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Identification of tropomyosin and arginine kinase as major allergens of *Portunus pelagicus* (blue swimming crab)

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Abstract. Crab is an important source of food allergen. Tropomyosin represents the main crab allergen and is responsible for IgE cross-reactivity between various species of crustaceans. Recently, other new crab allergens including arginine kinase have been identified. However, information on allergens of the local Portunidcrab is not available. Thus, the aim of this study was to identify the major allergens of *Portunus pelagicus* (blue swimming crab) using the allergenomics approach. Raw and cooked extracts of the crab were prepared from the crab meat. Protein profile and IgE binding pattern were demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using sera from 30 patients with crab allergy. The major allergens of the crab were then identified by two-dimensional electrophoresis (2-DE), followed by mass spectrometry analysis of the peptide digests. The SDS-PAGE of raw extract revealed approximately 20 protein fractions over a wide molecular weight range, while cooked extract demonstrated fewer protein bands. The raw extract also demonstrated a higher number of IgE reactive bands than the cooked extract. A heat-resistant protein of 36 kDa has been identified as the major allergen in both raw and cooked extracts. In addition, a heat-sensitive protein of 41 kDa was also recognized as a major allergen in raw crab. The 2-DE gel profile of the raw extract demonstrated about >100 distinct proteins spots and immunoblotting of the 2-DE profile demonstrated at least 12 different major IgE reactive spots with molecular masses between 13 to 250 kDa and isoelectric point (pI) values ranging from 4.0 to 7.0. The 36 and 41 kDa proteins were identified as the crab tropomyosin and arginine kinase, respectively by mass spectrometry. Therefore, this study confirmed that tropomyosin and arginine kinase are the major allergens of the local Portunid crab, *P. pelagicus*.

INTRODUCTION

Crabs are common constituents in the diet of many populations worldwide. With a global annual production of over 1.3 million tonnes, crab is among the most popular crustacean food item (FAO, 2010). However, as one of the eight major sources of food allergens proposed by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), reports on allergic reactions induced by ingestion of shellfish including crabs are frequent (Huang *et al.*, 2010), especially in coastal areas where seafood is common in the diet (Liang *et al.*, 2008). In sensitized individuals, crab allergens can provoke allergic symptoms including urticaria, rhinitis, angioedema, diarrhoea, asthma and even life-threatening anaphylaxis (Liang *et al.*, 2008; Lopata & Lehrer, 2009). A recent study in Taipei indicated that crab is the most common food allergen among school children with the prevalence of 88% (Wan *et al.*, 2010). It has also been reported that allergy to crab affects up to 16.2% of adults in Singapore (Thong *et al.*, 2007). In Malaysia, our previous study indicated that about 44%

of patients with asthma and allergic rhinitis were sensitised to at least one shellfish including crab (Shahnaz *et al.*, 2001).

To date, there are a number of reports on identification of crab allergens at a molecular level (Motoyama *et al.*, 2007; Liang *et al.*, 2008). Leung *et al.* (1998) first reported a 34 kDa protein, designated as *Cha f* 1, as the major allergen of red crab (*Charybdis feriatus*) and identified as tropomyosin based on nucleotide and amino acid sequence comparison. Subsequently, tropomyosin has also been recognized as the major allergens in numerous species of crab including horsehair crab *Erimacrus isenbekii*, king crab *Paralithodes camtschaticus* (Motoyama *et al.*, 2007), Chinese mitten crab *Eriocheir sinensis* (Liang *et al.*, 2008; Liu *et al.*, 2010a), snow crab *Chionoecetes opilio* (Motoyama *et al.*, 2007; Abdel Rahman *et al.*, 2010b, 2010c), mud crab *Scylla serrata* (Huang *et al.*, 2010) and mud crab *Scylla paramamosain* (Yu *et al.*, 2011).

Besides tropomyosin, arginine kinase has also been reported as a new potential pan-allergen in mud crab *S. serrrata* (Shen *et al.*, 2011) and snow crab *C. opilio* (Abdel Rahman *et al.*, 2011). Recently four additional new allergens of snow crab were identified as sarcoplasmic calcium-binding protein (20 kDa), troponin (23 kDa), α-actine (42 kDa) and smooth endoplasmic reticulum Ca2+ATPase (113 kDa) (Abdel Rahman *et al.*, 2011).

The blue swimming crab or blue crab, *Portunus pelagicus* belongs to the family Portunidae and is adapted to a life in warmer waters. Therefore, this species is widely distributed throughout the coastal waters of the tropical regions of the western Indian Ocean and the Eastern Pacific (Svane & Hooper, 2004). *P. pelagicus* is an important commercial species with over 350,000 tonnes being caught annually (FAO, 2010).

As there is no information on the allergens of portunid crab, this study was conducted to identify the major allergens of *P. pelagicus* among our local patients with crab allergy using the allergenomics approach. Allergenomics is one of the functional proteomics strategies that focus on potential allergen discovery (Abdel Rahman *et al.*, 2012). In this study, the protein profiles of the crude extracts of raw and cooked crab samples were revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and 2-DE electrophoresis, followed by identification of the major allergens by immunoblotting of the SDS-PAGE and 2-DE gels using sera from patients with crab allergy. The major allergens were then excised, tryptic digested and characterized by mass spectrometry analysis.

MATERIAL AND METHODS

Preparation of crab extracts

Crab was purchased fresh from a fish market in Port Klang, Selangor. The raw and boiled extracts were prepared by a minor modification of a protocol as described by Rosmilah *et al.* (2008). Briefly, the crab meat was minced, homogenized in phosphate buffer saline (PBS, pH 7.2) using a waring blender, and extracted overnight at 4°C under a constant mixing (50 rpm). After centrifugation (14,000 rpm for 30 minutes at 4°C), the supernatant was sterile-filtered, freeze-dried and then stored at -20°C. For preparation of the cooked extract, the homogenates were boiled for 15 minutes before extracted as described above. Determination of the protein concentration was performed using the Folin and Ciocalteu's phenol reagent kit (Sigma, USA).

Human sera

In this study, serum samples were collected from 30 patients with history of crab allergy and have positive skin prick test (SPT) to raw extract of blue crab. The SPT was performed by a medical officer. In addition, the patients sera were also tested for the presence of specific IgE test against crab allergen, f23 (classes 2 to 5) using a Phadia System (Phadia, Sweden). All sera were stored at -80°C until used. This study was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia.

Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE method was a modification of the procedure of Rosmilah *et al.* (2008). Briefly the crab extracts were heated at 97°C for 4 minutes in a denaturing Laemmli buffer before loading them onto lanes of a 12.5% separating gel and a 5% stacking gel and were run using a Mini Protean 3 System for 45 minutes at 120 mA and 200 V (Biorad, USA). Precision Plus Protein Dual Color Standard (Biorad, USA) was run as a reference, along with samples. After running, protein components were visualized by staining with Coomassie Brilliant Blue R-250. Molecular weight of the protein components were then estimated by comparing to the molecular weight markers using an imaging densitometer and Quantity One Software (Biorad, USA).

Immunoblotting

The immunoblotting procedure was carried out as described in our previous study (Rosmilah *et al.*, 2008) using sera from 30 patients as described above. Briefly, following SDS-PAGE, the unstained protein fractions of the extracts were electro-transferred from the gel to a 0.45µm nitrocellulose membrane, washed with tris-buffered saline (TBS) containing 0.05% Tween 20 (TTBS), blocked with blocking buffer containing 10% non-fat milk in TBS and probed with patients' sera as the primary antibody overnight at 4°C. After washing, the membrane was then probed with biotinylated goat-antihuman IgE (Kirkergaard and Perry Laboratories, UK) as a secondary antibody. Immunodetection was carried out by incubating the strips in streptavidin-conjugated alkaline phosphatase (BioRad, USA), followed by incubation in Alkaline Phosphatase Conjugate Substrate Kit (BioRad, USA). Serum from a non-allergic individual was used as a negative control. A strip without sample was used as a blank in this experiment.

2-Dimensional Electrophoresis (2-DE) and immunoblotting

For 2-DE gel electrophoresis, the lyophilized raw crab extract was resuspended in rehydration buffer. Fifty µg of protein extract was applied to 7 cm of immobilized pH 3-10 non-linear gradient strip (BioRad, USA) for rehydration overnight and focusing using IEF cell (BioRad, USA). The first dimensional electrophoresis was performed to separate the proteins by charge with 4 steps: 100 V for 1 minute, 250 V for 30 minutes, 4000 V for 2 hours and 4000 V for 10000 V-hr (Vhour). The strips were fractionated by molecular weight using 12% of separating gels of SDS-PAGE with 5% of stacking gels. Protein spots profile was visualized with the use of Coomassie brilliant blue R250. The major IgE-reactive protein spots of the crab were identified by 2-DE immunoblotting using sera of 10 subjects known to have specific IgE to crab inimmunoblotting as above.

Liquid Chromatography-ESI Mass Spectrometry (MS & MS/MS) and data analysis

The mass spectrometry analysis was conducted at Adelaide Proteomics Centre, Australia. Briefly, the Coomassie-stained protein spots corresponding to those recognized by the above sera were manually excised. The gel pieces were then rehydrated in 50 mM ammonium bicarbonate, diced to ~1mm^3 cubes, destained and digested with 100 ng of trypsin per sample according to the laboratory's Standard Operating Procedure 07. After concentration of the extracted peptides, they were diluted to approximately 10 µL with 3% acetonitrile (ACN)/0.1% formic acid (FA). Approximately 30% of each sample was chromatographed using an Agilent Protein ID Chip column assembly(40 nL trap column with 0.075 x 43 mm C-18 analytical column) housed in an Agilent HPLC-Chip Cube Interface connected to a HCT ultra 3D-Ion-Trap mass spectrometer (BrukerDaltonik GmbH). The column was equilibrated with 4% ACN/0.1% FA at 0.5 µL/min and the samples eluted with an ACN gradient (4-31% in 32 min). Ionizable species ($300 < m/z < 1,300$) were trapped and one or two the most intense ions eluting at the time were fragmented by collision-induced dissociation (CID). MS and all fragment ion (MS/MS) spectra were subjected to peak detection using Data Analysis (BrukerDaltonik GmbH)

then imported into BioTools (BrukerDaltonik GmbH). Here, the MS/MS spectra were finally submitted to the in-house Mascot database-searching engine (Matrix Science) against the proteome database of the Kingdom Metazoa. The search specifications were as follows: database: NCBI non-redundant 20090320 or 20090430; enzyme: trypsin; fixed modifications: carbaminodomethyl (C); variable modifications: oxidation (M); Mass tol MS: 0.3 Da; MS/MS tol: 0.4 Da; peptide charge: 1+, 2+ and 3+; and missed cleavages: 1.

RESULTS

Crab protein profiles

The SDS-PAGE gel of the crab extracts is shown in Figure 1. Overall, more protein bands were observed in the raw extract compared to the cooked extract. The raw extract exhibited 20 protein bands in the range of 13 to 250kDa. Several protein bands between 25 to 30 kDa and 41 to 250 kDa which were present in the raw extract were not detected in the cooked extract, and therefore were identified as heat-sensitive proteins. However, the intensity of low molecular weight protein bands (between 18 to 23 kDa) was increased in the cooked extract. Both raw and cooked extracts afforded a prominent heat-stabile protein band of about 36 kDa at almost the same position as crab tropomyosin.

IgE-binding proteins

Immunoblotting reactivity to both raw and cooked extracts was observed in all patients' sera and the negative control serum from a normal individual showed negative result (Figure 1 and Table 1). Multiple IgE-binding proteins were detected in both extracts in the molecular weight range of 18 to 250 kDa. All the patients had individual IgE-binding pattern. In general, more IgE-binding bands were detected in the immunoblotting of raw extract than in the cooked extract. Immunoblotting of both raw and cooked extracts identified a 36 kDa heat-resistant protein as the major allergen of blue crab with the frequencies of 90% in both extracts. However, in the immunoblotting of the cooked extract, more intense staining to this band was observed in most sera tested. Several sera (No. 3, 7, 9, 17, 20, 29 and 30)

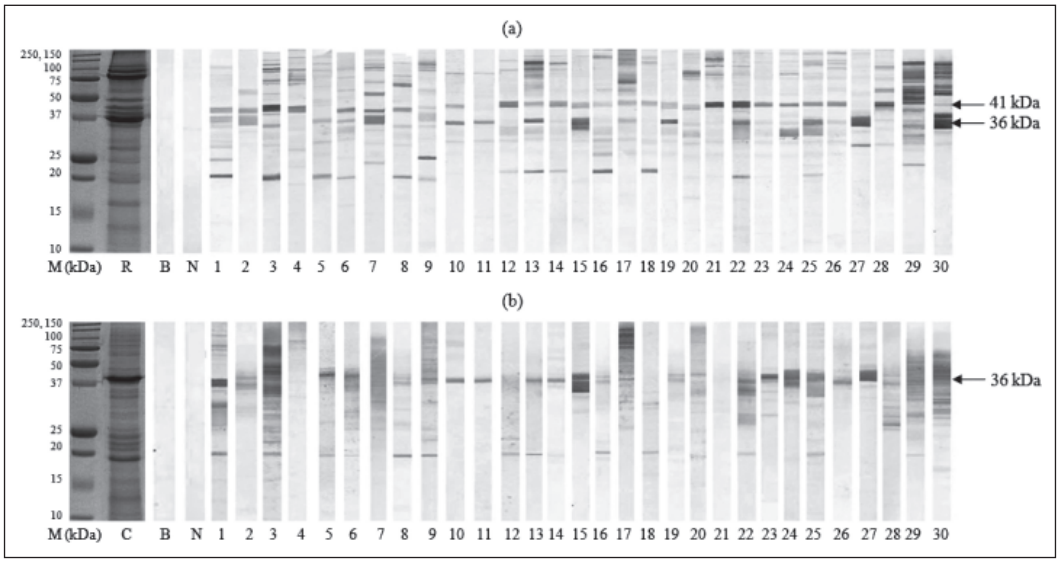

Figure 1. SDS-PAGE and immunoblotting profiles of raw (a) and cooked (b) extracts of blue crab. R and C are SDS-PAGE profiles of raw and cooked extracts, respectively. Lane 1 through 30 represents immunoblotting results of sera from 30 patients with blue crab allergy. Lane B and N represent a blank and a negative control of a normal serum, respectively. Lane M indicates molecular weight markers in kiloDalton (kDa)

Table 1. Immunoblotting results of raw crab (a) and cooked crab (b) extracts using 30 sera of patients with allergy to blue crab

(a)

M (kDa)	Subjects 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	%
18	▲		▲		▲	▲		▲	▲			▲	▲	▲		▲		▲				▲			▲						43
23	▲						▲		▲							▲													▲	▲	20
30	▲				▲											▲	▲			▲		▲	▲				▲	▲			30
36	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲		▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲	90#
41	▲	▲	▲	▲		▲	▲	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲		▲	▲	▲	90#
50		▲	▲				▲	▲	▲				▲				▲									▲		▲	▲	▲	36
65			▲										▲				▲									▲			▲	▲	20
75			▲	▲			▲	▲												▲	▲								▲	▲	26
95			▲	▲			▲		▲				▲				▲				▲	▲						▲	▲	▲	36
150	▲		▲				▲		▲				▲				▲				▲				▲	▲			▲	▲	36
250														▲			▲							▲	▲	▲					16

(b)

M (kDa)	Subjects 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	%
18	▲		▲		▲	▲		▲	▲			▲	▲	▲		▲		▲				▲			▲						43
23	▲																														20
30			□				□		□								□			□		▲	▲					▲	□	□	10
36	▲	▲	□		▲	▲	□	▲	□	▲	▲	▲	▲	▲	▲	▲	□		▲	□		▲	▲	▲	▲	▲	▲	▲	□	□	90#
41			□				□		□								□			□									□	□	23
50			□				□		□								□			□									□	□	23
65			□				□		□								□			□									□	□	23
75			□				□		□								□			□									□	□	23
95			□				□		□								□			□									□	□	23
150			□				□		□								□			□									□	□	23
250			□						□								□			□											13

M = molecular weight in kiloDalton (kDa), ▲ = IgE-binding protein, # =Major allergen, □ = Smear region

also demonstrated a smearing pattern without clearly defined band in the molecular weight region of 30 to 250 kDa of the cooked extract. In addition, a heat-sensitive protein of 41 kDa has also been recognized as a major allergen in raw extract. Moreover, a heat-resistant protein at18 kDa was detected as a potential minor allergen (frequency of 43%) in both raw and cooked extracts. Other heat-sensitive proteins of 30, 50, 75 and 95 and 150 kDa were also recognized as potential minor allergens (frequencies of more than 25%) only in raw extract.

2-DE profiles and IgE-binding spots

Figure 2 (a) shows a 2-dimensional map of the blue crab proteins. About 100 protein spots of molecular masses between 13 to 250 kDa and pI of 4.0 to 7.0 were visible with coomassie blue staining. The 2-DE IgE immunoblots using 10 different sera demonstrated that each subject had an individual IgE binding pattern with 12 to 40 different allergenic spots and 8 of these immunoblot results are shown in Figure 2(b). The most abundant IgE-binding spots of blue crab major allergens were labeled as spot number 1 (36 kDa) and 2 (41 kDa). These spots were selected for mass spectrometry analysis. No IgE-binding spots were detected by immunoblotting using a control serum from a non-allergic subject (result not shown).

Allergen identification

Two coomassie-stained protein spots (spot No. 1 and 2) were analyzed after tryptic

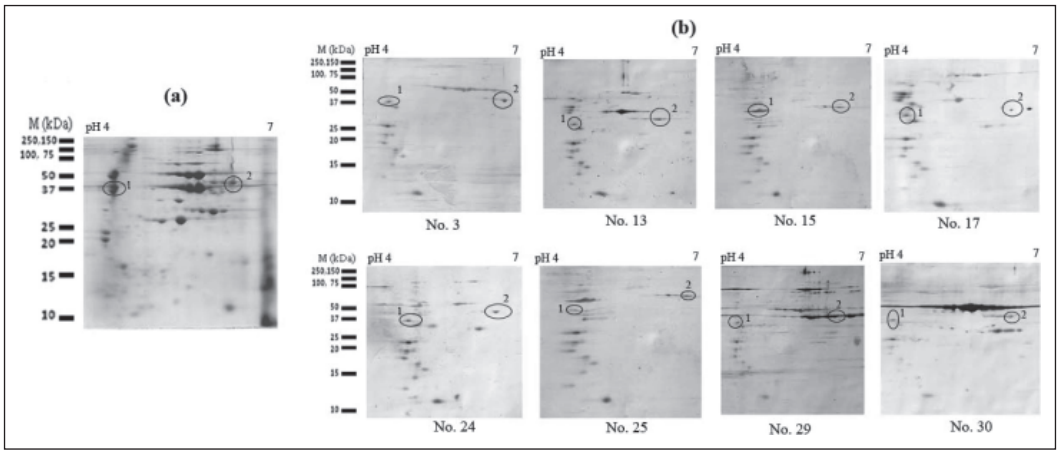

Figure 2. Two-dimensional electrophoresis (2-DE) and immunoblot analysis of 2-DE profile of raw extract of blue crab. (a) Coomassie blue stained of 2-DE profile. (b) Immunoblot with individual patients' sera. The *circle* represents the protein spot send for mass-spectrometry analysis. M, molecular mass markers in kiloDalton (kDa)

Table 2. Combined MS and MS/MS MASCOT search results summary of blue crab spots (spot 1 and 2)

Spot	Spot origin (species)	Observed MW(kDa)	Observed pI	Predicted MW (kDa)	Predicted pI	Accession no.	Combined Ion Score (threshold score/cut)	Protein Identification	Organism
1	*Portunus pelagicus* (blue crab)	36	4.7	32.8	4.71	*gi*|119674937	776/39	Allergen tropomyosin	*Portunus sanguinolentus* (Portunidae)
2	*Portunus pelagicus* (blue crab)	41	7.0	40.6	6.19	*gi*|25453074	1461/40	Arginine kinase	*Callinectes sapidus* (Portunidae)

digestion by mass spectrometry. The MS/MS spectra matched significantly with known crab proteins in the databases. The protein identities of the matched proteins are summarized in Table 2. Tropomyosin was clearly identified in spot No. 1, while in spot No. 2, arginine kinase has been strongly identified to be the major component of the spot. The observed MW and pI values of spot No. 1 and No. 2 were almost consistent with the predicted values for the protein from closely related species, *Portunus sanguinolentus* and *Callinectes sapidus*, respectively.

DISCUSSION

On a global basis, crab is a common cause of seafood allergy (Leung *et al.*, 1998; Lehrer *et al.*, 2003). The crab species of *Portunus pelagicus* or blue swimming crab is commonly consumed in our region.

Crustaceans generally contain a number of thermostable and heat-sensitive proteins (Herrero *et al.*, 2001; Samson *et al.*, 2004; Gill *et al.*, 2009). Similarly, as shown in SDS-PAGE gels, blue crab contains various heat-stabile and heat-labile proteins ranging in molecular weight from 13 to 250 kDa. At least 20 protein bands were identified in the raw extract while the cooked extract revealed smaller number of bands as most proteins with a molecular weight greater than 41 kDa and several proteins between 25 to 30 kDa were found to be heat-labile and thus were not detected in the gel. The disappearance of these bands might be related to the effects of heat on protein structure, since heat denaturation of globular proteins may disrupt their tertiary structure and lead to random-coiled aggregation and insolubility (Peng & Gygi, 2001). Therefore, alteration in protein structure by food processing can lead to epitope destruction, modification, masking, or unmasking thereby decreasing, increasing

or having no effect on allergenicity (Sathe *et al.*, 2005).

In accordance to the SDS-PAGE results, immunoblotting experiments also revealed that the raw crab extract had higher IgE-binding than the cooked extract, similar as reported in other food allergens (Bestler *et al.*, 2001; Samson *et al.*, 2004; Sathe *et al.*, 2005). Moreover, several studies proved that crustaceans are allergenic in both raw and cooked states. This is a significant finding as crustaceans are commonly consumed in both forms (Besler *et al.*, 2001; Samson *et al.*, 2004; Sathe *et al.*, 2005). This was also seen in our study as all sera demonstrated IgE-reactivity to both raw and cooked extracts, but with different IgE-binding patterns and profiles. These findings suggest that patients did not react to the same IgE-binding epitopes. It was reported that the difference in IgE-binding pattern may reflect different symptom patterns of allergy (Lucas *et al.*, 2003; Gill *et al.*, 2009). IgE epitope mapping is currently suggested to have the potential to become an additional tool for the diagnosis or prognosis of food allergy (Lin & Sampson, 2009).

In immunoblotting of raw extract, the majority of patients (90%) had IgE-binding to a prominent band of 36 kDa and a 41 kDa protein. The 36 kDa band was also identified as the major allergen in cooked extract, suggesting that this protein was resistant to heat denaturation. As expected, the digested peptide fragments isolated from this band were significantly identical to crab tropomyosin. To date, several crab tropomyosin including *Cha f* 1 (Leung *et al.*, 1998) *Eri s* 1 (Liang *et al.*, 2008), *Chi o* 1 (Abdel Rahman *et al.*, 2010b) and *Scy s* 1 (Huang *et al.*, 2010) have been well-characterized as major crab allergens at molecular level. This protein is a regulatory protein belonging to a family of highly conserved proteins with multiple isoforms found in both muscle and non-muscle cells of all animals, with a high degree of amino acid sequence identity among different species (Reese *et al.*, 1999; Santos *et al.*, 1999; Lehrer *et al.*, 2003). Presently, tropomyosin with a molecular mass of 34 to 38 kDa has been widely identified as a major and cross-reactive pan-allergen in various species of invertebrates including crustaceans, mollusks, mites and cockroaches (Leung *et al.*, 1998; Reese *et al.*, 1999; Santos *et al.*, 1999; Ayuso *et al.*, 2002; Chiou *et al.*, 2003; Lehrer *et al.*, 2003; Jeong *et al.*, 2006; Marihno *et al.*, 2006; Mikita & Padlan, 2007; Abdel Rahman *et al.*, 2010a; Yadzir *et al.*, 2012).

We found that the majority of the sera demonstrated markedly enhanced IgE-binding intensity to the tropomyosin protein (36 kDa) with the cooked extract. This is not surprising as most people in Malaysia consume cooked or processed crab. This finding is in accordance with other reports which suggested that tropomyosin is more allergenic in boiled form, as this protein is highly heat-stable and cooking or boiling process alters the epitopes of the allergen (Nagpal *et al.*, 1989; Bestler *et al.*, 2001; Liu *et al.*, 2010b;Yadzir *et al.*, 2010). In this study, we also noted smeared IgE-binding regions of ~30to 250 kDa in the cooked extract which could be due to protein aggregation or protein glycosylation (Calabozo *et al.*, 2003). Thermal treatment might have caused the tropomyosin to under protein – protein interactions resulting in aggregation causing enhanced and smeared IgE binding (Yu *et al.*, 2011). Furthermore, unmasking of boiled tropomyosin in the boiled extract may result in IgE epitopes being more surface accessible (Aalberse, 2000).

On SDS-PAGE of the cooked extract, we also noted that the band intensity of multiple heat-resistant proteins at low molecular weights (18 to 23 kDa) was slightly increased as compared with the raw extract. However, immunoblotting results identified these proteins only as minor allergens in both raw and cooked crab. Recently, a study by Abdel Rahman *et al.* (2011) has identified allergenic proteins of 20 and 23 kDa in snow crab (*C. opilio*) as sarcoplasmic calcium-binding protein (SCP) and troponin, respectively. Other studies have also identified new crustacean allergens at 20 kDa protein as SCP (Shiomi *et al.*, 2008; Ayuso *et al.*, 2009) and myosin light chain (Ayuso *et*

al., 2008; Abdel Rahman *et al.*, 2010a). However, whether the 18 and 23 kDa minor allergens found in our study are similar to those allergens is unknown.

Our study has also identified a 41 kDa protein as an additional major allergen in blue crab which differs from tropomyosin. Remarkably, those major allergen and several potential minor allergens were clearly detected only in the raw crab extract. These findings show that all those allergens were heat-sensitive proteins and boiling processing has decreased the allergenicity of the allergen extract, as a result of change in protein conformation (Samson *et al.*, 2004; Chatterjee *et al.*, 2006; van Boxtel *et al.*, 2008; Liu *et al.*, 2010b; Yadzir *et al.*, 2010). Sequence homology searches of the digested peptide fragments have revealed that this 41 kDa band is significantly homologous to crab arginine kinase. Notably enzymes including arginine kinase are sensitive to high temperature, and will lead to enzyme denaturation (Hendrickx *et al.*, 1998). Our finding is in accordance with recent studies which reported arginine kinase with a molecular mass of 40 kDa as a new potential allergen of snow crab (*C. opilio*) (Abdel Rahman *et al.*, 2011) and mud crab (*S. serrata*) (Shen *et al.*, 2011). At present, arginine kinase represents a new class of cross-reactive allergen in different crustacean and invertebrate species including shrimps or prawns (Yu *et al.*, 2003; Yao *et al.*, 2005; Garcia-Orozco *et al.*, 2007; Ortea *et al.*, 2009; Abdel Rahman *et al.*, 2010a; Yadzir *et al.*, 2012), moth (Binder *et al.*, 2001), house dust mite (Hales *et al.*, 2007), cockroach (Brown *et al.*, 2004; Sookrung *et al.*, 2006; Tungtrongchitr *et al.*, 2009) and spider (Bobolea *et al.*, 2011). This enzyme is a phosphagen kinase that is the key to energy metabolism in invertebrates, which catalyzes the reversible transfer of the high-energy phosphoryl group from arginine phosphate to ADP to form ATP, thereby regenerating ATP during bursts of cellular activity (Yao *et al.*, 2005).

We have identified two major allergens with different biochemical properties in the blue crab *P. pelagicus* which may play an important role in crab allergy among our local patients. One of the major allergens was a 36 kDa heat-resistant protein which was identified as crab tropomyosin and the other one was a 41 kDa heat-labile protein corresponds to crab arginine kinase. This data could provide general insights in the properties of epitopes being responsible for eliciting allergic reaction in patients with crab sensitization. Hence, we recommended that both allergens should be included for diagnostic and therapeutic strategies of crab allergy. Moreover, blue crab-allergic patients should avoid consumption of both raw and cooked crab.

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