Effect of high-pressure processing and frozen storage prior to canning on the content of essential and toxic elements in mackerel

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ABSTRACT

The mineral content of canned (115 ºC, 45 min; F_o = 7 min) Atlantic Chub mackerel (*Scomber colias*) previously subjected to different high-pressure processing (HPP) (200, 400, and 600 MPa for 2 min) conditions and frozen storage times (3, 10, and 15 months at –18 ºC) was studied. Prior processing steps modified extensively the contents of essential and toxic elements, so that substantial changes were produced in canned fish. Thus, canned mackerel showed higher levels of most essential (*Na, Ca, Fe, Co, Cu, Se*) and toxic (*Sn, As*) elements when compared with initial raw fish; contrary, some essential (*K, Mg, P*) and toxic (*Pb*) elements revealed lower values in canned samples. HPP led to increased levels of essential (*S, Se*) and toxic (*Cd*) elements; the opposite effect was produced on *Ca* and *Mn* (essentials) and *Ba* (toxic) elements. Scarce effects of frozen storage time could be concluded; remarkably, storage time increase led to increased *Ca* and *Mn* levels, while produced decreases of *K, Cd* and *Pb* contents. Changes in essential and toxic element contents are explained on the basis of protein denaturation, protein and lipid breakdown, water and liquor losses from the fish muscle, and muscle interaction with brine-packaging medium.

Keywords: Canned mackerel; high-pressure processing; frozen storage time; essential elements; toxic elements; physico-chemical modifications

Running title: Essential and toxic elements in pre-processed canned mackerel

INTRODUCTION

An increased attention is being accorded to seafood for their contribution to human requirement for minerals (Martínez-Valverde et al. 2000). Thus, the majority of the macroelements and trace elements considered essential for biological processes, including growth, reproduction, hormone metabolism and antioxidant defence, can be found in marine species (Fraga 2005; Oehlenschläger 2010). On the other side, seafood contribute to human exposure to toxic trace elements as a result of contamination of the food chain by human activities, including agricultural, industrial, and municipal wastes (Sioen et al. 2008; Noël et al. 2011). Consequently, both the scientific community and consumers have shown a growing interest in the potential risks of seafood consumption (Squadrone et al. 2016).

Canning is one of the most important means of fish and invertebrate species preservation by providing excellent nutritional products and protecting them from food-spoiling microorganisms (Horner 1997). However, marine species constituents have revealed to be highly sensitive to heat processing (Aubourg 2001; Mújica-Paz et al. 2011). Among the most important events, heat degradation, oxidation of constituents, leaching of water-soluble constituents and toughening and drying of fish muscle can be mentioned. Concerning the mineral content of fish muscle, the most important event would be protein denaturation, minerals release from the muscle, and resulting liquor losses including essential and toxic elements from the fish muscle into the packaging medium (Gokoglu et al. 2004; Mierke-Klemeyer et al. 2008). As a result, previous research has shown the loss of essential (Seet and Brown, 1983; Castrillón et al., 1996) and toxic (Ganjavi et al., 2010) elements from the muscle into the packaging medium.

According to canneries needs for raw material availability, frozen storage has been used abundantly as a prior preservative storage condition to canning process. However, fish deterioration is susceptible to continue during frozen storage (i.e., protein denaturation, hydrolysis and breakdown; lipid oxidation and hydrolysis) as a result of endogenous

77 enzyme activity, especially if long-term storage periods are encountered and if convenient
78 storage temperatures are not respected (Sista et al. 1997; Sokorski and Kolakowski 2000).
79 Consequently, different complementary technologies to frozen storage have been found
80 necessary to be applied to maintain the high-quality degree of raw material for the canning
81 process. Among such complementary technologies, high-pressure processing (HPP) has
82 proved its effectivity by inhibiting the most important damage mechanisms related to
83 endogenous enzyme activity in frozen fatty and lean fish species (Fidalgo et al. 2015;
84 Méndez et al. 2017; Vázquez et al. 2018). However, both frozen storage and HPP have
85 revealed an increasing effect on protein denaturation (Mackie 1993; Tabilo-Munizaga et al.
86 2016). Thus, the resulting loss of water holding capacity of denatured proteins can be
87 especially important for mineral presence, since liquor produced can lead to important
88 losses in mineral content in the muscle. Notably, no information concerning changes in
89 mineral content of fish muscle related to HPP and frozen storage is available.
90 Therefore, the basic objective of the current study was to analyse the effect that both
91 previous treatments (HPP and frozen storage) may have on the mineral content of canned
92 fish. Thus, the study focused on a fatty fish species (Atlantic Chub mackerel, *Scomber*
93 *colias*) subjected to different high-pressure processing (HPP; 200, 400, and 600 MPa for 2
94 min) and frozen storage times (3, 10, and 15 months at –18 ºC) before the canning process.
95 The content in canned fish of eleven essential macroelements and trace elements and five
96 toxic elements was studied. The working hypothesis was that mineral content (essential and
97 toxic elements) in canned fish can be influenced by previous processing (HPP and frozen
98 storage).
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100

MATERIALS AND METHODS

Initial raw fish, HPP, freezing and frozen storage

Mackerel specimens (78 individuals; length and weight ranges: 24.0-27.0 cm and 150-170 g, respectively) were obtained at Vigo harbour (North-Western Spain) and transported on ice to the laboratory. Then, 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 (12 bags; six individuals per bag), vacuum-sealed at 150 mbar (Vacuum Packaging Machine Culinary, Albipack, Águeda, Portugal) and divided into four batches (3 bags in each batch). Bags corresponding to one of such batches were directly stored at –30 ºC for 48 h (freezing treatment) and considered as control fish (CT batch). Bags corresponding to the other three batches were subjected to HPP (200, 400, and 600 MPa for 2 min, respectively) in a 55-L high pressure unit (WAVE 6000/55 HT; NC Hiperbaric, Burgos, Spain). For it, water was applied as pressurising medium at 3 $MPa \cdot s^{-1}$ yielding 67, 133, and 200 s as the come up times, respectively, decompression time being less than 3 s. After HPP, all bags were stored at –30 ºC for 48 h (freezing treatment).

Once the freezing step was accomplished in all batches, bags (3 bags per batch) were kept at –18 ºC for 3, 10, and 15 months, respectively. At each frozen storage time, one bag of each batch was thawed overnight at 4 ºC and then employed for the canning process.

126 **Canning and sampling procedure**

127 Thawed fish were beheaded, eviscerated and filleted. Then, 45-g portions of
128 mackerel fillets (from one fish individual) were placed in small flat rectangular cans
129 (105 × 60 × 25 mm; 150 mL), being filled with brine solution (2 % w/v). All cans were
130 vacuum-sealed and subjected to heat sterilisation treatment (115 ºC, 45 min; F_o = 7 min)
131 in a steam retort (CIFP Coroso, Ribeira, A Coruña, Spain). Once the heating time was
132 completed, steam was cut off, air was used to flush away the remaining steam, and cans
133 were cooled at reduced pressure.

134 Canned fish was stored for three months at room temperature (20 ºC). At this
135 time, the cans were opened, and the liquid part was carefully drained off gravimetrically
136 and filtered through a filter paper. Mackerel white muscle was separated, wrapped in
137 filter paper and used for analysis. For each batch, the fish white muscle corresponding
138 to two cans was pooled together to carry out the chemical analyses. Each batch was
139 analysed in triplicate (n=3).

140

141 **Moisture and lipid determination**

142 Moisture content was determined as the weight difference in homogenised
143 muscle (1-2 g) before and after 4 h at 105 ºC (AOAC 1990). Results were calculated as
144 $g \cdot kg^{-1}$ muscle.

145 Lipids from the mackerel white muscle were extracted following the Bligh and
146 Dyer (1959) method. In it, a single-phase solubilisation of the lipids is employed by
147 means of a chloroform-methanol (1:1) mixture. Results were calculated as g $lipid \cdot kg^{-1}$
148 canned mackerel muscle.

149

150

151 **Mineral analysis**

152 Content of essential macroelements (*Na, K, Mg, Ca, P,* and *S*), essential trace
153 elements (*Mn, Fe, Co, Cu,* and *Se*), and toxic elements (*Ba, Cd, Sn, Pb,* and *As*) was
154 analysed according to the following procedure based on EPA 3050B (US-EPA 1996).
155 About 1 g of ground sample was put into a digestion flask with 9 mL of 69% nitric acid
156 (TMA) Hiperpur, 3 mL of H_2O_2 (for ultratrace analysis) and 3 mL of Milli-Q water.
157 Samples, plus four blanks and four samples of certified reference material, were
158 digested in a microwave oven (Mars-Xpress CEM Corp. Matthews, NC, USA). After
159 complete digestion, solutions were transferred to 50 mL flasks. Handling of samples
160 was carried out inside a clean ISO 5 laminar flow cabinet (Cruma 670 FL, Barcelona,
161 Spain). The sixteen aforementioned elements were analysed by ICP-MS by means of an
162 Agilent 7900 equipment using external calibration with element standards traceable to
163 NIST standards. Detection limits were calculated from blank standard deviations (LD =
164 3·SD blanks). Procedural blanks always accounted for <1% of element concentrations
165 in the samples. Accuracy of the analytical procedures was ensured using certified
166 reference material DORM-2, prepared by the National Research Council of Canada
167 (NRCC), as the quality control material (Table 1). Since NRCC does not certificate
168 macroelements and *Ba* contents, these values in DORM-2 were obtained from Engström
169 et al. (2004). Results were calculated as $g \cdot kg^{-1}$ dry muscle (macroelements) and as
170 $mg \cdot kg^{-1}$ dry muscle (trace elements).

171

172 **Statistical analysis**

173 Data obtained were subjected to the ANOVA method to explore differences
174 resulting from the effect of the prior HPP, frozen storage time, and canning.
175 Comparison of means was performed using the least-squares difference (LSD) method.

In all cases, analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA); differences among batches were considered significant for a confidence interval at the 95% level ($p<0.05$).

RESULTS

Essential macroelements

A definite effect of prior pressure treatment on *Na* content could not be concluded ($p>0.05$) (Table 2); however, the lowest average values were obtained in canned fish corresponding to the highest pressure level at all storage times considered. Additionally, prior frozen storage time did not provide a general tendency on *Na* level in canned fish; however, in most batches the lowest levels were detected after a 3-month storage period. In agreement with the coating medium employed, all canned fish showed higher ($p<0.05$) *Na* contents than the initial raw fish.

The *K* content in fish did not reveal ($p>0.05$) a definite effect of HPP (Table 2). However, previous frozen storage time led to a progressive *K* content decrease that was found significant ($p<0.05$) in all pressure-treated batches after a 15-month storage. A substantial decrease ($p<0.05$) of *K* content was observed in all canned fish by comparison with the initial raw fish.

Levels of *Mg* in canned fish were included in all cases in the 0.15-0.18 $g \cdot kg^{-1}$ muscle range (Table 2). No effect ($p>0.05$) of HPP or frozen storage time was detected. However, canning led to a marked effect so that all canned fish revealed lower levels ($p<0.05$) than the initial raw fish.

Decreasing *Ca* levels were detected in canned fish with prior pressure-level increase (Figure 1); thus, all pressure-treated fish showed lower levels than canned fish corresponding to the control batch when a 15-month storage is considered. Prior frozen

storage time did not provide a general behaviour in all batches; in the case of canned fish corresponding to control and 200-MPa batches, a significant increase (p<0.05) with storage time was obtained. All kinds of canned samples revealed higher (p<0.05) *Ca* levels than the initial raw fish.

Content on *P* element showed some decrease by increasing the prior HPP (Table 2). Thus, fish corresponding to 400- and 600-MPa treatments provided lower average values than their counterparts corresponding to control and 200-MPa conditions; however, differences were not found significant (p>0.05). Concerning the effect of the frozen storage time, a definite trend could not be implied (p>0.05). Initial raw fish showed a substantial higher value (p<0.05) than any canned sample.

A progressive increase of *S* content was observed in canned mackerel muscle by increasing the pressure value applied (Table 2); remarkably, a higher (p<0.05) *S* level was observed in fish corresponding to the 600-MPa batch after 3 and 10 months of frozen storage. Related to the storage time effect, the highest average values were observed in samples corresponding to the shortest storage time; however, a definite effect throughout the whole storage period could not be implied (p>0.05). Comparison of canned fish with raw fish did not provide differences for the *S* content, except for fish corresponding to the 600-MPa batch; in such case, increased values were reached at all frozen storage times considered.

Essential trace elements

Prior HPP revealed a marked effect on *Mn* value in canned mackerel (Figure 2). Thus, an increased pressure level led to a progressive decrease of the presence of this element; levels obtained in canned fish from 600 MPa-batch were lower (p<0.05) than their counterparts corresponding to control and 200-MPa batches at all prior storage

times. No effect (p>0.05) of frozen storage time was implied at any of the batches under study for the *Mn* content; however, an increased average value was observed with frozen storage time. A decrease (p<0.05) of *Mn* level was observed in canned fish corresponding to 400- and 600-MPa prior treatments when compared with the initial raw fish; contrary, control and 200-MPa batches did not provide differences (p>0.05) with the starting fish material.

Fe content in canned fish is depicted in Table 3. No effect (p>0.05) of prior HPP or frozen storage time could be implied on values of this transition metal. However, a substantial increase (p<0.05) in all kinds of canned samples was observed by comparison with the starting raw fish.

An increased pressure level led to an increased *Co* content (p<0.05) in canned fish corresponding to a 3-month storage (Table 3). However, if longer storage periods are considered, a definite trend could not be concluded. An increase of the frozen storage period led to different tendencies according to the batch taken into account, so that a definite effect of storage time on *Co* level could not be concluded. A substantial increase (p<0.05) was detected in all canned samples by comparison with the initial raw fish.

No effect (p>0.05) of HPP was observed in the *Cu* content in canned fish although some differences (p<0.05) could be detected (Table 3). Similarly, a definite effect was not also concluded for the prior storage time; however, the highest average values were observed in fish corresponding to a 3-month frozen storage. A lower average value was obtained in raw fish for *Cu* content when compared with any canned sample.

Prior HPP led to a progressive increase of *Se* value in canned fish by increasing the pressure level applied (Table 3); comparison of canned control fish with

counterparts from 400- and 600-MPa batches showed significant differences (p<0.05) at all prior storage times considered in the study. A definite trend of storage time on the content of this metalloid element in canned fish could not be concluded; however, the highest average values were observed in fish corresponding to the longest storage time, differences being found significant (p<0.05) in control and 200-MPa batches. A substantial increase in *Se* content was observed in any canned sample when compared with values corresponding to the initial raw fish.

Toxic elements

Decreasing *Ba* levels were detected in most canned mackerel samples with pressure value applied (Table 4); thus, canned fish corresponding to the 600-MPa batch showed lower levels (p<0.05) than control fish in samples corresponding to all frozen storage times. No effect (p>0.05) of prior frozen storage time was implied on the presence of this alkaline earth element. A substantial decrease (p<0.05) in canned fish corresponding to 400- and 600-MPa batches was obtained for this toxic metal content when compared with the initial raw fish value; however, no differences (p>0.05) were detected for the control batch.

A progressive increase of *Cd* content in canned muscle was detected with pressure level applied (Figure 3). At all storage times, fish corresponding to 400- and 600-MPa batches revealed higher values (p<0.05) than control canned fish. Frozen storage time did not provide a general behaviour in all batches; however, fish corresponding to the two highest pressure treatments showed a decreasing tendency (p<0.05) with storage time for this toxic transition metal. Comparison with the initial value proved a substantial increase (p<0.05) in all canned samples that were previously subjected to HPP; contrary, control canned samples did not show differences (p>0.05).

Although some differences were detected, a definite trend could not be concluded on the *Sn* level in canned mackerel as a result of prior HPP or frozen storage time (Table 4). However, a marked effect can be signalled for canning, since most canned samples revealed an increase ($p<0.05$) of this toxic metal content when compared with the initial raw fish value.

Prior HPP did not provide a definite effect ($p>0.05$) on *Pb* level in canned mackerel, although some differences among canned samples were detected (Table 4). Contrary, an increased storage time led to a substantial *Pb* content decrease in samples corresponding to all canned batches; thus, values for control and 600-MPa batches were found lower ($p<0.05$) at the longest storage period considered. Furthermore, initial raw fish showed a higher ($p<0.05$) content than any canned sample.

Concerning the *As* level in canned fish, some differences could be observed as a result of HPP or frozen storage time (Table 4); however, a definite trend could not be concluded for any of both prior treatments to canning. Most canned samples revealed higher average values than the initial raw fish, although differences were hardly found significant ($p>0.05$).

Moisture and lipid values

A definite effect of HPP on moisture content of canned fish ($p>0.05$) was not obtained (Table 5); however, the lowest average levels were detected in fish corresponding to the 600-MPa batch at all frozen storage times. Except for 400-MPa fish, all batches showed an increasing tendency for moisture value in canned fish with prior frozen storage time; this increase was found significant ($p<0.05$) in canned fish corresponding to the prior highest pressure applied. Canned fish did not reveal significant differences ($p>0.05$) of moisture value with the initial raw fish.

Lipid content of the initial raw mackerel was 63.1±2.5 ($g \cdot kg^{-1}$ wet muscle). A slight decrease of the average lipid content was detected in canned fish (range: 53.5–65.5 $g \cdot kg^{-1}$ muscle); however, no significant effect ($p>0.05$) of prior HPP or frozen storage time was detected on the content of this constituent.

DISCUSSION

Changes in moisture and lipid values

In the current study, two basic and opposite effects can influence the moisture content in canned mackerel muscle. One side, denaturation of muscle proteins during the different steps of processing (HPP, freezing, frozen storage time, and canning) would lead to a decrease of water-holding capacity, so that a substantial discard of water from the muscle should be produced (Sikorski and Kolakowski 2000; Tabilo-Munizaga et al. 2016). On the other side, an important interaction between the canned muscle and the packaging medium is expected to occur during the canning process and subsequent canned storage (Castrillón et al. 1996; Aubourg and Medina 1997). As a result of this interaction and on the basis that a water-packaging medium was employed in the current study, fish muscle would be imbibed in the brine-packaging medium so that this effect would lead to a water content increase.

Current results have shown scarce differences of moisture level as a result of HPP, so that a balanced influence of both mentioned effects can be signalled in most cases. However, fish subjected previously to the highest pressure level showed a lower moisture content, this leading to the conclusion that the denaturation effect was more important than interaction with the packaging medium. Concerning the influence of prior frozen storage time, both effects showed to have a balanced significance; however, in the case of the 600-MPa batch, the water-gain effect resulting from the interaction with the packaging medium was found somewhat more important, so that increased values were detected with the storage period.

Concerning the lipid fraction, its relative content in muscle has shown to be influenced by variations of the moisture level, so that an inverse ratio between both constituents has been

328 described (Piclet 1987; Aubourg et al. 2007). However, current results have shown that lipid
329 content of canned fish was not affected by the different processing steps. Accordingly, previous
330 results have shown no variation of the lipid content in canned fish when a brine-packaging
331 medium was employed (Aubourg and Medina 1997). Contrary, a substantial increase was
332 produced by using an oil-packaging medium as a result of the interaction with the packaging
333 medium (Castrillón et al. 1996).
334
335 **Changes in mineral content in canned fish muscle**
336 Previous research has reported on abundant information on chemical changes related to
337 proteins and lipids during the different previous steps required for the canning process (Pérez-
338 Martín et al. 1988; Castrillón et al. 1996; Aubourg 2001). However, information concerning the
339 mineral composition changes related to this multi-step process can be considered scarce.
340 Current research has shown important changes in the content of essential and toxic elements in
341 canned fish as a result of the different processing steps.
342 Concerning the essential elements, HPP has shown to exert a positive effect on the
343 nutritional value by increasing the *S* and *Se* contents, while a negative effect could be implied
344 by decreasing the levels of *Ca* and *Mn*; both effects showed to increase with pressure level
345 employed. A definite effect of HPP on *Na, K, Mg, P, Fe, Co,* and *Cu* could not be proved.
346 Concerning the frozen storage, an increased nutritional value in canned fish was obtained on the
347 basis of the increase on *Ca* and *Mn* presence; contrary, a decrease on *K* level was observed.
348 Such changes on minerals content increased with frozen storage time. Frozen storage time did
349 not lead to a definite trend for most essential elements (*Na, Mg, P, S, Fe, Co, Cu,* and *Se*).
350 Finally, comparison of raw fish with control canned fish previously subjected to 3 months of
351 frozen storage showed a marked increase in essential elements such as *Ca, Na, Fe, Co, Cu,* and
352 *Se*; contrary, a nutritional value decrease was detected on the basis of a decrease in *K, Mg,* and
353 *P* levels. A definite effect on *Mn* and *S* could not be proved as a result of canning.
354 Related to the toxic elements presence, a negative effect on safety was produced by
355 HPP by means of increasing the *Cd* level; contrary, a decrease on *Ba* content was obtained.

356 Such effects increased with pressure level applied. Meantime, a definite effect on *Sn, Pb,* and *As*
357 content could not be proved for HPP. Concerning the frozen storage, none of the elements under
358 study provided a marked increase with frozen storage; notably, *Cd* and *Pb* levels showed to
359 decrease with frozen storage time. Finally, *Sn* and *As* presence in canned fish showed to
360 increase by comparison of raw fish with control canned fish subjected to 3 months of frozen
361 storage; meantime, *Pb* level decreased, while no effect was proved for *Cd* and *Ba* presence. In
362 spite of the fact that levels of toxic elements in muscle increased in certain cases, values
363 obtained for *Pb* and *Cd* are in all cases far below the accepted limits in EC regulations, 0.30 and
364 0.05 mg kg^{-1} fish muscle, respectively (EC 2014; EC 2015).

365 Modifications of essential and toxic elements presence have shown different trends
366 according to the processing step considered and the concrete element taken into account.
367 Several effects can be signalled to influence mineral content in canned fish. Thus, the resulting
368 trend (increase, decrease or no modification) observed for each element will be the result of
369 such effects.

370 One side, and according to the high susceptibility of marine constituents, each of the
371 different processing steps (HPP, freezing, frozen storage, thawing, sterilisation, and canned
372 storage) would lead to a partial damage of constituents consisting in breakdown and content
373 loss. This effect would be of special significance in proteins. Thus, protein denaturation
374 followed by breakdown or damage reactions have shown to be produced as a result of HPP
375 (Pazos et al. 2014; Carrera et al. 2018), freezing and frozen storage (Sista et al. 1997; Sikorski
376 and Kolakowski 2000), and canning (Pérez-Martín et al. 1988; García-Arias et al. 1994; Mújica-
377 Paz et al. 2011). It has been reported that denatured proteins become more reactive and can be
378 damaged easily by interacting with other constituents, especially if a strong processing such as
379 sterilisation is concerned. Related to the lipid fraction, a pro-oxidant effect of HPP (Tabilo-
380 Munizaga et al 2016), frozen storage (Kolakowska 2003) and heat treatment (Aubourg 2001)
381 has been signalled. Release of prooxidant elements such as non-heme *Fe* from heme-*Fe*
382 complexes as a result of protein denaturation may have important consequences in rancidity
383 stability of fish muscle (Buchowski et al. 1988; Turhan et al. 2004). Thus, a marked lipid

384 oxidation development may occur, this provoking lipid breakdown and production of low-
385 molecular-weight compounds susceptible to be lost from the muscle into the surrounding
386 medium, especially if a hydrophilic packaging is concerned (Aubourg and Medina 1997). As a
387 result of protein and lipid damage and breakdown, losses on such constituents would lead to
388 relative content increases of other constituents in canned fish muscle such as essential and toxic
389 elements.

390 On the other side, modifications of the different main constituents would lead to
391 breakdown of binding of minerals to other constituents. Among the different constituent
392 modifications, protein denaturation can be of special significance for mineral content in fish
393 muscle. Furthermore, liquor losses resulting from the different processing steps (i.e., thawing,
394 sterilisation and canned storage) would lead to a partial loss of minerals from the fish muscle
395 into the surrounding aqueous medium (thawing or packaging medium). The exception for this
396 would be the *Na* content; thus, since an aqueous 2% NaCl-packaging medium was used in the
397 current study, the fact that fish muscle may be imbibed in it would facilitate the observed value
398 increase in *Na* content in canned muscle.

399 An additional aspect to be taken into account would be the kind of binding of minerals
400 to other constituents of fish muscle, as well as the more or less hydrophilic/lipophilic behaviour
401 of molecules they are integrated in (Piclet 1987; Gordon 1988). Thus, hydrophilic molecules
402 will be likely to be lost during processing steps such as thawing, sterilisation and canned
403 storage. Notably, interaction of mineral elements with other fish constituents has shown a great
404 dependence on their chemical characteristics (Piclet 1987; Gordon 1988). Thus, alkali (*Na* and
405 *K*) and alkali earth (*Mg* and *Ca*) elements have shown to be present in the cellular medium as
406 chlorides, sulphates or organic salts (citrates, lactates, or pyruvates). Contrary, transition metals
407 (*Fe, Cu*, etc.) and non-positive elements (*S, P*, etc.) have shown to be strongly bound to other
408 muscle constituents and give rise to a wide number of functional molecules. Furthermore,
409 higher fat contents in the fish flesh showed to produce lower losses of minerals, this indicating a
410 kind of interaction between both kinds of constituents (Gall et al. 1983; Aubourg et al. 2007).

411 No previous studies addressing the effect of HPP on mineral content in seafood have
412 been reported. Concerning the effect of freezing and frozen storage, previous research can be
413 considered scarce. Thus, Karl et al. (2005) proved a marked reduction of *I* content in different
414 kinds of fish after deep-freezing and thawing. Furthermore, Pourashouri et al. (2009) proved an
415 increase of the non-heme *Fe* content due to release of *Fe* from heme-*Fe* complexes during the
416 frozen storage of Wels catfish. Notably, previous research related to minerals content in canned
417 seafood is more abundant. Thus, loss of minerals (*Na, K, Mg, Ca, P, Cu,* and *Fe*) from the
418 muscle into the dipping medium was proved by Seet and Brown (1983) in water-packaged
419 canned tuna (*Thunnus alalunga*). Later on, Castrillón et al. (1996) showed a decrease value in
420 some elements (*P*, *Mg,* and *K*), although others (*Zn, Cu, Fe, Na,* and *Ca*) did not modify their
421 contents as a result of albacore (*Thunnus alalunga*) steaming. Concerning toxic elements,
422 defrosting, cooking, and sterilisation reduced the contents of *Pb* and *Cd* considerably in
423 oil-canned yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*)
424 from the Persian Gulf and Oman Sea (Ganjavi et al. 2010).
425
426 ## CONCLUSIONS
427 Preliminary processing steps modified extensively the contents of essential and
428 toxic elements in canned mackerel, this effect leading to substantial changes in the
429 nutritive value of the final product, as well as in the potential risks of consumption.
430 Changes in the different essential and toxic elements content can be explained on the
431 basis of different effects such as fish protein denaturation, lipid and protein breakdown,
432 water and liquor losses from the muscle, interaction of the fish muscle with the brine-
433 packaging medium, the kind of binding of minerals to other constituents of the fish
434 muscle and the more or less hydrophilic/lipophilic behaviour of molecules elements are
435 integrated in.

436 Previous research concerning the effect of HPP and frozen storage, alone or
437 combined, has been focused on changes in the most abundant constituents (i.e. proteins
438 and lipids). Consequently, information related to minor constituents such as minerals
439 can be considered very scarce. The current study provides new information to fill this
440 knowledge gap by addressing the effect of prior HPP conditions and frozen storage
441 times on the mineral content of canned mackerel. On the basis of the need for the frozen
442 storage of raw material to be canned and the advantages demonstrated for previous HPP
443 on frozen fish quality, further research is found necessary to optimise the levels found in
444 essential and toxic elements in the resulting canned product. This optimisation ought to
445 take into account the marine species encountered, the HPP (pressure level and pressure
446 holding time), frozen storage (time and temperature) and canning (packaging medium,
447 *Fo* value) conditions. Furthermore, a greater knowledge would be necessary concerning
448 the kind of binding of the different minerals to other constituents and the hydrophilic or
449 lipophilic characteristics of molecules elements are integrated in.
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DECLARATIONS

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585
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587
588

589 **FIGURE LEGENDS**

590

591 **Figure 1**: Content on *Ca* ($g \cdot kg^{-1}$ dry muscle)* in raw and canned mackerel previously subjected to
592 different high-pressure processing (HPP) (CT, Control; 200, 400, and 600 MPa) conditions and frozen
593 storage times**

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

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

598

599 **Figure 2**: Content on *Mn* ($mg \cdot kg^{-1}$ dry muscle)* in raw and canned mackerel previously subjected to
600 different high-pressure processing (HPP) (CT, Control; 200, 400, and 600 MPa) conditions and frozen
601 storage times**

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

603 ** For each frozen storage time, different low-case letters (a-c) indicate significant differences (p<0.05)
604 as a result of HPP. No significant differences (p>0.05) could be observed as a result of frozen
605 storage time.

606

607 **Figure 3**: Content on *Cd* ($mg \cdot kg^{-1}$ dry muscle)* in raw and canned mackerel previously subjected to
608 different high-pressure processing (HPP) (CT, Control; 200, 400, and 600 MPa) conditions and frozen
609 storage times**

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

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

TABLE 1

Accuracy control of the analytical procedures for the determination of macroelements and trace elements*

Elements	Certified	Measured	Unit
Macroelements			
Na	5.06 ± 0.07	5.72 ± 0.17	$g \cdot kg^{-1}$
K	18.9 ± 1.1	17.0 ± 0.5	"
Mg	1.05 ± 0.05	1.15 ± 0.05	"
Ca	0.62 ± 0.05	0.62 ± 0.09	"
P	9.9 ± 0.1	10.6 ± 0.5	"
S	8.9 ± 0.5	8.5 ± 0.2	"
Trace elements			
Mn	3.66 ± 0.34	3.02 ± 0.29	$mg \cdot kg^{-1}$
Fe	142 ± 10	105 ± 15	"
Co	0.182 ± 0.031	0.16 ± 0.02	"
Cu	2.34 ± 0.16	1.92 ± 0.23	"
Se	1.40 ± 0.09	1.41 ± 0.12	"
Ba	2.34 ± 0.03	2.4 ± 0.3	"
Cd	0.043 ± 0.008	0.038 ± 0.002	"
Sn	0.023 ± 0.001	0.026 ± 0.009	"
Pb	0.065 ± 0.007	0.047 ± 0.007	"
As	18.0 ± 1.1	17.3 ± 1.8	"

* Data expressed as average values ± standard deviation (n=4). DORM-2 from NRCC was the certified reference material employed, except for macroelements and *Ba* values that were referenced according to Engström et al. (2004).

TABLE 2

Content (g·kg^{-1} dry muscle)* on essential macroelements in canned mackerel previously subjected to different high-pressure processing (HPP) conditions and frozen storage times**

Element	Frozen storage time (months)	HPP (MPa)			
		CT	200	400	600
Na	Raw fish	2.29 (0.05)			
	3	4.97 abA (0.10)	5.23 cA (0.06)	5.10 bcA (0.12)	4.70 aA (0.20)
	10	5.31 aB (0.13)	5.25 aA (0.05)	5.40 aB (0.09)	5.20 aB (0.24)
	15	5.47 bB (0.06)	5.15 abA (0.30)	5.22 abAB (0.40)	4.95 aAB (0.14)
K	Raw fish	3.64 (0.21)			
	3	1.31 aA (0.07)	1.42 aB (0.07)	1.36 aB (0.09)	1.33 aB (0.06)
	10	1.25 aA (0.12)	1.19 aA (0.09)	1.26 aAB (0.06)	1.24 aAB (0.10)
	15	1.17 aA (0.23)	1.18 aA (0.13)	1.17 aA (0.07)	1.10 aA (0.03)
Mg	Raw fish	0.50 (0.03)			
	3	0.16 aA (0.01)	0.18 aB (0.01)	0.17 aA (0.01)	0.16 aA (0.01)
	10	0.15 aA (0.01)	0.14 aA (0.01)	0.15 aA (0.01)	0.16 aA (0.01)
	15	0.16 aA (0.02)	0.15 aA (0.01)	0.16 aA (0.01)	0.15 aA (0.01)
P	Raw fish	2.63 (0.04)			
	3	1.51 aA (0.02)	1.52 aA (0.08)	1.37 aA (0.11)	1.46 aA (0.02)
	10	1.39 aA (0.11)	1.42 aA (0.14)	1.33 aA (0.04)	1.35 aA (0.02)
	15	1.59 aA (0.11)	1.64 aA (0.21)	1.36 aA (0.11)	1.42 aA (0.06)
S	Raw fish	2.70 (0.09)			
	3	2.81 aA (0.19)	2.83 aB (0.04)	2.85 aA (0.10)	3.27 bB (0.13)
	10	2.55 aA (0.12)	2.61 aA (0.06)	2.73 aA (0.13)	3.05 bAB (0.11)
	15	2.70 aA (0.27)	2.74 aAB (0.10)	2.75 aA (0.07)	2.94 aA (0.10)

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

** 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-B) indicate significant differences (p<0.05) as a result of frozen storage time.

TABLE 3

Content (mg·kg^{-1} dry muscle)* on essential trace elements in canned mackerel previously subjected to different high-pressure processing (HPP) conditions and frozen storage times**

Element	Frozen storage time (months)	HPP (MPa)			
		CT	200	400	600
Fe	Raw fish	3.41 (0.02)			
	3	5.86 aA (0.54)	6.91 aA (0.96)	6.69 aA (0.44)	6.23 aA (0.10)
	10	6.84 aA (0.80)	5.92 aA (0.81)	6.00 aA (0.15)	5.61 aA (0.77)
	15	6.39 aA (0.82)	6.33 aA (0.86)	6.14 aA (0.44)	6.19 aA (0.23)
Co	Raw fish	0.0019 (0.0004)			
	3	0.0053 aA (0.0003)	0.0063 bB (0.0001)	0.0062 bB (0.0003)	0.0080 cB (0.0001)
	10	0.0047 aA (0.0010)	0.0038 aA (0.0004)	0.0044 aA (0.0003)	0.0042 aA (0.0001)
	15	0.0064 bB (0.0002)	0.0044 aA (0.0009)	0.0063 abAB (0.0019)	0.0041 aA (0.0007)
Cu	Raw fish	0.35 (0.04)			
	3	0.75 aB (0.10)	0.61 aAB (0.14)	0.68 aB (0.06)	0.56 aA (0.09)
	10	0.44 aA (0.09)	0.40 aA (0.12)	0.41 aA (0.04)	0.46 aA (0.12)
	15	0.46 aA (0.02)	0.57 bB (0.01)	0.56 abAB (0.15)	0.52 abA (0.03)
Se	Raw fish	0.44 (0.03)			
	3	0.63 aA (0.01)	0.69 bA (0.03)	0.79 cA (0.03)	0.92 dB (0.04)
	10	0.61 aA (0.02)	0.65 abA (0.03)	0.71 bcA (0.04)	0.78 cA (0.05)
	15	0.75 aB (0.04)	0.78 abA (0.11)	0.93 bB (0.03)	0.94 bB (0.04)

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

** 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-B) indicate significant differences (p<0.05) as a result of frozen storage time.

TABLE 4

Content (mg·kg^{-1} dry muscle)* on toxic elements in canned mackerel previously subjected to different high-pressure processing (HPP) conditions and frozen storage times**

Element	Frozen storage time (months)	HPP (MPa)			
		CT	200	400	600
Ba	Raw fish	0.053 (0.002)			
	3	0.059 bAB (0.007)	0.053 abAB (0.019)	0.036 aA (0.001)	0.039 aB (0.007)
	10	0.046 bA (0.005)	0.045 bA (0.002)	0.038 abA (0.011)	0.018 aA (0.008)
	15	0.070 cB (0.013)	0.056 bcB (0.003)	0.034 aA (0.006)	0.047 abB (0.007)
Sn	Raw fish	0.0013 (0.0007)			
	3	0.0035 aA (0.0009)	0.0041 aA (0.0007)	0.0040 aA (0.0006)	0.0029 aA (0.0005)
	10	0.0040 abA (0.0006)	0.0037 aA (0.0004)	0.0047 bA (0.0003)	0.0041 abB (0.0003)
	15	0.0040 aA (0.0002)	0.0033 aA (0.0008)	0.0040 aA (0.0006)	0.0039 aAB (0.0006)
Pb	Raw fish	0.0129 (0.0038)			
	3	0.0060 bB (0.0012)	0.0051 abB (0.0005)	0.0062 abB (0.0017)	0.0042 aB (0.0003)
	10	0.0039 aA (0.0005)	0.0033 aAB (0.0018)	0.0058 aAB (0.0038)	0.0038 aB (0.0001)
	15	0.0037 cA (0.0001)	0.0017 aA (0.0004)	0.0024 bA (0.0001)	0.0032 cA (0.0003)
As	Raw fish	1.04 (0.04)			
	3	1.22 aA (0.02)	1.09 aA (0.11)	1.11 aAB (0.20)	1.23 aB (0.05)
	10	1.27 cA (0.04)	1.30 cA (0.13)	0.87 aA (0.05)	0.97 bA (0.01)
	15	1.18 aA (0.22)	1.18 aA (0.12)	1.41 aB (0.11)	1.22 aB (0.21)

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

** 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-B) indicate significant differences (p<0.05) as a result of frozen storage time.

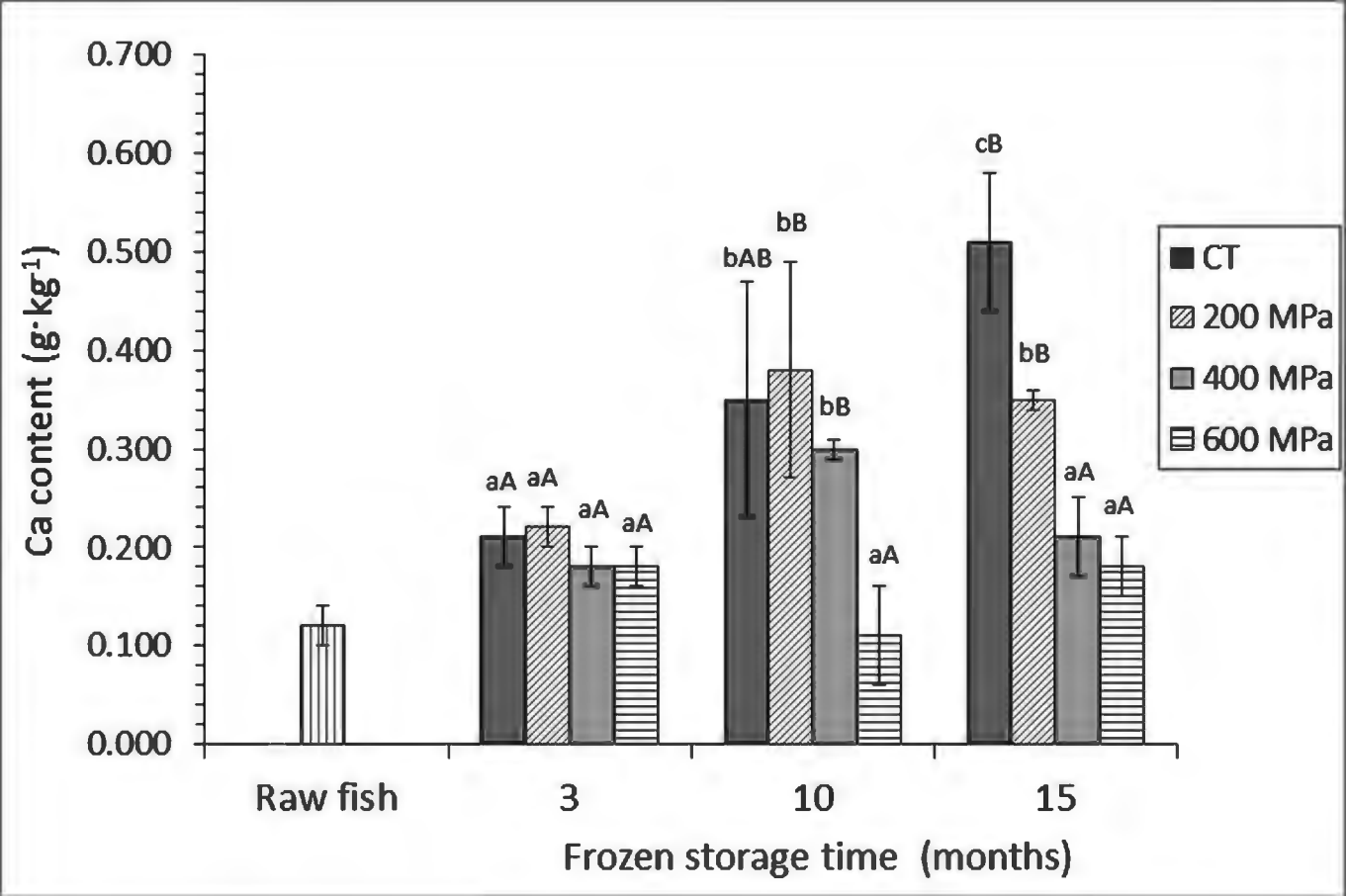
TABLE 5

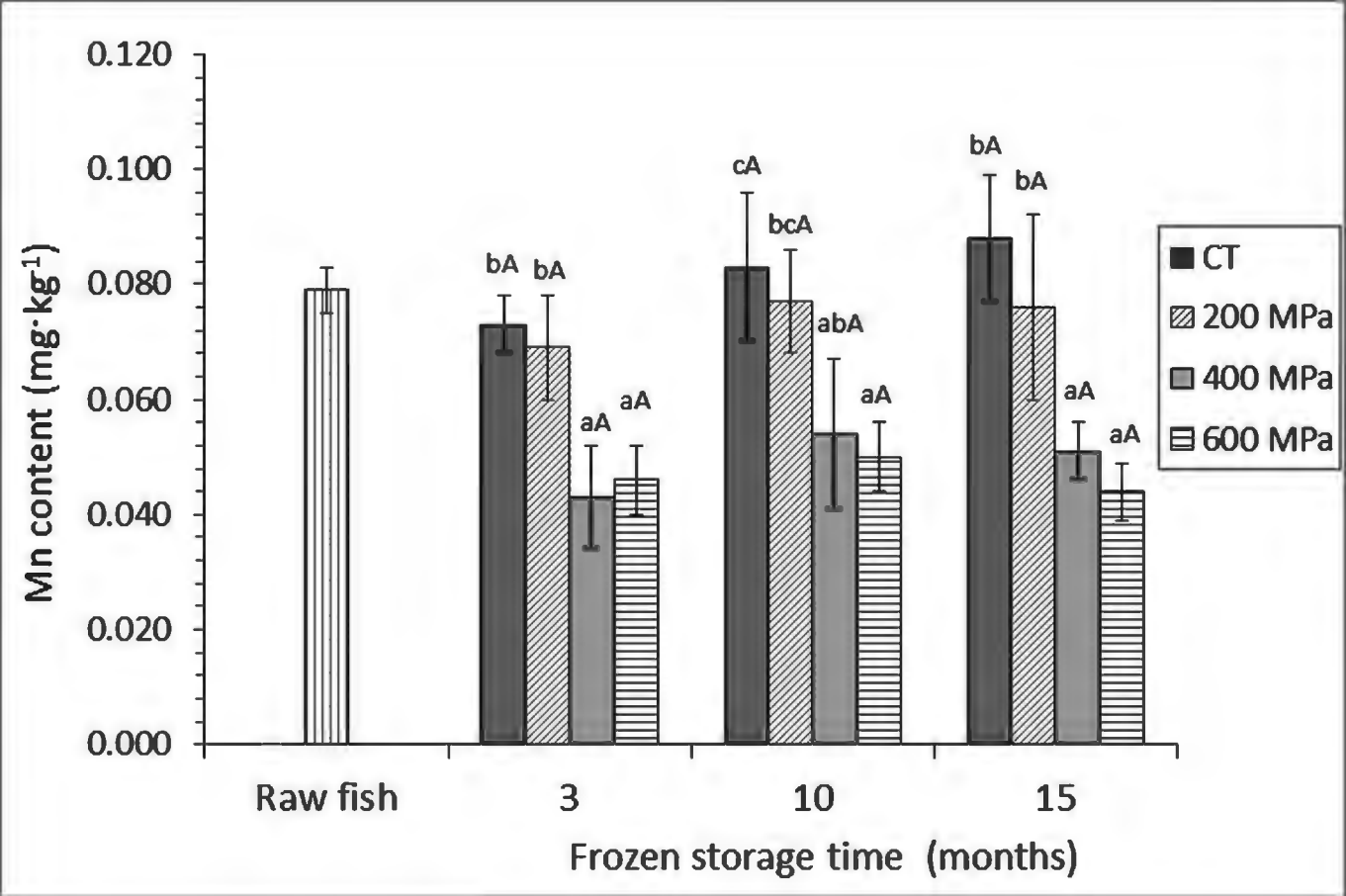
Moisture content (g·kg^{-1} muscle)* in canned mackerel previously subjected to different high-pressure processing (HPP) conditions and frozen storage times**

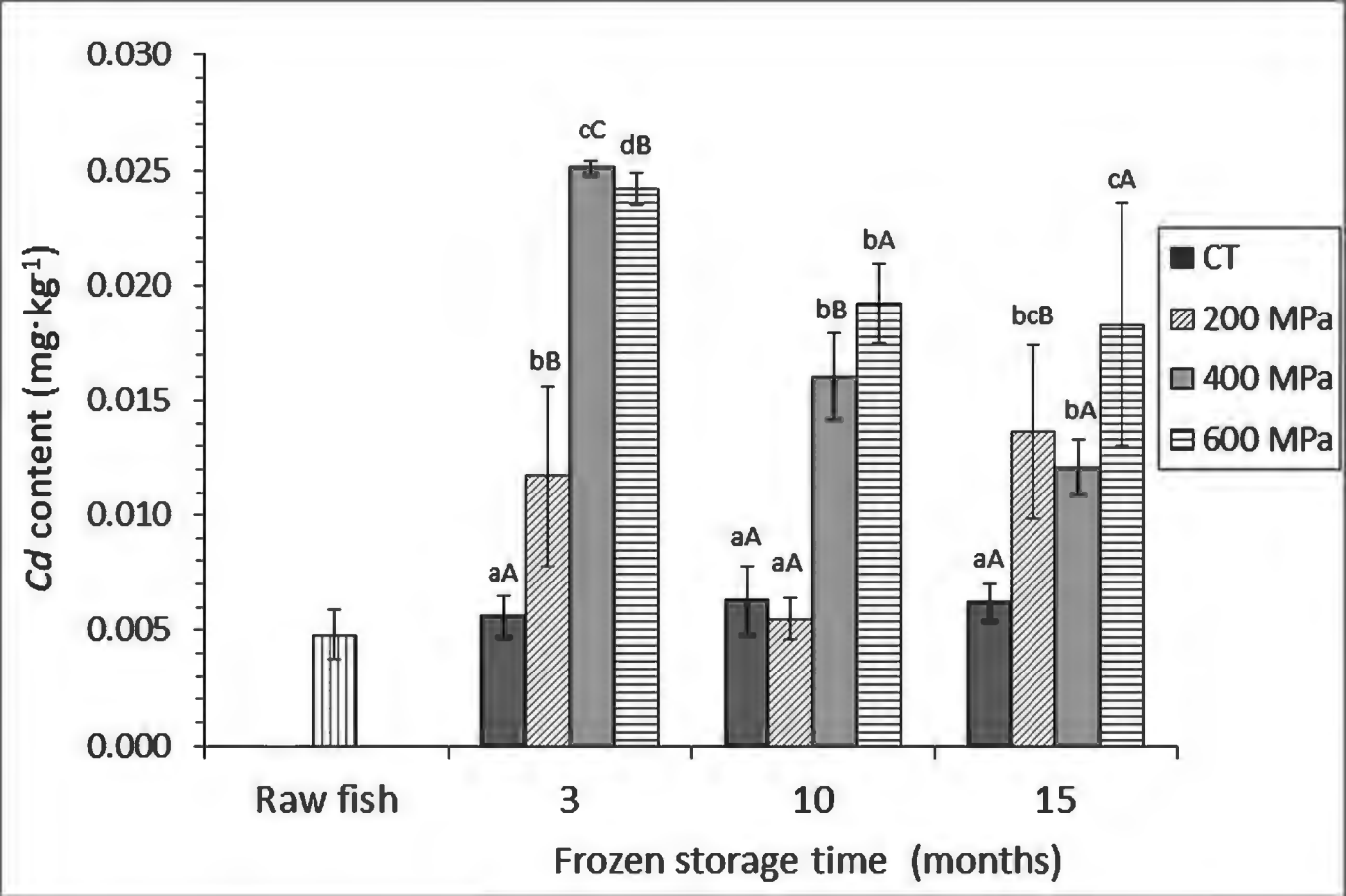
Frozen storage time (months)	HPP (MPa)			
	CT	200	400	600
Raw fish	719.4 (18.8)			
3	688.4 bA (10.2)	696.4 bA (7.3)	708.2 bAB (12.3)	655.5 aA (3.4)
10	697.7 abA (25.0)	697.1 abA (20.7)	717.2 bB (6.3)	686.4 aB (7.5)
15	699.5 abA (22.3)	719.2 abA (18.5)	706.8 bA (2.7)	694.1 aB (6.4)

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

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


0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Ca content (g·kg-1)
aA
aA
aA
aA
bAB
bB
bB
aA
cB
bB
aA
aA
CT
200 MPa
400 MPa
600 MPa
Raw fish
3
10
15
Frozen storage time (months)


0.120
0.100
0.080
0.060
0.040
0.020
0.000
Mn content (mg·kg-1)
bA
bA
aA
aA
cA
bcA
abA
aA
bA
bA
aA
aA
CT
200 MPa
400 MPa
600 MPa
Raw fish
3
10
15
Frozen storage time (months)


0.030
0.025
0.020
0.015
0.010
0.005
0.000
Cd content (mg·kg-1)
aA
bB
cC
dB
aA
aA
bB
bA
aA
bcB
bA
cA
CT
200 MPa
400 MPa
600 MPa
Raw fish
3
10
15
Frozen storage time (months)