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Chapter 11

IMPACT OF A SAPONIN-FREE QUINOA EXTRACT ON MICROBIAL SPOILAGE IN CHILLED LEAN AND FATTY FISH

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ABSTRACT

Inhibition of microbial activity in chilled fish was investigated. For it, an aqueous/ethanolic saponin-free quinoa extract was added to the icing system and applied to lean (megrim, *Lepidorhombus whiffiagonis*; 0.03 and 0.12 g lyophilised quinoa/L icing solution) and fatty (Atlantic chub mackerel, *Scomber colias*; 0.05 and 0.20 g lyophilised quinoa/L icing solution) fish. Microbial development was monitored for a 13-day chilled storage period by microbial and chemical indices. An inhibitory effect ($p < 0.05$) of the quinoa extracts present in the icing systems on microbial development in megrim (aerobe counts, pH and free fatty acid value) and mackerel (aerobe, psychrotroph, proteolytic and lipolytic counts; pH and free fatty acid values) was observed when considering the most concentrated presence of quinoa in the icing system. A novel icing system is proposed to obtain improvement in the quality of both lean and fatty fish species during commercialisation under chilled conditions.

Keywords: quinoa, saponin-free, *Lepidorhombus whiffiagonis, Scomber colias,* chilling, microbial activity

INTRODUCTION

Seafoods are highly perishable products produced from poikilothermic species that possess a high water and non-protein nitrogen content, as well as other characteristics such as soft muscle and skin structure, a low collagen content and a highly unsaturated lipid profile (Brown, 1986; Simopoulos, 1997). Consequently, rapid and efficient processing and storage are needed to avoid post-mortem changes which are due to a wide range of damage pathways (Whittle et al., 1990; Tantasuttikul et al., 2011; Biji et al., 2015). Among seafoods, chilled products have taken the market in the last few decades. In order to maintain the original properties of marine species and offer the consumer a high-quality fresh product, ice storage has been widely employed. However, due to the limited shelf life of marine species, icing is not always enough, and needs to be combined with other protection strategies (Nirmal and Benjakul, 2010; Campos et al., 2012; Mohan et al., 2017). Among them, the inclusion of preservative compounds in the icing

medium, such as ClO_2 (Shin et al., 2004), natural organic acids (García-Soto et al., 2013; García-Soto et al., 2014) and plant (Oral et al., 2008; Özyurt et al., 2012) or alga (Barros-Velázquez et al., 2016; Miranda et al., 2016) extracts has been revealed to be a promising strategy.

Quinoa (*Chenopodium quinoa* Willd.) is a highly appreciated pseudo-cereal of Andean origin showing a balanced composition in profitable constituents and having no gluten content (Vega-Gálvez et al., 2010). Concerning its employment in food application, quinoa-derived products have been successfully employed in the preparation of emulsion-type products and malted beverages, as fat/cream substitutes and to enhance the quality of baked foods, as well as for producing quinoa protein concentrates and hydrolysates (Bhargava et al., 2006; Graf et al., 2015). Furthermore, the antimicrobial activity of quinoa has recently been proved (Tremonte et al., 2017). Thus, the inclusion of quinoa products in active packaging systems has proven to enhance the antimicrobial properties of the corresponding films (Pagno et al., 2015; Caro et al., 2016), leading to an increase in the shelf life of various fruit products (Valenzuela et al., 2015; Abugoch et al., 2016).

However, saponin compounds located in the pericarp seeds constitute a drawback for utilization of quinoa as a practical food source. Quinoa saponins are bitter, interfere with quinoa's palatability and digestibility (Vega-Gálvez et al., 2010; Graf et al., 2015) and have even been reported to be toxic because of their haemolytic activity and ability to reduce surface tension (Khalil and El-Adawy, 1994; Stuardo and San Martín, 2008). Consequently, the employment of quinoa products after removal of saponin compounds could 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. In agreement with the lipid oxidation assessment and polyene content retention, saponin-free quinoa was shown to maintain the original preservative properties (Miranda et al., 2017).

The main goal of the present work was to inhibit microbial development by using an icing system with quinoa extract. For it, an aqueous/ethanolic

extract of saponin-free quinoa was included, to our knowledge for the first time, in an icing system and applied to lean (megrim, *Lepidorhombus whiffiagonis*) and fatty (Atlantic chub mackerel, *Scomber colias*) fish species. Microbial development was monitored for 13 days of chilled storage by means of microbial (Enterobacteriaceae, aerobes, psychrotrophs, proteolytic and lipolytic bacteria) and chemical (pH and free fatty acid content) indices.

MATERIAL AND METHODS

Quinoa Raw Material, Saponin Removal and Preparation of Lyophilised Quinoa Extracts

Cancosa 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.

Removal of saponin compounds was achieved in agreement with a previous work (Miranda et al., 2017). Thus, the grains were washed with distilled water (quinoa/water proportion of 1/10, w/w) at 20°C with constant stirring for 1 h, 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 ± 0.2 m/s. Samples were dried until they reached constant weight (equilibrium condition). Saponin-free quinoa grains were finally finely ground and subjected to a 500-micron sieve (U. S. Standard Sieve Series, Sieve N° 35; Dual Manufacturing Co., Chicago, IL, USA).

Extracts from saponin-free quinoa were obtained in agreement with the method of Miranda et al. (2017). 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. The mixture was homogenized 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 5,000 × g, the supernatant being filtered through a Whatman N° 1 filter. The filtrate was afterwards concentrated under reduced pressure at 40°C on a rotary evaporator (Büchi RE 121, Flawil, Switzerland) and then lyophilised in a freeze-dryer (Virtis Advantage Plus, Gardiner, NY, USA). The lyophilised saponin-free quinoa extract was kept under refrigerated conditions (4°C) to be employed throughout a 1-month period.

Chilling Systems

The current work includes two different and independent experiments, on megrim and mackerel, respectively.

In the megrim experiment, 0.20 and 0.80 g of the lyophilised extract was dissolved, respectively, in 80 mL of an 80% aq. (v/v) ethanol solution and then diluted to 6 L with distilled water. As a result, low-concentration (LCME solution; 0.03 g lyophilised quinoa/L solution) and high-concentration (HCME solution; 0.12 g lyophilised quinoa/L solution) quinoa solutions were obtained, respectively. Additionally, a batch control was considered (CME, megrim control solution). For that, 80 mL of an 80% aq. (v/v) ethanol solution was diluted to make a 6-L solution by addition of distilled water.

For the mackerel experiment, 0.30 and 1.20 g of the lyophilised extract was dissolved, respectively, in 80 mL of an 80% aq. (v/v) ethanol solution and then diluted to 6 L with distilled water. As a result, low-concentration (LCMA solution; 0.05 g lyophilised quinoa/L solution) and high-concentration (HCMA solution; 0.20 g lyophilised quinoa/L solution) quinoa solutions were obtained, respectively. Additionally, a batch control was considered (CMA, mackerel control solution). For that, 80 mL of an 80% aq. (v/v) ethanol solution was diluted to 6 L with distilled water.

In both experiments, all solutions were packaged in polyethylene bags, kept frozen at -18°C and later used as icing media. Prior to the addition to

fish specimens, the different kinds of ice system were ground to obtain ice flakes and employed as chilling systems.

The concentrations of lyophilised saponin-free quinoa employed in both experiments were chosen on the basis of various preliminary studies. For both species, a 0.01–2.00 g lyophilised quinoa/L solution range was tested. It could be observed that concentrations higher than 0.80 g/L modified some sensory descriptors (i.e., odour, colour or taste) in megrim fish. In a similar way, 1.20 g/L proved to be the highest concentration that did not modify sensory descriptors in mackerel. Accordingly, 0.80 and 1.20 g/L concentrations were considered in the present work for the megrim and mackerel experiments, respectively, together with less concentrated ones (0.20 and 0.30 g/L, respectively).

Fish Chilling Storage

In the megrim experiment, fish (78 specimens) were obtained at Vigo harbour (Northwestern Spain) and transported in an ice chest to the laboratory. The length and weight of the individual fish were 23.5 ± 1.7 cm and 115 ± 15 g, respectively. Upon arrival at the laboratory (about 10 h), six specimens were separated and analysed to explore their initial quality (day 0). These specimens were divided into three groups (two specimens per group) which were analysed independently (n = 3). The remaining fish specimens were divided into three batches (24 specimens in each batch) which were allocated to independent boxes which had different kinds of ice added to them (HCME, LCME and CME icing conditions, respectively).

For the mackerel experiment, 78 fish specimens were obtained at Vigo harbour and carried in an ice chest to the laboratory. The length and weight of the individual fish were in the ranges 26.5 ± 1.7 cm and 117 ± 11 g, respectively. Upon arrival at the laboratory (about 10 h), six specimens were separated and analysed to explore the initial quality on day 0. These specimens were divided into three groups (two specimens per group) which were analysed independently (n = 3). The remaining fish specimens were divided into three batches (24 specimens in each batch) which were placed

in independent boxes and directly surrounded by different kinds of ice (HCMA, LCMA and CMA icing conditions, respectively).

In both experiments, ice was added to fish at a 1:1 fish:ice ratio (w/w), and all batches were kept inside a refrigerated room (3 ± 1°C). Boxes allowed drainage of melted ice, and ice was renewed when required to maintain the 1:1 fish:ice ratio. Fish samples from both experiments were stored for a 13-day period, being sampled and analysed on days 2, 6, 9 and 13. At each sampling time, six specimens were taken from each batch and divided into three groups (two specimens per group) which were studied independently (n = 3).

Microbiological Evaluation of Chilled Megrim Muscle

Portions of 10 g of fish muscle were aseptically dissected, mixed with 90 mL of 0.1% peptone water (Oxoid Ltd, London, UK) and subsequently homogenized in a Stomacher blender (Seward Medical, London, UK), as described elsewhere (Ben-Gigirey et al., 1999). Serial dilutions of the microbial extracts in 0.1% peptone water were prepared. Total aerobic counts were determined by surface inoculation in plate count agar (PCA; Oxoid), incubation being carried out at 30°C for 72 h. Psychrotrophic bacteria were also investigated in PCA (Oxoid), after incubation at 7–8°C for 10 days. Enterobacteriaceae were determined in crystal violet neutral red bile glucose agar (VRBD agar; Merck, Darmstadt, Germany); plates were incubated at 37°C for 24 h. Bacteria exhibiting lipolytic or proteolytic phenotypes were investigated on tributyrin or casein agar media, respectively, incubation being carried out at 30°C for 48 h, as described elsewhere (Ben-Gigirey et al., 2000).

Bacterial numbers were transformed into log colony-forming units (CFU)/g muscle before statistical analysis. All analyses were done in triplicate.

Chemical Evaluation of Microbial Activity

All solvents and chemical reagents employed were of reagent grade (Merck, Darmstadt, Germany). The evolution of pH values in fish muscle during storage was determined using a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

Lipids were extracted from fish muscle by single-phase solubilisation with chloroform–methanol (1:1), as described by Bligh and Dyer (1959), and the results obtained were expressed as g lipids/kg muscle.

The free fatty acid (FFA) concentration of the fish 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 muscle and expressed as mg FFA/kg muscle.

Statistical Analysis

Data obtained (three replicates; $n = 3$) from the different microbiological and chemical analyses were subjected to the ANOVA method to explore differences resulting from the effect of the icing systems employed; comparison of means was performed using the least-squares difference (LSD) method. Analyses were carried out using 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.

Results and Discussion

Microbiological Results in Chilled Fish Muscle

The effects of the presence of quinoa extracts in the icing media on microbial activity in megrim muscle are shown in Table 1 and Figure 1.

The addition of quinoa extract to the icing media provided lower average values than in control with respect to aerobe growth (Figure 1), a result that was observed for all storage times and that reached statistical significance ($p < 0.05$) on days 2 (HCME batch) and 13 (LCME batch). In such cases, differences between the control batch and those including quinoa extracts rose up to 2.03 and 1.25 log CFU/g, respectively.

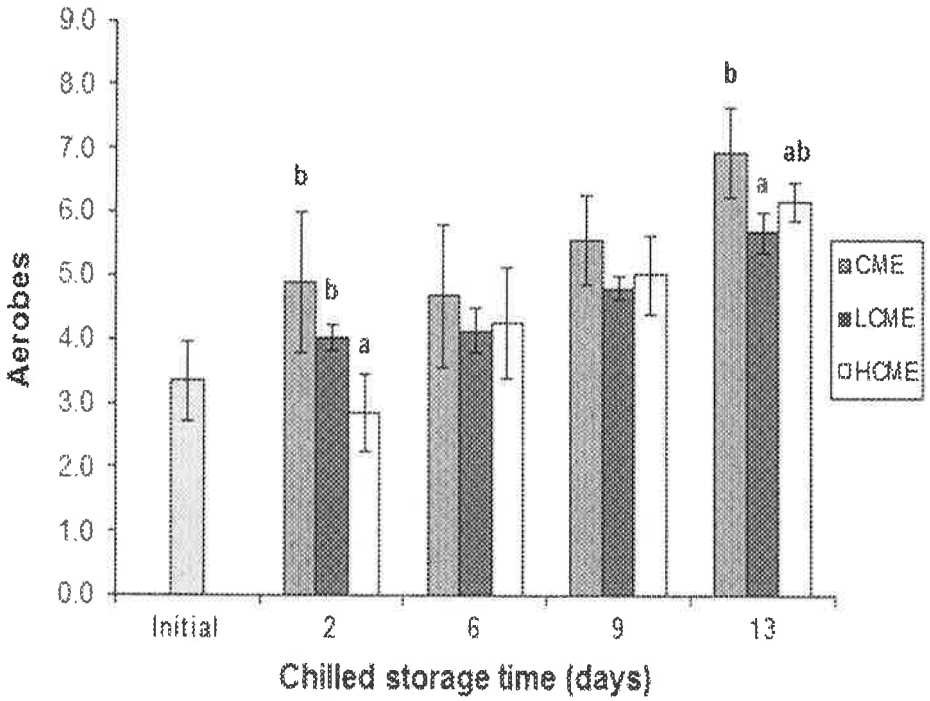

Figure 1. Effect of various icing conditions* including saponin-free quinoa extract on aerobe counts (log CFU/g muscle) in megrim muscle throughout chilled storage.**
* Abbreviations for icing conditions as expressed in Table 1.
** Average values of three replicates ($n = 3$). Standard deviations are 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 icing condition* including saponin-free quinoa extract on the development of various microbial groups (log CFU/g muscle) in megrim muscle throughout chilled storage**

Microbial parameter	Icing condition	Chilled storage time (days)				
		Initial	2	6	9	13
Enterobacteriaceae	CME	1.00 (0.00)	1.48 b (0.20)	1.00 (0.00)	1.93 (0.58)	1.00 (0.00)
	LCME		1.00 a (0.00)	1.00 (0.00)	2.16 (0.12)	1.26 (0.45)
	HCME		1.00 a (0.00)	1.67 (1.15)	1.97 (0.20)	1.00 (0.00)
Psychrotrophs	CME	4.17 (0.14)	5.00 (1.35)	5.28 (0.82)	6.74 (0.49)	7.47 (0.55)
	LCME		5.05 (1.03)	6.26 (0.67)	6.51 (0.28)	7.31 (0.08)
	HCME		4.24 (0.80)	6.13 (0.26)	6.32 (0.21)	7.19 (0.18)
Proteolytics	CME	4.24 (0.32)	3.63 b (0.50)	4.14 (0.35)	5.94 (0.79)	7.42 (0.23)
	LCME		2.79 a (0.27)	5.02 (0.67)	5.49 (0.23)	6.89 (0.29)
	HCME		2.61 ab (0.68)	4.51 (0.05)	5.87 (0.38)	7.19 (0.12)
Lipolytics	CME	2.69 (0.68)	3.29 (0.86)	3.83 (0.30)	4.44 (0.41)	6.25 ab (0.47)
	LCME		2.58 (0.51)	4.27 (0.52)	3.98 (0.86)	5.90 a (0.16)
	HCME		2.47 (0.57)	4.52 (0.64)	4.19 (0.77)	6.26 b (0.13)

* Abbreviations of icing conditions: CME (without quinoa extract; megrim control), LCME (low-concentrated quinoa extract in megrim experiment), HCME (high-concentrated quinoa extract in megrim experiment).

** Average values of three (n = 3) replicates; standard deviations are indicated in brackets. For each microbial group, average values followed by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the icing condition applied. No letters are included when significant differences were not found ($p > 0.05$).

Lower average values in treated megrim were also observed in most sampling times for proteolytic bacteria (Table 1), a microbial group with specific spoilage activity, and against which the LCME batch, including the

lower concentration of quinoa extract, provided a significant ($p < 0.05$) protective effect for short storage times (day 2); in this case, the differences in microbial number between LCME and the control batch were 0.84 log units. Concerning Enterobacteriaceae (Table 1), an inhibitory effect was observed on day 2 for both kinds of quinoa batch; however, this preservative effect was not maintained throughout the 6-13-day period. With respect to psychrotrophs, a definite trend could not be implied so that no significant ($p > 0.05$) differences among batches were observed (Table 1). Finally, and with respect to bacteria exhibiting a lipolytic phenotype, a significant effect ($p > 0.05$) of quinoa extract presence in ice could not be proved (Table 1); interestingly, fish corresponding to the LCME batch showed lower average values than their corresponding to control for the latest storage period (9-13 period).

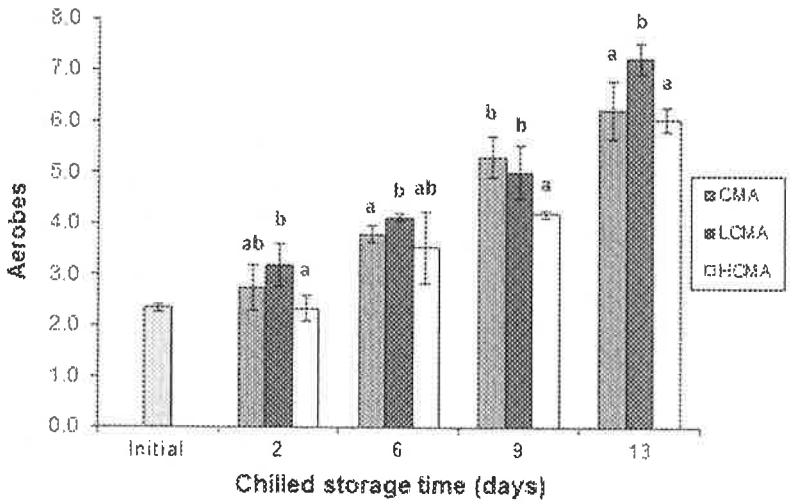

Figure 2. Effect of various icing conditions* including saponin-free quinoa extract on aerobe counts (log CFU/g muscle) in Atlantic chub mackerel muscle throughout chilled storage.**

* Abbreviations for icing conditions as expressed in Table 2.

** 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 2. Effect of the icing condition* including saponin-free quinoa extract on the development of various microbial groups (log CFU/g muscle) in Atlantic chub mackerel muscle throughout chilled storage**

Microbial parameter	Icing condition	Chilled storage time (days)				
		Initial	2	6	9	13
Enterobacteriaceae	CMA	< 1.00	< 1.00	< 1.00 a	2.98 (0.33)	4.33 (0.45)
	LCMA		1.23 (0.25)	0.99 a (0.02)	2.82 (0.44)	4.80 (0.71)
	HCMA		< 1.00	1.14 b (0.04)	2.60 (0.08)	4.31 (0.38)
Psychrotrophs	CMA	2.43 (0.19)	2.99 b (0.32)	4.67 a (0.27)	6.09 ab (0.37)	7.09 ab (0.64)
	LCMA		3.34 b (0.40)	5.38 b (0.13)	6.40 b (0.15)	7.90 b (0.44)
	HCMA		2.23 a (0.33)	4.33 a (0.30)	5.88 a (0.58)	5.66 a (0.76)
Proteolytics	CMA	2.34 (0.35)	2.32 (0.45)	3.27 (0.58)	4.86 b (0.34)	6.63 (0.17)
	LCMA		2.10 (0.14)	3.93 (0.38)	5.12 b (0.16)	6.61 (0.15)
	HCMA		1.98 (0.02)	3.09 (0.58)	3.90 a (0.41)	6.20 (0.29)
Lipolytics	CMA	< 2.00	2.66 b (0.08)	2.92 b (0.06)	3.72 (0.18)	5.33 b (0.47)
	LCMA		2.35 ab (0.33)	2.36 a (0.26)	4.05 (0.46)	4.65 ab (0.80)
	HCMA		< 2.00 a	2.52 a (0.20)	3.50 (0.65)	3.67 a (0.17)

* Abbreviations of icing conditions: CMA (without quinoa extract; mackerel control), LCMA (low-concentrated quinoa extract in mackerel experiment), HCMA (high-concentrated quinoa extract in mackerel experiment).

** Average values of three (n = 3) replicates; standard deviations are indicated in brackets. For each microbial group, average values followed by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the icing condition applied. No letters are included when significant differences were not found ($p > 0.05$).

In the case of mackerel, the effects of quinoa extract in the icing media on microbial activity are displayed in Table 2 and Figure 2. Remarkably, the presence of quinoa extract in the icing media led to a significant ($p < 0.05$) reduction in the growth of bacteria exhibiting a lipolytic phenotype, implying a remarkable protective effect of quinoa extracts on the development of lipid hydrolysis (Table 2). This effect was observed at all storage times except for day 9 and was especially intense in the case of the HCMA batch. Thus, the greatest differences between batches were observed for advanced storage periods (day 13), reaching 1.66 log CFU/g. With respect to aerobes (Figure 2), the batch including the highest quinoa extract content in the icing media revealed lower average values in microbial growth for all storage times and reached statistical significance ($p < 0.05$) on day 9 when differences between HCCM and the control batch raised up to 1.12 log units. In the case of psychrotrophs, the HCMA batch provided a lower average development of microbial activity throughout the whole experiment; such protection was significant ($p < 0.05$) on day 2. With respect to Enterobacteriaceae, scarce differences could be outlined so that a definite trend for quinoa presence in the icing medium could not be inferred. However, proteolytic counts revealed lower average values for fish corresponding to the HCMA batch when compared to their counterpart control (Table 2); differences were found significant ($p < 0.05$) at day 9.

The current research has shown an inhibitory effect on microbial activity during the chilled storage of two kinds of fish species as a consequence of the incorporation of the most concentrated quinoa extract in the ice system; contrary, a protective effect of the less concentrated extract could hardly be proved. In agreement with previous research (Gyawali and Ibrahim, 2014), phenolic compounds or other hydrophobic components of essential oils have been reported to be responsible for this antibacterial mechanism (Dorman and Deans, 2000; Stojković et al., 2013). Thus, great diversity of phenolic compounds has been reported in different kinds of quinoa-derived product (Vega-Gálvez et al., 2010; Graf et al., 2015). Interestingly, Miranda et al. (2017) showed that water-washing of quinoa leads to substantial losses of polyphenol and flavonoid content. However, and in agreement with the water extraction ability (Babakhani et al., 2015; Barros-Velázquez et al.,

2016), hydrophilic polyphenol and flavonoid molecules are likely to be extracted to a greater extent than lipophilic-type compounds. Since an antimicrobial effect has been observed in the current study, it can be concluded that saponin-free quinoa resulting from the washing step necessary for the removal of saponin compounds significantly retains its antimicrobial properties.

To the best of our knowledge, no previous research accounts of employing quinoa or quinoa-derived products to enhance seafood quality. However, previous studies reported 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, the results of our study agree with previous research where quinoa extracts were associated with antimicrobial activity in other kinds of food. Thus, the antimicrobial activity of aqueous extracts from quinoa pearling by-product was strong against different kinds of strain (i.e., Gram-negative and positive bacteria) (Tremonte et al., 2017). Additionally, quinoa extracts have been included in different kinds of biofilm with the aim of providing them with antimicrobial activity. Thus, a previous study reported that biofilms including quinoa starch exerted a strong inhibitory effect on *Staphylococcus aureus* (Pagno et al., 2015), and active films based on chitosan and quinoa proteins showed an inhibitory effect against *Listeria innocua*, *S. aureus*, *Salmonella typhimurium*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* (Caro et al., 2016). Additionally, coatings including quinoa protein–chitosan–sunflower oil reduced mould and yeast counts and increased the shelf life of refrigerated (0 ± 0.5°C) strawberries (*Fragaria* × *ananassa*) (Valenzuela et al., 2015), while edible films including quinoa protein–chitosan–sunflower oil extended the shelf life of refrigerated (4°C) blueberries (*Vaccinium corymbosum*) (Abugoch et al., 2016).

Concerning the strategy developed in the current work, several previous studies have accounted for the profitable effect on inhibition of microbial activity derived from the inclusion of a preservative compound in the icing medium employed during chilled storage. Thus, also related to vegetable extracts, the inclusion of wild-thyme (*Thymus serpyllum*) hydrosol in the icing medium led to lower microbial counts (aerobes, psychrotrophs,

Enterobacteriaceae and pseudomonads) in gutted and ungutted Transcaucasian barb (*Capoeta capoeta*) (Oral et al., 2008).

Furthermore, the presence of natural organic acids (i.e., citric and lactic acids) in the icing system led to lower aerobe and psychrotroph counts in chilled megrim (*L. whiffiagonis*) (García-Soto et al., 2014), as well as to lower counts of aerobes, anaerobes, psychrotrophs, Enterobacteriaceae and proteolytic bacteria in chilled hake (*Merluccius merluccius*) (García-Soto et al., 2013). Finally, the presence of aqueous and ethanolic extracts of the algae *Fucus spiralis* (Barros-Velázquez et al., 2016) and *Bifurcaria bifurcata* (Miranda et al., 2016) led to lower aerobe, psychrotroph, proteolytic and lipolytic bacteria counts in chilled hake (*M. merluccius*) and megrim (*L. whiffiagonis*), respectively.

Chemical Results of Microbial Activity in Chilled Fish

For both megrim and mackerel, a progressive increase in pH with chilling time was found in all batches (Table 3). In the case of megrim, slightly higher average values were derived from the presence of quinoa in the icing medium for the 2–6-day period. However, lower average pH values were obtained in treated megrim for advanced storage times (9–13-day period). Interestingly, a higher ($p < 0.05$) pH value was observed on day 13 in control fish when compared to both batches including quinoa presence. For mackerel, a lower average pH value was observed throughout the storage period as a result of the quinoa extract presence. Thus, a significantly lower pH ($p < 0.05$) was determined in fish specimens corresponding to the higher quinoa content on days 2, 9 and 13.

A tendency of increasing pH value in fish muscle generally indicates the accumulation of alkaline compounds, such as trimethylamine, ammonia and other nitrogen compounds, which mainly derive from microbial activity (Whittle et al., 1990; Campos et al., 2012). In a previous study, the inclusion of quinoa protein in an edible film led to lower pH values in

Table 3. Effect of the icing condition* including saponin-free quinoa extract on various chemical parameters in megrim and Atlantic chub mackerel muscle throughout chilled storage**

Fish species/ chemical parameter	Icing condition	Chilled storage time (days)				
		Initial	2	6	9	13
Megrim						
pH	CME	6.56 (0.06)	6.45 ab (0.19)	6.65 (0.04)	7.02 (0.09)	7.06 b (0.06)
	LCME		6.45 a (0.07)	6.80 (0.11)	6.89 (0.07)	6.94 a (0.02)
	HCME		6.59 b (0.04)	6.79 (0.08)	6.84 (0.32)	6.93 a (0.02)
Free fatty acids content (g/kg muscle)	CME	0.04 (0.01)	0.06 (0.02)	0.20 b (0.03)	0.41 b (0.05)	0.51 (0.11)
	LCME		0.07 (0.02)	0.13 ab (0.03)	0.29 a (0.02)	0.41 (0.11)
	HCME		0.07 (0.01)	0.09 a (0.03)	0.26 a (0.06)	0.43 (0.07)
Atlantic chub mackerel						
pH	CMA	5.88 (0.07)	5.94 b (0.08)	6.32 (0.16)	6.34 b (0.03)	6.37 b (0.07)
	LCMA		5.83 ab (0.14)	6.31 (0.13)	6.24 ab (0.07)	6.17 a (0.09)
	HCMA		5.64 a (0.06)	6.31 (0.09)	6.21 a (0.08)	6.12 a (0.03)
Free fatty acids content (g/kg muscle)	CMA	0.05 (0.01)	0.06 (0.03)	0.08 (0.01)	0.38 b (0.08)	0.46 b (0.09)
	LCMA		0.09 (0.06)	0.10 (0.02)	0.35 ab (0.11)	0.44 ab (0.18)
	HCMA		0.06 (0.02)	0.10 (0.00)	0.17 a (0.07)	0.38 a (0.06)

* Abbreviations of icing conditions as expressed in Table 1 (megrim experiment) and Table 2 (mackerel experiment).

** Average values of three (n = 3) replicates; standard deviations are indicated in brackets. For each chemical parameter, average values followed by different letters (a, b) denote significant differences ($p < 0.05$) as a result of the icing condition applied. No letters are included when significant differences were not found ($p > 0.05$).

refrigerated blueberries (*V. corymbosum*) when compared to a control batch (Abugoch et al., 2016). Concerning the icing strategy tested in the current study, previous studies have reported that the inclusion of other kinds of vegetable extract in ice systems can lead to lower pH and total volatile base values. Thus, oregano or rosemary extract exerted a protective effect on chilled jack mackerel (*Trachurus murphyi*) (Quitral et al., 2009), wild-thyme

(*T. serpyllum*) hydrosol exerted a protective effect on chilled Transcaucasian barb (*C. capoeta*) (Oral et al., 2008), and rosemary extract provided antimicrobial effects on chilled sardine (*Sardinella aurita*) (Özyurt et al., 2012). Moreover, the inclusion of lactic and citric acids in the icing medium led to lower pH and trimethylamine values in chilled hake (*M. merluccius*) (García-Soto et al., 2013) and megrim (*L. whiffiagonis*) (García-Soto et al., 2014). An antimicrobial effect was also determined in chilled megrim (*L. whiffiagonis*) when an ethanolic extract of the alga *B. bifurcata* was included in the ice system (Barros-Velázquez et al., 2016).

Concerning the FFA content, a progressive increase with storage time was observed in all batches and fish species (Table 3). For megrim, lower average values in quinoa-treated fish were obtained for the 6–13-day period. Differences were found to be significant ($p < 0.05$) for fish corresponding to the higher quinoa content on days 6 and 9. In the case of mackerel, lower average values for FFA content were observed in the 9-13-day period in fish chilled in the presence of quinoa extracts. Thus, differences were found to be significant ($p < 0.05$) in fish corresponding to the HCCM batch for such period. It is concluded that an inhibitory effect on FFA formation was observed in both species, despite the fact that they have a very different lipid content (i.e., 3.5–4.5 g/kg muscle for megrim and 46.5–68.5 g/kg muscle for mackerel).

Development of hydrolysis during chilled storage of fish has been reported to be the result of both endogenous enzyme and microbial activity (Whittle et al., 1990; Sikorski and Kolakowski, 2000). Before the end of the microbial lag phase (about 6–9 days), endogenous enzyme activity should be predominant but after that time, microbial activity should gain importance and be mostly responsible for the development of lipid hydrolysis for advanced storage periods (i.e., 9–13 days). Inhibition of lipid hydrolysis was more intense when the saponin-free quinoa extract concentration in the icing medium was increased.

To our knowledge, no previous research is available concerning the effect of quinoa-derived products on FFA formation in seafood in particular and in food in general. Previous research concerning the inclusion of other plant extracts in icing media has described an inhibitory effect on the

development of lipid hydrolysis. For example, a rosemary extract protected chilled sardine (*S. aurita*) against lipid hydrolysis (Özyurt et al., 2012), and the addition of rosemary or oregano extracts to icing media protected the lipids of chilled jack mackerel (*T. murphyi*) (Quitral et al., 2009). Furthermore, the presence of an extract of the alga *B. bifurcata* in the icing medium led to a lower FFA content in chilled megrim (*L. whiffiagonis*) (Miranda et al., 2016). On the contrary, the inclusion of citric and lactic acids in the icing medium did not provide any inhibitory effect on lipid hydrolysis in megrim (*L. whiffiagonis*) (García-Soto et al., 2014) and hake (*M. merluccius*) (García-Soto et al., 2013).

CONCLUSION

A novel icing system based on the inclusion of 80% aq. (v/v) ethanol extracts of saponin-free quinoa has been tested for the quality enhancement of two chilled lean and fatty fish species. As a result, an inhibitory effect ($p < 0.05$) of the quinoa extracts present in the icing systems on microbial development in megrim (aerobe counts, pH and FFA value) and mackerel (aerobe, psychrotroph, proteolytic and lipolytic counts; pH and FFA values) was observed when considering the most concentrated presence of quinoa in the icing system; contrary, a preserving tendency could hardly be implied by applying ice including the less concentrated presence of quinoa. Under the conditions tested in the present study, a novel icing system based on saponin-free quinoa is proposed as a suitable tool to be applied for the quality enhancement of both lean and fatty fish species during commercialization under chilled conditions. As a result of the previous washing step performed on quinoa grains, this preservative system would avoid the inconvenience derived from the presence of quinoa saponins. The proposed green preservation strategy matches with current interest in the search for effective antimicrobials from natural sources to replace synthetic preservatives in food in general. Further research is necessary to optimize the preservative

role of saponin-free quinoa in the icing system when applied to different kinds of seafood.

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