



Fish muscle processing into seafood products reduces β -parvalbumin allergenicity

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ARTICLE INFO

Keywords:

Fish processing
Seafood products
Fish allergy
IgE
 β -Parvalbumin

ABSTRACT

Fish is one of the eight major foods causing type-I food allergy, and the prevalence of its allergy is increasing in part due to changes in consumption habits. One of the main drivers for these changes has been the processing developments transforming the fish muscle into seafood products. Most fish allergic patients react to the Ca^{2+} -binding protein β -parvalbumin (β -PV) abundant in muscle. Here we have analyzed the effect of processing in the content and allergenic properties of the β -PV. We found that the transformation process decreases the β -PV content (4.7 ± 0.3 mg/g muscle, 0.24 ± 0.03 mg/g surimi, $\leq 0.003 \pm 0.001$ mg/g in seafood products), reduces the specific-IgE binding and prevents allergy relevant properties such the protease resistance and amyloid aggregation. These results suggest seafood products as potentially tolerable foods for fish allergic patients, but milk and egg allergic patients should be aware of the presence relevant additives.

1. Introduction

Fish allergy is an IgE-mediated hypersensitive reaction due to the failure of oral tolerance to fish proteins (Wild and Lehrer, 2005; Sharp and Lopata, 2014; Matricardi et al., 2016). Most fish allergic patients are sensitized to β -parvalbumin (β -PV), an intracellular EF-hand Ca^{2+} -binding acid protein family abundant in bony fish muscles (Kuehn et al., 2014; Sharp and Lopata, 2014; Matricardi et al., 2016). Despite its molecular simplicity, β -PV involves a family of isoforms displaying globular monomer and aggregated structures that modulates its allergenicity (Martínez et al., 2015; Sánchez et al., 2016, 2018; Pérez-Tavarez et al., 2019). Patients clinically diagnosed with fish allergy are advised to avoid fish in their diet, impairing their nutritional requirements and quality of life. However, β -PV-sensitized patients sometimes tolerate several fish species and fish products such as canned tuna (Bernhisel-Broadbent et al., 1992; Griesmeier et al., 2010; Calderón-Rodríguez et al., 2016; Stephen et al., 2017; Kalic et al., 2019).

Fish consumption has largely increased in recent years in part due to processing developments (FAO, 2020). One of these developments

consists in the transformation of surimi (SR) into seafood products such as kamaboko (KB) and the more elaborated baby eel imitation (EI) and crab-like sticks (CI). SR is made of stabilized myofibrillar proteins obtained from water-washed and cryoprotectant blended mechanically deboned fish flesh (Lee, 1984; Niwa, 1992; Park et al., 2013a). Worldwide SR production uses approximately 3 million MT of fish per year, with the best quality SR made from Alaska Pollock muscle flesh (M). Transformation of this onboard product involves heat treatment, enzymatic cross-linking and the inclusion of additives to accomplish distinct textures and flavors (Park et al., 2013b). Among the additives, proteins such as α -lactalbumin (ALA) and ovalbumin (OVA), and natural food colorings containing melanin, carminic acid, capstatin are commonly used (Park et al., 2013b; Lauro et al., 2013).

Given the relevance of seafood products in modern diets and their potential benefits for fish allergic patient diets, we have characterized the content, molecular and IgE-binding properties of β -PV along the transformation steps. Our results show that fish muscle transformations reduce the β -PV content to levels similar to those of low allergenic fish species such tuna and impedes allergy-relevant structure properties

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<https://doi.org/10.1016/j.foodchem.2021.130308>

Received 17 March 2020; Received in revised form 3 June 2021; Accepted 3 June 2021

Available online 16 June 2021

0308-8146/© 2021 The Author(s).

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(Kuehn et al., 2010; Kobayashi et al., 2016; Pérez-Tavarez et al., 2019). However, processing uses non-fish proteins which are relevant for egg and milk allergic patients.

2. Materials and methods

2.1. Ethics statement.

All experimental protocols and methods were performed in accordance with the University Hospital La Paz and the Spanish National Research Council guidelines and applied with approval from the Ethics Committee of the University Hospital La Paz (protocol number PI-3065). Anonymity was and will be preserved as established in the ethical permission.

2.2. Food allergic patient sera.

Sera were obtained from patients recruited from the Hospital La Paz in Madrid (Spain). Fish, milk, or egg allergic patients were selected based on case history, positive skin prick test with commercial extracts and determination of specific IgE (sIgE) by ImmunoCAP 100 (Thermo Fisher, Uppsala, Sweden) according to the manufacturer instructions. The clinical features and IgE profiles of the sera of the patients recruited for the study are displayed in Table 1.

2.3. Fish products.

Frozen samples of Alaska Pollock (*T. chalcogramma*) M and grade KA Alaska Pollock SR, gelified (gKB) and nongelified (KB) kamabokos of baby eel (gKBei, KBei) and crab stick (gKBci, KBci) imitations, and the final EI and CI products were supplied or produced by Angulas Aguinaga (Guipuzcoa, Spain). Additives such as OVA and ALA were also included.

2.4. Extract preparation and β -PV isolation

Samples (duplicates of 3 distinct batches of each sample) were blended at 20% W/V with an OMNI TH International rotor–stator homogenizer (Omni International, USA) (2 cycles of 1 min at 13,000 rpm on ice) in mQ H₂O followed by gentle agitation for 12 h at 4 °C and a centrifugation at 13,500×g for 20 min. The supernatant fractions (referred to as extracts) were collected and their protein concentration determined using the Bradford method with the BioRad protein assay kit. Extracts were divided into aliquots and store at −80 °C until their use. When required, the pellet fractions of the centrifugation at 13,500×g for 20 min were dispersed in 25 mM Tris-HCl pH 7.4, 150 mM NaCl (TBS) supplemented with 8 M urea and sonicated in a bath sonicator for 15 min at 50 °C for their SDS-PAGE analysis (see below). β -PV was isolated from muscle extracts by ultrafiltration. Briefly, freshly muscle extracts prepared as described above were filtered using Amicon

Ultra centrifugal 50 kDa filter units (Sigma-Aldrich, USA). The obtained filtrate containing the β -PV was then concentrated by centrifugation using an Amicon Ultra 10 kDa filter (Sigma-Aldrich, USA). After the determination of the protein concentration using the Bradford method, the β -PV solution was divided into aliquots and store at −80 °C until their use. Recombinant Gad m 1 (gmPV1) was produced as described (Pérez-Tavarez et al., 2019).

2.5. Preparation of protein aggregates

Extracts at 2.2 mg/ml total protein concentration and solutions of the isolated β -PV at 1.5 mg/ml, were diluted 9:1 with either 10 × 0.1 M Gly pH 1.5 or 10 × TBS. After 30 h of incubation at 37 °C, the samples were centrifuged for 1 h at 4 °C at 100,000×g using an Optima Tm^{Max} ultracentrifuge (Beckman, USA). The insoluble fractions were resuspended in their original buffer at protein concentrations >1.5 mg/ml and stored at room temperature for further analysis.

2.6. Circular dichroism spectroscopy

Circular dichroism (CD) measurements were performed using a Jasco J-820 spectropolarimeter equipped with a Peltier-controlled thermostatted cell holder (Jasco, Japan). Far-UV CD spectra were recorded using a 25 μ M protein concentration solution in either 25 mM Tris-HCl pH 7.4, 1 mM CaCl₂ (native) or 25 mM Gly pH 1.5 (aggregated). rate. Spectra were analyzed as described (Martínez et al., 2015; Sánchez et al., 2016).

2.7. Protease digestion

Extracts in 25 mM Tris-HCl pH 7.4, 150 mM NaCl or in 0.1 M Gly pH 1.5 were incubated for 1 h at 37 °C in the absence or presence of proteases at a 1:50 protease:protein ratio using pepsin ($\geq 3,200$ units/mg protein, Sigma-Aldrich, USA) at pH 1.5 and proteinase K (≥ 30 mAnson units/mg protein, Promega Biotech Ibérica, Spain) at pH 7.4. The use of proteinase K instead of conventional trypsin was guided by the Ca²⁺ independent activity. Reactions at pH 1.5 were stopped by the addition of 1 M Tris-HCl pH 8, whereas the proteinase K digestions were stopped with 5 mM final concentration of phenylmethylsulfonyl fluoride (Sigma-Aldrich, USA). When required, the soluble and insoluble (pellet) fractions of the reaction products were obtained by centrifugation at 100,000×g for 1 h using an Optima Tm^{Max} ultracentrifuge. The reaction products were analyzed by SDS-PAGE and immunoblotting.

2.8. SDS-PAGE and immunoblotting

Samples containing 2–14 μ g of total protein prepared in Laemmli buffer containing β -mercaptoethanol (BioRad, USA) and heated at 95 °C for 15 min were separated by SDS-PAGE using Mini-Protean® TGX

Table 1

Clinical features and relevant specific IgE profiles of the sera of food allergic patients recruited for the study.

Pool	Patient	Sex (M/F)	Age (y)	Ofending food	Symptoms ^a	ImmunoCap				
						Milk [ALA] (kU/L)	Egg white [OVA] (kU/L)	Cod (kU/L)	Hake (kU/L)	Swordfish (kU/L)
Milk	1	M	3	milk	GIS	10.8 [nd]	–	–	–	–
	2	M	1	milk	E, GIS	98.0 [99.2]	–	–	–	–
Egg	3	F	31	Egg (white, yolk), chicken meat	AE, RD	–	8.8 [34.4]	–	–	–
	4	M	13	Egg white	U	–	3.4 [1.7]	–	–	–
Fish	5	F	33	Egg (white, yolk)	AE, U	–	77.7 [77.1]	–	–	–
	6	F	12	Fish (except tuna), kiwi	AE, RD	–	–	3.0	3.7	0.3
	7	M	13	Fish	GIS, OAS, U	–	–	5.7	3.5	0.6
	8	F	13	Fish	AE, GIS, RD, U	–	–	8.5	10.4	0.7

^a AE, angioedema; E, eczema; GIS, gastrointestinal syndrome; OAS, oral allergy syndrome; RD, respiratory distress; U, urticaria.

Stain-Free™ gels (#456–8126, BioRad, USA). Bands were visualized using Coomassie Brilliant Blue R-250 (CBB) staining solution (BioRad, USA), and the images were recorded with the Molecular Imager ChemiDoc XRS-Plus (BioRad, USA). For the western blots, samples were first separated by SDS-PAGE and then electrotransferred onto 0.2 µm immunoblot PVDF membranes in Towbin buffer (25 mM Tris, 192 mM Gly, pH 8.3 with 10% methanol) for 90 min at 4 °C. After blocking for 1 h in TBS containing 0.5% Tween 20 (TBS-T) and 0.5% bovine serum albumin (Sigma-Aldrich) at room temperature, the membranes were incubated for 2 h with the mouse monoclonal anti-parvalbumin PARV19 antibody (Sigma-Aldrich, diluted 1:1000 in blocking buffer) or with distinct serum pools (diluted 1:10 in blocking buffer). The membranes were washed 3 times for 10 min each with TBS-T and then incubated with either horseradish peroxidase (HRP)-labeled goat anti-mouse IgG (Sigma-Aldrich, 1:5000 dilution) or HRP-labeled mouse monoclonal B3102E8 anti-human IgE (Abcam, 1:2000 dilution). After washing, the membranes were developed using Clarity Western-ECL (BioRad), and the signals were recorded with the ChemiDoc XRS-Plus imager. For dot blot analysis, samples of 0.1–1 µg protein/dot were loaded at least in duplicate onto nitrocellulose membranes. Membranes were developed as described for western blotting, including the conformational specific rabbit polyclonal anti-amyloid fibrils OC (AB2286 Merck Millipore, 1:2000 dilution) antibody as primary and HRP-labeled goat anti-rabbit IgG (Sigma-Aldrich, 1:5000 dilution) as the secondary antibody.

2.9. Quantitative and statistical analysis

SDS-PAGE and blot images were analyzed using the volume tools provided by ImageLab software (BioRad, USA). The obtained intensities were corrected for background contribution and, when required, referred to the signal generated by gmPV1 used as an internal control. Analyses were performed at least in duplicate and using two different experiments. The statistical analysis was performed using the variance tools provided by Origin 2019 (OriginLab Corp., USA). The level of statistical significance was taken as $p < 0.05$.

3. Results and discussion

Fig. 1A depicts the scheme of the processes used to transform and revalorize the Alaska Pollock muscles into EI and CI seafood products. Steps consisting of washes, thermal treatments and inclusion of additives for best texture and flavor can alter both the content and the structural properties (conformation and aggregation) of β -PV, as well as to provide fish-unrelated sources of food allergens.

3.1. The β -PV allergen load is modified along the transformation steps

To analyze the effect of the transformation chain on the allergen

content, the extraction procedure was first optimized to ensure the quantitative and reproducible extraction of the β -PV monomers in the soluble fraction of all samples. It must be noted that the extraction of transformed products resulted in high amounts of insoluble material that hampered their analysis. Among the variety of conditions tested for the dispersion of the pellets, the dispersion in TBS supplemented with 8 M urea at 50 °C followed by a sonication allowed their manipulation as a solution. Fig. 2A shows that the extraction procedure described in the methods section yielded β -PV monomers almost exclusively in the soluble fractions with no detectable trace in the corresponding pellets. The use of 3 distinct batches of each of the samples yielded similar results (data not shown), indicating the reproducibility of the extraction procedure. The protein content of the distinct extracts was on average: M = 8.5 mg/ml; SR = 6.7 mg/ml; KBci/ei = 3.0 mg/ml; gKBci/ei = 2.3 mg/ml; CI = 2.3 mg/ml; and EI = 2.3 mg/ml.

To determine the β -PV burden we used dot-blot assay based on the PARV-19 anti-PV antibody reactivity, adjusting the dot protein load for a similar β -PV monomer amount (12 kDa band observed in SDS-PAGE gels) and including gmPV1 (Gad m 1) as an internal control (displayed in original figures). Fig. 2B shows that the levels of β -PV in M amounted to 4.7 ± 0.3 mg/g tissue, which is in the range of the muscle burden of highly allergenic fish species such *G. morhua* (Kuehn et al., 2010; Pérez-Tavarez et al., 2019). The transformation of Alaska Pollock M into SR drastically reduced the β -PV amount from 4.7 ± 0.3 to 0.24 ± 0.03 mg/g tissue. This 19-fold reduction is consistent with the extensive washes used to remove soluble proteins such as β -PV in the SR preparation process. Importantly, the SR β -PV burden is similar to that the muscle of fish species classified as having a low allergen content such as tuna (Kuehn et al., 2010; Kobayashi et al., 2016; Pérez-Tavarez et al., 2019). Transformation of SR into CI and EI final seafood products further decreased the β -PV content below 0.003 ± 0.001 mg/g tissue (Fig. 2B). Notwithstanding, the transformation of SR into the manufactured products involves a first mixture of 45% weight SR with non-fish proteins and salt to generate protein pastes known as KB and a further thermal-aided gelification (gKB). Comparison of the β -PV contents of the corresponding KB and gKB extracts (0.07 ± 0.02 mg/g tissue and $\leq 0.003 \pm 0.001$ mg/g tissue, respectively) shows that the unexpected additional decrease results from the thermal-induced gelification step. Although a degradation process cannot be discarded, western blots of denatured extracts of SR and manufactured products probed with PARV19 supports that gelification steps provokes the conversion of β -PV into SDS-resistant polymers (Fig. 2C). Thus, these polymers could display an altered PARV19 that may affect quantitation.

Thus, these analyses showed that manufacturing of Alaska Pollock muscles into seafood products drastically reduces the β -PV content to levels below of that of fish species considered as low allergenic but provokes the apparition of novel polymeric β -PV forms of unknown allergenicity.

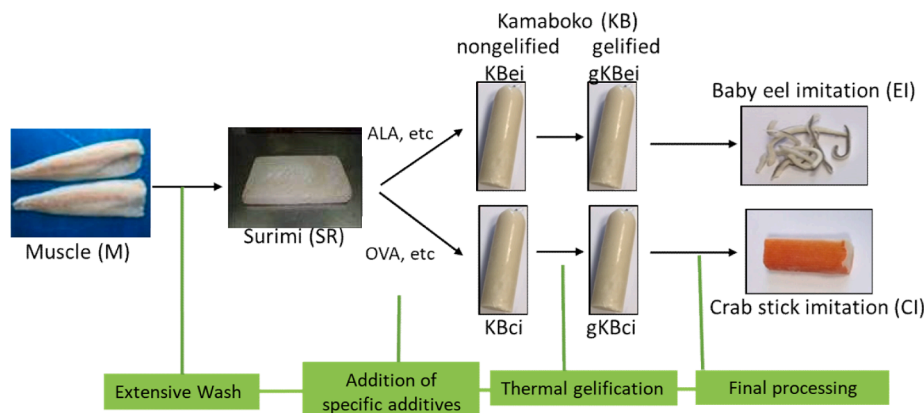


Fig. 1. Scheme of the transformations used to valorize Alaska pollock muscles as seafood products.

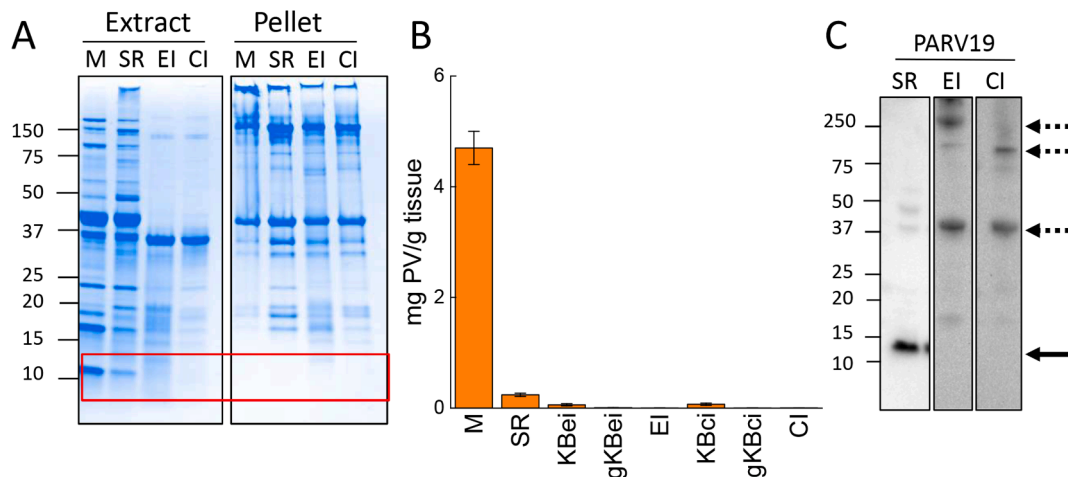


Fig. 2. Processing drastically reduces the β -PV burden of seafood products. (A) Typical SDS-PAGE gels of the soluble (extract) and insoluble (pellet) fractions of the homogenates of M, SR, EI and CI. Migration of β -PV monomer is indicated by a red rectangle. (B) Variation in the β -PV content along the manufacturing chain determined by quantitative dot-blot analysis using PARV19 antibody. (C) Western blots of SR, EI and CI extracts probed with PARV19 antibody. Monomers are polymers are indicated by solid and dotted arrays, respectively. Molecular weight markers in kDa units are displayed on the left in panels A and C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

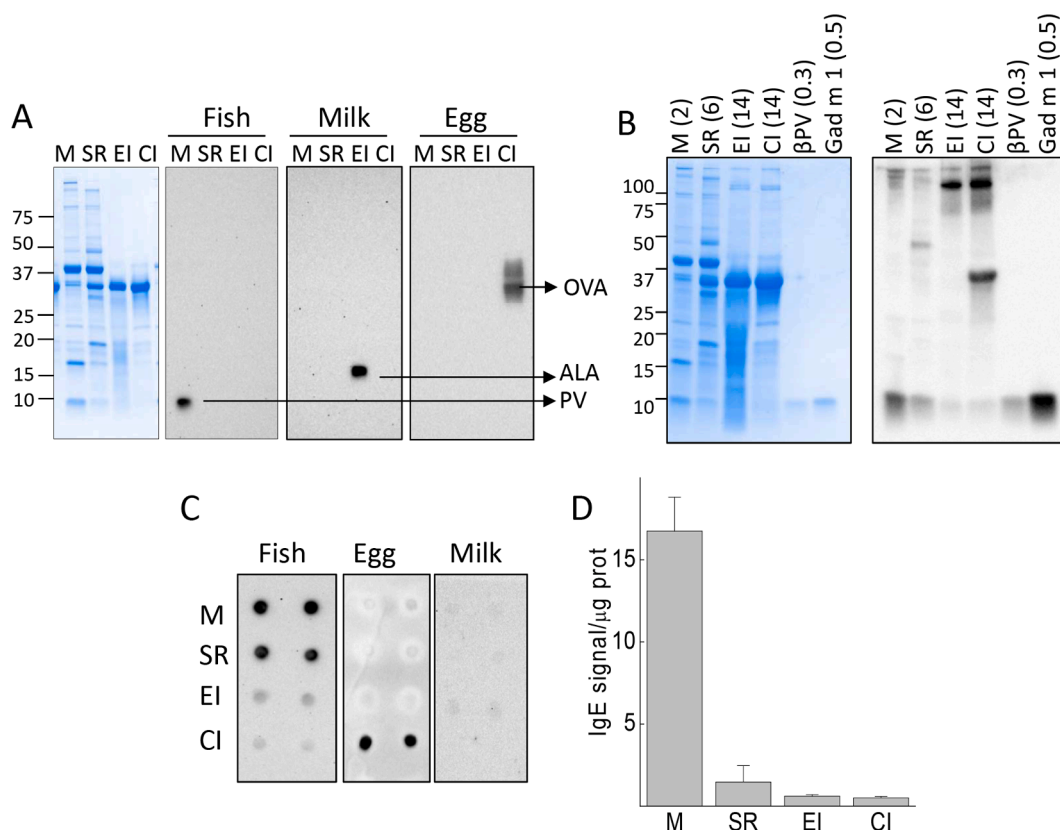


Fig. 3. Changes in IgE reactivity during fish muscle transformation. (A) Typical Coomassie blue-stained SDS-PAGE gel and immunoblots of fish product extracts probed with the IgE present in the sera of fish, milk and egg allergic patients using 3 μ g total protein load per lane. Molecular weight markers in kDa units are displayed on the left. The bands corresponding to β -PV, ALA and OVA are indicated by arrows. (B) SDS-PAGE gel and immunoblot of fish product extracts probed with the IgE present in the sera of fish allergic patients using different total protein loads. The protein load in μ g is indicated in brackets. (C) Typical dot blot analysis of the IgE reactivity of the distinct fish product extracts probed with the sera of fish, egg and milk allergic patients. Loading was performed using 0.1 (M), 0.3 (SR), 0.7 (EI) and 0.7 μ g (EI) of total protein per dot. (D) Specific anti-fish IgE binding of the different fish product extracts determined from the quantitative analysis of dot blots. Measurements were performed in duplicate using extracts of two distinct batches. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. The recognition by the sera IgE of fish allergic patients is altered along processing

To analyze the effect of fish muscle processing on IgE recognition, we used sera from fish allergic patients. Given that processing includes the incorporation of additives, some of which, such ALA (incorporated in EI) and OVA (included in CI), are food allergens, we included in the study the sera from egg and milk allergic patients (Table 1). The reactivity of the different fish foods during their processing with the sera of fish, milk and egg allergic patients is shown in Fig. 3. For a similar total protein load (2 μ g), the IgE reactivity of fish allergic sera was singly detected in muscle, whereas the IgE reactivity of milk and egg allergic patient sera were uniquely found in EI and CI products, respectively (Fig. 3A). The sera used identified the β -PV, OVA and ALA monomer bands as the fish, egg and milk relevant allergens in each of the allergic patient groups. Increasing the relative protein load of processed products compared to that of M shows that the IgE of fish allergic patient sera recognizes in all fish samples the 12 kDa band corresponding to the β -PV monomer and in EI and CI products high molecular weight bands (Fig. 3B). To quantify the observed recognition differences, dot-blot assays using similar loading protocol as described for PARV19 quantitation were performed (Fig. 3C). Fig. 3D shows that the specific allergenicity of the extracts varies similarly to that of the β -PV content shown in Fig. 2B. These results supports that muscle processing into seafood products attenuates but does not abolishes the anti- β -PV IgE binding. As for the other main allergenic additives, CI displays anti-OVA IgE binding both under denature and native conditions whereas the anti-ALA IgE binding of EI requires denaturation (Fig. 3A and 3C).

3.3. Events modulating β -PV allergenicity are prevented in seafood products

Although the seafood products have reduced both β -PV content and fish IgE binding, the processing steps used in their manufacturing may alter protein properties such as amyloid formation and protease resistance, both relevant in the modulation of allergic potency (Martínez et al., 2015; Akkerdaas et al., 2018; Cao and Mezzenga, 2019; Freidl et al., 2019; Pérez-Tavarez et al., 2019, 2021; Luo et al., 2020). In fact, most proteins containing in their sequence sticking motives and fulfilling the concentration requirements form amyloid fibrils under gastric-like conditions and acquire the characteristic protease-resistance of the amyloid structure (Martínez et al., 2015; Pérez-Tavarez et al., 2019; Ke et al., 2020). In addition to the preservation against digestion of the protein and/or its fragments, amyloid assembly provides a polymeric state that enhances binding avidity and confers ligand cross-

linking capacity, both features relevant in IgE-mediated reactions (Sharp and Lopata, 2014; Sánchez et al., 2016; Ke et al., 2020).

To gain insights these events the properties of the β -PV were first characterized. Two β -PV isoforms have been reported for Alaska Pollock (*T. chalcogramma*) muscle (Van Do et al., 2005). As shown for the Atlantic Cod Gad m 1, the β -PV isolated from Alaska Pollock muscle exhibited a dual conformation, a Ca^{2+} -bound α -helical folded state and an aggregated β -sheet rich structure with the conformational specific anti-amyloid fibril OC antibody reactivity (Fig. 4A and 4B) (Martínez et al., 2015; Pérez-Tavarez et al., 2019; Pérez-Tavarez et al., 2021). Of these two states, the amyloid aggregate displayed an enhanced IgE reactivity compared to the Ca^{2+} -bound native monomer when probed with the sera of fish allergic patients (Fig. 4B). Incubation of the extracts under acid conditions such as those used for the isolated β -PV, showed the formation of insoluble OC-reactive species that are also recognized by PARV19 and the IgE of fish allergic patient sera only in the M and SR extracts (Fig. 4C). The absence of these species in EI and CI supports both that their β -PV monomer content is below the concentration threshold required for amyloid assembly and that the gelification-induced β -PV polymers lack such property (Pérez-Tavarez et al., 2019). Thus, the amyloid-dependent IgE binding amplification found in isolated β -PV is impeded in seafood products (Fig. 4B).

Other relevant feature of allergens is their resistance to protease digestion (Martínez et al., 2015; Akkerdaas et al., 2018; Freidl et al., 2019; Luo et al., 2020). Both structural states of the isolated β -PV displayed significant pepsin resistance but were fully digested by proteinase K, used to avoid the interference of Ca^{2+} on the proteolytic activity, (Fig. 5A). Use of similar harsh digestion conditions with the extracts, SDS-PAGE shows that proteinase K resulted in complete digestion whereas pepsin digestions yielded some resistant low molecular weight bands (Fig. 5B). To allow the detection of all short epitopes containing fragments, the effect of pepsin digestion was analyzed using PARV19 and the IgE present in the sera of fish allergic patients in dot blots assays. Fig. 5C depicts that the digestion of the extracts with pepsin abolished their immunoreactivity, indicating that β -PV is largely sensitive to acid proteolysis in the extracts.

Taken together, these results show that processing prevents β -PV from displaying allergy-relevant properties (Akkerdaas et al., 2018; Freidl et al., 2019).

4. Conclusions

CI and EI are two examples seafood products resulting from restructuring technologies that accomplish nutritional and taste factors and revalorize otherwise discarded fish species. Despite being produced

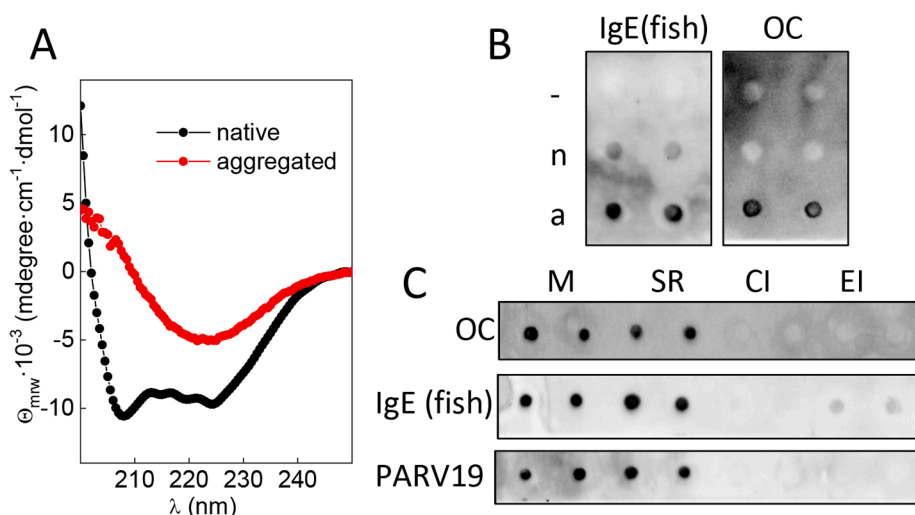


Fig. 4. Effect of processing on the amyloid aggregation of β -PV. (A) Far-UV CD spectra of the native (pH 7.4) and aggregated (pH 1.5) forms of the β -PV isolated from M extracts. (B) Dot-blot of the native (n) and aggregated structures of β -PV probed with the IgE of fish allergic patient sera and the anti-amyloid fibril specific OC antibody. Each dot was loaded with 0.1 μ g of protein. The control load (buffer) is indicated as “-”. (C) Dot blots of the protein aggregates isolated by ultracentrifugation from the extracts probed with anti-amyloid fibril OC antibody, the IgE of fish allergic patient sera and the anti-parvalbumin PARV19 antibody. Dot loads correspond to 0.1 μ g (M) or 0.5 μ g (SR, EI and CI) of total protein.

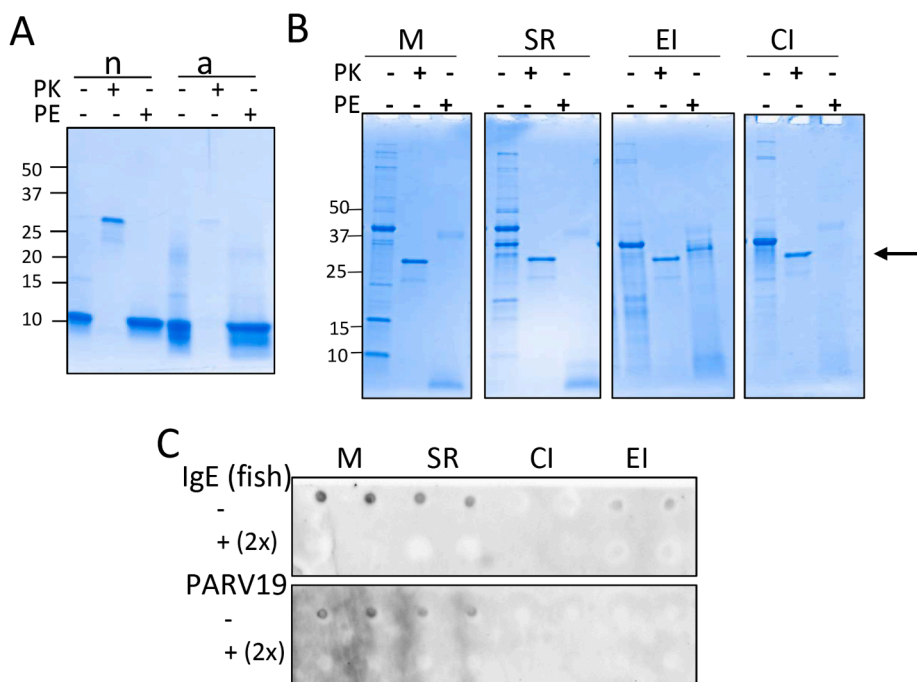


Fig. 5. Protease resistance of β -PV in fish products. (A) Typical SDS-PAGE gel of the pepsin (PE) and proteinase K (PK) digestions of the native (n) and aggregated (a) forms of β -PV isolated from M extracts. Molecular weight markers (kDa units) are displayed on the left. (B) Typical SDS-PAGE gels of proteinase K (PK) and pepsin (PE) digestions of fish product extracts. Loading was performed using 3 μ g of protein in lanes of undigested samples and 2x in lanes of the digests. Molecular weight markers in kDa units are displayed on the left side. The band at 29 kDa (arrow) correspond to the proteinase K. (C) Typical dot blots of undigested and pepsin-digested extracts probed with the IgE of fish allergic patient sera and PARV19. Protein load was 0.1 μ g (M) or 0.5 μ g (SR, EI and CI) for undigested samples. Dots of digested samples were loaded 2x the volume loaded for the undigested.

from fish muscles containing high levels of β -PV isoforms displaying structural and IgE binding properties shared with other highly allergenic β -PV, these seafood products displayed an attenuated β -PV allergenicity. The observed β -PV content and IgE binding reductions support seafood production as a fish transformation such as canning (Sletten et al., 2010; Mejrihit et al., 2018). Despite the necessity of individual testing for solid assessment, seafood products could display a tolerability as canned tuna for β -PV specific sensitized allergic patients. It must be stressed that other fish allergens such collagen, tropomyosin, aldolase A and β -enolase could be present in these products and exert their allergenicity (Ruethers et al., 2018). Also, both seafood products are enriched with egg and milk proteins, among others, with allergenic properties relevant for egg and milk allergic patients.

CRedit authorship contribution statement

Raquel Pérez-Tavarez: Investigation, Visualization, Formal analysis, Writing - review & editing. **Helena M. Moreno:** Investigation, Validation, Writing - original draft. **Javier Borderias:** Resources. **David Loli-Ausejo:** Resources. **María Pedrosa:** Resources, Writing - review & editing. **José Luis Hurtado:** Resources. **Rosa Rodríguez-Pérez:** Methodology, Resources, Writing - review & editing. **María Gasset:** Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Douglas V. Laurents is acknowledged for his critical comments. The financial support of this research is from the Spanish AEI/EU-FEDER BFU2015-72271-EXP and PID2019-103845RB-C21 grants and the Spanish Ministry of Science and Innovation CDTI-CIEN Tolera grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.130308>.

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