**The effect of glazing based on saponin-free quinoa
(*Chenopodium quinoa*) extract on the lipid quality of frozen
fatty fish**

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ABSTRACT

This study focussed on the rancidity stability of frozen fatty fish. Its basic objective was to investigate the effect of a glazing system including a saponin-free quinoa (*Chenopodium quinoa*) extract during the frozen storage of Atlantic mackerel (*Scomber scombrus*). For it, two different concentrations of 80% aq. (v/v) ethanol quinoa extracts (0.45 and 1.36 g lyophilised quinoa L⁻¹ solution, respectively) were tested and compared with two control treatments (water glazing and non-glazing conditions). Chemical and sensory changes were monitored for a 8-month frozen storage. An inhibitory effect (p<0.05) of the glazing system including the most concentrated saponin-free quinoa extract was observed on the lipid hydrolysis (free fatty acid formation) evolution and on the formation of secondary (thiobarbituric acid reactive substances) and tertiary (fluorescent compounds) lipid oxidation compounds. A sensory quality enhancement and shelf life increase was obtained in fish submitted to glazing conditions including the most concentrated quinoa extract in agreement to raw (gills and muscle odour) and cooked (muscle odour) descriptors. A novel quinoa-glazing system is proposed, this avoiding the inconveniences of the saponin compounds presence.

Keywords: Frozen mackerel; glazing; *Chenopodium quinoa*; saponin; rancidity stability

Running title: Saponin-free quinoa glazing and frozen fish rancidity

1. INTRODUCTION

During freezing and frozen storage, marine lipids are reported to undergo fast oxidation and lead to the production of off-flavours and odours and to a nutritional value decrease (Kolakowska, 2003; Lehmann and Aubourg, 2008). Consequently, different strategies and technologies have been tested to extend the shelf life time during the frozen storage of fatty seafood. One particular technology greatly used is the application of an ice layer to the surface of the frozen seafood, referred as glazing (Vanhaecke, Verbeke, & De Brabander, 2010; Yerlikaya & Gokoglu, 2010). Thus, adequate glazing of marine specimens prior to frozen storage can protect the final product from dehydration, oxidation and general quality loss. The amount of glaze applied would depend on various factors such as glazing time, seafood temperature, water temperature, product size and shape (Jacobsen & Fossan, 1999). Previous research has shown profitable effects of glazing by inhibiting lipid hydrolysis (Çoban, 2013) and oxidation (Sathivel, Huang, & Bechtel, 2008; Sundararajan et al. 2011; Cavonius & Undeland, 2017) development and microbial activity (Gonçalves & Gindri, 2017; Solval, Rodezno, Moncada, Bankston, & Sathivel, 2014).

Quinoa (*Chenopodium quinoa* Willd.) is a highly appreciated pseudo-cereal of Andean origin showing a balanced composition on amino acids, lipids, trace elements, fibre and vitamins, high contents of proteins, and absence of gluten (Vega-Gálvez et al., 2010). Quinoa-derived products have successfully been employed in the preparation of emulsion-type products and malted beverages, as fat/cream substitutes, to enhance the quality of baked foods, as well as for producing quinoa protein concentrates and hydrolysates (Bhargava, Shukla, & Ohri, 2006; Graf et al., 2015). Concerning food preservation by quinoa presence, an antimicrobial activity has recently been proved (Tremonte, Succi, Messina, Coppola, & Marconi, 2017), while its high content on

vitamin E and phenolic compounds has been found responsible for its antioxidant properties (Gómez-Caravaca, Iafelice, Verardo, Marconi, & Caboni, 2014; Carciochi, Manrique, & Dimitrov, 2015). Indeed, the inclusion of quinoa and quinoa-derived products in active packaging systems has shown to enhance the antimicrobial properties of the corresponding films (Pagno et al., 2015; Caro et al., 2016), this leading to an increase of the shelf life time in various fruit products (Valenzuela et al., 2015; Abugoch et al., 2016).

However, saponin compounds located in the pericarp seeds constitute a drawback for the quinoa utilisation as a practical food source. In spite of showing a wide range of beneficial effects such as hypertension prevention (Huang, Davidge, & Wu, 2014) and anti-inflammatory (Yao, Yang, Shi, & Ren, 2014) activities, saponins of quinoa are bitter, interfere with quinoa's palatability and digestibility (Vega-Gálvez et al., 2010; Graf et al., 2015) and have been reported to be toxic because of their hemolytic activity and ability in lowering surface tension (Khalil & El-Adawy, 1994; Stuardo & San Martín, 2008). Consequently, the employment of quinoa and quinoa-derived products after removal of saponin compounds can represent a promising choice leading to additional advantages. In this context, a comparative study of the antioxidant behaviour and properties between whole quinoa and saponin-free quinoa was carried out in a heated marine-oil model system (Miranda et al., 2017); in agreement to the lipid oxidation assessment (primary and secondary products) and polyene content retention, saponin-free quinoa showed to maintain the original preservative properties.

The present research was focussed on the rancidity stability of frozen Atlantic mackerel (*Scomber scombrus*). Its basic objective was to investigate the effect of a glazing system including an aqueous ethanolic extract of saponin-free quinoa during the frozen storage. For it, two different concentrations of quinoa extracts were tested and

compared with two control treatments (water glazing and non-glazing conditions). Quality changes were monitored for a 8-month frozen storage by chemical (lipid hydrolysis and oxidation development) and sensory (raw and cooked descriptors) analyses.

2. MATERIAL AND METHODS

2.1. Initial quinoa, saponin removal and preparation of ethanolic extracts

Ancovinto ecotype quinoa cultivated in the Elqui Valley (Vicuña, Chile; 700 m a.s.l.; 30°1'0''S; 70°42'0''W) and harvested in 2014 was used. Homogeneous grains were selected according to similar ripening conditions, colour and size; visual inspection was carried out to discard contaminant particles or impurities.

Saponin-compounds removal was achieved in agreement to previous research (Miranda et al., 2017). Thus, the grains were washed with distilled water (quinoa/water ratio of 1/10, w/w) at 20 °C with constant stirring for one hour, with water changes every 10 min. After draining the quinoa grains for 5 min, the drying process was carried out at 60 °C using a convective dryer with a constant air flow rate of $2.0 \pm 0.2 \text{ m s}^{-1}$. Samples were dried until they reached constant weight (equilibrium condition). Saponin-free quinoa grains were finally finely ground and subjected to a 500-micro sieve (Dual Manufacturing Co., U. S. Standard Sieve Series, Sieve N° 35, Chicago, IL, USA).

Extracts from saponin-free quinoa were obtained in agreement with Miranda et al. (2017). For it, a mixture of 100 g of ground and sieved samples and 1,000 mL of an 80% aq. (v/v) ethanol solution (dried grains/alcoholic solution ratio of 1/10, w/v) was prepared. Each mixture was homogenised and shaken for 24 h in an orbital shaker (BOECO, OS2, Hamburg, Germany), and thereafter centrifuged (Eppendorf, 5804 R,

Hamburg, Germany) for 15 min at 5,000xg, being the supernatant filtered through a Whatman filter N° 1. Then, the filtrate was concentrated under reduced pressure at 40 °C on a rotary evaporator (Büchi RE 121, Flawil, Switzerland) and afterwards lyophilised in a freeze dryer (Virtis, Advantage Plus, Gardiner, NY, USA). The lyophilised quinoa extract was kept under refrigerated conditions (4 °C) to be employed throughout a 1-month period.

2.2. Glazing systems

Two glazing systems were prepared including the lyophilised saponin-free quinoa. For it, 5 and 15 g of the lyophilised extract were dissolved, respectively, in 200 mL of an 80% aq. (v/v) ethanol solution and then diluted to a 11-L solution with distilled water. As a result, low-concentrated glazing (LCQ condition; 0.45 g lyophilised quinoa L⁻¹ solution) and high-concentrated glazing (HCQ condition; 1.36 g lyophilised quinoa L⁻¹ solution) systems were obtained, respectively.

Two kinds of batch controls were considered in this study. One side, 200 mL of an 80% aq. (v/v) ethanol solution were diluted to a 11-L solution with distilled water and employed as water glazing control (WG condition). Furthermore, a blank control batch with no glazing treatment (NG condition) was also taken into account.

Concentrations of lyophilised quinoa in the glazing solutions employed in the current study were chosen on the basis of various preliminary studies. Thus, a 0.20-2.00 g lyophilised quinoa L⁻¹ solution range was tested. As a result, concentrations higher than 1.36 g L⁻¹ could modify some sensory descriptors (i.e., odour, colour and taste) of the fish species chosen. Accordingly, this concentration was considered in the present study, together with a less concentrated one (0.45 g L⁻¹).

2.3. Fish sample collection, freezing, glazing and frozen storage

Fresh Atlantic mackerel (102 specimens) were obtained at the Vigo harbour (North-Western Spain) in April 2015 and transported to the laboratory. Throughout this process (10 h), the fish were maintained in ice. The length and weight of the fish specimens ranged from 24.0 to 27.5 cm and from 107 to 125 g, respectively.

Upon arrival to the laboratory, six specimens were separated and analysed as initial fish. These fish specimens were divided into three different groups (two individuals per group) that were analysed independently to achieve the statistical analysis (n=3). The remaining fish specimens were divided into four batches (24 individuals in each batch) that were immediately frozen at -40°C .

After 48 hours at -40°C , the first batch was packed in polyethylene bags (two pieces per bag) and stored at -18°C (blank control; non-glazed batch; NG batch). At the same time, the remaining three batches were immersed, respectively, in the above-mentioned WG, LCQ and HCQ glazing systems. In all cases, specimens were immersed for 30 s at 0°C , allowed to drain for 15 s, packaged in polyethylene bags (two pieces per bag) and stored at -18°C .

Sampling was undertaken at months 2, 4, 6 and 8 of frozen storage at -18°C . At each time and for each glazing condition, six individuals were taken that were divided into three groups (two individuals per group) and studied separately (n=3). Analysis of frozen material was undertaken after thawing; thawing was carried out by overnight storage in a cool room (4°C).

2.4. Chemical evaluation of lipid damage

All solvents and chemical reagents used were of reagent grade (Merck, Darmstadt, Germany). Chemical analyses were carried out on the white muscle of mackerel.

Lipids were extracted from mackerel muscle by single-phase solubilisation with chloroform-methanol (1:1), as described by Bligh and Dyer (1959).

The free fatty acid (FFA) concentration of the mackerel lipid extract was determined by colorimetric reaction with cupric acetate-pyridine and absorbance at 715 nm, according to Lowry and Tinsley (1976). Results were calculated as mg oleic acid kg⁻¹ muscle and expressed as mg FFA kg⁻¹ muscle.

The peroxide value (PV) of the mackerel lipid extract was determined by peroxide reduction with ferric thiocyanate and absorbance at 500 nm (Beckman Coulter, DU 640; London, UK), as described by Chapman and McKay (1949). Results are expressed as meq active oxygen kg⁻¹ lipids.

The thiobarbituric acid index (TBA-i) was determined as described by Vyncke (1970) and based on the reaction between a 5% trichloroacetic acid extract of the fish muscle and thiobarbituric acid (TBA). Content on thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and calculated from a standard curve using 1,1,3,3-tetraethoxy-propane. Results are expressed as mg malondialdehyde kg⁻¹ muscle.

The formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer España, Tres Cantos, Madrid, Spain) was determined by measurements at 393/463 nm and 327/415 nm as described by Aubourg, Rey-Mansilla and Sotelo (1999). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg ml⁻¹ in 0.05 M H₂SO₄) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463 \text{ nm}}/RF_{327/415 \text{ nm}}$. The FR value was determined using the aqueous phase that resulted from the lipid extraction of the fish muscle (Bligh & Dyer, 1959).

2.5. Sensory evaluation of frozen mackerel

Sensory analysis was carried out by a sensory panel consisting of four to six experienced judges. Before carrying out the present experiment, the judges received special training on frozen mackerel, focused on the evaluation of specimens that exhibited different qualities. Special attention was accorded to the evolution of the sensory descriptors that were found as limiting factors for the shelf life. Consequently, various raw (external odour, gills, and muscle odour) and cooked (muscle odour and taste) descriptors were analysed. The different descriptors were evaluated on a scale from 7.0 (stage of highest quality) to 0.0 (stage of lowest quality) in agreement with Lehmann and Aubourg (2008). According to European Council Regulation (1996), four rang categories were considered: 7.0-5.6 (excellent), 5.5-3.6 (good), 3.5-1.5 (fair) and 1.4-0.0 (rejectable).

At each sampling time, fish individuals from each batch were analysed. Evaluation began by the analysis of fish in the raw state and was followed by the analysis of samples in the cooked state. Cooking was accomplished at 95-100 °C for 7 min in a pre-warmed oven with air circulation and then submitted to the panel. At each sampling time, fish samples were coded with 3-digit random numbers and presented to the panellists in individual trays, which were scored individually. Each descriptor of each sample was scored a single time by each member of the panel. The panel members shared samples tested. Scores among panellists were averaged.

2.6. Statistical analysis

Data obtained (three replicates; n=3) from the different sensory and chemical analyses were subjected to the ANOVA method to explore differences resulting from the effect of the glazing system employed; comparison of means was performed using

the least-squares difference (LSD) method. Analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA); differences among batches were considered significant for a confidence interval at the 95% level ($p < 0.05$) in all cases.

3. RESULTS AND DISCUSSION

3.1. Chemical assessment of lipid hydrolysis in frozen mackerel

Lipid hydrolysis development was measured by the FFA formation. Results are depicted in Figure 1. A progressive FFA formation was observed in all kinds of samples throughout the frozen storage time. Fish samples corresponding to the glazing condition including the highest quinoa concentration showed the lowest average values for the whole storage period; comparison with both controls (i.e., NG and WG batches) showed significant differences ($p < 0.05$) for the 6-8-month period. An inhibitory effect ($p < 0.05$) on FFA formation was also implied in samples corresponding to the LCQ batch at the end of the experiment.

Lipid hydrolysis development is reported to be produced during the frozen storage as a result of lipase release from liposomes into the muscle, which then facilitates closer proximity between enzyme and substrate (Sista, Erickson, & Shewfelt, 1997; Aubourg et al., 1999). As a result of the quinoa extract presence in the glazing system, a lower FFA formation has been produced. This result could be explained on the basis of a modification of the lipase environment, this leading to a partial inhibition of its catalytic effect (Sikorski & Kolakowski, 2000; Aubourg & Ugliano, 2002). In agreement to the current study, the employment of a glazing system including essential oils (sage, thyme and clove) extracts led to an inhibitory effect on the formation of FFA in frozen (6 months at $-18\text{ }^{\circ}\text{C}$) rainbow trout (*Oncorhynchus mykiss*) fillets (Çoban,

2013); in such study, an inhibitory effect was also observed in fish samples corresponding to water glazing when compared with samples not glazed. In another related study (Taheri, 2015), the effect of a glazing system including rainbow sardine (*Dussumieria acuta*) protein hydrolysates was checked during subsequent frozen storage (6 months at -18°C) of black pomfret (*Parastromateus niger*) fillets; as a result, an inhibitory effect on FFA formation could be observed in fish including protein-hydrolysate glazing when compared with control conditions.

3.2. Chemical assessment of lipid oxidation in frozen mackerel

Lipid oxidation development was measured by assessment of the peroxides and TBARS values as well as the FR. In all kinds of samples, peroxides formation can be considered low, since values did not overpass the 5.57 score (Table 1). The highest average scores were obtained in all cases at month 6, this result being followed by a general decrease at the end of the experiment. Such decrease can be explained on the basis of peroxide breakdown at advanced frozen storage times, thus leading to content increases in secondary and tertiary lipid oxidation compounds (Howell, 1995; Aubourg & Ugliano, 2002). Comparison among samples showed some significant differences ($p<0.05$); however, the glazing system including the presence of a quinoa extract did not lead to a definite effect on the peroxide content of frozen mackerel.

A progressive formation of TBARS was observed in all kinds of samples throughout the storage period (Table 1). Samples corresponding to the HCQ batch showed lower average values than fish corresponding to both controls, differences being found significant ($p<0.05$) at the end of the experiment. Likely, TBARS formation in fish from batch LCQ showed lower average values than both controls at months 2, 6

and 8. Interestingly, no significant differences ($p > 0.05$) were implied between samples corresponding to both **glazing systems with quinoa extracts presence**.

Interaction compounds formation between primary and secondary lipid oxidation compounds and nucleophilic molecules present in the fish muscle were assessed by means of the fluorescent compounds formation (i.e., FR). Results show a progressive increase of such index in all kinds of samples with storage time increase (Table 1). Comparison among samples showed lower average values in fish corresponding to the glazing medium **including the highest content of quinoa extract**; this difference was found significant ($p < 0.05$) at month 2 by comparison to both controls. Furthermore, lower average values were obtained in samples corresponding to the LCQ batch when compared to their counterpart controls at months 2, 6 and 8.

Lipid oxidation development has been recognised as a complex process in which different kinds of molecules are produced, most of them unstable and susceptible to breakdown or react with other molecules present in the reaction medium (Howell, 1995; Aubourg & Ugliano, 2002). According to the results obtained, an inhibitory effect on both secondary (i.e., TBARS) and tertiary (fluorescent molecules) compounds formation has been **observed in** fish where the glazing condition with the highest quinoa content was applied. Consequently, an increased rancidity stability has been achieved during the frozen storage of the current fatty fish species. This antioxidant **effect is** in agreement to a previous research carried out on a heated (50 °C for 30 days) marine-oil model system (Miranda et al., 2017). In it, a comparative study between the antioxidant abilities of whole quinoa and saponin-free quinoa was undertaken. As a result, saponin-free quinoa showed to retain the antioxidant properties of the original quinoa, so that a marked inhibition of lipid oxidation development and retention of the polyunsaturated fatty acids content was implied in the heated-marine oil. Furthermore, the antioxidant

behaviour found in the current study for the aqueous-ethanolic extracts of saponin-free quinoa employed (i.e., Ancovinto ecotype) is in agreement with the above-mentioned study (Miranda et al., 2017) concerning contents on total polyphenols (142.85 ± 0.08 mg gallic acid equivalents g^{-1} dry quinoa), total flavonoids (31.83 ± 0.20 quercetin equivalents g^{-1} dry quinoa), α -tocopherol (58.43 ± 2.76 mg kg^{-1} dry quinoa), γ -tocopherol (51.29 ± 2.39 mg kg^{-1} dry quinoa), and δ -tocopherol (9.25 ± 0.61 mg kg^{-1} dry quinoa), as well as the antioxidant activity measured by the DPPH assay (114.99 ± 0.75 μ moles trolox equivalents g^{-1} dry quinoa).

Antioxidant efficacy is often difficult to predict since the antioxidant activity has shown to depend on the antioxidant structure, the food system itself and the conditions of storage/processing (Pokorný, 2007; Farvin & Jacobsen, 2013). No previous research is available, to our knowledge, about the effect of a quinoa-derived product (with or without saponin compounds) on the rancidity stability of seafood. Consequently, the present research could serve as a basis for further studies addressing the quinoa employment in different kinds of frozen fatty seafood. Interestingly, previous research has proved marked antioxidant properties of quinoa products when other kinds of foods are involved. This preservative effect has been explained on the basis of a relevant presence of different kinds of compounds such as polyphenols (Gómez-Caravaca et al., 2014), flavonoids (Carciochi et al., 2015) and tocopherols (Graf et al., 2015).

In agreement to the current study, previous related research accounts for lipid oxidation inhibition (peroxides and TBARS) as a result of applying a glazing system including various preservative compounds. This accounts for the employment of essential oils (sage, thyme or clove) extracts in the glazing system of frozen (6 months at -18 °C) rainbow trout (*Oncorhynchus mykiss*) fillets (Çoban, 2013), the addition of rainbow sardine (*Dussumieria acuta*) protein hydrolysates during the frozen storage (6

months at -18°C) of black pomfret (*Parastromateus niger*) fillets (Taheri, 2015) and the application of a glazing system with herring (*Clupea harengus*) muscle press juice to frozen ($-18/-20^{\circ}\text{C}$ for 52 weeks) herring fillets (Cavonius & Undeland, 2017). Additionally, inhibition of secondary lipid oxidation compounds (i.e., TBARS) formation was detected in various studies related to the employment of a glazing system including different kinds of preserving compounds. This result accounts for chitosan nano particles combined with sodium tripolyphosphate in frozen (30 days at -21°C) shrimp (*Litopenaeus setiferus*) (Solval et al., 2014), green tea and grape seed extracts in frozen (5 months at -18°C) bonito (*Sarda sarda*) (Yerlikaya & Gokoglu, 2010), green tea extract in frozen (180 days at -21°C) shrimp (*Litopenaeus setiferus*) (Sundararajan et al., 2011) and pollock (*Theragra chalcogramma*) skin hydrolysates in frozen (4 months at -35°C) pink salmon (*Oncorhynchus gorbusha*) (Sathivel et al., 2008).

3.3. Sensory evaluation of quality loss in frozen mackerel

Sensory quality was measured by evaluation of raw (Table 2) and cooked (Figures 2-3) descriptors. For all kinds of samples and descriptors, a progressive quality decrease could be observed with the frozen storage time increase.

A higher average score was observed in fish corresponding to the HCQ batch in the 4-8-month period for the evaluation of the external odour, the gills, and the raw muscle odour (Table 2). Comparison with both controls showed significant differences ($p<0.05$) at month 6 for the gills evaluation and the raw muscle odour development. In agreement to raw descriptors, fish corresponding to NG, WG and LCQ batches were considered unacceptable at month 8 in agreement to the evaluation of the external and raw muscle odours. On the contrary, fish belonging to the HCQ condition was acceptable at the end of the experiment.

Related to cooked descriptors, fish corresponding to the highest quinoa presence in the glazing system showed higher average scores throughout the whole experiment for cooked odour (Figure 2) and taste (Figure 3). Differences were found significant ($p<0.05$) at month 6 (cooked muscle odour) and at month 4 (cooked muscle taste) when compared to their counterparts from both control batches. According to cooked descriptors, all kinds of samples were found acceptable throughout the whole experiment.

In agreement to the above-mentioned chemical assessment of lipid damage development, a sensory quality increase can be implied by employment of a glazing system including the most concentrated extract of saponin-free quinoa. No previous research is actually available, to our knowledge, showing the effect of quinoa or quinoa-derived products on the shelf life or sensory quality of seafood. However, previous research accounts for a wide range of studies concerning the employment of quinoa to improve the sensory quality of baked foods via substitution of cereal grains by quinoa flour (Graf et al., 2015). Furthermore, quinoa-derived products have been found profitable to improve functionality of biofilms (Pagno et al., 2015; Caro et al., 2016); this strategy has been successful during the refrigeration storage of strawberry (*Fragaria x ananassa*) (Valenzuela et al., 2015) and blueberries (*Vaccinium corymbosum*) (Abugoch et al., 2016) by increasing the shelf life time (i.e., firmness and colour evaluation).

In agreement to the current study, a sensory quality enhancement of frozen seafood has been obtained as a result of previous glazing including various natural preservatives. Thus, the employment of a glazing system including rainbow sardine (*Dussumieria acuta*) protein hydrolysates led to a longer shelf life time (higher scores in colour, odour, taste, firmness and general appearance) of frozen (6 months at $-18\text{ }^{\circ}\text{C}$)

black pomfret (*Parastromateus niger*) fillets (Taheri, 2015). Furthermore, average score values of rancid odour development were found slightly higher for frozen (−18/−20 °C for 52 weeks) herring (*Clupea harengus*) control fillets than for their counterpart fillets previously submitted to glazing treatment including the herring muscle press juice (Cavonius & Undeland, 2017). Also related to the present study, a previous phosphate dipping to freezing and frozen (15 days at −25 °C) storage led to a sensory quality enhancement in red shrimp (*Xiphopenaeus kroyeri*) (Gonçalves & Gindri, 2009). Contrary to the current results, the application of a glazing system including essential oils (sage, thyme or clove) extracts did not increase the shelf life of frozen (6 months at −18 °C) rainbow trout (*Oncorhynchus mykiss*) fillets (Çoban, 2013).

4. CONCLUSIONS

A novel glazing system based on the inclusion of 80% aq. (v/v) ethanol extracts of saponin-free quinoa has been tested for the quality enhancement of frozen Atlantic mackerel. As a result, an inhibitory effect ($p < 0.05$) of the glazing system including the most concentrated quinoa extract was observed on lipid hydrolysis (free fatty acid formation) evolution and on the formation of secondary (TBARS) and tertiary (fluorescent compounds) lipid oxidation compounds; interestingly, no effect ($p > 0.05$) on peroxide content was detected. A sensory quality enhancement and shelf life increase was obtained in fish submitted to glazing conditions including the most concentrated quinoa extract in agreement to raw (gills and muscle odour) and cooked (muscle odour) descriptors. Under the conditions tested in the present study, a glazing system is proposed to be applied for the quality enhancement of fatty fish species during commercialisation under frozen conditions. Such tool can be considered a profitable and practical strategy in agreement with the availability and commercial cost of the

materials employed. On the basis of the readiness with which saponin compounds can be removed from whole quinoa, this preservative system would avoid the inconveniences of the saponin compounds presence. Further research would be necessary to optimise the preservative role of saponin-free quinoa in the glazing system when applied to different kinds of fatty seafood.

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538

FIGURE LEGENDS

Figure 1: Effect of various glazing conditions* on the free fatty acids (FFA) formation in mackerel muscle throughout frozen storage**

* Abbreviations of glazing conditions as expressed in Table 1.

** Average values of three replicates (n=3). Standard deviations are indicated by bars.

Average values accompanied by different letters (a, b) denote significant differences ($p < 0.05$). No letters are included when significant differences were not found ($p > 0.05$).

Figure 2: Effect of various glazing conditions* on the cooked muscle odour throughout frozen storage**

* Abbreviations of glazing conditions as expressed in Table 1.

** Average values of three replicates (n=3). Standard deviations are indicated by bars.

Average values accompanied by different letters (a, b) denote significant differences ($p < 0.05$). No letters are included when significant differences were not found ($p > 0.05$).

Figure 3: Effect of various glazing conditions* on the cooked muscle taste throughout frozen storage**

* Abbreviations of glazing conditions as expressed in Table 1.

** Average values of three replicates (n=3). Standard deviations are indicated by bars.

Average values accompanied by different letters (a, b) denote significant differences ($p < 0.05$). No letters are included when significant differences were not found ($p > 0.05$).

TABLE 1

Effect of the glazing treatment* including saponin-free quinoa extract on the lipid oxidation development in mackerel muscle throughout frozen storage**

Lipid oxidation index	Frozen storage time (months)	Glazing condition			
		NG	WG	LCQ	HCQ
Peroxide value (PV; meq active oxygen kg ⁻¹ lipids)	2	3.93 (1.15)	3.42 (1.15)	3.04 (1.51)	4.08 (1.17)
	4	4.19 b (1.35)	2.25 ab (0.59)	1.69 a (0.53)	2.58 ab (1.73)
	6	5.57 (0.63)	4.34 (0.68)	4.29 (1.23)	4.18 (1.34)
	8	1.67 ab (0.23)	1.91 ab (0.35)	2.14 b (0.73)	0.99 a (0.35)
Thiobarbituric acid index (TBA-i; mg malondialdehyde kg ⁻¹ muscle)	2	0.96 (0.29)	0.86 (0.41)	0.75 (0.12)	0.82 (0.30)
	4	1.01 (0.36)	0.95 (0.40)	0.97 (0.19)	0.84 (0.19)
	6	1.33 (0.24)	1.14 (0.18)	0.94 (0.17)	0.83 (0.38)
	8	1.65 b (0.11)	1.75 b (0.24)	1.34 ab (0.68)	1.33 a (0.11)
Fluorescence ratio (FR)	2	0.56 b (0.12)	0.61 b (0.07)	0.55 ab (0.25)	0.48 a (0.03)
	4	0.68 (0.03)	0.69 (0.09)	0.77 (0.22)	0.66 (0.03)
	6	1.02 (0.16)	0.90 (0.21)	0.88 (0.19)	0.74 (0.23)
	8	1.20 (0.33)	1.15 (0.34)	1.12 (0.15)	0.91 (0.15)

* Abbreviations of glazing conditions: NG (without glazing; blank control), WG (water glazing), LCQ (low-concentrated quinoa extract glazing) and HCQ (high-concentrated quinoa extract glazing) in agreement with the material and methods section.

** Average values of three (n=3) replicates; standard deviations are indicated in brackets. For each quality index, average values followed by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the glazing condition applied. No letters are included when significant differences were not found ($p > 0.05$). Initial fish values: 0.73 ± 0.64 (PV), 0.72 ± 0.03 (TBA-i) and 0.37 ± 0.05 (FR).

TABLE 2

Effect of the glazing treatment* including saponin-free quinoa extract on the sensory acceptability (raw descriptors) of mackerel throughout frozen storage**

Sensory descriptor	Frozen storage time (months)	Glazing condition			
		NG	WG	LCQ	HCQ
External odour	2	5.0 (1.4)	4.5 (0.7)	5.5 (0.7)	5.0 (0.0)
	4	3.3 a (1.2)	4.3 ab (1.2)	4.3 ab (1.5)	5.3 b (0.6)
	6	1.5 a (0.7)	3.0 ab (1.4)	2.0 ab (0.3)	4.0 b (1.4)
	8	1.3 (0.5)	1.3 (0.6)	1.0 (1.0)	2.3 (0.7)
Gills	2	5.0 (0.0)	5.0 (0.0)	5.0 (0.0)	5.0 (0.7)
	4	4.7 (0.6)	4.7 (0.6)	4.7 (0.6)	5.0 (0.2)
	6	2.0 a (0.2)	2.0 a (0.2)	2.5 ab (0.7)	4.0 b (1.4)
	8	2.3 (0.6)	2.0 (1.0)	2.0 (1.0)	2.7 (0.6)
Raw muscle odour	2	5.0 ab (0.3)	6.0 b (0.3)	4.5 a (0.7)	5.5 ab (0.7)
	4	4.0 (1.0)	4.0 (0.0)	4.3 (1.5)	5.7 (0.6)
	6	2.5 a (0.7)	2.5 a (0.7)	1.5 a (0.7)	5.0 b (1.4)
	8	2.3 b (0.6)	0.7 a (0.6)	1.3 ab (0.8)	2.7 b (0.6)

* Abbreviations of glazing conditions as expressed in Table 1.

** Average values of three (n=3) replicates; standard deviations are indicated in brackets. For each descriptor, average values followed by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the glazing condition applied. No letters are included when significant differences were not found ($p > 0.05$). Initial fish values: 7.0 ± 0.0 for all descriptors.

Figure 1

Figure 2

Figure 3