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EFFECT OF VINEGAR TREATMENT ON ALLERGENICITY OF *Macrobrachium rosenbergii*

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Abstract: The purpose of this study was to determine the allergenicity of vinegar treated prawn extract. Prawn proteins were extracted from the raw and vinegar treated prawn. Both extracts were then fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine their protein profiles, and tested by immunoblotting test using sera from 20 prawn-allergic patients to determine their IgE-binding capacities. The raw prawn has 27 protein bands between 6 to 207 kDa, while the vinegar treated prawn has only a few bands between 6 to 38 kDa. The major allergens were identified at 72, 65, 48, 38, 36, and 30 kDa in the raw prawn. Vinegar treated extract only contains a few IgE-binding bands mainly at 30 and 36 kDa. As a conclusion, this study indicated that *M. rosenbergii* has numerous allergenic proteins, but mostly are sensitive to low pH environment, suggesting that vinegar treatment might be used to reduce prawn allergenicity. These data would assist the clinicians in management of prawn-allergic patients in this country.

Keywords: *Macrobrachium rosenbergii*, prawn, allergy, SDS-PAGE, immunoblotting.

1. Introduction

Seafood is one of the essential foods in the human dietary and nutrition plan [1]. Globally, the production and consumption of seafood have continuously grow in the past few decades. However, seafood has the tendency to cause most of the common food allergies relating to fish and shellfish, being the two big significant food allergens [2]. Among seafood, an extra attention was given to prawn as the most potent seafood allergens. After consuming prawn, the occurrence of allergy will be induced by the allergens in the prawn. Allergic reactions can also be induced even when someone inhale or had any skin contact either while working nearer or cooking prawns [3]. Therefore, sensitized individuals can easily develop allergic reactions, such as urticaria, angio-oedema, asthma, or life-threatening anaphylactic reactions [4].

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Prawns are classified in the phylum Arthropoda, subphylum Crustacean, class Malacostraca, and order Decapoda. The term "prawn" is largely used in the description of any large shrimps. Prawns are basically associated with approximately 33 genera with the estimation of at least 2500 species and less than 300 of the species are of economic interest worldwide. In Malaysia, the number of different species of prawns are known to be more than 20. 60,000 tonnes prawns are landed every year as reported by our local data. It is also known that the number of prawns imported increases every year [1]. *Macrobrachium rosenbergii* (giant freshwater prawn) is widely disbursed in Southeast Asia. In Malaysia, this prawn has been extensively consumed. Among local sufferers with atopic sicknesses, this prawn is likewise considered as the most common allergenic prawn [5].

The essential allergen for prawns has been shown to be a 34 to 38 kDa heat-stable protein referred to as tropomyosin [6]. Tropomyosin is associated with thin filaments in muscle and performs a function in regulating muscle contraction. A previous local study has identified the major allergens of *M. rosenbergii* as the proteins of 36 and 42 kDa, identical as tropomyosin and arginine kinase, respectively [5].

Various type of heat and chemical treatments while preparing food can alter the structure of its protein. The alteration might then lead to masking or unmasking, epitope management and modification of allergen. It will also results in increase or decrease or no effect on allergenicity [7, 8]. Considering that there had been not many studies been carried out on *M. rosenbergii* species in terms of allergen stability, this study was conducted to characterize the allergenicity of these allergens after given a vinegar treatment.

2. Materials and Method

M. rosenbergii Extracts

Live *M. rosenbergii* was purchased from a local supplier in Tanjong Malim, Perak, Malaysia. The extracts of this prawn proteins were prepared from the prawn meat as explained previously [5]. In brief, the raw prawn were homogenized in phosphate buffer saline (PBS) pH 7.2, extracted overnight at 4 ºC, centrifuged, filtered, lyophilized and stored at -20 ºC until use. Meanwhile for the vinegar treated extract, the prawn meat was treated in vinegar overnight before subjected to the above extraction method.

Serum Samples

This study used 20 sera from prawn-allergic patients from previous study [5]. Serum from a non-allergenic individual was used as a negative control. An ethical approval was endorsed by Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia [5].

SDS PAGE

The protein profile in the prepared extracts was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, the extracts were treated with Laemmli buffer and loaded into wells containing 12% of resolving gel and 5% of stacking gel using a Mini Protean 3 Apparatus (Bio-Rad, USA). The prawn proteins and a prestained molecular weight markers were then separated at 120 mA for 50 minutes in the Tris-HCl buffer. After completed, the gels were stained by Coomassie Brilliant Blue R-250. The molecular weights of the proteins were then estimated using an imaging densitometer (Bio-Rad, USA).

Immunoblotting

Immunoblotting was done as described previously [5]. Briefly, the prawn proteins in SDS-PAGE gel were electro-transferred to a nitrocellulose membrane using Mini Transblot System (Bio-Rad, USA) at 250 mA for 70 minutes. After completed, the membrane was stained by Ponceau S, cut into small strips and washed with TTBS solution. After blocking with 5% low-fat milk in TBS solution, the strips were incubated with the sera overnight at 4˚C. IgE binding proteins on the strips were identified by detection systems containing of biotinylated Goat antihuman IgE (KPL, UK), conjugated streptavidin-alkaline phosphatase (Bio-Rad, USA) and alkaline phosphatase conjugate substrate (Bio-Rad, USA).

3. Results and Discussion

Protein Profiles of Raw and Vinegar Treated Prawn Extracts

Figure 1 shows the comparison of protein profiles between raw and vinegar treated extracts of *M. rosenbergii*. A visible protein bands and complex protein pattern, mostly within the molecular weight of 6 to 207 kDa was displayed by the raw extract of prawn. Prominent protein bands between 30 to 38 kDa was possibly corresponding to tropomyosin [9].

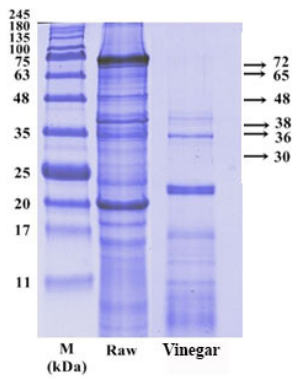

Figure 1: Protein profiles of raw and vinegar treated extracts of *Macrobachrium rosenbergii*. Lane M is molecular weight markers in kiloDalton (kDa). Arrows indicated major allergen in kDa.

Compared to the raw extract, the intensity of protein bands in the vinegar treated extract became less visible. Bands at molecular weight of 36-38 kDa showed relatively low intensity, while all higher molecular weight bands above 40 kDa disappeared. The few bands that can be seen in vinegar treated prawn extract are 34, 18, 17, 14, 12, 11 and 10 kDa. The reduction of number of bands in the vinegar-treated extract can be explained by an alteration in the allergen structures after treatment with vinegar due to partial loss of protein structures as the result of proteolytic degradation of proteins by the activity of acidic protease at acidic pH [10].

Immunoblotting of Raw and Vinegar-treated Prawn Extracts

The IgE-binding protein components of raw and vinegar treated extracts were detected by immunoblotting as shown in Figure 2. Numerous IgE-binding proteins were detected in the raw extract with six proteins at 30, 36, 38, 48, 65 and 72 kDa of *M. rosenbergii* could tie to IgE antibodies of at least half of the tested sera, thus were identified as the major allergens for *M. rosenbergii.*

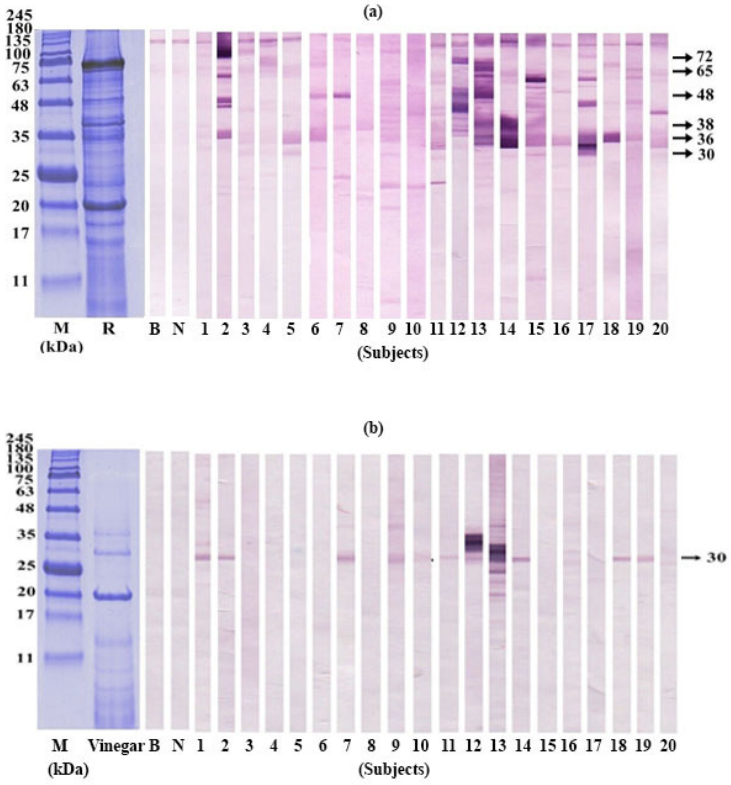

Figure 2: Immunoblotting results of raw (a) and vinegar treated extracts of *M. rosenbergii* using sera from 20 prawn-allergic patients (lane 1 to 20). Lane M is molecular mass markers in kilo Dalton (kDa); lane R is raw extract; lane B is blank and lane N is immunoblot using a negative control serum. Arrows indicated the major allergens molecular weight in kDa.

Meanwhile, most of the patients showed less or no reaction to immunoblotting test of vinegar treated extract. The reduction of the immunoreactivity may be caused by proteolysis of the antigenic protein by acidic proteases in prawn muscle, which is activated by vinegar. However, more than half of the tested sera retain the IgE-binding to the 30 kDa band. It can be clearly seen that at the band of 36 kDa, strong reaction elicited by Patients No. 12 and 13. This is because of the nature of this band, assumed as tropomyosin, to retain its antibody reactivity even at acidic condition. Similarly, Lasekan et al. (2017) [11] reported that the extractability of soluble myofibrillar proteins in prawn was reduced significantly among shrimp marinated in vinegar at pH 1.0–3.5, and a substantial amount of tropomyosin was retained in the insoluble pellets. Consequently, the IgE-binding capacity of tropomyosin was significantly lower in the soluble protein fraction of shrimp marinated at pH 1.0–3.5, compared the control. However, tropomyosin in the insoluble protein fraction of all marinated shrimp showed strong IgE-binding capacity at all marinating conditions [11].

The rest of the patients who showed reaction to the IgE binding of the vinegar treated extract only revealed 1 to 3 bands, relatively low when compared to raw immunoblotting. Most of the IgE binding reaction occurred at 30 kDa. It can be said that the band of 30 kDa does not dissolve in an acidic pH environment since it exerts IgE reaction to majority of the tested sera. This band might be triose phosphate isomerase at molecular weight of 28 kDa (close with the molecular weight of 30 kDa). Triose phosphate isomerase has been characterized as a new allergen in shrimp *Crangon crangon* (Cra c 8), crayfish (Arc s 8), and cockroach (Bla g TPI). However, the clinical and immunological cross-reactivity of triose phosphate isomerase among various invertebrate species are not well understood and amino acid sequences have not been performed [6].

These findings suggest that the chemical reactions by vinegar could cause the decrease in allergenicity of *M. rosenbergii*. The reduction might be due to the precipitation of prawn allergens in an acidic pH environment. Acid denaturation may also be the reason as it is responsible for the decrease in the allergenicity of prawn other than allergen precipitation. Hence, a dramatic decrease in IgE-binding reactivity was showed after the prawn was treated with vinegar. This result is in accordance with other studies which reported acid ingredients used as everyday food flavorings may have the possibility to decrease the allergenicity of several food allergens including shrimp [12], egg and lentil [13] and peanuts [14].

5. Conclusion

This study demonstrated that vinegar treatment revealed a decrease in recognition of the proteins bands but retained minimal IgE-binding at several highly stable bands mainly at 30 and 36 kDa of *M. rosenbergii*. Based on this finding, vinegar treatment can be used to reduce the prawn allergenicity. This study will be useful for clinicians in management of prawn-allergic patients.

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