Lipid damage inhibition by previous high pressure processing in frozen horse mackerel

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SUMMARY

This work focuses on the effect of a previous high pressure processing (HPP) on the lipid damage development occurring during the frozen storage (-10ºC; up to 3 months) of Atlantic horse mackerel (*Trachurus trachurus*). HPP conditions included different pressure (150, 300, 450 MPa) and pressure holding time (0.0, 2.5, 5.0 min) values. During frozen storage, horse mackerel muscle was analysed for lipid hydrolysis (free fatty acid assessment) and oxidation (formation of peroxides, thiobarbituric acid reactive substances and fluorescent compounds), and polyene content. An inhibition of lipid hydrolysis development was observed; thus, both an increasing pressure level and pressure holding time led to a marked inhibition of FFA content throughout the frozen storage. Concerning the lipid oxidation development, a partial inhibition was also produced during the frozen storage (months 1 and 3) by increasing the pressure level applied (namely, fluorescent and peroxide compound formation); however, pressure holding time did not led to a definite trend. No effect of HPP treatment was concluded on the polyene content of the fish muscle lipids. Present research provides novel information concerning the employment of HPP technology focused on the inhibition of lipid damage during a subsequent frozen storage.

Running Title: Frozen storage of high pressure-treated horse mackerel

Keywords: *Trachurus trachurus*; high pressure; frozen storage; lipid hydrolysis; lipid oxidation

PRACTICAL APPLICATIONS

Frozen storage of fatty and medium-fat fish species is known to be strongly limited by lipid damage development, which is a drawback to its commercialisation as such or to its subsequent employment as raw material in other kinds of processing (canneries, smoking, etc.). Present research provides valuable information concerning the employment of the high pressure technology to inhibit lipid damage development during the subsequent frozen storage of Atlantic horse mackerel (*Trachurus trachurus*). Thus, both an increasing pressure level (from 150 to 450 MPa) and pressure holding time (from 0 to 5 min) led to a marked inhibition of lipid hydrolysis during frozen storage. Additionally, inhibition of lipid oxidation was produced by increasing the pressure level. Since the response to high pressure processing of marine species has been reported to vary with species, a preliminary study is recommended to be carried out before applying the high pressure-frozen storage combining strategy.

1. INTRODUCTION

Fish and other marine species give rise to products of great importance to the economies of many countries. Freezing technology is often used to retain the sensory and nutritional properties of fish products for direct consumption or as raw materials for other technological processes. However, marine species with a highly unsaturated lipid composition, and an important presence of prooxidant molecules such as endogenous enzymes and transition metals, suffer even under frozen conditions the development of rancidity resulting in a loss of quality and shelf life [1,2]. Due to this important drawback for frozen fish commercialization, new and advanced treatments are required to inhibit lipid oxidation development.

High pressure processing (HPP) has been shown to inactivate microbial development and extend shelf life. This technology has shown potential application in the seafood industry in surimi production [3], cold-smoked fish preparation [4], pressure-assisted thawing [5], and thermal processing [6]. An additional positive HPP effect is that hydrolytic (namely, lipases and phospholipases) and oxidative (peroxidases, lipoxygenases, etc.) endogenous enzymes could be inactivated before storage or subsequent processing of fish products [7, 8]. Thus, a positive effect on quality retention has been observed when HPP was employed before refrigeration [9, 10] or chilled [11] storage; however, research related to frozen storage of HPP-treated fish products has been limited [12]. Additionally, previous research concerning lipid changes as a result of HPP treatment in fish is also limited when compared to information related to microbial activity and protein deterioration studies.

The fish industry is suffering from a dwindling availability of traditional species increasing the commercial interest in the exploitation of unconventional sources of raw material. One such species is Atlantic horse mackerel (*Trachurus trachurus*), a medium-fat content fish abundant in the Atlantic Northeast. Efforts have been made to utilize it for chilled [13] and restructured [14] products. When stored frozen, previous research has shown a marked lipid oxidation and quality loss [15]; accordingly, great efforts have been made to enhance its shelf life in the frozen state as whole [16] or fillet [17] products.

The aim of this study was to investigate the potential benefits during the frozen storage of horse mackerel by HPP treatment prior to freezing. To this end, lipid hydrolysis and oxidation were analysed in horse mackerel muscle throughout the frozen

storage as a function of the pressure level and pressure holding time conditions of the HPP treatment.

2. MATERIALS AND METHODS

2.1. Raw fish, processing, storage and sampling

Atlantic horse mackerel (65 kg) caught near the Bask coast in Northern Spain was obtained at the Ondarroa harbour (Bizkaia, Spain) and transported in ice to the AZTI Tecnalia (Derio, Spain) pilot plant for HPP treatment within 6 h after catch. Whole horse mackerel individuals (25-30 cm and 200-250 g range) were placed in flexible polyethylene bags (three individuals per bag) and vacuum sealed at 400 mbar.

HPP treatments at 150-450 MPa (pressure levels) and 0-5 min (pressure holding times) were conducted in a 55-L high pressure unit (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain) according to the experiment design that included the following treatments: 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 HPP treatment at room temperature (20ºC).

After HPP treatments, horse 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 HPP treatment and subjected to the same freezing and frozen storage conditions was used as control (T-0 treatment); starting fresh fish was also analysed. Three batches or replicates (n=3) for each treatment (T-0 to T-12; starting fresh fish) were analysed independently. Each analysis was based on the lipid fraction extracted from the fish white muscle pooled from two individual fish.

The response to the HPP treatment of marine species has been reported to vary with species, chemical composition and size [7, 18]. Consequently, a preliminary study was undertaken to elucidate the pressure conditions to be applied in the present study. For it, a wide range of pressure (600, 500, 400, 350, 300, 250, 200 and 100 MPa) values was tested for 5 minutes as pressure holding time and compared to untreated horse mackerel by means of sensory analysis. Thus, different sensory descriptors (eyes,

external colour, hardness, external odour, blood, skin and gills) were analysed by a sensory panel, according to guidelines concerning fresh and refrigerated fish [19]. At each pressure condition, the fish were presented to panellists in individual trays and scored individually. The panel members shared samples tested. 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 0-300-MPa range while at higher pressure, blood coagulated and the gills colour was markedly lighter. Accordingly, 300 MPa was chosen as the mid pressure point to be studied in the present research which included also a lower and a higher pressure value of 150 and 450 MPa, respectively.

2.2. Lipid hydrolysis analysis

Lipids were extracted following the Bligh and Dyer [20] method, i.e., a single-phase lipid solubilisation with a (1:1) chloroform-methanol mixture and expressed as g lipid kg^{-1} muscle.

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle following the Lowry and Tinsley [21] 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 kg^{-1} lipids.

2.3. Lipid oxidation assessment

The peroxide value (PV) was determined on the lipid extract by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay [22] method. Results were expressed as meq active oxygen kg^{-1} lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke [23] based on the reaction between a trichloracetic acid extract of the fish muscle and thiobarbituric acid. Content of thiobarbituric acid reactive substances (TBARS) was 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 from measurements at 393/463 nm and 327/415 nm for the aqueous phase obtained when extracting lipids [24] and quantified as relative fluorescence (RF) calculated as $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum and F_{st} is the fluorescence

intensity of a standard quinine sulphate solution (1 μg ml^{-1} in 0.05 M H_2SO_4) at the corresponding wavelength. Results were expressed as the fluorescence ratio (FR), which was calculated as the ratio of the RF values at each excitation/emission maximum according to the following equation: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$.

2.4. Polyene index assessment

Lipid extracts were converted into fatty acid methyl esters (FAME) by employing acetyl chloride and then analysed by gas chromatography [11]. FAME were analysed by means of a Perkin-Elmer 8700 chromatograph employing a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, Supelco Inc., Bellefonte, PA, USA), nitrogen at 10 psi as carrier gas, and a flame ionisation detector (FID) at 250ºC. Peaks corresponding to fatty acids were identified by comparison of their retention times with those for standard mixtures (Qualmix Fish, Larodan, Malmö, Sweden; FAME Mix, Supelco, Bellefonte, PA, USA). Peak areas were automatically integrated with 19:0 fatty acid being used as internal standard for quantitative analysis. Finally, the polyene index (PI) was calculated as the (C20:5ω3 + C22:6ω3)/C16:0 fatty acid ratio.

2.5. Statistical analysis

An experimental design taking into account that samples from each treatment were analysed after 0, 1 and 3 months of frozen storage was obtained using the Design Expert® 7.1.1 software (Stat-Ease, Inc., Minneapolis, MN, USA). The model used was validated through a multifactor ANOVA test. The experiments T-1 to T-12 followed a three-level factorial design for two factors (pressure level and pressure holding time) [25]. Analyses were repeated for each frozen storage time and the whole set of data was fitted together to obtain the mathematical models. This strategy allows both to determine the effect of each variable of the HPP (pressure level and pressure holding time) and the frozen storage time on the lipid damage indices.

3. RESULTS AND DISCUSSION

3.1. Lipid hydrolysis development

Lipid content (10.5-15.5 g kg^{-1} muscle) was consistent with published values for this medium fat content fish [26]. The evaluation of the FFA content in fresh and in frozen control fish showed a progressive lipid hydrolysis development as a result of freezing and subsequent frozen storage (Table 1). The sharp FFA increase observed

after 3 months is consistent with previous research [15, 16]. In the case of HPP-treated samples (Table 2), higher mean FFA values were observed for all pressure levels with 0 holding time as compared to samples treated with longer pressure holding times. Differences were important in most cases for fish samples treated at 300 and 450 MPa.

The analysis of the pressure effect led to different conclusions depending on the storage time. Samples corresponding to frozen storage time 0 and showing only the HPP effect on the freezing step had lower mean FFA scores when treated at 150 MPa. However, after 1 and 3 months of frozen storage, the 300 and 450 MPa HPP-treated samples had lower FFA content than the control and the 150 MPa treated samples.

Since the three independent variables (pressure, pressure holding time and frozen storage time) showed a marked effect on FFA formation, a multifactor ANOVA analysis was necessary to assess their relative influences. A significant ($p<0.0001$) model with an F-value of 69.09 was used to confirm the significant effect of each independent variable. FFA formation was strongly affected by frozen storage time (F-value = 464.84; p-value probability > F was $p \leq 0.0001$), although an important effect of pressure and pressure holding time could also be concluded, according to their F-value scores (26.48 and 12.99, respectively; p-value probability > F were $p \leq 0.0001$ and 0.0014, respectively). The correlation value of the model was $r^2 = 0.9613$ with adjusted and predicted r^2 values of 0.9474 and 0.9195, respectively, in addition to a signal/noise ratio of 25.33. All these statistical parameters confirmed that an empirical coded equation could be used to model the effect of HPP pre-treatment and frozen storage on the FFA formation. The model prediction for the effect of the two variables that exerted the most influence on FFA formation (frozen storage time and pressure level) is shown in Figure 1.

Previous research concerning the effect of HPP treatment on FFA formation is scarce and can be considered as non-existent in studies concerning the frozen storage of fish. An increasing FFA formation was observed during chilled storage of turbot [27] and carp [28] muscle after applying low pressure levels (from 100 to 200 MPa). He et al. [9] did not observe inhibition of lipase activity in refrigerated (4ºC) oysters previously pressurized at 207-310 MPa for 1-2 min. The same conclusion was reached by Gómez-Estaca et al. [29] when studying cold-smoked sardine stored at 5ºC for up to 21 days when previously treated at 300 MPa for 15 min. On the other hand, Ohshima et al. [30] found that enzymatic degradation of phospholipids in cod muscle was

successfully inhibited during storage at -2ºC for up to 6 days when previously treated at 400 or 600 MPa for 15 or 30 min.

Accumulation of FFA in fish muscle has no nutritional significance, but is found undesirable due to secondary reactions, such as muscle texture changes, lipid oxidation enhancement and interrelation with off-odour development [1, 2]. Different kinds of fish species have shown an important development of lipid hydrolysis during frozen storage as a result of endogenous enzyme (namely, lipases and phospholipases) activity [24, 31]. Present results show that the employment of the HPP technology prior to freezing and frozen storage steps can lead to a significant reduction of the FFA formation in the frozen product, this likely resulting from an inhibitory effect on the hydrolytic behaviour of the above-mentioned enzymes.

3.2. Lipid oxidation development

In this study, peroxide formation was relatively low and all measurements remained below 8 meq active oxygen kg^{-1} lipids (Tables 1-2). At time 0 months, the comparison of peroxide values for starting fresh fish and frozen control samples showed no peroxide formation caused by freezing, whereas peroxide values increased during frozen storage (Table 1). Concerning HPP-treated fish (Table 2), pressure holding time showed some influence on peroxide values during frozen storage such as the inhibitory effect observed after 3 months for samples treated at 150 MPa; however, its effect on peroxide values was not consistent. Concerning the pressure effect, higher mean peroxide values were observed after 1 and 3 months of frozen storage in control fish individuals as compared to the corresponding HPP-treated samples.

The multifactor ANOVA analysis to assess the relative influence of the three variables yielded an F-value of 27.24 implying that the model was significant with a p-value probability > F of 0.0001. The effect of frozen storage time (F-value = 79.69; p-value probability > F of 0.0001) was higher than the one observed for the pressure holding time (F-value = 8.159; p-value probability > F of 0.0078) and pressure (F-value = 0.0114; p-value probability > F of 0.9156). The correlation value of the model was r^2 = 0.7381 and the adjusted and predicted r^2 values were 0.7110 and 0.6532, respectively, while the signal/noise ratio was 15.62. The prediction of the model obtained for the effect of the two variables exerting the most influence on peroxide formation (frozen storage time and pressure holding time) is expressed in Figure 2.

Concerning secondary lipid oxidation, an increase in the TBA-i due to freezing and frozen storage was not observed (Table 1). Related to HPP-treated samples (Table 2), the pressure holding time effect on the TBA-i showed no general trend. For example, TBA-i increased with pressure holding time after 0 and 1 month frozen storage when samples were treated at 300 MPa, whereas an inhibitory effect of pressure holding time was observed after 0 and 3 months of storage when samples were treated at 150 and 450 MPa, respectively. The pressure effect on TBA-i (Table 2) was also inconsistent with higher mean values in most 450 MPa treated samples when compared to other HPP-treated and control samples, while TBA-i values in fish samples treated at 150 and 300 MPa was generally lower than in controls.

The multifactor ANOVA analysis taking into account the comparative effect of the three variables (frozen storage time, pressure and pressure holding time) on the TBARS formation yielded a relatively low F-value (4.86), although the model was found significant (p-value probability > F of 0.0012). F-values obtained for the pressure level and pressure holding time (10.27 and 6.436, respectively) were found significant (p-value probability > F where $p \leq 0.0038$ and 0.0181, respectively) showing that these process variables had a stronger effect on TBARS formation than frozen storage time (F-value = 0.2939; p-value probability > F of 0.5928). The correlation value of the model was $r^2 = 0.6183$ with an adjusted and predicted r^2 values of 0.4910 and 0.3291, respectively, while the signal/noise ratio was 8.307.

Previous research reporting the effect of HPP treatments on the formation of primary oxidation compounds is scarce. Ohshima et al. [30] showed an increase in cod and mackerel muscle when the pressure increased from 200 to 600 MPa (15 and 30 min treatments). A similar conclusion was obtained using a model system containing sardine lipids [32]; thus, peroxide values increased throughout storage at 5ºC for up to 4 days when treated at 150 MPa for 15 or 30 min. On the other hand, peroxide formation was partially inhibited in Coho salmon during chilled storage when previously treated at 170 and 200 MPa for 30 s [11].

An increase of TBA-i as a result of HPP treatments has been observed for carp [28] and turbot [27] fillets, both showing an increasing effect with pressure holding time. However, no differences in TBARS formation were observed in Atlantic salmon [33] after applying a 50-200 MPa treatment for 15 min. No effect in TBARS formation in horse mackerel was also observed by Erkan et al. [34] after 220, 250 and 330 MPa treatments for 5 and 10 min; additionally, no effect was observed in such study on b*

307 values (yellowness/blueness), a colour parameter closely related to lipid oxidation.
308 Previous research has shown an increase in TBARS as a result of HPP treatment,
309 followed by subsequent fish storage/processing. This was the case of chilled rainbow
310 trout [18], cold-smoked salmon [4], and cod or mackerel muscle stored at -2ºC [30]. On
311 the other hand, an inhibitory effect has been reported in other storage studies. Thus, a
312 lower TBARS formation was found in HPP-treated fish than in control ones when
313 minced albacore muscle was HPP-treated (275 and 310 MPa; 2-6 min) and then
314 refrigerated at 4ºC [35]; additionally, an inhibitory effect on TBA-i score was also
315 attained in red mullet (*Mullus surmelutus*) muscle during storage at 4ºC [10].
316 The formation of fluorescent compounds (Tables 1-2) is reflected in FR values
317 in the low 0.17-0.59 range, consistent with the relatively low peroxide and TBARS
318 values previously mentioned [15]. The analysis of untreated fresh fish and frozen
319 control samples showed a small FR increase caused by freezing and frozen storage
320 (Table 1). Higher mean values were obtained after 1 and 3 months of frozen storage in
321 control samples when compared to any HPP-treated samples (Tables 1-2); additionally,
322 lower mean values were obtained in samples corresponding to 300 and 450 MPa
323 treatments when compared to their counterpart control samples. After 3 months of
324 frozen storage, the following decreasing sequence in FR was observed: control > 150
325 MPa > 300- and 450-MPa.
326 A multifactor ANOVA analysis was also carried out to take into account the
327 comparative effect of the three variables (frozen storage time, pressure and pressure
328 holding time) on the FR. The F-value obtained (11.96) implied that the model was
329 significant (p-value probability > F of 0.0001). F-values obtained for both pressure level
330 and frozen storage time (32.04 and 23.48, respectively) were found significant (p-value
331 probability > F were $p \leq 0.0001$ in both cases). However, the F-value (1.982) obtained
332 for the pressure holding time was not significant (p-value probability > F of 0.1710).
333 The correlation value of the model was $r^2 = 0.7864$ with adjusted and predicted r^2 values
334 of 0.7207 and 0.6290, respectively, while the signal/noise ratio was 12.21. The model
335 prediction for the effect of the two variables with the higher influence on fluorescent
336 compound formation (pressure and storage time) is shown in Figure 3.
337 Lipid oxidation is a complex process producing many different compounds,
338 most of them unstable and thus breaking down into smaller molecular weight ones or
339 reacting with other compounds, mostly nucleophilic-type, present in fish muscle. This is
340 the case of peroxides and TBARS, widely reported to give rise to tertiary (or interaction

compounds) lipid oxidation compounds [24, 36]. In this study, TBARS formation throughout frozen storage was found to be negligible, while peroxide values reached only 3-8 meq active oxygen kg^{-1} lipids (Tables 1-2). However, a marked increase of the interaction compound formation was found to be important for samples with the longest frozen storage time. As a result, samples corresponding to 3-month storage showed an inhibitory effect of pressure level applied on the FR value obtained, this likely resulting from an inhibitory effect on the pro-oxidant behaviour of the endogenous enzymes (peroxidases, lipoxygenases, etc.).

3.3. Polyene index evolution

All polyene index values fell within a very small range (1.44-1.76; Tables 1-2). A comparison of values for fresh and frozen control samples showed that freezing and frozen storage did not cause important changes in this parameter (Table 1). Pressure treatments cause almost no differences and thus general trends concerning pressure and pressure holding time effects on the polyene content in horse mackerel lipids could not be inferred (Table 2). This was confirmed by multifactor ANOVA analysis yielding a low F-value (1.21) implying that the model was not significant (p-value probability > F of 0.3301).

Previous research has shown an important detrimental effect of lipid oxidation on the polyunsaturated fatty acid content, i.e., a decrease in the polyene index [2]. As lipid oxidation observed in the present study was relatively minor (negligible TBARS formation and low peroxide values), such low development is in agreement with the minor differences observed in the polyunsaturated fatty acid content. Previous reports on the effect of HPP treatments on changes in the fatty acid composition during storage are limited, but are consistent with the results here presented. For example, Ohshima et al. [30] did not find differences in saturated, monounsaturated and polyunsaturated fatty acid content in cod and mackerel muscle after 6 days of storage at -2ºC when previously treated under HPP conditions (200, 400 and 600 MPa for 15 min). Additionally, Aubourg et al. [11] did not find differences in Coho salmon muscle polyene index as a result of HPP treatments (135, 170 and 200 MPa for 30 s) followed by chilled storage for up to 20 days.

4. CONCLUSIONS

The effect on lipid damage of HPP treatment prior to freezing was analysed in horse mackerel muscle during frozen storage for up to 3 months. Lipid damage was assessed using complementary analytical tools to obtain a comprehensive description of the lipid damage evolution. As a result, an inhibition of lipid hydrolysis was observed. Increasing pressure (from 150 to 450 MPa) and pressure holding time (from 0 to 5 min) resulted in a marked inhibition of FFA formation during frozen storage (see data for months 1 and 3). Increasing pressure (from 150 to 450 MPa) caused a partial inhibition of lipid oxidation during frozen storage (see the FR and PV indices for months 1 and 3), while pressure holding time showed no definite trend on oxidation development. Finally, no HPP effect was observed on the polyene content of the fish muscle lipids. The research here presented provides valuable and novel information concerning the employment of HPP technology to inhibit lipid damage (hydrolysis and oxidation) during frozen storage and accordingly, increase the shelf life.

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Conflict of interest

The authors have declared no conflict of interest.

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FIGURE LEGENDS

Figure 1

Model prediction for the effect of frozen storage time (months) and pressure (MPa) on the free fatty acid (FFA) value (g kg^{-1} lipids)*

* Pressure holding time was fixed at 2.5 min

Figure 2

Model prediction for the effect of frozen storage time (months) and pressure holding time (min) on the peroxide value (PV) score (meq active oxygen kg^{-1} lipids)*

* Pressure level was fixed at 450 MPa

Figure 3

Model prediction for the effect of pressure (MPa) and frozen storage time (months) on the fluorescent ratio (FR) value*

* Pressure holding time was fixed at 5 min

TABLE 1

Lipid damage assessment* in samples not submitted to high pressure processing (starting fresh fish and frozen control fish; T-0 treatment)**

Frozen storage time (months)	Lipid damage index				
	FFA	PV	TBA-i	FR	PI
Starting fresh fish	6.9 (1.0)	0.90 (0.32)	0.44 (0.19)	0.17 (0.07)	1.63 (0.10)
0	12.2 (8.9)	0.92 (0.27)	0.60 (0.26)	0.25 (0.06)	1.76 (0.15)
1	31.3 (12.5)	2.54 (1.93)	0.47 (0.12)	0.46 (0.12)	1.53 (0.11)
3	112.2 (21.6)	7.99 (1.05)	0.68 (0.27)	0.59 (0.06)	1.56 (0.17)

* Mean values of three (n = 3) replicates; standard deviations are indicated in brackets.

** Abbreviations and units: FFA (free fatty acids; g kg^{-1} lipids), PV (peroxide value; meq active oxygen kg^{-1} lipids), TBA-i (thiobarbituric acid index; mg malondialdehyde kg^{-1} muscle), FR (fluorescence ratio) and PI (polyene index).

546 **TABLE 2**

547

548 **Effect on lipid damage parameters of horse mackerel* of experimental factors**
549 **concerning high pressure processing and subsequent frozen storage**

550

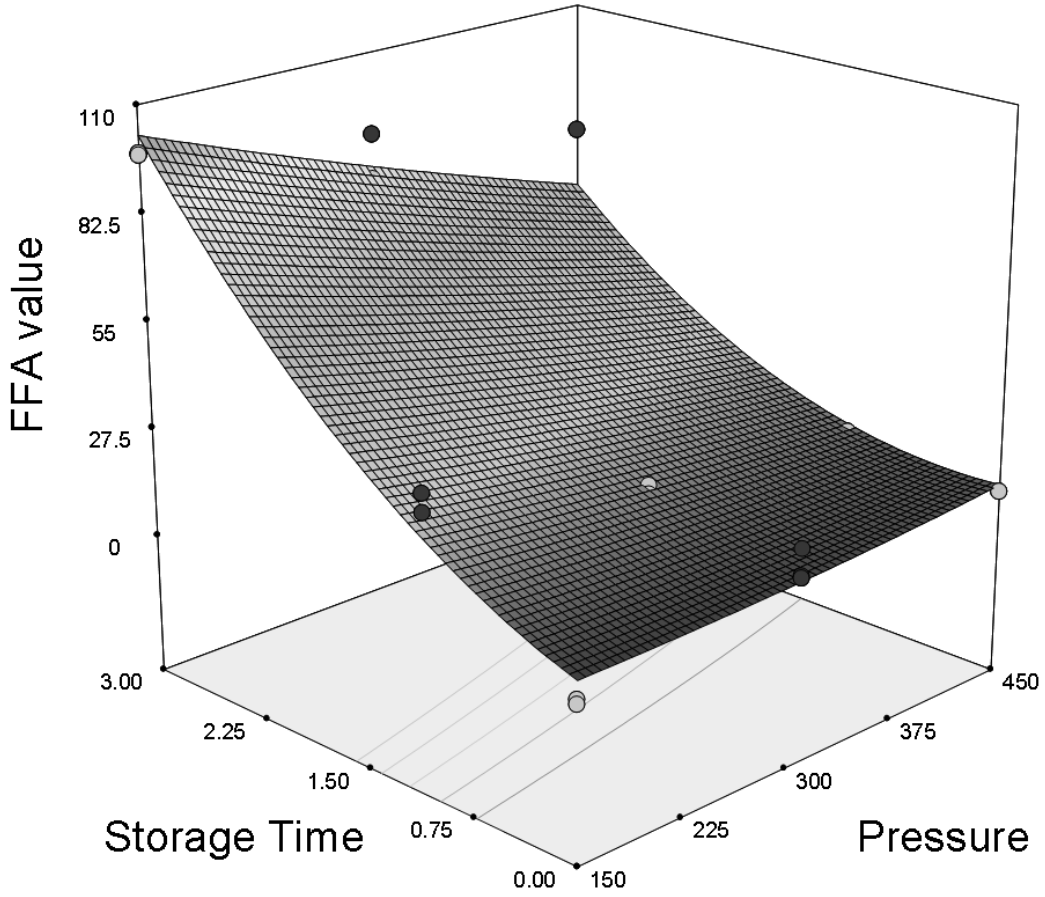
Experimental factors			Lipid damage parameters				
Pressure (MPa)	**Pressure holding time** (min)	**Frozen storage time** (months)	**FFA**	**PV**	**TBA-i**	**FR**	**PI**
450	0.0	0	23.6	2.63	1.19	0.22	1.69
	2.5		11.5	1.03	0.88	0.15	1.44
	5.0		12.8	1.41	0.56	0.18	1.66
300	0.0		24.9	0.43	0.27	0.18	1.67
	2.5		11.0	0.81	0.18	0.10	1.55
	2.5		10.5	0.62	0.16	0.14	1.55
	2.5		17.8	0.71	0.46	0.27	1.67
	5.0		1.6	1.32	0.93	0.20	1.99
150	0.0		4.0	0.31	0.30	0.27	1.64
	2.5		4.1	1.29	0.31	0.28	1.65
	2.5		2.9	1.58	0.62	0.31	1.74
	5.0		5.2	2.20	0.43	0.29	1.75
450	0.0	1	16.8	1.56	0.75	0.44	1.80
	2.5		14.3	2.29	0.76	0.28	1.58
	5.0		4.0	3.24	0.78	0.27	1.51
300	0.0		25.9	1.35	0.31	0.14	1.49
	2.5		17.7	1.99	0.32	0.15	2.01
	2.5		16.8	1.95	0.25	0.13	1.46
	2.5		19.9	1.35	0.25	0.21	1.64
	5.0		17.5	1.64	0.75	0.16	1.75
150	0.0		36.6	1.34	0.28	0.23	1.70
	2.5		33.8	0.93	0.47	0.38	1.50
	2.5		38.5	1.76	0.29	0.27	1.66
	5.0		26.8	1.57	0.48	0.29	1.57
450	0.0	3	75.6	2.39	0.69	0.21	1.57
	2.5		75.5	3.90	0.48	0.22	1.57
	5.0		42.1	2.39	0.66	0.27	1.51
300	0.0		85.1	1.79	0.39	0.29	1.75
	2.5		76.7	5.75	0.72	0.23	1.49
	2.5		88.5	4.47	0.56	0.24	1.70
	2.5		64.2	4.41	0.47	0.25	1.56
	5.0		80.3	3.48	0.52	0.36	1.66
150	0.0		116.9	4.00	0.67	0.49	1.52
	2.5		97.5	2.63	0.56	0.42	1.49
	2.5		98.1	4.86	0.59	0.41	1.53
	5.0		62.8	1.48	0.47	0.49	1.47

551

552

553 * Abbreviations and units as expressed in Table 1.

554 Figure 1
555



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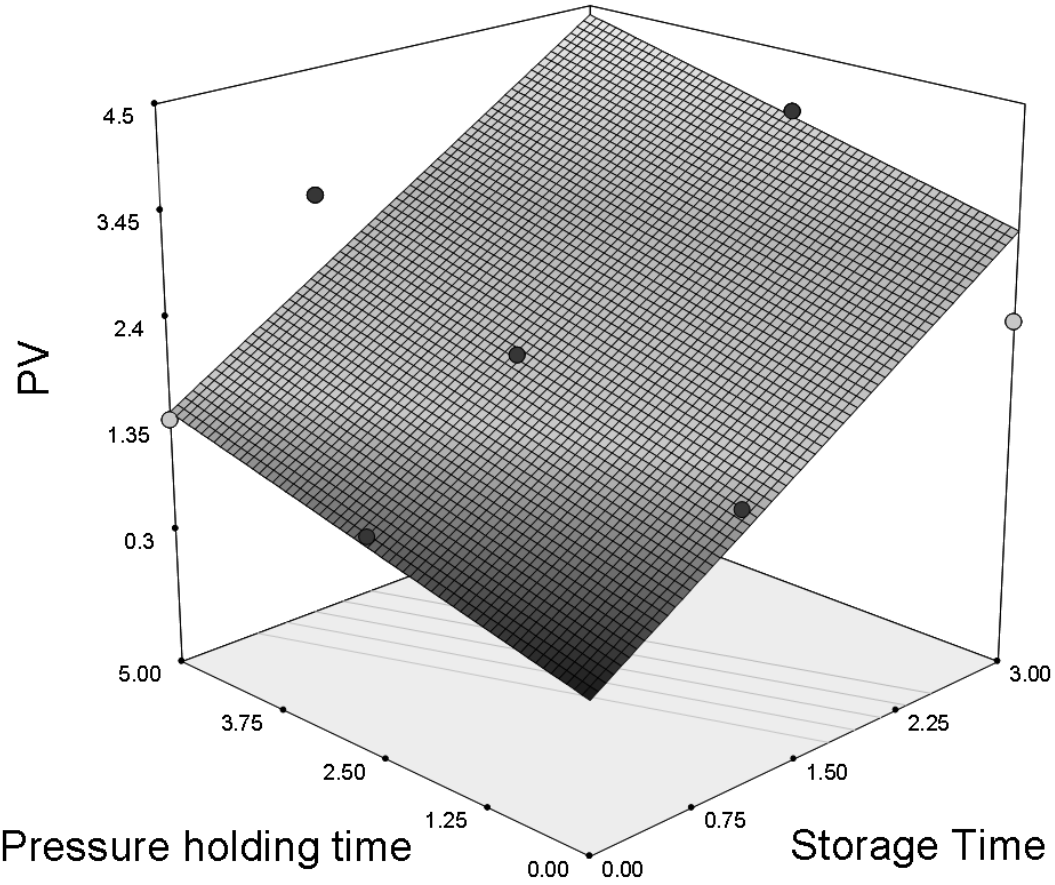
558 Figure 2



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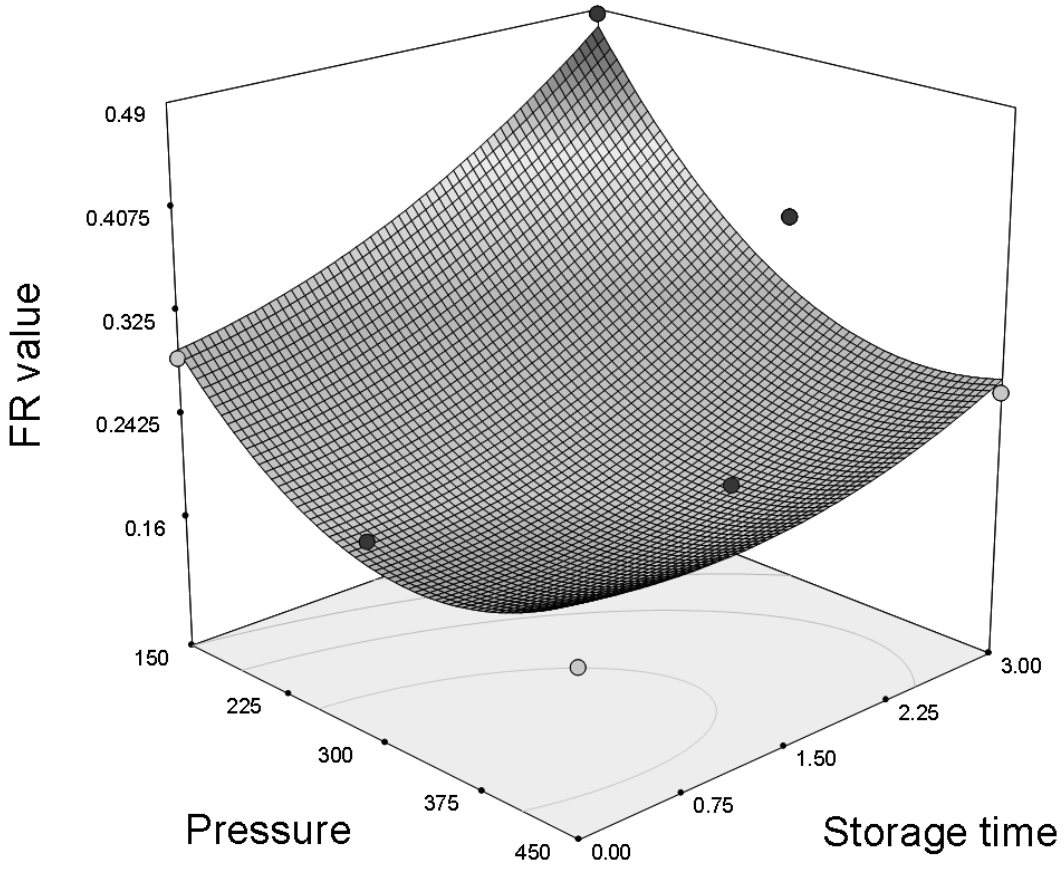
561 Figure 3



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