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Characterization of Immunoglobulin E-Binding Proteins (IgE) of *Scomberomorus commerson* Lacepede

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ABSTRAK

Objektif kajian ini adalah untuk mengenal pasti protein pengikat-Imunoglobulin E (IgE) dan alergen major Scomberomorus commerson Lacepede (Narrow-barred Spanish mackerel). Ekstrak alergen dihasilkan daripada ikan yang tidak dimasak dan yang dimasak melalui peng-homogenisasian di dalam larutan penimbal berfosfat diikuti dengan pengekstrakan secara berterusan pada suhu 4°C atau di dalam ais. Profil protein dan corak pengikatan IgE seterusnya dikesan dengan menggunakan elektroforesis gel natrium dodesil poliakrilamida (SDS-PAGE) and pemblotan imuno dengan menggunakan serum subjek yang sensitif terhadap ikan ini. SDS-PAGE ekstrak ikan yang tidak dimasak telah mengasingkan sebanyak 26 komponen protein dalam julat di antara 11 hingga >175 kD, manakala ekstrak ikan yang telah dimasak pula kurang mengandungi komponen protein. Pemblotan imuno telah mengesan sebanyak 17 komponen protein pengikat-IgE, dalam julat berat molekul di antara 11 hingga 151 kD. Sebanyak dua komponen protein dengan berat molekul protein ~50 and 42 kD menunjukkan frekuensi pengikatan IgE paling tinggi (masing-masing 62.2 dan 51.4%) dan telah dikenal pasti sebagai alergen major dalam alergi ikan ini. Protein pengikat-IgE yang lain termasuk protein bersaiz ~12 kD yang mempunyai saiz yang sama seperti parvalbumin pula telah dikenal pasti sebagai alergen minor.

Kata kunci: Alergi ikan, Scomberomorus commerson Lacepede, alergen major, protein pengikat-IgE, elektroforesis, pemblotan imuno

ABSTRACT

The objective of this study was to determine the Immunoglobulin E-binding proteins (IgE) and major allergens of Scomberomorus commerson Lacepede (Narrow-barred Spanish mackerel). Allergen extracts were obtained from uncooked and cooked fish by homogenization in phosphate-buffered saline followed by continuous extraction at 4°C or on ice. Protein profiles and IgE-binding patterns were then detected by means of sodium dodecyl polyacrylamide

gel electrophoresis (SDS-PAGE) and immunoblotting using sera from patients sensitized to the fish. SDS-PAGE of the uncooked fish extracts revealed 26 protein bands in the range of about 11 to >175 kD, while the cooked extracts produced fewer protein bands. Immunoblotting demonstrated 17 IgE-binding bands, ranging in molecular weight from 11 to 151 kD. Two components with molecular weight of about ~50 and 42 kD showed the highest frequency of IgE-binding (62.2 and 51.4% respectively) and were identified as the major allergens of this fish allergy. Other IgE-binding proteins including a protein at ~12 kD which was equivalent in size to parvalbumin were identified as the minor allergens.

Key words: Fish allergy, Scomberomorus commerson Lacepede, major allergen, IgE-binding proteins, electrophoresis, immunoblotting

INTRODUCTION

Fish and fish products play an important role in human nutrition. Fish is a valuable source of highly assimilated proteins and it contains large amounts of polyunsaturated fatty acids and fat-soluble vitamins (Harel et al. 2001). However, it also represents one of the most important causes of IgE-mediated hypersensitivity, especially in countries where the majority of the populations lives on fishing and where fish is the mainstay of the diet (Samartin et al. 2001). In allergic individuals, fish ingestion can provoke diarrhea, angioedema, or severe anaphylactic reactions. Inhalation of vapors generated during the cooking of fish may cause asthma, and skin contact can lead to urticaria and dermatitis (Lopata & Potter 2000). Fish allergy has been reported in 1 of 1000 subjects in Norway, and in 39% and 30% of pediatric patients with food allergy in Sweden and Spain, respectively. Fish was found to be the second most important food allergen after eggs in these patients (Lopata & Potter 2000). In Malaysia, among local patients with allergic rhinitis and asthma, shellfish and fish are the most common food allergens, as seafood forms a major component of the local diet (Shahnaz et al. 2001). Fish allergy has also been reported in the occupational setting. The prevalence of occupational asthma due to fish and shellfish varies from 7 to 36% among different groups of workers including seafood processing and fishmeal workers, fishermen and restaurant cooks (Lopata & Potter 2000).

Extensive studies on the codfish *Gadus callaris* have identified the major allergen (known as *Gad c* 1) as a calcium-binding sarcoplasmic protein in the ordinary muscle known as ß-parvalbumin, with a low molecular mass (~12.5 kD) (O'Neil & Lehrer 1995). Recently, allergens of several species of fish, Atlantic salmon (*Sal s* 1), Atlantic cod (*Gad m* 1), carp (*Cyp c* 1) and mackerel (*Sco j* 1, *Sco a* 1 and *Sco c* 1) have also been identified as parvalbumin at the molecular level (Bugajska-Schretter et al. 2000; Das Dores et al. 2002a; Hamada et al. 2003b; Van Do et al. 1999).

It has also been reported that *Gad c* 1 was the major cross-reactive allergen among certain fish species such as cod, catfish, bass, perch, mackerel, salmon, herring, plaice, trout, flounder and snapper (Lopata & Potter 2000; O'Neil & Lehrer 1995). However, it is interesting to note that other allergens besides *Gad c* 1 are involved in fish allergy. In two species of tuna fish (albacore and yellowfin), parvalbumin was recognized by only one of the eight tuna-allergic patient sera (Yamada et al. 1999). Monosensitivity to swordfish has been shown to be due to a species specific allergen at 25 kD and monospecific allergy to tropical sole was associated with IgE-binding to proteins at 6-7 and 40 kD (Asero et al. 1999; Kelso et al. 1995). More recent, aldehyde phosphate dehydrogenase has been characterized as a new codfish allergen (Das Dores et al. 2002b). Collagen, a high molecular weight protein has also been identified as a new fish allergen and demonstrated as a cross-reactive allergen among various species of fish (Hamada et al. 2001; Hamada et al. 2003a).

Tenggiri batang, the local name for Narrow-barred Spanish mackerel, *Scomberomorus commerson* Lacepede (Family Sciaenidae) is one of the most frequently consumed fish in Malaysia. It is found in the Indo-West Pacific from South Africa and the Red Sea through the Indo-Australian Archipelago to Australia and Fiji and north to Malaysia, Thailand, China and Japan. It also migrates to the eastern Mediterranean Sea by way of the Suez Canal. World catch for this fish increased from 55,452 tonnes in 1978 to 72,281 tonnes in 1981 (Mohsin & Ambak 1996). The aim of this study was to identify the IgE-binding proteins and major allergens of this fish.

MATERIALS AND METHODS

ALLERGEN PREPARATION

Extracts of *Scomberomorus commerson* were prepared according to the methods of Porcel et al. (2001) and Yamada et al. (1999) with slight modifications. A total of four extracts were prepared; two extracts of uncooked fish and two extracts of cooked fish. Each extract was prepared by homogenization of fish meat in 0.1 M phosphate-buffered saline (PBS, pH 7.2) at a 20% wt/vol solution, followed by either an overnight extraction with constant mixing at 4°C or a one hr extraction on ice. After centrifugation, the fish extracts were sterile filtered, freeze-dried and then stored at -20°C. Protein content of all extracts was determined by the Lowry-Biuret method following the manufacturer's instructions (Sigma Diagnostics, USA).

SODIUM DODECYL POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Protein profile for all the extracts was determined by SDS-PAGE using the procedures described by Hansen et al. (1997) and Yamada et al. (1999) with minor

modifications. The fish extract samples were denatured and heated in SDS reducing buffer. Each sample and the prestained molecular weight markers were resolved in a 12.0% separating gel with a 4% stacking gel using a Mini Protean 3 apparatus (BioRad, USA). The individual protein bands were identified with a Coomassie blue stain, and destained with a solution containing 40% methanol and 7% acetic acid. The molecular weight of each protein was estimated by comparison of the protein's gel position to those of broad range prestained molecular standards (Biolab, UK) in the same gel.

IMMUNOBLOTTING FOR DETECTION OF SPECIFIC IGE

To identify the IgE-binding components of *S. commerson*, immunoblotting was performed using sera of 37 patients sensitized to the fish. Serum from a non-allergic individual was used as a negative control. This study was approved by The Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia. The separated fish proteins were electrophoretically transferred from unstained SDS-PAGE gel to 0.45 mm pore size nitrocellulose membrane using a Mini Transblot System (BioRad, USA) in an electroblotting buffer. After blotting, the membrane was stained with Ponceau S (Sigma Diagnostics, USA) to verify the transfer of separated proteins. The nitrocellulose blot was then cut into 3 mm wide strip, washed with tris-buffered saline (TBS, pH 7.5) containing 0.05% Tween 20 (TTBS) and then blocked with 10% non fat milk in TBS. The strips were then incubated with individual patient's serum. The bound IgE on the strips were detected by incubation with biotinylated goat antihuman IgE (Kirkergaard and Perry Laboratories, UK), followed by incubation in streptavidin-conjugated alkaline phosphatase (BioRad, USA). Finally, the Alkaline Phosphatase Conjugate Substrate Kit was used to detect the bound IgE (BioRad, USA).

RESULTS

Figure 1 shows the Coomassie stained protein bands of the different preparation of fish extracts. The raw or uncooked extracts produced the most protein bands. At least 26 protein bands in the range of 11 to >175 kD were observed in the uncooked extracts while cooked fish extracts demonstrated fewer protein bands. The protein bands in the range of 40 to 90 kD were sensitive to denaturation by heat and were not present in the extracts cooked at 100°C.

Total protein contents of uncooked fish extracts with overnight and 1 hr extraction were 385 and 350 mg/dl, respectively, while the cooked extracts at 60°C and 100°C were 285 and 220 mg/dl, respectively. The uncooked fish extracts with overnight extraction had the highest protein content and the most protein bands. Hence, this extract was selected for use in skin testing and immunoblotting.

Results of IgE immunoblots performed using serum samples from 37 subjects with positive SPT to *S. commerson* are given in Figure 2 and Table 1. A total of

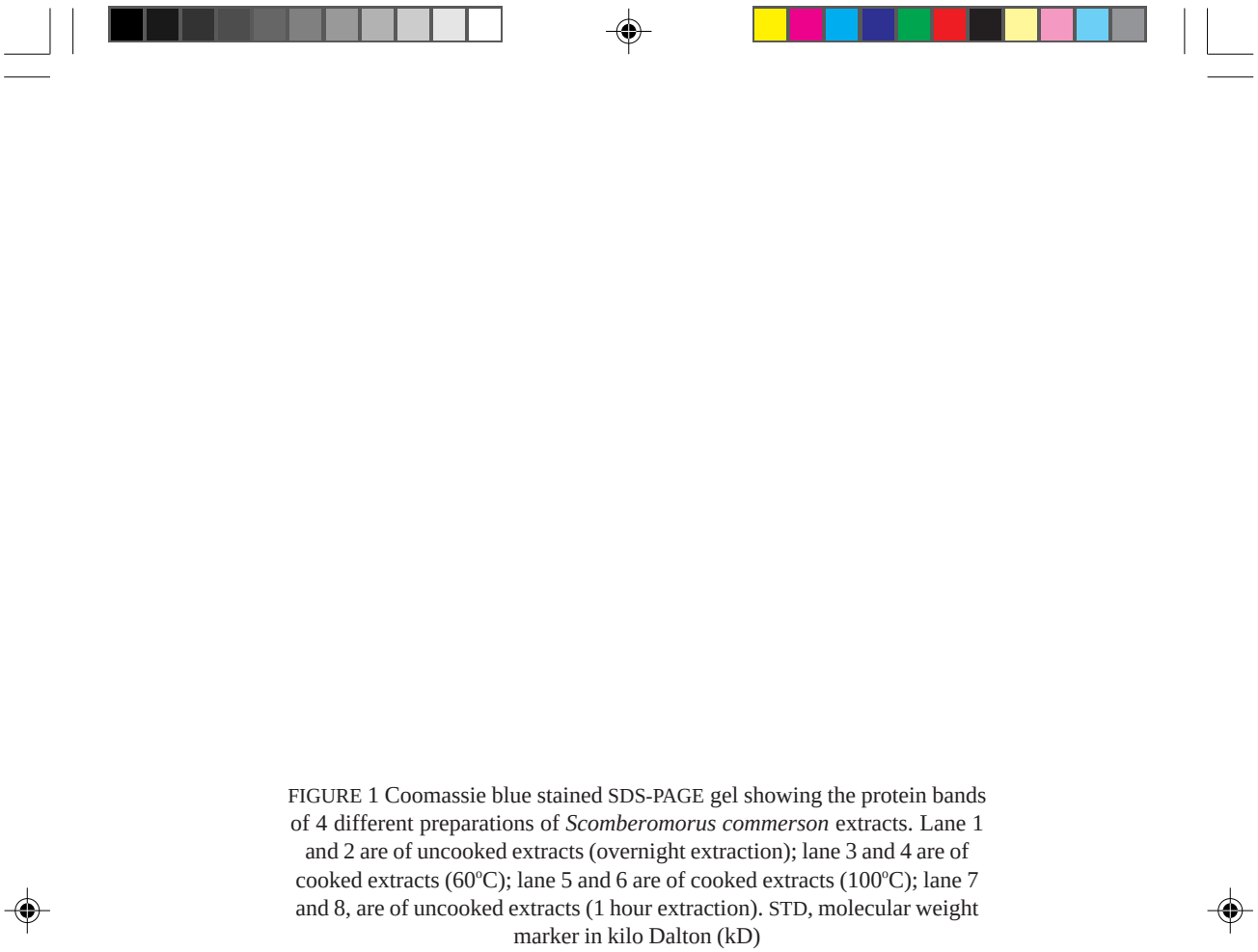

FIGURE 1 Coomassie blue stained SDS-PAGE gel showing the protein bands of 4 different preparations of *Scomberomorus commerson* extracts. Lane 1 and 2 are of uncooked extracts (overnight extraction); lane 3 and 4 are of cooked extracts (60^{o}C); lane 5 and 6 are of cooked extracts (100^{o}C); lane 7 and 8, are of uncooked extracts (1 hour extraction). STD, molecular weight marker in kilo Dalton (kD)

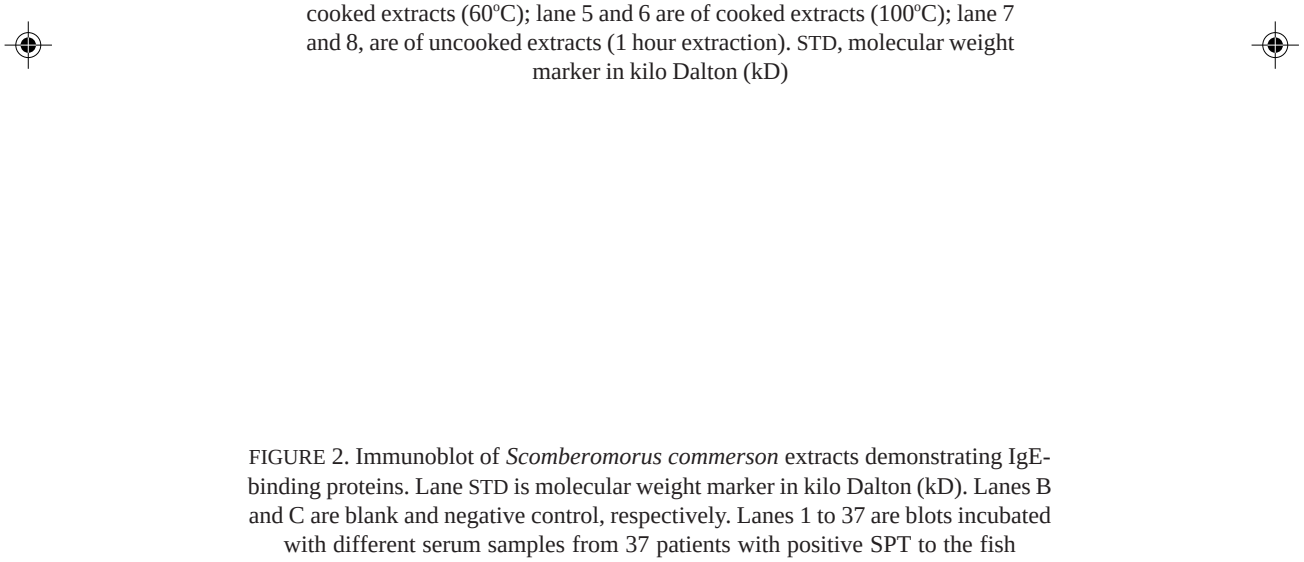

FIGURE 2. Immunoblot of *Scomberomorus commerson* extracts demonstrating IgE-binding proteins. Lane STD is molecular weight marker in kilo Dalton (kD). Lanes B and C are blank and negative control, respectively. Lanes 1 to 37 are blots incubated with different serum samples from 37 patients with positive SPT to the fish

17 different IgE-binding proteins between 11 to 151 kD were detected by the various sera. Proteins of ~50 kD and 42 kD demonstrated the highest frequency of IgE binding (62.2% and 51.4%, respectively) and was therefore identified as the major allergens of this fish. The major allergen is defined as the protein

TABLE 1. The frequency of specific IgE-binding proteins of the 37 patients with positive SPT to *Scomberomorus commerson* extracts

SUBJECTS	IgE-BINDING PROTEINS (kD)																
	151	125	90	80	70	60	~50	48	46	42	38	36	26	25	21	17	~12
1						X				X	X		X			X	X
2							X		X	X				X			
3		X				X	X		X	X			X	X	X	X	X
4		X							X	X							X
5							X			X							
6							X			X		X	X				
7						X	X		X	X						X	
8							X		X					X			
9							X		X	X		X	X				
10							X				X						X
11		X					X			X	X						
12										X	X						X
13						X	X			X			X				
14						X	X			X			X				
15	X	X					X			X			X	X			X
16						X	X		X				X	X			
17	X	X								X	X						
18							X		X		X	X					X
19	X					X				X	X	X	X				X
20											X						
21					X	X	X	X	X	X	X	X	X	X			
22										X		X	X				
23								X	X			X	X	X			
24													X				
25		X				X					X	X					
26							X										
27							X										
28							X										
29	X	X	X	X	X												
30									X	X			X				
31							X		X								
32							X										
33									X				X				
34												X					
35							X			X							
36							X										
37							X			X			X				
FREQUENCY (%)	10.8	21.6	2.7	2.7	5.4	29.7	**62.2***	5.4	35.1	**51.4***	27.6	29.7	37.8	18.9	2.7	8.1	21.6

* Major allergen

component(s) against which at least 50% of the tested sera demonstrate specific IgE. The other potential allergenic proteins were detected at 125 (21.6%), 60 (29.7%), 46 (35.1%), 38 (27.6%), 36 (29.7%), 26 (37.8%) and ~12 kD (21.6%).

DISCUSSION

The results from this study demonstrated that *S. commerson* contains 26 protein bands ranging from 11 to >175 kD. Although fish contains many proteins, only a few are allergenic (Porcel et al. 2001). For this fish, only 17 IgE-binding proteins were detected using sera of patients sensitized to the fish. These IgE-binding proteins ranged from 11 to 151 kD. Proteins at ~50 and 42 kD were identified as major allergens of this fish. Other minor allergens (detected by more than 20% sera) at 125, 60, 46, 38, 36, 26 and ~12 kD were also detected. Other studies have also demonstrated that fish generally contain a wide variety of IgE-binding proteins (Asero et al 1999; Hamada et al. 2001; Hansen et al. 1997; James et al. 1997; Kelso et al. 1995; Sten et al. 2004; Yamada et al. 1999).

Several studies have demonstrated that different species of fish have shared allergenic determinants and hence demonstrate considerable degree of cross-reactivity. Due to this cross-reactivity, a large number of patients are allergic to more than one species of fish (O'Neil & Lehrer 1995). The most widely reported cross-reactive allergen is the 12.5 kD parvalbumin, *Gad c* 1. However, we found only 21.6% of our patients had IgE-binding to the ~12 kD protein.

Some recent studies have also reported and characterized fish allergens of other molecular weights. A study on codfish identified a new parvalbumin allergen, a 24 kD protein named *Gad m* 1 (Das Dores et al. 2002a). The same investigators also found a 41 kD IgE-reactive protein purified from raw cod which is homologous to aldehyde phosphate dehydrogenase (APDH), an enzyme which is located within the cell (Das Dores et al. 2002b). It could be possible that the 42 kD IgE-reactive protein found in our study was similar to the 41 kD protein described by Das Dores et al. (2002b). Collagen, a high molecular weight protein (>100 kD) has also been identified as a new fish allergen (Hamada et al. 2001).

In this study we found that most of the IgE-binding proteins except for the proteins in the range of 40 to 90 kD were resistant to denaturation and were found in the cooked fish extracts. Generally, most seafood allergens are stable molecules and resist the effects of cooking, processing or digestive process (Lopata & Potter 2000). Other studies also reported that fish proteins in the range of 40 to 90 kD were sensitive to heat denaturation (Bernhisel-Broadbent et al. 1992; Porcel et al. 2001).

The major allergens for *Scomberomorus commerson* were the ~50 and 42 kD proteins. The ~12 kD protein commonly described as the major allergen for fish in other studies was only seen as a minor allergen for this fish.

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