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Lipid hydrolysis and oxidation development in frozen mackerel (*Scomber scombrus*): Effect of a high hydrostatic pressure pre-treatment

Manuel Vázquez[1], J. Antonio Torres[2], José M. Gallardo[3], Jorge Saraiva[4] and Santiago P. Aubourg[3,*]

[1] Department of Analytical Chemistry, Facultad de Ciencias Veterinarias, Universidad de Santiago de Compostela, Lugo, Spain

[2] Food Processing Engineer Group, Department of Food Science and Technology, Oregon State University, Corvallis, OR, USA

[3] Department of Food Technology, Instituto de Investigaciones Marinas (CSIC), Vigo, Spain

[4] Research Unit of Organic Chemistry, Natural and Agro-food Products (QOPNA), Chemistry Department, Aveiro University, Campus Universitário de Santiago, Aveiro, Portugal.

[*] Correspondent: saubourg@iim.csic.es, +34986292762 (fax), +34986231930 (phone)

ABSTRACT

This work focused on assessing lipid damage during the storage of frozen Atlantic mackerel (*Scomber scombrus*) subjected to a high hydrostatic pressure (HHP) treatment (150, 300, 450 MPa with holding times of 0.0, 2.5, and 5.0 min) prior to freezing. The extent of lipid hydrolysis (free fatty acids) and oxidation (peroxide, thiobarbituric acid reactive substance and fluorescent and browning compound formation) as well as the polyene content were analysed during 3 months of accelerated storage at -10ºC. A marked inhibition ($p<0.05$) of free fatty acids and tertiary lipid oxidation compounds formation during storage was observed when increasing the pressure level or the pressure holding time of the HHP treatment. However, only minor differences in the polyene index and no effect ($p>0.05$) in the content of primary and secondary oxidation compounds were observed .

Keywords: *Scomber scombrus*, high pressure, freezing, lipids, hydrolysis, oxidation

Running Title: Frozen mackerel lipids and high pressure

1. INTRODUCTION

Fish species provide important components to human nutrition but deteriorate rapidly post-mortem unless subjected to an appropriate treatment. Freezing followed by frozen storage constitute one of the best methods to retain sensory and nutritional properties of fish products (Erickson, 1997). However, the presence of highly unsaturated fatty acids and a large content of pro-oxidant molecules can lead to substantial enzymatic and non-enzymatic rancidity strongly influencing product quality after freezing and frozen storage (Harris & Tall, 1994; Richards & Hultin, 2002; Kolakowska, 2003).

Among advances in food processing technologies, high hydrostatic pressure (HHP) technology has shown to retain the sensory and nutritional properties of foods while inactivating microbial populations and leading to shelf-life extension and food safety enhancement (Torres & Velázquez, 2005; Norton & Sun, 2008; Bermúdez-Aguirre, Guerrero-Beltrán, Barbosa-Cánovas, & Welti-Chanes, 2011). This technology has shown potential application in the seafood industry for surimi and kamaboko production (Uresti, Velázquez, Vázquez, Ramírez, & Torres, 2005), for cold-smoked fish preparation (Lakshmanan, Parkinson, & Piggott, 2007) and as assisting freezing (Alizadeh, Chapleau, de Lamballerie, & Le-Bail, 2007), thawing (Rouillé, Le Bail, Ramaswamy, & Leclerc, 2002) and thermal (Ramírez, Saraiva, Pérez-Lamela, & Torres, 2009) processing. An additional positive effect of HHP treatment is that deteriorative hydrolytic endogenous enzymes, namely, lipases, phospholipases, peroxidases, and lipoxygenases among others can be inactivated for a further stabilization of the fish product (Murchie et al., 2005). Thus, a beneficial effect on quality retention has been observed when an HHP treatment is used prior to refrigerated (He, Adams, Farkas, & Morrissey, 2002; Erkan, Üretener, & Alpas, 2010) or chilled (Hurtado, Montero, &

Borderías, 2001; Ortea, Rodríguez, Tabilo-Munizaga, Pérez-Won, & Aubourg, 2010) storage. However, studies on the potential benefit of HHP treatments prior to the frozen storage of fish products are very scarce (Pérez-Won et al., 2006).

Small pelagic fish species could constitute food products of great economic importance in many countries (FAO, 2007). Unfortunately, some of these species are captured in large volumes during times when their demand is relatively low and thus a large portion of these resources is underutilised and transformed into fish meals for animal feed. One such abundant species on both North Atlantic coasts is the Atlantic mackerel (*Scomber scombrus*) belonging to the Scombridae family (FAO, 2007). Although it is recognised as a healthy food, it remains underutilised (Martelo-Vidal, Mesas, & Vázquez, 2012) reflecting mainly its poor frozen shelf life. Previous research has shown an important endogenous pro-oxidant activity (Decker & Hultin, 1990; Saeed & Howell, 2001) and significant quality loss during its frozen storage (Saeed & Howell, 2002; Aubourg, Rodríguez, & Gallardo, 2005).

The present work concerns the assessment of lipid damage during the frozen storage of Atlantic mackerel previously subjected to HHP treatment at three pressure levels and three pressure holding time conditions. The extent of lipid hydrolysis and oxidation was analysed in mackerel muscle during frozen storage at -10ºC to accelerate the detection of the HHP effect.

2. MATERIALS AND METHODS

2.1. Raw fish, processing, storage and sampling

Atlantic mackerel (70 kg) caught near the Bask coast in Northern Spain was obtained at the Ondarroa harbour (Bizkaia, Spain) and transported under ice to the AZTI Tecnalia (Derio, Spain) pilot plant for HHP treatment within 6 hours after catch. Whole

mackerel individuals were placed in flexible polyethylene bags (three individuals per bag) and vacuum sealed at 400 mbar. The length and weight of the specimens ranged 28-33 cm and 230-280 g, respectively.

HHP treatments at 150-450 MPa and 0-5 min pressure holding times were performed in a 55-L high pressure unit (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain) according to the following experiment design: T-1 (450 MPa, 0.0 min), T-2 (450 MPa, 2.5 min), T-3 (450 MPa, 5.0 min), T-4 (300 MPa, 0.0 min), T-5 (300 MPa, 2.5 min), T-6 (300 MPa, 2.5 min), T-7 (300 MPa, 2.5 min), T-8 (300 MPa, 5.0 min), T-9 (150 MPa, 0.0 min), T-10 (150 MPa, 2.5 min), T-11 (150 MPa, 2.5 min), T-12 (150 MPa, 5.0 min). Water applied as the pressurising medium at 3 MPa/s yielded 50, 100 and 150 s as the come up time for the 150, 300 and 450 MPa treatments, respectively, while decompression time was less than 3s. Cold pressurising water was used to maintain temperature conditions during HHP treatment at room temperature (20ºC).

After HHP treatments, mackerel individuals were kept at –20ºC for 48 hours and then stored at –10ºC with samples analysed after 0, 1 and 3 months of storage. Fish without HHP treatment and subjected to the same freezing and frozen storage conditions was used as control (T-0 treatment). Three batches or replicates (n=3) for each treatment (T-0 to T-12) were analysed independently. Each analysis was based on the lipid fraction extracted from the fish white muscle pooled from two individual fish.

A frozen storage temperature (-10ºC) higher than commercial practice (-18ºC) was chosen as an accelerated test condition since no published research was available to estimate the extent of the HHP treatment effect on the lipid damage development of a frozen fish species. In addition, the response to the HHP treatment of marine species has been reported to vary with species, chemical composition and size (Murchie et al., 2005; Yagiz, Kristinsson, Balaban, & Marshall, 2007). Consequently, a preliminary study was

undertaken to confirm the above mentioned pressure conditions. Sensory parameters (eyes, external colour, hardness, external odour, blood, skin and gills) were analysed after testing a wide range of pressure (600, 500, 400, 350, 300, 250, 200 and 100 MPa) values for 5 minutes as pressure holding time and compared to those observed for untreated mackerel. Most attributes showed quality losses increasing with the pressure applied as compared to control samples. On the other, the appearance of blood and gills remained unchanged in the range 0-300 MPa range while at higher pressure, blood coagulated while the gills colour was markedly lighter. Accordingly, 300 MPa was chosen as the mid pressure point in the present study which included also a lower and a higher pressure value of 150 and 450 MPa, respectively.

2.2. Lipid hydrolysis analysis

Lipids were extracted by the Bligh, and Dyer (1959) method employing a chloroform-methanol (1:1) mixture for the single-phase solubilisation of lipids in the fish muscle. Quantification results were expressed as g lipid/ 100g muscle. Free fatty acid (FFA) content in this lipid extract was determined by 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 lipids.

2.3. Lipid oxidation assessment

The peroxide value (PV) in the lipid extract was determined by peroxide reduction with ferric thiocyanate, according to the Chapman, and McKay (1949) method. Results were expressed as meq active oxygen/ kg lipids. The thiobarbituric acid index (TBA-i) was determined as described by Vyncke (1970) and based on the reaction

between a trichloracetic acid extract of the fish muscle and thiobarbituric acid. Content on thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and results were expressed as mg malondialdehyde/ kg muscle.

2.4. Formation of interaction compounds

The formation of fluorescent compounds was determined in the aqueous phase obtained during the lipid extraction (Bligh, & Dyer, 1959) by measurements at 393/463 nm and 327/415 nm (Aubourg, 1999). A relative fluorescence (RF) was defined as the F/F_{st} ratio where F is the fluorescence measured at each excitation and emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 µg/ ml in 0.05 M H_2SO_4) at the corresponding wavelength. A fluorescence ratio (FR) was calculated as the ratio between the two RF values. i.e., $FR = RF_{393/463\ nm} / RF_{327/415\ nm}$. Browning formation was measured in the lipid extract at 450 nm and 400 nm to define browning ratio (BR) as the 450 nm/400 nm absorbance ratio suggested by Hassan, Khallaf, Abd-El Fattah, and Yasin (1999).

2.5. Polyene index assessment

Lipid extracts were converted into fatty acid methyl esters (FAME) by reaction with acetyl chloride and then analysed using a Perkin-Elmer 8700 gas chromatograph equipped with a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, 0.20 µm film, Supelco Inc., Bellefonte, PA, USA) and using nitrogen at 10 psi as carrier gas (linear flow rate of 1.0 ml/min), a flame ionisation detector (FID) at 250ºC, and 19:0 fatty acid as internal standard for quantitative analysis (Aubourg, Medina, and Pérez-Martín, 1996). Peaks corresponding to fatty acids were identified by comparison of the retention times of two standards mixtures (Qualmix Fish, Larodan, Malmo, Sweden;

FAME Mix, Supelco Inc., Bellefonte, PA, USA). Peak areas were automatically integrated. The polyene index (PI) was calculated as the following fatty acid ratio: (C 20:5ω3 + C 22:6ω3)/ C 16:0.

2.6. Statistical analysis

The statistical experiment design was formulated using the Design Expert® 7.1.1 software (Stat-Ease, Inc., Minneapolis, MN, USA). The model was validated through a multifactor ANOVA test. The set of experiments (T-0 to T-12) followed the Box-Behnken design (Box & Behnken, 1960), and combined two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but with a fraction of the experiments required when using a three-level factorial design.

Fish samples corresponding to each treatment (T-0 to T-12) were analysed after 0, 1 and 3 months frozen storage time. Data (n = 3) obtained from the different lipid damage indices were subjected to one-way ANOVA method ($p<0.05$) to explore differences as a result of pressure level, holding time of pressure and frozen storage time. Comparison of means was performed using a least-squares difference (LSD) method (Statsoft, Statistica, version 6.0, Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

3.1. Lipid hydrolysis development

The lipid content of Atlantic mackerel muscle ranged from 8.5 to 11.5 g/ 100g and was consistent with previous research reflecting a fatty fish species (Aubourg et al., 2005; Zotos & Vouzanidou, 2012). Comparison of fresh raw fish and frozen control samples showed significant lipid hydrolysis ($p<0.05$) caused by freezing and by the

increasing frozen storage time (Table 1). When comparing samples corresponding to the same pressure and holding time values, an increasing ($p<0.05$) FFA content with frozen storage time was observed for HHP-treated fish. However, at any frozen time considered, the HHP treatments tested caused a remarkable inhibition of FFA formation (Table 1). Increasing the holding time resulted in a partial inhibition of FFA formation at 300 or 450 MPa while at the lowest pressure level (150 MPa), the inhibitory effect could be observed only after the longest storage time studied (3 months). Concerning the effect of pressure, a significant FFA content decrease was observed by increasing the pressure value applied. This trend was significantly stronger for longer frozen storage times and pressure holding times.

Since the three independent variables (pressure, holding time and frozen storage time) showed an important effect on FFA formation, a multifactor ANOVA analysis was necessary to assess their relative influence yielding a significant ($p<0.0001$) model with an F-value of 60.47. The evaluation of the F-values for the three independent variables confirmed their individual significant effect. Thus, FFA formation was highly affected by frozen storage (F-value = 379.24; p-value probability > F was $p \leq 0.0001$), although an important effect of pressure and holding time could also be observed (F-value of 72.59 and 23.56, respectively; p-value probability > F were $p \leq 0.0001$ in both cases). The correlation value of the model was $r^2 = 0.9544$ with adjusted and predicted r^2 values of 0.9386 and 0.8836, respectively, and a signal/noise ratio of 26.80. These statistical parameters confirmed that an empirical coded equation could be used to model the effect of HHP pre-treatment and frozen storage time on the FFA formation and is expressed in Figure 1.

Previous research concerning the effect of HHP treatment on FFA formation is scarce and almost unavailable for refrigerated and frozen marine products, respectively.

A FFA content increase was observed in coho salmon (Ortea et al., 2010), turbot (Chevalier, Le Bail, & Ghoul, 2001) and carp (Sequeira-Muñoz, Chevalier, Le Bail, Ramaswamy, & Simpson, 2006) fillets after applying relatively low pressures (100-200 MPa). Higher pressure values were applied when a subsequent storage period was intended. Thus, He et al. (2002) did not observe inhibition of lipase activity in refrigerated (4 ºC up to 27 days) oysters previously pressurized at 207-310 MPa for 1-2 min. A similar observation was made by Gómez-Estaca, Montero, Giménez, & Gómez-Guillén (2007) when studying the storage (5 ºC up to 21 days) of cold-smoked sardines previously treated at 300 MPa for 15 min. However, and consistent with the research here reported, Ohshima, Nakagawa, and Koizumi (1992) found that enzymatic degradation of phospholipids in cod muscle was successfully inhibited during storage at -2 ºC for 6 days when previously treated at pressures above 400 MPa applied for 15 and 30 min.

Accumulation of FFA in fish muscle has no nutritional significance, but it has undesirable secondary effects including muscle texture changes (Sikorski & Kolakowska, 1994), acceleration of lipid oxidation (Mackie, 1993), and off-odour development (Refsgaard, Brockhoff, & Jessen, 2000). Both fatty and lean fish species show significant lipid hydrolysis during frozen storage (Aubourg, 1999; Aubourg et al., 2005) and is one of the most important product damage pathways leading to a remarkable shelf life reduction. This study showed that HHP as a treatment prior to freezing and frozen storage can lead to a significant reduction of FFA formation and consequently to product quality enhancement.

3.2. Lipid oxidation development

Peroxide formation showed to be very low with values in the 0.17-2.97 score range (Table 2). Comparison of fresh raw fish and frozen control samples corresponding to month 0 showed a slight increase ($p<0.05$) as a result of the freezing step; control fish also showed an increasing peroxide value (PV) throughout the frozen storage time. HHP-treated samples showed higher mean values than in raw fish; however, a definite trend concerning the frozen time effect on HHP-treated fish cannot be ascertained ($p>0.05$) by comparison of samples with the same pressure and holding time.

HHP conditions had only minor effects on peroxide formation (Table 2); thus, a general pattern could not be determined for the holding time nor the pressure level when analysing the one-way ANOVA results. A complementary multifactor ANOVA analysis did not show a clear trend either. Although an F-value of 3.83 implied that the model was significant with a p-value probability > F of 0.0188, low F-values were obtained for the pressure level and holding time (1.083 and 0.003, respectively) and only the storage time affected peroxide formation (F-value = 9.63; p-value probability > F of 0.004). Therefore, it can be concluded that the HHP pre-treatment before frozen storage did not affect the level of peroxide values.

The assessment of secondary oxidation is summarized in Table 3. The comparison of mean values for fresh and frozen control fish showed increasingly higher values as a result of the freezing step and the frozen storage time. For HHP-treated fish, an increased TBARS formation was observed in all cases when compared to raw fish; however, TBARS formation in HHP-treated fish as a result of frozen storage showed no definite trend ($p>0.05$) when comparing samples treated at the same pressure and holding time. The effect of HHP treatment conditions showed only minor differences (Table 3). Thus, lower mean TBA-i values were reached for shorter holding times at

intermediate pressure (300 MPa). However, a definite effect on TBARS formation by pressure and holding time could not be established. A multifactor ANOVA analysis was also carried out to take into account the comparative effect of the three variables (frozen storage time, pressure and holding time) on the TBARS formation. The low F-value obtained (1.38) implied that the model was not significant (p-value probability > F of 0.2436). It can be concluded that the HHP pre-treatment before frozen storage did not affect the level of TBARS formation.

An increase in the levels of primary and secondary lipid oxidation compounds has been reported to result from pressure treatments (Gudmundsson, & Hafsteinsson, 2002; Lakshmanan, Piggott, & Patterson, 2003). However, a partial inhibition of peroxide formation has been observed in coho salmon throughout chilled storage after HHP treatments at 170 and 200 MPa for 30 s (Aubourg, Tabilo-Munizaga, Reyes, Rodríguez, & Pérez-Won, 2010). An increase in secondary lipid oxidation compounds (TBA-i) as a result of HHP treatments has also been observed for carpet (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 (*Trachurus trachurus*) (Erkan, Üretener, Alpas, Selçuk, Özden, & Buzrul, 2011) and Atlantic salmon (Amanatidou, Schlüter, Lemkau, Gorris, Smid, & Knorr, 2000). Different results have been observed when evaluating the effect of HHP treatment on TBARS formation throughout storage; thus, an increase was observed in cod and mackerel muscle stored at -2ºC (Ohshima et al., 1992), while an inhibitory effect on TBA-i score was observed in red mullet (*Mullus surmelutus*) muscle throughout the refrigerated storage at 4ºC (Erkan et al., 2010).

Literature data on the effect of HHP treatment on lipid oxidation development in fish can be considered somehow controversial. Although most research has shown an

increase in lipid oxidation as a result of the HHP treatment, extracted lipids have shown to be relatively stable against oxidation under HHP conditions and during further storage. Additionally, the possible prooxidant 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 complexation compound (EDTA, for example) was added (Gudmundsson, & Hafsteinsson, 2002; Lakshmanan et al., 2003). Consequently, iron-bound protein denaturation during HHP treatment has been reported to facilitate a free metal ion content increase which would be responsible for lipid oxidation in fish meat after HHP treatment.

3.3. Interaction compound formation

Mean values obtained for the FR assessment in raw and frozen control fish did not show significant differences ($p>0.05$) as a result of freezing and frozen storage time (Table 4). Concerning HHP-treated samples, a definite effect of frozen storage could not be concluded ($p>0.05$) when comparing samples corresponding to the same values for pressure and holding time. In most cases, control fish showed higher mean FR values than their corresponding HHP-treated samples. Such differences were found significant at month 3; at that time, samples corresponding to 450 MPa led to a lower FR value than their counterparts from 150 MPa. Additionally, fluorescence inhibition was also observed at months 1 and 3 by increasing the holding time at 450 MPa.

Although the Multifactor ANOVA F-value of 5.85 implied that the model with the three variables (frozen storage time, pressure and holding time) was significant (p-value probability > F of 0.0005), the correlation value ($r^2 = 0.56$) and the predicted (0.46) and adjusted (0.35) r^2 values can be considered as very low. It was observed that the effect exerted on the FR value by the pressure level (F-value = 23.75; p-value

probability > F of 0.0001) was higher than that of holding time (F-value = 1.14; p-value probability > F of 0.2941) and frozen storage time (F-value = 0.89; p-value probability > F of 0.3544). Additionally, an adequate precision (signal/noise) ratio was attained (12.25). The prediction of the model obtained for the effect of the two variables that exerted a higher influence on the FR value (pressure level and holding time) is shown in Figure 2.

The BR values were all within the small 0.57-0.79 range (Table 5). Values for fresh and frozen control fish showed no effect ($p>0.05$) of freezing and frozen storage time. Values for HHP-treated fish followed the same trend. Very small differences in the BR value were observed as a result of the pressure and holding time. Multifactor ANOVA analysis confirmed that none of the three variables (frozen storage time, pressure and holding time) exert a significant effect (F-value = 1.33; p-value probability > F of 0.2363) on the BR value.

Lipid oxidation is a complex process involving the formation of different classes of compounds, most of them unstable, and thus susceptible to breakdown and form lower weight compounds or react with other molecules, mostly nucleophilic type, present in fish muscle. This would be the case of peroxides and TBARS, widely reported to breakdown and give rise to tertiary (or interaction compounds) lipid oxidation compounds (Aubourg, 1999; Tironi, Tomás, & Añón, 2002). Peroxide and TBARS formation observed in this study were relatively low and this explains the low levels of fluorescence and browning compound formed, observed mostly at the longest frozen storage time when lipid oxidation was most significant.

3.4. Polyene index evolution

Mean PI values of frozen control samples showed no significant effect ($p>0.05$) of frozen storage time (Table 6). Concerning the effect of HHP conditions applied, the one-way ANOVA analysis showed no effect of holding time on the PI value (Table 6). Mean values for the control fish were lower than their equivalent storage time samples treated at 150, 300 and 450 MPa. Differences were significant for samples at month 3 and holding time 2.5 min; however, no significant differences ($p>0.05$) were found among samples applied different high pressures.

The multifactor ANOVA analysis led to an F-value of 4.28, which implied that the model was significant (p-value probability > F of 0.0026). The evaluation of the F-values of the different independent variables showed that PI score was mainly affected by the holding time (F-value = 12.92) and less by the frozen time (F-value = 1.59) and pressure (F-value = 0.24). However, the correlation value of the model ($r^2 = 0.64$) as well as the predicted and adjusted r^2 values (0.29 and 0.48, respectively) can be considered as very low. Additionally, an adequate precision (signal/noise) ratio was obtained (8.97). The prediction of the model obtained for the effect of the two variables that exerted a higher influence on PI score (holding time and frozen storage time) is shown in Figure 3.

Previous research has shown an important detrimental effect of lipid oxidation on the polyunsaturated fatty acid content (PI decrease). Since lipid oxidation (peroxide and TBARS formation) was relatively minor, losses of polyunsaturated fatty acid content would be expected to be minor too and consequently, yielding small PI differences among treatments. Previous research is consistent with this observation. For example, Ohshima et al. (1992) did not find differences in saturated, monounsaturated and polyunsaturated fatty acid groups in cod and mackerel stored 6 days at –2ºC when

previously treated at 200, 400 and 600 MPa for 15 min. Additionally, Aubourg et al. (2010) found no differences in coho salmon muscle PI as a result of the previous HHP treatment (135, 170 and 200 MPa for 30 s) and further chilled storage up to 20 days.

4. FINAL REMARKS

The effect of a previous HHP treatment on lipid damage in mackerel muscle was analysed throughout frozen storage at -10ºC. Lipid damage assessed by complementary determinations showed an important inhibition ($p<0.05$) of lipid hydrolysis increasing with pressure (from 150 to 450 MPa) and holding time (from 0 to 5 min) resulting in a remarkable inhibition ($p<0.05$) of FFA formation during frozen storage. Lipid oxidation analysis showed an inhibition ($p<0.05$) of tertiary lipid oxidation compound formation (fluorescence assessment) increasing with pressure level and pressure holding time. No effect ($p>0.05$) on primary (peroxide formation) or secondary (thiobarbiturtic acid index) oxidation compounds content was observed. Finally, the polyene index showed only minor HHP treatment differences.

The frozen storage of fatty fish species is strongly limited by damage to lipids reducing its commercialisation as is or as raw material for further processing (canning, smoking, etc.). Consequently, great attention is being devoted by manufacturers to find technological treatments increasing the shelf-life time of frozen fatty species and accordingly, its trading value. The work here presented provides for the first time information concerning the employment of HHP technology to inhibit lipid damage (hydrolysis and oxidation) during frozen storage. Additional research should examine the shelf life of fatty fish species kept frozen under commercial conditions (–18ºC) and include sensory and nutritional aspects.

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FIGURE LEGENDS

Figure 1

Prediction of the model obtained for the effect of frozen storage time (months) and pressure level (MPa) on the free fatty acid (FFA) content (g/100g lipids)*.

* Holding time was fixed at 2.5 min.

Figure 2

Prediction of the model obtained for the effect of pressure level (MPa) and holding time (min) on the fluorescence ratio (FR) value*.

* Frozen storage time was fixed at 3 months.

Figure 3

Prediction of the model obtained for the effect of holding time (min) and frozen storage time (months) on the polyene index content*.

* Pressure level was fixed at 450 MPa.

REFERENCES

Alizadeh, E., Chapleau, N., de Lamballerie, M., & Le-Bail, A. (2007). Effect of different freezing processes on the microstructure of Atlantic salmon (*Salmo salar*) fillets. *Innovative Food Science and Emerging Technologies, 8,* 493-499.

Amanatidou, A., Schlüter, O., Lemkau, K., Gorris, L., Smid, E., & Knorr, D. (2000). Effect of combined application of high pressure treatment and modified atmospheres on the shelf life of fresh Atlantic salmon. *Innovative Food Science and Emerging Technologies, 1*, 87-98.

Aubourg, S. (1999). Lipid damage detection during the frozen storage of an underutilized fish species. *Food Research International, 32*, 497-502.

Aubourg, S., Medina, I., & Pérez-Martín, R. (1996). Polyunsaturated fatty acids in tuna phospholipids: Distribution in the sn-2 location and changes during cooking. *Journal of Agricultural and Food Chemistry*, *44*, 585-589.

Aubourg, S., Rodríguez, A., & Gallardo, J. (2005). Rancidity development during frozen storage of mackerel (*Scomber scombrus*): Effect of catching season and commercial presentation. *European Journal of Lipid Science and Technology, 107,* 316-323.

Aubourg, S., Tabilo-Munizaga, G., Reyes, J., Rodríguez, A., & Pérez-Won, M. (2010). Effect of high-pressure treatment on microbial activity and lipid oxidation in chilled coho salmon. *European Journal of Lipid Science and Technology*, *112*, 362-372.

Bermúdez-Aguirre, D., Guerrero-Beltrán, A., Barbosa-Cánovas, G., & Welti-Chanes, J. (2011). Study of the inactivation of *Escherichia coli* and pectin methylesterase

in mango nectar under selected high hydrostatic pressure treatments. *Food Science and Technology International, 17*, 541-547.

Bligh, E., & Dyer, W. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Box, G., & Behnken, D. (1960). Some new three level designs for the study of quantitative variables. *Technometrics, 2*, 455-475.

Chapman, R., & McKay, J. (1949). The estimation of peroxides in fats and oils by the ferric thiocyanate method. *Journal of the American Oil Chemists' Society, 26*, 360-363.

Chevalier, D., Le Bail, A., & Ghoul, M. (2001). Effects of high pressure treatment (100-200 MPa) at low temperature on turbot (*Scophthalmus maximus*) muscle. *Food Research International, 34*, 425-429.

Decker, E., & Hultin, H. (1990). Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *Journal of Food Science, 55*, 947-950, 953.

Erickson, M. (1997). Lipid oxidation: Flavor and Nutritional Quality Deterioration in Frozen Foods. In M. Erickson, & Y.-C. Hung (Eds.), *Quality in Frozen Food* (pp. 141-173). New York, USA: Chapman & Hall.

Erkan, N., Üretener, G., & Alpas, H. (2010). Effect of high pressure (HP) on the quality and shelf life of red mullet (*Mullus surmelutus*). *Innovative Food Science and Emerging Technologies, 11*, 259-264.

Erkan, N., Üretener, G., Alpas, H., Selçuk, A., Özden, Ö., & Buzrul, S. (2011). Effect of high hydrostatic pressure (HHP) treatment on physicochemical properties of horse mackerel (*Trachurus trachurus*). *Food and Bioprocess Technology, 4*, 1322-1329.

FAO (2007) Fishery statistics. *Capture Production. Yearbook 2005*, 100/1, pp. 200-209, 242-267. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gómez-Estaca, J., Montero, P., Giménez, B., & Gómez-Guillén, M.C. (2007). Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*). *Food Chemistry, 105*, 511-520.

Gudmundsson, M., & Hafsteinsson, H. (2002). Minimal processing in practice: seafood. In T. Ohlsson, & N. Bengtsson (Eds.), *Minimal processing technologies in the food industry* (pp. 245-266). Boca Raton, Fl, USA: CRC Press.

Harris, P., & Tall, J. (1994). Rancidity in fish. In J. Allen, & R. Hamilton (Eds.), *Rancidity in foods* (pp. 256-272). London, UK: Chapman and Hall.

Hassan, I., Khallaf, M., Abd-El Fattah, L., & Yasin, N. (1999). Quality criteria, expiration period and marketing loss estimation of pre-treated and cold stored mullet fish. *Grasas y Aceites, 50*, 208-217.

He, H., Adams, R., Farkas, D., & Morrissey, M. (2002). Use of high-pressure processing for oyster shucking and shelf-life extension. *Journal of Food Science, 67*, 640-645.

Hurtado, J., Montero, P., & Borderías, A. J. (2001). Chilled storage of pressurized octopus (*Octopus vulgaris*) muscle. *Journal of Food Science, 66*, 400-406.

Kolakowska, A. (2003). Lipid oxidation in Food Systems. In Z. Sikorski, & A. Kolakowska (Eds.), *Chemical and functional properties of food lipids* (pp. 133-165). London, UK: CRC Press.

Lakshmanan, R., Parkinson, J., & Piggott, J. (2007). High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *Food Science and Technology, 40*, 544-551.

Lakshmanan, R., Pigott, J., & Paterson, A. (2003). Potential applications of high pressure for improvement in salmon quality. *Trends in Food Science and Technology, 14*, 354-363.

Lowry, R., & Tinsley, I. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society, 53*, 470-472.

Mackie, I. (1993). The effects of freezing on flesh proteins. *Food Reviews International, 9*, 575-610.

Martelo-Vidal, M. J., Mesas, J. M., & Vázquez, M. (2012) Low-salt restructured fish products from Atlantic mackerel (*Scomber scombrus*) with texture resembling turkey breast. *Food Science and Technology International, 18*, 251-259.

Murchie, L., Cruz-Romero, M., Kerry, J., Linton, M., Patterson, M., Smiddy, M., & Kelly, A. (2005). High pressure processing of shellfish: A review of microbiological and other quality aspects. *Innovative Food Science and Emerging Technologies, 6*, 257-270.

Norton, T., & Sun D.-W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology, 1*, 2-34.

Ohshima, T., Nakagawa, T., & Koizumi, C. (1992). Effect of high hydrostatic pressure on the enzymatic degradation of phospholipids in fish muscle during storage. In E. Bligh (Ed.), *Seafood Science and Technology*, Chapter 8 (pp. 64-75). Oxford, UK: Fishing News Books.

Ortea, I., Rodríguez, A., Tabilo-Munizaga, G., Pérez-Won, M., & Aubourg, S. (2010). Effect of hydrostatic high-pressure treatment on proteins, lipids and nucleotides in chilled farmed salmon (*Oncorhynchus kisutch*) muscle. *European Food Research and Technology, 230*, 925-934.

Pérez-Won, M., Barraza, M., Cortés, F., Madrid, D., Cortés, P., Roco, T., Osorio, F., & Tabilo-Munizaga, G. (2006). Textural characteristics of frozen blue squat lobster (*Cervimunida johni*) tails as measured by instrumental and sensory methods. *Journal of Food Engineering, 29,* 519-531.Ramírez, R., Saraiva, J., Pérez Lamela, C., & Torres, J. A. (2009). Reaction kinetics analysis of chemical changes in pressure-assisted thermal processing. *Food Engineering Reviews, 1*, 16-30.

Refsgaard, H., Brockhoff, P., & Jensen, B. (2000). Free polyunsaturated fatty acids cause taste deterioration of salmon during frozen storage. *Journal of Agricultural and Food Chemistry, 48,* 3280-3285.

Richards, M., & Hultin, H. (2002). Contributions of blood and blood components to lipid oxidation in fish muscle. *Journal of Agricultural and Food Chemistry, 50,* 555-564.

Rouillé, J., Le Bail, A., Ramaswamy, H., & Leclerc, L. (2002). High pressure thawing of fish and shellfish. *Journal of Food Engineering, 53,* 83-88.

Saeed, S., & Howell, N. (2001). 12-lipoxygenase activity in the muscle tissue of Atlantic mackerel (*Scomber scombrus*) and its prevention by antioxidants. *Journal of the Science of Food and Agriculture, 81,* 745-750.

Saeed, S., & Howell, N. (2002). Effect of lipid oxidation and frozen storage on muscle proteins of Atlantic mackerel (*Scomber scombrus*). *Journal of the Science of Food and Agriculture, 82,* 579-586.

Sequeira-Muñoz, A., Chevalier, D., Le Bail, A., Ramaswamy, H., & Simpson, J. (2006). Physicochemical changes induced in carp (*Cyprinus carpio*) fillets by high pressure processing at low temperature. *Innovative Food Science and Emerging Technologies, 7,* 13-18.

Sikorski, Z., & Kolakowska, A. (1994). Changes in protein in frozen stored fish. In Z. Sikorski, B. Sun Pan, & F. Shahidi (Eds.), *Seafood proteins* (pp. 99-112). New York, USA: Chapman and Hall.

Tironi, V., Tomás, M., & Añón, MªC. (2002). Structural and functional changes in myofibrillar proteins of sea salmon (*Pseudopercis semifasciata*) by interaction with malondialdehyde (RI). *Journal of Food Science, 67*, 930-935.

Torres, J. A., & Velázquez, G. (2005). Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering, 67*, 95-112.

Uresti, R., Velázquez, G., Vázquez, M., Ramírez, R., & Torres, J. A. (2005). Effects of sugars and polyols on the functional and mechanical properties of pressure-treated arrowtooth flounder (*Atheresthes stomias*) proteins. *Food Hydrocolloids, 19*, 964-973.

Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. *Fette Seifen Anstrichmittel, 72*, 1084-1087.

Yagiz, Y., Kristinsson, H., Balaban, M., & Marshall, M. (2007). Effect of high pressure treatment on the quality of rainbow trout (*Oncorhynchus mykiss*) and mahi mahi (*Coryphaena hippurus*). *Journal of Food Science, 72*, C509-C515.

Zotos, A., & Vouzanidou, M. (2012). Seasonal changes in composition, fatty acid, cholesterol and mineral content of six highly commercial fish species of Greece. *Food Science and Technology International, 18*, 139-149.

TABLE 1

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Free fatty acid (g/ 100g lipids) assessment* in frozen mackerel muscle previously processed under different high hydrostatic pressure conditions**

Frozen storage time (months)	**Holding time** (min)	**Pressure** (MPa)			
		Control	**150**	**300**	**450**
0	0.0	y 1.34 (0.10)	z 0.63 (0.01)	z 0.71 b (0.13)	z 0.66 b (0.16)
	2.5	y 1.34 (0.10)	z 0.66 (0.20)	z 0.52 ab (0.09)	z 0.64 b (0.14)
	5.0	x 1.34 (0.10)	y 0.62 (0.12)	zy 0.47 a (0.10)	z 0.38 a (0.06)
1	0.0	y 3.46 (0.35)	z 1.73 (0.22)	z 1.72 b (0.35)	z 1.51 c (0.12)
	2.5	x 3.46 (0.35)	y 1.87 (0.28)	zy 1.30 ab (0.18)	z 1.10 b (0.26)
	5.0	w 3.46 (0.35)	x 2.20 (0.33)	y 1.09 a (0.22)	z 0.77 a (0.02)
3	0.0	x 4.10 (0.19)	y 3.69 b (0.15)	z 2.48 b (0.23)	z 2.71 b (0.12)
	2.5	w 4.10 (0.19)	x 3.01 a (0.43)	y 2.13 ab (0.27)	z 1.14 a (0.23)
	5.0	x 4.10 (0.19)	y 2.79 a (0.30)	z 1.98 a (0.25)	z 1.39 a (0.35)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. Starting raw fish value: 0.20±0.10.

- ** For each frozen storage time and pressure, mean values followed by different letters (a, b, c) indicate significant ($p<0.05$) differences as a result of holding time. For each frozen storage time and holding time, mean values preceded by different letters (z, y, x, w) indicate significant ($p<0.05$) differences as a result of pressure. No letters are indicated when significant differences are not found ($p>0.05$).

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TABLE 2

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- **Peroxide value (meq active oxygen/ kg lipids) assessment* in frozen mackerel muscle previously processed under different high hydrostatic pressure conditions****

Frozen storage time (months)	**Holding time** (min)	**Pressure** (MPa)			
		Control	**150**	**300**	**450**
0	0.0	y 1.10 (0.51)	z 0.50 a (0.06)	zy 0.83 (0.45)	zy 1.24 (0.74)
	2.5	1.10 (0.51)	0.79 b (0.18)	1.12 (0.87)	0.66 (0.08)
	5.0	1.10 (0.51)	0.86 b (0.25)	0.87 (0.28)	0.54 (0.24)
1	0.0	1.12 (0.41)	0.65 (0.48)	0.62 (0.12)	1.16 (0.46)
	2.5	1.12 (0.41)	1.24 (0.47)	0.98 (0.48)	1.23 (0.60)
	5.0	1.12 (0.41)	0.97 (0.44)	0.91 (0.31)	1.02 (0.37)
3	0.0	y 2.97 (1.81)	y 2.32 (1.03)	z 0.45 a (0.19)	zy 0.92 (0.77)
	2.5	2.97 (1.81)	1.88 (0.87)	1.59 b (0.45)	1.05 (0.34)
	5.0	2.97 (1.81)	1.10 (0.57)	0.85 ab (0.43)	1.03 (0.10)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. Starting raw fish value: 0.17±0.25.

** For each frozen storage time and pressure, mean values followed by different letters (a, b) indicate significant ($p<0.05$) differences as a result of holding time. For each frozen storage time and holding time, mean values preceded by different letters (z, y) indicate significant ($p<0.05$) differences as a result of pressure. No letters are indicated when significant differences are not found ($p>0.05$).

- **TABLE 3** -

Thiobarbituric acid value (mg malondialdehyde/ kg muscle) assessment* in frozen mackerel muscle previously processed under different high hydrostatic pressure conditions**

Frozen storage time (months)	**Holding time** (min)	**Pressure** (MPa)			
		Control	**150**	**300**	**450**
0	0.0	0.50 (0.22)	0.62 a (0.04)	0.59 a (0.10)	0.65 (0.06)
	2.5	0.50 (0.22)	0.82 b (0.13)	0.95 ab (0.53)	0.82 (0.13)
	5.0	z 0.50 (0.22)	y 0.97 b (0.09)	y 0.91 b (0.17)	z 0.58 (0.14)
1	0.0	0.70 (0.26)	0.68 ab (0.22)	0.47 a (0.08)	0.93 (0.20)
	2.5	0.70 (0.26)	0.97 b (0.16)	0.82 b (0.19)	0.65 (0.19)
	5.0	0.70 (0.26)	0.60 a (0.19)	0.95 b (0.17)	0.72 (0.07)
3	0.0	zy 0.88 (0.24)	y 0.88 (0.05)	z 0.48 a (0.01)	zy 0.70 a (0.14)
	2.5	0.88 (0.24)	1.01 (0.28)	0.91 b (0.22)	1.11 b (0.18)
	5.0	0.88 (0.24)	0.97 (0.09)	0.81 b (0.12)	0.74 a (0.16)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. Starting raw fish value: 0.22±0.11.

** For each frozen storage time and pressure, mean values followed by different letters (a, b) indicate significant ($p<0.05$) differences as a result of holding time. For each frozen storage time and holding time, mean values preceded by different letters (z, y) indicate significant ($p<0.05$) differences as a result of pressure. No letters are indicated when significant differences are not found ($p>0.05$).

TABLE 4

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Fluorescence formation assessment* in frozen mackerel muscle previously processed under different high hydrostatic pressure conditions**

Frozen storage time (months)	**Holding time** (min)	**Pressure** (MPa)			
		Control	**150**	**300**	**450**
0	0.0	0.77 (0.50)	0.29 a (0.11)	0.22 (0.04)	0.36 (0.14)
	2.5	0.77 (0.50)	0.36 a (0.22)	0.52 (0.68)	0.35 (0.10)
	5.0	zy 0.77 (0.50)	y 0.68 b (0.04)	zy 0.53 (0.46)	z 0.36 (0.15)
1	0.0	zy 1.05 (0.85)	zy 0.38 ab (0.05)	z 0.24 (0.10)	y 0.45 b (0.07)
	2.5	zy 1.05 (0.85)	y 0.43 b (0.05)	y 0.32 (0.09)	z 0.17 a (0.04)
	5.0	zy 1.05 (0.85)	y 0.32 a (0.03)	zy 0.22 (0.02)	z 0.15 a (0.05)
3	0.0	x 0.82 (0.10)	y 0.58 (0.06)	zy 0.45 (0.20)	z 0.28 b (0.02)
	2.5	y 0.82 (0.10)	y 0.87 (0.30)	z 0.26 (0.22)	z 0.14 a (0.01)
	5.0	x 0.82 (0.10)	y 0.57 (0.08)	z 0.17 (0.03)	z 0.18 a (0.04)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. Starting raw fish value: 0.58±0.13.

** For each frozen storage time and pressure, mean values followed by different letters (a, b) indicate significant ($p<0.05$) differences as a result of holding time. For each frozen storage time and holding time, mean values preceded by different letters (z, y, x) indicate significant ($p<0.05$) differences as a result of pressure. No letters are indicated when significant differences are not found ($p>0.05$).

TABLE 5

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Browning development assessment* in frozen mackerel muscle previously processed under different high hydrostatic pressure conditions**

Frozen storage time (months)	**Holding time** (min)	**Pressure** (MPa)			
		Control	**150**	**300**	**450**
0	0.0	0.70 (0.05)	0.63 (0.09)	0.70 (0.11)	0.57 (0.13)
	2.5	0.70 (0.05)	0.67 (0.10)	0.53 (0.24)	0.63 (0.08)
	5.0	0.70 (0.05)	0.62 (0.05)	0.73 (0.19)	0.70 (0.04)
1	0.0	y 0.79 (0.08)	zy 0.57 (0.11)	zy 0.61 (0.08)	z 0.55 (0.12)
	2.5	y 0.79 (0.08)	z 0.57 (0.08)	zy 0.64 (0.19)	z 0.57 (0.11)
	5.0	y 0.79 (0.08)	zy 0.65 (0.10)	z 0.54 (0.07)	zy 0.66 (0.07)
3	0.0	0.64 (0.07)	0.64 (0.07)	0.66 (0.04)	0.70 (0.03)
	2.5	0.64 (0.07)	0.52 (0.07)	0.57 (0.10)	0.60 (0.08)
	5.0	0.64 (0.07)	0.63 (0.10)	0.69 (0.03)	0.68 (0.11)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. Starting raw fish value: 0.69±0.07.

** No significant ($p>0.05$) differences were obtained as a result of holding time. For each frozen storage time and holding time, mean values preceded by different letters (z, y) indicate significant ($p<0.05$) differences as a result of pressure; no letters are indicated when significant differences are not found ($p>0.05$).

TABLE 6

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Polyene index assessment* in frozen mackerel muscle previously processed under different high hydrostatic pressure conditions**

Frozen storage time (months)	**Holding time** (min)	**Pressure** (MPa)			
		Control	**150**	**300**	**450**
0	0.0	1.05 (0.08)	1.18 (0.09)	1.10 (0.13)	1.02 (0.07)
	2.5	1.05 (0.08)	1.13 (0.19)	1.04 (0.09)	1.01 (0.04)
	5.0	1.05 (0.08)	1.09 (0.07)	1.10 (0.05)	1.10 (0.04)
1	0.0	0.97 (0.12)	1.06 (0.09)	0.96 (0.17)	0.98 (0.07)
	2.5	0.97 (0.12)	1.05 (0.08)	1.01 (0.11)	0.98 (0.14)
	5.0	0.97 (0.12)	0.98 (0.07)	0.95 (0.12)	1.09 (0.17)
3	0.0	0.92 (0.05)	0.96 (0.06)	0.99 (0.04)	0.96 (0.05)
	2.5	z 0.92 (0.05)	y 1.11 (0.08)	y 1.07 (0.05)	zy 1.05 (0.16)
	5.0	0.92 (0.05)	1.26 (0.33)	1.00 (0.24)	1.01 (0.13)

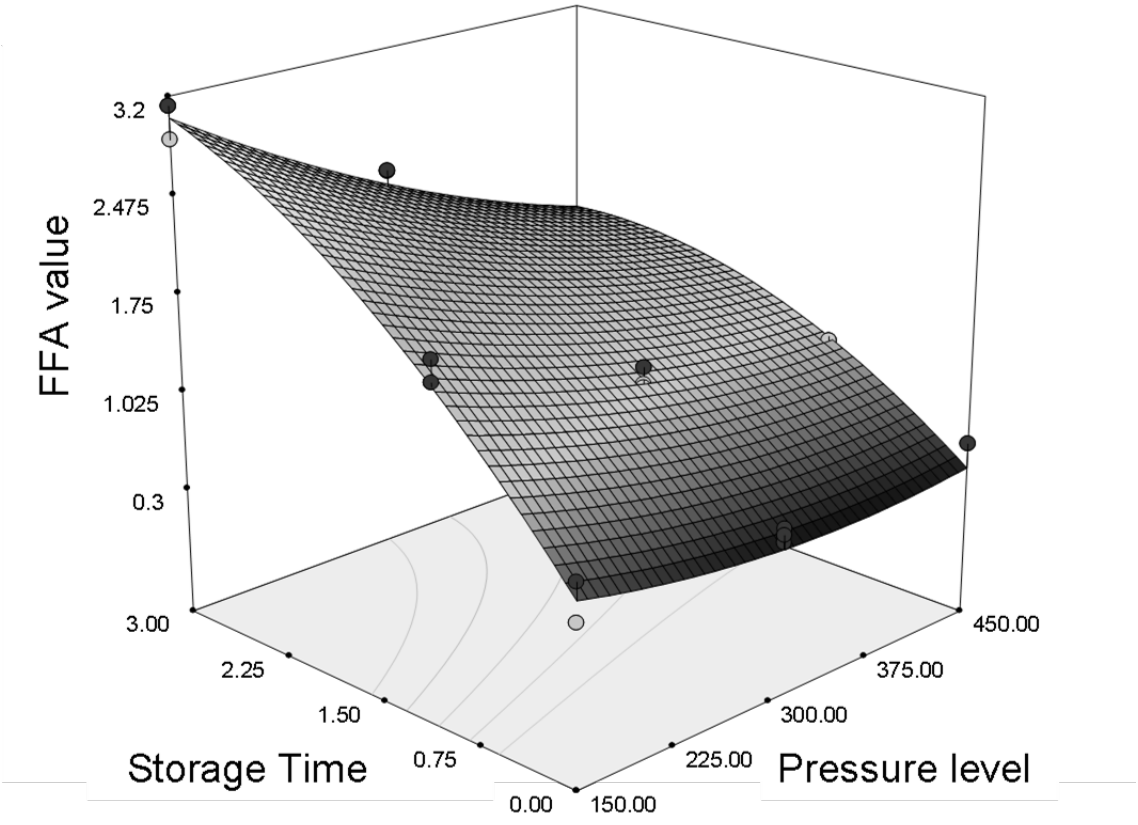
* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets. Starting raw fish value: 1.05±0.14.

** No significant ($p>0.05$) differences were obtained as a result of holding time. For each frozen storage time and holding time, mean values preceded by different letters (z, y) indicate significant ($p<0.05$) differences as a result of pressure; no letters are indicated when significant differences are not found ($p>0.05$).

1

1 Figure

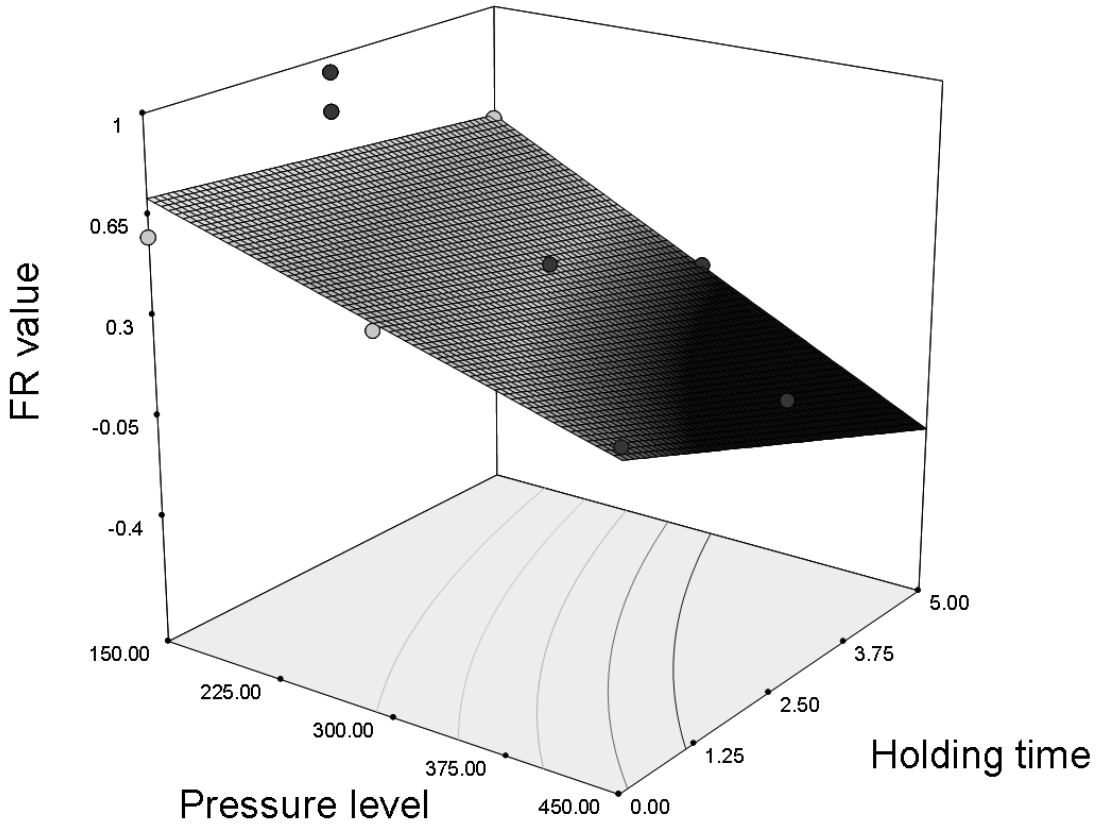
2 1



3

4

1 Figure 2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0.3
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150.00
225.00
300.00
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Pressure level
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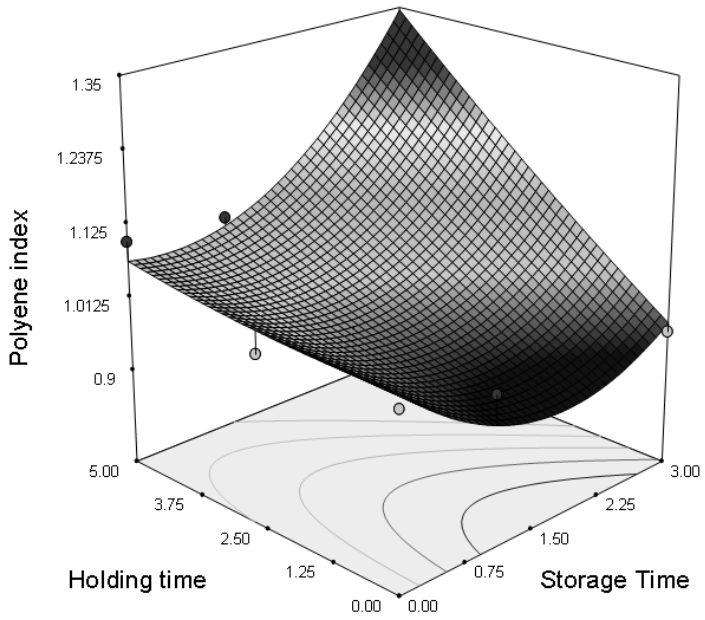
1.25
2.50
3.75
5.00
Holding time

2

3

1 Figure 3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1.125
1.0125
0.9
5.00
3.75
2.50
1.25
0.00
Holding time
0.00
0.75
1.50
2.25
3.00
Storage Time

2