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Extraction of crab (*Scylla serrata*) myofibrillar and sarcoplasmic proteins to create a skin prick test reagent for crab allergy diagnosis

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Abstract

The major and minor allergens in fish and shellfish that have been identified are broadly spread in the myofibrillar and sarcoplasmic proteins. This study aimed to create a skin prick test reagent of crab myofibrillar and sarcoplasmic proteins to improve sensitivity and specificity diagnosis of allergy. Myofibrillar protein fraction was composed of 19 protein bands with the molecular weights of 9.6-111.6 kDa and sarcoplasmic protein fraction was composed of 19 protein bands with the molecular weights of 9.5-104.6 kDa. The allergenic myofibrillar protein has the molecular weights of 13.9-155.4 kDa while the allergenic sarcoplasmic protein has the molecular weights of 8.4-129.4 kDa. Crab myofibrillar and sarcoplasmic reagents met the requirements of the European Pharmacopoeia Monograph on Allergen Products 7 (2010:1063) for the parameters of moisture content, protein content, sterility and microbiology. A skin prick test to the respondents indicated that the sensitivity value of crab myofibrillar reagent was 85% and crab sarcoplasmic reagent was 69% with a negative error rate for each myofibrillar and sarcoplasmic reagents was 15% and 31%, respectively. The result diagnosis of crab myofibrillar and sarcoplasmic sensitivities would generate sensitivity value at 100%. The separation of crab proteins into myofibrils and sarcoplasm for skin prick test could improve its sensitivity value and lowered the negative misdiagnosis. The specificity of each myofibrillar and sarcoplasmic proteins was 100% with an error rate of positive diagnosis occurrence at 0%.

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Keyword: allergy diagnosis; crab; myofibrillar; sarcoplasmic; skin prick test.

Introduction

Crab (*Scylla serrata*) is a seafood favored by people in Indonesia and the rest of the world because of its nutritional content and flavor. In 2012, Indonesia produced 8.212 tons of crab, increasing its 2011 production volume by 22.19% [1]. For some people, consuming crab can

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generated a type allergic reaction mediated by IgE antibodies which can cause fatal effect. Allergic reactions are mainly triggered by allergen content in the crab. In fact, crab was ranked as top 7 as causes of food allergic reactions in the world [2] and the cause of most allergies of Indonesian adults [3].

The Skin Prick Test (SPT) is a reliable method for diagnosing IgE-mediated allergies in patients with rhino conjunctivitis, asthma, urticaria, anaphylaxis, atopic eczema, food and drug allergy [4]. The SPT technique was first published by Helmtraud Ebruster in 1959 and has been used as the primary diagnostic method for the detection of type I hypersensitivity reactions [5]. The main material used for the SPT is allergen extracts or SPT reagents. Allergen extract is a mixture of different variety of proteins, glycoproteins and polysaccharides. This extract should contain major and minor allergens since not all patients are allergic to each antigen in the extract. Although derived from the same allergen, SPT manufacturers produce different reagents quality and specifications, which may give different SPT results [6]. In addition, variations in the quality of SPT reagents are caused by different raw materials, extraction method, and formulation of SPT production [7]. Raw material factors, which could affect the quality of SPT reagents, are the origin and varieties of raw materials, transportation, food processing, and changes during storage [8]. Although derived from the same species, raw materials taken from different regions may have different allergen composition and reactivity against IgE [9]. Generally, PBS solution was used in the extraction method for animal-derived food such as eggs and marine products. Gautrin et al. [10] isolate the meat protein of snow crab using PBS solution (pH 7.2, 0.01mol/l). Similarly, Bernhisel-Broadbent et al. [11] extract various kinds of raw and processed marine products (such as catfish, tuna, salmon, trout, and codfish) using PBS solution. Meanwhile, Wittman et al. [12] using distilled water (pH 8) to extract protein from oyster, crab, and mussels.

Structurally, the protein constituent of fish and shellfish consist of sarcoplasmic, myofibrillar and stromal protein. Studies showed that the major and minor allergens in fish and shellfish are spread between myofibrillar and sarcoplasmic proteins. In crabs, major allergens listed in WHO/IUIS Allergen Nomenclature can be classified as myofibrillar protein group are Cha f 1, tropomyosin with molecular weight (MW) of 34 kDa and Por p 1, tropomyosin with MW of 39 kDa whereas the major allergen of sarcoplasmic protein group is phospholipase A1B with MW of 34 kDa [13].

The aim of this study is to create a skin prick test reagents by separating the myofibrillar and sarcoplasmic protein which is expected to improve the sensitivity and selectivity of the diagnosis, thus reducing the frequency of misdiagnosis. The quality requirements of the reagent refer to European Pharmacopoeia 7 Monograph on Allergen Products (2010:1063) [14]. Crabs used in this research are those consumed by people in Indonesia so that SPT reagent produced contains alergen

that causes allergy to crab in Indonesia. This will avoid miss-diagnosis and to improve the sensitivity and selectivity of the diagnosis. If using imported reagen, then the composition of its alergen might be different with local crab even though the type of the crabs are the same so that miss-diagnosis might occur.

Materials and Methods

Materials

The main material used in this study was fresh male crabs (*Scylla serrata*) obtained from the Muara Angke fish market, North Jakarta. The main chemicals used were BSA (bovine serum albumin), acrylamide, glycine, 2-Mercaptoethanol, 0.05 M carbonate-bicarbonate buffer at pH 9.6. coomasie brilliant blue R-250, coomasie brilliant blue G-250, IgE antibody, anti-IgE human antibody labeled with HRP (Horse radish Peroxidase) enzyme, DAB (3,3'-Diaminobenzidine) substrate, TMB (3,3',5,5'-Tetramethylbenzidine) substrate, N,N'-methylenebisacrylamide, low molecular weight proteins (LMW) Fermentas® (containing 7 types of protein, which are β -galactosidase standards (MW: 116 kDa), bovine serum albumin (MW: 66.2 kDa), ovalbumin (MW: 45 kDa), lactase dehydrogenase (MW: 35 kDa), REase BSP 981 (MW: 25 kDa), β -lactoglobulin (MW: 18.4 kDa), and lysozyme (MW: 14.4 kDa). Other chemicals were purchased from Sigma.

Equipments

The equipments used were high speed micro centrifuge, SDS-PAGE Bio-Rad Mini-Protean II tool, immunoblotting Mini Trans-Blot® Electrophoretic Transfer Cell Bio-Rad tool, Costar® 96-well ELISA microplates, Lab System Multiskan EX ELISA reader, UV-VIS spectrophotometer, freeze drier, nitrocellulose membranes for blotting pore size 0.45 μ m, size 15 cm x 15 cm (Sigma N8267), pH meter, sonicator, vortex mixer, stirrer, 0.5 μ l to 1000 μ l micropipettes, 0.2 μ m SFCA syringe filter, Whatman #1 filter paper, and other glass wares.

Extraction of Crab Myofibrillar and Sarcoplasmic Proteins

Weight 20 grams of cleaned crab meat was put into a blender, added with 200 mL of phosphate buffer (pH 7.5) with ionic strength (I) = 0.05 M (15.6 mM Na_2HPO_4 , 3.5 mM KH_2PO_4) then added with 0.5 mL aprotinin (protease inhibitor) and homogenized 3 x @ 1 minute. The formed mixture was centrifuged at 4 °C with 5000 $\times$ g speed for 25 min. The centrifuge result consisted of liquid at the top layer (supernatant) and pellet (solid) at the bottom layer. The supernatant was collected in a 50 mL centrifuge tube and the pellets remained in a centrifuge tube were re-added with 200 mL of phosphate buffer (pH 7.5, I = 0.05 M) to be re-extracted. The supernatant was then combined in a 50 mL centrifuge tube (here in after called the sarcoplasmic protein fraction), while the pellets were further processed for myofibrillar protein extraction. The

pellets were added with 200 mL of phosphate buffer (pH 7.5, I = 0.5 M; 0.45 M KCl, 15.6 mM Na_2HPO_4 , 3.5 mM KH_2PO_4) and homogenized 3 x @ 1 minute. The formed mixture was centrifuged at 4 °C with 5000×g speed for 25 min. The obtained supernatant was the myofibrillar protein extracts and the obtained pellets were re-extracted [15].

Serum Preparation

Serum collection was conducted in two steps. The first step was conducted toward 25 respondents who have food allergy (discovered from interviews). This serum was later used to conduct the allergenicity test of crab myofibrillar and sarcoplasmic proteins using ELISA and immunoblotting methods prior to the SPT. The second step of serum collection was conducted toward 13 respondents with crab allergy and 11 respondents without crab allergy which later used to conduct allergenicity test of SPT reagent. Second-step respondents attended SPT in allergy clinic. Serum collection and SPT were conducted by medical personnel and allergologist physician who have obtained permission from the Health Research Ethics Committee, Faculty of Medicine Indonesia University, Cipto Mangunkusumo Hospital with number of ethical approval is 199/H2.F1/ETIK/2014. 10 mL of each respondent's blood was withdrawn then placed in a bottle containing no EDTA. The withdrawn blood was allowed to stand for 1 hour, centrifuged for 20 minutes at 2500 rpm (1250 g). The obtained supernatant was a serum containing IgE antibodies and stored at -20 °C [16].

SDS-PAGE Electrophoresis and Immunoblotting

Electrophoresis was conducted according to Laemmli [17] with 12% concentration of separating gel and stained with coomassie brilliant blue R-250. The unstained gel from electrophoresis result was transferred to 0,45µm nitrocellulose membrane using transblotting equipment with 90 V for 90 minutes. After being transferred, the membrane was removed from equipment and soaked with 50% methanol for 2 minutes, then blocked with 5% skim milk in PBST (Phosphate Buffered Saline with 0.05 % Tween® 20) for 1 hour at room temperature. The membrane was washed with PBST 3 times, each for 5 minutes. After being washed, the membrane was added with the allergic patient serum with 1:10 dilution in PBST, incubated for 2 hours at room temperature. Washing was conducted again (3 times) with PBST, each for 5 minutes, then added with HRP conjugated monoclonal mouse anti-human IgE antibody (1:3000 dilution in PBST) and incubated for 1 hour while shaken. After that, the membrane was re-washed with PBST (3 times) each for 5 minutes, and added with DAB substrate. Positive detection results were marked with the formation of brown-colored band on nitrocellulose membrane [18].

Determination of Total IgE

Dilute 100 μ l serum/well with a 1:10 dilution (in 0.05 M carbonate-bicarbonate buffer at pH 9.6) was attached to the microtiter plates and incubated for 1 night at 4 °C. Normal human sera (NHS) were used as control. The remaining sample was removed and the microtiter plates were washed 5 times with PBST (250 μ l/well). After that, 200 μ l/well of 5% skim milk in PBST was added and incubated for 1 hour at 37 °C. Furthermore, it was washed 5 times with PBST (250 μ l/well) and added with 100 μ l/well of HRP conjugated monoclonal mouse anti-human IgE antibody (with 1:6000 dilution in PBST), then incubated at 37 °C for 1 hour. The microtiter plates were washed with PBST (250 μ l/well) for 10 times, then added with 100 μ l/well of TMB substrate. Positive results were marked when blue color developed. After 5 minutes, the reaction was stopped by adding 100 μ l/well of 2 M H₂SO₄, and the solution would turn bright yellow. Optical density (OD) was measured using ELISA reader at 450 nm. Mean $\pm$ 2SD of normal controls was taken as cut-off for ELISA positive results [16, 19].

Determination of Specific IgE

Dilute 100 μ l/well of each crab myofibrillar and sarcoplasmic proteins 10 μ g/mL (in 0.05 M carbonate-bicarbonate buffer, pH 9.6) was attached to the microtiter plates, then incubated at 4 °C for 1 night. The remaining sample was removed and the microtiter plates were washed for 5 times with PBST (250 μ l/well). After that, 200 μ l/well of 5% skim milk in PBST was added and incubated for 1 hour at 37 °C. Furthermore, it was washed 5 times with PBST. 100 μ l/well of allergic patient serum with 1:10 dilution in PBST was added and incubated for 1 hour at 37 °C. Normal human sera (NHS) were used as control. After incubation, the microtiter plates were washed with PBST (250 μ l/well) for 5 times, then added 100 μ l/well of HRP conjugated monoclonal mouse anti-human IgE antibody (diluted 1:6000 in PBST), then incubated for 1 hour at 37 °C. Microtiter plates were washed with PBST for 10 times, and 100 μ l/well of TMB substrate was added. Positive results were marked when blue color developed. After 5 minutes, the reaction was stopped by adding 100 μ l/well of 2 M H₂SO₄, and the solution would turn bright yellow. Optical density (OD) was measured using ELISA reader at 450 nm. Mean $\pm$ 2SD of normal controls was taken as cut-off for ELISA positive results [16, 19].

Creating Skin Prick Test Reagent

Each 0.2 g of myofibrillar and sarcoplasmic proteins was dissolved in 2 mL of phosphate buffer saline (PBS) at pH 7.4, sonicated 5 X @ 1 minute in cold condition and then centrifuged at 11780 g for 15 minutes. The obtained supernatant was filtered using 0.2 μ m syringe filter for sterilization in order to obtain a sterile protein stock solution. The procedure was conducted in a

sterile and aseptic room. The protein content of stock solution was determined using the Bradford method (Bradford, 1976). Stock solution was then dissolved in 50% glycerol-saline solution containing 0.4% sterile phenol, thus the concentration became 1 mg/ml. The obtained solution was then called “crab myofibrillar and sarcoplasmic SPT reagent” which then tested chemically, sterilitically and microbiologically. If it was qualified from chemical, sterility and microbiological tests, the reagent would be used for SPT in humans [20].

Skin Prick Test

The SPT was conducted by allergologist physician who has received permission from the Health Research Ethics Committee with number of ethical approval is 199/H2.F1/ETIK/2014. SPT was conducted on the volar forearm. The area that dropped with reagent was marked with a ballpoint and disinfected with alcohol. 1 mg/ml histamine was used as a positive control and 50% glycerol-saline solution was used as a negative control. Positive and negative controls were dropped on the opposite area then other reagents were dropped. Skin prick that has been dropped with histamine, control buffer, and allergen extracts using the brown marrow needle. The pricking process was conducted slowly with 45° slope the penetrated the epidermal layer without causing bleeding. The diameter of the wheal (urtica) on the skin was measured after 15-20 minutes by measuring the developed wheal (urtica). The test results were transferred to the millimeter block paper by making line surrounding the wheal border with marker pen (size 0.2), taped with masking tape and masking tape was affixed to the millimeter paper block. Each circle diameter on the tape was then measured. The result expressed as “0” when the wheal size is equal to the negative control (no wheal formed), “+1” if the wheal size is 25% -50% greater than the negative control (<3 mm), “+2” if the wheal size is 50%-75% greater than the negative control (3 mm-5 mm) and “+3” if the wheal size is equal to the histamine (5 mm-7 mm), and “+4” if the wheal size is 25%-50% greater than the histamine and “>+4” when the wheal size is more than 50% greater than the histamine. From the SPT results, sensitivity and selectivity of each reagent were measured [20].

Results and Discussion

Analysis of Total and Specific IgE Antibodies

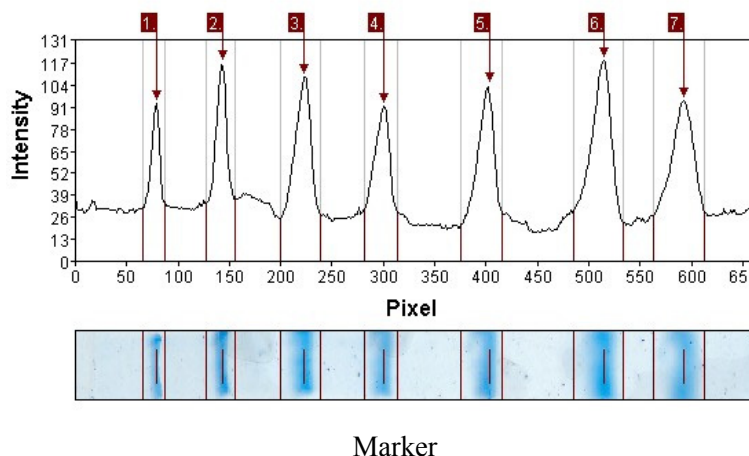
Total and specific IgE antibodies were analyzed from 25 respondents (respondent 1-25) who have allergy history based on interview results. Total IgE antibodies were analyzed to determine the positive or negative allergy status of respondents, while specific IgE antibodies were analyzed to determine the ability of myofibrillar and sarcoplasmic crab protein to bind to specific IgE antibodies in the serum and also to determine the positive or negative crab allergy of respondents. These analyses showed that all 25 respondents had positive allergy tests. Specific IgE analysis

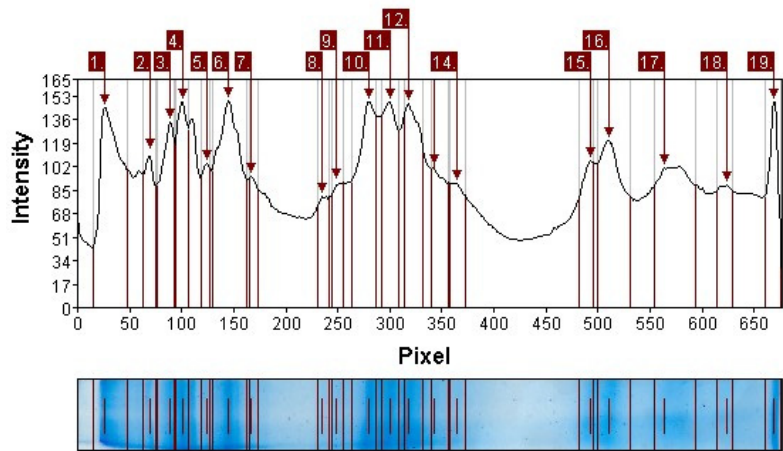
revealed that 11 respondents (44%) had positive myofibrils allergy, while 12 respondents (48%) had positive sarcoplasmic allergy. For respondents that had positive allergy on one of the proteins, sarcoplasmic or myofibrils, the diagnosis result was stated as positive allergy of crab, since they did not consume myofibrillar or sarcoplasmic proteins alone. The results showed that 14 respondents (56%) were diagnosed to have crab allergy. Serum that has high allergenicity was used to determine the allergens protein profile by immunoblotting.

Protein and Crab Myofibrils Allergens Profile

Figure 1 shows the protein profile of crab myofibrils by SDS-Page electrophoresis (A) and allergen protein profile by immunoblotting of respondent's serum who had allergy (B). Bands identification using Gel Analyzer 2010a software showed that the myofibrillar protein of crab is composed of 19 bands with different intensities. The molecular weight of identified myofibrillar proteins were 111.6, 94.6, 87.7, 83.7, 76.7, 71.0, 65.0, 50.2, 47.8, 42.3, 39.2, 36.5, 33.4, 30.6, 18.7, 17.6, 14.3, 11.4, and 9.6 kDa. The 111.6 kDa protein showed the highest intensity (16.9%), followed by the 65.0 kDa protein (15.1%) and the 14.3 kDa protein (14.3%). Comparing to the allergen data on WHO/IUIS (International Union of Immunological Societies), the protein in the band 11 was suspected as allergen proteins Por p1 [13].

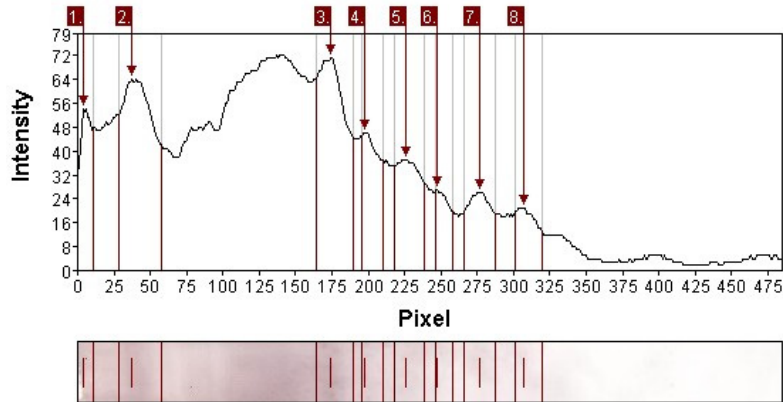
Figure 1B shows the allergen profile of crab myofibrils that can bind to specific IgE antibodies in four serums of respondents with high degree of allergenicity on crab. Allergen proteins that can bind to the specific IgE antibodies in the serum 12, 14, 16, and 24 are 8, 2, 11 and 8 bands, respectively.



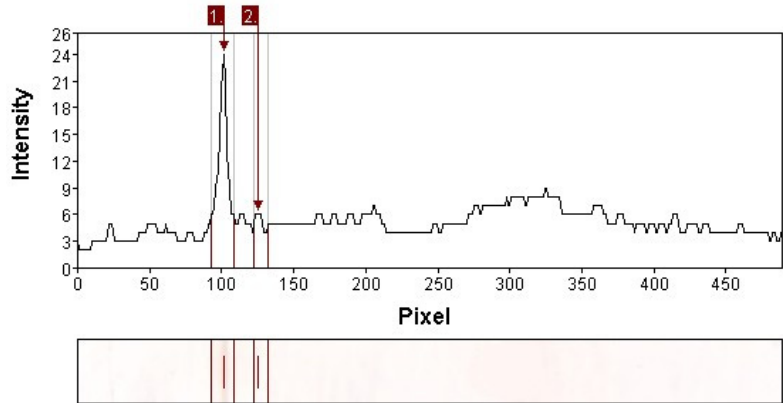


Myofibrillar Protein

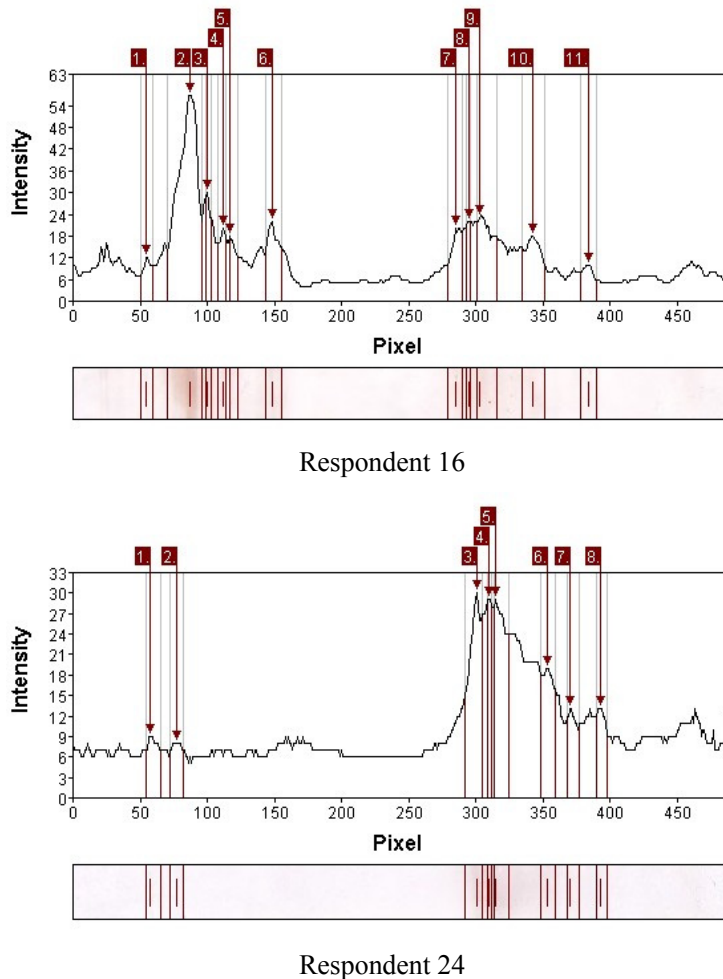
A. SDS-Page Electrophoresis results of crab myofibrillar protein



Respondent 12



Respondent 14



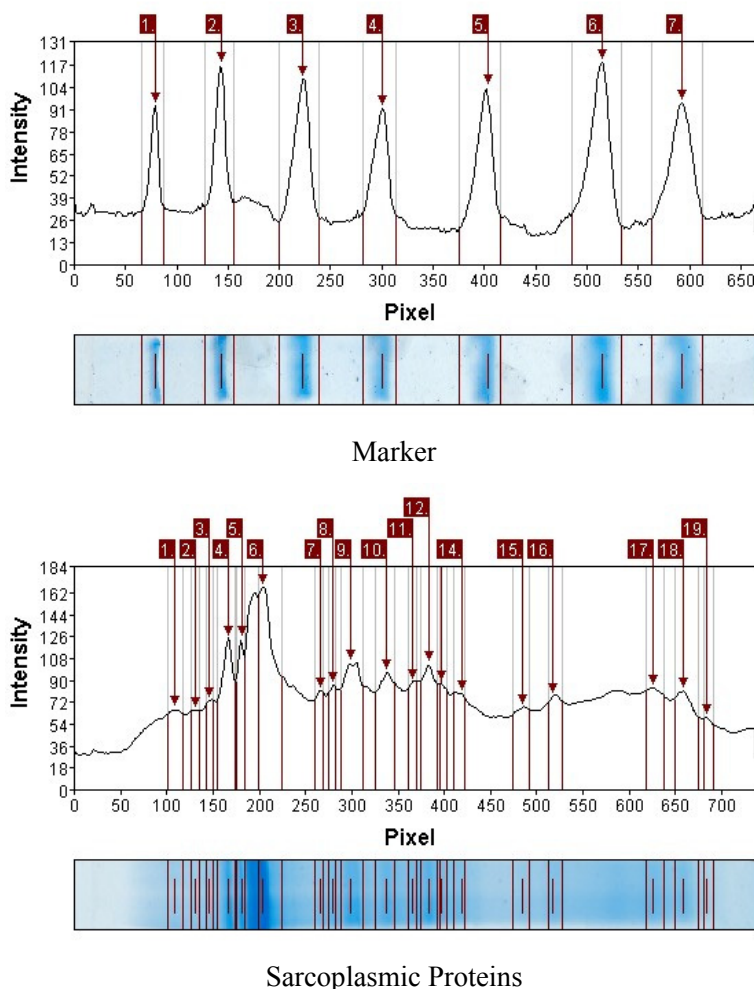
B. Immunoblotting result of crab myofibrillar protein

Fig. 1. SDS-Page Electrophoresis results (A) (marker and crab myofibrils) and immunoblotting (B) (Respondent 12, 14, 16, 24)

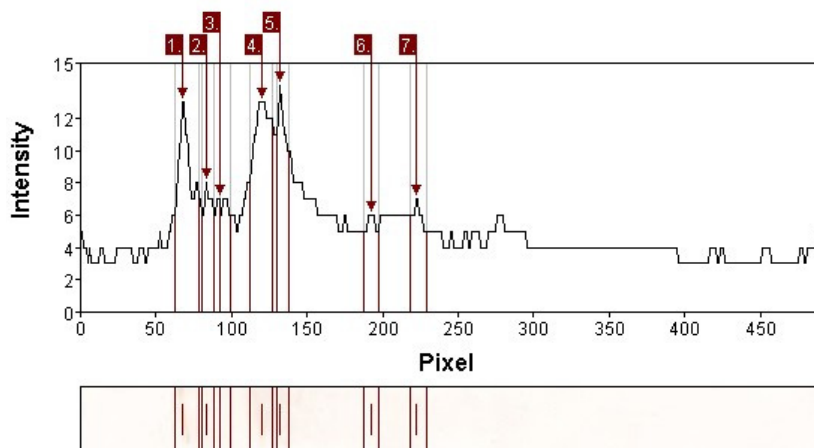
Myofibrils that can bind to specific IgE of the serum 12 were the 155.4 kDa, 126.3 kDa, 53.4 kDa, 46.0 kDa, 38.5 kDa, 33.8 kDa, 28.0 kDa, and 23.2 proteins. The specific IgE antibodies in the serum 14 that can bind to the crab's myofibrillar protein were the 84.3 kDa and 73.0 kDa, while to the serum 16 were the 113.9 kDa, 92.9 kDa, 85.6 kDa, 79.5 kDa, 77.1 kDa, 63.7 kDa, 27.2 kDa, 25.5 kDa, 24.3 kDa, 19.0 kDa and 14.7 kDa whereas to the serum 16 were the 111.9 kDa, 98.7 kDa, 24.6 kDa, 23.3 kDa, 22.5 kDa, 17.8 kDa, 16.0 kDa, and 13.9 kDa. The 5th band in respondent 12 is suspected to be the allergens proteins Por p1. Myofibrillar proteins that react positively was troponin-1 (MW 23 kDa), which reacted with serum 12 (the 8th band) and serum 24 (the 4th band). Troponin-C protein with a molecular weight of 18 kDa can bind to serum 24 in the 6th band 6. Paramiosin protein with a molecular weight of 111.9 kDa can bind to serum 24 in the 1st band.

Protein and Crab Sarcoplasmic Allergens Profile

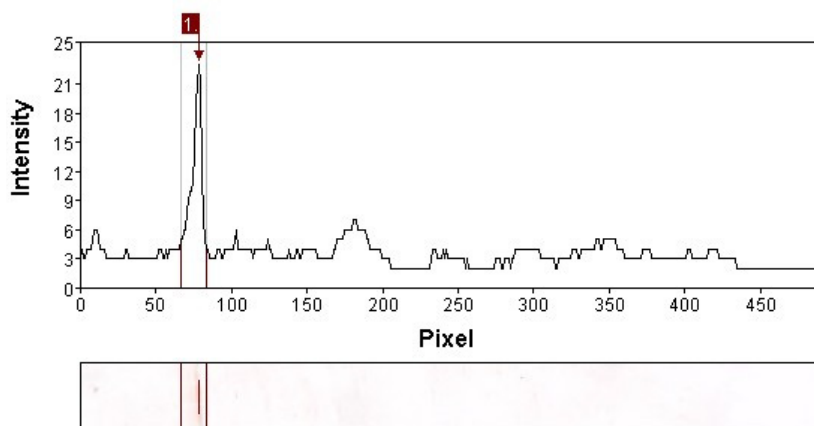
The detected sarcoplasmic proteins are 19 bands including the 104.6 kDa, 94.8 kDa, 89.5 kDa, 82.1 kDa, 77.0 kDa, 70.2 kDa, 54.1 kDa, 51.1 kDa, 47.3 Da, 40.1 kDa, 35.8 kDa, 33.3 kDa, 31.5 kDa, 28.6 kDa, 21.8 kDa, 19.0 kDa, 12.1 kDa, 10.5 kDa, and 9.5 kDa proteins (Fig. 2A). Protein with the highest intensity is the 6th band with MW of 70.2 kDa (36%) followed by the 4th band with MW of 82.1 kDa (13.5%) and the 9th band with MW of 47.3 kDa (10.9 %).



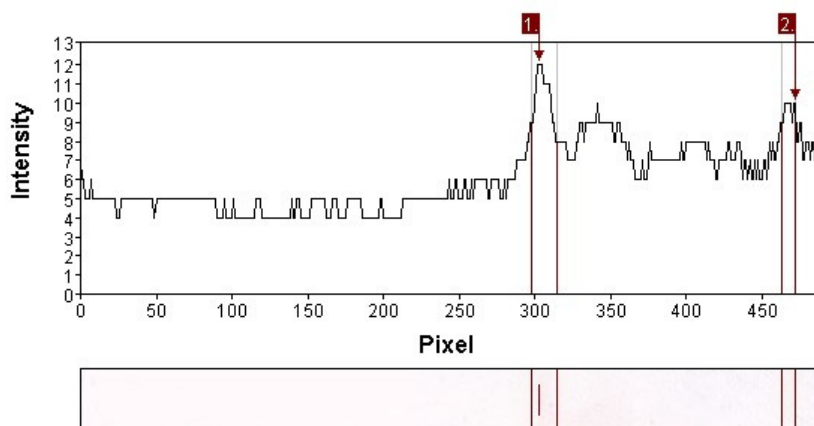
A. SDS-Page Electrophoresis results of crab sarcoplasmic proteins



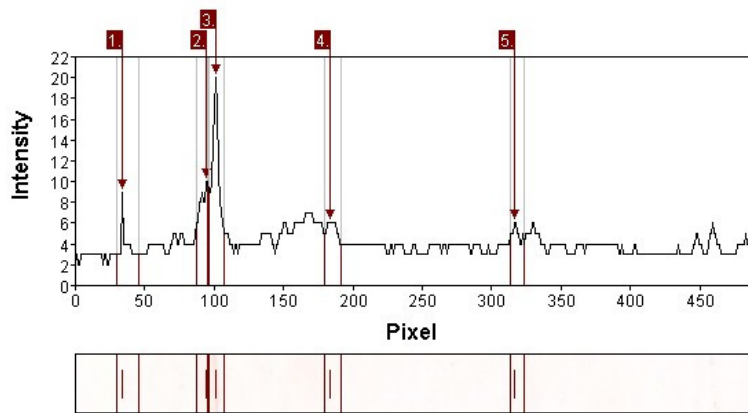
Respondent 12



Respondent 13



Responden 14



Responden 16

B. Immunoblotting result of crab sarcoplasmic protein

Fig. 2. SDS-Page Electrophoresis results (A) (marker and crab myofibrils) and immunoblotting (B) (Respondent 12, 13, 14 and 16)

Figure 2B shows the immunoblotting results of crab sarcoplasmic proteins using serum of respondents 12, 13, 14, and 16. The number of protein bands that can bind to specific IgE of respondent 12, 13, 14, and 16 are 7, 1, 2 and 5 bands, respectively. The sarcoplasmic proteins that can bind to specific IgE antibodies of serum 12 was the 105.4 kDa, 95.3 kDa, 90.2 kDa, 75.8 kDa, 70.4 kDa, 48.4 kDa, and 40.2 kDa proteins, while of serum 13 is the 98.3 kDa protein. Sarcoplasmic proteins that can bind to specific IgE antibodies of serum 14 was the 24.2 kDa and 8.4 kDa proteins, whereas of serum 16 was the 105.4 kDa, 95.3 kDa, 90.2 kDa, 75.8 kDa, 70.4 kDa, 48.4 kDa, and 40.2 kDa proteins.

Several seafood sarcoplasmic proteins which can cause allergy is the sarcoplasmic calcium binding protein with molecular weight of 20 kDa [21], hemocyanin with MW of 72 kDa [22], arginine kinase with MW of 42 kDa [23] and amylase [24]. Band with MW of 40 kDa reacted positively with the IgE of serum 1 and is thought to be the Scy s 2 [25].

Formulation and the Quality Requirement of Allergen Product for Skin Prick Test

Allergen products or SPT reagents made in this study is a product of glycerinated extract containing 50% glycerin. Figure 3 shows the sterile crab sarcoplasmic and myofibrils reagents for the skin prick test. Table 1 shows the requirements of the *European Pharmacopoeia 7 Monograph on Allergen Product (2010:1063)* and the analysis results of allergen product of crab myofibrils and sarcoplasm.



Fig. 3. Sterile crab myofibrils and sarcoplasm reagents for skin prick test

Results showed that the crab myofibrils and sarcoplasmic allergen product used for crab allergy diagnosis by skin prick test method has met the requirements of the *European Pharmacopoeia 7 Monograph on Allergen Products* (2010:1063).

Table 1. Requirements of European Pharmacopoeia 7 Monograph on Allergen Product (2010:1063) and the analysis results of allergen products of crab myofibrils and sarcoplasm.

No.	European Pharmacopoeia 7		Analysis Results	
	Parameters	Requirements	Myofibrils	Sarcoplasm
1	Moisture content (%)	Maximum 5% for freeze-dried products and can be more than 5% for liquid products	62,41 ± 0,07 (liquid products)	60,55 ± 0,06 (liquid products)
2	Protein content (µg/µl)	80-120% from the stated concentration (1 µg/µl)	0,85	0,99
3	Sterility	Sterile, if it is not sterile then refers to chapter 5.1.4. on European Pharmacopoeia 7 01/2011:50104	Sterile	Sterile
4	Total plate count*)	Max 10 ² CFU/g or CFU/mL	0	0
5	Staphylococcus aureus*)	Not detected in 1g or 1 mL	0	0
6	Pseudomonas aeruginosa*)	Not detected in 1g or 1 mL	0	0
7	Fungi*)	Max 10 ¹ CFU/g or	0	0
8	Yeast*)	CFU/mL	0	0

*) According to European Pharmacopoeia 7 01/2011:50104

Skin Prick Test Results

Table 2 shows that 13 respondents had allergy based on the test results of total IgE by ELISA method. Analysis of specific IgE of crab myofibrils shows that 11 respondents gave positive crab myofibrils allergic results and 9 respondents gave positive crab sarcoplasmic allergic results. Overall, all respondents are positively allergic to crab.

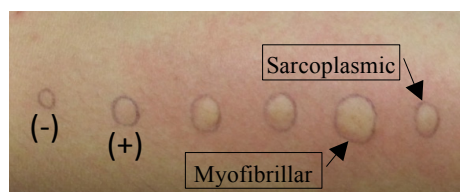


Fig. 4. The results of crab myofibrils and sarcoplasm skin prick tests on crab allergy respondents

The results of skin prick test allergy of respondents using crab myofibril and sarcoplasmic reagents are shown in Figure 4. The skin prick test results in Table 2 revealed variation between the myofibrillar and sarcoplasmic proteins. For example, respondent with code 9 shows negative allergy result to myofibrillar proteins (0) but shows positive allergy result (+1) to sarcoplasmic proteins. In contrast, respondent with code 36 shows very strong positive allergy result to myofibrillar proteins (>+4) but shows negative allergy result to sarcoplasmic proteins (0). There were 8 respondents (61.5%) which gave positive allergic results to myofibrils and sarcoplasmic proteins. Based on the skin prick test, all respondents in Table 2 had a crab allergy although some of them showed negative results on myofibrillar and sarcoplasmic proteins. The positive allergy status is given to a person who consumes crabs without separating the myofibrillar and sarcoplasmic proteins.

Table 2. Total IgE (tIgE), specific IgE (sIgE) and skin prick test result of crab myofibrils and sarcoplasm on the respondents with crab allergy

No	Resp. Code	tIgE Result	sIgE Result			SPT Result		
			Myofibrils	Sarcoplasm	Diagnosis result	Myofibrils	Sarcoplasm	Diagnosis result
1	2	+	+	-	+	+1	0	+
2	3	+	+	-	+	+3	+3	+
3	4	+	+	+	+	>+4	+4	+
4	9	+	-	+	+	0	+1	+
5	10	+	+	-	+	+2	0	+
6	11	+	+	+	+	0	+1	+
7	13	+	+	+	+	+2	0	+
8	19	+	+	+	+	>+4	+2	+
9	22	+	+	+	+	+4	+3	+
10	23	+	+	+	+	>+4	+3	+
11	28	+	+	+	+	+2	+2	+
12	30	+	+	+	+	>+4	+4	+
13	36	+	+	-	+	>+4	0	+

+: Positive, - and 0: Negative, Res. code: respondent code

Table 3. Total IgE (tIgE), specific IgE (sIgE) and skin prick test result of crab myofibrils and sarcoplasm on the respondents without crab allergy

No	Res. Code	tIgE Result	Myofibrils	sIgE Result		Diagnosis result	SPT Result		
				Sarcoplasm			Myofibrils	Sarcoplasm	Diagnosis result
1	1	+	-	-	-	-	0	0	-
2	7	-	-	-	-	-	0	0	-
3	8	+	-	-	-	-	0	0	-
4	12	+	-	-	-	-	0	0	-
5	14	+	-	-	-	-	0	0	-
6	16	+	-	-	-	-	0	0	-
7	18	+	-	-	-	-	0	0	-
8	26	-	-	-	-	-	0	0	-
9	27	-	-	-	-	-	0	0	-
10	29	-	-	-	-	-	0	0	-
11	34	+	-	-	-	-	0	0	-

+: Positive, - and 0: Negative, Resp. code: respondent code

Table 3 shows that the SPT results of all respondents who were not allergic to the proteins of myofibrillar and sarcoplasmic proteins is negative. It can be seen that although the respondent had allergy, based on total IgE, it was not necessarily a crab allergy. The specific IgE results of crab myofibrils and sarcoplasm by ELISA and SPT indicate that respondents are not allergic to crab. Respondent with code 12, for example, had allergy status but shows no crab allergy.

Table 4. Sensitivity and specificity of crab reagents for skin prick test

No	SPT Reagent	Allergy, Positive SPT, Sum (%) Sensitivity)	Allergy, Negative SPT, Sum (%) Negative Error)	Non-allergy, Positive SPT, Sum (%) Positive Error)	Non-allergy, Negative SPT, Sum (%) Specificity)
1	Myofibrils	11/13 (85)	2/13 (15)	0/11 (0)	11/11 (100)
2	Sarcoplasm	9/13 (69)	4/13 (31)	0/11 (0)	11/11 (100)
	Diagnosis result	13/13 (100)	0/13 (0)	0/11 (0)	11/11 (100)

Table 4 shows the sensitivity and specificity of the reagents for SPT test, which calculated based on SPT results in Table 2 and 3. The results indicate that the myofibrils reagent has higher sensitivity (85%) than the sarcoplasmic reagent (69%) with negative error rate for each reagent were 15% and 31%, respectively. If the sensitivity results of myofibrils and sarcoplasmic were combined, the sensitivity of crab protein is 100%. This indicates that the separation of crab proteins into sarcoplasmic and myofibrils for SPT may increase its sensitivity and reduce the occurrence of misdiagnosis. The myofibrils and sarcoplasmic proteins show the same specificity (100% each) with an error rate of 0% occurrence of a positive diagnosis.

Conclusion

Crab myofibrillar and sarcoplasmic proteins extracts may be used to create SPT reagent in accordance to the requirements of European Pharmacopoeia 7 Monograph on Allergen Products (2010:1063). The separation of crab proteins into myofibrils and sarcoplasm for SPT reagents could improve its sensitivity and specificity value and lowered the negative and positive misdiagnosis.

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