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Effect of a previous high hydrostatic pressure treatment on lipid damage in chilled Chilean jack mackerel (*Trachurus murphyi*)

By **D. Maluenda**[1], **T. Roco**[1], **G. Tabilo-Munizaga**[2], **M. Pérez-Won**[1] and **S.P. Aubourg**[3, ✉]

[1] Department of Food Engineering. University of La Serena. La Serena (Chile)
[2] Department of Food Engineering. University of Bío-Bío. Chillán (Chile).
[3] Department of Food Technology. Marine Research Institute (CSIC). Vigo (Spain)
✉ Corresponding author: saubourg@iim.csic.es

RESUMEN

Efecto de un tratamiento previo por altas presiones hidrostáticas sobre la alteración lipídica de jurel chileno *(Trachurus murphyi)* refrigerado

Se estudió la evolución de la alteración lipídica en jurel chileno *(Trachurus murphyi)* refrigerado previamente tratado a altas presiones hidrostáticas (HHP). Se aplicaron distintos valores de presión y tiempo de presurización; asimismo, se analizó de forma comparativa la respuesta al proceso del pescado inicial en estados pre- y post-rigor mortis (RM). El tratamiento previo por HHP produjo inhibición de la hidrólisis lipídica en pescado refrigerado, siendo más intenso el efecto de la presión que el del tiempo de presurización. De acuerdo con el análisis de distintos índices de oxidación, no se concluyó un efecto determinante sobre la oxidación lipídica por parte del tratamiento previo de HHP. En relación al efecto del estado de RM del pescado inicial, se observó una oxidación primaria y secundaria mayor en jurel correspondiente a la condición post-RM durante la conservación en refrigeración; sin embargo, no se detectó un efecto claro sobre la hidrólisis lipídica.

PALABRAS CLAVE: Altas presiones – Hidrólisis lipídica – Oxidación lipídica – Refrigeración – Rigor mortis – Trachurus murphyi.

SUMMARY

Effect of a previous high hydrostatic pressure treatment on lipid damage in chilled Chilean jack mackerel *(Trachurus murphyi)*

Lipid damage evolution was analyzed in chilled Chilean jack mackerel *(Trachurus murphyi)* previously treated with high hydrostatic pressure (HHP) technology. Different pressure levels and pressure holding times were tested. In addition, fish corresponding to pre- and post-*rigor mortis* (RM) stages were comparatively studied. Previous HHP treatment led to a marked lipid hydrolysis inhibition in chilled fish. Increasing the pressure level and pressure holding time led to a lower free fatty acid content, with the effect of pressure being more relevant. According to the analysis of different types of lipid oxidation indexes, no effect of the previous HHP treatment on the lipid oxidation development could be determined in chilled jack mackerel. Concerning the effect of the RM stage of raw fish, a higher primary and secondary lipid oxidation development was observed in fish corresponding to the post-RM condition throughout the chilled storage; although a definite effect on lipid hydrolysis could not be found.

KEY-WORDS: Chilling – High pressure – Lipid hydrolysis – Lipid oxidation – Rigor mortis – Trachurus murphyi.

1. INTRODUCTION

Marine species are known to deteriorate rapidly postmortem due to the effects of a variety of degradation mechanisms (Whittle *et al*., 1990). The current increasing consumer demand for high quality fresh products has led to the development of advanced technologies. Among them, high hydrostatic pressure (HHP) technology has been reported to maintain sensory and nutritional properties, while inactivating microbial development and leading to a shelf-life extension and a safety improvement (Torres and Velázquez, 2005; Norton and Sun, 2008; Sánchez *et al*., 2012). This technology has shown a potential application in the seafood industry for the production of surimi and kamaboko (Montero *et al*., 1998), for cold-smoked fish preparation (Lakshmanan *et al*., 2007) and for aiding in freezing (Alizadeh *et al*., 2007), thawing (Rouillé *et al*., 2002) and thermal (Ramírez *et al*., 2009) processing. An additional positive effect of HHP treatment is that deteriorative hydrolytic (namely, lipases and phospholipases) and oxidative (peroxidases, lipoxygenases, etc.) endogenous enzymes can be inactivated for a further storage of the fish product (Ohshima *et al*., 1992). Thus, a profitable effect on quality retention has been observed when employed previously to a refrigerated (He *et al*., 2002; Erkan *et al*., 2010) or chilled (Hurtado *et al*., 2001; Ortea *et al*., 2010) storage.

Small pelagic fish species can constitute food products of great economic importance in many countries. Some of these fish species are captured in high proportions when their demand is relatively low, so that a large portion of their catches is underutilized and transformed into fish

meals for animals. One such abundant species is Chilean jack mackerel (*Trachurus murphyi)*. This underutilized fish is captured in large volumes in countries like Chile, China and Peru (FAO, 2007). Although a great interest has been accorded to its commercialization (Simpson *et al*., 2004), most efforts have been focused on its employment as a surimi-type product (Ortiz *et al*., 2004) and as a fish meal source (Bórquez and González, 1994). Studies on quality changes during refrigerated and chilled storage of this species appear to be scarce. Thus, microbiological activity during storage at room temperature or under refrigeration conditions has been studied (Schoebitz *et al*., 1985), and the inhibition of lipid damage and microbial activity during chilling storage was monitored by means of the application of essential oil (oregano and thyme) extracts (Quitral *et al*., 2009).

The quality characteristics of processed fish originating from pre- and post-*rigor mortis* (RM) raw material have been studied comparatively (Roth *et al*., 2006; Birkeland and Akse, 2010). However, the quality analysis has mainly been focused on sensory and physical properties, while lipid damage differences have been scarcely studied (Antipova and Smirnova, 1982; Duran *et al*., 2008). The present work concerns the lipid damage (hydrolysis and oxidation) development of Chilean jack mackerel during chilled storage. The study was aimed at investigating the effect of a previous HHP treatment, including different pressure levels and pressure holding times. In addition, the effect of the RM condition of the initial raw fish was analyzed.

2. MATERIALS AND METHODS

2.1. Raw fish, processing and sampling

Fresh Chilean jack mackerel fish (360 individuals; weight range: 0.8-1.0 kg; length range: 28-30 cm) were obtained at the Coquimbo (IV Región, Chile) harbor, sacrificed in a water-ice mixture and transported to our laboratory within one hour after catch. Before the RM process was initiated, one half of the total fish (180 individuals) was beheaded, gutted, filleted and placed in individual flexible polyethylene bags in order to be submitted to the HHP process. The other half of the fish (180 individuals) was maintained in ice (1:1, fish:ice ratio); once the RM process was finished (12 hours), individuals were processed the same way as their counterparts from the pre-RM condition to be further submitted to the HHP process.

The high-pressure equipment employed consisted of a cylindrical loading container provided with a 2 liter-pilot unit (Avure Technologies Incorporated, Kent, WA, USA). For both kinds of fish individuals (pre- and post-RM stages), different pressure levels (250, 450 and 550 MPa) and pressure holding times (3 and 4 minutes) were applied at room temperature (15 ± 2 °C). In all cases, water was employed as the pressurizing medium, working at a 17 MPa/s ramp rate; decompression time was less than 5s.

Once the HHP treatment was carried out, the fish were kept in ice (1:1, fish:ice ratio) in a temperate room (4 °C) and analyzed at days 0, 2, 6, 10 and 14 of chilled storage. The boxes used allowed for draining and the ice was renewed when needed. The fish without previous HHP treatment was also submitted to chilled storage and considered as the control. For each type of fish sample, three different batches or replicates (n = 3) were considered and analyzed independently throughout the chilled storage. Analyses were carried out on the lipid fraction extracted from the white muscle of the fish. For each sampling point, two individuals were considered, and their white muscle portions were pooled together.

The response to the HHP treatment of marine species has been reported to vary with species, chemical composition and size (Yagiz *et al*., 2007). On the basis of conditions developed (150-450 MPa for 0-5 min) in a previous research carried out on a related species (Atlantic mackerel, *Scomber scombrus*; Vázquez *et al*., 2013), the above mentioned conditions were chosen for the present investigation.

2.2. Lipid extraction and lipid hydrolysis analysis

Lipids were extracted according to the Bligh and Dyer (1959) method, by employing a single-phase solubilization of the lipids using a chloroform-methanol (1:1) mixture. Quantification results were expressed as g lipid $100g^{-1}$ muscle.

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle according to the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment (Beckman Coulter DU 640, London, UK). Results were expressed as g FFA $100g^{-1}$ lipids.

2.3. Lipid oxidation analysis

The peroxide value (PV) was determined in the lipid extract by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay (1949) method. Results were expressed as meq active oxygen kg^{-1} lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). This method is based on the reaction between a trichloracetic acid extract of the fish muscle and thiobarbituric acid. The contents of thiobarbituric acid reactive substances (TBARS) were spectrophotometrically measured at 532 nm and calculated from a standard curve using 1,1,3,3-tetraethoxy-propane (TEP). Results were expressed as mg malondialdehyde kg^{-1} muscle.

The formation of fluorescent compounds was determined by measurements at 393/463 nm and

327/415 nm as described by Aubourg and Pérez-Martín (1996). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulfate solution (1 μg mL^{-1} in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463nm}/RF_{327/415nm}$. The FR value was determined in the aqueous phase resulting from the lipid extraction (Bligh and Dyer, 1959).

2.4. Statistical analysis

Data (n = 3) obtained from the different lipid damage indexes were subjected to the one-way ANOVA method ($p < 0.05$) to explore differences as a result of the following parameters: pressure level, pressure holding time, RM stage and chilled storage time (Statsoft, Statistica, version 6.0, 2001); comparison of means was performed using a least-squares difference (LSD) method.

3. RESULTS AND DISCUSSION

3.1. Lipid content assessment

The lipid content of Chilean jack mackerel white muscle employed was found to be in the range of 1.25-1.75 g $100g^{-1}$ muscle, which can be considered lower than the one previously reported for the same species (3.0-5.0 g/100g muscle) (Aranda *et al*., 2006; Quitral *et al*., 2009). No effect ($p > 0.05$) on the lipid content could be observed as a result of the HHP conditions (pressure level and pressure holding time) applied or the RM stage of fish individuals employed as starting raw material.

3.2. Lipid hydrolysis development

The results obtained for the FFA formation are included in Table 1. For both pre- and post-RM fish, a marked lipid hydrolysis inhibition ($p < 0.05$) throughout the chilled storage could be observed as a result of previously applying the two strongest pressures tested; for pre-RM samples, FFA values

Table 1
Free fatty acid (g $100g^{-1}$ lipids) assessment* in chilled jack mackerel previously processed under different high pressure conditions**

Chilling storage time (days)	Pressure holding time (minutes)	Pressure (MPa)							
		Pre-*rigor mortis* stage				Post-*rigor mortis* stage			
		Control	250	450	550	Control	250	450	550
0	3	1.84 c (0.03)	1.63 b (0.12)	0.41 a (0.25)	y 0.45 a (0.01)	2.41 b (0.47)	2.62 b (0.35)	y 1.09 a (0.01)	1.12 a (0.11)
	4	1.84 d (0.03)	1.30 c (0.45)	0.46 b (0.18)	z 0.16 a (0.02)	2.41 b (0.47)	2.39 b (0.55)	z 0.81 a (0.13)	0.90 a (0.11)
2	3	2.68 d (0.12)	y 1.97 c (0.06)	0.20 a (0.05)	y 0.51 b (0.09)	2.02 c (0.02)	1.43 b (0.13)	0.32 a (0.12)	0.38 a (0.01)
	4	2.68 d (0.12)	z 0.96 c (0.15)	0.18 a (0.04)	z 0.35 b (0.01)	2.02 c (0.02)	1.59 b (0.30)	0.17 a (0.05)	0.29 a (0.33)
6	3	4.89 d (0.35)	y 3.44 c (0.02)	y 0.28 a (0.01)	y 0.86 b (0.14)	3.17 c (0.15)	y 2.15 b (0.06)	0.32 a (0.12)	0.43 a (0.07)
	4	4.89 d (0.35)	z 1.15 c (0.45)	z 0.16 a (0.01)	z 0.43 b (0.11)	3.17 c (0.15)	z 1.66 b (0.45)	0.57 a (0.56)	0.57 a (0.39)
10	3	5.78 d (0.08)	y 4.32 c (0.04)	0.39 a (0.05)	0.66 b (0.00)	4.03 c (0.24)	2.81 b (0.11)	0.83 a (0.41)	y 0.89 a (0.25)
	4	5.78 d (0.08)	z 1.84 c (0.23)	0.33 a (0.10)	0.76 b (0.09)	4.03 d (0.24)	2.97 c (0.10)	0.89 b (0.11)	z 0.32 a (0.26)
14	3	6.86 d (0.35)	y 4.73 c (0.23)	0.54 a (0.10)	1.19 b (0.17)	5.88 c (0.47)	3.45 b (0.84)	0.93 a (0.03)	0.84 a (0.53)
	4	6.86 d (0.35)	z 2.35 c (0.03)	0.70 a (0.10)	1.07 b (0.07)	5.88 c (0.47)	2.96 b (0.08)	0.98 a (0.12)	1.06 a (0.05)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets.
** For each chilling storage time, pressure holding time and *rigor mortis* stage, means followed by different letters (a-d) indicate significant ($p < 0.05$) differences as a result of pressure. For each chilling storage time, pressure and *rigor mortis* stage, mean values preceded by different letters (z, y) indicate significant ($p < 0.05$) differences as a result of pressure holding time. No letters are indicated when significant differences are not found ($p > 0.05$).

were found lower in 450-MPa fish than in their counterpart belonging to the 550-MPa batch. A lower FFA content was also evident at chilling time 0 in HHP-treated fish, when compared to their counterpart control. This effect could be explained as a result of FFA interaction with other fish constituents during the HHP treatment, so that their extraction during the lipid fraction extraction could be partly prevented. This effect at chilling time 0 was found to increase with pressure and was more evident in pre-RM fish than in post-RM samples. This possible interaction would imply a volume decrease and agree with the Le Chatelier principle, which stipulates the chemical changes produced during HHP application.

The analysis of FFA values also shows an inhibitory effect ($p < 0.05$) on lipid hydrolysis development by means of increasing the pressure holding time in individuals corresponding to a pressure value of 250 MPa and to the pre-RM stage. No effect ($p > 0.05$) on samples previously treated under higher pressures (450 and 550 MPa) could be concluded, probably as a consequence of the strong inhibitory effect developed by the pressure.

FFA have been reported to be mostly produced during the first stage of a chilling process (up to days 6-9, depending on several factors) as a result of endogenous enzyme (namely lipases and phospholipases) activity (Whittle *et al*., 1990). Later on, microbial activity should gain importance, so that FFA formation is then mostly produced as a result of bacterial catabolic processes (Chaouqy *et al*., 2008; Quitral *et al*., 2009). According to this profile, the present results on FFA formation in Chilean jack mackerel showed that a marked inhibition of both endogenous enzyme and microbial activities was produced by HHP conditions (namely, pressure level). On the other hand, FFA production as a direct effect of HHP treatment on higher-molecular-weight lipids (namely triglycerides and phospholipids) would not be expected to occur. Thus, a covalent bound breakdown would lead to a volume increase that should not be facilitated by an HHP treatment, according to the Le Châtelier principle (Norton and Sun, 2008).

Previous research concerning the effect of HHP treatment on FFA formation is scarce. An increased FFA content was observed in Coho salmon (Ortea *et al*., 2010), turbot (Chevalier *et al*., 2001) and carp (Sequeira-Muñoz *et al*., 2006) fillets after applying relatively low pressure values (100-200 MPa range). Higher pressure values are reported to be employed in experiments where a further storage was encountered; thus, He *et al*. (2002) did not observe inhibition of lipase activity in refrigerated (4 °C) oysters, which were previously pressurized at 207-310 MPa for 1-2 min. The same conclusion was attained by Gómez-Estaca *et al*. (2007) when studying cold-smoked sardine storage (5 °C up to 21 days) previously treated under 300 MPa for 15 min. However, in agreement with the present research, Ohshima *et al*. (1992) found that enzymatic degradation of phospholipids in cod muscle was successfully inhibited during storage (–2 °C, 6 days) when a previous pressure of 400 MPa and over was applied for 15 and 30 min.

The accumulation of FFA in fish muscle has no nutritional significance, but it is found undesirable due to secondary reactions, such as muscle texture changes, lipid oxidation enhancement and relation to off-odor development (Sikorski and Kolakowska, 1994; Refsgaard *et al*., 2000). In agreement to the present results obtained in the control samples, previous research on Chilean jack mackerel has shown an important lipid hydrolysis development during chilling (Roth *et al*., 2006) and frozen (Aranda *et al*., 2006) storage. Present results show that the employment of the HHP technology as a previous treatment to chilling storage can lead to a significant reduction in FFA formation and accordingly, to a quality enhancement.

Concerning the effect of the RM stage on FFA formation in the present experiment, a higher ($p < 0.05$) FFA content was obtained in post-RM individuals than in their pre-RM counterparts in all types of samples at chilling time 0; differences were higher in HHP-treated fish than in the control. In agreement with this difference, a considerable hydrolysis of triglycerides and phospholipids has already been reported to occur in stellate sturgeon during RM development (Antipova and Smirnova, 1982). Throughout the present chilled storage, differences found as a result of the RM stage were scarce and did not lead to a definite trend in any of the types of samples except for the control fish, where higher ($p < 0.05$) values were obtained in pre-RM fish.

3.3. Primary lipid oxidation assessment

This oxidation stage was analyzed by means of the PV assessment (Table 2). In all cases, a progressive peroxide formation could be observed throughout the chilled storage time. Lower mean PV could be observed in pre-RM fish when 250- and 450-MPa pressures were previously applied; differences with the control were found significant ($p < 0.05$) for the 0-2-day and 0-6-day periods, respectively. Meanwhile, a pro-oxidant effect ($p < 0.05$) could be depicted in pre-RM fish previously treated under 550 MPa in the 10-14-day period. For post-RM samples, a definite effect of the previous pressure applied on the peroxide formation could not be concluded.

Concerning the effect of the pressure holding time applied, pre- and post-RM samples corresponding to 250- and 450-MPa conditions showed a pro-oxidant effect ($p < 0.05$) by increasing the pressure holding time in fish corresponding to the 6-14-day chilling time; a definite effect of this factor could not be concluded for 550-MPa samples.

Previous research focusing on the effect of HHP treatment on the formation of primary oxidation compounds is scarce and unclear. Thus, Ohshima

Table 2
Peroxide value (meq oxygen kg^{-1} lipids) assessment* in chilled jack mackerel previously processed under high pressure conditions**

Chilling storage time (days)	Pressure holding time (minutes)	Pressure (MPa)							
		Pre-*rigor mortis* stage				Post-*rigor mortis* stage			
		Control	250	450	550	Control	250	450	550
0	3	2.50 c (0.14)	y 1.15 a (0.07)	y 1.13 a (0.31)	z 1.55 b (0.05)	1.90 a (0.04)	2.51 b (0.23)	z 2.89 b (0.29)	y 3.73 c (0.11)
	4	2.50 b (0.14)	z 0.60 a (0.07)	z 0.58 a (0.04)	y 2.97 c (0.05)	1.90 b (0.04)	2.48 c (0.56)	y 4.85 d (1.59)	z 1.68 a (0.02)
2	3	4.40 c (0.39)	2.50 a (0.03)	2.13 a (0.62)	z 3.43 b (0.30)	4.77 a (0.53)	5.12 ab (0.73)	z 4.37 a (0.38)	y 6.37 b (0.90)
	4	4.40 c (0.39)	2.46 a (0.18)	2.89 b (0.11)	y 4.62 c (0.18)	4.77 b (0.53)	4.78 b (0.16)	y 8.79 c (0.11)	z 3.12 a (0.27)
6	3	8.63 c (0.02)	z 7.50 b (0.18)	z 6.32 a (0.02)	y 9.18 c (1.89)	14.53 b (2.05)	z 8.59 a (0.25)	z 9.12 a (0.51)	y 12.83 b (1.88)
	4	8.63 c (0.02)	y 9.03 bc (1.14)	y 7.77 ab (0.54)	z 6.61 a (1.28)	14.53 c (2.05)	y 11.19 b (0.68)	y 15.35 c (0.85)	z 6.56 a (0.51)
10	3	10.38 a (2.32)	z 10.27 a (1.20)	z 9.20 a (0.34)	y 18.42 b (0.31)	15.49 b (1.38)	z 9.77 a (0.54)	z 9.27 a (1.22)	y 14.75 b (1.71)
	4	10.38 a (2.32)	y 15.56 bc (1.68)	y 13.05 ab (1.30)	z 16.11 c (0.28)	15.49 b (1.38)	y 15.31 b (0.87)	y 16.59 b (2.06)	z 8.22 a (1.94)
14	3	18.08 b (1.13)	z 16.83 a (0.22)	z 15.98 a (0.25)	26.39 c (0.04)	25.12 c (3.36)	z 19.06 b (0.09)	z 13.73 a (0.99)	z 12.57 a (0.35)
	4	18.08 a (1.13)	y 22.94 b (0.21)	y 18.04 a (0.29)	28.24 c (1.99)	25.12 bc (3.36)	y 26.94 c (1.24)	y 19.78 ab (3.25)	y 20.76 a (0.39)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets.
** For each chilling storage time, pressure holding time and *rigor mortis* stage, means followed by different letters (a-d) indicate significant ($p < 0.05$) differences as a result of pressure. For each chilling storage time, pressure and *rigor mortis* stage, mean values preceded by different letters (z, y) indicate significant ($p < 0.05$) differences as a result of pressure holding time. No letters are indicated when significant differences are not found ($p > 0.05$).

et al. (1992) showed that the peroxide content of cod and mackerel muscle increased by applying an increasing pressure value (a 200-600 MPa range for 15 and 30 min). A similar conclusion was obtained in a model system including sardine lipids (Tanaka *et al.*, 1991); thus, an increasing PV was attained throughout storage (5 °C up to 4 days) as a result of a previous HHP treatment (150 MPa for 15 and 30 min). On the contrary, a partial inhibition of peroxide formation was observed in Coho salmon during the chilled storage when a previous HHP treatment (170 and 200 MPa for 30 s) was applied (Aubourg *et al.*, 2010).

Concerning the effect of the RM stage of the initial raw fish, a higher PV ($p < 0.05$) was obtained in pre-RM control individuals than in their post-RM counterparts (chilling time 0). On the other hand, higher peroxide levels ($p < 0.05$) were obtained at that time in post-RM fish when 250- and 450-MPa pressures (both for 3 or 4 minutes) were previously applied. Throughout the chilled storage, post-RM fish showed higher mean peroxide levels when control, 250- and 450-MPa samples were considered; in the case of 550-MPa fish, higher ($p < 0.05$) peroxide levels were found for pre-RM fish in the 10-14-day period.

Pre-RM fish showed a PV lower than score 10 for the 0-6-day period for all types of samples. In fact, fish samples corresponding to 450-MPa and 3-min conditions were still under this mark after 10 days of chilled storage. In the case of post-RM fish, a PV under 10 was only obtained for the 0-2-day interval, with samples from 250- and 450-MPa and 3-min still under this value for the 0-10-day period.

3.4. Secondary lipid oxidation assessment

Results related to the formation of TBARS can be observed in Figures 1-2. Samples corresponding to a previous 250-MPa treatment were lost so that analyses could not be done. In all types of samples analyzed, a progressive formation of such type of compounds could be observed throughout the chilling time. This result agrees with the above mentioned general increase detected for peroxide formation (Table 1).

No effect ($p > 0.05$) of the pressure applied could be concluded at chilling time 0 for pre-RM

fish (Figure 1). However, a higher mean TBARS formation was detected at that time in HHP-treated post-RM fish when compared to control samples (Figure 2), and this effect is the result of HHP treatment on the lipid fraction; significant differences were found ($p < 0.05$) for 550-MPa-treated fish. Throughout the chilled storage, all kinds of HHP-treated fish led to a higher mean TBA-i than their corresponding control; differences were found to be significant ($p < 0.05$) at day 6 and days 2 and 6 for samples previously treated under 450 and 550 MPa, respectively. A definite effect of the pressure holding time (comparison between 3 and 4 minute treatments) could not be concluded ($p > 0.05$) for the TBARS formation in either pre- or post-RM fish, whatever the pressure applied was.

Contradictory conclusions have been reported concerning the effect of HHP treatment on the TBA-i. Thus, an increase as a result of HHP treatment has been observed for carp (Sequeira-Muñoz *et al*., 2006) and turbot (Chevalier *et al*., 2001) fillets, both showing an increasing effect with pressure holding time. However, no differences in TBARS formation were observed for horse mackerel (Erkan *et al*., 2011) or Atlantic salmon (Amanatidou *et al*., 2000).

Contradictory results have also been observed when evaluating the effect of a previous HHP treatment on TBARS formation throughout a further storage. Thus, an increase was observed in chilled rainbow trout (Yagiz *et al*., 2007) and Coho salmon (Aubourg *et al*., 2010), cold-smoked salmon (Lakshmanan *et al*., 2007), refrigerated (4 °C) cod muscle (Angsupanich and Ledward, 1998) and stored (–2 °C) cod and mackerel muscle (Ohshima *et al*., 1992). Opposite results were obtained however,

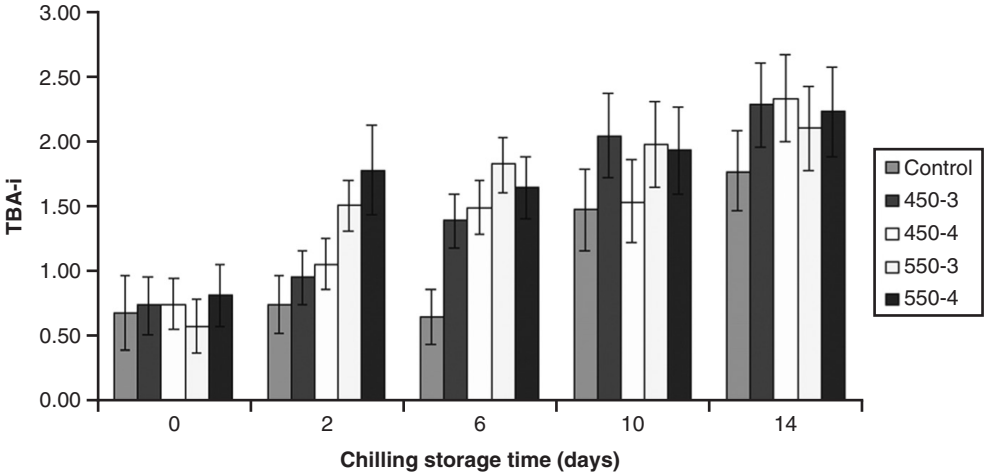

Figure 1
Evolution of the thiobarbituric acid index (TBA-i) (mg malondialdehyde kg^{-1} muscle)* in chilled pre-rigor mortis Chilean jack mackerel previously processed under different high hydrostatic pressure (HHP) conditions**

* Mean values of three (n = 3) replicates; standard deviations are indicated by bars.
** HHP treatment abbreviations: 450-3 (450 MPa/3 min), 450-4 (450 MPa/4 min), 550-3 (550 MPa/3 min), 550-4 (550 MPa/4 min). Control: No HHP treatment.

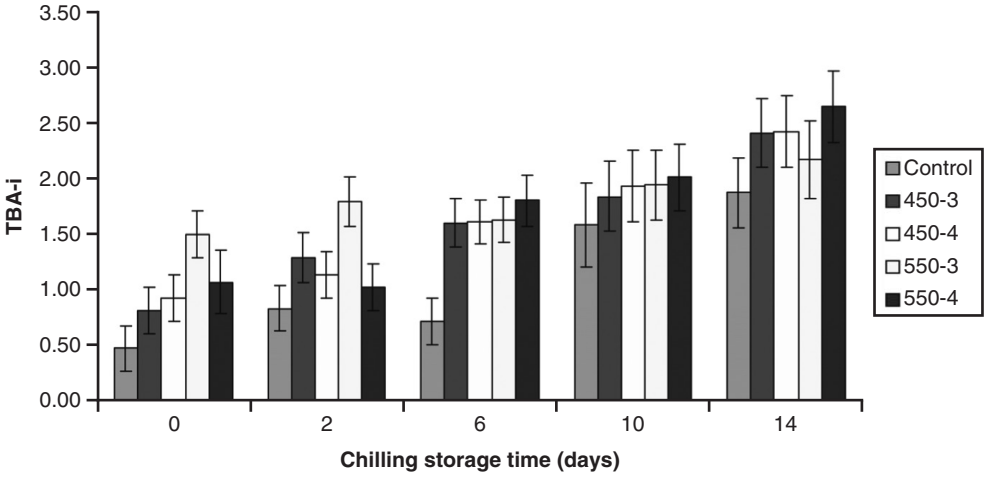

Figure 2
Evolution of the thiobarbituric acid index (TBA-i) (mg malondialdehyde kg^{-1} muscle)* in chilled post-rigor mortis Chilean jack mackerel previously processed under different high hydrostatic pressure (HHP) conditions**

* Mean values of three (n = 3) replicates; standard deviations are indicated by bars.
** HHP treatment abbreviations as expressed in Figure 1.

by Ramírez-Suárez and Morrisey (2006) when minced albacore muscle was HHP-treated and then refrigerated at 4 °C; thus, a lower TBARS formation was found in HHP-treated fish than in control ones. An inhibitory effect on TBA-i score was also attained in red mullet muscle throughout refrigerated (4 °C) storage (Erkan *et al*., 2010).

Regarding the effect of the RM stage in the present research, a higher mean TBARS formation was detected in pre-RM control samples when compared to their post-RM counterparts at chilling time 0. However, if HHP-treated fish are considered at that time, a higher mean formation in post-RM fish was found in all cases; this difference was found to be significant ($p < 0.05$) in the case of 550-MPa-3-min treatment. Throughout the chilled storage, higher mean TBA-i values were obtained in most cases in samples belonging to the post-RM condition; however, differences were not found to be significant ($p > 0.05$). In agreement with the present research, lower TBA-i values were reported for fillets obtained from pre-RM rainbow trout and carp than in their post-RM counterparts (Duran *et al*., 2008).

3.5. Assessment of interaction compound formation

The formation of interaction compounds (FR assessment; Table 3) throughout the chilled storage was found lower than for primary (Table 2) and secondary (Figures 1-2) lipid oxidation compounds; thus, a significant ($p < 0.05$) FR increase with time was only found in the longest storage period (10-14-day interval) for all types of samples. This lower formation can be explained on the basis that interaction compounds would mainly be formed once primary and secondary oxidized lipids (namely, electrophilic compounds) have been produced and have then reacted with protein-type molecules (namely, nucleophilic compounds) (Aubourg, 1999; Tironi *et al*., 2002).

Lower mean FR values were obtained in HHP-treated fish than in their counterpart controls at chilling time 0, this effect being attributable to chemical modifications by HHP treatment of the fluorescent compounds measured; and significant differences were found ($p < 0.05$) in most cases, whatever the pressure, pressure holding time or RM stage condition is considered. However,

Table 3
Fluorescent compound formation* in chilled jack mackerel previously processed under different high pressure conditions**

Chilling storage time (days)	Pressure holding time (minutes)	Pressure (MPa)							
		Pre-*rigor mortis* stage				Post-*rigor mortis* stage			
		Control	250	450	550	Control	250	450	550
0	3	0.44 b (0.07)	0.21 a (0.06)	0.23 a (0.02)	0.25 a (0.08)	0.32 c (0.11)	0.20 bc (0.02)	0.18 b (0.00)	0.14 a (0.02)
	4	0.44 b (0.07)	0.30 a (0.02)	0.38 ab (0.11)	0.33 ab (0.19)	0.32 b (0.11)	0.14 a (0.03)	0.18 a (0.02)	0.14 a (0.01)
2	3	0.37 b (0.08)	z 0.20 a (0.02)	z 0.19 a (0.03)	y 0.40 b (0.00)	0.26 (0.01)	0.23 (0.03)	0.22 (0.03)	0.24 (0.00)
	4	0.37 (0.08)	y 0.37 (0.06)	y 0.31 (0.07)	z 0.15 (0.06)	0.26 b (0.01)	0.19 a (0.04)	0.28 ab (0.08)	0.26 ab (0.04)
6	3	0.34 (0.02)	0.32 (0.06)	0.30 (0.04)	0.25 (0.00)	0.46 b (0.08)	0.37 a (0.00)	0.36 a (0.06)	0.34 a (0.02)
	4	0.34 (0.02)	0.41 (0.05)	0.35 (0.07)	0.31 (0.05)	0.46 (0.08)	0.34 (0.10)	0.42 (0.04)	0.39 (0.03)
10	3	0.43 a (0.02)	z 0.49 b (0.01)	z 0.39 a (0.00)	y 0.55 b (0.04)	0.63 (0.03)	0.61 (0.03)	y 0.61 (0.06)	0.59 (0.02)
	4	0.43 a (0.02)	y 0.67 c (0.02)	y 0.51 b (0.01)	z 0.45 a (0.03)	0.63 c (0.03)	0.54 bc (0.05)	z 0.47 b (0.01)	0.41 a (0.00)
14	3	0.73 b (0.11)	z 0.62 a (0.02)	z 0.61 a (0.04)	0.74 b (0.14)	0.69 b (0.11)	0.58 a (0.01)	y 0.70 b (0.03)	0.47 a (0.12)
	4	0.73 ab (0.11)	y 0.85 b (0.02)	y 0.73 ab (0.04)	0.68 a (0.08)	0.69 c (0.06)	0.62 bc (0.06)	z 0.38 a (0.04)	0.52 b (0.05)

* Mean fluorescent ratio values of three (n=3) replicates; standard deviations are indicated in brackets.
** For each chilling storage time, pressure holding time and *rigor mortis* stage, means followed by different letters (a-c) indicate significant ($p < 0.05$) differences as a result of pressure. For each chilling storage time, pressure and *rigor mortis* stage, mean values preceded by different letters (z, y) indicate significant ($p < 0.05$) differences as a result of pressure holding time. No letters are indicated when significant differences are not found ($p > 0.05$).

throughout the storage time, a definite pattern related to the effect of the pressure applied could not be concluded ($p > 0.05$). Previous research has shown an increasing FR value with pressure applied throughout the further chilling storage of Coho salmon (Aubourg *et al*., 2010); thus, pressure values of 200 and 175 MPa led to higher fluorescent scores than those obtained for the control and 135-MPa-treated fish.

Concerning the pressure holding time effect, an increasing mean FR value could be depicted by increasing it in pre-RM samples when the previous pressure applied was 250 and 450 MPa; and differences were found to be significant ($p < 0.05$) at days 2, 10 and 14. On the contrary, an inhibitory effect was observed in pre-RM fish when the pressure applied was 550 MPa; in such case, significant differences were found ($p < 0.05$) at days 2 and 10.

The effect of HHP treatment on lipid oxidation development (primary, secondary and interaction compound formation) in fish can be considered somewhat controversial. Although most research has shown that an increased lipid oxidation development is to be produced as a result of the HHP treatment, isolated extracted lipids have shown to be relatively stable against oxidation under HHP conditions and during further storage (Ohshima *et al*., 1993; Angsupanich and Ledward, 1998). Additionally, the possible pro-oxidant effect of HHP treatment on muscle lipids was shown to be eliminated if a previous water washing of the muscle was applied or if a complex compound (EDTA, for example) was added. Consequently, iron-bound protein denaturation during HHP treatment has been reported to facilitate a free metal ion content increase and to be responsible for this lipid oxidation development in fish meat after HHP treatment.

According to the present study, two main HHP-treatment effects can be distinguished in lipid oxidation development during the chilled storage. According to previously mentioned research, HHP may lead to a pro-oxidant effect as a result of a free metal ion content increase, so that peroxide and TBARS formation could be favored. Additionally, HHP treatment has been reported to induce damages in the protein structure (Ramírez-Suárez and Morrisey, 2006), leading to an increasing reactivity of proteins towards electrophilic compounds and fluorescent compound formation (Aubourg, 1999; Tironi *et al*., 2002). On the other hand, the pressure treatment conditions (pressure level and pressure holding time) can lead to an inhibition of the pro-oxidant properties of fish endogenous enzymes (lipoxygenases, peroxydases, etc.) throughout a further processing or storage, and lead to a damage inhibition and shelf- life increase. In addition, a lower FR value has been observed to be produced after HHP treatment (day-0 fish). The results obtained in the present research for the three oxidation levels can be considered as the resulting values of both inverse effects.

Regarding the effect of the RM stage in the present research, lower mean FR values were observed at chilling time 0 in post-RM fish than in their pre-RM counterparts; significant differences were found ($p < 0.05$) in most HHP-treated fish. However, a definite pattern could not be concluded ($p > 0.05$) throughout the chilled storage for the RM stage whatever the HHP conditions applied were.

4. CONCLUSIONS

The present research provides a valuable lipid damage study concerning the employment of HHP technology followed by a subsequent chilling storage of an underutilized fish species. In addition, new information related to lipid hydrolysis and oxidation development is given concerning the effect of the RM stage of the initial raw material employed.

Thus, previous HHP treatment has led to a marked lipid hydrolysis inhibition in chilled Chilean jack mackerel. Both an increasing pressure level as well as an increasing pressure holding time led to a lower FFA content in fish muscle, with the effect of pressure being more relevant. As a result of the different factors encountered in lipid oxidation development, a definite effect of HHP conditions (pressure level and pressure holding times) on lipid oxidation evolution (primary, secondary and interaction compound formation assessment) in chilled Chilean jack mackerel could not be concluded. Concerning the effect of the RM stage of the starting raw fish, the differences found showed that in most cases processing of fish before the RM stage takes place is more convenient.

According to the present study, the suggestion of better HHP conditions to be employed before a subsequent chilled storage of Chilean jack mackerel would be based mostly on the lipid hydrolysis results. Thus, a relatively high-pressure value (namely, 450 MPa) and the longest pressure holding time tested (namely, 4 min) would be recommended. In addition, the employment of pre-RM fish would be highly recommended. However, and in order to definitely choose the best HHP conditions to be applied to the actual fish species, complementary analyses concerning sensory acceptance and microbiological development ought to be carried out.

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