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THE EFFECTS OF THERMAL AND NON-THERMAL TREATMENTS ON PROTEIN PROFILES OF *SCYLLA TRANQUEBARICA* (PURPLE MUD CRAB)

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Abstract

Crab is the most frequent cause of allergic reactions in countries where crab consumption is high. *Scylla tranquebarica* was reported as one of the dominant mud crabs in Malaysia. Thus, the aim of this study was to determine the protein profiles of this crab after subjected to various food-processing techniques. Raw, thermal and non-thermal treated extracts were prepared and then resolved by SDS-PAGE. Approximately 38 protein bands between 10 to 245 kDa were detected in the raw extract, while all treated extracts revealed fewer protein bands. In general, dried, salted, steamed and boiled crabs presented higher numbers of protein bands compared to the acid treated, microwaved, roasted and fried crabs. Two prominent of heat and chemical-resistant bands at 34 to 38 kDa were seen in all extracts, which hypothesized to be tropomyosin. Other prominent bands between 20 to 26 kDa and 42 to 48 kDa were not present in almost all treated crabs, indicating that those bands are sensitive to high temperature and extreme pH conditions. The results showed that the microwaving and acid treatments were the most effective methods in denaturing the protein bands of *S. tranquebarica* while boiling, steaming and drying have the least effect on the protein profile of this crab. As a conclusion, this study indicated that *S. tranquebarica* contains numerous proteins with either sensitive or resistant to thermal or non-thermal treatments, which might play an important role in crab allergy. Further study is currently conducted to characterize the allergenic properties of these protein bands.

Keywords: Crab, Thermal, non-thermal, SDS-PAGE, protein

Introduction

As a high-protein and a low-fat food source, crabs play an important role in human nutrition and health. Consumption of seafood including crab has increased in many countries in Europe and Asia in recent years (Ruethers *et al*., 2018). The highest consumption of crab in Asia was in China and Malaysia (Bahna, 2016). However, the crab consumption could trigger crab allergy in hypersensitive individuals. In Malaysia, 44% of allergic patients suffered an allergy to crustaceans including crab (Rosmilah *et al*., 2012).

Various types of food processing methods can be applied to seafood including crab to maintain or improve their qualities for human consumption (Jiménez-Saiz *et al*., 2015). Both thermal and non-thermal treatments are usually applied (Jiménez-Saiz *et al*., 2015; Hu *et al*., 2017). However, these treatments may produce many modifications, including the denatured of the protein, the hydrolysis of the peptide bonds, clustered by the covalent bonds, disulfide bonds and reactions with other food molecules such as lipids and carbohydrates. As a consequence, the allergenicity of the seafood may also be altered by either reducing or enhancing it (Jiménez-Saiz *et al*., 2015; Han *et al*., 2018). Tropomyosin, the major allergenic protein in shellfish including crabs has been well-documented as highly stable to both thermal and non-thermal processing techniques while arginine kinase (Rosmilah *et al*., 2012; Kamath *et al*., 2013) and sarcoplasmic calcium-binding protein (Hu *et al*., 2017), the other shellfish major allergens were identified as thermal-sensitive proteins (Rosmilah *et al*., 2012; Kamath *et al*., 2013; Hu *et al*., 2017).

Scylla tranquebarica is an important mud crab found in mangrove areas in Malaysia especially in Sabah coastal areas and other Indo-Pacific regions (Sharif *et al*., 2016). Thus, the aim of this study was to determine the effects of several thermal and non-thermal treatments on protein profiles of this species of crab.

Materials and Methods

Preparation of Protein Extracts

Live S. *tranquebarica* samples were purchased from a local supplier in Tawau, Sabah. Raw, thermal treated (boiled, steamed, microwave heating, roasted, fried) and non-thermal treated (salted, dried and acid treated) extracts of *S. tranquebarica* were prepared from the crab flesh according to the methods by other studies (Rosmilah *et al*., 2012; Jiménez-Saiz *et al*., 2015; Yadzir *et al*., 2012; Aberoumand, 2014) with slight modifications.

SDS-PAGE Analysis

To determine the protein profile of this species of crab was performed (SDS-PAGE) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis the extracts prepared by using the method described previously (Rosmilah *et al*., 2012; Yadzir *et al*., 2012).

Results and Discussion

SDS- PAGE fractionated the *S. tranquebarica* proteins to numerous protein bands (Figure 1). In general, as shown in Table 1, all treated extracts expressed lesser protein bands compared to the raw extract.

The raw extract has the most protein bands, with approximately 38 bands. This is not surprising as the raw extract contains all types of proteins, either sensitive or resistant to the food treatments applied. This result was in line with another report, which also detected numerous protein bands in raw local crab (Sharif *et al*., 2016). This study showed that different methods of preparing crabs produce varieties of protein profiles. Among the heated extracts, the boiled and steamed crabs presented more protein

bands than the other heat-treated crabs (18 and 19 bands, respectively); while the microwave-heated extract has the least bands (4 bands). Some protein bands in microwaved, fried and acidic crabs demonstrate to be clearly reduced compared with those of the boiled and steamed forms. Frying and microwaving presents fewer protein bands than boiling, steaming and roasting. This is in probability because of the aggregation of the protein and the derangement of protein conformation by high temperature (Jiménez-Saiz *et al*., 2015; Sharif *et al*., 2016).

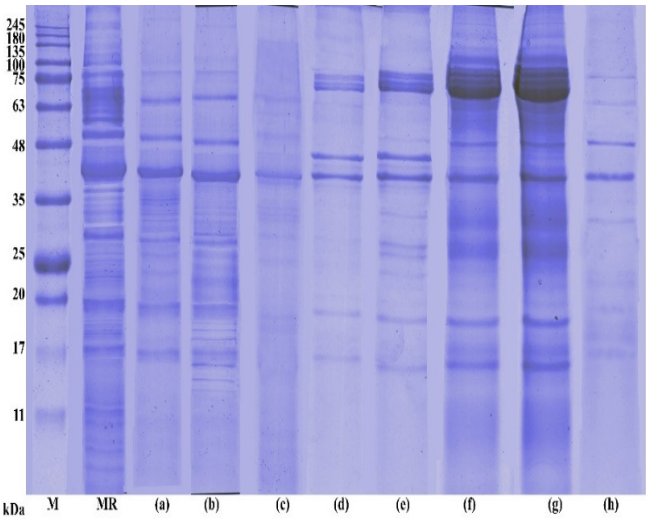

Fig. 1 : The SDS-PAGE analysis of raw (MR), boiled (a), steamed (b), microwave heated (c), fried (d), roasted (e), salted (f), dried (g) and acid treated (h) treated extracts. M is molecular weight markers in kiloDalton (kDa).

In most proteins at temperatures above 80°C, thermal modification ruptures the finely balanced intermolecular forces and reorganizes all levels of protein structures. Where occurs loss in the results of almost secondary and tertiary structures. This causes the protein to adopt a configuration that approaches a fully unfolded, random-coil conformation (Hu *et al*., 2017). In this study, the temperature applied in microwave heated was the highest (220 to 250°C), compared to the fried (190°C), roasted (180°C), boiled (100°C) and steamed (100°C). This might explain the reason for frying and microwave present fewer protein bands than the other thermal treatments in this study. Steaming of crab revealed an almost similar number of protein bands to boiling but the pattern of the protein bands was slightly different from each other. Meanwhile, the protein profiles of fried crab almost identical to the roasted crab. However, in all heated extracts our study detected a prominent heat-resistant protein at 31-38 kDa, which might be hypothesized to be tropomyosin.

Meanwhile, among the non-thermal treated crab, the acid treated crab expressed the least bands (6 bands) than the other non-thermal treated extracts. The reduction of the number of bands in the acid-treated extract can be explained by an alteration in the protein structures after acid treatment due to partial loss of protein structures as the result of proteolytic Degradation of proteins by the activity of acidic protease at acidic pH (Aberoumand, 2014; Khan *et al*., 2018). Meanwhile, the protein profile of salted and dried extracts was almost similar, with only a few proteins were not detected in the SDS-PAGE gel. In the salted crab, the protein losses might because a lot uptake of salt (NaCl) by the muscle, give rise to competition with muscle protein for water molecules, thus it causes the denaturation and aggregation of these proteins by a process of "salting out". Generally, during salting processes, the number of bands decreases owing to protein denaturation (Martínez-Alvarez & Gómez-Guillén, 2006). Meanwhile, food drying is a method of food conservation that doing by removing water from the food, which inhibits the growth of bacteria. This study revealed that the protein profile of the crab was more resistant to change upon drying as this process occurred only at low temperatures using sun drying (33 to 45°C) (Zhang *et al*., 2006). The raw extract of the crab presented the most complex protein pattern with 38 visible protein bands between the molecular weight of 10 to 245 kDa. Not detected in most of the treated extracts, bands between10 to 22 kDa and 50 to 245 kDa, which were present in the raw extract, were and therefore were identified as the heat and chemical sensitive protein bands. The most prominent protein bands in the range of 34-38 kDa have appeared in all extracts, which might correspond to tropomyosin (Rosmilah *et al*., 2012; Yadzir *et al*., 2012).

Tropomyosin, a myofibrillar protein consisting of 2 subunits with molecular masses of 31 to 38 kDa has been well documented as the major and cross-reactive allergen of various species of crabs (Ruethers *et al*., 2018; Zhang *et al*., 2006).is present in much higher quantity than other identified shellfish allergens, because, Tropomyosin is played a primary role in muscle function (Ruethers *et al*., 2018). The major allergen in shellfish heats stable and highly water-soluble is tropomyosin (Ruethers *et al*., 2018; Yadzir *et al*., 2015; Kamath *et al*., 2014). The ability of tropomyosin to withstand heats treatment and extreme pH conditions because tropomyosin does not easily denature and aggregated (Yadzir *et al*., 2015; Kamath *et al*., 2014). This may explain why this protein was still present although after all heated and acid treatments in this study. Bands of 20-26 kDa were also seen in raw and treated crab extracts except for the microwave heated, fried and acid treated extracts. It found the sarcoplasmic calcium-binding protein; myosin light chain and troponin C (≈21 kDa) is consistent with recognized shellfish allergens in this status (Ruethers *et al*., 2018). In contrast, a protein of 42-48 kDa appeared only in raw, boiled and steamed extracts with different intensity and this might be homologous to arginine kinase (≈42 kDa) (Rosmilah *et al*., 2012). Extreme pH condition thus were not present in almost all treated crabs, except for boiled and steamed crabs. Therefore, based on the number of protein bands, this result showed that the microwaving and acid treatments were the most effective methods in denaturing the protein bands of *S. tranquebarica* while boiling, steaming and drying have the least effect on protein profile of *S. tranquebarica*. These protein profile variation may also influence the allergenicity of the crabs.

Table I : Molecular weight of protein bands of raw, thermal and non-thermal treated extracts of *S. tranquebarica*.

Protein Molecular Weight (kDa)	Raw	Boil	Steam	Microwaving	Fry	Roasted	Salted	dried	Acid-treated
245	√								
150	√								
107								√	
100	√						√	√	
84	√						√	√	
75	√	√	√		√		√	√	
74	√	√	√				√	√	
73	√	√	√	√		√	√	√	√
72	√	√	√		√		√		
70	√	√	√				√		
69	√						√		
67	√						√		
63	√	√	√	√	√	√	√	√	√
60	√								
54	√								
53	√		√						
52	√								
51	√		√						
50	√	√	√	√	√	√	√	√	√
48	√	√	√						
42	√	√	√						
41	√								
38	√	√		√		√	√	√	√
37	√	√	√		√				
36	√								
35	√	√							
34	√	√	√						
33			√						
32			√						
31	√	√	√		√	√	√	√	√
30	√	√			√				
27						√	√	√	
26	√	√	√			√		√	
25	√	√	√			√	√	√	
24	√	√	√			√	√	√	
23	√	√	√						
22	√		√						
20	√	√				√		√	√
18	√								
16	√				√	√		√	
15	√								
13	√	√							
10	√		√		√				
Total, n	38	18	19	4	7	12	17	16	6
%	100	47	55	10	18 31		44	42	15

√, Protein band

Conclusion

This study revealed that *S. tranquebarica* has numerous protein bands with properties of either sensitive or resistant to heat and chemical processes, indicating that this species of crab might have varied allergenicity after subjected to different food processing techniques. Further study to identify the effect of the different processing methods on the allergenicity of *S. tranquebarica* are currently conducted by an allergenic approach.

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References

Aberoumand, A. (2014). Studies on the effects of processing on food quantity of two selected consumed marine fishes in Iran. International Food Research Journal, 21(4): 1429.

Agrahar-Murugkar, D. and Jha, K. (2010). Effect of drying on nutritional and functional quality and electrophoretic pattern of soyflour from sprouted soybean (*Glycine max*). Journal of food science and technology, 47(5): 482-487.

Bahna, S.L. (2016). Not every seafood "allergy" is allergy!. Annals of Allergy, Asthma & Immunology, 117(5): 458-461.

Han, X.Y.; Yang, H.; Rao, S.T.; Liu, G.Y.; Hu, M.J.; Zeng, B.C. and Liu, G.M. (2018). The maillard reaction reduced the sensitization of tropomyosin and arginine kinase from scylla paramamosain, simultaneously. Journal of agricultural and food chemistry, 66(11): 2934-2943.

Hu, M.J.; Liu, G.Y.; Yang, Y.; Pan, T.M.; Liu, Y.X.; Sun, L.C. and Liu, G.M. (2017). Cloning, expression, and the effects of processing on sarcoplasmic-calcium-binding protein: an important allergen in mud crab. Journal of agricultural and food chemistry, 65(30): 6247-6257.

Jiménez-Saiz, R.; Benedé, S.; Molina, E. and López-Expósito, I. (2015). Effect of processing technologies on the allergenicity of food products. Critical reviews in food science and nutrition, 55(13): 1902-1917.

Kamath, S.D.; Rahman, A.M.A.; Komoda, T. and Lopata, A.L. (2013). Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies. Food chemistry, 141(4): 4031-4039.

Kamath, S.D.; Rahman, A.M.A.; Voskamp, A.; Komoda, T.; Rolland, J.M.; O'hehir, R.E. and Lopata, A.L. (2014). Effect of heat processing on antibody reactivity to allergen variants and fragments of black tiger prawn: A comprehensive allergenomic approach. Molecular nutrition & food research, 58(5): 1144-1155.

Khan, M.U.; Ahmed, I.; Lin, H.; Li, Z.; Costa, J.; Mafra, I. and Wu, Y.N. (2018). Potential efficacy of processing technologies for mitigating crustacean allergenicity. Critical reviews in food science and nutrition, 1-24.

Khora, S.S. (2016). Seafood-associated shellfish allergy: a comprehensive review. Immunological investigations, 45(6): 504-530.

Martínez-Alvarez, O., and Gómez-Guillén, M.C. (2006). Effect of brine salting at different pHs on the functional properties of cod muscle proteins after subsequent dry salting. Food Chemistry, 94(1): 123-129.

Rosmilah, M.; Shahnaz, M.; Zailatul, H.M.; Noormalin, A. and Normilah, I. (2012). Identification of tropomyosin and arginine kinase as major allergens of *Portunus pelagicus* (blue swimming crab). Trop Biomed, 29(3): 467-78.

Ruethers, T.; Taki, A.C.; Johnston, E.B.; Nugraha, R.; Le, T.T.; Kalic, T. and Lopata, A.L. (2018). Seafood allergy: a comprehensive review of fish and shellfish allergens. Molecular immunology, 100: 28-57.

Sharif, N.A.M.; Kahar, N.A.S.; Rodrigues, K.; Ransangan, J. and Kian, A.Y.S. (2016). Genetic diversity of mud crabs, *Scylla tranquebarica* in Sabah, Malaysia based on Cytochrome C Oxidase (COI) gene sequence. Songklanakarin Journal of Science & Technology, 38(4).

Yadzir, M.; Hani, Z.; Misnan, R.; Bakhtiar, F.; Abdullah, N. and Murad, S. (2015). Tropomyosin and actin identified as major allergens of the carpet clam (*Paphia textile*) and the effect of cooking on their allergenicity. BioMed research international.

Yadzir, Z.H.M.; Misnan, R.; Abdullah, N.; Bakhtiar, F.; Arip, M. and Murad, S. (2012). Identification of the major allergen of *Macrobrachium rosenbergii* (giant freshwater prawn). Asian Pacific journal of tropical biomedicine, 2(1): 50-54.

Zhang, Y.; Matsuo, H. and Morita, E. (2006). Cross-reactivity among shrimp, crab and scallops in a patient with a seafood allergy. The Journal of dermatology, 33(3): 174-177.