RESEARCH ARTICLE

Effects of Different Heat Processing on Molecular Weight and Allergenicity Profile of White Shrimp (*Litopenaeus vannamei*) and Mud Crab (*Scylla serrata*) from Indonesian Waters

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Abstract

Shrimp and crab are fishery products with high nutritional value, especially as protein sources. However, they belong to the crustacean group known to cause many allergies in Southeast Asian countries. Not only affecting the nutritional composition, processing also has the potential in reducing the allergenicity of a food ingredient. This study aims to analyze the effects of various heat processing on nutrient composition, soluble protein content, protein molecular weight profile, and allergenicity of white shrimp and mud crab. The processing was carried out by boiling, frying, and autoclaving. Changes in macronutrient content were determined using proximate analysis. Soluble protein content was analyzed using the Bradford method, while molecular weight and allergenicity profiles were analyzed using SDS-PAGE electrophoresis and ELISA techniques. Results showed that processing treatment could significantly reduce protein solubility. SDS-PAGE profile showed that heating shrimp and crab using autoclave at 121.1 °C and pressure of 0.2 MPa for 10 min was able to remove protein bands of 35-38 kDa, which were suspected as the allergen bands. However, boiling (100 °C for 10 min) and deep frying in palm oil (160 °C for 10 min) treatments of the samples gave stronger 35-38 kDa bands intensity than the unprocessed sample. Autoclaving was able to significantly reduce IgE reactivity to shrimp extract better than the boiling and frying process. In contrast, IgE reactivity of crab extract was increased by heat processing especially frying. Thus, autoclaving can be used in the white shrimp processing to produce hypoallergenic food.

Keywords: allergens, ELISA, crustaceans, processing, SDS-PAGE

Introduction

Marine products, especially crustaceans, are a source of protein with high nutritional value. The Indonesian Ministry of Marine Affairs and Fisheries (2020) data shows that shrimp and crab production in 2020 reached 1.3 million tonnes and 10 thousand tonnes, respectively. The increasing trends of interest and awareness to consume healthy and highly nutritious food increase the consumption of marine products. Shrimp and crab are the most consumed crustaceans in Indonesia as cooked products. Based on the Indonesian Food Consumption Survey Data (SKMI) in 2014 released by the Health Research and Development Agency, the Indonesian Ministry of Health, the daily consumption of shrimp, crab and their processed products reached 2.6 g/day, the third-largest consumption of fishery products after marine and freshwater fish. Hence, food allergies are also increasing along with the increasing seafood consumption.

Similar trends are also observed in other countries, such as in Vietnam, Philippines, and Singapore. Data showed that the prevalence of allergy cases is increasing from year to year. The prevalence of food allergy symptoms experienced by adults in Vietnam reached 4.6%, with crustaceans being the main cause (Le et al., 2020). Moreover, regional cases of allergies due to crustaceans are also experienced by children in Vietnam with a prevalence of 3.83% (Le et al., 2019), and adolescents in the Philippines and Singapore with

a prevalence of 5.12% and 5.23%, respectively (Shek et al., 2010).

The main cause of allergy in crustaceans is related to the content of specific proteins (called allergen protein) found in the meat, such as tropomyosin, arginine kinase, myosin light chain, sarcoplasmic calcium-binding protein, and triosephosphate isomerase (Fernandes et al., 2015). Tropomyosin is the primary allergen protein responsible for 72-98% of crustacean allergy cases (Gámez et al., 2011). Moreover, allergen protein differs from other proteins due to groups or regions called epitopes that can react to immune cells and respond differently to sensitive individuals. Epitopes can bind to immune cells and induce IgE production, resulting in an allergic response in atopic individuals. The number of epitopes in one allergen molecule is not always the same as in other allergens (Kresno, 2001).

Food processing, such as boiling and frying, involves high temperatures that increase the safety and palatability of a food product (Suleman et al., 2020) and will affect the molecular weight composition of protein (Qixing et al., 2014). Concerning protein allergens, processing can change the conformation of a protein and potentially change its allergenicity. Recent studies have shown that processing can either increase or decrease food allergenicity. The boiling process of *Litopenaeus vannamei* shrimp extract reduced its reactivity to IgE compared to the unprocessed product, but it also actually increased the reactivity of allergen tropomyosin (Liu et al., 2010). Long et al. (2015) showed that high-pressure processing (HPP) at 500 MPa with a combination of high temperature at 55 °C were better than the regular boiling process to reduce tropomyosin reactivity in *L. vannamei* extract. A similar observation was shown in the study by Lasekan and Nayak (2016). The intensity of tropomyosin band and myofibrillar proteins of tiger prawns (*Penaeus monodon*) decreased by pressurized steaming processing.

However, data regarding changes in the allergenicity of various types of crustaceans due to high temperatures and pressure combinations process are limited. Further studies on changes in the allergenicity of crustaceans, especially shrimp and crab, due to various processing treatments (such as boiling, frying, and pressurized heating) are much needed to obtain a complete picture of their allergenicity profile. This study aims to analyze the effects of various types of high-temperature processes and combination with pressure on macronutrient value, solubility profile, molecular weight profile, and changes in the allergenicity of white shrimp and mud crab extracts. Furthermore, to demonstrate the potential of processing treatment in reducing allergenicity of shrimp and crab products.

Material and Methods

Material

The fresh white shrimp (*L. vannamei)* and mud crab (*Scylla serrata)* samples were obtained and stored in ice from the Muara Angke fish market, Jakarta. The sample was then transported to the laboratory in a cooler box full of ice gels to maintain the temperature below 5 °C, then stored at -20 °C until use.

Methods

Processing

The processing treatments used in this study were: 1) boiling at 100 °C for 10 min.; 2) deep frying in palm oil at 160 °C for 10 min.; and 3) autoclaving at 121.1 °C, 0.2 MPa in pouches for 10 min., following the processes performed by Yadzir et al. (2015) and Abramovitch et al. (2017). Prior to processing, the shrimp samples were cleaned and separated from the heads, tails, and outer shells, while the mud crab carapace was opened.

Proximate Analysis

Analyses of nutritional contents (water, ash, fat, protein, and carbohydrate) were carried out using the proximate method according to AOAC (2012) in duplicates.

Protein Extraction

Protein extraction was proceeded following the methodology suggested by Rolland et al. (2018) with modification in the centrifugation speed, 10,000 *g*. A total of 20 g of shrimp and crab meat samples that have been separated from the shell and bones were put into a blender, then added with 200 mL of phosphate buffer saline (PBS 0.1 M, pH 7.2). The mixture was then blended for 1 min and incubated in an a 300 mL-Erlenmeyer at 4 °C overnight. The solution was then centrifuged at 10,000 *g* at 4 °C for 30 min. The supernatant was taken, aliquoted and stored in the freezer at -20 °C until used.

Soluble Protein Analysis

A microtiter plate (microplate) was used to analyze the soluble protein content with Bradford assay (1976). About 5 µL of protein extract solution was taken and added with 250 µL Bradford reagent, then stirred using a vortex. The absorbance of the mixture was measured using a microplate absorbance reader (BIO-RAD, Hercules, CA, USA) at 595 nm. The standard used

was bovine serum albumin (BSA) solution ranged from 62.5 g/µL to 1500 g/µL.

Molecular Weight Analysis (SDS-PAGE Electrophoresis Method)

Molecular weight analysis using SDS-PAGE electrophoresis was performed following the Laemmli method (1970). This was using the Mini-PROTEAN Tetra Cell electrophoresis device (BIO-RAD, Hercules, CA, USA), with modifications on stacking gel of 5% and separating gel of 12%. One part of sample extract (approx. 1 µg/µL protein) was added to one part of Laemmli sample buffer (0.0658 M tris hydrochloric acid pH 6.8, 2.1% sodium deodecyl sulfate, 26.3% glycerol, 5% 2-mercaptoethanol and 0.01% bromophenol blue) and heated at 95 °C for 3 min. The sample was then pipetted into the wells, while a 10 µL standard protein marker Spectra Multicolor Broad Range Protein Ladder with the molecular weight of 10-260 kDa was placed in one of the wells (Thermo Scientific, Waltham, MA, USA, 26634). The electrophoresis process was carried out at 90 V for 90 min until the dye migration remained 0.5 cm from the base of the gel. It was continued with gel staining using Coomassie brilliant blue G-250 (Merck, Darmstadt, Germany) with a 1 mg/mL concentration for 30 min before rinsing with distilled water. The de-staining solution was added until a blue protein band appeared on the gel. The molecular weight of the sample was calculated by comparing it with protein markers, while the protein band intensity was calculated by measuring the area under the curve produced from gel analyzer software (Equation 1) as follow.

$$\text{Protein band intensity (area/µg) per µl samples} = \frac{\text{the area under the curve}}{\text{protein concentration (µg)}} \quad (1)$$

Allergen Protein Analysis (Immunoblotting Method)

The immunoblotting test was performed following the method from Bollag and Edelstein (1991). The electrophoretic gel without staining was transferred to a nitrocellulose membrane (0.45 µm) using a transblot device (BIO-RAD, Hercules, CA, USA). The blotting process was carried out for 90 min at 90 V. The transferred membrane was soaked with 50% methanol for 2 min, then blocked using 5% skim milk in phosphate-buffered saline containing 0.05% tween, pH 7.2 (PBST) for 1 h at room temperature. The membranes were washed with PBST three times. The mixture of human sera in PBST solution (1:10 ratio) with 10 mL was added to the membrane and incubated for 2 h at room temperature. PBST was then used for the rinsing process three times. HRP labeled mouse anti-human IgE monoclonal antibody (ICL Lab, ME-80P-24A) was diluted in PBST solution with a ratio of 1:3000 and incubated for 1 h. The membrane was then washed with PBST three times and added with DAB substrate ((3,3'-diaminobenzidine) until a protein band with a brown color complex appeared, which indicated a positive result.

Allergenicity Testing (ELISA Method)

Preparation of patient sera

This research has obtained ethical clearance from LPPM Ethics Commission, IPB University, with letter number 115/IT3.KEPMSM-IPB/SK/2018. Sera were taken from four respondents who were allergic to shellfish and one non-allergic as the negative control. Skin Prick Test (SPT) testing and blood collection were carried out at dr. Indrajana Allergy and Asthma Clinic - Tanah Abang, Jakarta (Indonesia), with the assistance from medical personnel. Prior to blood sampling, respondents underwent SPT on eight major allergenic foods to obtain confirmation of the type and level of allergy. The protein isolates used in the SPT test were protein from milk, soy, tuna, crab, shrimp, shellfish, and squid. The results of the SPT test were expressed by the bump size that appeared after the test. SPT value was determined relative to the diameter of positive control and negative control. SPT value was declared negative (-) if the diameter was the same as a negative control, while the value was declared +3 if the size was the same as the positive control. The maximum value of the SPT was +4, i.e., when the bump size was larger than the positive control. Each respondent has been asked for permission to donate 10 mL of blood with a consent form. The serum was then separated by centrifugation (Kubota KA-1000) at 2,610 *g* for 5 min. The supernatant obtained was serum which contained IgE. Serum samples were stored at -20 °C until the test period.

Analysis of total IgE serum

Total IgE serum analysis was performed according to Wijaya et al. (2015). A total of 100 µL serum was dissolved using carbonate-bicarbonate buffer (0.05 M pH 9.6) with a ratio of 1:10, then plated on a microtiter plate and incubated for 17 h at 4 °C. The plates were washed five times with rinse solution (PBST 250 µL/ well) in each step. The respondent of non-allergy sera was used as a control. Approximately 200 µL skim milk (5%) in PBST was added to each well and incubated for 1 h at 37 °C as blocking agent. 100 µL/ well HRP labeled mouse anti-human IgE monoclonal antibody in PBST (1:6000 in PBST) was added and

then incubated for 1 h at 37 °C. A total of 100 µL/well TMB substrate (3,3',5,5'-tetramethylbenzidine) was added. The formation of blue color indicated a positive result. After 5 min, the reaction was stopped by adding 100 µL/well of H_2SO_4 (2 M) so that the color changed to bright yellow. Optical density (OD) was measured using an ELISA reader (BIO-RAD, Hercules, CA, USA) at λ=450 nm.

IgE reactivity test

IgE reactivity test was referred to Wijaya et al. (2015). Sample protein (10 µg/mL) in carbonate-bicarbonate buffer (0.05 M, pH 9.6) was added to a microtiter plate (100 µL/well), then incubated overnight at 4 °C. The plates were washed five times with rinse solution (PBST 250 µL/well) each step. As much as 200 µL/well of 5% skim milk in PBST was added as a blocking agent and incubated for 1 h at 37 °C. As much as 100 µL/well of sera (1:10 in PBST) was added and incubated for 1 h at 37 °C. Blood serum of non-allergic respondents was used as a control. About 100 µL/well HRP labeled mouse anti-human IgE monoclonal antibody (1:6000 in PBST) was added and incubated for 1 h at 37 °C. A total of 100 µL/well TMB substrate was added. The formation of a blue color indicated a positive result. After 5 min, the reaction was stopped by adding 100 µL/well of H_2SO_4 (2 M) until the solution turned bright yellow. Optical density (OD) was measured using an ELISA reader (BIO-RAD, Hercules, CA, USA) at λ=450 nm.

Statistical Analysis

Data of proximate analysis, soluble protein concentration, and sample allergenicity with two replication of each treatment were statistically processed using a one-way analysis of variance (One-Way ANOVA) at a 95% confidence level (significant level 0.05). Duncan's test was carried out post-hoc if the analysis of variance yields substantial results. The software used was IBM SPSS Statistics 24. The results are reported as mean ± standard deviation (SD).

Results and Discussion

Nutritional Composition of Unprocessed and Processed Products

White shrimp (*L. vannamei*) and mud crab (*S. serrata*) are widely consumed protein sources, especially in Indonesia. Venugopal and Gopakumar (2017) mentioned that the protein content of shrimp and crab ranges from 17.00-21.00 and 15.00-18.40 g/100 g of material, respectively. This study also stated that the difference in the proximate content of crustaceans is strongly affected by environment, age, and type of feed. According to Lasekan et al. (2016), the boiling process of shrimp for at least 5 min has exceeded the minimum standard of cooking time per recommendations of the US Food and Drug Administration (FDA). Test results in this study showed that unprocessed mud crab had higher water content than white shrimp. The boiling and autoclaving processes were not significant in reducing the water content of white shrimp but were significant for mud crab. The frying process significantly water content more than boiling and autoclaving in white shrimp (p<0.05) but not significantly in mud crab. This is due to the condition where mud crab was fried without separating claws and legs meat from the shells, while the body only opened from the carapace shell.

The boiling and autoclaving in the current study significantly reduced the protein content of mud crab but insignificantly in the white shrimp. According to Venugopal and Gopakumar (2017), heat treatment processes such as boiling and steaming only affect the proximate composition of shellfish, including crustaceans. The deep-frying technique in this study reduced the protein content of white shrimp significantly to 49.00 ± 4.76%. This immense reduction is caused by protein denaturation and leaching out of the nitrogenous substance due to high temperatures during the frying process. The overall changes in proximate composition in the samples before and after treatments are shown in Table 1.

Changes in macronutrients also occurred in the fat contents of shrimp and crab. It is known that marine products are rich in essential fats, both saturated fatty acids (SFA) and unsaturated fatty acids (PUFA and MUFA). The boiling and autoclaving process reduced the percentage of white shrimp and mud crab fat contents, but it was not significant (p>0.05). Generally, high-temperature processing can damage volatile components, thereby causing a decrease in fat content. It will also oxidize fat components. Kong et al. (2007) stated that thermal processing, such as cooking, reduces the fat content because it is being leached out from the muscle due to myosin's denaturation and shrinkage of the myofibrils. In contrast, the fried shrimp fat content (based on dry basic) increased significantly to 26.82 ± 8.77%. The increase occurred because of oil absorption from the frying media during the process, followed by water release from the sample. However, the frying process reduced the fat content of unprocessed mud crab, equivalent to the decrease in the boiling and autoclaving processes. This happened because the frying process for crab meat was carried

Table 1. Nutritional value of unprocessed and processed white shrimp and mud crab

Sample	Processing treatment	Moisture content (%) (wb)	Ash content (%) (db)	Protein content (%) (db)	Fat content (%) (db)	Carbohydrate content (%) (db)
Shrimp	Unprocessed	76.78 ± 0.09^{b}	5.64 ± 0.11^{d}	81.04 ± 0.80^{b}	8.41 ± 0.01^{a}	4.91 ± 0.69^{a}
	Boiling	71.50 ± 0.52^{b}	3.04 ± 0.00^{b}	80.90 ± 1.71^{b}	3.36 ± 0.01^{a}	12.70 ± 1.73^{b}
	Frying	43.30 ± 5.97^{a}	4.22 ± 0.15^{c}	49.00 ± 4.76^{a}	26.82 ± 8.77^{b}	19.96 ± 3.85^{c}
	Autoclaving	71.42 ± 0.05^{b}	1.96 ± 0.05^{a}	74.91 ± 1.34^{b}	4.32 ± 0.54^{a}	18.80 ± 1.94^{bc}
Crab	Unprocessed	86.66 ± 0.83^{c}	16.32 ± 0.37^{d}	73.22± 0.68^{b}	10.00 ± 0.05^{b}	0.46 ± 0.36^{a}
	Boiling	71.46 ± 0.35^{a}	5.70 ± 0.21^{b}	71.75 ± 0.53^{ab}	4.04 ± 0.68^{a}	18.52 ± 0.36^{b}
	Frying	73.79 ± 0.20^{b}	5.08 ± 0.03^{a}	72.94± 0.76^{ab}	3.62 ± 1.69^{a}	18.36 ± 2.43^{b}
	Autoclaving	71.84 ± 0.08^{a}	7.95 ± 0.01^{c}	70.99 ± 1.01^{a}	4.85 ± 0.64^{a}	16.22 ± 1.66^{b}

Note: The difference in letters in the same column in each sample shows a significant difference at $p<0.05$. wb=wet bas; db=dry basis

out without opening and separating the shell, which led to oil penetration that was not too deep.

Soluble Protein Profile of the Unprocessed and Processed Product

According to Scopes (1994), the protein of marine products consists of 66 - 77% myofibril protein, 30% of sarcoplasmic protein, and the rest is stromal protein. Most allergens are found in the myofibril proteins. Myofibril proteins can be extracted using buffers with high ionic strength and high salt content (Nugraha et al., 2021). The extract obtained from the current research was dominated with myofibril protein. The extracted protein consisted of allergen and non-allergen proteins. Soluble protein concentration in unprocessed white shrimp and mud crab extract was 639.77 mg/g and 834.48 mg/g samples, respectively.

The results showed that the boiling process generally reduced more soluble protein content of shrimp and crab extracts rather than the autoclaving and frying processes. The highest reduction of dissolved protein content was obtained by boiling and frying white shrimp. This is in accordance with the research by Lasekan et al. (2016), which stated that the boiling process resulted in the highest reduction of soluble protein extracts in tiger shrimp (*P. monodon*) samples compared to other heat processing (i.e., roasting, steaming, and autoclaving). During processing, the decrease in protein solubility is due to muscle protein denaturation and protein aggregation resulting in insoluble aggregates. Meanwhile, the pressure-induced solubilization of myofibrillar proteins (Ma et al., 2011). Data on the soluble protein content of white shrimp and mud crab are presented in Table 2.

Protein Molecular Weight (MW) Profile based on SDS-PAGE Electrophoresis

The results of protein extract electrophoresis testing using SDS-PAGE can be seen in Figures 1 and 2. A total of 21 protein bands were detected in unprocessed white shrimp extract with molecular weights in the range of 17.3–188 kDa (Figure 1). Several thick protein bands with strong intensity could be clearly seen based on qualitative analysis with bands sizes of 20.4 kDa (255,300 area/µg protein), 22.4 kDa (18,200 area/ug protein), 33.6 kDa (79,600 area/µg protein), 36.2 kDa (83,900 area/µg protein), and 40.2 kDa (114,700 area/ µg protein). According to the data, the protein bands obtained were suspected to be myosin light chain protein (20.4 kDa), SCBP-sarcoplasmic calcium-binding protein (22.4 kDa), tropomyosin (33.6 kDa and 36.2 kDa), and arginine kinase (40.2 kDa). These results were in accordance to the allergen proteins in white shrimp as classified by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS 2021). Autoclaving removed all protein bands of white shrimp extract in the range of 1-260 kDa. Boiling and frying reduced the protein bands of white shrimp, from 21 to seven and six protein bands in the range of 3-41.1 kDa MW, respectively. This shows that the higher the temperature and pressure during the processing, the more protein bands were reduced. Changes in the intensity of white shrimp extract protein band (Table 3) showed that the boiling process could reduce the intensity of allergen protein arginine kinase (-36%) and myosin light chain (-18%), but increased the intensity of allergen protein tropomyosin (+528%). The frying process decreased the intensity of allergen protein arginine kinase (-46%) and myosin light chain (-54%), but increased the intensity of allergen tropomyosin (+554%). The missing protein bands may be degraded into smaller MW or aggregated into larger sizes. Fragmented protein smaller than the resolution limit of gel may travel fast through the gel and release into the buffer (Yadzir et

Table 2. Soluble protein concentration extract (mg/g sample) of unprocessed and processed product

Processing Treatment	White Shrimp	Mud Crab
Unprocessed	639.77 ± 0.57^{c}	834.48 ± 9.76^{b}
Boiled	31.34 ± 1.56^{a}	35.75 ± 1.34^{a}
Fried	28.43 ± 0.71^{a}	43.45 ± 1.06^{a}
Autoclaving	49.49 ± 1.64^{b}	51.45 ± 6.30^{a}

Note: *The difference in letters in the same column shows significant differences at $p<0.05$

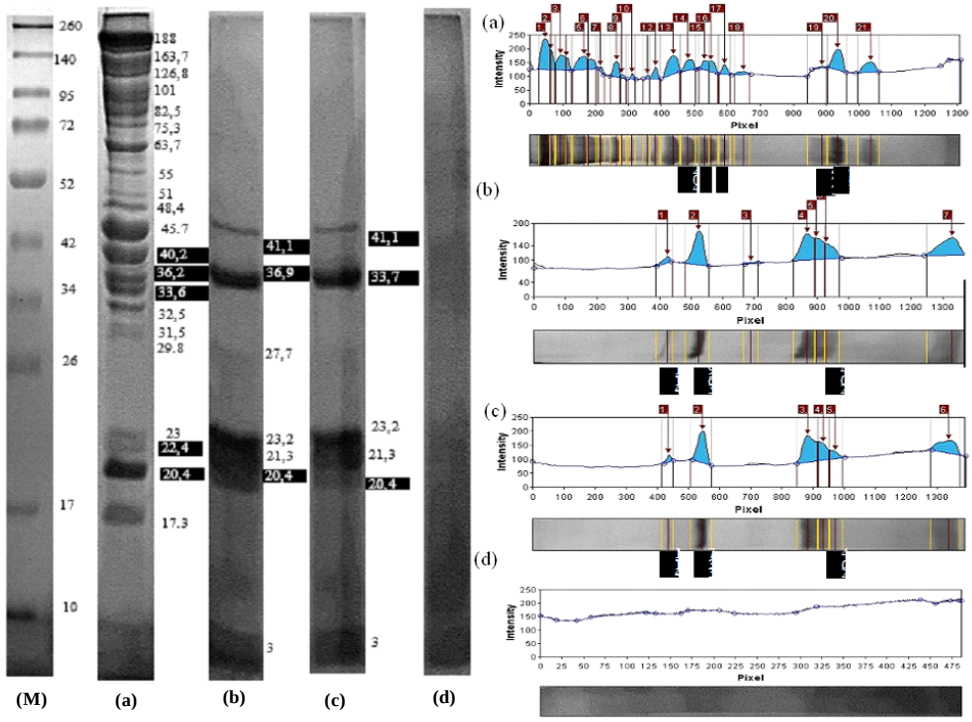

Figure 1. Profile of molecular weight and protein band intensity of white shrimp extracts (a) unprocessed, (b) boiled, (c) fried, (d) autoclaved compared with (M) marker. Molecular weight marked with a black square on SDS-PAGE results and an asterisk on gel analyzer results indicates allergen suspected protein bands.

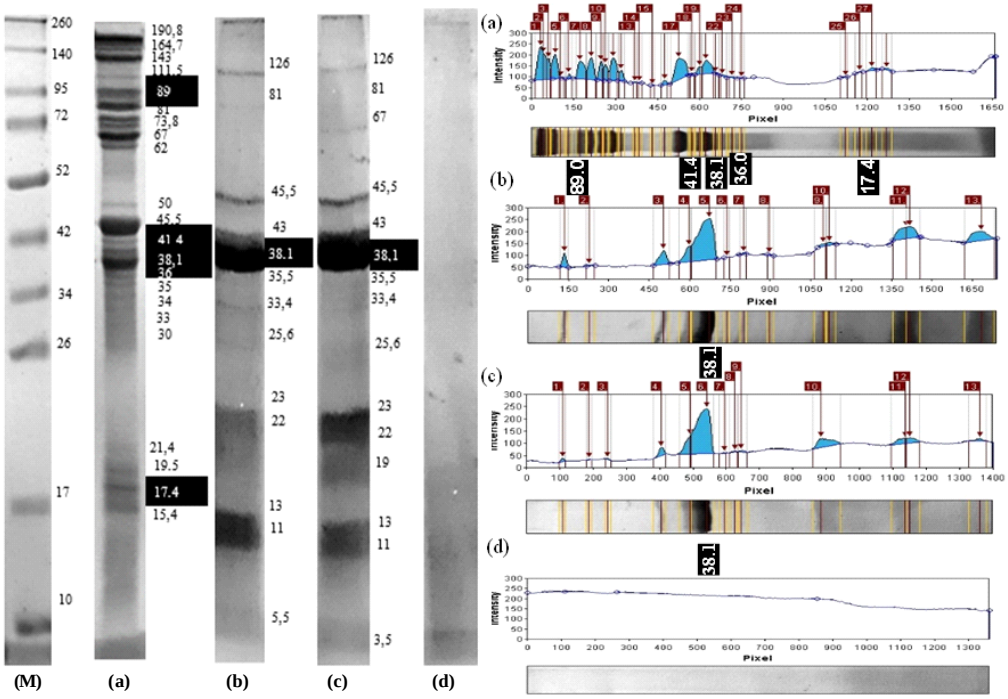

Figure 2. Profile of molecular weight and protein band intensity of mud crab extracts (a) unprocessed, (b) boiled, (c) fried, (d) autoclaved compared with (M) marker. Molecular weight marked with a black square on SDS-PAGE results and an asterisk on gel analyzer results indicates allergen suspected protein bands.

al., 2015). However, the main allergen in white shrimp, tropomyosin, with an MW of 35-38 kDa from the test results, persisted after boiling and frying processes and became stronger in intensity.

A total of 27 protein bands were detected in mud crab extract with an MW range of 15.4-190.8 kDa (Figure 2). Based on qualitative analysis of the band, several thick protein bands with strong intensity could

Table 3. The changes in suspected allergen protein bands intensity of white shrimp and mud crab

Allergen	White shrimp			Mud crab		
	Boiling	Frying	Autoclaving	Boiling	Frying	Autoclaving
Arginine kinase	-36%	-46%	-100%	346%	197%	-100%
Tropomyosin	528%	554%	-100%	529%	390%	-100%
Myosin light chain	-18%	-54%	-100%	-100%	-100%	-100%

Note: The number indicates the percentage change in band intensity compared to the unprocessed product. The sign (+) indicates an increase, while the sign (–) indicates a decrease in intensity.

be seen on the bar with sizes of 17.4 kDa (30,500 area/µg protein), 38 kDa (189,100 area/µg protein), 41.1 kDa (10,200 area/µg protein), and 89 kDa (22,600 area/µg protein). According to the data, the protein bands obtained in this study were suspected to be filamine C (89 kDa), arginine kinase (41.1 kDa), tropomyosin (38 kDa), and myosin light chain (17.4 kDa). Izzah et al. (2015) identified two major allergen proteins with MW of 36 kDa and 41 kDa and several minor allergens with MW of 25, 28, 65, 75, and 95 kDa in mud crab (*S. serrata*). There was also a reduction in the number and intensity of protein bands after boiling and frying mud crab. The process of boiling and frying reduced protein bands that previously amounted to 27 in unprocessed mud crab to 13 protein bands with MW of 5.5–126 kDa. The changes can be significantly seen in that protein band intensity of 41.1–43 kDa increased to 346% due to the boiling process and 197% due to the frying process. The intensity of the protein band of 38.1 kDa also increased significantly by several folds. Autoclaving process removed all protein bands of mud crab extract in 1-260 kDa. Yu et al. (2011) also stated that the autoclave process (pressure 0.14 MPa at 121 °C, for 20 min) was the best method in reducing the intensity of protein bands in crab (*S. paramamosain*) compared to the ordinary boiling process.

Overall, the higher the processing temperature and the greater the pressure applied, the more protein bands will be reduced. The reduced protein bands were most likely degraded into protein bands with smaller molecular weights (Kim et al., 2011). Some protein bands suspected to be allergen were presented/ interacted during the processing, while others were missing. The main allergen (suspected as tropomyosin) in white shrimp and mud crab with an MW of 35-38 kDa was still detected after the boiling and frying processes but disappeared after autoclaving. Tropomyosin is a heat-resistant allergen, and in fact, its intensity increases due to heating process (Kamath et al., 2013). Lasekan et al. (2016) also stated that heat processing (i.e., boiling, steaming, frying, microwave heating, and roasting) increased the intensity of the tropomyosin protein band.

Profile of Allergen Protein Band based on Immunoblotting Test

The results of the immunoblotting test using respondent's serum against protein extract of white shrimp and mud crab are presented in Figure 3. Reactive IgE was detected against five unprocessed white shrimp extract protein bands with MW of 189, 141, 118, 87, and 43 kDa. Only one protein band reactive to IgE was detected after the boiling and frying process with an MW of 43 kDa. This band protein was not shown in the electrophoresis result of fresh, boiled, and fried shrimp. But there were protein bands with an MW close to 43 kDa, which were 45.7 kDa in fresh shrimp extract and 41.1 kDa in both boiled and fried shrimp extracts. Protein bands with these molecular weights may experience a shift due to protein aggregation and degradation. The allergen protein band with MW 43 has also been previously reported by Akimoto et al. (2021) as allergen protein derived from white shrimp known as fructose 1,6-bisphosphate aldolase (FBPA), which is reactive to eight serums of the respondent with shrimp-FDEIA conditions.

The same allergen protein bands were also found in the immunoblotting results of unprocessed, boiled and fried mud crab extracts, along with two others. There were three protein bands reactive to IgE with MW 141 and 118 kDa. This allergen was also found in the electrophoresis result of boiled and fried mud crab extract, except in the fresh crab, where the band was overshadowed by smeared band protein with MW 45.5 kDa. A protein band with an MW of 43 kDa identified as arginine kinase was also reported from mud crab (*S. tranquebarica*) by Jasim et al. (2021) using MALDI-TOF spectrometry. No reactive protein band was seen from the extract of both samples after autoclaving processing. This confirms the results of SDS-PAGE that autoclaving likely degrades protein bands to a smaller size than one kDa.

Allergenicity Profile of White Shrimp and Mud Crab

The total IgE testing was carried out to verify that the serum from the respondent was reactive. According to the SPT result, the serum is declared positive and considered reactive if the absorbance value is greater than the mean absorbance value ±2 standard deviations of the negative control (Calenoff et al., 1993). Results of the respondent's serum test showed that the total IgE absorbance value of four respondents was positively reactive and could be used for specific IgE tests (Figure 4).

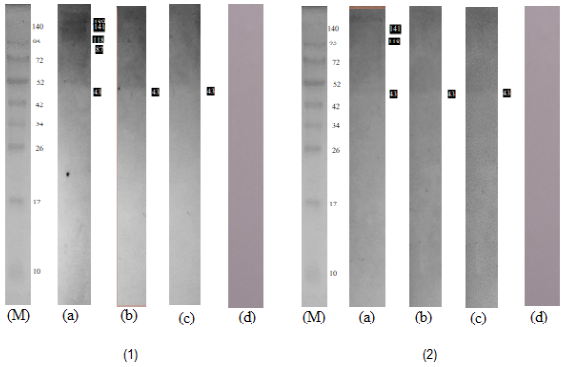

Figure 3. Immunoblotting result of white shrimp (1) and mud crab (2) (M) protein marker, (a) unprocessed, (b) boiled, (c) fried, and (d) autoclaved against serum of respondent 1. Figures in the image marked with a black square are allergen suspected protein bands.

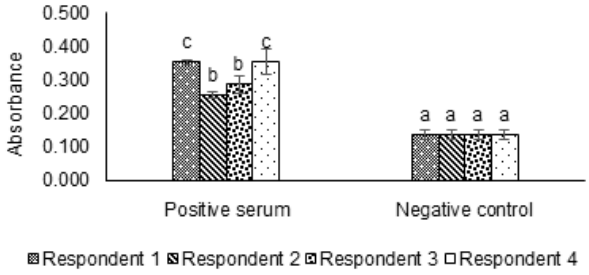

Figure 4. Total IgE reactivity to allergy sufferer respondent's serum.

Results of the allergenicity test for white shrimp and mud crab can be seen in Figure 5. It can be observed that the heat treatment process can reduce the allergenicity of white shrimp in respondents, both individually and on average. This is in line with the study by Liu et al. (2010), who also reported that the boiling process of white shrimp reduced IgE binding reactivity in a crude extract of boiled shrimp by 28% compared to unprocessed shrimp extract.

The decrease in IgE reactivity of boiled and fried white shrimp might be related to the disappearance of several reactive protein bands with MW of 189, 141, 118, 87 kDa, which could be seen in the immunoblotting result. In addition, there was a decrease in several protein bands intensity suspected to be allergen protein which is not reactive to respondent's sera with sizes of 40.2 kDa (-58%), 33.6 kDa (-100%), 20.4 kDa (-46%), and 17.3 kDa (-100%). A similar trend was also shown in the frying process, in which IgE reactivity decreased by 23%. There was also a decrease in the band intensity of allergen suspected protein with a size of 40.2 kDa (-62%), 36.2 kDa (-100%), 20.4 kDa (-67%), and 17.3 kDa (-100%) which were not reactive to respondent's sera. This speculates that boiling and frying might change the conformation of these potential allergen proteins and alter IgE binding epitopes. The test results also showed that a combination of high temperature and pressure with an autoclave is the most effective in reducing IgE reactivity compared to boiling and frying. The decrease in IgE reactivity by autoclaving reached 51% compared to the unprocessed sample. When compared to frying and boiling, autoclaving removes more protein bands. This result is in line with the results of Lasekan et al. (2016) that stated the intensity of tropomyosin band and myofibrial proteins in the extract of shrimp heated at a higher pressure in the autoclave was reduced compared to those in the raw extract. However, the loss of the protein bands due to autoclaving may not eliminate allergenicity of white shrimp extract completely due to the presence of reactive epitopes that have smaller molecular weight, resulting from the defragmentation of the protein bands. The results of other studies revealed that the allergenic reactivity of purified tropomyosin extract of white shrimp treated by boiling (Liu et al., 2010) and tiger prawn (*P. monodon*) extract (Lasekan et al., 2016) treated by both boiling and frying were increased. However, its IgE binding epitopes structure was not altered significantly. Kamath et al. (2013) also stated that the heating process in shellfish increased antibody reactivity of tropomyosin type allergen protein and caused changes in tropomyosin molecular weight compared to unprocessed products.

In contrast to shrimp extract, results from the ELISA test for mud crab protein extract showed that processing did not decrease serum IgE reactivity to mud crab protein extract and instead increased its reactivity. Compared to unprocessed extract, boiling and autoclaving treatments increased IgE reactivity by 4%. Meanwhile, frying treatment increased IgE reactivity by 23%. This was supported by the SDS-PAGE result, which showed an increase of the intensity of protein bands with MW of 38.1 kDa (suspected to be tropomyosin), 41.1 kDa (suspected to be arginine kinase), and the formation of a new protein band with a molecular weight of 23 kDa (suspected to be troponin). The highest band intensity was observed in the extracts treated by the frying process. The test results showed that frying significantly increased IgE reactivity to mud crab extract. This finding was supported by Rahman et al. (2011) which successfully identified allergen proteins with a size of 20-23 kDa in snow crab (*Chionoecetes opilio*). Meanwhile, the undetected of protein bands with a size of 1–260 kDa after the autoclave process may not necessarily eliminate the allergenicity of mud crab. It was possible that the remaining protein band fragments were small, undetectable from the SDS-PAGE assay, and were reactive to IgE. Our study results were different from

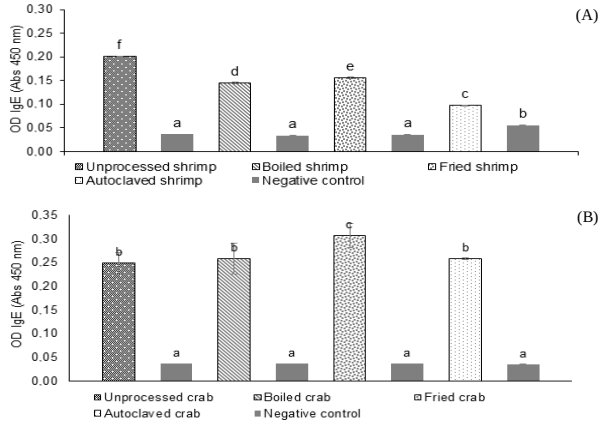

Figure 5. Analysis of specific IgE antibodies against protein extracts (A) white shrimp and (B) mud crab in various processes (RS = respondent). The different letters in each group of respondents showed a significant difference at p <0.05

Yu et al. (2011), who stated that autoclaving was able to increase the rate of tropomyosin degradation in digestive enzymes and significantly reduce its allergenicity. This occurred due to the extracts tested in this study were a whole extract, not a purified allergen (tropomyosin). The difference in IgE reactivity test results in the processing of mud crab and white shrimp is because the processing of mud crab did not separate the meat from the shells. This treatment might have resulted in a lower heat penetration into the crab meat than in the shrimp meat, leading to lower allergen protein denaturation. The crab processing used in this study was similar to the cooking process commonly performed by consumers/households.

Conclusion

The different heat processing (i.e., boiling, frying, and autoclaving) applied in the current study variably reduced the protein contents of white shrimp and mud crab and significantly reduced their protein solubility. Compared to boiling and frying, the autoclaving reduced all protein bands suspected as allergen proteins in both samples. Significantly, autoclaving was also more effective in reducing the allergenicity of IgE to white shrimp extract than boiling and frying. In contrast, the processing of mud crab insignificantly reduced the allergenicity of IgE in respondents' serum, except for the frying process. Thus, the autoclaving process can be used in the white shrimp processing technique for hypoallergenic food products.

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Supplementary Material

Supplementary material is not available for this article.

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