

The Burden of Allergens in Surimi Based Products Diminish With Industrial Processing

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Surimi products are a mixture of the flesh of small fish of many species minced, thoroughly washed and gelled [1]. To improve the gel properties and to give surimi products different textures and flavors, other proteins are added to the mixture, mainly soy isolated protein, whey protein from milk and egg ovalbumin [2,3].

There is evidence in the literature of allergic reactions after consuming surimi based products in fish allergic patients [4,5]. However, there are no published studies addressing the IgE recognition of egg or milk allergens in surimi-derived products nor the variation of allergen content throughout the industrial processing.

We have investigated the allergenicity of baby eel surimi (BE) that includes milk in its formulation and crabstick surimi (CS) which includes egg. Prick by prick (PPT) and basophil activation test (BAT) were performed in patients allergic to fish, egg and milk.

We included 11 patients with fish allergy, 5 patients with milk allergy, 7 patients with egg allergy and 3 patients allergic to both fish and egg; referred to Hospital la Paz, (Madrid, Spain). Furthermore, we studied the effects of industrial processing of surimi on the IgE recognition in two critical steps of manufacturing (before and after the gelation process by heat) and in the final product by ELISA. We used sera from a group of 56 patients (18 fish, 14 egg, 14 milk, 7 fish and egg and 3 egg and milk) for the ELISA experiments. The local Ethics Committee of Hospital la Paz approved the study (PI-3065) and written informed consent was obtained. Data on age, total IgE and fish, egg and milk specific IgE of patients are presented in supplementary Table 1.

We prepared 0.1 M PBS extracts of BE, CS and two intermediate processing products of them (step 1: before the gelation process, and step 2: after that process) for ELISA experiments. PPT was performed according to European guidelines, using products BE and CS directly without any manipulation. PPT was considered positive when the wheal diameter was >3 mm. BAT was performed with PBS extracts, using BD FastImmuneCD63/CD123/HLA-DR mixture (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions, samples were acquired in a FACS flow cytometer (BD Biosciences) [6]. The IgE reactivity of the extracts was determined by ELISA as described elsewhere [7]. The ELISA results were evaluated with Wilcoxon signed-rank non-parametric test. Two-tailed contrast analysis was performed, α was set at <0.05 .

Thirteen fish allergic patients (13/14; 92.8%) showed positive PPT results with BE (median 6.0 mm, IQR 4.5-7.8) and CS (median 6.0 mm, IQR 4.9-9.0), indicating the presence of fish allergens in both products. The egg allergic patients (10/10, 100%) showed a positive prick test with CS (median 5.8 mm, IQR: 4.9-9.2) whereas reactivity to BE was lower (median 2.8 mm, IQR: 2.5-5.0). Results are concordant with the presence of egg in CS formula and not in BE. Last, 75% (3/4) of milk allergic patients studied were positive with BE (median: 12.0 mm; IQR: 3.8-20.5) and rendered negative results with CS, according to the presence of milk in BE, but not in CS (Supplementary Fig 1A).

Regarding BAT results performed with BE extract, the median %CD63⁺ basophils was 27.8% (IQR: 8.4%-63.6%) for the 12 fish allergic patients included. Egg allergic patients gave lower activation percentages (n=9; median: 5.9%; IQR: 0.8%-27.8%) whereas milk allergic patients tested positive with BE (n=5; median: 22.1%; IQR: 14.8%-46.7%). BAT results with CS were similar to BE for fish allergic patients (median:

28.7%; IQR 15.7%-71.5%) but not for egg and milk patients, reflecting the difference in their composition. The %CD63+ for egg allergic patients with CS was positive in 8/9 (88.8%; median: 26.9%; IQR: 3.9%-58.7%). Conversely, milk allergic patients showed lower activation with CS than with BE (n=5; median: 16.6%; IQR:3.0%-27.4%) (Supplementary Fig 1B).

We studied the variation in allergenicity along the processing of these products by ELISA. Allergens were extracted from step 1 (before gelation) and step 2 (after gelation) and the final products BE and CS. We analyzed sera from patients allergic to fish, egg and milk. Overall, manufacturing process lead to a drop in the IgE recognition. CS seems to retain more fish allergens than BE in the three steps studied. The reduction of IgE recognition reached statistical significance between steps 1 and 2 in BE ($p < 0.001$), and a reduction can be observed in both products and in the two final steps (Fig 1A and 1B). Regarding the egg allergen content, BE gave negative results in all the steps as this product does not include egg in its formulation (Supplementary Fig 2A). Conversely CS showed high absorbance with a significant decrease from step 1 to 2 ($p = 0.024$, Supplementary Fig 2C). We did not detect milk allergens in CS as expected (Supplementary Fig 2D). However, final BE and its previous steps were strongly recognized by milk allergic sera ($p = 0.023$; Supplementary Fig 2B).

β -Parvalbumin is a major fish panallergen, present in most fish and recognized by 80% of allergic patients [8]. We confirmed the presence of β -parvalbumin by western-blotting inhibition with recombinant mackerel β -parvalbumin (Sco j 1) before gelation process, but its IgE recognition disappears after the gelation process (Supplementary Fig 3).

Some of the steps of surimi production can mitigate allergenicity by removal of the allergenic proteins, either during the leaching step or by structural modifications by heating during gelation process[9,10,11]. This fact explains the bigger drop in the IgE recognition by ELISA before and after gelation and the presence of allergens in the final products observed.

We can conclude that milk and egg as additives not lose IgE binding ability throughout the processing, so they constitute a real danger for allergic patients. Regarding fish allergens, we have observed that β -parvalbumin is undetectable in the final products although enough IgE reactivity is retained in the final products. Recently it has been published the decrease in sIgE to parvalbumin rGad c 1 and the mean wheal diameter of SPT for hake and salmon can be used as markers of prognosis in the acquisition of tolerance by fish-allergic patients [12]. In line with this study, our results suggests that some fish allergic patients could tolerate surimi products, although previous supervised oral food challenge is necessary.

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Conflict of interests

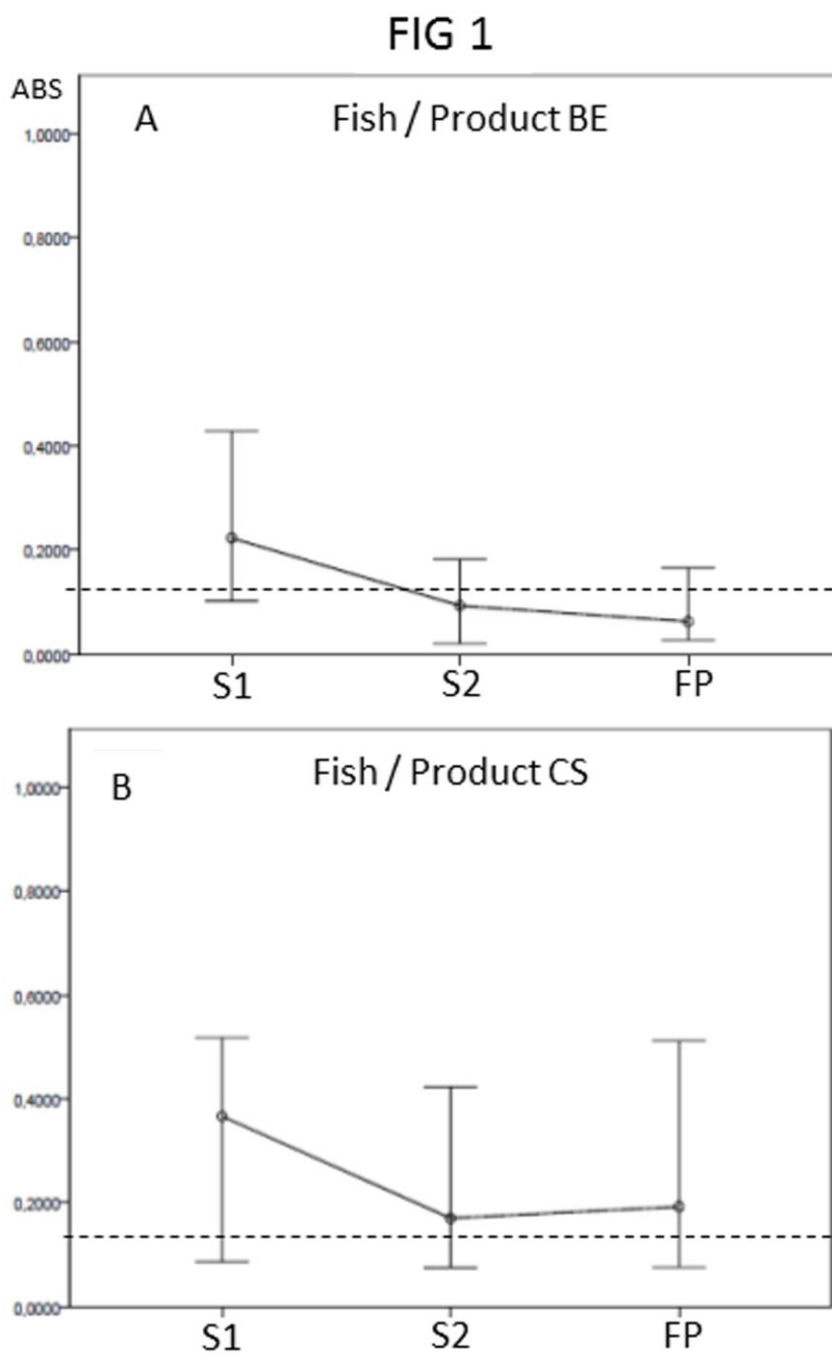
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Figure 1. Variation of fish specific IgE recognition during processing tested by ELISA.



Y axis shows absorbance units for fish allergic patients tested with the different extracts. Median and error bars (95% CI) are shown. S1: step 1, before surimi gelation; S2: step 2, after surimi gelation, FP: final product. **A**, fish allergic sera tested with product BE (baby eel surimi steps). **B**, fish allergic sera tested with product CS (crab stick surimi steps). Dotted line: cut-off ≥ 0.14 Absorbance Units