The impact of quinoa (*Chenopodium quinoa* Willd.) ethanolic extracts in the icing medium on quality loss of Atlantic chub mackerel (*Scomber colias*) under chilling storage

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ABSTRACT

The employment of an ethanolic extract of quinoa (*Chenopodium quinoa* Willd.) as a novel preservation medium and as a source of bioactive compounds was tested during fish chilled storage. For this purpose, specimens from an under-valued pelagic species (Atlantic chub mackerel, *Scomber colias*) were stored in ice including quinoa at two concentrations: 80% aq. (v/v) ethanol extracts (0.05 and 0.20 g lyophilised quinoa extract·L^{-1} icing solution; Q-1 and Q-2 batches, respectively) and compared to a control icing condition (Q-0 batch). The evolution of fish quality was assessed during a 13-day chilled storage period. The presence of the most concentrated quinoa extract (i.e., Q-2 batch) in the icing medium implied lower ($p<0.05$) lipid oxidation (thiobarbituric acid and fluorescence values) and hydrolysis (free fatty acids formation and lipolytic bacteria counts), as well as to a decrease in pH and trimethylamine values. Such effect was more pronounced at advanced storage times. Furthermore, fish specimens from Q-0 and Q-1 batches did not exhibit acceptable quality at the end of storage time, while those from the Q-2 batch still exhibited acceptable quality at that moment; interestingly, quality limit aspects were skin, eyes and external odour.

PRACTICAL APPLICATIONS

Under the conditions tested, a novel quinoa-based system is proposed as a suitable tool for the improvement of a chilled fatty fish species and consequent extension of its shelf-life. This preservative strategy matches with current interest in the search for effective antioxidants and antimicrobials from natural sources to replace synthetic preservatives in the food sector. Additionally, this preservative strategy has proven to increase the technological aptitude and, accordingly, the commercialisation possibilities, of a currently under-valued fish species. The innovative treatment reported in this study

opens the way to the employment of this strategy to currently commercial seafoods (namely, lean and fatty fish). Further research should be undertaken to gain a deeper knowledge on the benefits derived from the presence of quinoa extracts in the icing system on fish quality as well as to apply it as a potential way to incorporate healthy biomolecules into seafood.

Keywords: *Chenopodium quinoa*; *Scomber colias*; icing system; ethanolic extract; quality; shelf-life

Running title: Quinoa and chilled Atlantic chub mackerel quality

1. INTRODUCTION

Due to a wide variety of damage pathways, seafoods are highly perishable products that need rapid and efficient processing and storage to avoid post-mortem changes [1, 2]. Among seafoods, chilled ones have taken the market in the last few decades. In order to maintain the best quality of marine species and provide the consumer a high-quality product, refrigerated storage in ice has been widely employed. However, due to the short commercial life of fish species, ice has often been combined with additional preservation methods [3, 4].

One particular and promising strategy has been the incorporation of natural preservative extracts in the ice system. Thus, the inclusion of natural organic acids such as lactic and citric acids in the icing medium allowed to slow down lipid damage events and microbial activity in hake specimens (*Merluccius merluccius*) [5], a similar result being observed in megrim (*Lepidorhombus whiffiagonis*) [6]. Furthermore, the presence of wild-thyme hydrosol [7] or rosemary extract [8] in ice led to an extension of shelf-life time in Transcaucasian barb (*Capoeta capoeta*) and sardine (*Sardinella aurita*), respectively. Moreover, icing conditions including algae *Fucus spiralis* or *Bifurcaria bifurcata* during chilled storage of hake [9] and megrim [10], respectively, exerted relevant inhibitory effects on lipid oxidation and microbial activity.

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal from the Andean region which has recently received increasing attention due to its optimal content of a wide variety of nutrients such as amino acids, lipids, vitamins and fibre, and due also to its absence of gluten [11]. Quinoa-derived products have been considered for the production of malted beverages and emulsion-type products, as fat substitutes, as enhancers of the quality of baked foods and as a source of both protein hydrolysates and concentrates [12, 13]. Concerning food preservation strategies based on quinoa, its

92 protective effect against microbial growth has been reported [14]. Moreover, an
93 antioxidant activity has also been reported, probably due to its remarkable contents on
94 tocopherols and phenolic compounds [15, 16]. In fact, quinoa and its derived products
95 have been successfully employed in antimicrobial packaging strategies [17, 18] that
96 implied significant extensions of commercial life of different fruits [19, 20].
97 Furthermore, an antioxidant effect of quinoa ethanolic extracts in a model system of
98 marine oil has been recently reported [21].

99 As a consequence of the more limited availability of traditional species, the fish
100 industry has turned its attention to unconventional sources [22, 23]. Thus, small pelagic
101 fish may represent valuable products of economic relevance in different geographic
102 areas. One of these fish species is Atlantic chub mackerel (*Scomber colias*), which has
103 been found in the Atlantic Ocean, the Mediterranean Sea, and the Black Sea, being
104 especially abundant in the Eastern Mediterranean. Previous research on this species has
105 proven a marked interest because of its remarkable vitamins content [24], as well as on
106 its polyunsaturated fatty acids (PUFA) composition and availability as a source of
107 protein hydrolysates [25]. Because of its under-valued nature, great efforts have been
108 carried out to differentiate it from other related fish species of higher commercial value
109 [26, 27]. Concerning technological aptitude, recent research has shown the quality loss
110 evolution of this species during a freezing/thawing process [28], a refrigerated storage
111 (3 and 6 ºC) under modified atmosphere and vacuum packaging [29] and a glazing
112 treatment followed by frozen storage [30].

113 This study was aimed at assessing the potential benefits of the employment of an
114 ethanolic extract of quinoa for the preservation of chub mackerel and as a source of
115 bioactive compounds. For this purpose, specimens from Atlantic chub mackerel were
116 stored in ice prepared with quinoa at two different concentration levels of 80% aq. (v/v)

ethanol extracts and compared to a control icing condition. Quality changes were evaluated during 13-days of storage under refrigeration conditions.

2.MATERIAL AND METHODS

2.1. Preparation of lyophilised quinoa extracts

Quinoa (Cancosa ecotype) from the Elqui Valley (Vicuña, Chile; 700 m a.s.l.; 30° 1' 0" S; 70° 42' 0" W) was considered in this study. The cereal was harvested in 2014. Grains exhibiting similar ripening, size and colour were selected.

As it was previously reported [11], quinoa grains were dried at 60 °C by means of a convective drying system at a flow rate of 2.0 ± 0.2 m s^{-1} until constant weight. Afterwards, dried grains were ground and passed through a 500-micron sieve (Sieve Nº 35; Dual Manufacturing Co., Chicago, IL, USA).

Quinoa extracts were obtained by using the method developed by Miranda et al.[21]. Briefly, a 100 g sample, processed as described above, was mixed with 1 L of an 80% aq. (v/v) ethanol solution. After 24 h of homogenisation in an orbital shaker (BOECO OS2, Hamburg, Germany) and spinning (Eppendorf 5804 R, Hamburg, Germany) for 15 min at 5,000 × *g*, the supernatant was filtered through a Whatman Nº 1 paper. Then, the filtrate portion was concentrated at 40 °C on a rotary evaporator (Büchi RE 121, Flawil, Switzerland) and finally subjected to lyophilisation (Virtis Advantage Plus, Gardiner, NY, USA). The dried extract obtained in this way was stored at low temperature (4 °C) prior to its use before one month.

2.2. Chilling systems

As a first step, a 3.75 g portion of the dried quinoa extract was dissolved in 50 ml of distilled water. Then, 4 ml and 16 ml of such solution were picked up and diluted to 6 L

142 with distilled water, respectively. As a result, low-concentration (Q-1 solution; 0.05 g
143 extract·L^{-1}) and high-concentration (Q-2 solution; 0.20 g extract·L^{-1}) quinoa solutions
144 were prepared. Additionally, a control solution was considered (Q-0), this consisting of
145 6 L of distilled water.

146 Quinoa and control solutions were introduced in polyethylene packs, freezed and
147 maintained frozen below −18 °C until required. Then, all solutions were ground until ice
148 flakes were obtained. Such flakes were used as icing media for the chilled storage of
149 chub mackerel specimens.

150 The concentrations of lyophilised quinoa employed had been previously selected
151 as follows. Briefly, a 0.01-2.00 g L^{-1} quinoa solution range was preliminary assayed. It
152 could be observed that quinoa levels above 0.20 g·L^{-1} negatively affected the sensory
153 quality (mainly odour, colour or taste) of fish. Thus, 0.20 g·L^{-1} proved to be the most
154 concentrated quinoa extract not affecting sensory quality. As a consequence of this, 0.20
155 g· L^{-1} and a less concentrated quinoa extract (0.05 g·L^{-1}) were chosen for this study.
156

157 **2.3. Fish chilling and storage**

158 A total of 78 Atlantic chub mackerel fish specimens were purchased at Vigo (North
159 Western Spain) harbour and transported under refrigerated conditions to our lab. The
160 length and weight ranges of fish were 25-30 cm and 115-135 g, respectively. As soon as
161 the fish material arrived to the lab (before 8 h), six specimens were divided in 3 groups
162 of two specimens each and analysed (n = 3) to assess the initial quality at day 0. All
163 remaining chub mackerel specimens were divided in three different groups of 24 fish
164 specimens each. Each fish batch was placed in contact with each type of ice at an
165 ice:fish ratio of 1:1 (w/w) to conform Q-0, Q-1 and Q-2 batches, respectively, and
166 stored for 13 days in a cold room at 3 ± 1 °C. Samples (six specimens from each batch,

167 divided in three groups, n = 3) were taken for analysis on days 2, 6, 9. The analyses
168 were performed in triplicate.
169
170 **2.4. Quality parameters related to lipid hydrolysis in chilled mackerel**
171 Fish lipids were obtained from fish muscle by the method of Bligh and Dyer [31], the
172 results being calculated as g lipids·kg^{-1} muscle.
173 The free fatty acids (FFA) were determined at 715 nm in a DU 640
174 spectrophotometer (Beckman Coulter; London, UK), as described by Lowry and
175 Tinsley [32], the results being calculated as mg oleic acid kg^{-1} muscle and expressed as
176 mg FFA·kg^{-1} muscle.
177 For the investigation of lipolytic bacteria, portions of white muscle (10 g) of fish
178 were mixed with 90 mL of 0.1% peptone water (Merck, Darmstadt, Germany) as
179 described elsewhere [33, 34]. Dilutions were also prepared in 0.1% peptone water.
180 Bacteria exhibiting lipolytic activity were directly detected for their ability to generate
181 halos on tributyrin-agar after 48 h of incubation at 30 °C, as previously described [35].
182
183 **2.5. Quality parameters related to lipid oxidation in chilled mackerel**
184 The determination of conjugated dienes (CD) was carried out at 233 nm (Beckman
185 Coulter, DU 640) according to Kim and Labella [36] procedure. The results were
186 calculated as follows: CD = B x V·w^{-1}, with B being the absorbance at 233 nm, V the
187 volume in mL and w the mass in mg of the lipid extract.
188 The investigation of the peroxide value (PV) was carried out by measuring the
189 absorbance at 500 nm according to Chapman and McKay [37], the results being
190 calculated as meq active oxygen·kg^{-1} lipids.

191 The determination of the thiobarbituric acid index was performed by
192 spectrophotometric assessment at 532 nm according to Vyncke [38], with the aid of a
193 standard curve using 1,1,3,3-tetraethoxy-propane. The results were calculated as mg
194 malondialdehyde·kg^{-1} muscle.

195 Fluorescent compounds were measured at 393/463 nm and 327/415 nm in a
196 Fluorimeter LS 45 (Perkin Elmer, Madrid, Spain) according to Aubourg [39]. The
197 relative fluorescence (RF) was the F/F_{st} ratio, where F was the fluorescence determined
198 at each excitation/emission maximum and F_{st} the fluorescence intensity of 1 µg·mL^{-1}
199 quinine sulphate solution (prepared in 0.05 M H_2SO_4) at the corresponding wavelength.
200 The fluorescence ratio (FR) was determined as the following ratio; $RF_{393/463\ nm}/RF_{327/415}$
201 $_{nm}$.

202

203 **2.6. Assessment of parameters indicative of microbial quality in Atlantic chub**
204 **mackerel**

205 The pH value of fish muscle was monitored by means of a 6-mm electrode (Crison,
206 Barcelona, Spain).

207 Trimethylamine-nitrogen (TMA-N) was quantified by spectrophotometric
208 assessment at 410 nm according to the picrate method, as described elsewhere [40]. All
209 results were calculated as mg TMA-N·kg^{-1} flesh.

210

211 **2.7. Sensory evaluation in chilled mackerel**

212 Sensory evaluation was performed by a trained panel formed by four to six persons and
213 included the examination of eyes, skin, gills, external odour, flesh odour (raw and
214 cooked) and flesh taste (cooked) as well as consistency. In agreement with the European

215 Council Regulation [41], each parameter was evaluated and assigned to any of these
216 quality levels: highest (E), good (A), fair (B), and quality (C).

217 The fish specimens were first evaluated raw, and then subjected to cooking at
218 95-100 ºC for 7 min in an oven (Digitheat, J. P. Selecta S. A., Abrera, Barcelona,
219 Spain). All fish samples were presented to the panellists as blind samples harbouring a
220 3-digit random code of numbers.

221

222 **2.8. Statistical analysis**

223 A one-way ANOVA method was considered to determine the differences derived from
224 the different ice systems employed in this study. The means were compared by the
225 least-squares difference (LSD) method. The PASW Statistics 18 software for Windows
226 (SPSS Inc., Chicago, IL, USA) was used to explore the significance of the differences
227 among batches at the 95% level ($p < 0.05$).

228

229 **3. RESULTS AND DISCUSSION**

230 **3.1. Lipid hydrolysis assessment**

231 The investigation of lipid hydrolysis is depicted in Figure 1. A marked increase was
232 detected in all batches chilling time increased. Comparison among batches showed an
233 inhibitory effect exerted by the quinoa extract present in the ice systems. Thus, the Q-2
234 batch, corresponding to the highest concentration of quinoa, exhibited lower ($p < 0.05$)
235 levels of FFA than the counterpart control batch at advanced storage times (days 6 to
236 13). Interestingly, FFA content was also lower ($p < 0.05$) in fish from the Q-1 batch, as
237 compared to the control batch on days six to nine. It is concluded that the presence of
238 quinoa in the ice system exerts benefits concerning lipid hydrolysis in chilled mackerel.

Development of hydrolysis in refrigerated fish may be caused by two mechanisms, i.e., endogenous enzyme and microbial activities [1, 42]. Thus, the activity of endogenous enzymes, such as lipases released from liposomes into the muscle, is more relevant on the first 6-9 days, while microbial activity on lipids is more intense at advanced storage periods (i.e., > 6–9 days). Current data indicate that quinoa effect has been important in the 6-13-day period, this indicating a remarkable inhibition on both lipolytic mechanisms. Related to the former, previous studies have reported the inhibition caused by polyphenols on lipase activity. Thus, berry [43] and white and green tea [44] polyphenols extracts have shown to inhibit pancreatic lipase during the development of *in vitro* experiments.

A progressive increase of lipolytic counts could be observed in fish muscle along the 13 days of chilled storage in all batches (Figure 2). Higher microbial counts were determined in the control batch when compared to mackerel iced in the presence of both quinoa extracts. These differences had statistical significance ($p < 0.05$) on days 2, 9 and 13 (comparison to Q-2 batch) and on the 2-6-day period (comparison to Q-1 batch). Accordingly, the presence of quinoa extract in the icing system inhibited the growth of lipolytic bacteria. Interestingly, a comparative analysis of FFA values and lipolytics counts revealed a good relationship between both quality parameters ($r = 0.84$-0.91, linear correlation).

This is the first study reporting the effect of quinoa-derived compounds on FFA formation in seafood in particular, and in food in general. However, previous research concerning the inclusion of other natural compounds in ice systems has described the inhibition of lipid hydrolysis. Thus, a rosemary extract protected chilled sardine (*S. aurata*) against lipid hydrolysis [8], while the addition of rosemary or oregano extracts to icing media inhibited FFA formation in the lipids of chilled jack mackerel (*T.*

264 *murphyi*) [45]. Furthermore, extracts of the alga *B. bifurcata* in the ice system implied
265 an inhibitory effect on FFA formation in chilled megrim (*L. whiffiagonis*) [10]. On the
266 contrary, the inclusion of natural organic acids in the ice system was not effective
267 against FFA formation in megrim (*L. whiffiagonis*) [6] and hake (*M. merluccius*) [5].
268
269 **3.2. Lipid oxidation assessment**
270 A slight increase in CD value was detected in all three batches as refrigeration time
271 progressed (Table 1). However, a significant effect of the quinoa extracts on this
272 parameter was not observed ($p > 0.05$). As being one of the first oxidation compounds
273 produced, CD can be broken down easily and give rise to other lipid oxidation
274 compounds so that its assessment is often not valuable when a fish storage study is
275 concerned [46].
276 A marked peroxide formation was determined in all three batches as
277 refrigeration time progressed (Table 1). Such formation resulted to be continuous
278 throughout the whole storage period for the batches containing quinoa extracts. On
279 contrary, the control batch displayed a maximum value after 9 days of storage, showing
280 a lower average value on day 13. This result may be due to the breakdown of peroxides
281 at prolonged chilling times, this leading to secondary and tertiary oxidation compounds
282 [39, 47]. However, the presence of quinoa did not cause a significant ($p > 0.05$)
283 reduction of primary lipid oxidation compounds. With respect to TBARS (secondary
284 lipid oxidation compounds), progressive increases were determined in all batches as
285 refrigeration time progressed (Table 1). Although scarce, the differences resulted to be
286 significant ($p < 0.05$) (namely, at day 13), indicating that the batches including a quinoa
287 extract exhibited lower TBARS values as compared to the control batch.

The formation of tertiary oxidation compounds (namely, FR assessment) during storage was observed (Table 1). However, this formation was not as strong as in the case of peroxides and TBARS. In agreement with the secondary compounds determination, scarce significant differences were observed among batches. However, an inhibitory effect ($p < 0.05$) at day 13 was observed in mackerel corresponding to Q-2 batch. Accordingly, the presence of the quinoa extract led to a lower formation of interaction compounds.

As a consequence of this, the Q-2 batch exhibited more stability against rancidity in Atlantic chub mackerel (lipid content: 65.0-95.0 $g \cdot kg^{-1}$ muscle), this effect being higher at advanced storage times. Such antioxidant activity confirms other work performed on a heated marine-oil model system [21]. In such work, lipid oxidation (i.e., CD, peroxides and TBARS determinations) was remarkably inhibited and the PUFA content was better preserved in the heated marine oil due to the presence of a quinoa extract. Furthermore, the antioxidant behaviour found in the current study agrees with such work [21] concerning the contents of compounds responsible for antioxidant behaviour such as polyphenols, flavonoids and tocopherols (Table 2). Additionally, such study revealed an antioxidant activity of the aq. 80% ethanolic extract as determined by the DPPH assay (Table 2).

No study has been performed to date concerning the effect of quinoa on the development of rancidity in fish. Accordingly, this study may open the way to other works involving quinoa in other types of commercial seafoods (namely, lean and fatty fish). It should also be considered that previous studies have pointed out the potential antioxidant activity of quinoa extracts due to its content in polyphenols [15], flavonoids [16] and tocopherols [13].

312 Previous reports indicated that the inclusion of natural compounds in the icing
313 system exerted an inhibitory effect on lipid oxidation. Thus, an extract of the alga *F.*
314 *spiralis* included in the ice system inhibited lipid oxidation in refrigerated hake (*M.*
315 *merluccius*) [9]. An extract of alga *B. bifurcata* in the ice medium inhibited the
316 formation of fluorescent compounds in refrigerated megrim (*L. whiffiagonis*) [10].
317 Concerning plant sources, the preparation of ice systems including oregano or rosemary
318 extracts inhibited peroxide and TBARS formation in jack mackerel (*Trachurus*
319 *murphyi*) [45].
320
321 **3.3. Chemical assessment of microbial activity**
322 The study on pH evolution revealed that all pH values were included in a narrow (i.e.,
323 5.91-6.04) range (Table 3). An increasing pH trend as storage time progressed could not
324 be concluded ($p > 0.05$) in any batch. Comparison among samples provided lower
325 average values for the Q-2 batch, the differences with respect to the control batch being
326 significant ($p < 0.05$) on day 9. Consequently, the inclusion of quinoa in the ice system
327 allowed a better control of pH value in mackerel muscle.
328 Concerning TMA-N formation, a relevant and progressive increase was
329 determined in all batches as storage time progressed (Table 3). The batches including
330 quinoa extracts exhibited comparatively lower TMA-N values as compared to the
331 control batch, being such differences significant ($p < 0.05$) at the end of the storage time
332 for mackerel belonging to the Q-2 batch. Accordingly, the presence of quinoa in the ice
333 system inhibited TMA-N formation in mackerel muscle.
334 The present research has shown that the inclusion of quinoa extracts in the ice
335 media inhibited microbial activity (as determined by pH, TMA-N values and lipolytics
336 counts) in an under-valued species such as Atlantic chub mackerel. In agreement with

337 previous research, phenolic compounds and other hydrophobic components of essential
338 oils have been reported to exert antibacterial activity [48, 49]. Thus, a great diversity of
339 phenolic compounds has been reported in different kinds of quinoa-derived products
340 [11, 13]. Interestingly, and as far as we know, no previous research accounted for
341 employing quinoa or quinoa-derived products to enhance the microbial quality of
342 chilled seafood. In agreement with the lipid damage section, this novel treatment may
343 be applied to commercial marine species.

344 Present results are in agreement with other studies referring the antimicrobial
345 activity of quinoa in other kinds of foods. Thus, the antimicrobial activity of aqueous
346 extracts from quinoa pearling by-product resulted to be strong against different kinds of
347 bacterial strains (i.e., Gram-negative and -positive bacteria) [14]. Additionally, quinoa
348 extracts have been included in different kinds of biofilms with the aim of providing
349 them with antimicrobial activity. Thus, a previous study reported that biofilms including
350 quinoa starch exerted a strong inhibitory effect on *Staphylococcus aureus* [17], and
351 active packaging films including chitosan and quinoa proteins showed an inhibitory
352 effect against a wide variety of Gram-positive and Gram-negative pathogenic bacteria
353 [18]. Additionally, coatings including quinoa protein-chitosan-sunflower oil reduced
354 mould and yeast counts and increased the shelf-life of refrigerated (0 ± 0.5 °C)
355 strawberries (*Fragaria* × *ananassa*) [19], while edible films including quinoa protein-
356 chitosan-sunflower oil extended the shelf-life of refrigerated (4 °C) blueberries
357 (*Vaccinium corymbosum*) [20].

358 Concerning the icing strategy tested in the current study, previous studies have
359 described that the application of other kinds of vegetable extracts in ice systems can
360 lead to lower pH, TMA-N and total volatile base values. Thus, oregano or rosemary
361 extracts inhibited the formation of volatile amines formation in refrigerated jack

362 mackerel (*T. murphyi*) [45], wild-thyme (*T. serpyllum*) hydrosol exerted a protective
363 effect on chilled Transcaucasian barb (*C. capoeta*) [7], and rosemary extract provided
364 antimicrobial effects on chilled sardine (*S. aurata*) [8]. Taking into account natural
365 compounds from marine sources, the inclusion of an extract of the alga *B. bifurcata* in
366 the ice medium exhibited a remarkable antimicrobial effect in refrigerated megrim (*L.*
367 *whiffiagonis*) when was included in the icing system [10]. Moreover, the inclusion of
368 lactic and citric acids in the ice system allowed a better control of pH and TMA-N
369 formation in refrigerated hake (*M. merluccius*) [5] and megrim (*L. whiffiagonis*) [6].
370

371 **3.4. Evaluation of sensory quality**

372 Sensory analysis was performed by assessing raw and cooked descriptors in fish
373 specimens from all batches (Table 4). A progressive decrease of quality was detected in
374 all batches in parallel to refrigeration time. The control batch was found to be
375 unacceptable at day 13 according to the following parameters: skin (yellowish colour
376 development), eyes (milky cornea development) and external odour (rancid odour
377 development). Additionally, fish from Q-1 batch were also found unacceptable at that
378 time according to the evaluation of the external odour (rancid odour development). On
379 contrary, fish specimens corresponding to the most concentrated quinoa extract were
380 still acceptable on day 13. A better maintenance of sensory quality was observed when
381 the most concentrated quinoa extract (i.e., Q-2 batch) was present in the icing system,
382 this being in agreement with the results of the chemical and microbial analyses.

383 No previous research referring the benefits of quinoa or its products on the
384 commercial life and sensory quality of any type of seafood is, to our knowledge,
385 currently available. On contrast to this lack of information, a variety of reports have
386 described the use of quinoa flour to enhance the quality of baked foods, by the

387 substitution of other cereal flours [13]. Moreover, products derived from quinoa have
388 been used as novel compounds for the preparation of active packaging films [17, 18].
389 Such strategy has been successfully applied to strawberry (*Fragaria* x *ananassa*) [19]
390 and blueberries (*V. corymbosum*) [20] by increasing their shelf-lives under refrigeration.
391 Considering the preservation strategy evaluated in the present work (i.e., the
392 inclusion of natural extracts in the icing system), previous research accounted for
393 various profitable examples, so that an increase in the shelf-life time was attained in all
394 cases. Concerning plant sources, such studies include the employment of a rosemary in
395 an ice system used for the preservation of sardine (*S. aurita*) [8] and the preservation of
396 Transcaucasian barb (*C. capoeta*) in an ice system including wild-thyme hydrosol [7].
397 Finally, the use of natural organic acids as components of an ice system for the
398 preservation of hake (*M. merluccius*) [5] and megrim (*L. whiffiagonis*) [6] provided a
399 higher retention of their sensory acceptance.
400
401 **4. CONCLUSIONS**
402 The presence of an ethanol extract of quinoa (Q-2 batch) in an ice system for the
403 preservation of Atlantic chub mackerel inhibited ($p < 0.05$) lipid oxidation (TBARS and
404 FR determinations) and hydrolysis (FFA formation and lipolytic bacteria counts), and
405 microbial activity as inferred by chemical parameters (pH value and TMA-N content).
406 Such inhibition was more relevant as storage time progressed. Furthermore, fish from
407 both Q-0 and Q-1 batches were not acceptable at day 13, while counterparts from the Q-
408 2 batch exhibited acceptable quality throughout storage time. The quality limiting
409 parameters resulted to be skin (yellowish colour development), eyes (milky cornea
410 development) and external odour (rancid odour development).

411 Under the conditions tested, a novel ice system including a quinoa extract is
412 recommended as a suitable tool for improving the quality and extending the shelf-life of
413 fatty fish. This novel and practical strategy matches with current interest in the search
414 for effective antioxidants and antimicrobials from natural sources to replace synthetic
415 preservatives in the food sector. Additionally, this innovative strategy has proven to
416 increase the technological aptitude and accordingly, the commercialisation possibilities,
417 of a currently under-valued fish species.

418 Further research is needed to explore the potential of quinoa extracts for the
419 preservation of other commercial seafoods (i.e., lean and fatty fish species). Such
420 optimisation should take into account the biopreservative compounds extraction as well
421 as the characteristics of the seafood species encountered (composition, catching season
422 and location, etc.). For this purpose, a deep analytical research ought to be addressed to
423 identify the bioactive compounds included in the current aq. ethanolic extract.
424 Moreover, and on the basis of the highly valuable nutritional composition of quinoa,
425 more attention should be paid to its potential role as a functional food. In this sense, the
426 strategy reported in the present work opens the way to the incorporation of potentially
427 healthy biomolecules present in quinoa extracts into the seafood.

428

429

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434

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581 **FIGURE LEGENDS**

582 **Figure 1**: Effect of various icing conditions* on the free fatty acids (FFA) formation in
583 chilled Atlantic chub mackerel muscle**

584 * Abbreviations of icing conditions: Q-0 (without quinoa extract; batch control), Q-1
585 (batch including a low-concentrated quinoa extract), and Q-2 (batch including a
586 high-concentrated quinoa extract).

587 ** Average values of three replicates (n = 3). Standard deviations are indicated by bars.
588 Average values accompanied by different letters (a, b, c) denote significant
589 differences ($p < 0.05$) as a result of the icing condition. No letters are included
590 when significant differences were not found ($p > 0.05$).

591

592 **Figure 2**: Effect of various icing conditions* on lipolytic bacteria counts in chilled
593 Atlantic chub mackerel muscle**

594 * Icing conditions as expressed in Figure 1.

595 ** Average values of three replicates (n = 3). Standard deviations are indicated by bars.
596 Average values accompanied by different letters (a, b) denote significant
597 differences ($p < 0.05$) as a result of the icing condition. No letters are included
598 when significant differences were not found ($p > 0.05$).

599

600

TABLE 1

Effect of various icing conditions* on the development of lipid oxidation in chilled Atlantic chub mackerel**

Quality index	Chilling storage time (days)	Icing condition		
		Q-0	Q-1	Q-2
Conjugated dienes value***	Initial		0.67 (0.12)	
	2	0.81 (0.11)	0.77 (0.15)	0.76 (0.10)
	6	0.90 (0.14)	0.87 (0.12)	0.82 (0.09)
	9	0.92 (0.11)	0.94 (0.09)	0.89 (0.12)
	13	0.93 (0.11)	0.95 (0.13)	0.93 (0.07)
Peroxide value (meq. active oxygen·kg^{-1} lipids)	Initial		1.53 (1.30)	
	2	3.26 (1.16)	2.74 (0.69)	2.84 (0.12)
	6	7.58 (1.92)	5.56 (1.22)	6.20 (0.96)
	9	13.21 (3.22)	8.23 (2.30)	9.32 (5.55)
	13	10.48 (2.52)	11.65 (3.01)	12.61 (2.32)
Thiobarbituric acid index (mg malondial-dehyde·kg^{-1} muscle)	Initial		0.42 (0.05)	
	2	1.96 (0.16)	1.91 (0.40)	1.64 (0.14)
	6	4.39 (1.14)	3.12 (0.41)	2.56 (0.71)
	9	5.01 (0.82)	4.12 (0.33)	4.02 (0.53)
	13	8.23 b (1.15)	7.14 ab (0.72)	5.36 a (0.47)
Fluorescence ratio	Initial		0.38 (0.19)	
	2	0.56 (0.09)	0.47 (0.13)	0.49 (0.02)
	6	0.48 (0.01)	0.54 (0.05)	0.51 (0.04)
	9	0.68 (0.08)	0.47 (0.04)	0.48 (0.13)
	13	0.95 b (0.21)	0.73 ab (0.16)	0.58 a (0.08)

* Icing conditions as expressed in Figure 1.

** 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 icing condition. No letters are included when significant differences were not found ($p > 0.05$).

*** Units as expressed in the Materials and Methods section.

TABLE 2

Contents on bioactive compounds* of quinoa aq. ethanolic extracts**

Bioactive compound	Content
Total polyphenols (mg gallic acid equivalent)	147.9 (0.6)
Total flavonoids (mg quercetin equivalent)	31.8 (0.5)
α-tocopherol ($mg \cdot kg^{-1}$ lyophilised quinoa)	48.6 (3.7)
β-tocopherol ($mg \cdot kg^{-1}$ lyophilised quinoa)	ND***
γ-tocopherol ($mg \cdot kg^{-1}$ lyophilised quinoa)	43.2 (1.2)
δ-tocopherol ($mg \cdot kg^{-1}$ lyophilised quinoa)	8.9 (0.3)

* Average values of three (n = 3) replicates; standard deviations are indicated in brackets.

** Adapted from Miranda et al. [21].

*** ND: Not detected

TABLE 3

Effect of various icing conditions* on pH and trimethylamine-nitrogen (TMA-N) values in chilled Atlantic chub mackerel**

Quality index	Chilling storage time (days)	Icing condition		
		Q-0	**Q-1**	**Q-2**
pH	Initial		5.91 (0.01)	
	2	6.02 (0.08)	5.98 (0.07)	5.94 (0.03)
	6	6.04 (0.01)	6.03 (0.05)	6.01 (0.08)
	9	5.99 b (0.03)	6.04 b (0.07)	5.91 a (0.01)
	13	5.95 (0.04)	5.97 (0.04)	5.89 (0.03)
TMA-N ($mg \cdot kg^{-1}$ muscle)	Initial		0.23 (0.02)	
	2	0.73 (0.24)	0.51 (0.05)	0.56 (0.04)
	6	3.36 (0.06)	2.44 (0.85)	2.75 (0.56)
	9	4.22 (0.44)	3.56 (0.45)	3.71 (0.32)
	13	10.35 b (3.08)	10.22 b (1.28)	6.63 a (0.26)

* Icing conditions as expressed in Figure 1.

** 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 icing condition. No letters are included when significant differences were not found ($p > 0.05$).

TABLE 4

Assessment of sensory acceptance* in chilled Atlantic chub mackerel under various icing conditions**

Descriptor	Chilling storage time (days)	Icing condition		
		Q-0	Q-1	Q-2
Skin	2	A	A	A
	6	B	A	A
	9	B	A	A
	13	C	B	B
Eyes	2	A	A	A
	6	B	A	A
	9	B	B	B
	13	C	B	B
External odour	2	A	A	A
	6	A	A	A
	9	B	B	B
	13	C	C	B
Gills	2	A	A	A
	6	B	A	A
	9	B	B	A
	13	B	B	B
Consistency	2	A	A	A
	6	A	A	A
	9	B	B	B
	13	B	B	B
Raw flesh odour	2	A	A	A
	6	A	A	A
	9	B	B	A
	13	B	B	B
Cooked flesh odour	2	A	A	A
	6	A	A	A
	9	B	B	B
	13	B	B	B
Cooked flesh taste	2	A	A	A
	6	A	A	A
	9	B	B	A
	13	B	B	B

* Quality categories: E (excellent), A (good), B (fair) and C (unacceptable). Starting fish (day 0) was category E in all descriptors.

** Icing conditions as expressed in Figure 1.

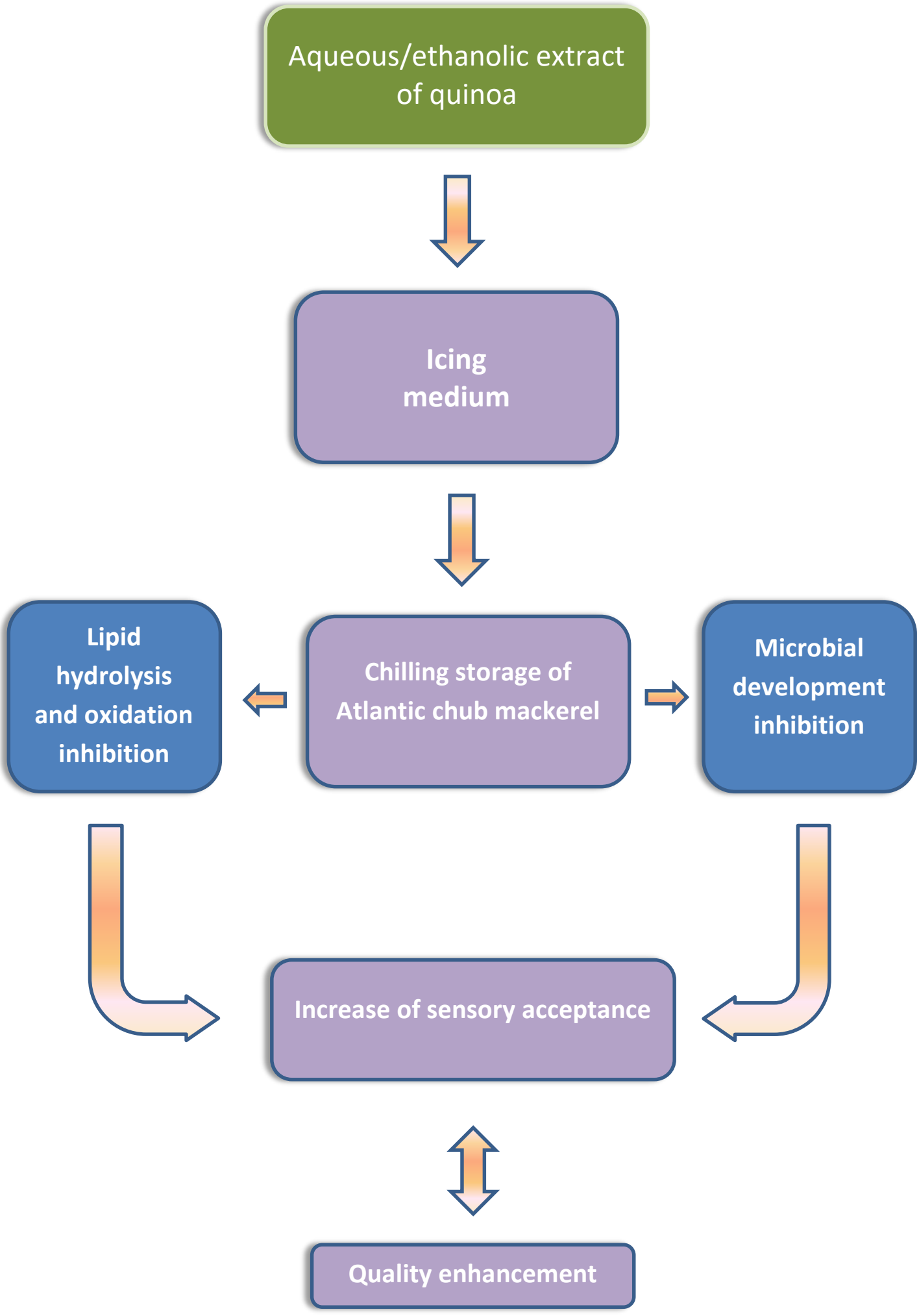

Aqueous/ethanolic extract of quinoa
Icing medium
Chilling storage of Atlantic chub mackerel
Lipid hydrolysis and oxidation inhibition
Microbial development inhibition
Increase of sensory acceptance
Quality enhancement

Figure 1

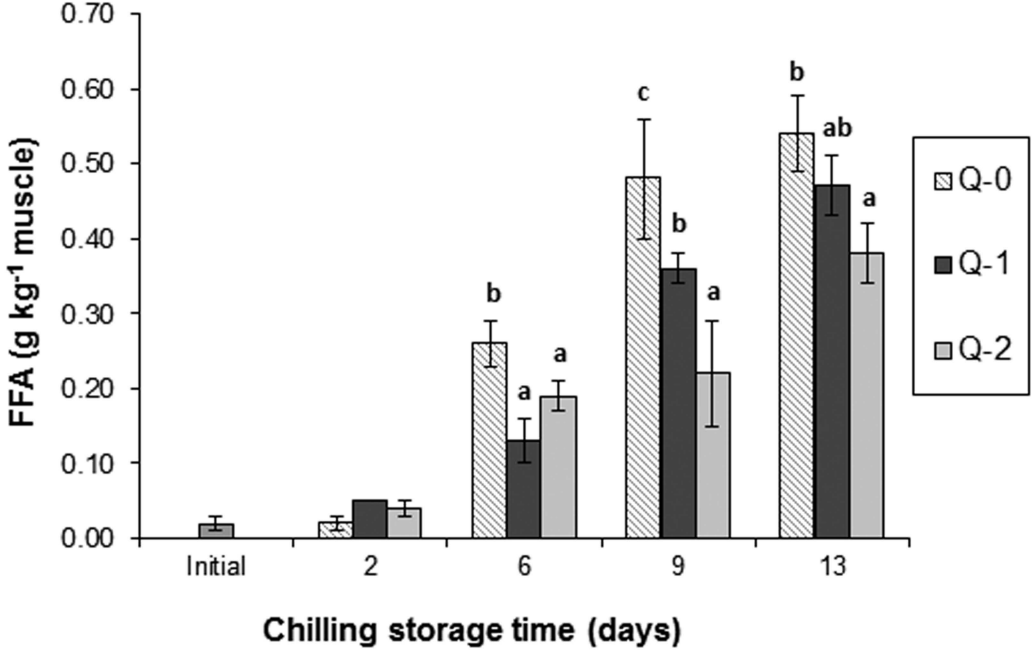
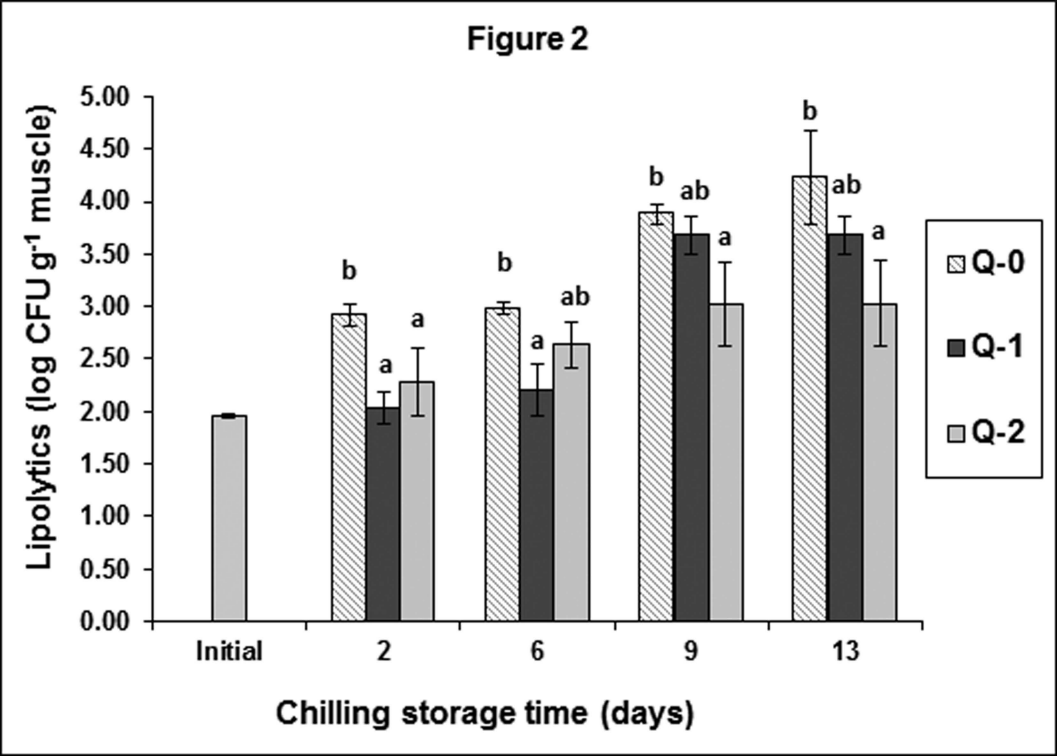

0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
FFA (g kg-1 muscle)
b
a
a
c
b
a
b
ab
a
Initial
2
6
9
13
Chilling storage time (days)
Q-0
Q-1
Q-2

Figure 2


Lipolytics (log CFU g-1 muscle)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
b
a
a
b
a
ab
b
ab
a
b
ab
a
Q-0
Q-1
Q-2
Initial
2
6
9
13
Chilling storage time (days)