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Original article

Application of amaranth protein isolate and hydrolysate on a reduced salt fish restructured product: antioxidant properties, textural and microbiological effects

Susan F. García Fillería & Valeria A. Tironi*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) – CCT-La Plata-CONICET, UNLP, 47 y 116, 1900 La Plata, Argentina

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Summary This work evaluates amaranth protein ingredients [isolate (*I*) and alcalase-hydrolysate (*H*)] acting as antioxidants and binders in restructured fish products. Gel products were obtained after thermal treatment (40 °C, 30 min; 90 °C, 30 min) of different formulations from fish muscle pastes, where salt (2%) was partially or totally replaced by *I* or *H*. Antioxidant activity was assessed by conjugated dienes and thiobarbituric acid-reactive substances (TBARs) measurements during the chilled storage. Textural properties, water-holding capacity, colour and microbiological quality were evaluated. Reduced-salt content products containing 2% w/w of *I* or *H* partially inhibited lipid oxidation especially at the level of the decomposition of hydroperoxides into secondary products, due to about 50% and 60% of inhibition of TBARs, respectively, was registered. Also, these products showed acceptable microbiological quality and technological characteristics with only minimal changes in properties as gel hardness and colour parameters compared with control products (2% w/w salt).

Keywords Alcalase-hydrolysate, amaranth protein isolate, antioxidant activity, binding capacity, restructured fish products, salt content.

Introduction

Amaranth (*Amaranthaceae* family) is an ancestral American pseudocereal that grows under tough conditions (dryness, high temperatures, saline soils), becoming an interesting crop particularly in poor regions. Seeds have high nutritional value, high protein content (15–17%) and excellent amino acid balance (Segura-Nieto *et al*., 1994). Antioxidant properties of amaranth have been attributed to the presence of polyphenolic compounds and squalene (Conforti *et al*., 2005; Nsimba *et al*., 2008). Also, the antioxidant activity of amaranth proteins and peptides has been studied. *Amaranthus mantegazzianus* seeds presented some naturally occurring peptides and polypeptides with free radical scavenging activity and ability to inhibit the linoleic acid oxidation in an emulsion model, which were distributed into the different protein fractions (albumins, globulins and glutelins). Moreover, alcalase hydrolysis improved the radical scavenging activity of the protein isolate and the protein fractions (Tironi & Añon, 2010). Likewise, hydrolysates obtained after a simulated gastrointestinal digestion process of isolate or alcalase-hydrolysate presented interesting activity against diverse reactive species suggesting the potentially of both – the amaranth protein isolate and their alcalase-hydrolysate – as antioxidant functional ingredients (Orsini Delgado *et al*., 2011; Orsini Delgado *et al*., 2015). The present work deals with another aspect of the antioxidant activity of amaranth proteins and peptides, that is the effect of the isolate or the alcalase-hydrolysate on the lipid oxidation in a food matrix as fish restructured products. Lipid oxidation is an important cause of sensorial, nutritional and functional quality loss in fish products (Frankel, 1998). In addition, primary and secondary lipid oxidation products are highly reactive and can produce deleterious effects in the human health (Shahidi, 1997). Various literature works evaluate the antioxidant activity of peptides from different sources added to meat products (Peña-Ramos & Xiong, 2003; Hogan *et al*., 2009). Also, antioxidant activity of peptides has been established in emulsion systems (Cheng *et al*., 2010).

Restructured meat products are produced from meat sources after some structural disintegration process took place. They can be marketed as refrigerated, frozen or cooked (gelified) products (Mandigo, 1988). They entail some advantages such as obtaining high-value products

*Correspondent: Fax: +54-221-4254853;
e-mail: vtironi@quimica.unlp.edu.ar

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from trimmings of the meat industry and from underutilised meat sources, as well as the possibility to change ingredients or add bioactive compounds in the formulation (Jiménez Colmenero *et al*., 2006; Sánchez-Alonso & Borderías, 2008). In gelified products, salt addition (1–3% w/w) is necessary to increase the binding capacity of muscle proteins and the water-holding capacity of the gel (Uresti *et al*., 2004; Min & Green, 2008). However, other binding compounds such as alginates, carrageenans, modified starches, flours, nonmeat animal proteins (total milk solids, casein, blood plasma proteins, egg albumin, gelatin, etc.) and vegetable proteins (soya, wheat gluten, etc.) have been studied. These compounds, as well as the microbial transglutaminase (MTG), or the combinations of them, are used to reduce salt content in the products, with technological purposes, improving gelification and textural aspects (Hand *et al*., 1981; Kuraishi *et al*., 1997). It is known that hypertension and cardiovascular and renal diseases are closely linked to high salt ingestion – particularly that of high processed food ingestion (He *et al*., 2010). So, salt consumption should be diminished to prevent these chronic diseases.

This work aimed at assessing the effect of the incorporation of amaranth protein ingredients (isolate and alcalase-hydrolysate) on the lipid oxidation of chilled restructured fish products. In addition, with the aim to obtain more healthy products, the capacity of these ingredients to partially or totally replace salt was evaluated through textural, colour and microbiological parameters.

Materials and methods

Raw materials and amaranth protein ingredients

Fish muscle

Merluccius hubbsi was caught by commercial vessels in the south-west Argentinean sea. Fillets (weight: 100–150 g per fillet, length: 20–30 cm per fillet) were purchased (about 4 days after capture) in the local market and kept on ice until processed within 6 h.

Protein isolates (I)

Amaranthus mantegazzianus (*Pass. cv Don Juan*) was grown at Facultad de Agronomía, Universidad Nacional de La Pampa, Argentina. Flour was obtained by grinding the whole seeds in an Udy mill, 1 mm mesh, screened by 0.092 mm mesh, and defatted by extraction with hexane (24 h, 4 °C). Protein isolates (*I*) were obtained from the defatted flour by extraction at pH 9, isoelectric precipitation (pH = 5), neutralisation and freeze-drying (Martínez & Añón, 1996).

Protein hydrolysates (H)

Alcalase-hydrolysate was obtained from *I* according to Tironi & Añon (2010). Briefly, 1% w/v isolate suspensions in 1 mM NaOH were prepared adjusting to pH = 10. Suspensions were agitated (1 h, 37 °C) maintaining the pH = 10 by adding 0.1 M NaOH. After that, alcalase 2.4 L (protease of *Bacillus licheniformis*, Novozyme Corp ≥2.4 U g^{-1}, Anson Units; Sigma Chemical Co., St Louis, MO, USA) was added (8 μL/100 mg sample). Reaction mixture was incubated at 37 °C for 4 h. Enzyme activity was stopped by heating at 85 °C for 10 min, and the suspension was freeze-dried. The hydrolysis grade (HD) was measured by reaction of free amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Adler-Nissen, 1979) as was previously described (Tironi & Añon, 2010). HD % value was 27.0 ± 0.9%.

Composition of ingredients

Centesimal composition of the ingredients was determined using the following methods: water: drying at 105 °C up to constant weight using an air-drying oven (Hart & Fisher, 1971); proteins: Kjeldahl method (AOAC14.067, 1984; $F = 5.85$); lipids: Soxhlet method (Hart & Fisher, 1971); ash: 550 °C (AOAC 14.006, 1984); and carbohydrates: Antrona method after complete acid hydrolysis (Southgate, 1976). Measures were done by duplicate.

Preparation of restructured fish products

Gelified products were prepared from fish muscle, cold water (to obtain a product as moisture as the original muscle), NaCl (0, 10 or 20 g kg^{-1}), and *I* or *H* (0, 10 or 20 g kg^{-1}), according to the formulations shown in Table 1. Fish fillets (about 500 g) were chopped in a domestic processor for 1 min. Homogenising the mixture, additives and water were added to the same processor for 2 min, obtaining a paste. Portions of the paste were placed in glass tubes (6 cm height and 2.5 cm diameter). They were closed and treated at 40 °C for 30 min (pretreatment, gel setting). After that, gelification was finished at 90 °C (30 min). After cooling in a water bath at room temperature, gels were

Table 1 Formulation (g/100 g) of products with the addition of amaranth protein ingredients (API): isolate (*I*) or hydrolysate (*H*)

Product	Muscle	Salt	API	Water
S_1	95	1	0	4
S_2	90	2	0	8
S_1I_1	90.4	1	1	7.6
S_2I_2	80	2	2	16
I_2	90.8	0	2	7.2
S_1H_1	90.5	1	1	7.5
S_2H_2	80.9	2	2	15.1
H_2	90.9	0	2	7.1

removed from the tubes, placed in polyethylene bags and stored at 4 °C (Ramírez *et al*., 2006). Due to our limitations in the total amount of sample to be simultaneously processed, five independent experimental batches were prepared, evaluating the following aspects in each one: (i) effect of I on lipid oxidation; (ii) effect of H on lipid oxidation; (iii) effect of I on texture, colour and water-holding capacity; (iv) effect of H on texture, colour and water-holding capacity; and (v) effect of the I and H on microbiological count. It is important to mark that the adequate control systems were prepared in each case.

Texture profile analysis

Texture profile analysis (TPA) determinations were performed on gel sections (1 cm height) using a TA-TX2i Texture Analyzer, with the data analysis software package Texture Expert for Windows, version 1.2 (Stable Micro Systems, Godalming, UK). A cylindrical probe with a flat contact surface (3.5 cm diameter) exerted compression. Texture profile analysis was performed applying 20% compression, a compression rate of 1 mm s^{-1}, and a 5-s interval between the two compression cycles. Values for hardness (H), instantaneous recoverable springiness (S_{ins}), retarded recoverable springiness (S_{ret}), cohesiveness (C), gumminess (G) and chewiness (M) were obtained (Fiszman *et al*., 1998).

Water-holding capacity

About 2–3 g of gel was accurately weighed, placed in centrifugation tubes and centrifuged at 10000 ***g*** for 15 min at 20 °C (Beckman Coulter J-25 Avanti Centrifuge, Indianapolis, IN, USA). After centrifugation, tubes were inverted (30 min) allowing the liquid to drain. Liquid loss was determined by weight difference. Water-holding capacity (WHC) was expressed as the percentage of retained liquid compared to the initial water content (determined by drying at 105 °C, 24 h) (Tironi *et al*., 2007a).

Colour

Colour parameters on the CIELAB system a^*, b^* and L^* were determined using a Minolta CR400 colorimeter (Osaka, Japan). From the mentioned parameters, the whiteness (W) value was calculated according to Min & Green (2008): $W = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.

Microbiological counts

Samples were analysed at different stages of chilled storage at 4 °C. A total amount of 10 g from several gels was collected and placed in a sterile plastic bag with 90 mL of buffered 0.1% peptone water; mixtures were homogenised using a stomacher machine for 1 min. After the adequate dilutions, total plate counts were performed by spreading 0.1 mL of the sample onto plate count agar followed by incubation at 30 °C for 48 h. Duplicate plates were used for each dilution (Amiza & Kang, 2013).

Lipid oxidation evolution

Lipid oxidation evolution during the chilled storage was evaluated by means of the following:

Conjugated dienes

Determination was adapted from Peña-Ramos & Xiong (2003). An exactly weighed mass of product was treated with a mixture of hexane:isopropanol (3:2) with agitation at 1100 rpm for 90 min, 15 °C (Thermomixer; Eppendorf, Hamburg, Germany). After that, the mixtures were centrifuged (2000 ***g***, 5 min, 15 °C) and the absorbance at 233 nm of the extract was measured. Results are expressed as μmol hydroperoxides g^{-1} lipids in the product ($\varepsilon = 25200\ M^{-1}\ cm^{-1}$).

2-Thiobarbituric acid assay (TBA)

TBA-reactive substances (TBARs) value was obtained according to Tironi *et al*. (2007b) by performing an acid extraction with trichloroacetic acid (TCA). Extracts were reacted with a 0.5% w/v TBA solution. Results are expressed as mg malonaldehyde kg^{-1} lipids in the product ($\varepsilon = 1.56 \times 10^5\ M^{-1}$).

Statistical analysis

Data from each experimental batch were evaluated by means of ANOVA according to the general lineal model [categorical variables: product, storage time (when it corresponds)] using a SYSTAT 12 software (SYSTAT Software Inc., San José, CA, USA). When significant differences ($P < 0.05$) were detected, mean value differences were analysed carrying out the Tukey test.

Results and discussion

Composition of the ingredients used for the preparation of the restructured products was obtained. As was previously mentioned, various experimental batches were performed. Composition of the different muscles used ranged between the following values: 79.5 ± 0.9% and 82.5 ± 0.1% w/w water, 14.9 ± 0.1% and 17.9 ± 0.1% w/w proteins, 0.81 ± 0.05% and 2.14 ± 0.02% w/w lipids, and 1.1 ± 0.1% and 2.1 ± 0.2% w/w ash. Composition of I was 77 ± 3 g proteins/100 g, 10.5 ± 0.2 g carbohydrates/100 g, 7.7 ± 0.7 g water/100 g, 3.5 ± 0.1 g ash/100 g and 1.27 ± 0.01 g lipids/100 g, while composition of H was 78.5 ± 0.6 g proteins/

100 g, 11 ± 3 g carbohydrates/100 g, 9.2 ± 0.3 g water/100 g, 5.2 ± 0.3 g ash/100 g and 1.06 ± 0.01 g lipids/100 g.

Lipid oxidation

Effect of the partial or total replacement of salt by I

Taking into account that 2% w/w (product S_2) is the more common salt content level used in restructured products, the effect of the partial (1%) or total (2%) replacement of salt by *I* (products S_1I_1 and I_2, respectively) on lipid oxidation during the chilled storage was studied. Additionally, other control products (S_1 and S_2I_2) were analysed in this case to evaluate the influence of the salt on lipid oxidation. As a measure of the formation of primary lipid oxidation products, the conjugated dienes (CD) content was determined. All the tested products (S_1, S_1I_1, S_2, S_2I_2 and I_2) presented statistically similar values ($P > 0.05$) at day 1 of the storage (Figure 1a). No significant increases in the CD content ($P > 0.05$) were registered during the chilled storage of any of the products, with the exception of S_1 which showed a significant increment ($P < 0.05$) after 6 days of storage (Figure 1a). When secondary lipid oxidation products were evaluated by the TBA number assay, results presented a different behaviour with respect to CD content. All the formulations showed similar values ($P > 0.05$) at day 1, but in this case, TBARs values increased as storage time increased (Figure 1b). After 6 days of storage, TBA number presented significant ($P < 0.05$) differences between formulations, showing product S_1 the highest value followed by S_2 and S_2I_2. After 9 days of chilled storage, products containing only salt (S_1 and S_2) presented TBARs levels significantly higher ($P < 0.05$) than products containing *I* (I_2, S_2I_2, S_1I_1), with no differences due to the *I* (1 or 2%) or salt (0, 1 or 2%) contents. According to the present results, the addition of 1 or 2% of *I* in the fish restructured products produced a diminution of about 50% in the formation of secondary lipid oxidation products (Figure 1b).

Effect of the partial or total replacement of salt by H

A similar experimental design was performed to evaluate the addition of *H* into the fish restructured products. Similar to the previously described results, CD content did not present significant differences ($P > 0.05$) at day 1 and there were not significant modifications during the chilled storage for any of the products; only the product S_2 showed a significantly higher ($P < 0.05$) CD content than the other products at 9 days of storage (Figure 2a). Figure 2b shows the TBARs values evolution. The incorporation of 1% of *H* (S_1H_1 product) and 2% of *H* (in the absence -H_2- as well as in the presence -S_2H_2- of salt) produced an important inhibition ($P < 0.05$) in the secondary lipid oxidation products (about 66% after 3 and 6 days of storage). At longer storage time (9 days), products with 2% salt and 2% *H* (S_2H_2) showed an inhibition of the lipid oxidation (about 33%) lower ($P < 0.05$) than in the case of products containing only 2% *H* (H_2) (about 50%), suggesting some effect of the presence of salt in these conditions.

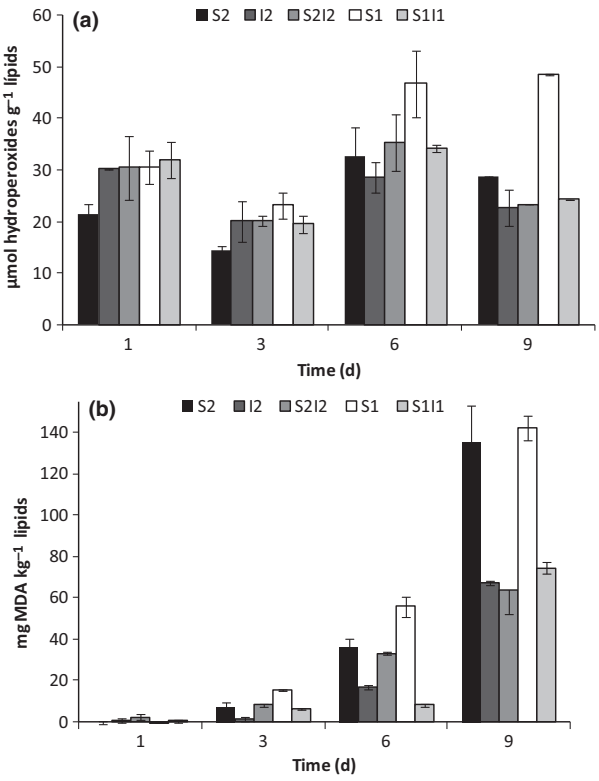

Figure 1 Lipid oxidation evolution for products with the replacement of salt by amaranth isolate *I*. (a) Conjugated dienes content (mean ± SD, $n = 3$); (b) thiobarbituric acid number (mean ± SD, $n = 4$). Products were prepared using a muscle containing 1.7 ± 0.2% w/w lipids.

According to the results previously exposed, CD content did not experience important changes for any of the products during the chilled storage. As an attempt to explain the lack of CD increment during the chilled storage, an additional assay was performed, evaluating the effect of the thermal treatment on the CD content for a control product (S_2). Thermal treatment (40 °C, 30 min and then 90 °C, 30 min) produced an increase (about 60%) in the CD content as demonstrated the values obtained at day 0 for thermally treated and nontreated samples (Figure S3). Newly, thermally treated product showed only minimal variations in the CD content during chilled storage. Meanwhile, not-thermally treated product presented an important increase (about 83%) in the CD content during storage (Figure S3). These results

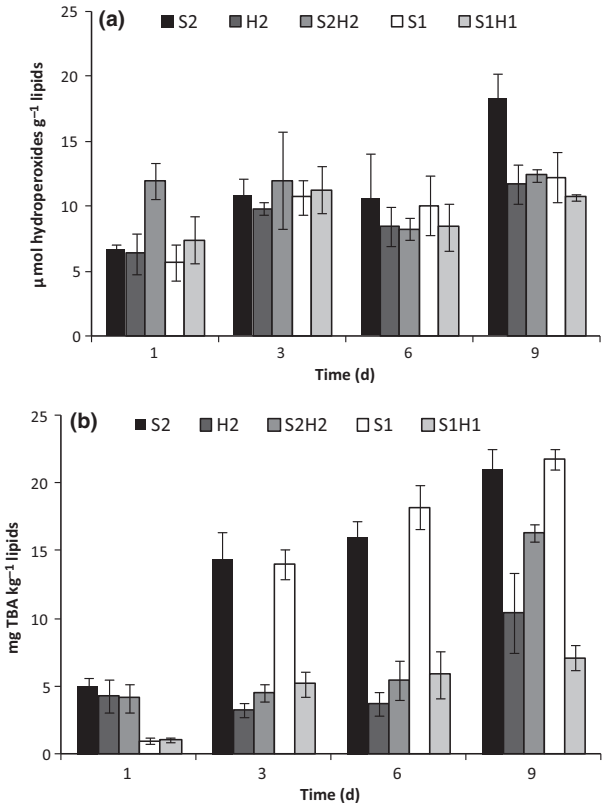

Figure 2 Lipid oxidation evolution for products with the replacement of salt by amaranth alcalase-hydrolysate *H*. (a) Conjugated dienes content (mean ± SD, $n = 3$); (b) thiobarbituric acid number (mean ± SD, $n = 4$). Products were prepared using a muscle containing 2.14 ± 0.02% w/w lipids.

indicated that thermal treatment induced a notorious formation of CD. According to the results obtained in the previous experiments, where no differences were registered between products at day 1 (Figures 1a and 2a), we can hypothesised that the presence of *I* or *H* could not inhibit the formation of hydroperoxides in these products.

In other way and according to TBA number results, both *I* and *H* in concentrations of 1 and 2% w/w would be capable to inhibit the lipid oxidation process, especially at the level of the decomposition of hydroperoxides to secondary oxidation products. It is known that salt could affect the lipid oxidation evolution. While some studies about the effect of salt on the lipid peroxidation have demonstrated its antioxidant effect (Srinivasan *et al.*, 1996), other works indicate that salt can act as a prooxidant (Osinchak *et al.*, 1992). This fact has been attributed to an increase in the solubilisation of metals that catalyse the lipid oxidation process, especially Fe^{+3}. In our case, diminution in the formation of lipid oxidation substances in products containing *I* or *H* was observed even in the presence of salt, suggesting that this decrease is not related to the elimination of salt but to the antioxidant capacity of amaranth protein ingredients.

Peña-Ramos & Xiong (2003) demonstrated that the addition (2% w/w) of soya protein isolate (SPI) or whey protein isolate (WPC) and their respective hydrolysates in cooked pork patties inhibited the lipid oxidation during refrigerated storage. Some WPC hydrolysates had a higher capability to inhibit the hydroperoxides (CD) as well as TBARs formation; meanwhile, the hydrolysis of SPI rendered products with higher capability to prevent hydroperoxides but not their decomposition to secondary products. These facts suggest the presence in the diverse protein sources of different molecules that could act in the lipid oxidation process inhibition by a variety of mechanisms. In the case of amaranth proteins and peptides, we have observed – in experiments where different fractions from amaranth protein isolate or hydrolysate were separated – that some of them presented radical scavenging activity, other ones showed inhibition of linoleic acid oxidation, and in some cases both activities were present (Tironi & Añon, 2010). Among the possible mechanisms of action, amaranth peptides could inhibit the initiation or propagation of radical reactions, acting as metal chelators or donating a hydrogen atom (Orsini Delgado *et al.*, 2015). The last one could be responsible for the action observed in the present products. In addition, the incorporation of *H* (10% w/w) into sunflower and canola oils presented antioxidant effects related to an increase in the oxidation induction period, inhibition of the decomposition of primary to secondary oxidation products and heat stabilising effects at high temperatures, as was demonstrated by differential scanning calorimetry (Tironi & Añon, 2014). These facts are in agreement with the present results with respect to the possible mechanism of action of the peptides. However, in the case of vegetable oils only *H* demonstrated to have antioxidant activity, while in the fish restructured products both *I* and *H* presented activity. These different behaviours could be related to diverse factors, such as the nature of the matrix, the behaviour and interaction of the proteins and peptides with the matrix, the different assays used to evaluate the lipid oxidation, between others. We cannot discard the existence in the amaranth protein ingredients of other antioxidant components such as phenolic compounds.

Restructured products characterisation

In addition to the antioxidant activity of the amaranth ingredients, their effect on other quality parameters were evaluated in products where salt was partially (S_1I_1 and S_1H_1) or totally replaced (I_2 and H_2). As

expected, a significant diminution ($P < 0.05$) in the ash content of the products was observed as a function of the salt replacement by the amaranth ingredients: about 30% for S_1I_1 and S_1H_1 products, and about 55% for I_2 and H_2 products, with respect to the control S_2.

Texture profile analysis

Determinations were performed after 1 day and after 1 week of chilled storage. Textural parameter values are shown in Table 2. The present products did not present adhesivity (negative area after the first compression cycle). The only primary parameter that was modified due to the salt replacement by amaranth ingredients was the hardness. The addition of I (1% and 2%) as well as of H (1%) produced a significant increase ($P < 0.05$) in the gel hardness measured at day 1 (Table 2). However, after 1 week of chilled storage, this parameter diminished for S_1I_1, I_2 and S_1H_1 (Table 2), all products reaching a similar value of hardness than the control S_2. In addition, S_{inst} presented a significant diminution ($P < 0.05$) for S_2 and S_1I_1 products (Table 2) due to the storage. Gumminess (G) showed a similar behaviour as hardness, while chewiness (M) presented a significant decrease ($P < 0.05$) after chilled storage of S_2, S_1I_1, I_2 and S_1H_1 products (Table 2).

Ramírez *et al*. (2006) studied the effect of microbial transglutaminase (MTG) and whey protein concentrate (WPC) as binders in Mexican flounder restructured products. The authors observed that hardness increased as a function of the salt content increment (between 1% and 2%). WPC (1%) addition did not change this parameter, while MTG (0.3%) addition increased hardness at two salt levels. Other works carried out on surimi (Ramírez *et al*., 2000) and stripped mullet products (Ramírez *et al*., 2007) also reported this last fact. However, it has not been possible to obtain restructured fish products with the solely addition of MTG, without salt. For that, combinations of the enzyme with nonmeat proteins, such as dairy proteins, have been evaluated, obtaining good results (Uresti *et al*., 2004). Likewise, in the present work, an increase in the gel hardness has been obtained with respect to control product (2% salt) by adding amaranth protein isolate or hydrolysate. Similar to us, the aforementioned authors did not find relevant differences in other textural parameters such as springiness or cohesiveness parameters after the addition of MTG or non-meat proteins. The gel hardness increments registered suggest that an interaction of the amaranth proteins (in the case of S_1I_1 and I_2 products) with the myofibrillar proteins strengthens the matrix network. These interactions seem to be nondependent of the presence of salt and would be modified during the chilled storage. It has been informed that other nonmeat proteins such as egg white, soya beans and whey can enhance gel characteristics; however, there usually is a lack of interaction between nonmeat proteins and muscle proteins and, in these cases, nonmeat proteins may not participate in the protein structure and can negatively affect texture by interfering with the gelation of the myofibrillar proteins (Sun & Holley, 2011). In the case of the amaranth peptides present in the hydrolysate, strengthening was only evident in product S_1H_1, suggesting that is necessary the presence of salt to achieve this effect. Feng & Xiong (2003) investigated the effect of soya protein hydrolysates on myofibrillar proteins gelation properties and concluded that it was dependent on the hydrolysates composition (used protease). Anyway, it is important to remark that neither amaranth protein isolate nor hydrolysate produced negative effects on the texture of restructured products.

Water-holding capacity

Water-holding capacity value measured at day 1 for control product S_2 was 91 ± 2%. A small (about 3%)

Table 2 TPA parameters of restructured products with the addition of amaranth isolate I or amaranth alcalase-hydrolysate H

Storage time (d)	Product	H	S_{ins}	S_{ret}	C	G	M
1	S_2	3.8 (0.8)^{a}	0.75 (0.08)^{a}	0.99 (0.2)^{a}	0.72 (0.02)^{a}	2.8 (0.5)^{a}	2.8 (0.4)^{a}
	S_1I_1	4.9 (0.5)^{b}	0.73 (0.08)^{a}	0.97 (0.02)^{a}	0.72 (0.01)^{a}	3.6 (0.4)^{b}	3.5 (0.2)^{a}
	I_2	5.1 (0.7)^{b}	0.68 (0.09)^{a,b}	0.95 (0.03)^{a}	0.72 (0.02)^{a}	3.7 (0.5)^{b}	3.5 (0.6)^{a}
	S_1H_1	5.7 (0.6)^{b}	0.69 (0.08)^{a}	0.94 (0.03)^{a}	0.72 (0.01)^{a}	4.1 (0.5)^{b}	3.8 (0.2)^{b}
	I_2	4 (1)^{a}	0.70 (0.06)^{a}	0.96 (0.03)^{a}	0.72 (0.01)^{a}	2.9 (0.8)^{a}	2.8 (0.6)^{a}
7	S_2	4 (1)^{a}	0.6 (0.2)^{b}	0.9 (0.2)^{a}	0.72 (0.03)^{a}	2.5 (0.8)^{a}	2.3 (0.8)^{b}
	S_1I_1	3.2 (0.9)^{a}	0.5 (0.1)^{b}	0.8 (0.1)^{a}	0.71 (0.02)^{a}	2.3 (0.5)^{a}	1.9 (0.6)^{b}
	I_2	4.0 (0.9)^{a}	0.61 (0.09)^{b}	0.88 (0.08)^{a}	0.72 (0.03)^{a}	2.9 (0.7)^{a}	2.6 (0.7)^{b}
	S_1H_1	3 (1)^{a}	0.73 (0.05)^{a}	0.94 (0.06)^{a}	0.72 (0.03)^{a}	2.8 (0.8)^{a}	2.4 (0.6)^{a}
	I_2	4 (1)^{a}	0.7 (0.1)^{a}	0.95 (0.05)^{a}	0.72 (0.03)^{a}	3.0 (0.7)^{a}	2.9 (0.7)^{a}

H, hardness; S_{ins}, instantaneous recoverable springiness; S_{ret}, retarded recoverable springiness; *C*, cohesiveness; G, gumminess; *M*, chewiness. Results are expressed as the mean ± SD (in parentheses) of at least seven measures. Different superscripts in the same column indicate significant differences ($P < 0.05$).

but significant decrease ($P < 0.05$) was registered for the S_1I_1 product. However, I_2 presented a similar value ($P > 0.05$) as S_2, indicating that total replacement of salt by I had no effect on WHC. In the case of products with H, no significant differences ($P > 0.05$) were recorded among S_2, S_1H_1 and H_2 products.

Diminution in the NaCl content of restructured products produced a lower WHC of the meat proteins causing synaeresis. One of the objectives of the non-meat proteins in these formulations is to compensate the WHC loss. Ramírez *et al.* (2006) reported a lower WHC in products with low salt content (1%) with MTG (0.3%) or WPC (1%) in comparison with products with 2% salt. These results were explained by a low exposition of hydrophilic groups, which would induce a low protein–water interaction. Moreover, the presence of NaCl in meat products could be a problem when some globular denature proteins are used as gelificant agents at high temperatures because of the ionic force which produces an increase in the hydrophobic interactions between these proteins, providing a higher hardness level but a lower WHC level (Pearce, 2005). This could be a possible explanation for the small WHC diminution of the S_1I_1 product. According to the present results, the presence of amaranth proteins in absence of salt (I_2) or small amaranth polypeptides/peptides (S_1H_1 and H_2) and their interactions with the fish protein matrix would not produce modifications in the WHC of the restructured products.

Colour

Table 3 shows L^*, a^*, b^* and whiteness (W) values for the different products, measured at the beginning of the chilled storage. L^* presented a significant decrease ($P < 0.05$) as a function of I or H concentration, which indicates that the addition of protein ingredients produced less bright products. A possible explanation for this would be the changes induced by the amaranth proteins and peptides in the gel network, suggesting the formation of more aggregated matrix. The parameter a^* shows a significant increase ($P < 0.05$) in I_2, S_1H_1 and H_2 products compared to their corresponding controls, showing a shift to redness due to the I or H addition, In the case of b^*, this parameter was only modified by the addition of 2% I, shifting to a more yellow tone. These changes could be probably related to the typical colour of the amaranth protein ingredients (light brown). The whiteness of the products was significantly reduced ($P < 0.05$) for products I_2 and H_2.

Table 3 Colour parameters of restructured products with the addition of amaranth isolate I or amaranth alcalase-hydrolysate H

Product	L^*	a^*	b^*	W
S_2	70 ± 1^{a}	-0.9 ± 0.2^{a}	9.9 ± 0.2^{a}	68 ± 1^{a}
S_1I_1	68.4 ± 0.9^{b}	-0.6 ± 0.3^{a}	10.3 ± 0.3^{a}	66 ± 1^{a}
I_2	66.8 ± 0.7^{c}	-0.3 ± 0.2^{b}	11.1 ± 0.5^{b}	65 ± 1^{b}
S_2	79 ± 3^{A}	-1.8 ± 0.4^{A}	10 ± 2^{A}	76 ± 4^{A}
S_1H_1	77 ± 2^{B}	-1.1 ± 0.6^{B}	11 ± 2^{A}	74 ± 3^{AB}
H_2	72 ± 1^{C}	-0.8 ± 0.6^{B}	12 ± 2^{A}	71 ± 1^{B}

Results are expressed as the mean ± SD of at least seven measures. Different superscripts in the same column indicate significant differences ($P < 0.05$).

Microbiological counts

In addition to its binding property, salt is considered as a preservative, although some authors have proposed that salt levels lower than 4% would not have effect on the microbial load evolution (Moreno *et al.*, 2013). To evaluate the possibility of a microbiological quality diminution due to the replacement of salt by amaranth ingredients, microbiological counts were performed along the chilled storage. As is shown in Figure S4, there were no significant differences ($P > 0.05$) neither in the initial bacterial load nor along the chilled storage of the different products. All products reached the maximal limit (10^6 UFC g^{-1}) for safety of fish products (Institute of Food Science and Technology, 1999) after 8–9 days of chilled storage. These facts are in agreement with other results reported in gelified fish products (Amiza & Kang, 2013). The present results show that the partial or total replacement of salt by I or H had no deleterious effects on the microbiological quality of the restructured products.

Conclusions

Both the amaranth protein isolate and their alcalase-hydrolysate could act as binders replacing the salt, producing restructured gelified fish products with only minimal textural and colour modifications and without changes in the WHC and microbiological counts with respect to the conventional products containing NaCl. Also, they act as natural antioxidants that partially inhibit the lipid oxidation process in restructured fish products in comparable levels, although isolate and hydrolysate probably involved in different active compounds and mechanisms of action. The addition of 2% w/w of any of the amaranth protein ingredients (without salt addition) rendered acceptable gel products with two important benefits: the inhibition of the formation of secondary lipid oxidation products which have negative effects in the human body in addition to their deleterious effects on the food quality; and the diminution of salt content in the product which is very important to prevent hypertension and cardiovascular diseases among the population. These results contribute to the consideration of these amaranth protein ingredients as promising from a technological and functional point of view.

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Conflict of interest

The authors Tironi and García Fillería have nothing to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Conjugated dienes content (mean ± SD, $n = 2$) during the chilled storage for products S2: w/ TT,without thermal treatment; TT, with thermal treatment (40 °C, 30 min, 90 °C, 45 min).

Figure S2. Microbiological counts evolution for products with the replacement of salt by amaranth isolate I or amaranth alcalasehydrolysate *H* (mean ± SD, $n = 2$).