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Effect of jumbo squid (*Dosidicus gigas*) skin extract on the microbial activity in chilled mackerel (*Scomber scombrus*)
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ABSTRACT

During the industrial processing of jumbo squid (*Dosidicus gigas*), large amounts of by-products containing biological active compounds are generated. In this study, aqueous solutions including acetic acid-ethanol extracts of jumbo squid skin (JSS) were tested at three different concentrations as icing media. The effects of the JSS extracts on the quality evolution of chilled mackerel (*Scomber scombrus*) were monitored. A significant inhibition ($p<0.05$) of microbial activity was determined in the fish batch corresponding to the icing condition including the highest JSS concentration. Additionally, fish specimens corresponding to batches including any of the JSS concentrations tested showed lower ($p<0.05$) proteolytic counts and pH values than control mackerel. Sensory analysis revealed a marked shelf life extension in chilled mackerel stored in ice including the highest JSS concentration; specimens from such batch were found to be still acceptable after 13 days of storage, while all other mackerel batches were rejectable. The marked microbial activity inhibition observed could be explained on the basis of the presence in ice of lipophilic-type compounds obtained by acetic acid-ethanol extraction of JSS.

Keywords: *Dosidicus gigas*; skin extract; *Scomber scombrus*; chilling; microbial activity

Running title: Jumbo squid skin extract and chilled mackerel quality

57

1. INTRODUCTION

58 Aquatic food products suffer a rapid *post-mortem* quality loss as a result of a wide range
59 of biochemical and microbial breakdown mechanisms (Ashie, Smith, & Simpson, 1996;
60 Ozen, & Floros, 2001). To maintain good quality and retard fresh fish spoilage as much
61 as possible, a wide number of preservative strategies have been combined to flake ice
62 and tested satisfactorily. Among them, the inclusion of natural preservatives in the icing
63 medium such as low-molecular weight organic acids (García-Soto, Aubourg, Calo-
64 Mato, & Barros-Velázquez, 2013), plant extracts (Quitral et al., 2009) and algae extracts
65 (Miranda, Ortiz, Barros-Velázquez, & Aubourg, 2016) has shown a remarkable quality
66 loss inhibition.

67 By-products of aquatic species are body parts that are removed before they reach
68 the final consumer in order to improve their preservation qualities, reduce the shipping
69 weight or increase the value of the main product (Linder, Fanni, & Parmentier, 2005;
70 Blanco, Sotelo, Chapela, & Pérez-Martín, 2007; Rustad, StorrØ, & Slizyte, 2011). By-
71 products can comprise blood, viscera, heads, bellies, bones, skin, trimmings and fins.
72 Along with reduction of waste production, discards have been used as sources of
73 valuable bio-ingredients such as proteins, minerals and lipids that could be used for
74 human nutrition, as well as for their functional properties (Gbogouri, Linder, Fanni, &
75 Parmentier, 2006; Falch, Rustad, &Aursand, 2006; Ferraro, Cruz, Ferreira Jorge, &
76 Malcata, 2010). Among by-products, squid skin has recently attracted a great attention
77 (Messenger, 2001; Mäthger, Denton, Marshall, & Hanlon, 2009; Deravi et al., 2014).
78 Nowadays, it is known that although squid skin is translucent, its coloration comes from
79 red, yellow or brownish-black pigments contained in elastic sacculus of thousands
80 neuromuscular organs, called chromatophores, which are found throughout the outer
81 skin layer (Demski, 1992; Simpson, 2006). Furthermore, squid skin pigments have been

82 described as ommochrome compounds which have been reported as having good
83 functional properties such as antioxidant activity (Van den Branden, & Decleir, 1976;
84 Aubourg, Torres-Arreola, Trigo, & Ezquerra-Brauer, 2016).

85 Jumbo flying squid (*Dosidicusgigas*) is one of the largest known cephalopods
86 and has shown an increasing economic interest in a wide number of countries such as
87 Chile, Peru, China, Mexico and Japan (FAO, 2014). During its processing, large
88 amounts (up to 60% of whole weight) of by-products that may contain high
89 concentrations of biological active compounds related to antimicrobial (Na et al., 2015)
90 and antioxidant (Giménez, Gómez-Estaca, Alemán, Gómez-Guillén, & Montero, 2009;
91 Jiangjia et al., 2015) properties are generated. With the aim of improving the
92 exploitation properties, most research has been focused on collagen and gelatin
93 extraction and characterization (Alemán, Giménez, Pérez-Santin, Gómez-Guillén, &
94 Montero, 2011; Uriarte-Montoya et al., 2011; Ramírez-Guerra et al., 2015).

95 The present work considers Atlantic mackerel (*Scomber scombrus*) traded as a
96 chilled product. This small pelagic fish species can constitute food products of great
97 economic importance in many European countries, although it remains underutilized
98 because of its limited chilled shelf life (up to 9-10 days) (Bennour, El Marrackchi,
99 Bouchriti, Hamama, & El Ouadaa, 1991; Sanjuás-Rey, Gallardo, Barros-Velázquez, &
100 Aubourg, 2012). In order to extend its shelf life, jumbo squid skin (JSS) was employed
101 in this work as a source of pigmented preservative compounds. For it, aqueous solutions
102 including acetic acid-ethanol extracts of JSS were tested at three different
103 concentrations as icing media. The effects of the JSS extract on the quality evolution
104 were monitored for up to 13 d of chilled storage by means of microbiological, chemical
105 and sensory analyses.

106

107 **2. MATERIALS AND METHODS**

108 **2.1. Preparation of jumbo squid extracts and icing systems**

109 Fifty jumbo squid specimens were caught by local fishermen using the jigging fishing
110 method at the Guaymas harbor (Sonora, Mexico; 8.75°N⁄112.25°W, 15–18 °C) in May
111 2013. The length and weight of the squid specimens ranged from 40 to 45 cm and from
112 2.0 to 3.0 kg, respectively. Squids were transported to the Seafood Laboratory at the
113 University of Sonora 8 h after being captured. The skin was manually removed from the
114 mantle and fins, cut into small pieces (about 15 cm length) and freeze-dried for two
115 days (LABONCO Freeze Dry, Kansas City, MO, USA). The freeze-dried skin (100-mg
116 portions) was placed in polyethylene-sealed bags, which were stored at -25 ± 2 °C.

117 Acetic acid-ethanol pigment extracts from freeze-dried skin were prepared
118 according to the method developed previously (Aubourg et al., 2016). Briefly, 1 g of
119 freeze-dried skin was blended in a 0.5% acetic acid-ethanol solution (v/v) with a
120 skin/solution ratio of 1/10 (w/v) at 0 °C for 1 min using an Ultra-Turrax equipment
121 (IKA-UltraTurrax T-25, Staufen, Germany). The blended mixture was then submitted to
122 an ultrasound bath at room temperature (18-20ºC) (Ultrasons, Selecta, Barcelona,
123 Spain) for 3 min. Afterwards, the homogeneous mixture was centrifuged at 3,500 x *g* at
124 4°C for 10 min, being then the supernatant recovered. This process was repeated three
125 times, so that the supernatant recovered after the last centrifugation process was
126 colorless. Extracts were pooled together and carried out to a 220-mL volume solution
127 with the acetic acid-ethanol solution.

128 After that, 4, 12 and 36 mL of the 220-mL solution were diluted to 6 L,
129 respectively, with distilled water. In order to maintain the same quantity of the acid-
130 ethanol solution in all 6-L solutions (i.e., 36 mL), 32 and 24 mL of the acid-ethanol
131 solution were employed for the preparation of the first and second 6-L solutions,

132 respectively. Then, all 6-L solutions were packaged in polyethylene bags, kept frozen at
133 -18ºC for 48 h and further used as icing media (C-1, C-2 and C-3 batches, respectively).
134 Besides, 36 mL of the 0.5% acetic acid-ethanol solution were carried to 6 L with
135 distilled water, packaged in polyethylene bags, kept frozen at -18ºC for 48 h and used as
136 icing control condition (C-0 batch). Before employment for the chilling storage of fish,
137 the different icing systems were ground to obtain ice flakes.

138 Experimental conditions (namely, content of the squid skin extract in the ice)
139 employed in the present study were based on previous preliminary tests carried out at
140 our laboratory. Thus, a 2-80 mL range of the 220-mL solution corresponding to the
141 extraction of 1-g lyophilized skin was considered. An increasing presence of the squid
142 skin extract in the icing medium provided better sensory acceptance (namely, inhibition
143 of rancid and putrid odor development of fish). However, a 36-mL volume of the extract
144 resulted in the highest concentration without modifying the sensory descriptors
145 (external odor and color, as well as flesh odor, flavor and color) of mackerel;
146 additionally, it did not modify the color of the resulting ice. Accordingly, this volume
147 (36 mL) was considered for the present study, together with two lower volumes of the
148 skin extract (4 and 12 mL).

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150 **2.2. Fish material, processing and sampling**

151 Fresh mackerel (153 specimens) were caught near the Galician Atlantic coast (North-
152 Western Spain) and transported to the laboratory. Throughout this process (10 h), the
153 fish were maintained in ice. The length and weight of the fish specimens ranged from
154 22.0 to 25.0 cm and from 104 to 119 g, respectively.

155 Upon arrival to the laboratory, nine individuals were separated and analyzed as
156 initial fish (day 0). These fish specimens were divided into three different groups (three

157 individuals per group) that were analyzed independently to achieve the statistical
158 analysis; n=3). The remaining fish specimens were divided into four batches (36
159 individuals in each batch), that were placed in independent boxes and directly
160 surrounded by different kinds of ice (C-0, C-1, C-2 and C-3 batches, respectively),
161 prepared as previously described. Ice was added at a 1:1 fish:ice ratio, and all batches
162 were placed inside a refrigerated room (2±1ºC). Boxes allowing draining of melted ice
163 were used for fish storage. The ice of all batches was renewed when required to
164 maintain the mentioned fish:ice ratio. Fish samples from all batches were stored for a
165 13-day period, being sampled and analyzed on days 4, 7, 11 and 13. At each sampling
166 time, nine specimens were taken from each batch for analysis and divided into three
167 groups (three individuals in each group) that were studied independently (n=3).
168
169 **2.3. Microbiological analyses related to quality loss**
170 Portions of 10 g of fish white muscle were dissected aseptically from refrigerated fish
171 specimens, mixed with 90 mL of 0.1% peptone water (Merck, Darmstadt, Germany)
172 and homogenized in sterilized stomacher bags (AES, Combourg, France) as described
173 elsewhere (Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velázquez, 1998;
174 Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velázquez, 1999). Serial
175 dilutions from the microbial extracts were prepared in 0.1% peptone water.
176 Total aerobes were assessed by surface inoculation on plate count agar (PCA,
177 Oxoid Ltd., London, UK) after incubation at 30ºC for 48 h. Psychrotrophs were also
178 determined in PCA, after an incubation period of 7 days at 7-8ºC. Enterobacteriaceae
179 were investigated by pour plating using Violet Red Bile Agar (VRBA) (Merck,
180 Darmstadt, Germany) after an incubation period of 24 h at 37±0.5ºC. Bacteria
181 exhibiting proteolytic or lipolytic phenotypes were determined on casein-agar or

182 tributyrine-agar, respectively, after incubation at 30ºC for 48 h, as described elsewhere
183 (Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velázquez, 2000).
184 In all cases, microbial counts were transformed into log CFU g^{-1} muscle before
185 undergoing statistical analysis. All analyses were conducted in triplicate.
186
187 **2.4. Chemical analyses related to quality loss**
188 All solvents and chemical reagents used were of reagent grade (Merck, Darmstadt,
189 Germany). Chemical analyses related to fish quality were carried out on the white
190 muscle of mackerel.
191 The evolution of pH values in mackerel muscle along storage time was
192 determined using a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).
193 Trimethylamine-nitrogen (TMA-N) values were determined using the picrate method,
194 as previously described by Tozawa, Erokibara and Amano (1971). This method
195 involves the preparation of a 5% trichloroacetic acid extract of fish muscle (10 g/25 ml),
196 which is made to react with formaldehyde at 30ºC for 5 min in the presence of toluene
197 and KOH. The resulting organic phase is made to react with picric acid, being the
198 resulting absorbance read spectrophotometrically at 410 nm (Beckman Coulter DU 640,
199 London, UK). The results were expressed as mg TMA-N kg^{-1} muscle.
200
201 **2.5. Sensory analysis**
202 Sensory analysis was carried out by a sensory panel consisting of four experienced
203 judges who adhered to traditional guidelines concerning fresh and refrigerated fish
204 (European Council Regulation, 1996). The panelists had participated in sensory analysis
205 of various fish and seafood products for the previous 15 years. Before carrying out the
206 present experiment, the judges received special training on refrigerated mackerel,

207 focused on the evaluation of refrigerated mackerel specimens that exhibited different
208 qualities. Special attention was paid to the evolution of the sensory descriptors that were
209 found as limiting factors for the shelf life.

210 According to the European Council Regulation (1996), four categories were
211 used to rank the samples (Table 1): highest quality (E), good quality (A), fair quality (B)
212 and unacceptable quality (C). Sensory assessment of the fish included the following
213 descriptors in the raw state: skin and mucus development, eyes, external odourodor,
214 gills appearance and odor, consistency and flesh odor. For cooked fish, the two
215 following descriptors were considered: flesh odor and taste.

216 At each sampling time, three fish individuals from each batch were removed and
217 analyzed. Evaluation began by the analysis of fish in the raw state and was followed by
218 the analysis of samples in the cooked state. Cooking was accomplished at 95-100ºC for
219 7 min in a pre-warmed oven with air circulation and then submitted to the panel. At
220 each sampling time, whole fish specimens were coded with 3-digit random numbers and
221 presented to the panelists in individual trays, which were scored individually. Each
222 descriptor of each sample was scored a single time by each member of the panel. The
223 panel members shared samples tested.

224

225 **2.6. Statistical analysis**

226 Data obtained from the different microbiological and chemical analyses were subjected
227 to the ANOVA method to explore differences resulting from the effects of both the
228 icing condition and the chilling time; the comparison of means was performed using the
229 least-squares difference (LSD) method. Data obtained from the sensory evaluation were
230 analyzed by the non-parametric Kruskal-Wallis test. In all cases, analyses were carried
231 out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL,

232 USA); differences among batches and among chilling times were considered significant
233 for a confidence interval at the 95% level ($p<0.05$) in all cases.

234 Correlation analysis among parameters (chilling storage time, microbiological
235 values and chemical indices) was also carried out. In it, linear fittings are mentioned;
236 otherwise, the kind of fitting is expressed.

237

238 **3. RESULTS AND DISCUSSION**

239 **3.1. Assessment of the quality evolution by microbiological analysis**

240 The evolution of the aerobic mesophiles in all four mackerel batches is displayed in
241 Table 2. As expected, a progressive increase was observed for all batches as storage
242 time progressed (r^2 = 0.86-0.93). The inclusion of acid-ethanol squid extract in the icing
243 medium of the C-3 batch exerted a better control of the aerobes as compared with the
244 control batch. Such effect was significant ($p<0.05$) on day 6, where microbial inhibition
245 reached a level of 0.89 log units as compared with the control batch. In the 10-13-day
246 period, aerobe mean concentrations were above 6 log units in all batches except for C-3
247 and C-1 batches on day 10.

248 The investigation of the psychrotrophs in mackerel batches is also presented in
249 Table 2. This bacterial group includes specific fish spoilage bacteria such as members of
250 genera *Pseudomonas, Shewanella, Acinetobacter, Moraxella* or *Flavobacterium*.
251 Remarkably, the evolution of psychrotrophs in the different batches exhibited a similar
252 behavior as in the case of aerobes, a progressive increase being observed in all four
253 batches as storage time progressed (r^2 = 0.86-0.92). Thus, the C-3 batch, this including
254 the most concentrated squid extract, exhibited significantly ($p<0.05$) lower
255 psychrotrophs counts on days 10 and 13. The highest difference between C-3 and
256 control batches rose up to 0.84 log units on day 10. It should also be remarked that C-3

batch was the only in which psychrotrophs concentrations were below 6 log units on day 10. This result indicates that the presence of the squid extract at the highest concentration tested provided a better protection of mackerel against this bacterial group.

The comparative evolution of Enterobacteriaceae is also shown in Table 2. This microbial group also showed a progressive formation with chilling time in all cases (r^2 = 0.82-0.91). Lower mean values were obtained in samples corresponding to the C-3 batch throughout the 6-13-day period. Remarkably, the highest differences (0.97 log CFU g^{-1}) were found between C-3 and control batches on day 13. Other batches including squid extracts at lower concentrations did not provide a significant ($p>0.05$) protection of mackerel against Enterobacteriaceae growth as compared with the control batch.

The evolution of proteolytic microorganisms is presented in Figure 1. This microbial group is especially relevant, since bacteria exhibiting a proteolytic phenotype have been reported to cause degradation and spoilage of fish muscle (Rodríguez, Barros-Velázquez, Ojea, Piñeiro, & Aubourg, 2003). Progressive and significantly ($p<0.05$) increasing counts were observed in all four batches as storage time progressed (r^2 = 0.85-0.95). The investigation of proteolytic bacteria also indicated a significant ($p<0.05$) inhibitory effect derived of the presence of acid-ethanol extract of squid skin in the refrigeration medium. Thus, significant ($p<0.05$) differences were determined between C-3 and control batches at days 6, 10 and 13. The maximum microbial inhibition was 1.54 log units on day 13.

Figure 2 includes the results of the investigation of lipolytic bacteria in all four mackerel batches. In a similar way to the previously described microbial groups, a progressive increase ($p<0.05$) was observed in the numbers of lipolytic bacteria

282 throughout storage time (r^2 = 0.91-0.95, quadratic fitting). Likewise, and as in the case
283 of proteolytic bacteria, the presence of acid-ethanol extracts of squid in the refrigeration
284 medium provided a better protection of mackerel muscle with respect to lipolytic
285 bacteria as compared with the control batch. This result was observed at medium and
286 advanced storage times, on days 6, 9 and 13. Differences between C-3 and control
287 batches reached a maximum of 1.08 log CFU g^{-1} after 13 days of storage.

288 The results presented in this work demonstrated a significant antimicrobial effect
289 on mackerel muscle as a result of the inclusion of an acid-ethanol extract of squid skin
290 in the icing medium (namely, C-3 batch). Previous studies had evaluated the effects
291 derived of the presence in ice of natural compounds with antimicrobial activity. Such
292 reports accounted for the inclusion of citric, lactic and ascorbic acids during the chilled
293 storage of Atlantic mackerel (García-Soto et al., 2013), as well as for the presence of an
294 extract of the alga *Fucus spiralis* in the icing media during the chilled storage of
295 megrim (*Lepidorhombus whiffiagonis*) (Miranda et al., 2016).

296 A previous study also investigated the preservative effect of an acetic acid-
297 ethanol extract of JSS (Aubourg et al., 2016). In such work, an antioxidant effect (lower
298 formation of primary and secondary lipid oxidation compounds, as well as higher
299 polyunsaturated fatty acids retention) was observed during the heating of cod liver oil in
300 a model system. Characterization analyses (solubility in different solvents, and
301 absorption UV-VIS and FT-IR spectra) showed that pigment compounds belonging to
302 the ommochrome family would be responsible for this behavior; indeed, a characteristic
303 xanthammatin peak was found in the JSS extract (Aubourg et al., 2016).

304 Ommochrome compounds include several biological pigments that occur in the
305 eyes of crustaceans and insects, as well as in chromatophores of cephalopods (Demski,
306 1992; Mäthger et al., 2009; Deravi et al., 2014). Ommochromes are metabolites of

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307 tryptophan, which are responsible for a wide variety of colors ranging from brown over
308 black and yellow to red. Additionally, an antioxidant effect of ommochromes
309 compounds has also been reported (Dontsov, Fedorovich, Lindström, &Ostrovsky,
310 1999; Romero, &Martínez, 2015). Furthermore, xanthommatin, an ommochrome
311 pigment that consists of a pyrido[3,2-*a*]phenoxazine ring system, has been reported to
312 be selectively extracted with dihydroxanthommatin in acidified (i.e., HCl) n-butanol
313 (Ferré, Silva, Real, & Ménsua, 1986).

314 Recent research accounted for the chemical characterization of collagen and
315 gelatin from JSS (Alemán et al., 2011; Uriarte-Montoya et al., 2011; Ramírez-Guerra et
316 al., 2015). Thus, special attention was paid to the antioxidant properties of such
317 compounds (Giménez et al., 2009; Jiangjia et al., 2015). Additionally, enzymatic
318 hydrolysates prepared from JSS showed antimicrobial (Na et al., 2015) and antioxidant
319 (Yuhong, Wenge, Dalun, Guohuang, & Jinjie, 2014) activities. In contrast to such
320 previous studies, the present research was focused on an acetic acid-ethanol extract of
321 JSS. As a result, an antimicrobial activity resulting from the presence of such lipophilic
322 extract was observed, to our knowledge for the first time. Further research is envisaged
323 to establish the possible antimicrobial role of ommochromes compounds present in the
324 acetic acid-ethanol extracts (Aubourg et al., 2016); as electron donors, ommochromes
325 could be responsible for inducing an imbalance in metabolic pathways of
326 microorganisms (Romero & Ramirez, 2015).

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328 **3.2. Assessment of the quality evolution by chemical indices**

329 Results concerning the pH value are shown in Table 3. For all batches, slight
330 differences can be mentioned, all values being below 6.30. This behavior can be
331 explained as a result of the presence of acetic acid in the icing medium, which might

332 have influenced the pH of the fish muscle. Concerning the comparison between batches,
333 a lower value ($p<0.05$) was observed in the C-3 batch when compared with the control
334 batch in the 6-13-day period. These results are in agreement with the above-mentioned
335 results on microbial counts, implying an inhibitory effect on microbial activity.

336 A sharp and progressive trimethylamine (TMA) formation ($p<0.05$) was
337 observed in all batches as chilling time increased ($r^2 = 0.91$-0.94) (Table 3). Comparison
338 among batches showed a lower ($p<0.05$) TMA content in the 6-13-day period in
339 specimens corresponding to the C-3 batch when compared with the control batch. This
340 result is in agreement with the above-mentioned results on pH value and microbial
341 activity. Thus, accurate correlation values between TMA content and all microbial
342 groups investigated were also observed ($r^2 = 0.86$-0.95). TMA-N values obtained were
343 in all cases below 20 mg kg^{-1}, in agreement with previous research on this fish species
344 (Sanjuás-Rey et al., 2012) and hake (*Merluccius merluccius*) (García-Soto et al., 2013),
345 but far lower than values found in megrim (*Lepidorhombus whiffiagonis*) (Miranda et
346 al., 2016).

347 In agreement with the evolution of microbial activity, chemical assessment (i.e.,
348 pH and TMA-N) has also proved an antimicrobial effect derived of the presence of the
349 lipophilic JSS extract (namely, C-3 batch) in the icing medium. As stated before,
350 ommochromes pigments extracted frompresent in the JSS by acid-ethanol
351 solutionsextract would probablymay be responsible for this preservative effect.

352

353 **3.3. Assessment of the quality evolution by sensory evaluation**

354 The evolution of sensory quality is depicted in Table 4. Initial fish specimens were
355 found E quality both in raw and cooked descriptors. Throughout storage time, a
356 progressive quality loss was observed in the different sensory descriptors for all batches.

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After 3 days of storage, specimens corresponding to the C-3 batch showed a higher quality and were still considered in the E category. This higher quality retention was maintained at day 6 when compared with fish samples corresponding to the control batch. Remarkably, mackerel belonging to C-1 and C-2 batches showed at that time a higher quality than their control counterparts when considering the raw-state descriptors.

A higher quality value was also observed in fish (descriptors of raw and cooked states) corresponding to the C-3 batch when compared with the control fish for the 10-13-day period. Indeed, mackerel corresponding to the C-0 batch was considered not acceptable at the end of the experiment in agreement with previous studies that reported a shelf life time of 9 days for this species (Bennour et al., 1991). Analyses carried out on raw cooked samples at advanced storage periods (10-13 days), showed that fish specimens belonging to C-1 and C-2 batches were not acceptable.

This increase of sensory acceptance and shelf life for fish stored under C-3 conditions is in agreement with the above-mentioned results concerning the microbiological and chemical indices related to microbial development. Previous research demonstrated an increased shelf life and a sensory quality enhancement in chilled mackerel by means of including preservative compounds (i.e., citric, lactic and ascorbic acids) in the icing system (Sanjuás-Rey et al., 2012). Also related to the current research, Indian mackerel (*Rastrelliger kanagurta*) previously dipped into pomegranate peel and tea leaf extracts showed a shelf life time of 17 d during chilled storage (Shinde, Reddy, & Patange, 2015).

4. CONCLUSIONS

A significant inhibition ($p<0.05$) of microbial activity (aerobes, psychrotrophs, Enterobacteriaceae, lipolytic bacteria; TMA formation) was observed in mackerel corresponding to the batch with the highest JSS extract presence (namely, C-3 condition) in the icing medium. Additionally, fish corresponding to batches including any of the JSS concentrations tested showed lower ($p<0.05$) counts of proteolytic bacteria and a better maintenance of pH value than their control counterpart. Sensory analysis revealed a marked increase of shelf life time in chilled mackerel corresponding to ice including the highest JSS content. Such fish was found to be still acceptable at the end of the storage period, while all other batches were considered rejectable. The marked microbial activity inhibition observed could be explained on the basis of the presence in ice of lipophilic-type compounds obtained by acetic acid-ethanol extraction of the JSS. Further research is envisaged to analyze the possible active molecules (i.e., ommochromes compounds) involved and to optimize their presence in ice during the chilling storage of different kinds of marine fish species.

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FIGURE LEGENDS

Figure 1: Proteolytic counts assessment (log CFU g^{-1} muscle)* in mackerel muscle stored under different icing conditions**

* Mean values of three replicates (n=3); standard deviations are indicated by bars. Values accompanied by different letters (a, b, c, d) indicate significant differences ($p<0.05$) as a result of the icing condition.

** Abbreviations of icing conditions (C-0 ▒, C-1 ▨, C-2 □ and C-3 ■) as expressed in Table 2.

Figure 2: Lipolytic counts assessment (log CFU g^{-1} muscle)* in mackerel muscle stored under different icing conditions**

* Mean values of three replicates (n=3); standard deviations are indicated by bars. Values accompanied by different letters (a, b) indicate significant differences ($p<0.05$) as a result of the icing condition.

** Abbreviations of icing conditions (C-0 ▒, C-1 ▨, C-2 □ and C-3 ■) as expressed in Table 2.

FIGURE LEGENDS

Figure 1: Proteolytic counts assessment (log CFU g^{-1} muscle)* in mackerel muscle stored under different icing conditions**

562

563 * Mean values of three replicates (n=3); standard deviations are indicated by bars.

564 Values accompanied by different letters indicate significant differences ($p<0.05$)

565 as a result of the icing condition.

566 ** Abbreviations of icing conditions (C-0 ■ ,C-1 ■ C-2 □ and C-3 ■ as expressed in

567 Table 2.

568

569

570 **Figure 2**: Lipolytic counts assessment (log CFU g^{-1} muscle)* in mackerel muscle stored

571 under different icing conditions**

572

573 * Mean values of three replicates (n=3); standard deviations are indicated by bars.

574 Values accompanied by different letters indicate significant differences ($p<0.05$)

575 as a result of the icing condition.

576 ** Abbreviations of icing conditions (C-0 ■ ,C-1 ■, C-2 □ and C-3 ■ as expressed in

577 Table 2.

578

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HIGHLIGHTS

- Jumbo squid (*Dosidicus gigas*) skin (JSS) was used for fish preservation purposes
- Icing media including JSS acid-ethanolic extracts were tested for mackerel chilling
- The shelf life of chilled mackerel was extended in JSS-containing ice batches
- An antimicrobial effect of JSS extract in mackerel muscle was concluded
- The antimicrobial effect was more intense as JSS concentration increased

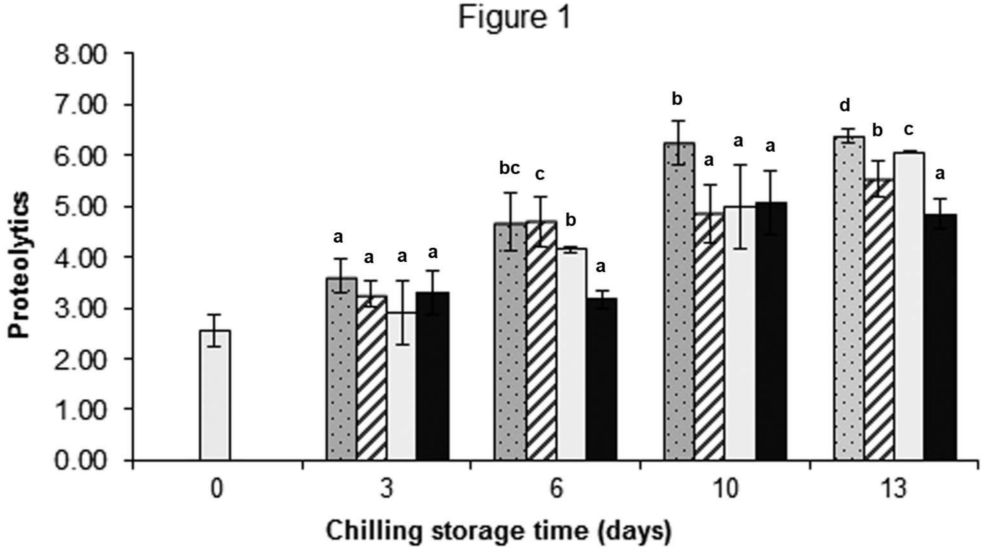
Figure 1
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Chilling storage time (days)

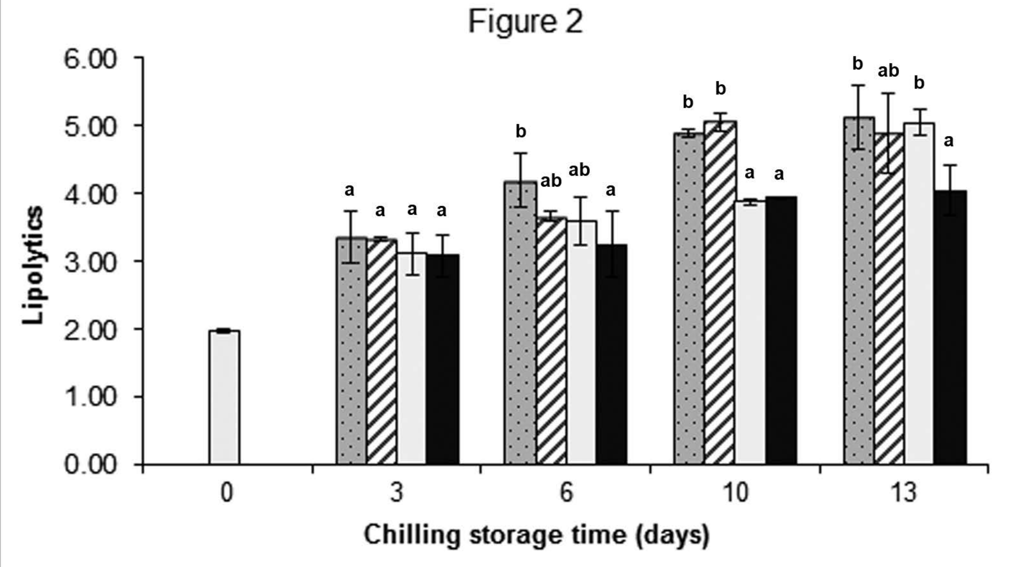
Figure 2
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Chilling storage time (days)

TABLE 1

Scale employed for evaluating the sensory quality of chilled mackerel

Descriptor	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin and mucus development	Very intense pigmentation; transparent mucus	Milky mucus; insignificant pigmentation losses	Slightly greyish mucus; pigmentation without shine	Widely opaque mucus; important pigmentation losses
Eyes	Convex; transparent cornea; bright and black pupil	Convex and slightly sunken; slightly opalescent cornea; black and cloudy pupil	Flat; opalescent cornea; opaque pupil	Concave and milky cornea; Internal organs blurred
External odor	Sharply seaweed and shellfish smell	Weakly seaweed and shellfish smell	Incipiently putrid or ammonia odor	Putrid or ammonia odor
Gills appearance and odor	Brightly red; lamina perfectly separated; without odor	Rose colored; lamina adhered in groups; without odor	Slightly pale; lamina adhered in groups; incipient fishy odor	Grey-yellowish color; lamina totally adhered; intense ammonia odor
Consistency	Presence of partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
Flesh odor (raw fish)	Sharply seaweed and shellfish	Weakly seaweed and shellfish	Incipiently putrid or ammonia odor	Putrid or ammonia odor
Flesh odor (cooked fish)	Sharply fresh and agreeable	Weakly fresh and agreeable	Incipiently putrid or ammonia odor	Putrid or ammonia odor
Flesh taste	Sharply fresh and agreeable	Weakly fresh and agreeable	Incipiently putrid or ammonia odor	Putrid or ammonia odor

TABLE 2

Aerobe, psychrotroph and Enterobacteriaceae counts assessment (log CFU g^{-1} muscle)* in mackerel muscle stored under different icing conditions**

Chilling time (days)	Aerobes				Psychrotrophs				Enterobacteriaceae			
	C-0	**C-1**	**C-2**	**C-3**	**C-0**	**C-1**	**C-2**	**C-3**	**C-0**	**C-1**	**C-2**	**C-3**
0	2.56 (0.90)				2.50 (0.41)				0.95 (0.00)			
3	3.43 a (0.39)	3.33 a (0.01)	3.64 a (0.22)	3.59 a (0.41)	3.21 a (0.18)	3.25 a (0.13)	3.24 a (0.23)	3.02 a (0.09)	1.93 b (0.04)	1.15 a (0.21)	1.60 ab (0.42)	1.85 b (0.00)
6	4.08 b (0.15)	4.48 c (0.19)	4.11 bc (0.36)	3.19 a (0.47)	3.19 a (0.13)	4.24 b (0.39)	4.04 b (0.06)	3.46 a (0.08)	2.04 a (0.27)	1.95 a (0.50)	2.13 a (0.25)	1.87 a (0.12)
10	6.54 a (0.12)	5.75 a (0.73)	6.51 a (0.15)	5.90 a (0.54)	6.64 b (0.30)	6.57 b (0.08)	6.60 b (0.28)	5.80 a (0.16)	4.53 a (0.46)	4.43 a (0.37)	4.11 a (0.39)	4.00 a (0.11)
13	6.85 a (0.50)	6.04 a (0.40)	7.35 a (0.62)	6.32 a (0.85)	6.84 b (0.17)	6.04 a (0.40)	7.35 b (0.62)	6.32 ab (0.85)	4.36 bc (0.35)	3.95 b (0.23)	4.37 c (0.09)	3.39 a (0.13)

* Mean values of three replicates (n=3); standard deviations are indicated in brackets. Mean values followed by different letters (a, b, c) indicate significant differences ($p<0.05$) as a result of the icing condition.

** C-0, C-1, C-2 and C-3 abbreviations denote icing conditions corresponding to 0, 4, 12 and 36 mL of the squid skin extract according to the Material and Methods section.

TABLE 3

Evolution of pH value and trimethylamine (TMA) content (mg TMA-N kg^{-1} muscle)* in mackerel muscle stored under different icing conditions**

Chilling time (days)	pH				TMA-N			
	C-0	C-1	C-2	C-3	C-0	C-1	C-2	C-3
0	5.95 (0.08)				0.55 (0.33)			
3	6.02 a (0.10)	6.07 a (0.02)	5.96 a (0.13)	5.88 a (0.16)	2.68 ab (1.46)	3.14 ab (0.61)	4.16 b (0.33)	2.53 a (0.95)
6	6.29 b (0.06)	6.20 ab (0.11)	6.14 a (0.03)	6.12 a (0.04)	6.05 b (1.80)	6.31 b (1.70)	6.54 b (0.56)	3.87 a (1.02)
10	6.29 b (0.11)	6.06 ab (0.03)	6.05 ab (0.01)	5.98 a (0.05)	15.44 c (1.36)	13.40 bc (2.02)	10.08 ab (1.94)	10.22 a (0.27)
13	6.26 b (0.03)	6.17 ab (0.14)	6.11 ab (0.11)	6.16 a (0.04)	16.11 b (0.44)	18.18 b (3.10)	16.94 b (1.55)	12.32 a (0.50)

* Mean values of three replicates (n=3); standard deviations are indicated in brackets. Mean values followed by different letters (a, b, c) indicate significant differences ($p<0.05$) as a result of the icing condition.

** Abbreviations of icing conditions (C-0, C-1, C-2 and C-3) as expressed in Table 2.

TABLE 4

Sensory assessment* of mackerel fish stored under different icing conditions**

Chilling time (days)	Descriptors corresponding to the raw state				Descriptors corresponding to the cooked state			
	C-0	**C-1**	**C-2**	**C-3**	**C-0**	**C-1**	**C-2**	**C-3**
0		E				E		
3	A	A	A	E	A	A	A	E
6	B	A	A	A	B	B	B	A
10	B	C	C	A	B	C	C	A
13	C	C	C	B	C	C	C	B

* Freshness categories: E (excellent), A (good), B (fair) and C (unacceptable).
** Abbreviations of icing conditions (C-0, C-1, C-2 and C-3) as expressed in Table 2.