The Allergenicity Potential of Edible Freshwater Fish

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Abstract: A freshwater fish is a popular type of fish consumed in Indonesia besides seawater fish. However, until now the most available information regarding the potential allergenicity of fish is only related to seawater fish. The potential allergenicity of freshwater fish has not been studied much, so it has not been identified and characterized. Based on this, this review aims to collect information regarding the potential allergenicity of protein in freshwater fish and the effect of processing on this potential. The potential allergenicity of proteins in several freshwater fish has been confirmed by immunoblotting and ELISA, namely mujair, nile, catfish, mas, toman, janjan, tambakan, tiger scat, barramundi, and eel. The identified allergenic proteins include parvalbumin, tropomyosin, enolase, aldolase, creatine kinase, triosephosphate isomerase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate isomerase, and glyceraldehyde-3-phosphate dehydrogenase. In addition, several proteins in other freshwater fish also have allergenicity potential but have not been confirmed through research, namely pomfret, gourami, snakehead, catfish, eel, betutu, and cetol. The potential allergenicity of fish protein is also influenced by the processing process. Changes in the potential allergenicity are related to the characteristics of each allergen protein. Therefore, it is necessary to select the appropriate processing so that potential allergenicity can be suppressed.

Keywords: Allergenicity, Freshwater Fish, Processing, Protein

1. INTRODUCTION

Since the last few years, the incidence of allergies globally has continued to increase, causing a considerable medical and socio-economic burden [1]. This is related to clinical manifestations of allergies that interfere with activities, reduce quality of life and require appropriate medical treatment [2]. The clinical manifestations of allergy in question are very diverse, ranging from mild reactions in the form of urticaria, atopic dermatitis angioedema, gastroenteritis, bronchial asthma to severe reactions in the form of anaphylaxis (a critical, life-threatening systemic hypersensitivity reaction) [3-4].

Food is known to be one of the causes of allergies. Generally, food allergies are caused by the shrimp, peanut and fish families [5]. Among Ige-mediated sources of food allergy triggers, it is known that the most common is fish [6]. In addition, fish are known to cause allergies in children and persist into adulthood, even for life [7-8].
Most of the fish that cause allergies are known to be bony fish of the Teleostei order. This order generally has the beta parvalbumin protein isoform as the main allergen, and other minor allergen proteins such as enolase, aldolase, collagen, gelatine, tropomyosin, and vitellogenin [6]. However, several studies have found that parvalbumin in certain fish species is not allergenic, instead other allergenic proteins are dominant in causing allergies [9-10].

The type of allergy related to fish consumption that has been widely reported and researched is allergy to seawater fish/seafood [11]. In fact, apart from seawater fish, freshwater fish are also popularly consumed in Asia, including Indonesia [12]. Freshwater fish are known to be a trigger for allergic rhinitis (39.29%) [13]. But, until now, the existing studies have only covered small of freshwater fish species, more reported types of seafood [14]-[18].

Food processing is known to affect the allergenicity of a food by suppressing the IgE binding ability of the allergenic protein contained in raw food [19]. This process is closely related to the characteristics of the allergen protein. Some of the characteristics of allergenic proteins are stable to heat (tropomyosin, parvalbumin, enolase), stable to acids and digestive enzymes (tropomyosin) [10], [20]. This review aims to collect information regarding allergenic proteins and the allergenic potential of freshwater fish protein in Indonesia and the effect of processing on the allergenic potential of fish protein. This study discussed the freshwater fish that have known to induce clinically allergic reaction with well characterized allergen or potential allergen that is still not well characterized.

2. MATERIALS AND METHODS

This review using the method of literature study. The references used are sourced from Google, Google Scholar, online protein database (Uniport.org), allergen protein online databases (allergen.org and allergome.org). The writing stage begins with the collection of literature related to fresh water fish consumed in Indonesia. The next stage is the collection of literature related to potential allergenicity based on empirical reports (articles on the e-health web, such as ALODOKTER, Ministry of Health, and Hospitals), research related to freshwater fish protein profiling and research related to freshwater fish protein allergenicity. The keywords used specifically mention the intended freshwater fish, "protein profile" “allergenic protein”, and "IgE". The number of articles used is 30 articles.

3. RESULTS AND DISCUSSION

3.1. Allergic Potential of Freshwater Fish

3.1.1. Mujair (Oreochromis mossambicus)

The presence of allergenic protein in tilapia fish/mujair protein extract was confirmed by immunoblotting using the serum of tilapia allergic patients. In this study, 3 allergenic proteins were found, namely phosphopyruvate hydratases (52 kDa), enolase (47-52 kDa) and fructose bisphosphate aldolase (37 kDa). In addition, it was confirmed that parvalbumin in tilapia is not allergenic [21]. Subsequent research found that there was an IgE bond with tilapia fish allergen protein measuring 32 kDa. The protein was identified using Mass Spectrometry as tropomyosin (TM) [16]. In another study, it was found that purified Ore m allergen was able to bind to IgE patients with confirmed tilapia allergy and was able to cross-react with shrimp tropomyosin [22]. TM of tilapia species has been registered as an allergen in fish in the WHO/IUIS database [23].
3.1.2. Nile (Oreochromis niloticus)

Several allergenic proteins in nile have been identified, one of which is parvalbumin and tropomyosin. The allergenicity of nile parvalbumin was confirmed through quantitative lateral flow immunoassay (LFIA), western blot and ELISA tests, while the tropomyosin reactivity of nile was confirmed through ELISA [18], [24]. It is also known that the allergenicity of nile protein with a molecular weight of 18 and 45 kDa, however, the type of this protein has not been determined [9].

Based on immunoblotting testing using 8 sera from food allergy sufferers, it was found that there was an IgE bond with the baby nile allergenic protein. There are differences in sensitivity between allergy sufferers, indicating the presence of IgE binding to proteins with various molecular weights. In the raw baby nile test, it was positive for protein with a molecular weight of 31 kDa, while the boiled and fried baby fish varied from 43 to >250 kDa, actually increase allergenicity. The types of allergenic proteins obtained were not further identified [25].

3.1.3. Eels (Monopterus sp)

In testing the eel protein extract from Cisalopa Village, Caringin District, Kab. Bogor, obtained several proteins that can bind with IgE from the serum of food allergy sufferers through immunoblotting testing. These proteins have molecular weights of 23, 25, 29, 51, 78 and 101 kDa, but the types of these proteins have not yet been identified [26]. In another study, based on the mouse model sensitized by turbot parvalbumin, it is known that parvalbumin eel can cross-react with parvalbumin turbot [27]. In addition, in 2018 there was a report on the incidence of atopic dermatitis which was triggered by an allergy to eel collagen [28].

3.1.4. Catfish (Pangasius sp)

Allergenic protein in catfish has been confirmed by immunoblotting and ELISA. Several known allergenic proteins and theirs capacity binding; Parvalbumin, 11kDa, 10-49%; Tropomyosin, 35kDa, 6-32%; Triosephosphate Isomerases (TPI), 25kDa, 19-34%; and other allergent include: Enolase/Beta (50kDa), Aldolase (40kDa), Creatine kinase (43kDa), Pyruvate Kinases (65kDa), Lactate Dehydrogenase (34kDa), Glucose-6-Phosphate Isomeraser (60kDa), Glyceraldehyde-3-phosphate dehydrogenases (36kDa), that showed 6-13% IgE-binding capacity [14], [29], [30]. So, the major allergens associated with IgE are parvalbumin, TPI and tropomyosin.

Based on the identification of the allergen protein content above, it is known that catfish has the most diverse protein allergens compared to other freshwater fish, the data is presented in Table 1. This certainly increases the allergenic potential of this fish. So, must be more careful if people with allergies to freshwater fish want to consume this fish. In addition, further research is needed to determine the effect of handling, storage, transportation, and processing on the allergenic potential of this fish.

3.1.5. Mas/Karper (Cyprinus carpio)

Identification of the presence of allergenic proteins in carp through immunoblotting has been reported in the form of parvalbumin (12kDa), β-enolase (~50kDa) and proteins with a molecular weight of 25kDa which have not been identified [29], [31]–[33]. Parvalbumin and β-enolase from carp are known to be homologous to several other fish species. Carp β-enolase protein has >90% homology with salmon and catfish β-enolase, while mas/karper parvalbumin is homologous and can cross-react with cod parvalbumin [33], [34].
3.1.6. Other Freshwater Fish

In general, the allergenicity of several other types of freshwater fish is related to the allergenicity of the protein parvalbumin, the data is shown in (Table 1) and (Figure 1) [8]. In addition, several species of freshwater fish are empirically known to cause allergies, but the types and characteristics of the allergenic proteins in them have not been identified. The potential allergenicity of these freshwater fish species is estimated to be related to the protein content which is generally allergenic, but there is no evidence regarding the reactivity of these allergenic proteins in each species, the data is shown in (Table 2). Based on this potential, more scientific evidence is needed regarding the allergenicity of proteins in these species.

![Freshwater Fish Contain Allergen](image)

**Figure 1. Freshwater Fish Contain Allergen [35]**

3.1.7. Parvalbumin, The Major Fish Allergen

Parvalbumin is a calcium binding protein with an EF handed structure, including an acidic intracellular protein and low molecular weight (10-12kDa) that regulates calcium homeostasis in muscle fibbers [22]. Parvalbumin is found in various parts of the fish. In general, fish are in the form of β-parvalbumins which are allergenic, while α-parvalbumins have homology with humans.

β-Parvalbumin has many variants (isoallergens) with high similarity and allows for cross-react. Several chemical reactions can increase the capacity of IgE-binding, included the process of protein aggregation and the process of glycation with glucose, mannose, ribose. However, in the 3D/4D conformation, the IgE-binding capacity decreases. In addition, parvalbumin bonds with Ca2+ and Mg2+ to form a stable conformation related to its allergenic potential. Parvalbumin is generally stable to light/radiation, chemical denaturation, mechanical processes, heat, digestive enzymes (protection by amyloid fibers and lipids) and resistant to protease, but is labile to pressure and chemical reaction. More than that, parvalbumin is a class 1 allergen, which can expose as a food and also an aero-allergen [36].
Table 1. Freshwater Fish Allergen

<table>
<thead>
<tr>
<th>Jenis Protein</th>
<th>Molecular Weight (kDa)</th>
<th>Mujair</th>
<th>Nile</th>
<th>Catfish*</th>
<th>Mas/Karper</th>
<th>Toman</th>
<th>Janjan</th>
<th>Tambakan</th>
<th>Tiger Scat</th>
<th>Barramundi</th>
<th>Eel*</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate Kinases</td>
<td>65</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14]</td>
</tr>
<tr>
<td>Glucose-6-Phosphate Isomerase</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14]</td>
</tr>
<tr>
<td>Enolase/Beta Enolase</td>
<td>47-52</td>
<td>v</td>
<td>-</td>
<td>v</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14], [21], [33]</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14]</td>
</tr>
<tr>
<td>Aldolase</td>
<td>37-40</td>
<td>v</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14], [21]</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14]</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>33-36</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14], [16], [18]</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>34</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14]</td>
</tr>
<tr>
<td>Triosephosphate Isomerases</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[14]</td>
</tr>
<tr>
<td>Parvalbumin</td>
<td>10-12</td>
<td>-</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>V</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>[24], [29], [30]</td>
</tr>
<tr>
<td>Not yet identified</td>
<td>45</td>
<td>-</td>
<td>v</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[9]</td>
</tr>
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<td></td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>-</td>
<td>v</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[9]</td>
</tr>
</tbody>
</table>

Fish species: mujair (Oreochromis mossambicus); nile (Oreochromis niloticus); catfish (Pangasius sp); mas/karper (Cyprinus carpio); toman (Channa micropeltes); janjan (Pseudapocryptes elongatus); tambakan (Helostoma temminckii); tiger scat (Scalophagus argus); barramundi (Lates calcarifer); eel (Monopterus albus)
Table 2. Potential Freshwater Fish Allergen

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight(kDa)</th>
<th>Pomfret</th>
<th>Gourami</th>
<th>Cork</th>
<th>Catfish</th>
<th>Eel</th>
<th>Betutu</th>
<th>Cetol</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitellogenin</td>
<td>~300</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>[37]</td>
</tr>
<tr>
<td>Collagen</td>
<td>180</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>[37]</td>
</tr>
<tr>
<td>Enolase/Beta Enolase</td>
<td>47-52</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>[38], [39]</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>[40]</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>[38], [39]</td>
</tr>
<tr>
<td>Aldolase</td>
<td>37-40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>[37]</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>[37]</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>33-36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>[37]</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14-15</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>[38], [39]</td>
</tr>
<tr>
<td>Parvalbumin</td>
<td>10-12</td>
<td>V</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>[33], [37], [38]</td>
</tr>
</tbody>
</table>

Fish species: pomfret (Colossoma macropomum); gourami (Osphronemus goramy); cork (Channa striata); catfish (Clarias batrachus); eel (Trichogaster trichopterus); betutu (Oxyeleotris marmorata); cetol (Poeclilia reticulata)

3.2. Effect of Processing on Protein Allergenicity

3.2.1. Heating and Steaming

There was a change in the molecular weight profile of nile bone protein after boiling at 100 °C for 5 minutes. In profiling the protein bands resulting from boiling compared to the raw sample, there are protein bands that disappear or just appear, and there is a change in protein intensity, that is, there is an increase or decrease [43]. Similar results were obtained in testing vannamei shrimp for 10 minutes. These changes are caused by denaturation of proteins by the heating process resulting in conformational changes including epitope changes. The boiling process can also dissolve water-soluble proteins, so that the protein content decreases. In addition, it was also found that there was a decrease in the level of allergenicity of the boiling sample, but it was not yet of significant value. These results were influenced by the characteristics of the main allergenic protein in the sample (tropomyosin) which is heat stable, but its intensity decreases due to being dissolved in water [44].

Different results were obtained in testing the snakehead fish, with various treatments in the form of boiling and steaming at 75 °C for 20 minutes, where there was an increase in protein levels after boiling and steaming. This change occurs due to shrinkage of water content, so that the structure of fish meat is more compact and denser. The greater the shrinkage of the water content, the greater the change in protein content in fish [45]. The heating process triggers structural changes in the tropomyosin such as unfolding that can expose the hidden linear epitopes. In addition, heating causes the formation of aggregates in tropomyosin and parvalbumin which also play a role in increasing IgE-binding capacity [36].

Protein profile of catfish with fried treatment for 3 and 6 minutes to control/without frying showed different results. There was a change in the number of protein bands, in the control sample, 5 major and 16 minor protein bands were obtained, whereas after 3 minutes of frying, 1 major protein and 10 minor proteins remained and after 6 minutes only 4 minor proteins remained [46]. In another study, it was found that the protein profile of shrimp in frying, roasting and roasting was generally
like the control sample but there was a decrease in the intensity of some bands. This shows the characteristics of a heat stable protein. There is also a decrease in total soluble protein and heat-stable protein, but there has not been a significant reduction in their allergenicity potential [47].

Dissolved protein content and heat resistance reached the highest value in the high-pressure steaming treatment (autoclave) when compared to other heating methods. However, in this treatment there was a decrease in the intensity of the tropomyosin band and the results of competitive ELISA inhibition also showed a decrease in tropomyosin allergenicity. Similar results were obtained in testing the allergenicity of tropomyosin and parvalbumin which decreased through a combination of heat and pressure [47]. So that the high-pressure steaming or combination with heat method is recommended for processing allergic proteins with heat-resistant characteristics.

3.2.2. Salting

Gourami protein profile in the presence of salt treatment for 12 hours with variations in salt content of 10, 20, 30 and 40% showed different results. There is a change in the number of protein bands, the more concentrated the salt content, the fewer protein bands remaining. This is caused by denaturation due to the salting process. The salting process will form cross-links between disulphides, thereby causing a decrease in protein solubility [48].

The pattern of changes in allergenicity in processing with salt (salted fish) is specific to each fish. In mackerel, the preparation of wet or dry salted fish was not able to remove the parvalbumin protein band. The allergenicity of pindang mackerel is known to be higher than salted mackerel, even after continued frying [49]. Similar results were obtained in testing freshwater fish, trout [50].

3.2.3. Surimi

One of the processed fish products that is known to reduce allergenic protein levels is the manufacture of surimi. The process of making surimi involves washing several times, one of which is to remove water-soluble proteins. This process can remove sarcoplasmic proteins. When measuring the intensity of protein bands on SDS-PAGE using densitometry, it was found that parvalbumin levels in surimi decreased by up to 95%. Reducing parvalbumin levels has the potential to reduce fish allergenicity [51].

3.2.4. Terasi

Terasi is made through the process of fermenting shrimp or fish. This process causes degradation of the tropomyosin allergenic protein and the tropomyosin band is not detected on the SDS-PAGE profile. In addition, in allergenicity testing through immunoblotting, the IgE binding response to tropomyosin decreased gradually upon making shrimp paste, and even disappeared. These changes vary depending on the type of shrimp used. Based on this, shrimp paste production is effective in reducing the potential allergenicity of shrimp products [19]. However, there has been no similar test for terasi made from fresh water fish.

3.3. How to Avoid the Freshwater Fish Allergy

Based on the explanation above, it is known that some freshwater fish have allergenic potential through the content of major allergen proteins in the form of parvalbumin and or tropomyosin. In general, the characteristics of these allergen proteins are stable to heat but labile to pressure and some
According to the characteristics, several processing methods were tested to determine the relationship between processing and allergenic potential. Several types of processing that have been known to reduce the allergenic potential of freshwater fish protein in general include processing with pressure or a combination of heating, salting, making surimi and fermenting it into terasi. These studies still use a small population of freshwater fish species, in which the protein diversity in fish can affect the correlation [20]. However, in general, this type of processing can be applied to freshwater fish species.

There was no effect of storage on fish allergens. Storage at -20 and -80°C for up to 112 days did not show a significant change in allergen protein levels [52]. There was no data on the effect of storage/handling at room temperature on potential allergens, but it was reported that the resistance of tilapia at storage temperatures of 4°C and 30°C only lasted a maximum of 18 hours, due to levels of biogenic amines such as putrescine, cadaverine and histamine which reached toxic levels, includes histamine poisoning, which causes allergy-like effects [53], [54]. Thus, to maintain quality, in general the distribution of fresh water fish in conditions of living in water or stunning (cold distribution), and frozen conditions [55].

In addition to reduce the potential for allergies, it is necessary to give attention the closeness of genetic-relationship between the freshwater fish associated with the potential for cross reactions. Fish allergy is a type of allergy with the potential for cross reactions, both among fish and other allergens [52]. So, if someone is allergic to types of freshwater fish, it is necessary to be careful with fellow freshwater fish and pay attention to the ingredient label on the food product packaging.

4. CONCLUSION

Based on the explanation above, several freshwater fish have been confirmed to have allergenic protein. The potential allergenic protein of several other freshwater fish needs to be confirmed through further research. In general, the characteristics of these allergen proteins are stable to heat but labile to pressure and some chemical reactions. Based on the characteristics, processing that can reduce the allergenic potential of freshwater fish protein in general include processing with pressure or a combination of heating, salting, making surimi and fermenting it into terasi. In addition, to reduce the potential for allergies and fish quality, it is necessary to carry out appropriate handling and storage.

Further in-vivo research with antibody formation methods is also needed. Information related to the type and characteristics of the allergen protein can be used as a basis for selecting the appropriate processing process to reduce potential allergenicity, prevent and develop allergy therapy. Furthermore, need to compare the allergenicity of marine and freshwater fish proteins, by knowing their characteristics to determine why marine fish are higher in allergenicity, and the factors that make the allergenicity different so we can suppress allergic incident.
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References


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