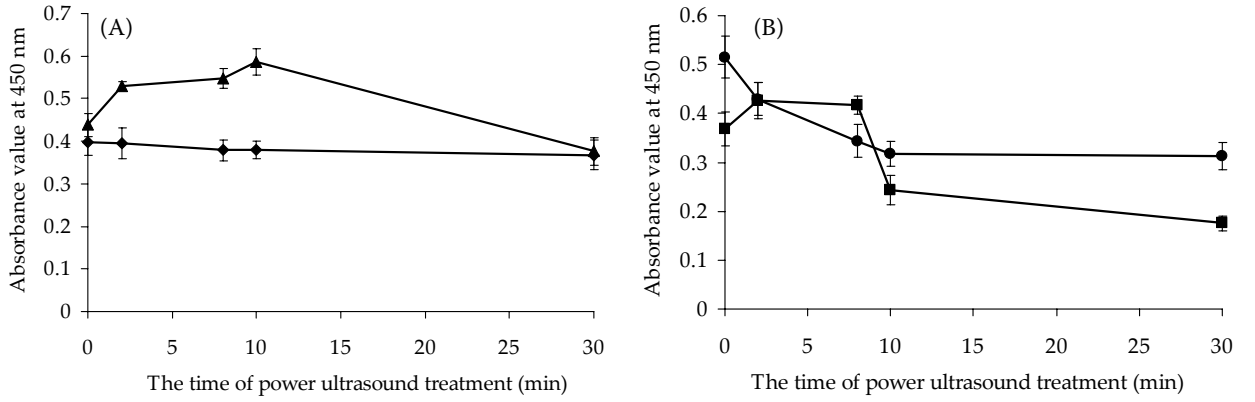
The protein concentration was adjusted to 2 mg/ml. Lane A_1–A_5 = PE from treatment 1 shrimp; Lane A_6–A_{10} = PE from treatment 2 shrimp; Lane B_1–B_5 = PE from treatment 3 shrimp; Lane B_6–B_{10} = PE from treatment 4 shrimp; Lane M = molecular weight marker. Different treatments refer to: treatment 1: treated with ultrasonic (30 kHz, 800 W) at 0°C for 0, 2, 8, 10, 30 min; treatment 2: treated with ultrasonic (30 kHz, 800 W) at 50°C for 0, 2, 8, 10, 30 min; treatment 3: heated with boiling water for 15 min, then treated with ultrasonic (30 kHz, 800 W) at 0°C for 0, 2, 8, 10, 30 min; treatment 4: heated with boiling water for 15 min, then treated with ultrasonic (30 kHz, 800 W) at 50°C for 0, 2, 8, 10, 30 minutes

Figure 1. SDS-PAGE/Coomassie blue-staining analysis of protein extracts from *Penaeus vannamei*

In present study, we treated raw and boiled shrimps respectively, with power ultrasound. The results suggested that only the boiled shrimp treated with power ultrasound showed a significant decrease in allergenicity. After the treatments for 30 min at 0°C and 50°C, the absorbance values at 450 nm were reduced to half and 60% of the control value, respectively. As reported in the previous study, the major shrimp allergen is a kind of thermally stable protein. Figure 1 also shows that the, primary allergen was maintained during the treatment in boiling water while the proteins conformation was altered, thusthe allergen epitopes were exposed (Davis & Williams 1998); and some proteins which may inhibit ultrasonic absorption by allergen epitopes were denatured. Both of the changes above may result in a better interaction between the power ultrasound waves and allergen epitopes. Raw shrimp treated by power ultrasound showed no significant decrease or even a slight increase in allergenicity, which may be explained by the exposure of new intra-molecule allergen epitopes induced by power ultrasound.

Texture profile analysis

Table 1 shows the TPA parameters of shrimps after different treatments. No significant differ-



Each point represented the average value of three experiments (n = 3); (▲) allergic activity of PE from treatment 1; (◆) allergic activity of PE from treatment 2; (■) allergic activity of PE from treatment 3; (●) allergic activity of PE from treatment 4

Figure 2. Changes of allergic activity of *Penaeus vannamei* without heat-treated (A) or with heat-treated (B)

Table 1. Mean ± SD TPA parameters of raw and boiled shrimp after power ultrasound treatments

Temperature (°C)	TPA parameters	Treatment time (min)				
		0	2	8	10	30
Raw shrimp						
	hardness (N)	0.59 ± 0.13	0.64 ± 0.12	0.72 ± 0.18	0.75 ± 0.09	0.90 ± 0.11
	cohesiveness	0.59 ± 0.03	0.56 ± 0.17	0.49 ± 0.07	0.48 ± 0.06	0.45 ± 0.08
0	adhesiveness (N·s)	–0.33 ± 0.04	–0.50 ± 0.16	–0.35 ± 0.05	–0.27 ± 0.03	–0.23 ± 0.06
	springiness (m)	0.61 ± 0.15	0.59 ± 0.16	0.54 ± 0.08	0.58 ± 0.10	0.60 ± 0.17
	chewiness (J)	0.18 ± 0.02	0.19 ± 0.03	0.23 ± 0.08	0.21 ± 0.05	0.29 ± 0.10
	hardness (N)	1.39 ± 0.17	1.48 ± 0.14	1.66 ± 0.10	1.74 ± 0.09	1.77 ± 0.04
	cohesiveness	0.60 ± 0.03	0.67 ± 0.06	0.66 ± 0.04	0.63 ± 0.02	0.62 ± 0.04
50	adhesiveness (N·s)	–0.34 ± 0.03	–0.18 ± 0.03	–0.11 ± 0.01	–0.03 ± 0.00	–0.05 ± 0.00
	springiness (m)	0.70 ± 0.04	0.73 ± 0.02	0.72 ± 0.02	0.73 ± 0.10	0.71 ± 0.07
	chewiness (J)	0.62 ± 0.11	0.76 ± 0.09	0.79 ± 0.10	0.84 ± 0.16	0.77 ± 0.03
Boiled shrimp						
	hardness (N)	2.07 ± 0.28	2.22 ± 0.10	2.26 ± 0.25	2.27 ± 0.10	2.39 ± 0.03
	cohesiveness	0.62 ± 0.01	0.72 ± 0.03	0.57 ± 0.05	0.69 ± 0.03	0.72 ± 0.04
0	adhesiveness (N·s)	–0.04 ± 0.00	–0.08 ± 0.01	–0.06 ± 0.02	–0.02 ± 0.00	–0.07 ± 0.00
	springiness (m)	0.70 ± 0.02	0.82 ± 0.02	0.60 ± 0.03	0.87 ± 0.11	0.82 ± 0.07
	chewiness (J)	0.99 ± 0.02	1.25 ± 0.04	1.29 ± 0.07	1.32 ± 0.07	1.43 ± 0.10
	hardness (N)	2.43 ± 0.23	2.49 ± 0.03	2.54 ± 0.11	2.63 ± 0.17	2.78 ± 0.06
	cohesiveness	0.68 ± 0.03	0.71 ± 0.03	0.67 ± 0.03	0.70 ± 0.03	0.71 ± 0.03
50	adhesiveness (N·s)	–0.05 ± 0.01	–0.05 ± 0.01	–0.03 ± 0.00	–0.05 ± 0.01	–0.08 ± 0.01
	springiness (m)	0.71 ± 0.04	0.79 ± 0.05	0.73 ± 0.03	0.78 ± 0.03	0.76 ± 0.08
	chewiness (J)	1.15 ± 0.08	1.37 ± 0.06	1.38 ± 0.23	1.41 ± 0.05	1.68 ± 0.02

ences in the texture parameters were found as compared to controls except for hardness. After the treatment with ultrasound for 30 min, the hardness of the sample in treatment 1 showed the greatest increase, 1.5-fold higher than the control, compared with 27% increase in treatment 2 and 15% increase in treatment 3 and treatment 4. The shrimp revealed greater hardness after having been treated with power ultrasound, which caused an increase in chewiness. Figure 3 shows a typical texture profile in force-time analysis obtained from the TMS-PRO Texture Analyser. The results showed that the hardness of shrimp increased on the treatment with power ultrasound.

According to Rongrong *et al.* (1998), a lower water content would tend to increase hardness. In this study, the increase of hardness probably resulted from the loss of water; the power ultrasound may promote osmosis of shrimp cells. Although the detailed mechanism is still unknown, the dehydration of some other foods during the ultrasound treatment has been confirmed. Carcel *et al.* (2007) demonstrated that the sonicated meat showed the dehydration phenomenon at a low ultrasound intensity (< 30W/cm^2). Fernandes *et al.* (2009) studied the effects of osmosis and ultrasound on pineapple cell tissue structure, and demonstrated that ultrasound increased water diffusivity because of the formation of microscopic channels, which offered a lower resistance to water diffusion.

The enhancement of shrimp hardness may also be induced by the heat converted from ultrasound waves absorbed by shrimp as a result of cavitation. For the raw shrimps, heat induced protein denaturation and aggregation more obviously than with the boiled shrimps. Myogen may be extruded from myofibrils by thermal effect and solidified in the gaps of myofibrils, therefore a tougher texture is formed (Hatae *et al.* 1986).

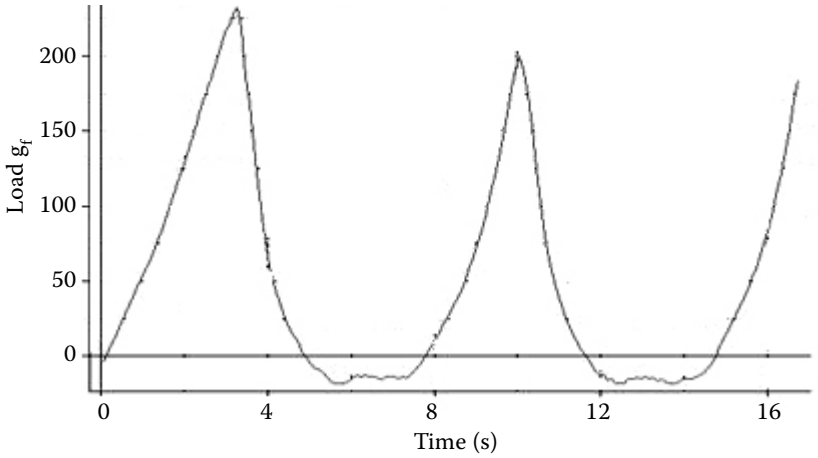


Figure 3. A typical texture profile analysis force-time obtained from the TMS-PRO Texture Analyser

CONCLUSION

The effects of power ultrasound treatment on the allergenicity and texture of both raw and boiled shrimps were investigated. The results showed that after the treatment for 30 min with power ultrasound, the boiled shrimps exhibited a significant decrease in allergenicity with a 15% increase of hardness, whereas the raw shrimps presented a small reduction in allergenicity and a greater increase in hardness, up to 50% increase in treatment 1. Although more research is needed to evaluate the changes in allergenicity, the results presented in this work indicate that power ultrasound might be a useful method to reduce the allergenicity of boiled shrimps with little alteration in the texture.

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