

# CHARACTERIZATION OF MAJOR ALLERGENS OF *MACROBRACHIUM ROSENBERGII* AND THEIR THERMOSTABILITY IN DIFFERENT COOKING METHODS

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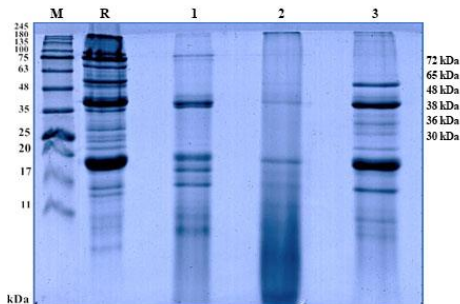
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## Graphical abstract



## Abstract

Prawn allergy is a common cause of allergic reactions in countries where crustacean is a famous seafood. *Macrobrachium rosenbergii* (giant river prawn) is declared as a local major food allergens, causes severe symptoms like asthma and anaphylactic shock. Therefore, the goal concerning this research is to identify the allergenicity of heat treated of *M. rosenbergii*. Prawn extracts have been prepared using fresh raw prawn and three heat-treated prawn flesh (boiled, steamed, fried), then afterward analyzed by the usage of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Allergenic proteins were identified by means of immunoblotting by the use of sera from 30 prawn-allergic patients. The identities of selective major allergens were then determined by mass-spectrometry analysis. The raw prawn has 27 protein fractions between 6 to 207 kDa, while the heat-treated prawns have reduced number of protein bands. Six prominent bands at 72, 65, 48, 38, 36, and 30 kDa were considered as the major allergens of raw extract. Meanwhile, after heat remedies applied, most of the IgE-binding bands were disappeared except for three major allergens at 38, 36 or 30 kDa which were still remain to be seen, suggesting as highly thermostable allergens. Among heat-treated prawns, steamed and boiled elicited more allergenic bands, contrast to fried prawn extract. Mass spectrometry analysis identified two selected major allergens at 36 and 48 kDa as tropomyosin and arginine kinase, respectively. As a conclusion, this study indicated that both thermostable and thermolabile proteins from *M. rosenbergii* were allergenic. Tropomyosin and arginine kinase were identified as the most important major allergens. Thus, the knowledge on thermostability of prawn allergens are crucial for improving diagnosis and management of prawn allergic patients in this county.

**Keywords:** *Macrobrachium rosenbergii*, SDS-PAGE, immunoblotting, allergenomics, thermostability

## Abstrak

Alahan terhadap udang adalah penyebab kepada tindakbalas alergi di kebanyakan Negara di mana krustasia merupakan makanan laut yang popular. *Macrobrachium rosenbergii* (udang galah) telah dilaporkan sebagai alergen makanan utama tempatan, yang menyebabkan simptom seperti asma dan renjatan anafilaksis. Oleh itu, kajian ini bertujuan untuk mengenalpasti alergeniti *M. rosenbergii* yang telah dirawat haba. Ekstrak

udang disediakan daripada udang mentah dan udang yang telah dirawat dengan tiga jenis rawatan haba (rebus, kukus dan goreng), diikuti dengan analisis menggunakan elektroforesis natrium dodesil sulfat-berpoliakrilamida (SDS-PAGE) untuk mengesan profil protein. Protein yang alergenik telah dikenalpasti melalui pembloatan imuno menggunakan serum daripada 30 pesakit yang alergic kepada udang. Identiti alergen major yang terpilih kemudiannya ditentukan dengan analisis spektrometri jisim. Udang mentah mempunyai 27 pecahan protein di antara 6 hingga 207 kDa, sementara udang yang dirawat haba mempunyai kurang jaluran protein. Sebanyak enam protein utama pada 72, 65, 48, 38, 36 dan 30 kDa telah dikesan sebagai alergen major pada ekstrak mentah. Sementara itu, selepas rawatan haba diaplikasikan, kebanyakan protein pengikat IgE utama telah menghilang kecuali tiga alergen major pada 38, 36 dan 30 kDa yang masih kekal kelihatan, mencadangkan sebagai alergen yang tahan haba. Dalam kalangan udang yang dirawat haba, rawatan wap dan rebus menunjukkan lebih banyak protein alergenik, berbanding dengan ekstrak udang yang digoreng. Analisis spektrometri jisim terhadap alergen major terpilih pada 36 dan 48 kDa masing-masing sebagai tropomyosin and arginin kinase. Kesimpulannya, kajian ini menunjukkan kedua-dua jenis protein yang tahan dan tidak tahan haba daripada *M. rosenbergii* adalah allergenik. Tropomyosin and arginin kinase telah dikenalpasti sebagai alergen major yang paling penting. Oleh itu, pengetahuan mengenai kestabilan haba alergen udang adalah penting untuk mengukuhkan pendiagnosis dan pengurusan pesakit alergi udang di negara ini.

**Kata kunci:** *Macrobrachium rosenbergii*, SDS-PAGE, pembloatan imuno, alergenomik, termostabiliti

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## 1.0 INTRODUCTION

Seafood acts as an extensive part of human nutrition, health and fitness. The increasing global trade of seafood has led to increase the frequency of seafood consumption across the countries. The increased production and consumption of seafood has been associated with seafood-related illness issues among customers, including seafood allergy [1, 2].

Prawn allergy has become a global issues. A recent survey in the United States stated up to 1 of 50 Americans has shellfish allergy including prawn [3]. Prawn allergens can be exposed to the hypersensitive individuals via diet of prawn meals, by respiration and skin contact through working and cooking. Sensitized individuals will develop various symptoms such as asthma, angio-oedema, urticaria and also fatal anaphylactic reactions [4]. To date, no specific therapy has been found to cure allergy. Avoidance is the most effective way to prevent allergic reactions [4].

Few prawn allergen have been identified based on IgE reactivity of prawn-allergic patients, but the molecular studies of this seafood allergens are still limited. Tropomyosin, the major prawn allergen, has been shown to induce CD4<sup>+</sup> T cell proliferation in allergic patients. The same reaction has been identified towards arginine kinase [5]. To date, 34 allergens have been identified and sequenced from various crustacean and mollusk species and registered in the International Union of Immunological Societies (IUIS) Allergen Database. Typically the biochemical criteria's of seafood allergens are of high water solubility, low molecular weight, high heat stability and an acidic isoelectric point. It is found that almost all the characterized allergens are edible

portions of shellfish species [6-8]. But, there are some protease-based allergens, which are non-IgE-mediated, been found in the gastrointestinal regions of the shellfish species [6].

Food processing are generally applied to preserve the food from microbial contamination, and thermal treatments are the most common method. However, food proteins that have been treated with heat might also have distinct effects on proteins such as denaturation, modification of non-covalent and disulphide bonds causing the hydrolysis of peptide bonds and also interaction with some other food molecules such as carbohydrates and lipids. As a outcome on this reactions, processing by thermal treatments might cause a strong effect on the allergenicity of the proteins by means of either lowering the allergenicity due to the loss of allergenic epitopes, or improving the allergenicity by generating new epitopes or unmasking the epitopes [1]. The impacts of food processing by thermal treatments on the allergenicity of foods have been extensively studied. Most of studies reported that thermal processing significantly decreased the IgE-binding reactivity of several heat-sensitive food allergens such as allergen in celery (Api g 1), apple allergen (Mal d 1) and hazelnut allergen (Cor a 1.04) [3]. After thermal processing, for example boiling, food allergens including prawns may lose some or all of their allergenicity [9]. This reduction of allergenicity can be explained by the alteration of the conformation of heat-labile proteins when exposed to heat, and hence causing the loss of antigenic epitopes [10].

The use of various preparations of prawn extracts could identify the effect of cooking methods on their protein profiles as well as their allergenic potencies. For example, food processing strategies appeared to

enhance IgE-binding to tropomyosin [7], as tropomyosin was reported as highly heat stable thus could retain its allergenicity at high temperature [8].

Reports on thermal properties of allergenic proteins of local giant river prawn or *Macrobrachium rosenbergii* is very limited. Thus, this study aimed to identify the major allergens of *M. rosenbergii* by allergenomic approach and subsequently determine the allergenicity of raw and heat treated extracts. In this current study, three different cooking methods have been applied including boiling, steaming and frying.

## 2.0 METHODOLOGY

### 2.1 Preparation of Allergen Extracts

Live samples of *M. rosenbergii* were collected from a local supplier in Tanjung Malim, Perak. Raw, boiled, steamed and fried extracts of *M. rosenbergii* were prepared from the prawn flesh. For extraction of raw prawn, the prawn flesh was first homogenized with ratio of 1:10 (weight/volume) in phosphate buffered saline (PBS), pH 7.2, using a blender, and then extracted overnight at 4°C. The homogenate was centrifuged at 4°C for 15 min at 14 000 rpm. After filtration with filter paper, the clear supernatant was sterilized using a 0.22 µm syringe filter. The extract was then dried in freeze dryer for 2 days. The lyophilized extract was stored at - 20°C until usage (Rosmilah et al., 2012). For preparation of boiled extract, the flesh was boiled in distilled water (100°C) for 20 minutes, while for preparation of steamed extract, the flesh was treated on a boiling water of 100°C in 20 minutes. Meanwhile, the fried extract was prepared by treating the flesh in vegetable oil for 10 minutes at 180°C [2, 9]. All the boiled, steamed and fried prawns were then extracted using the same method of extraction of raw prawn as mentioned above.

### 2.2 Human Sera

Serum samples used in this study were collected from 30 prawn-allergic patients from Allergy Clinic, Kuala Lumpur General Hospital. The prawn-allergic status of the patients was confirmed by skin prick test and ImmunoCAP test (Phadia, Uppsala, Sweden) to prawn allergens. Meanwhile, for negative control, a serum sample from a non-allergic volunteer was used. All sera were kept at - 20°C until utilized. This research was endorsed by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (Ethical Approval: NMRR-08-763-1918).

### 2.3 SDS-PAGE

To investigate the protein profile in the prepared extracts, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed following the method described by Rosmilah et al. [11]. Briefly, the prawn proteins were heated in a denaturing

Laemmli buffer at 97°C for 3 minutes. After that, the protein components in the prawn extracts and prestained molecular weight markers (Biorad, USA) were separated in a 12.5% separating gel with 5% stacking gel using a Mini Protean 3 System at 120 mA for 45 min (BioRad, USA). The isolated protein bands were visualized by staining with Coomassie brilliant blue R-250. Protein molecular weights were estimated by comparing the prawn protein bands with the molecular weight markers using the Quantity One Software (BioRad, USA) on an imaging densitometer (BioRad, USA).

### 2.4 Immunoblotting

The unstained prawn proteins isolated by SDS-PAGE were electro-exchanged from the gel to a nitrocellulose membrane utilizing the Mini Transblot System (BioRad, CA, USA) at 100 volt for 70 minutes. Exchange of the proteins to the membrane was affirmed by staining with Ponceau S. After washing in TTBS, a tris-buffered saline (TBS) containing 0.05% Tween 20 (pH 7.4), the membrane was blocked for 2 hours at 4°C with 10% skimmed milk in TBS. Subsequent to washing with TTBS, the membrane was probed overnight at 4°C with individual patient serum as the primary antibody (dilution 1:5) and followed by incubation in biotinylated goat-antihuman IgE (dilution 1:10 000) as the secondary antibody (KPL, Maryland, USA) for 30 minutes at 4°C. Then, the membrane were incubated in streptavidin-conjugated alkaline phosphatase and Alkaline Phosphate Conjugate Substrate Kit (BioRad, CA, USA) for discovery of IgE-binding protein. The molecular weight of the allergenic protein bands was evaluated by comparing with the protein marker utilized.

### 2.5 Two-dimensional (2-DE) gel electrophoresis and Immunoblotting

The prawn extract in rehydration buffer was applied to an immobilized non-linear gradient strip, pH 3-10 (Biorad, USA) at room temperature overnight. The protein was first separated by charge using IEF cell (BioRad, USA), followed by the second dimension separation by SDS-PAGE as described above. The prawn protein spots were then stained with Coomassie brilliant blue R250 and their pI and molecular weight were analyzed by PDQuest Software using an imaging densitometer (BioRad, USA). Meanwhile, for detection of IgE-binding spots, immunoblotting of 2-DE map was performed using the same methods as described above. Sera from 10 selected prawn-allergic patients were used.

### 2.6 Mass Spectrometry

The major IgE-binding spots of selective major allergens were analyzed using Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) by First Base Laboratories Sdn. Bhd., Malaysia. The spectra were

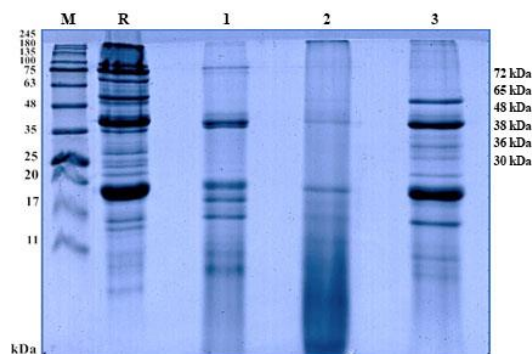
analyzed using Matrix Science software with Ludwig NR Database to identify the protein spots.

### 3.0 RESULTS AND DISCUSSION

#### 3.1 Protein Profile Analysis

The protein profiles in raw, boiled, steamed and fried extracts of *M. rosenbergii* were demonstrated by SDS-PAGE as shown in Figure 1. The cooked extracts contained less protein bands compared to the raw extract, while among the cooked extracts, the fried prawn presented the least protein bands. The raw extract displayed a visible protein bands and complex protein pattern, mostly within the molecular weight of 6 to >207 kDa. Out of them, several prominent protein bands in the range of 32-38 kDa and 17-20 kDa were seen in all cooked prawns thus were identified as heat resistant proteins. Additionally, proteins of 24-27 and 40-42 kDa were also declared as heat resistant but only in the boiled and steamed extracts. In contrast, the other bands were identified as heat sensitive protein bands which in the cooked extracts particularly the protein band above 42 kDa. These results showing different methods of cooking prawns produce varieties of protein profiles. Frying presents fewer protein bands than boiling and steaming. This is probably because of the aggregation of the protein at temperature above 90°C [2] and the disruption of protein conformation by high temperature [11]. Most proteins are sensitive to thermal treatment which causes degradation of the finely balanced intra molecular forces and reorganizes all levels of protein structures causing the loss of secondary and tertiary structures and subsequently changes the protein configuration to a random-coil conformation and fully unfolded [2].

This study showed that steaming exhibited higher protein bands than boiling. Unlike boiling, steaming is a fat-free cooking method that keeps the natural moisture and nutrients inside the seafood, while boiling will result in the loss of some of their nutrients in the cooking water [2, 3]. This condition might explained the reduced number of the band in the boiled prawn than the steamed prawn in this study.



**Figure 1** Protein profile analysis of raw (R), boiled (1), fried (2) and steamed (3) prawn extracts. M is protein markers in kiloDalton (kDa)

#### 3.2 Major Allergen Identification

Results of immunoblotting experiments demonstrated that more than half of the proteins in raw extract were competent to bind IgE antibodies from prawn-allergic patients (Figure 2). All sera used in this study exhibited profoundly heterogeneous IgE-binding towards prawn proteins, indicating that IgE reactivity to prawn allergens are vary between people due to both hereditary and the ecological factors [12]. Allergenic proteins are assigned as a major allergen if at least half of the tested patients have IgE reactivity to the allergen [13]. In many cases major allergens serve as a major diagnostic marker allergen for allergic patients [13]. Six proteins at 30, 36, 38, 48, 65 and 72 kDa of *M. rosenbergii* could bind to IgE antibodies of at least half of the sera as shown in Figure 2, and thus were identified as the major allergens for this prawn.

This research showed that two major allergens of 36 and 38 kDa have the highest frequencies of IgE binding thus were identified as the most important major allergens of *M. rosenbergii*. Mass spectrometry analysis has identified the 36 kDa major allergen as tropomyosin (Figure 3 and Table 1). However, we believed that the 38 kDa protein was also a tropomyosin. Tropomyosin which comprises 2 subunits with molecular weight in the range of of 36–38 kDa is a myofibrillar protein that has been very much reported as the significant allergen which responsible in cross-reactivity reactions between different types of shrimps and prawns, such as black tiger shrimp (Pen m 1), brown shrimp (Pen a 1) and white leg pacific shrimp (Lit v 1). Tropomyosin is an essential protein in muscle contraction which is found in abundant in both invertebrates and vertebrates, and because of this, tropomyosin is available in substantially higher amount than other recognized shellfish allergens [7, 9]. Tropomyosin was widely reported as a significant allergen, being the major cause for reported clinical hypersensitive responses in shellfish allergy [9].

Besides tropomyosin, shellfish also has other significant allergens at different molecular weights. A protein at molecular weight of 40 kDa, identified as arginine kinase has been portrayed as a noteworthy allergen in crab, shrimp and other species of invertebrates. More recent, a protein at 42 kDa, identified as actin was additionally recognized as an allergen in both crustacean (mainly shrimp and crab) and molluscan shellfish [11-13]. Arginine kinase is a phosphokinase enzyme which has an important role in regulation of ATP level in muscles of invertebrates [9], whereas actin, a component of muscle cells in all eukaryotes is an essential contractile protein [11]. Our study has identified the second most important major allergen of *M. rosenbergii* at 48 kDa as arginine kinase, although the molecular weight is slightly higher than the theoretical molecular weight at 40-42 kDa (Figure 3 and Table 1).

This study additionally distinguished one lower molecular weight major allergen at 30 kDa and two higher molecular weight proteins at 65 and 72 kDa as



significant major allergens. However, further identification of the other major allergens at 30, 38, 65 and 75 kDa by mass-spectrometry analysis were not conducted due to limited number of available sera for immunoblotting of 2-DE maps.

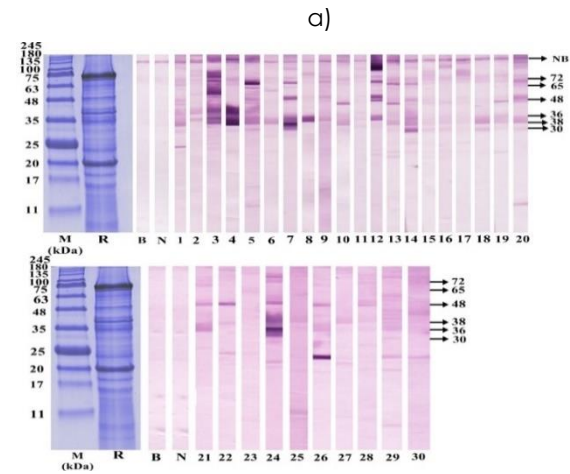
Protein at 30 kDa in shellfish was rarely portrayed as a noteworthy allergen. Nonetheless, this band was perceived by 70% of the tested sera in immunoblotting of raw *M. rosenbergii*. To date, there has been only one study reported shellfish allergens at 28 kDa, which were close with the molecular weight of the 30 kDa major allergen found in this study. Those 28 kDa band was identified as triose phosphate isomerase in shrimp *Crangon crangon* [12, 14]. Similarly, the major allergens of 65 and 72 kDa were also rarely reported as allergenic proteins in literatures, and the identification have not yet been described [12, 14].

This study additionally showed that a large proportion of the tested sera had minor IgE-binding to different proteins at different molecular weights with various frequencies. Minor allergens with molecular weights of 10 to 250 kD have been recognized in shellfish by various reports, but the bands are remain unidentified [15]. Minor allergens were only proposed as clinically important allergens on certain patients [16].

One of the minor allergen is a protein at 107 kDa with frequency of binding at 47%. Up until this point, there has been just a single report has recognized paramyosin, a novel mollusc allergen other than tropomyosin in abalone *Haliotis discus discus* at high molecular weight of ~100 kDa [17]. Proteins may also form oligomers as a dimer or trimer at high molecular weight regions in the range of 150 to 200 kDa. These oligomers may have a higher capability to induce allergic responses than monomers as they might have extra epitopes [18]. Thus, we believed that the 107 kDa allergenic protein in blotting of raw *M. rosenbergii* may be either a paramyosin or an oligomers.

In this study, a few responses has also been seen at lower molecular weight bands of 18 and 20 kDa. We believed these bands might be either sarcoplasmic calcium binding protein, myosin light chain or troponin C, the new reported shellfish allergens at ~20 kDa [17].

This study found all sera demonstrated a non-specific band at 135 kDa in immunoblotting of raw *M. rosenbergii*. This band might be formed because of the aggregation of the primary and/or secondary antibodies with the blot membrane [19, 20].



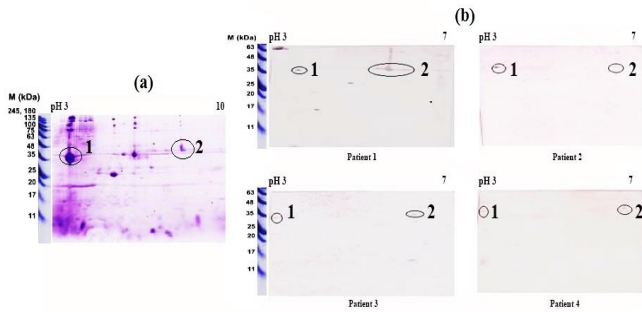
(a)Raw

No.	150	107	95	72	65	61	58	52	48	42	40	38	36	30	26	24	22	14	No. of bands
1				√	√				√	√	√	√	√	√		√			8
2	√			√	√				√				√	√					7
3			√	√	√			√	√			√	√	√					8
4				√	√	√			√	√	√	√	√	√					6
5				√	√	√	√	√	√	√	√	√	√	√				√	12
6					√				√										3
7		√		√	√	√			√			√	√	√					8
8				√	√							√	√	√					3
9				√	√				√	√			√	√					5
10				√	√				√				√	√					4
11		√		√	√			√				√	√	√					7
12		√	√	√	√				√	√		√	√	√		√	√		10
13				√	√					√	√	√	√	√				√	7
14		√		√	√				√	√		√	√	√	√	√	√		11
15		√	√	√	√				√	√		√	√	√		√	√		10
16	√			√						√			√	√					5
17		√	√	√					√	√			√	√					7
18				√	√							√	√	√					5
19				√	√				√			√	√	√		√			6
20		√	√	√	√				√	√	√	√	√	√				√	11
21				√	√				√			√	√	√					6
22	√							√				√	√	√		√			4
23	√				√						√	√			√				5
24	√	√		√	√				√	√	√	√	√	√	√	√	√		12
25	√	√		√	√							√	√	√					4
26		√		√				√				√	√	√		√			7
27	√	√										√	√	√		√	√		6
28		√	√	√				√	√	√		√	√	√		√	√		10
29	√	√	√	√			√	√	√	√	√	√	√	√		√	√		13
30	√	√	√	√	√				√	√	√	√	√	√		√	√		12
N	9	14	11	22	19	5	3	11	16	13	9	23	26	17	3	12	7	2	
%	30	47	37	73*	63*	17	10	37	53*	43	30	77*	87*	57*	10	40	23	7	

\*= Major Allergen

√ = IgE-binding protein

**Figure 2** Immunoblotting results of raw *M. rosenbergii* using sera from 30 prawn-allergic patients (a) and their frequency of IgE-binding bands (b). Lane M is molecular mass markers in kiloDalton (kDa); lane R is raw; lane B is blank and lane N is immunoblot using a negative control serum



**Figure 3** Two-dimensional gel electrophoresis (a) and immunoblot analysis of *M. rosenbergii* using sera from 4 patients (b). Lane M, molecular mass markers. Circles indicate the IgE-binding spots of major allergens subjected to mass spectrometry analysis

**Table 1** Summary of MASCOT search results of *M. rosenbergii* from digested peptides of spot 1 and 2

Spot	Observed MW, pI	Theoretical MW, pI	Protein Identification	Organism	Accession No.	% Coverage MS/MS	Match Peptide
1	36, 4.5	32830 4.72	Tropomyosin	<i>Penaeus monodon</i>	sp A1KYZ2	13	4
		32830 4.72	Tropomyosin	<i>Penaeus japonicus</i>	fr A2V731	13	4
		32826 4.73	Tropomyosin	<i>Macrobrachium rosenbergii</i>	fr D3XNR9	13	4
2	48, 6.3	40,142, 6.19	Arginine kinase	<i>Litopenaeus vannamei</i>	fr B0FRF9	11	3
		40,115	Arginine kinase	<i>Penaeus monodon</i>	sp C7E3T4 fr E7CGC2	11	3
		40,104, 5.92	Arginine kinase	<i>Fenneropenaeus chinensis</i>	fr Q4KY22	10	3

**3.3 Thermostability of Prawn Allergens**

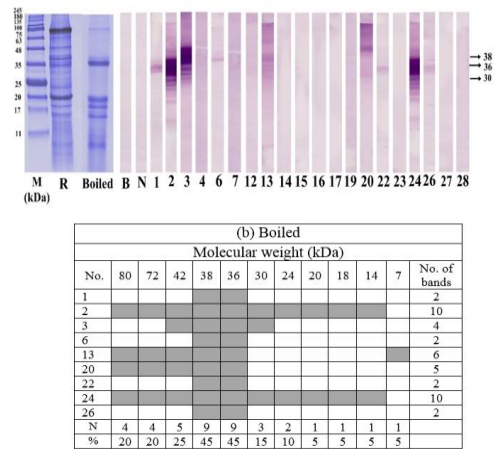
In contrast with raw extract, immunoblotting tests of boiled, steamed and fried extracts of *M. rosenbergii* did not show remarkable IgE-reactivity pattern (Figure 4 to 6). Only a few bands retained their capabilities to bind the IgE in certain sera, and mostly appeared as smears. Other study also reported the allergenic capability of the food proteins was not completely diminished after boiling or steaming but incited a weaker immune reaction than raw food [21].

Generally, steamed and boiled elicited more IgE binding than the fried prawn extract. A total of 9 and 7 sera retain their IgE-binding capabilities in boiled and steamed prawns, respectively, while only 3 sera were responded to the fried prawn. Fried prawn extract has less IgE-binding bands as this cooking method treated the prawn with higher temperature than boiling and roasting. During frying, physical and chemical changes occur in fats under the influence of high temperature and prolonged heating. This result is in concordance with the SDS-PAGE results that revealed that the fried prawn demonstrated the least number of bands.

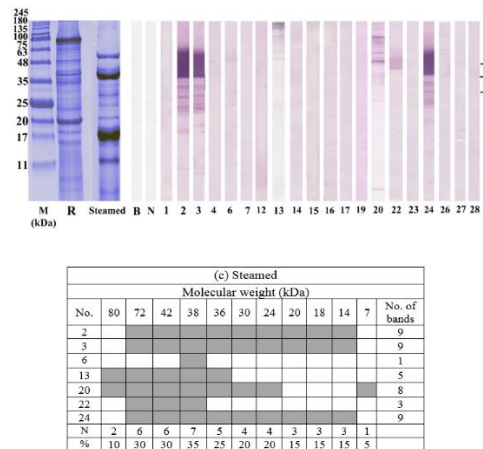
Generally, conformation of proteins will be altered after subjected to cooking process such as boiling, roasting, steaming or frying, and subsequently might

destroy, modify or generate new allergenic epitopes [9, 11]. This study revealed that most of the IgE-binding proteins including the major allergens of 48, 65 and 72 kDa were heat-sensitive, showing that cooking process could reduce the allergenicity of *M. rosenbergii*.

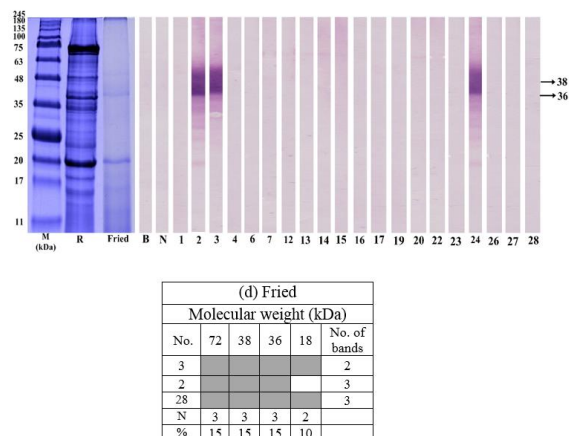
As an enzyme which generally sensitive to high temperatures, arginine kinase at 48 kDa was confirmed as a highly heat-sensitive major allergen as it only appeared in the raw extract. Thus, this major allergen unable to retain its IgE-binding capacity in immunoblotting of all cooked extracts. Other studies also reported arginine kinase as heat-sensitive allergens in crustaceans and mollusk [9, 11]. However, the 72 kDa major allergen still could responses to IgE antibodies in some sera as weak smears, showing that this band is not highly heat-sensitive.



**Figure 4** Immunoblotting results of boiled *M. rosenbergii* using sera from 20 prawn-allergic patients and their frequency of IgE-binding bands. Lane M is molecular mass markers in kiloDalton (kDa); lane R is raw; lane B is blank and lane N is immunoblots using a negative control serum



**Figure 5** Immunoblotting results of steamed *M. rosenbergii* using sera from 20 prawn-allergic patients and their frequency of IgE-binding bands. Lane M is molecular mass markers in kiloDalton (kDa); lane R is raw; lane B is blank and lane N is immunoblots using a negative control serum



**Figure 6** Immunoblotting results of fried *M. rosenbergii* using sera from 20 prawn-allergic patients (a) and their frequency of IgE-binding bands (b). Lane M is molecular mass markers in kiloDalton (kDa); lane R is raw; lane B is blank and lane N is immunoblots using a negative control serum

In contrast, the major allergens of 30, 36 and 38 kD in this study has been described as a heat stable protein in SDS-PAGE, which appear in all extracts of *M. rosenbergii*. As mentioned earlier, the 36 kDa major allergen was confirmed as tropomyosin by MALDI-TOF, however, we also believed that the 30 and 38 kDa were also might be tropomyosin but appeared at different molecular weights. Tropomyosin was broadly reported as highly water soluble and highly heat-stable allergen between molecular weights of 32 to 38 kDa due to the thermostability of alpha helical coils in its secondary structure [5, 6, 9]. The capacity of tropomyosin to withstand heat treatments causes tropomyosin to resist denaturation as seen in the immunoblotting of cooked extracts in this study. Interestingly, some sera (sera no. 2, 3 and 24) demonstrated enhancement in the IgE-binding intensities of 30 to 38 kDa region in all cooked extracts, indicating that cooking process could also increase the allergenicity of some allergens, most probably because of Maillard reactions [9, 11].

Our results showed that cooking treatments of boiling, steaming and frying could modified the allergenicity of *M. rosenbergii*, by either decreasing or increasing its allergenicity, depending on the sera used, most probably due to both environmental and genetic factors as mentioned earlier [12].

#### 4.0 CONCLUSION

Six major allergens of *M. rosenbergii* have been identified at 30, 36, 38, 48, 65 and 72 kDa with either heat-stable or heat-labile properties. Two of the major allergens at 36 and 48 kDa were identified as tropomyosin and arginine kinase, respectively, by MALDI-TOF analysis. Meanwhile, this study is the first report in Asian countries to determine the stability of

the prawn allergens after subjected to three different thermal treatments usually applied prior prawn consumption (boiling, steaming and frying). It is found that raw prawn capable to react more with IgE antibodies of local prawn-allergic patients than the treated prawns. Among the thermal treated prawns, fried prawn has the least allergenic bands, suggesting that frying treatment is the most effective method to reduce prawn allergenicity. Thus, this study indicated both raw and thermal-treated prawns are crucial to be included in diagnosis and management of prawn allergic patients.

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