

Changes in the Allergenicity during Different Preparations of Pomfret, Hilsa, Bhetki and Mackerel Fish as Illustrated by Enzyme-Linked Immunosorbent Assay and Immunoblotting

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Key Words

Bhetki • Enzyme-linked immunosorbent assay • Hilsa • IgE reactivity • Immunoblot • Mackerel • Pomfret

Abstract

Background: Although the identification and characterization of several fish allergens have already been reported, there is almost no data on Indian fish allergens and the effect of thermal processing on their allergenicity. This study aimed at the evaluation of the changes in the level of allergenicity of 4 highly consumed Indian fishes, i.e. pomfret, hilsa, bhetki and mackerel, that occurred after boiling and frying. **Methods:** In this study 110 patients with fish hypersensitivity as evidenced by clinical history and symptoms were recruited based on their positive skin prick test results. The raw, boiled and fried muscle extracts of the 4 fishes were prepared, and each extract was tested by ELISA and immunoblotting with patients' sera. **Results:** ELISA and immunoblotting studies demonstrated that the raw muscle extracts of pomfret, hilsa, bhetki and mackerel were allergenic. While the allergenicity of boiled and fried extracts of pomfret and hilsa was considerably reduced, maximum allergenicity of bhetki was demonstrated in the fried extract. The degree of allergenicity of

bhetki was demonstrated in the order fried > boiled > raw while that of mackerel followed the order raw > boiled = fried. **Conclusion:** The specific IgE-binding activity and immunoblot profile clearly showed that pomfret and hilsa fish allergens are heat-labile, while allergens of bhetki and mackerel maintained strong reactivity even after thermal treatment.

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Introduction

The potential cause of IgE-mediated fish hypersensitivity is due to ingestion of fish [1] or inhalation of vapors during processing or cooking of fish [2]. Consumption of fish products could lead to several allergic symptoms, i.e. skin rash, dermatitis, urticaria, angioedema, diarrhea, vomiting, respiratory distress and even fatal systemic anaphylactic reactions. Since allergens are mainly proteins or glycoproteins, their structure may be changed by various types of thermal treatment resulting in alteration of allergenicity. Certain food-allergenic proteins, such as milk, eggs, fish, peanuts and products thereof, are heat-stable, while others like soybean, cereals and celery are partially stable and still others, e.g. fruit of the Rosaceae family and carrots, are heat-labile [3, 4]. Many allergens are mostly resistant to proteolysis or digestive enzymes [5]. For food

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allergic populations most allergenic food proteins require extensive alteration to decrease their allergenicity. Hypoallergenic infant formulas are produced for infants with cow's milk allergy by heat denaturation and enzymatic hydrolysis of caseins or whey proteins [6]. The well-characterized fish allergen from codfish, Gad c 1, a parvalbumin, showed that the primary structure was resistant to thermal degradation [7, 8]. Although Gad c has always been described as a digestion-stable allergen, Untersmayr et al. [9, 10] recently reported that it was degraded immediately when subjected to peptic digestion. It was shown that different forms of heat affected the allergenicity in different ways. While roasting increased the allergenicity of peanut protein or left it unaltered, Ara h 1, boiling or frying reduced its allergenic potential [11]. There are reports that the IgE-binding capacity of the allergen increased relatively on storage due to the formation of new peptides [12]. Although the effects of thermal processing on eggs, milk and milk products have been studied extensively, only a few have focused on seafood proteins [13]. Fish processing like canning and lyophilization altered some of the major fish allergens responsible for IgE-mediated fish allergy [14].

Our earlier study [15] showed hypersensitivity to hilsa (*Tenualosa ilisha*) and pomfret (*Pampus argenteus*) fish allergens. Fish are usually subjected to some form of heat treatment, especially boiling or frying, prior to consumption. Since there is little information available about heat-sensitive or heat-resistant fish allergens, the objective of our present study is to evaluate the effect of thermal processing on the level of allergenicity of 4 highly consumed Indian fishes, viz. pomfret (*P. argenteus*), hilsa (*T. ilisha*), bhetki (*Lates calcarifer*) and Indian mackerel (*Rastrelliger kanagurta*). The significance of the results is interpreted statistically.

Materials and Methods

Patients

A total of 161 patients who suffered from allergic symptoms caused by different foods visited the Allergy and Asthma Research Clinic Kolkata. Among them 110 patients who had a clinical history of fish hypersensitivity as well as reported allergic symptoms after ingestion of fish and showed positive skin prick test (SPT) results with any of the 4 fish allergens (raw extract) were recruited in this study with their informed consent. Of them 92 were found to be hypersensitive to all 4 fishes. To make the sample size substantial 50 out of 92 patients were chosen for this study by random selection. Among these 50 patients 12 were recruited upon receipt of their consent for an SPT study with boiled and fried extracts of the 4 fishes. Sera from these patients were

used, and those from nonallergic individuals served as negative controls. The Human Ethical Committee of our Institute approved this study.

Preparation of Fish Extracts

Fresh fish species, i.e. hilsa, pomfret, bhetki and mackerel, were procured from the city municipal market. The raw extract of each fish was prepared by homogenization of boneless fish muscles in 0.1 M of PBS (pH 7.2) at 4°C followed by overnight stirring. The supernatant from each preparation was collected by centrifugation at 10,000 rpm for 20 min and concentrated by YM 10 membrane filtration (Amicon). The boiled extract of each fish was prepared by boiling raw boneless muscles with 0.1 M of PBS (pH 7.2) for 10 min at 90°C. The supernatant was collected and concentrated as above. The fried extract of each fish species was prepared by lightly frying fish muscles with mustard oil for 5 min and subsequently placed on filter paper to remove the oil. The dried fish muscles were homogenized in 0.1 M of PBS and centrifuged as above. All the extracts were preserved at -20°C for further experiments. The protein concentration of the extracts was determined by the method of Lowry et al. [16].

Skin Prick Tests

The SPTs were performed with the raw, boiled and fried extracts of the 4 fishes in the Allergy and Asthma Research Clinic Kolkata by an expert clinical technician under the supervision of Dr. Arijit Das, director of the clinic. The extract was prepared in PBS-glycerol (1:20 w/v) and sterilized by millipore filtration (0.22 µm). A drop (10 µl) of the extract was placed on the volar aspect of the forearm with a disposable (26-gauge) hypodermic needle and scratched. The skin reaction was measured after 20 min, and a wheal of ≥ 3 mm in diameter surrounding the erythema was regarded as a positive response. As negative control 50% glycerol in PBS was taken and histamine (1 mg/ml) as positive control.

Enzyme-Linked Immunosorbent Assay

The specific IgE reactivity of different fish extracts, which were prepared under different conditions, was tested by enzyme-linked immunosorbent assay (ELISA). Each well of a microtiter plate was incubated with 100 µl of different fish extracts (2 µg/well) in 10 mM of carbonate buffer (pH 9.6) overnight at 4°C, washed with PBS-T (10 mM of PBS, pH 7.2, containing 0.05% Tween 20) and incubated again with 1% BSA in PBS at 37°C for 1 h. After usual washing the wells were incubated with patients' sera (100 µl, 1:20) for 1 h at 37°C and then with 100 µl of goat antihuman IgE-horse-radish peroxidase (1:1,000) in PBS for 1 h at 37°C. After washing the wells were incubated with 100 µl of *o*-phenylenediamine (1 mg/ml in 0.05 M of citrate phosphate buffer with 0.01% H₂O₂, pH 5) for 20 min in the dark at 25°C. The absorbance was measured at 492 nm after adding 3 N sulfuric acid in an ELISA reader (Flow Laboratory, UK). At each step washing was performed thrice, each of 5 min duration. Absorbance ≥ 0.08 suggested hypersensitivity. Serum from nonallergic individuals was used as negative control according to Calenoff et al. [17]. A panel of serum samples from nonallergic subjects was estimated for specific IgE. The mean of these values was regarded as control value, and thus a threshold sensitivity was established. Sera from nonallergic subjects gave 0. D values ranged between 0.04 and 0.07 and were used as a background control. The ELISA readings were the average of the triplicate assays [18].

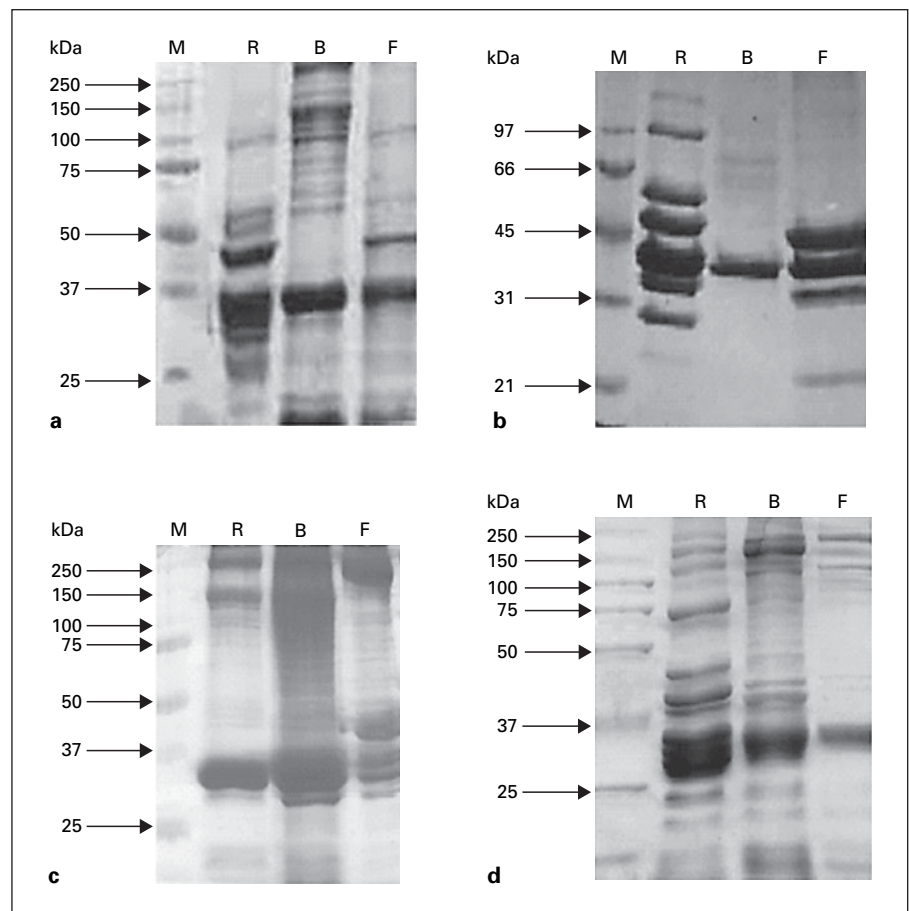


Fig. 1. 10% SDS-PAGE profiles of pomfret (a), hilsa (b), bhetki (c) and mackerel (d) extracts. The bands were stained with Coomassie blue (R250). Lanes M: Precision plus proteinTM standards (BioRad), R: raw extract, B: boiled extract, F: fried extract. Each well was loaded with 10 µg of protein.

Statistical Analysis

The results of specific IgE were evaluated statistically. From a population of 51 patients who were allergic to food except fish, 20 (taken by random sampling) served as a negative control. Among the population of 92 patients (positive to all 4 fishes) 50 were considered for statistical analysis by random sampling. The mean values were compared by Student's *t* test for unpaired samples [19].

SDS-PAGE and Immunoblotting

After treatment under different conditions the different fish extracts were mixed with electrophoresis sample buffer (10% SDS, 40% glycerol, 5% β -mercaptoethanol and 0.1% bromophenol blue in Tris buffer, pH 6.8), digested at 100°C for 5 min and separated on 10% SDS-PAGE (protein: 10 µg/lane). Protein bands were visualized with Coomassie brilliant blue (R250) [20]. Immunoblotting was performed by electrophoretic transfer of protein bands from polyacrylamide gel to nitrocellulose membrane followed by blocking with 1% BSA [21]. After washing the strips were incubated with pooled sera (1:20 in saline) for 1 h. The strips were again washed and incubated with anti-human IgE-horseradish peroxidase (1:1,000) for 1 h. After washing the protein bands were developed with diaminobenzidine and 0.01% H_2O_2 in sodium acetate buffer (pH 5). At each step washing was done thrice with saline-Tween 20, each of 5 min duration.

Results

SDS-PAGE Profile of Raw, Boiled and Fried Pomfret, Hilsa, Bhetki and Mackerel Extracts

Figure 1 shows polypeptide band profiles of pomfret extracts. Most of the bands in the raw pomfret extract were in the range of 25–60 kDa, the prominent ones being approximately at 31, 34, 35, 36, 37 and 43 kDa, respectively. While in boiled extract, some of the low-molecular-weight bands disappeared or faded away, several additional bands of high molecular weight above 60 kDa appeared. Most of the bands in raw and boiled extract disappeared on frying. The bands at 35, 36, 60 and 100 kDa were present in all the 3 extracts. The 43-kDa band though was present in raw and fried extracts, but absent in boiled extract.

The raw extract of hilsa showed 6 major bands between 28 and 97 kDa (fig. 1). Contrarily the boiled extract displayed only 1 prominent band of approximately 40 kDa and some minor bands of higher molecular weight. The

Table 1. Clinical summary of 50 fish-hypersensitive patients taken by random sampling

Subject	Age years	Sex	Symptoms	SPT (raw extracts)				Family history
				P	H	B	M	
1	18	M	skin rash	++	+++	+	++	none
2	65	F	breathlessness	++	+++	+++	+++	none
3	36	M	skin rash	+++	+++	++	++	none
4	19	F	skin rash	++	+	+	+	asthma
5	53	M	breathlessness, diarrhea	+++	++	+++	+++	asthma
6	43	F	breathlessness	+	++	++	++	none
7	50	M	severe skin rash	+	+	+	+	none
8	46	F	breathlessness, skin rash	+++	+++	++	+++	asthma
9	36	F	breathlessness	++	+	+++	++	asthma, eczema
10	35	F	breathlessness, diarrhea	++	++	++	+	eczema
11	65	M	asthma, reddening of eyes	++	+++	+++	+++	asthma, urticaria, eczema
12	40	F	urticaria, vomiting	+	+	++	+	none
13	45	M	reddening of eyes, sneezing, skin rash	++	++	++	+	none
14	33	F	skin rash, reddening of eyes	++	+	++	++	none
15	11	M	breathlessness, sneezing	++	+++	++	++	asthma, eczema
16	23	F	diarrhea, vomiting	+	+	+	+	asthma, urticaria
17	14	M	skin rash, asthma	+++	+++	+++	++	asthma, rhinitis, urticaria
18	40	M	breathlessness, skin rash	+++	+++	+	+++	eczema, urticaria, asthma
19	49	F	diarrhea, urticaria	++	++	+++	+	asthma
20	24	M	sneezing, urticaria	+	+	++	+	urticaria
21	14	M	skin rash	++	++	++	+	rhinitis
22	16	M	breathlessness, diarrhea	++	+++	+++	+++	urticaria, asthma
23	85	F	reddening of eyes	+++	++	++	++	rhinitis, asthma
24	2	F	sneezing, breathlessness	++	+++	+	+++	urticaria, eczema
25	20	M	skin rash, diarrhea	+	++	++	++	eczema, urticaria
26	45	M	skin rash, reddening of eyes	+	+	+	+	none
27	29	M	skin rash	+++	++	++	++	asthma, urticaria
28	43	M	urticaria	+++	+++	++	+++	none
29	51	F	sneezing, skin rash	++	+++	++	++	none
30	9	M	skin rash	++	++	++	+	urticaria, asthma
31	10	F	breathlessness, vomiting	+	+	+	+	asthma
32	31	M	urticaria, diarrhea	++	++	++	++	none
33	14	F	sneezing	+	+	+	++	asthma, eczema
34	32	F	breathlessness, reddening of eyes	++	+++	++	+++	asthma, eczema, urticaria
35	40	M	breathlessness, vomiting	++	+++	++	+	none
36	18	F	breathlessness, sneezing, skin rash	+++	++	++	++	asthma, eczema, urticaria
37	30	F	reddening of eyes, diarrhea	++	++	+	++	asthma
38	30	F	skin rash	++	+	+	+	asthma, eczema, urticaria
39	35	F	breathlessness, vomiting	+++	+++	+++	++	asthma, eczema, urticaria
40	7	F	skin rash	++	+	++	+++	asthma, eczema
41	47	F	skin rash	+	++	+	+	none
42	28	M	diarrhea, urticaria	+++	++	+++	++	asthma
43	16	M	diarrhea	+	+	+	++	none
44	20	F	vomiting, urticaria	++	++	++	++	none
45	37	M	skin rash, diarrhea	+++	+++	++	+++	urticaria
46	35	M	sneezing, diarrhea, reddening of eyes	++	+	+	+	eczema, urticaria
47	3	M	skin rash, breathlessness, diarrhea	++	+++	+	++	asthma
48	21	M	skin rash, reddening of eyes	+	+	++	+	asthma
49	15	F	skin rash	++	+++	+++	+++	urticaria, eczema
50	42	M	skin rash, reddening of eyes, diarrhea	++	++	+++	+	none

Wheal diameter: + = 3–5 mm; ++ = >6 mm; +++ = >6 mm with pseudopodia; P = pomfret extract; H = hilsa extract; B = bhetki extract; M = mackerel extract.

Table 2. SPT results of patients with boiled and fried preparations of the 4 fish extracts

Serial	Subjects No. ¹	Boiled fish extract				Fried fish extract			
		pomfret	hilsa	bhetki	mackerel	pomfret	hilsa	bhetki	mackerel
1	2	++	++	++	+++	+	+	+++	++
2	4	++	+	+++	+++	++	+	+++	+
3	8	+	++	+++	++	++	+	++	++
4	12	++	++	++	+++	+	++	++	+++
5	15	+	+	++	+	+	++	+++	++
6	21	++	++	+++	++	++	+	++	+++
7	26	+++	+++	++	+	+	++	+++	+
8	29	+	+	+	++	++	+	++	++
9	37	++	++	++	+++	+	++	+	+++
10	40	++	+	+++	+++	++	+	+++	++
11	43	+	+	++	++	+	+++	+	+
12	48	+++	+	+++	+++	++	+	++	+

Wheal diameter: + = 3–5 mm; ++ = >6 mm; +++ = >6 mm with pseudopodia.

¹ Same as table 1.

fried extract contained 4 polypeptides of approximately 21, 30, 31, 40 and 43 kDa, respectively.

In bhetki the raw extract (fig. 1) consisted of 5 major polypeptides at 34, 35, 36, 150 and 250 kDa. Besides there were some minor bands ranging from 40 to 50 and 75 to 100 kDa, respectively. The 34- and 35-kDa bands became more prominent in the boiled extract. Many bands of high molecular weight and 1 of 32 kDa appeared in the boiled preparation. The polypeptide bands of 32, 34, 35 and 36 kDa were also present in the fried preparations. Interestingly the 22-kDa band, which was present in the raw and boiled extracts, faded away in the fried extract. A band around 40 kDa appeared to be prominent in the fried extract in comparison to the raw and boiled ones. The band of 150 kDa almost faded away, whereas the 250-kDa band intensified in the fried extract.

Figure 1 also shows the SDS-PAGE band profile of different preparations of mackerel. Several conspicuous bands ranging between 20 and 250 kDa appeared in the raw extract, 3 being intense at 31, 34 and 36 kDa, respectively. Upon boiling most of the polypeptides seen in the raw extract lost their intensities, but 3 polypeptide bands of 20, 125 and 225 kDa, respectively, intensified. In the fried preparation all bands of polypeptides except 2 (125 and 225 kDa) appeared very faint.

SPT Reactivities

Clinical reports showed that out of 161 patients, 110 (68.32%) gave positive SPTs with 1 or more fish extracts

and 92 (83.63%) of them showed positive results for all 4 fish extracts. Among these 92 patients, 50 underwent SPT with raw extracts of all 4 fishes. Their clinical history and SPT results are summarized in table 1. Among the 50 patients 12 were subjected to SPT with boiled and fried extracts of the 4 fishes. The results are presented in table 2. Among the 110 patients with positive SPT results, 62% showed skin rash, 63% breathlessness, 66% reddening of the eyes, 49% sneezing, 21% asthma, 9% diarrhea, 9% urticaria and 3% vomiting. Of them 62 patients reported allergic symptoms within 30 min, 37 of them within 30–60 min and 11 patients after 1 h following the ingestion of fish.

Specific IgE of Patients

ELISA was performed to measure specific IgE levels in the patients who had a positive SPT. The absorbances were compared with those of the control individuals according to the criteria of Calenoff et al. [17]. Patients showing values (mean $\pm$ 2 SD) greater than that of the controls were considered positive (the cutoff value being 0.08). Figure 2 represents the ELISA results of the 4 fish extracts under different conditions with pooled sera. The results were in concordance with the clinical symptoms and the patients' SPT results. No significant increase in IgE level was detected in the control subjects.

Figure 3 represents the distribution pattern of specific IgE-binding affinity within a population of 50 patients to raw and thermally processed fish allergens. The

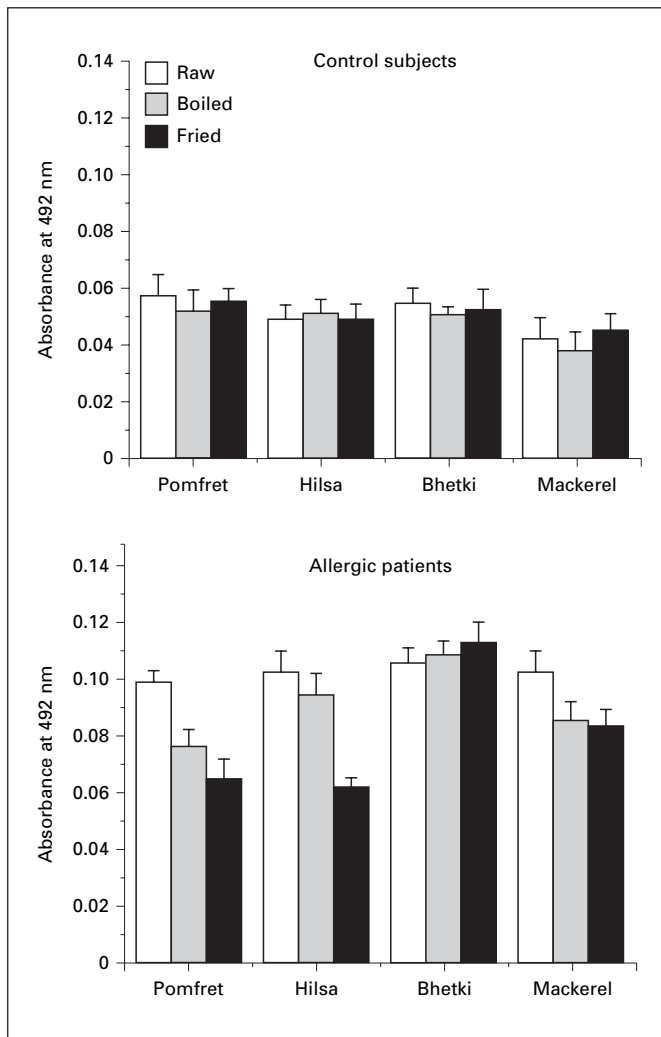


Fig. 2. Comparison of specific IgE-binding activity between raw, boiled and fried extracts of pomfret, hilsa, bhetki and mackerel by using pooled sera of patients and normal individuals by ELISA.

distribution curve of the histogram revealed that the region where the patients' specific IgE values (cutoff limit 0.08) clustered was markedly different from those obtained with control subjects. The specific IgE values obtained for the 4 fish allergens under different conditions showed a high significance, with a confidence limit greater than 99.99% ($p < 0.001$; table 3). There was a marked decrease in specific IgE reactivities in the boiled as well as fried extracts in pomfret and hilsa. However, few hilsa-allergic patients showed a marginal activity towards fried extract.

In bhetki and mackerel all the extracts showed a positive IgE-binding reactivity. In bhetki boiling and frying

Table 3. Comparison of specific IgE-binding activity to raw and thermally processed pomfret, hilsa, bhetki and mackerel extracts between control and allergic subjects

Subject	Statistic value	Raw	Boiled	Fried
Control, n = 20	mean	0.058	0.051	0.048
	SD	0.012	0.015	0.020
	SE	0.002	0.002	0.003
Pomfret, n = 50	mean	0.099	0.076	0.065
	SD	0.020	0.021	0.010
	SE	0.003	0.004	0.003
Hilsa, n = 50	mean	0.103	0.075	0.062
	SD	0.033	0.020	0.017
	SE	0.003	0.004	0.003
Bhetki, n = 50	mean	0.106	0.108	0.113
	SD	0.020	0.025	0.021
	SE	0.003	0.0026	0.003
Mackerel, n = 50	mean	0.103	0.086	0.084
	SD	0.032	0.020	0.018
	SE	0.004	0.003	0.002

n = Number of patients.

gradually increased the binding capacity of the allergen in the order fried > boiled > raw, whereas in mackerel the binding pattern was raw > boiled ≈ fried.

Immunoblot Study

Figure 4 shows the immunoblot results of allergenic bands of all 4 fish extracts prepared under different conditions. Immunoblotting of raw pomfret extract showed allergenic bands at 36, 37, 43, 48, 50 and 82 kDa (fig. 4). In boiled and fried extracts only 1 IgE reactive band at 100 kDa was noticed.

Among the 3 hilsa preparations raw extract showed IgE-binding activity which presented polypeptide bands nearly at 37, 40, 43, 50, 64, 65 and 94 kDa, respectively. No immunoreactive band was observed in boiled or fried extract. However, the fried one showed a few specific IgE-binding polypeptides with some other patients' sera (data not shown).

The immunoblot profile of bhetki was different from those of pomfret and hilsa. In bhetki the fried preparation showed the maximum IgE-binding activity, since it produced many IgE-binding bands between 35 and 150 kDa. The boiled one showed moderate binding, whereas weak IgE-reactive bands were observed in the raw extract. The band of approximately 35 kDa was present in all 3 types of preparations and showed maximum intensity in the boiled preparation. The same prepara-

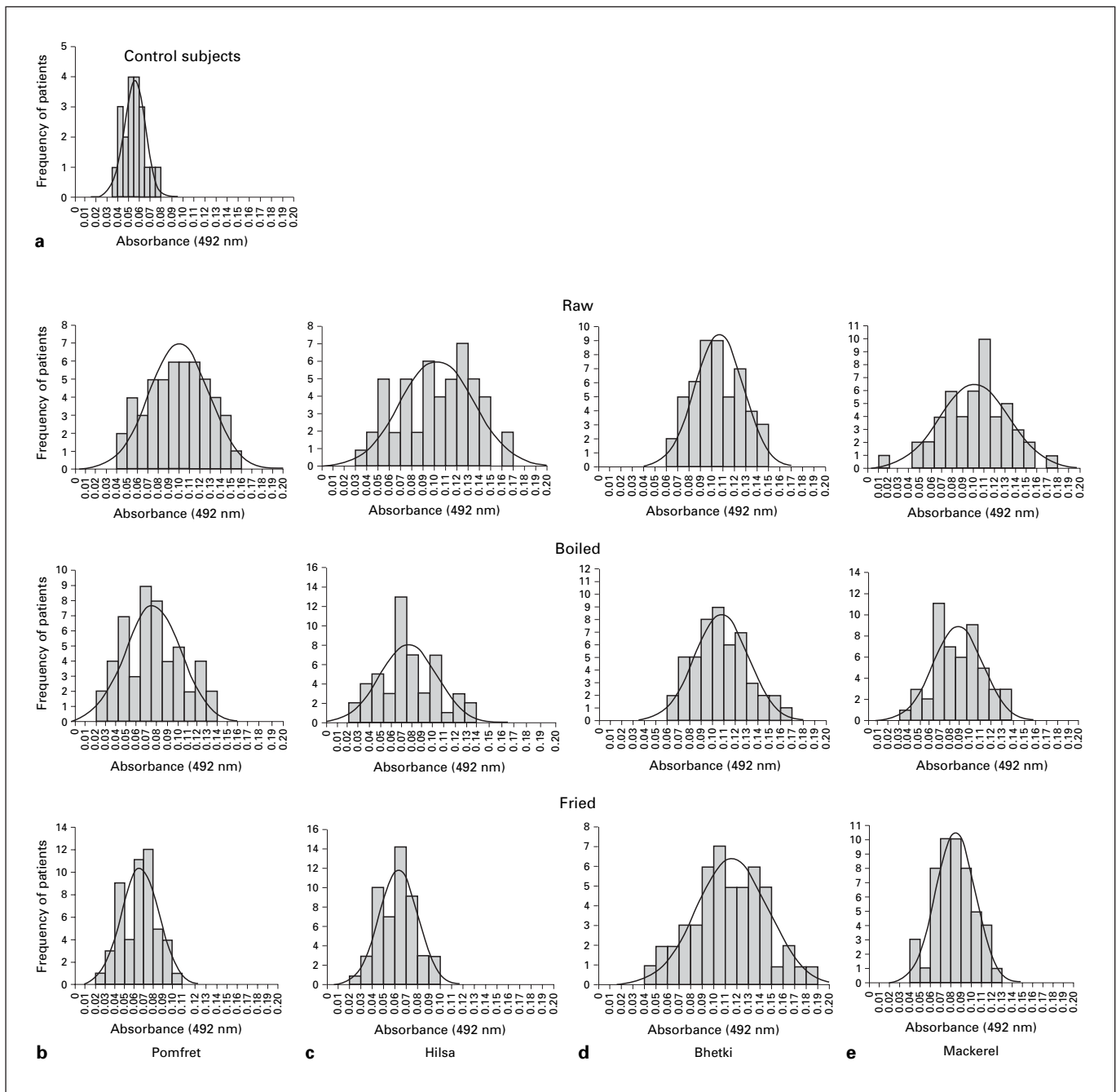


Fig. 3. Statistical distribution curves of patients in raw, boiled and fried extracts of pomfret, hilsa, bhetki and mackerel compared with control subjects. **a** Control subjects. **b** Pomfret. **c** Hilsa. **d** Bhetki. **e** Mackerel extract.

tion also contained 2 major polypeptides of 55 and 70 kDa, respectively.

In mackerel maximum IgE binding was observed in the raw extract. The prominent IgE-binding bands were of 30, 37, 43, 50, 60 and 80 kDa, respectively. The boiled

extract showed almost all the reactive bands present in the raw extract. One prominent band of approximately 23 kDa appeared in the boiled extract, whereas it was faint in the raw and fried extracts. The fried extract revealed a striking loss of many of the protein bands.

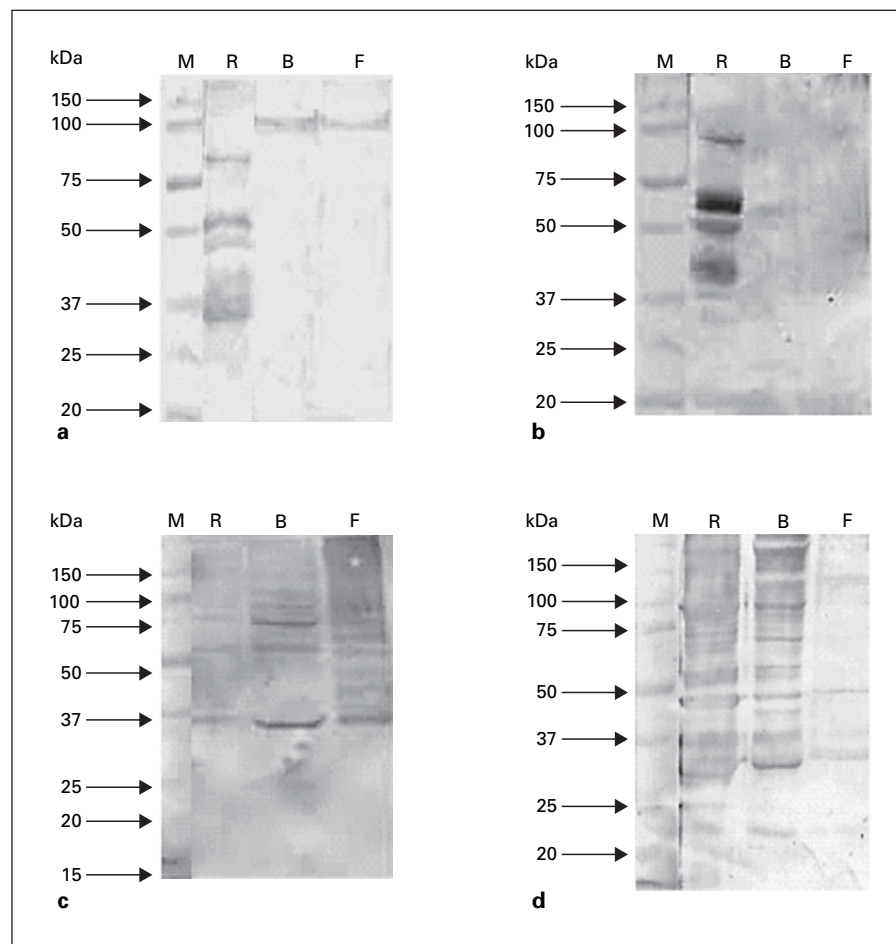


Fig. 4. Identification of IgE-reactive polypeptide by immunoblot with pomfret (**a**), hilsa (**b**), bhetki (**c**) and mackerel (**d**) extracts. Lanes M: Precision plus protein standards (BioRad), R: raw extract, B: boiled extract, F: fried extract.

Discussion

This study is the first attempt to compare the levels of allergenicity in raw and thermally processed extracts (boiled and fried preparations) of 4 different kinds of Indian fish.

Fish is one of the most common foods causing allergy, especially in coastal countries. The major fish allergen in codfish is parvalbumin (Gad c 1), a 12-kDa thermostable, calcium-binding protein which is also present in other fish species [22, 23]. However, several studies were inconsistent with the foregoing report on fish allergens. Das Dores et al. [24] reported an allergen of 41 kDa from codfish. Fish gelatin (type 1 collagen) was identified as a new allergen [25]. Tuna extract lacked the 12-kDa protein band but contained a protein of ~40 kDa [14]. Previously we [15] identified several allergens in hilsa and pomfret. However, immunoblot experiments did not reveal any low-molecular-weight protein like parvalbumin.

It is a matter of concern how the IgE-binding properties of a food allergen are modified after cooking, as there is no general rule regarding the effects of thermal treatment on allergenicity. Any type of processing like boiling, frying, grilling, salting, drying or freezing might create new allergenic epitopes as well as abolish existing ones. Whether and how heat treatment may significantly alter the allergenicity of a food is therefore a difficult question. Roasting can cause the Maillard reaction, which increases the allergenicity of some foods like peanut [26]. Heating reduces the allergenicity of cow's milk, but does not completely eliminate it [27]. Sometimes heating totally eliminates the allergenicity of chitinase-containing food [28]. In contrast, nonspecific lipid transfer proteins are highly stable to pepsin treatment and thermal processing and show a higher allergenic potency [29].

Our study demonstrated that boiled or fried preparation yielded markedly different SDS-PAGE profiles than those observed in the raw extracts. Many polypeptide bands pres-

ent in the raw extract of pomfret and hilsa disappeared in the boiled preparation, while the appearance of many new high-molecular-weight bands was observed in all the fishes except hilsa. After frying some other changes in polypeptide patterns were observed. All of them must be a reflection of a progressive denaturation of the proteins present in the raw extract with the consequent formation of aggregates resulting in some high-molecular-weight bands.

Our immunoblot study revealed that the fried or boiled pomfret and hilsa allergens showed almost no IgE-binding activity and could be considered as heat-labile. Contrarily the allergenicity of the boiled and fried bhetki was somewhat increased, suggesting that some new epitopes might have been exposed for IgE binding. Immunoblotting showed that the raw extract of Indian mackerel was more allergenic than the boiled and fried preparations, both of which showed almost the same allergenicity. In mackerel a low-molecular-weight ~23-kDa allergen was detected in all the 3 extracts. It is interesting to note that except mackerel our result could not detect any parvalbumin-like low-molecular-weight protein in the fishes studied. There is a loss of conformational IgE-binding epitopes upon heating. Like peanut protein allergens bhetki and mackerel allergens were found to be resistant to thermal denaturation. This indicated that conformational epitopes may not always be vital and play a minor role in their allergenicity [11]. Also it may be possible that the epitopes are present at the sites of the protein that are not heat-sensitive. In the heat-labile allergens of hilsa and pomfret extracts, it may be assumed that their allergenicity is an intrinsic property based on structural features.

The statistical analysis also contributed to the knowledge of allergenicity of the 4 fishes. The specific IgE data were analyzed statistically by taking significant numbers of negative controls. From the various distribution curves it is well understood that the specific IgE value was remarkably decreased for pomfret and hilsa allergens on thermal processing. There was no significant change in the level of specific IgE for the thermally treated bhetki and mackerel allergens.

The changes in the allergenicity of fish protein during heating showed variation in individual patients. Immunoblotting revealed that there was no patient having an IgE-binding activity with fried hilsa, though 2 exhibited binding (data not shown). In conclusion, our findings show that boiling or frying may decrease or increase the allergenicity depending on the individual IgE reactivity to the protein involved. This study contributes to the knowledge of the allergenicity of highly consumed Indian fish and suggests that some fish allergens are not necessarily heat-stable.

Acknowledgments

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