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ORIGINAL ARTICLE **Allergens**

# Identification of sole parvalbumin as a major allergen: study of cross-reactivity between parvalbumins in a Spanish fish-allergic population

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**Summary**

*Background* Fish allergy is becoming an important health problem in Spain, a country with the third highest level of fish consumption after Japan and Portugal. The most common fish allergens are parvalbumins. In our area, the most widely consumed fish species are lean, such as whiff (*Lepidorhombus whiffiagonis*) and sole (*Solea solea*). Adverse reactions to fish are usually related to these species, a fact that is largely unknown to allergists in other countries.
*Objective* The aim of this study was to identify and purify the major allergen implicated in allergic response to sole and evaluate the IgE cross-reactivity of purified parvalbumins from whiff and sole, which are phylogenetically close, and more distant species (i.e. cod and salmon).
*Methods* Eighteen Spanish fish-allergic patients with a positive history of type I allergy to fish were recruited from the clinic. Total protein extracts and purified parvalbumins from whiff and sole were tested for their IgE-binding properties by combining two-dimensional Western blotting and mass spectrometry. The extent of cross-reactivity between these parvalbumins along with cod and salmon parvalbumins was investigated by IgE ELISA inhibition assay.
*Results* An IgE-binding spot of approximately 14 kDa was identified as parvalbumin and confirmed as a major allergen in sole extract, which is recognized by almost 70% of the patients. Whiff parvalbumin was recognized by 83.4% of the patients. High cross-reactivity was determined for all purified parvalbumins by IgE inhibition assay.
*Conclusions and Clinical Relevance* Sole and whiff parvalbumin were confirmed as major allergens. The parvalbumins of sole, whiff, cod and salmon were highly cross-reactive, thus suggesting a high amino acid sequence identity between them.

**Keywords** cross-reactivity, fish allergy, sole parvalbumin, two-dimensional immunoblotting
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## Introduction

Food allergies have been increasing in prevalence over the last 10 years. Currently, about 2–8% of the population of Western countries suffers from some type of food allergy whose impact ranges from a mere inconvenience to a life-threatening condition [1].

Fish and fish-derived products are an important component of human nutrition, and constitute a valuable source of polyunsaturated fatty acids such as ω-3, vitamins and high-assimilation proteins. However, fish represent one of the most important groups of allergens in the induction of immediate (type I) food hypersensitivity, along with egg, cow's milk and peanut [2].

High intake of fish and seafood are traditional dietary components in a number of populations world-wide, such as in Mediterranean countries. Prevalence of fish allergy is proportional to the amount of fish intake in the local diet [3]. Therefore, fish allergy is an important health problem in Spain, the third-highest country in fish consumption after Japan and Portugal.

In our surrounding area, the most common fish in children's diets are those which are lean; the two most frequently consumed species are whiff (*Lepidorhombus whiffiagonis*) and sole (*Solea solea*), which belong to the order Pleuronectiformes. Adverse reactions to fish are usually linked to these species, which are largely unknown to allergists in other countries.

Other species, such as codfish (*Gadus morhua*), which belongs to the family Gadidae and salmon (*Salmo salar*), which belongs to the family Salmonidae, are consumed in great quantity elsewhere and have been the subject of

numerous studies. Most fish-allergic patients do not tolerate cod, which is why cod is commonly used as a reference point for the study of other fish allergens [4]. The major allergen of Baltic cod (*Gadus callarias*), Gad c 1, which has been very well characterized, was found to be a parvalbumin [5] and is considered to be a fish and amphibian pan-allergen [3]. The major allergenic protein in salmon and whiff was also found to be a parvalbumin and was given the name Sal s 1 [6] and Lep w 1 [7], respectively.

Fish parvalbumins are major allergens for individuals who suffer from IgE-mediated hypersensitivity to fish products [2, 5, 8, 9]. Significant homology was observed between cod parvalbumin and other fish species [2, 4, 8–10], but very little is known about the main allergens implicated in allergic reaction to sole or its parvalbumin. Triose phosphate isomerase β has only recently been described in a case report as being an allergen in sole [11]. In the case of whiff, parvalbumin has been recently described as a major allergen (Lep w 1) and its cDNA sequence has been reported [7]. Based on the phylogenetic relationship between whiff and sole, parvalbumin is also expected to represent a major allergen in sole.

The aim of this study was to use proteomic approaches to identify the major allergen implicated in allergic response to sole, one of the most consumed fish species in the Mediterranean diet. We also wanted to confirm whiff parvalbumin as an allergen by using two-dimensional (2D) immunoblotting, which has never been tried before. In this study, we also examined the IgE cross-reactivity of purified parvalbumins from whiff and sole as examples of species from the same taxonomical order as well as natural purified parvalbumins from Atlantic cod (*G. morhua*, Gad m 1) and Atlantic salmon (*S. salar,* Sal s 1) as examples of taxonomically distant species.

## Methods

### *Patient characteristics*

Sera from 18 Spanish fish-allergic patients (four adults, 14 children) with a positive history of type I allergy to fish were selected from Hospital Fundación Jiménez Díaz and Hospital Niño Jesús of Madrid. Allergy diagnosis was based on the criteria described by Wood [12]. All patients had a history of allergic symptoms appearing when eating fish (mainly, whiff, sole, cod and salmon) in addition to positive skin prick tests to fish extracts, which were carried out by following the technique described by Dreborg et al. [13], according to EAACI guidelines [14]. Diagnosis of IgE-mediated fish allergy was verified by determination of fish-specific IgE antibodies using the Pharmacia CAP-FEIA System (Pharmacia, Uppsala, Sweden). All of them had specific IgE antibodies against purified fish parvalbumin. The criteria for selection were (1) a convincing clinical history of allergic reactions after fish ingestion, (2) a positive prick by prick test response to fish and (3) specific IgE levels to any of the four purified fish parvalbumins (whiff, sole, cod and salmon purified parvalbumins). Sera from five healthy volunteers with negative history for any type I allergy were included as controls. All subjects provided informed consent and all protocols received local institutional review board approval.

### *Preparation of fish extracts*

All fish species were purchased from a fish market in Madrid. Thirty grams of cooked whiff and sole filets (100 °C for 30 min) were extracted in 10% (w/v) of phosphate-buffered saline (PBS), pH 7.2, with 1 mM PMSF at 4 °C undergoing constant stirring for 24 h. After centrifugation at 12 000 **g** for 30 min at 4 °C, the supernatants were delipidated with diethyl ether and dialysed against 0.1 M ammonium bicarbonate. Protein extracts were lyophilized and stored at 4 °C. Total protein content was determined by Coomasie Plus (Bradford) Assay (Pierce, Rockford, IL, USA).

### *Purification of parvalbumins*

Cooked muscle from fish was used as a starting material to purify parvalbumins. Purification of whiff, sole, cod and salmon parvalbumins was carried out based on the protocol described by Bugajska-Schretter et al. [15] with some modifications. The parvalbumin-enriched fraction was lyophilized, redissolved in Tris 10 mM, pH 7.5, and applied to a MonoQ column (MonoQ 5/50 GL, GE Healthcare, Uppsala, Sweden) in an ÄKTA System (GE Healthcare). Fractions containing purified parvalbumin isoforms were eluted with a linear salt gradient from 0 to 1 M NaCl. We analysed parvalbumin fraction from chromatography by mass spectrometry (MS) to check for purity. Fractions containing more than 90% of parvalbumin were used for experiments. In these fractions, only parvalbumin was recognized by patient sera.

### *Sodium dodecyl sulphate-polyacrylamide gel electrophoresis*

SDS-PAGE was carried out according to Laemmli [16] under reducing conditions using the Hoofer SE 600 electrophoresis system (GE Healthcare). Twenty micrograms of protein extract and 5 μg of purified parvalbumin were applied per lane. To ensure proper protein separation and visualization, the gels were stained with PageBlue Protein Staining Solution (Fermentas International Inc., Burlington, ON, Canada), or used for immunoblotting.

*Two-dimensional gel electrophoresis and staining*

Final samples of whiff and sole extracts were solubilized to a final concentration of 350 μL in rehydration buffer: 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM dithiothreitol (DTT) and 0.5% (v/v) carrier ampholyte cocktail (Bio-Lyte® 3/10 ampholyte, Bio-Rad, Madrid, Spain). The protein sample was loaded using immobilized pH gradient strips (Immobiline™ DryStrip, 7 cm long, pH 4–7; and 11 cm long, pH 3–10, GE Healthcare) and active rehydration was carried out at 50 V for 12 h. Isoelectric focussing (IEF) was performed according to the following running conditions: from 50 to 300 V in 1 min, from 300 to 1000 V in 30 min, from 1000 to 5000 V in 1 h30 min and finally 5000 up to 29 000 V accumulated. After IEF, the strips were equilibrated in two steps of 20 min each in equilibration buffer containing 6 M urea, 50 mM Tris-HCl pH 8.8, 30% glycerol, 4% SDS and 0.065 M DTT (first equilibration step) or 0.135 M indole aceticacid (IAA) (second step). The second dimension was carried out on homogenous running gels (14%) and the gels were then silver-stained (GE Healthcare).

*Immunoblot analysis and inhibition assays*

Protein bands and spots separated by SDS-PAGE were transferred by semi-dry blotting onto nitrocellulose sheets according to the method of Towbin et al. [17]. Immunoblots were carried out as described previously [18]. To perform immunoblotting inhibition assays, 1 mL of serum pool was incubated previously with 100 μg of each fish extract at room temperature for four hours under constant stirring. The serum pool was diluted 1/10 in NET buffer for assays.

*Protein identification and characterization by mass spectrometry*

Identification of proteins by MS and/or MS/MS was performed as described previously [18, 19].

Protein identification was performed by searching a non-redundant protein sequence database (NCBI) using the Mascot program (http://www.matrixscience.com).

**Immunoglobulin E enzyme-linked immunosorbent assay and inhibition assays**

For ELISA and inhibition ELISA assays, 96-well flat-bottom plates were used (Immunolon 4HBX, Thermo, Waltham, MA, USA) and procedure was followed as described previously [20]. Briefly, the plates were coated overnight at 4 °C. The proteins were diluted in coating buffer (0.05 M carbonate bicarbonate buffer pH = 9.6). The plates were blocked for 1 h at room temperature (RT) and incubated for 2 h at room temperature with patients' sera diluted 1/10 in blocking buffer. Plates were washed and incubated with the secondary antibody (goat anti-human IgE/PO, Nordic Cultek, Madrid, Spain) diluted 1/5000 with 2% bovine serum albumin (BSA), 0.05% PBS-T for 1 h at RT. IgE reactivity was detected by the addition of TMB (Chemicon, Bangalore, India) and measured at 620 nm. Sera from five non-atopic patients were used as negative controls.

For inhibition ELISA, cod and salmon purified parvalbumins were added to the experiment. Sera from allergic patients were pre-incubated for 2 h at 37 °C with 10-fold dilutions from 10 to 0.00001 μg of the inhibitor (purified parvalbumin) per mL of serum.

## Results

*Patient characteristics*

The fish-allergic patients had a mean age of 16.4 years (range 5–40 years), 55.5% were males and 44.4% females. All patients showed at least one of the typical clinical symptoms (i.e. urticaria, angioedema, asthma and anaphylactic reaction) after eating fish proteins. The clinical data on allergy and serum-specific IgE values as well as the CAP-FEIA classes are provided in Table 1.

*Immunoglobulin E recognition patterns to fish extracts and purified proteins*

One hundred percent of the patients had positive ($\geqslant$0.35kUA/L) ImmunoCAP results to whiff and 94.4% (17 out of the 18 patients) to sole.

A serum pool of five patients with fish allergy (see Table 1: patients 2, 6, 8, 10 and 15) were used for the immunoblotting experiments. The criteria for inclusion of patients in the serum pool were (1) high level of specific IgE to parvalbumin and (2) serum availability. IgE-binding patterns of extracts are presented in Fig. 1. The extracts were resolved into a number of bands with molecular weight ranging from 6 to 97 kDa, approximately (Figs 1a and b, lane 1). Immunoblotting revealed clearly stained bands for both extracts (Figs 1a and b, lane 2). A predominant band in whiff was observed around 6 kDa, indicating parvalbumin. In the case of sole, there were two predominant bands at 6 and 14 kDa, respectively. Analysis by MS of these bands confirmed that both corresponded to the same protein named parvalbumin. Weakly stained IgE-binding proteins were shown at the high-molecular-weight region in sole extract (Fig. 1b, lane 2).

Purified parvalbumins are also shown in Figs 1a and b, lane 3. The IgE-binding capability of purified parvalbumins remained constant throughout the process, as confirmed by immunoblot testing performed with the serum pool from fish-allergic patients (Figs 1a and b, lane 4). Control serum from non-atopic patients did not show IgE-binding bands (lane 5).

**Table 1.** Summary of clinical histories and laboratory data

| | | | Whiff | | Sole | | |
|---|---|---|---|---|---|---|---|
| Patient no. | Age (years) | Sex | CAP (kU/L) | ELISA* (OD) | CAP(kU/L) | ELISA* (OD) | Fish allergy symptoms |
| 1 | 13 | M | 0.4 | 0.363 | 0.37 | 0.647 | U |
| 2 | 31 | F | 3.44 | 0.782 | 3.88 | 0.3065 | U, A, AE, CU |
| 3 | 14 | M | 1.05 | 0.12 | 0.5 | 0.087 | U, AE |
| 4 | 17 | F | 8.23 | 0.101 | 9.59 | 0.101 | U, AE |
| 5 | 14 | F | 1.36 | 0.217 | 2.25 | 0.1315 | U |
| 6 | 40 | F | 16.6 | 0.7675 | 36.2 | 0.77 | AE |
| 7 | 10 | M | 2.03 | 0.344 | 1.77 | 0.254 | OAS |
| 8 | 38 | F | 54.9 | 18.615 | 43.7 | 0.6965 | U |
| 9 | 7 | F | 1.92 | 0.171 | 2.46 | 0.336 | U |
| 10 | 12 | F | 17 | 0.8335 | 14.2 | 0.448 | U |
| 11 | 15 | M | 8.69 | 0.376 | 6.17 | 0.1035 | OAS, AE |
| 12 | 20 | M | 7.28 | 0.612 | 6.7 | 0.2855 | OAS, A, E |
| 13 | 9 | M | 0.61 | 0.2505 | $<0.35$ | ND | U |
| 14 | 9 | M | 5.95 | 0.68 | 3.83 | 0.283 | OAS |
| 15 | 14 | M | 10.6 | 0.721 | 7.85 | 0.344 | GIS |
| 16 | 5 | F | 0.87 | 0.293 | 0.46 | 0.1535 | OAS, U |
| 17 | 15 | M | 78.8 | 10.875 | 47.3 | 0.5205 | ANA |
| 18 | 13 | M | 19.9 | 1.91 | 21.5 | 0.7535 | ANA |

*IgE reactivity was considered positive when OD 620 nm $\geqslant 2\times$OD of negative controls (0.19).

A, asthma; AE, angioedema; ANA, anaphylaxia; CU, contact urticaria; U, urticaria; GIS, gastrointestinal syndrome; OAS, oral allergy syndrome; ND, not done.

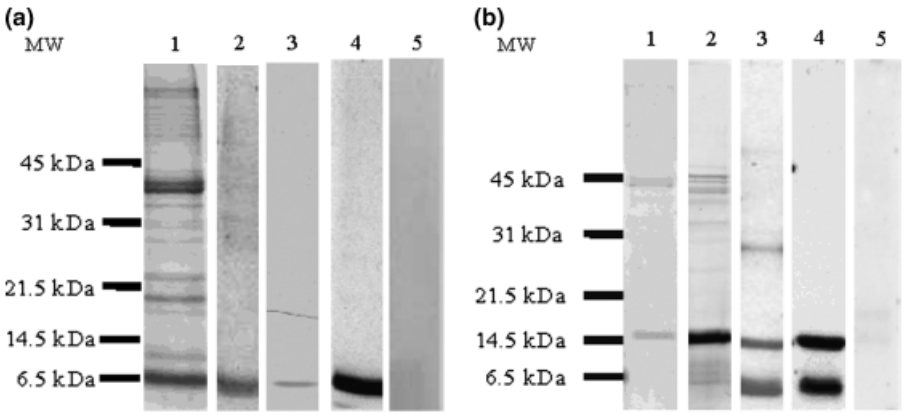


**Fig. 1.** Purification and identification of parvalbumins in whiff (a) and sole (b). Coomassie blue-stained gels of fish-protein extracts (lane 1) and immunoblotting with serum pool of fish-allergic patients (lane 2). Coomassie staining of purified parvalbumins (lane 3) and immunoblotting with serum pool of fish-allergic patients (lane 4). In lane 5, serum from a non-atopic patient was used as a negative control.

### *Two-dimensional gel electrophoresis and protein identification by mass spectrometry*

Two-dimensional gel electrophoresis (2-DE) and subsequent immunoblot of cooked whiff and sole extracts were performed and parvalbumin was confirmed as allergen in both fish species (Fig. 2). Several spots with molecular masses of 6.5–45 kDa and isoelectric point (*p*I) values ranging from 4.0 to 7.0 were shown in cooked whiff extract (Fig. 2, top). In the case of cooked sole extract, several spots were observed in the 2D gel, ranging from 6 to 65 kDa and with *p*I values from 3.0 to 10.0. In this case, only three main spots were visualized by immunoblotting (Fig. 2, bottom). In Fig. 1a, lane 2, several weakly specific IgE-binding bands were observed. These same bands were better resolved in 2-DE gel electrophoresis (Fig. 2a), confirming this as the state-of-the-art technique in protein resolution and identification. No positive spots were detected using serum from non-allergic individuals (data not shown).

Prominent IgE-binding spots seemingly at the parvalbumin molecular level were selected from whiff and sole gels (spots #1 and 2, respectively). In the case of whiff, both spots, at 17 and 6.5 kDa, were confirmed as parvalbumin from *Lepidorhombus whiffiagonis*. To identify the 14 and 6.5 kDa proteins from sole (spots #1 and 2,

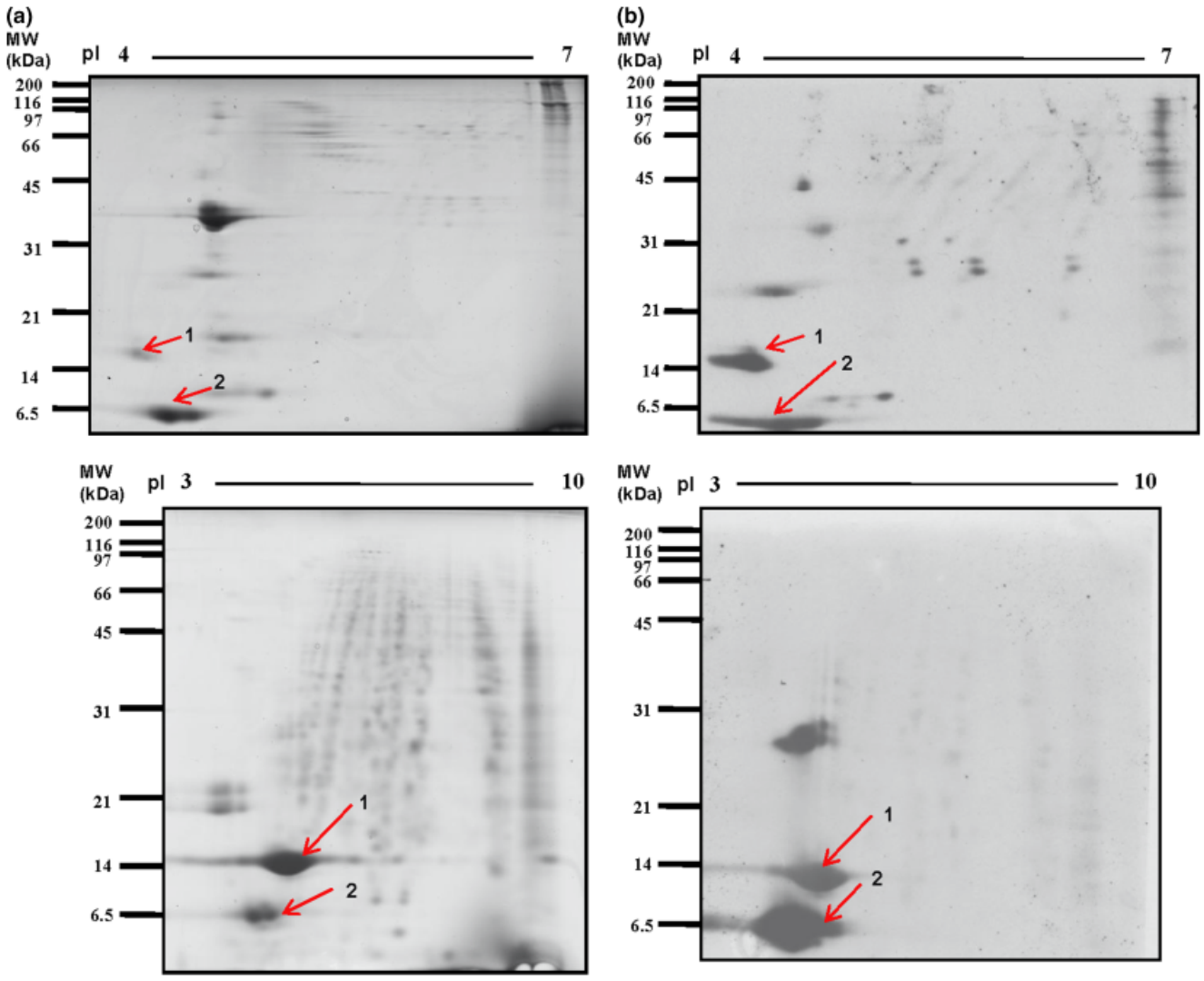


Fig. 2. Two-dimensional electrophoresis (2-DE) and immunoblot analysis of whiff (top) and sole (bottom) extracts. Coomassie blue-stained 2-DE (a). IgE immunoblots probed with pooled sera from five fish-allergic patients (b) diluted 1/5 in Net buffer.

respectively), the following sequences of internal peptides were obtained: LFLQNFK and IGIDEFAAMIK for 14 kDa protein and AFLAAGDSDGDGKIGVDEFAALVKA for the 6.5 kDa protein. The peptide sequences of the 14 kDa protein were highly consistent with parvalbumin from related species such as chub mackerel (*Scomber japonicus*) and parvalbumin-like protein from gilthead bream (*Sparus aurata*).

### *Immunoglobulin E reactivity of purified parvalbumins*

All of the patients showed sensitization to at least one of the purified parvalbumins tested (Fig. 3). Seventeen out of the 18 patients tested (94.4%) showed sensitization to whiff parvalbumin. In the case of sole parvalbumin, 16 out of the 18 patients (88.9%) were sensitized. These results confirmed parvalbumin as a major allergen in whiff and sole fish species. Sensitization to parvalbumin purified from cod and salmon was 94.4% and 66.7%, respectively.

Significant correlations were observed between the ELISA values of purified parvalbumins from whiff and sole ($r = 0.75$) and an even stronger correlation existed between ImmunoCap values of whole extracts ($r = 0.91$). When comparing ELISA and CAP values among species, correlation was still significant and very similar in both cases ($r = 0.65$ in whiff and $r = 0.67$ in sole).

To demonstrate that the ability of IgE binding of the purified proteins was the same as the binding found in the fish extracts, immunoblot inhibition assays were performed with pre-incubated pooled sera obtained from patients allergic to fish (Fig. 4). The IgE-binding pattern of whiff and sole extracts was very similar (Figs 4a and b, lane 1). The inhibition immunoblotting experiment showed complete inhibition of IgE using 100 μg of each extract (Figs 4a and b, lane 2). Nearly total inhibition was reached in whiff

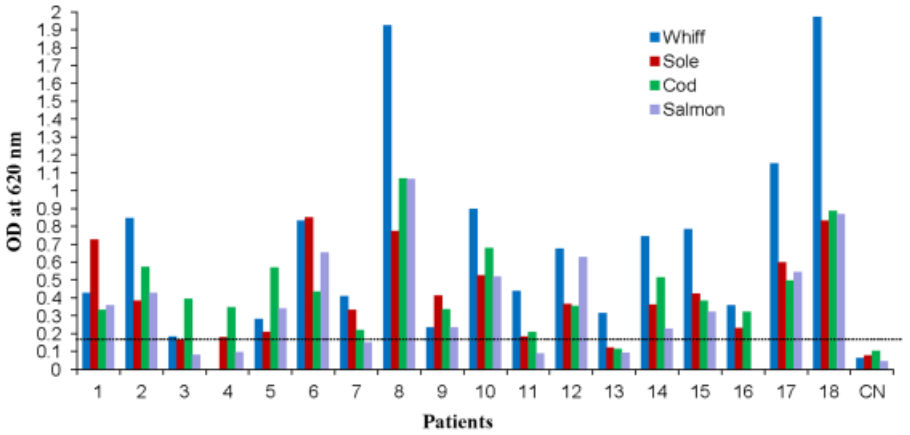


**Fig. 3.** IgE-ELISA analysis of specific IgE reactivity to purified parvalbumins. IgE sensitization of fish-allergic patients to whiff (light-blue column) and sole (dark-blue column) purified parvalbumins. IgE reactivity was considered positive above dotted line, when optical density (OD) at 620 nm $\geqslant$OD 620 nm of the negative control$\times$2 (0.14). All tests were performed in duplicate.

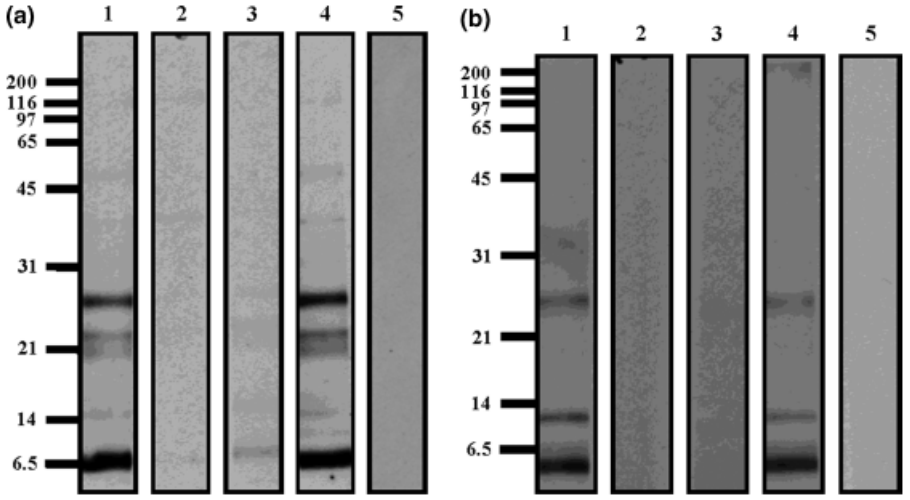


**Fig. 4.** Immunoblot inhibition assay using cooked extracts from whiff (a) and sole (b). IgE immunoreactivity of cooked extracts with a serum pool (lane 1). Immunoreactivity of the serum pool inhibited with whiff extract (lane 2a) and sole extract (lane 2b), with sole extract (lane 3a) and whiff extract (lane 3b) and with bovine serum albumin (lane 4). Lane 5 represents IgE immunoblot with a non-atopic patient used as a negative control.

and sole extracts when the sera were pre-incubated with the other extract (Figs 4a and b, lane 3). This experiment suggests a high degree of homology between whiff and sole parvalbumins. No inhibitory effect was detected in the control experiment using BSA (Fig. 4, lane 4). Serum from a non-atopic individual was used as a negative control (Fig. 4, lane 5), showing no IgE-binding bands.

### *Immunoglobulin E enzyme-linked immunosorbent assay inhibition*

The same serum pool used for immunoblot experiments was pre-incubated with different concentrations of purified parvalbumin. A high degree of inhibition in the fourth purified parvalbumins was shown (Fig. 5). IgE binding from the serum pool to whiff parvalbumin was inhibited by more than 72% following sera pre-incubation with purified sole parvalbumin (Fig. 5a). Eighty-two percent inhibition to sole parvalbumin was shown when the serum pool was pre-absorbed with whiff parvalbumin (Fig. 5b). The percentage of inhibition reached between cod parvalbumin and whiff extract was the same as that reached between salmon parvalbumin and whiff extract (approximately 65%). However, there was higher inhibition of salmon parvalbumin to sole extract (94%) than cod parvalbumin to the same extract (84%). One hundred percent inhibition was not reached due to an insufficient amount of parvalbumin for complete inhibition of specific IgE to any of the parvalbumins, including the one used for coating the plate. Use of more than 10 µg of purified parvalbumins would likely have led to total inhibition. Cross-reactivity between the fourth parvalbumins tested has been demonstrated, and thus data indicate that parvalbumins had a high degree of identity.

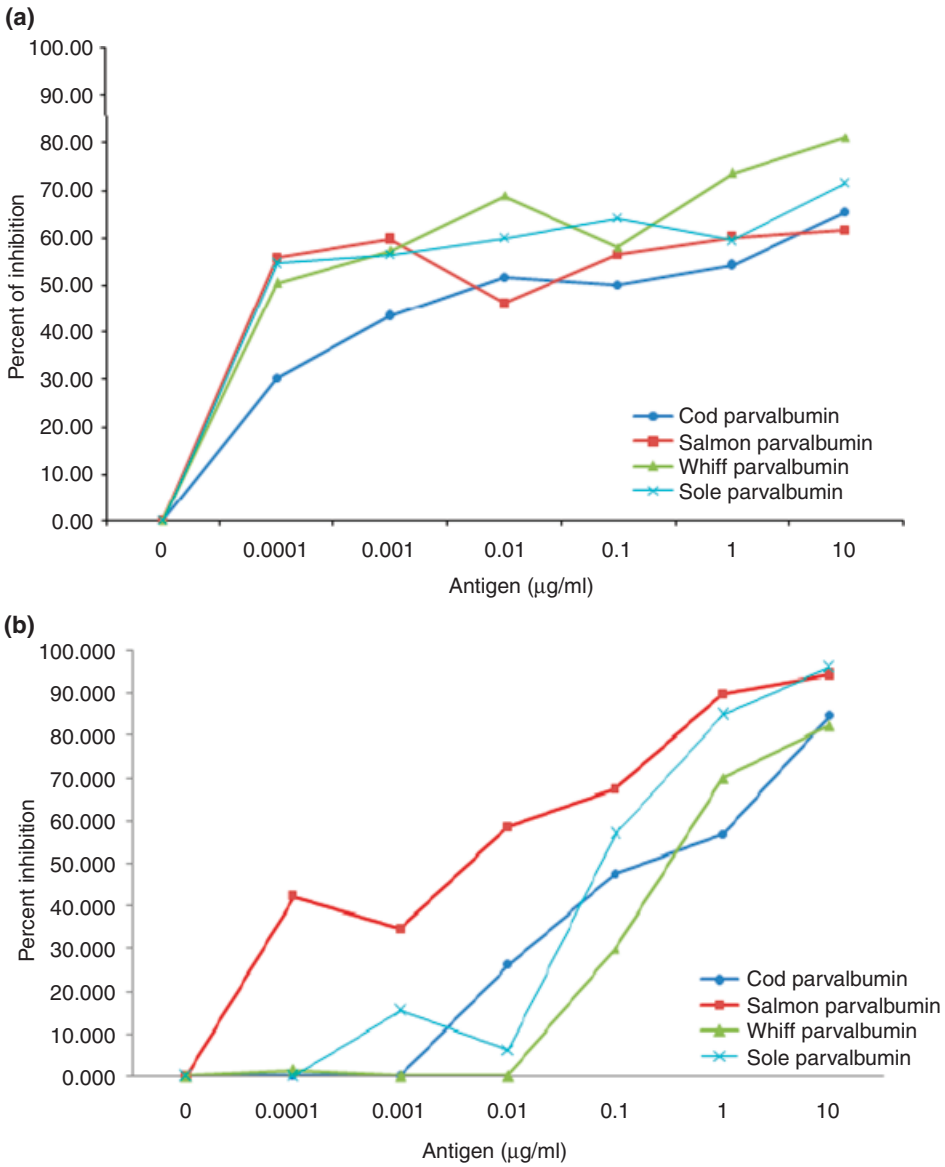


**Fig. 5.** IgE-ELISA inhibition assay. Purified parvalbumin from whiff (a) and sole (b) were used as a solid phase and the serum pool was pre-incubated with increasing concentrations of purified parvalbumin from cod (dark blue), salmon (red), whiff (green) and sole (light blue). All tests were performed in duplicate.

## Discussion

To date, only a handful of studies have been carried out on fish allergy, and mostly in northern European countries, were the fish industry uses a large part of the population and fish is a staple food. In Spain, the most heavily consumed species are whiff, sole and hake, among others. In fact, whiff and sole are two of the first lean species introduced in Spanish children's diets [3].

In the present study, we used proteomic approaches to evaluate the IgE reactivity of cooked *L. whiffiagonis* and *S. solea* extracts. IgE-binding bands obtained in unidimensional immunoblotting might be constituted by several proteins with similar molecular weight. However, in 2D-immunoblotting, each spot corresponds to a unique protein, which is translated into a better protein resolution, thus avoiding the identification of proteins with similar molecular weight as IgE-binding band. Moreover, in recent years, proteomic approaches had provided a powerful tool in molecular characterization of complex allergen sources and a rapid increase in allergy-related data, including allergen sequences and cross-reactivity. Protein profiling using 2-DE, allergen detection by IgE and MS analysis have become useful methods for analysing allergens. MS analysis has revealed the presence of new IgE reactive components in other allergic sources such as wheat [21], ryegrass pollen [22] and maize [23] among others. Eighteen fish-allergic patients were

recruited from the clinic based on their proven allergic symptoms after ingestion of at least one of the fish species tested. This is the first time parvalbumin has been identified as a major allergen implicated in allergic reaction to sole. This conclusion, which shows that 88.9% of the allergic patients were sensitized to sole purified parvalbumin (Fig. 3), was arrived at using IgE ELISA. We have also shown that 94.4% of patients were sensitized to purified parvalbumin from whiff, thus confirming parvalbumin as a major allergen in both extracts.

On 2D immunoblots, parvalbumin spots were seen around 6.5 and 14 kDa, which happened to be different forms of the same allergen. These results were confirmed by MS according to internal sequence peptides obtained by MS/MS. We have also confirmed that parvalbumins from whiff and sole are highly cross-reactive (Fig. 5), arriving at this conclusion by conducting an IgE-ELISA inhibition experiment. Whiff and sole species are very close taxonomically and their parvalbumins appeared to be very similar, as confirmed by the high percentage of inhibition reached between them (Fig. 4). Cross-reactivity was also confirmed by the inhibition immunoblot experiment. Whiff IgE-binding bands were almost completely inhibited when the serum pool was pre-absorbed with sole extract and vice versa (Fig. 4). In addition, we wanted to evaluate cross-reactivity between these parvalbumins and others that were phylogenetically more distant, such as Atlantic cod parvalbumin (Gad m 1) and Atlantic salmon parvalbumin (Sal s 1). Results confirmed the high degree of inhibition between fish parvalbumins. When whiff extract was incubated with the serum pool that had been preabsorbed with the 4 parvalbumins, a similar degree of inhibition was reached by all of them, ranging from 60% inhibition in salmon parvalbumin to 81% in whiff parvalbumin. In the case of sole extract, the percentage of inhibition was even higher, ranging from 82% for whiff parvalbumin – the closest taxonomically – to 95% inhibition for sole parvalbumin. Remarkably, salmon parvalbumin demonstrated 94% inhibition, leading us to the conclusion that even though sole and salmon are not very close taxonomically, their parvalbumins might share a higher degree of identity. This similarity could be explained by the fact that proteins are encoded by the same gene in the two different species. Indeed, Das Dores et al. [24] reached similar conclusions when studying the case of Gad m 1 and Sal s 1, whose identity was higher (75%) than the one between Gad m1 and Gad c 1 (62.3%), which are two major allergens from much closer fish species. However, it will be necessary to clone sole parvalbumin and compare it with Sal s 1, Lep w 1 and Gad m 1 in order to confirm the exact degree of identity.

In conclusion, we have purified and identified sole parvalbumin as a major allergen in sole for the first time. We have also confirmed cross-reactivity between parvalbumins from different fish species such as whiff, sole, cod and salmon. We have also confirmed Lep w 1 as a whiff allergen by applying proteomic approaches never used before.

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