Impact of prior high-pressure processing on lipid damage and volatile amines formation in mackerel muscle subjected to frozen storage and canning

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ABSTRACT

This research describes a first approach to analyse the effects of using high-pressure processing (HPP) as a pre-treatment before frozen storage and subsequent canning of fish. For it, Atlantic Chub mackerel (*Scomber scombrus*) was subjected to HPP (200, 400 and 600 MPa for 2 min) followed by freezing (–30 ºC for 48 h), frozen storage (–18 ºC for 0, 3, 6, 10 or 15 months), canning and canned storage (3 months at 20 ºC). Lipid damage development (hydrolysis and oxidation) and volatile amines formation (total and trimethylamine) were determined in canned mackerel. As a result, a marked inhibitory effect ($p<0.05$) on free fatty acids content was observed in canned mackerel previously subjected to different frozen storage times. This effect increased with pressure applied. Furthermore, higher average polyene values were obtained in most canned fish previously subjected to any high-pressure treatment when compared with Control canned fish. No effect ($p>0.05$) on thiobarbituric acid reactive substances and trimethylamine values was noticed related to HPP. However, increasing ($p<0.05$) peroxides and fluorescent compounds contents were obtained when applying the two highest pressure levels.

Key words: Canned mackerel; high-pressure processing; frozen storage; lipid damage; volatile amines

Running title: High-pressure processing and canned mackerel quality

1. INTRODUCTION

Canning represents one of the most important means of fish preservation. The extensive heat treatment involved in it substantially alters the nature of the raw material. Therefore, a product with different characteristics is formed (Horner, 1997; Lukoshkina & Odoeva, 2003). As a result of heat treatment, both enzymes and bacteria should be permanently inactivated. Unfortunately, most species destined for canning are caught in large quantities and canneries have to store the raw material before it is processed. Consequently, most of the problems with canned fish acceptance can be related to quality of the raw material, which continuously changes during storage prior to processing (Aubourg, 2008; Naseri & Rezaei, 2012).

Since chilled fish have a short shelf life, most excess material is kept frozen prior to canning. Freezing followed by frozen storage has been used increasingly both on shore and on board fishing vessels (Erickson, 1997) to increase shelf life by inhibition of microbial growth and by the slowdown of enzyme activity. However, when long storage periods are required and/or relatively high temperatures are applied, quality assessment demonstrates that fish deterioration continues during frozen storage as undesirable changes associated with lipids, proteins and other constituents are produced (Sotelo & Rehbein, 2000; Richards & Hultin, 2002; Kolakowska, 2003).

Among recent preserving technologies, high-pressure processing (HPP) has shown to inactivate microbial growth and lead to an extended shelf life in seafood (Murchie et al., 2005; Bermúdez-Aguirre & Barbosa-Cánovas, 2011). Additionally, hydrolytic (*i.e.*, lipases and phospholipases) and oxidative (lipoxygenases, peroxidases, and so on) endogenous enzymes can be inactivated before storage or subsequent processing of fish products (Norton & Sun, 2008; Campus, 2010). Consequently, recent

research has demonstrated a marked inhibition of lipid oxidation and hydrolysis in frozen fatty fish species such as mackerel (*Scomber scombrus*) (Vázquez, Torres, Gallardo, Saraiva, & Aubourg, 2013) and sardine (*Sardina pilchardus*) (Méndez et al., 2017) if they are previously subjected to HPP. Furthermore, pressure-shift technology revealed advantages in protein properties such as lower denaturation and toughening extent and an increased water-holding capacity in turbot (*Scophthalmus maximus*) (Chevalier, Sequeira-Muñoz, Le Bail, Simpson, & Goul, 2000) and sea bass (*Dicentrarchus labrax*) (Tironi, de Lamballerie, & Le Bail, 2010) muscle.

In this research, the effects of using HPP as a pre-treatment before frozen storage and subsequent canning of fish were evaluated, to our knowledge, for the first time. In it, lipid damage development (hydrolysis and oxidation) and volatile amines formation (total and trimethylamine) were determined in canned Atlantic Chub mackerel (*Scomber colias*).

2. MATERIALS AND METHODS

2.1. Initial fish, HPP, freezing, frozen storage, canning and sampling procedure

Specimens (126 fish) of mackerel (length range: 24.5-28.0 cm; weight range: 157-175 g) were obtained at Vigo harbour (North-Western Spain) in November 2017 and transported on ice to the laboratory. Once at the laboratory, 6 fish specimens were selected and divided into three groups (two specimens per group). Such specimens (initial raw fish) were beheaded, eviscerated, filleted and the white muscle analysed independently within each group (n = 3).

The remaining fish individuals were placed in flexible polyethylene bags (20 bags; six individuals per bag), vacuum-sealed at 150 mbar (Vacuum Packaging Machine

104 Culinary, Albipack Packaging Systems Solutions, Águeda, Portugal) and divided into
105 four batches (5 bags in each batch). The bags corresponding to one of the four batches
106 were directly placed in a single layer inside a static freezer at –30 ºC for 48 h (freezing
107 treatment) and considered as the Control treatment.

108 Bags corresponding to the other three batches were subjected to HPP (200, 400
109 and 600 MPa for 2 min, respectively) in a 55-L high pressure unit (WAVE 6000/55 HT;
110 NC Hiperbaric, Burgos, Spain). A wide pressure levels range was tested in order to
111 analyse the effect on final canned fish. For it, water was applied as the pressurising
112 medium at 3 $MPa \cdot s^{-1}$ yielding 67, 133, and 200 s as the come up times, respectively,
113 decompression time being less than 3 s. After HPP, all bags were placed in a single
114 layer inside a static freezer at –30 ºC for 48 h (freezing step).

115 Then, fish corresponding to one bag of each batch (Control, 200-, 400- and 600-
116 MPa batches) were air-thawed overnight in a refrigerated room at 4 ºC and then canned
117 (month-0). The remaining bags of all batches (4 bags per batch) were kept at –18 ºC for
118 3, 6, 10 and 15 months, respectively. At each subsequent sampling time (month-3, -6,
119 -10 and -15), one bag of each batch was air-thawed overnight in a refrigerated room at 4
120 ºC before canning.

121 The canning process was performed on the frozen fish corresponding to all
122 batches. For it, frozen fish were thawed, beheaded, eviscerated and filleted. Then, 45-g
123 portions of mackerel fillets (from one fish) were placed in small flat rectangular cans
124 (105 × 60 × 25 mm; 150 mL), all cans being filled with brine solution (2 % w/v). Cans
125 were vacuum-sealed and subjected to heat sterilisation treatment in a steam retort (115
126 ºC, 45 min; F_o = 7 min) (CIFP Coroso, Ribeira, A Coruña, Spain). Once the heating
127 time was completed, steam was cut off, air was used to flush away the remaining steam,
128 and cans were cooled with water at reduced pressure. After 3 months of storage at room

129 temperature (20 ºC), the cans were opened, and the liquid part was carefully drained off
130 gravimetrically and filtered through a filter paper. Mackerel white muscle was
131 separated, wrapped in filter paper and used for analysis. For each sample point, the fish
132 muscle of two cans was pooled together to carry out the different chemical analyses.
133 Each batch was analysed in triplicate (n = 3).

134 All solvents and chemical reagents used were of reagent grade (Merck,
135 Darmstadt, Germany).

136

137 **2.2. Lipid damage analyses**

138 Lipids were extracted from the mackerel white muscle by the Bligh and Dyer
139 (1959) method, which employs a chloroform-methanol (1:1) mixture. Lipid content was
140 calculated as g lipid·kg^{-1} muscle.

141 Free fatty acids (FFA) content was determined on the muscle lipid extract by the
142 Lowry and Tinsley (1976) method, which is based on complex formation with cupric
143 acetate-pyridine followed by spectrophotometric (715 nm) assessment (Beckman
144 Coulter DU 640 spectrophotometer). Results were calculated as g FFA·kg^{-1} muscle.

145 Peroxide value (PV) was determined spectrophotometrically (520 nm) on the
146 lipid extract by peroxide reduction with ferric thiocyanate (Chapman and McKay,
147 1949). Results were calculated as meq. active oxygen·kg^{-1} lipids.

148 Thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970).
149 For it, content of thiobarbituric acid reactive substances (TBARS) was
150 spectrophotometrically measured at 532 nm and calculated from a standard curve using
151 1,1,3,3-tetraethoxy-propane (TEP). Results were calculated as mg malondialdehyde·kg^{-1}
152 muscle.

153 The formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer
154 España; Tres Cantos, Madrid, Spain) was determined in the lipid extract of the fish
155 muscle as described previously (Losada, Rodríguez, Ortiz, & Aubourg, 2006). The
156 relative fluorescence (RF) was calculated as follows: RF = F/F_{st}, where F is the
157 fluorescence measured at each excitation/emission wavelength pair and F_{st} is the
158 fluorescence intensity of a quinine sulphate solution (1 μg·mL^{-1} in 0.05 M H_2SO_4) at the
159 corresponding wavelength pair. Results were calculated as the fluorescence ratio (FR),
160 which was calculated as the ratio between the two RF values: FR = $RF_{393/463\ nm}/RF_{327/415}$
161 $_{nm}$.

162 Lipid extracts were converted into fatty acid methyl esters (FAME) by using
163 acetyl chloride and then analysed using a Perkin-Elmer 8700 gas chromatograph
164 (Madrid, Spain) equipped with a fused silica capillary column SP-2330 (0.25 mm i.d. x
165 30 m, 0.20 μm film, Supelco Inc., Bellefonte, PA, USA) (Vázquez et al., 2013). Peaks
166 corresponding to FAME were identified by comparing their retention times with those
167 of standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME mix, Supelco,
168 Inc., Bellefonte, PA, USA). Peak areas were automatically integrated, C19:0 fatty acid
169 being used as the internal standard for quantitative purposes. The polyene index (PI)
170 was calculated as the following fatty acids contents ratio: (C20:5ω3 + C22:6ω3)/C16:0.
171

172 **2.3. Volatile amines formation**

173 Total volatile base-nitrogen (TVB-N) values were measured as reported by
174 Antonacopoulos (1960), with some modifications. Briefly, fish muscle (10 g) was
175 extracted with 60 g·L^{-1} perchloric acid in water (30 mL) and brought up to 50 mL. An
176 aliquot of the acid extracts was rendered alkaline to pH 13 with 200 g·L^{-1} aqueous

177 NaOH and then steam-distilled. Finally, the TVB-N content was determined by titration
178 of the distillate with 10 mM HCl. Results were calculated as mg TVB-N·kg^{-1} muscle.

179 Trimethylamine (TMA)-nitrogen (TMA-N) values were determined using the
180 picrate spectrophotometric (410 nm) method (Tozawa, Erokibara, & Amano, 1971).
181 Results were calculated as mg TMA-N·kg^{-1} muscle.

182

183 **2.4. Statistical analysis**

184 Data obtained were subjected to the ANOVA method to explore differences
185 resulting from the effect of HPP, freezing and frozen storage time. The comparison of
186 means was performed using the least-squares difference (LSD) method. In all cases,
187 analyses were carried out using the PASW Statistics 18 software for Windows (SPSS
188 Inc., Chicago, IL, USA); differences among batches were considered significant for a
189 confidence interval at the 95-% level ($p < 0.05$).

190

191 **3. RESULTS AND DISCUSSION**

192 **3.1. Determination of lipid hydrolysis development**

193 Lipid hydrolysis in the current canned fatty species (lipid content: 63.0 g·kg^{-1}
194 muscle) was measured by determination of the FFA content. All canned samples
195 corresponding to a frozen storage of 0 months showed a significantly ($p < 0.05$) higher
196 value of FFA content when compared with the initial raw fish value (Figure 1), so that
197 an increased formation was proved as a result of the freezing step and the canning
198 process. In all batches, a progressive increase of the FFA content ($p < 0.05$) was
199 observed by increasing the frozen storage time. Concerning the effect of HPP, FFA
200 content was found lower ($p < 0.05$) in canned fish previously subjected to 600 MPa

when compared with all other canned batches, this result being observed at all frozen storage times. Furthermore, canned mackerel previously pressurised at 400 MPa showed a lower ($p < 0.05$) lipid hydrolysis development than fish corresponding to Control batch when taking into account samples corresponding to the 6-15-month frozen storage period. Interestingly, Control canned fish provided a higher FFA level ($p < 0.05$) than any other batch when considering the 10-15-month frozen period. Finally, comparison between Control and 600-MPa batches showed a reduction of FFA content round 50 % when canned fish corresponding to the 6-15-month frozen storage period is considered. Consequently, a marked inhibitory effect of HPP on lipid hydrolysis development has been proved in canned fish. This effect was found higher by increasing the pressure level and was evident in canned samples corresponding to all frozen storage times.

Accumulation of FFA via lipid hydrolysis in fish muscle has no nutritional significance. Nevertheless, it has been recognised as a most important event during the frozen storage of fish species as leading to muscle texture changes, acceleration of lipid oxidation compounds formation, and off-odour and off-taste development (Refsgaard, Brockhoff, & Jensen, 2000). This degradative pathway has been signalled as the result of an increased hydrolytic endogenous enzyme (lipases, phospholipases) activity during the frozen storage (Sikorski & Kolakowski, 2000). However, previous research has shown an inhibitory effect on lipid hydrolysis development during the frozen storage as a result of prior HPP in lean (Vázquez, Fidalgo, Saraiva, & Aubourg, 2018) and fatty (Vázquez et al., 2013) fish species; this effect showed to be more important by increasing the pressure applied. Concerning the canning process, a marked lipid hydrolysis development has been reported as a result of thermal treatment, this effect being higher by decreasing the quality of the starting raw fish employed for canning

(Aubourg, 2008). However, to our knowledge, no previous research has been addressed to the effect of HPP on the FFA content in canned fish.

3.2. Lipid oxidation assessment

Compared with the initial raw fish, only marginally significant ($p < 0.05$) changes of PV could be observed in canned samples (Table 1). Throughout this study, average values remained below a 5.27 score in all cases. Compared with month-0 canned fish, a general increase of average values was observed in canned samples corresponding to a frozen storage period of 3 months that was followed by a decrease in month-6 canned samples; after that time, no marked tendency could be outlined as a result of the frozen storage time. Concerning the effect of HPP, higher average values were obtained at most sampling times in fish corresponding to 600-MPa treatment (frozen storage of 0, 6 and 15 months); thus, differences were found significant ($p < 0.05$) in canned samples corresponding to a frozen storage time of 0 and 15 months when compared with all other batches. Except for month-10 samples, average values obtained in canned fish corresponding to 400- and 600-MPa treatments were higher than their counterparts from Control and 200-MPa batches; remarkably, differences were found significant ($p < 0.05$) in month-3 samples.

A low peroxides formation has already been described in different kinds of canned fish species such as bluefin tuna (*Thunnus thynnus*) and sardine (*Sardina pilchardus*) (Selmi, Monser, & Sadok, 2008), Atlantic salmon (*Salmo salar*) (Ortiz, Vivanco, & Aubourg, 2014) and the current species (Barbosa, Trigo, Campos, & Aubourg, 2019). Peroxides are known to be produced during the frozen storage time and the subsequent thermal sterilisation step (Aubourg, 2008). However, this thermal

250 treatment has also been reported to destroy primary oxidation compounds and give rise
251 to low-molecular-weight molecules formation (*i.e.*, carbonyl compounds) and also to
252 interact with nucleophilic compounds present in the muscle leading to fluorescent
253 compounds formation (Tironi, Tomás, & Añón, 2002; Losada et al., 2006).

254 Average TBARS values remained in all cases below a 2.07 score (Figure 2). The
255 raw fish showed higher average values than most canned samples of all kinds of batches
256 considered; consequently, a significant increase could not be detected ($p > 0.05$) in any
257 canned fish. According to average values, a general decrease was observed by
258 comparing the initial fresh fish and canned fish muscle corresponding to month-0 frozen
259 storage. After this time, canned muscle did not provide a general trend about the effect
260 of the frozen storage period. No effect ($p > 0.05$) could also be attributed to the pressure
261 level applied.

262 Secondary oxidation compounds are reported to be produced during fish frozen
263 storage and as a result of the fish sterilisation step (Aubourg, 2008). As expressed
264 above, their formation should arise especially from peroxides breakdown. However, and
265 according to their hydrophilic degree, TBARS may be lost partly into the current brine
266 packaging medium and not be measured when analysing the canned fish muscle.
267 Additionally, TBARS are reported to interact with nucleophilic compounds present in
268 the muscle and facilitate the fluorescent compounds formation (Tironi et al., 2002;
269 Losada et al., 2006). In agreement with the current data, a marked decrease of the
270 TBARS value has already been described for canned fish when different kinds of water-
271 coating media were tested such as tomato-sauce (Selmi et al., 2008), brine (Barbosa et
272 al., 2019) and water (Barbosa, Trigo, Fett, & Aubourg, 2018). Contrary, an increased
273 formation of secondary lipid oxidation compounds was observed in canned sardine

(*Sardina pilchardus*) by employing brine packaging as well as olive-oil packaging (Aubourg & Medina, 1997).

A marked increase ($p < 0.05$) of the FR could be observed in all canned samples corresponding to month-0 frozen storage when compared with the initial raw fish (Table 1). Furthermore, a progressive increase of the FR value was detected in all batches with the frozen storage time. As the pressure level is concerned, comparison with the Control batch revealed higher average values in samples corresponding to the two highest pressure levels applied for samples corresponding to the 3-15-month frozen period; interestingly, such differences were found significant ($p < 0.05$) when considering 3, 10 and 15 months of frozen storage. Fluorescent compounds formation was found to be especially important in the 600-MPa batch, since higher values ($p < 0.05$) than in Control samples were obtained at all frozen storage times.

Seafood research has shown a marked increase of the FR as a result of the fish frozen storage time (Aubourg & Medina, 1999), as well as by increasing the heating conditions of the canning process (Aubourg et al., 1992; Naseri & Rezaei, 2012). Concerning HPP as a prior processing to canning, no previous research accounts for its influence on the FR value in canned fish. In the present study, results obtained for this quality parameter can be considered the result of two different effects. One side, HPP should inhibit the pro-oxidative effect of fish endogenous enzymes during the frozen storage (*i.e.*, enzymatic pathway of lipid oxidation development); this effect should be higher by increasing the pressure level (Vázquez et al., 2013). On the other, denaturation of metal-bound proteins (*i.e.*, iron) as a result of HPP has been reported to increase the free metal ion content (Richards & Hultin, 2002; Lakshmanan, Parkinson, & Piggott, 2003; Campus, 2010), which during the subsequent frozen storage, and especially during the sterilisation step, would lead to an increase of lipid oxidation

299 development (*i.e.*, non-enzymatic pathway of lipid oxidation development). According
300 to the current results on FR value, this second effect showed to be more important in
301 400- and 600-MPa batches. Furthermore, this pro-oxidative effect resulting from the
302 increased free metal ion content was also observed in primary lipid oxidation
303 development (*i.e.*, peroxides formation) (Table 1). However, canned fish corresponding
304 to the 200-MPa batch did not show an increased FR value when compared with the
305 Control batch. In order to avoid the current increase observed in lipid oxidation
306 development in canned fish, further research ought to be addressed to optimise the HPP
307 conditions (pressure level and pressure holding time) to be combined with the frozen
308 storage and the canning process.

309 Comparison of PI values of initial raw fish with canned fish corresponding to
310 month-0 frozen storage did not provide significant differences ($p > 0.05$) (Figure 3).
311 Frozen storage time led to a progressive decrease in all canned batches; thus, a marked
312 decrease ($p < 0.05$) of the PI values was proved by comparing month-0 and month-15
313 samples in all canned batches. Related to the effect of HPP, negligible significant
314 differences were detected. However, higher average PI values were obtained in most
315 canned fish previously subjected to any HPP condition when compared with Control
316 canned fish; thus, differences between Control and 600-MPa batches were found
317 significant ($p < 0.05$) when a 15-month frozen period is considered.

318 Minimal changes in the PI value were obtained in frozen fatty (Vázquez et al.,
319 2013) and lean (Vázquez et al., 2018) fish species previously treated under HPP
320 conditions. In such cases, pressure levels of 150-450 MPa were applied and a frozen
321 storage at –10 ºC for 3 (Atlantic mackerel, *S. scombrus*) and 5 (hake, *Merluccius*
322 *merluccius*) months were employed. Concerning the effect of the canning process, a
323 decrease of the average PI score was detected by comparing the initial raw fish with the

corresponding canned samples when a brine dipping medium, as in the current case, was employed (Barbosa et al., 2019).

3.3. Assessment of volatile amines formation

Data corresponding to the determination of total volatile amines are shown in Table 2. Results do not provide a definite trend about the effect of frozen storage time on the TVB-N value. Additionally, HPP pre-treatment did not lead to a marked effect on this quality parameter in canned mackerel. Remarkably, average values corresponding to the initial fish showed to be higher than those of most canned samples. To explain the current results, it has to be taken into account that total volatile compounds content can be considered as the result of different effects. In agreement with previous research, formation of volatile amines would be expected to occur especially as a result of the sterilisation treatment, by means of breakdown of different kinds of constituents present in the fish muscle (Losada et al., 2006; Ortiz et al., 2014). Furthermore, some formation would also be expected to occur during the frozen storage by endogenous enzyme activity (Sotelo & Rehbein, 2000), although this effect should be inhibited partly by increasing the pressure employed (Vázquez et al., 2018). Finally, it has to be considered that most of all volatile amines produced are water-soluble compounds (*i.e.*, ammonia, methylamine, dimethylamine, etc.) that can be extracted partly by the coating medium and not be measured when analysing the canned fish muscle. According to the results obtained, this effect has shown to be especially

347 important in the current study and lead to relatively low values in canned fish when
348 compared with those of initial raw fish.

349 Research on canned Atlantic mackerel (*S. scombrus*) (Barbosa et al., 2018) and
350 Chub mackerel (*S. colias*) (Barbosa et al., 2019) has already shown lower total volatile
351 amine levels in canned fish than in the initial raw fish when a hydrophilic coating
352 medium was employed. However, opposite results were reported when an oil-packaging
353 medium was employed. Thus, TVB-N content showed to increase after the canning
354 process in canned salmon (*Oncorhynchus kisutch*) (Rodríguez, Carriles, & Aubourg,
355 2010), tuna (*T. thynnus*) and sardine (*S. pilchardus*) (Losada et al., 2006; Selmi et al.,
356 2008).

357 Contrary to TVB-N detection, TMA-N content showed a marked increase as a
358 result of freezing and frozen storage followed by canning (Table 2). Thus, canned fish
359 corresponding to any batch from month-0 frozen storage showed higher levels ($p <$
360 0.05) than the initial raw fish. Furthermore, a progressive increase of TMA content was
361 detected in all canned batches by increasing the frozen time; in all cases, comparison
362 between samples corresponding to month-0 and month-6-15 period showed significant
363 differences ($p < 0.05$). Related to the high-pressure effect, a lower ($p < 0.05$) formation
364 of TMA was implied in samples corresponding to 400 and 600 MPa batches when
365 considering a month-0 frozen period; contrary, such batches showed a higher ($p < 0.05$)
366 formation than Control and 200-MPa batches if a frozen storage of 6 months is taken
367 into account. Consequently, a definite effect of HPP on TMA content in canned fish
368 could not be concluded.

369 Since microbial activity should be inhibited in the current study, formation of
370 TMA can be explained especially as a result of trimethylamine N-oxide and protein-like

371 compounds breakdown during the sterilisation step (Losada et al., 2006; Rodríguez et
372 al., 2010). Differently to most volatile amines, TMA is a non-water-soluble tertiary
373 amine. Consequently, it is not likely to be leached into the present brine packaging
374 medium. According to the current study results, a marked TMA-N content increase was
375 observed after canning in tuna (*T. thynnus*) and sardine (*S. pilchardus*) muscle (Selmi et
376 al., 2008), as well as in the current species (Barbosa et al., 2019). Previous research on
377 frozen fatty fish species (sardine, *S. pilchardus*; mackerel, *S. scombrus*) showed an
378 inhibitory effect of HPP (125-200 MPa for 0 min) on TMA formation only after the
379 freezing step (Méndez et al., 2017), while no effect could be observed during a
380 subsequent frozen storage (9 months at –18 ºC). However, when applying higher
381 pressure levels (200, 300, and 400 MPa) and longer pressure holding times (5 and 15
382 min), Senturk and Alpas (2013) observed a higher TMA formation as a result of the
383 high-pressure treatment when compared with Control fish.

384

385 **4. CONCLUSIONS**

386 In this research, the effects of using HPP as a pre-treatment before frozen
387 storage and subsequent canning of fish were evaluated, to our knowledge, for the first
388 time. As a result, an increased quality loss of canned fish was observed by increasing
389 the frozen storage time in agreement with an increase of FFA, fluorescent compounds
390 and TMA contents, as well as a decrease of the polyene value. Nevertheless, such
391 tendency was notably influenced by the HPP. Thus, a marked inhibitory effect ($p <$
392 0.05) on FFA formation was observed in canned mackerel corresponding to all frozen
393 storage times. This effect increased with pressure applied. Remarkably, comparison
394 between Control and 600-MPa batches showed a reduction of FFA content of
395 approximately 50 % when canned fish corresponding to the month-6-15 frozen storage

period was considered. Furthermore, higher average polyene values were obtained in most cases in canned fish previously subjected to any HPP condition when compared with Control canned fish; thus, differences between Control and 600-MPa batches were found significant ($p < 0.05$) when a 15-month frozen period is considered. Concerning lipid oxidation development, most canned samples showed a decrease of peroxides and TBARS contents by comparison with the initial raw fish. However, an increasing effect ($p < 0.05$) on peroxides and fluorescent compounds levels was noticed due to HPP by increasing the pressure applied. Finally, no effect ($p > 0.05$) on TBARS formation and TMA values was concluded by HPP treatment.

Present results indicate that prior HPP can constitute a novel and promising strategy to enhance the quality of canned fish previously subjected to freezing and different times of frozen storage (–18 ºC). As being a multi-step process, different effects on canned fish quality ought to be taken into account, sometimes with opposite results. However, quality performances obtained in the present study would justify additional research with a commercial overview. Consequently, and on the basis of being applied on canned fish in general, further research is needed to optimise HPP conditions (pressure level and pressure holding time) to be combined with frozen storage and canning. To carry out such optimisation, sensory analysis of canned products (*i.e.*, colour, odour, texture) ought to be taken into account in addition to the measurement of chemical changes. Interestingly, development of optimised conditions could open the way to the application of HPP as a pre-treatment before temporary frozen storage of high value fish before canning.

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FIGURE LEGENDS

Figure 1: Determination of free fatty acids (FFA) content ($g \cdot kg^{-1}$ muscle)* in canned mackerel** previously subjected to different high-pressure processing (HPP) conditions followed by freezing (–30 ºC) and frozen storage (–18 ºC)***

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

** Sample names abbreviations: IRF (initial raw fish) ( ), M-0 ( ), M-3 ( ), M-6 ( ), M-10 ( ) and M-15 ( ) correspond to canned samples previously stored under frozen conditions for 0, 3, 6, 10 and 15 months, respectively.

*** For each frozen storage time, different low-case letters (a-c) indicate significant differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters (A-F) indicate significant differences ($p < 0.05$) as a result of frozen storage time.

563 **Figure 2**: Determination* of thiobarbituric acid (TBA) value (mg malondialdehyde·kg^{-1}
564 muscle) in canned mackerel** previously subjected to different high-pressure
565 processing (HPP) conditions followed by freezing (–30 ºC) and frozen storage (–18
566 ºC)***

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

568 ** Sample names abbreviations: IRF (initial raw fish) ( ), M-0ᵃ ( ), M-3ᵃ ( ),
569 M-6ᵃ ( ), M-10ᵃ ( ) and M-15ᵃ ( ) correspond to canned samples previously
570 stored under frozen conditions for 0, 3, 6, 10 and 15 months, respectively.

571 *** For each frozen storage time, different low-case letters (a-c) indicate significant
572 differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters
573 (A-C) indicate significant differences ($p < 0.05$) as a result of frozen storage
574 time.

575 **Figure 3**: Determination* of polyene index (PI) in canned mackerel** previously
576 subjected to different high-pressure processing (HPP) conditions followed by freezing
577 (–30 ºC) and frozen storage (–18 ºC)***

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

579 ** Sample names abbreviations: IRF (initial raw fish) ( ), M-0ᵃ ( ), M-3ᵃ ( ),
580 M-6ᵃ ( ), M-10ᵃ ( ) and M-15ᵃ ( ) correspond to canned samples previously
581 stored under frozen conditions for 0, 3, 6, 10 and 15 months, respectively.

582 *** For each frozen storage time, different low-case letters (a-b) indicate significant
583 differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters
584 (A-D) indicate significant differences ($p < 0.05$) as a result of frozen storage
585 time.

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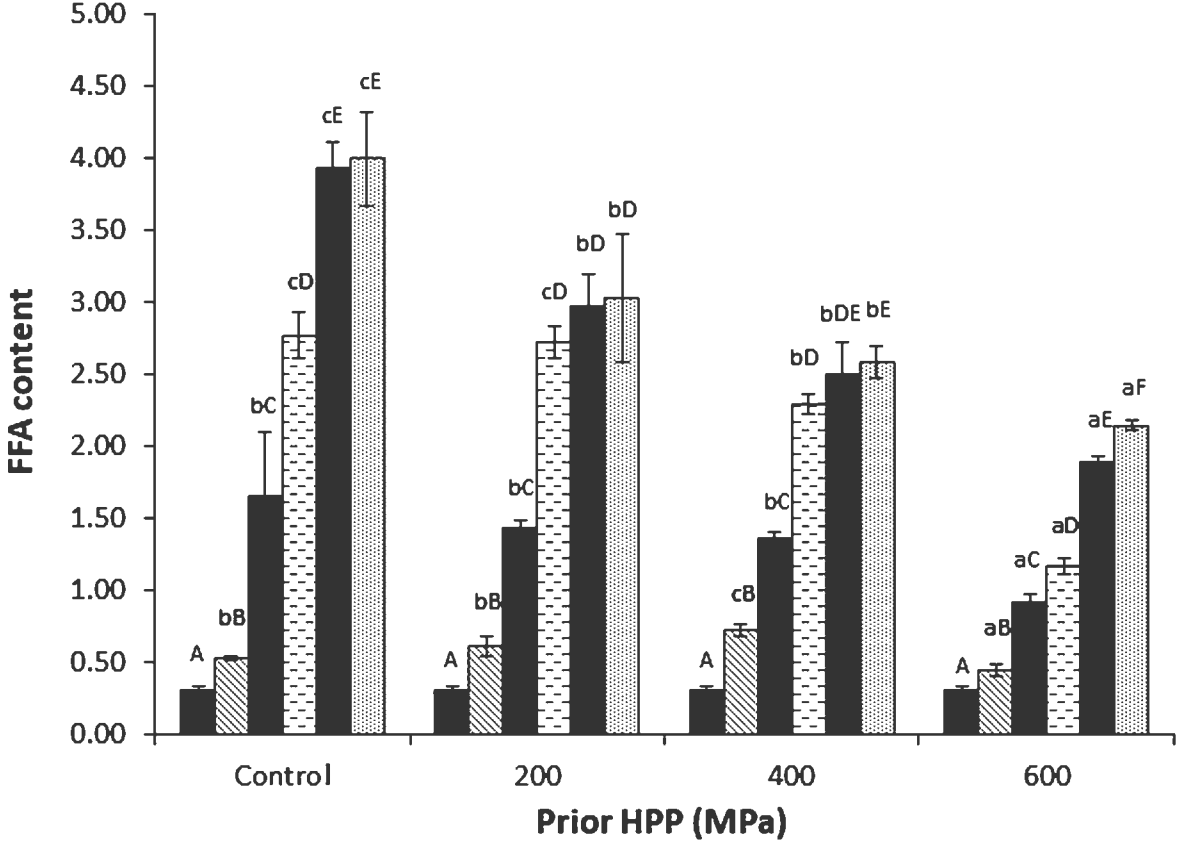
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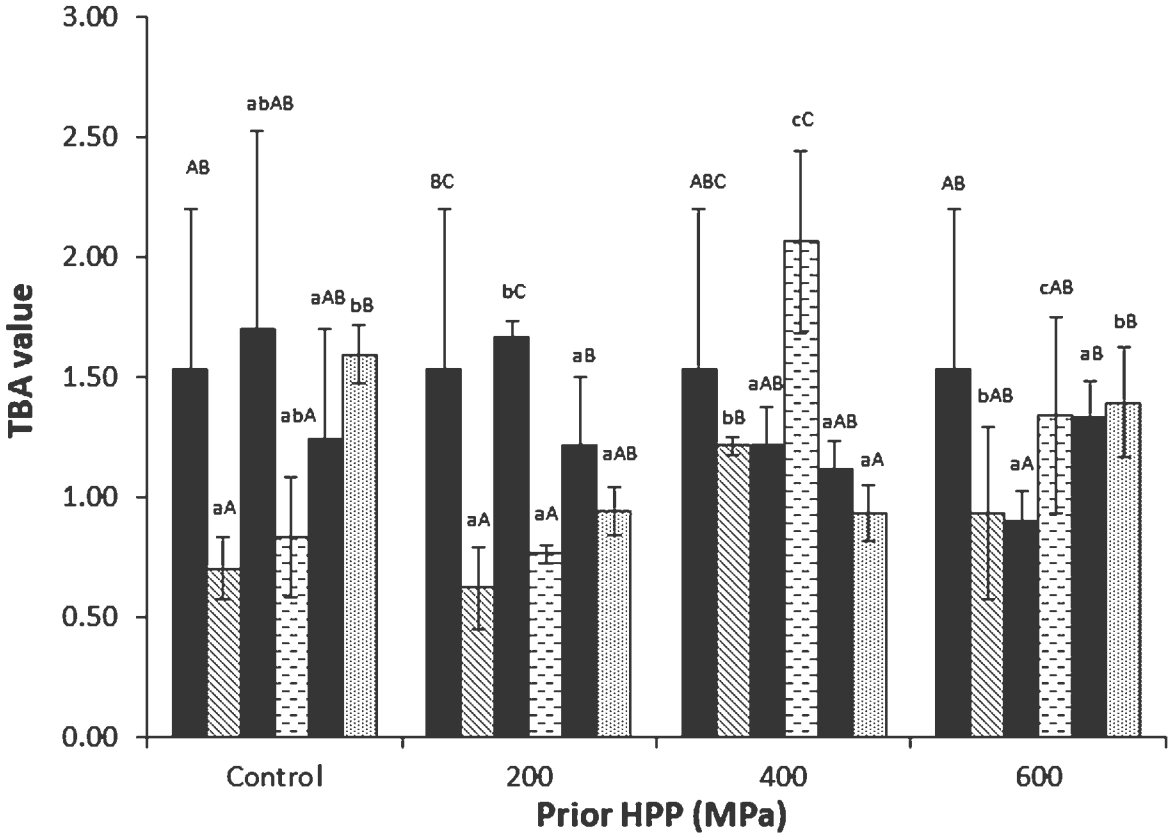
Prior HPP (MPa)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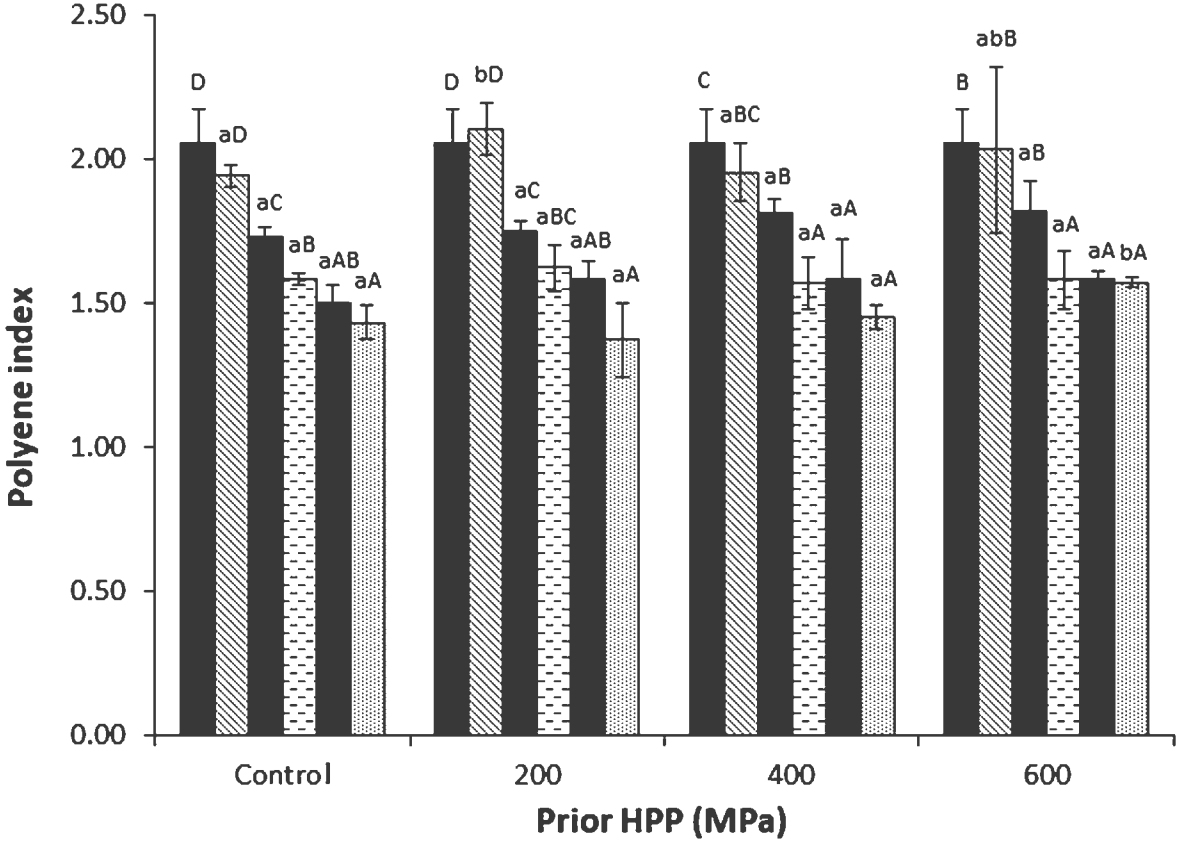
Prior HPP (MPa)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Prior HPP (MPa)

TABLE 1

Determination* of peroxide value and fluorescent compounds formation (fluorescence ratio) in canned mackerel previously subjected to different high-pressure processing (HPP) conditions followed by freezing (–30 ºC) and frozen storage (–18 ºC)**

Quality index	Prior HPP (MPa)	Initial raw fish	Prior frozen storage time (months)				
			0	**3**	**6**	**10**	**15**
Peroxide value (meq. active oxygen·kg^{-1} lipids)	Control	2.86 A (0.79)	1.93 aA (0.62)	2.42 aA (0.46)	2.09 abA (0.90)	2.04 aA (0.16)	2.01 aA (0.29)
	200	2.86 B (0.79)	1.50 aA (0.12)	2.31 aB (0.48)	1.46 aA (0.31)	2.56 bB (0.08)	2.62 bB (0.17)
	400	2.86 AB (0.79)	1.98 aA (0.47)	5.26 bC (0.93)	3.01 bB (0.37)	2.35 abAB (0.78)	2.78 bB (0.15)
	600	2.86 AB (0.79)	3.77 bBC (0.95)	5.09 bC (0.35)	3.56 bB (0.51)	1.91 aA (0.19)	4.92 cC (0.61)
Fluorescence ratio	Control	2.23 A (0.70)	4.42 aB (0.12)	4.63 aBC (0.21)	4.93 aCD (0.17)	4.96 aCD (0.31)	5.28 aD (0.36)
	200	2.23 A (0.70)	4.21 aB (0.27)	4.45 aB (0.22)	5.22 abBC (0.87)	5.26 aC (0.20)	5.66 aC (0.27)
	400	2.23 A (0.70)	4.32 aB (0.06)	5.67 bC (0.16)	6.21 bCD (0.51)	6.71 bD (0.43)	7.07 bD (0.31)
	600	2.23 A (0.70)	6.09 bB (0.24)	6.34 cBC (0.42)	6.41 cC (0.02)	7.21 bD (0.11)	7.90 bD (0.55)

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

** For each frozen storage time, different low-case letters (a-c) indicate significant differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters (A-D) indicate significant differences ($p < 0.05$) as a result of frozen storage time.

TABLE 2

Determination* of total volatile amines and trimethylamine in canned mackerel previously subjected to different high-pressure processing (HPP) conditions followed by freezing (–30 ºC) and frozen storage (–18 ºC)**

Quality index	Prior HPP (MPa)	Initial raw fish	Prior frozen storage time (months)				
			0	**3**	**6**	**10**	**15**
Total volatile base-N value ($mg \cdot kg^{-1}$ muscle)	Control	239.9 B (0.6)	251.3 bC (6.3)	183.9 aB (14.1)	151.8 aA (2.8)	218.3 abB (24.1)	188.7 aB (15.9)
	200	239.9 C (0.6)	269.1 bD (18.6)	200.9 aB (8.1)	172.2 cdA (12.5)	204.3 aB (8.7)	191.7 aAB (20.3)
	400	239.9 B (0.6)	248.1 abB (7.9)	204.3 aA (16.1)	193.7 dA (9.3)	235.9 bB (5.3)	198.4 aA (16.8)
	600	239.9 C (0.6)	212.5 aB (19.8)	209.3 aB (4.0)	167.9 bcA (3.6)	232.0 bBC (18.1)	218.0 aB (10.9)
Trimethylamine -N value ($mg \cdot kg^{-1}$ muscle)	Control	8.8 A (1.2)	33.9 bB (0.7)	39.4 aBC (8.2)	41.1 aC (7.8)	50.9 aCD (5.4)	59.4 aD (9.8)
	200	8.8 A (1.2)	33.1 bB (1.9)	35.7 aBC (5.2)	43.0 aC (5.7)	51.6 aC (6.2)	55.0 aC (9.8)
	400	8.8 A (1.2)	26.9 aB (2.1)	42.7 aC (2.9)	56.9 bD (4.2)	56.2 aD (2.8)	58.5 aD (4.7)
	600	8.8 A (1.2)	25.0 aB (4.6)	45.9 aC (5.9)	55.4 bCD (4.8)	56.6 aCD (13.2)	68.6 aD (11.4)

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

** For each frozen storage time, different low-case letters (a-d) indicate significant differences ($p < 0.05$) as a result of HPP. For each HPP condition, capital letters (A-D) indicate significant differences ($p < 0.05$) as a result of frozen storage time.