

1 **Effect of high-pressure pretreatments applied before freezing and frozen storage**
2 **on the functional and sensory properties of Atlantic mackerel (*Scomber scombrus*)**

3

4 Santiago P. Aubourg¹, J. Antonio Torres², Jorge A. Saraiva³, Esther Guerra-Rodríguez⁴,
5 Manuel Vázquez^{4*}

6

7 ¹Department of Food Technology, Instituto de Investigaciones Marinas (CSIC), 36208-
8 Vigo, Spain

9 ²Food Processing Engineering Group, Department of Food Science & Technology,
10 Oregon State University, Corvallis, OR 97331, USA

11 ³Research Unit of Organic Chemistry, Natural and Agro-food Products (QOPNA),
12 Chemistry Department, Aveiro University, Campus Universitário de Santiago, 3810-
13 193 Aveiro, Portugal.

14 ⁴Department of Analytical Chemistry, Faculty of Veterinary Science, University of
15 Santiago de Compostela, 27002-Lugo, Spain

16

17 * Corresponding author: manuel.vazquez@usc.es. Tel: +34 982822420, FAX: +34
18 982822420

19

20 **Keywords:** *Scomber scombrus*, high pressure, high hydrostatic pressure processing,
21 freezing, stored frozen

22

23 **ABSTRACT**

24 The frozen storage of Atlantic mackerel (*Scomber scombrus*) is limited by lipid damage
25 causing sensory quality losses, an important drawback to its commercialisation. This
26 work deals with changes in functional and sensory properties during freezing and frozen
27 storage of Atlantic mackerel pre-treated by high hydrostatic pressure processing (HPP).
28 Three levels of pressure (150, 300, and 450 MPa), holding time (0.0, 2.5, and 5.0 min)
29 and frozen storage time (0, 1, and 3 months) were tested. Expressible water, CIE colour
30 parameters, mechanical texture parameters and sensory parameters were evaluated.
31 Results showed that HPP at low levels (150 MPa) yielded raw samples with expressible
32 water lower than 40%, improving the quality of frozen muscle. During frozen storage,
33 the flesh colour of the controls (no HPP) tended to yellowness, while low-pressure
34 treatments (150 MPa) yielded samples with lightness similar to fresh muscle. HPP
35 effects on the colour parameters were negligible. Hardness and chewiness values of
36 HPP-treated samples and those for no-HPP controls were similar. Sensory analysis
37 suggested that 150 MPa did not affect the flesh odour. Most importantly, the sensorial
38 acceptability of HPP-treated samples was better than that of frozen fillet controls and
39 similar to that of fresh mackerel.

40 **1. Introduction**

41

42 Atlantic mackerel (*Scomber scombrus*) is a small pelagic fish species captured in
43 large amounts during periods of relatively low demand, and thus a large portion of the
44 catch is underutilised and transformed in non human feed. Freezing followed by frozen
45 storage is one of the best methods to retain the sensory and nutritional properties of fish
46 products (Erickson, 1997). Although mackerel is recognised as a healthy food, it
47 remains underutilised (Martelo-Vidal, Mesas, & Vazquez, 2012) because its frozen
48 shelf life is limited by a rapid deterioration of sensory quality (Aubourg, Rodriguez, &
49 Gallardo, 2005). The presence of highly unsaturated fatty acid and pro-oxidant
50 molecules causes during frozen storage substantial enzymatic and non-enzymatic
51 rancidity that strongly influences product quality (Richards & Hultin, 2002).

52 To extend shelf life as long as possible, high hydrostatic pressure processing
53 (HPP) has been shown to retain sensory and nutritional properties, while inactivating
54 microbial load, leading to shelf-life extension and safety enhancement (Alvarez-
55 Virrueta, Garcia-Lopez, Montalvo-Gonzalez, Ramirez, Mata-Montes-de-Oca, & Tovar-
56 Gomez, 2012; Escobedo-Avellaneda, Pateiro-Moure, Chotyakul, Torres, Welti-Chanes,
57 & Perez-Lamela, 2011; Mujica-Paz, Valdez-Fragoso, Tonello Samson, Welti-Chanes, &
58 Torres, 2011; Rios-Romero, Tabilo-Munizaga, Morales-Castro, Reyes, Perez-Won, &
59 Araceli Ochoa-Martinez, 2012; Téllez-Luis, Ramírez, Pérez-Lamela, Vázquez, &
60 Simal-Gándara, 2001). This technology has shown potential application in the seafood
61 industry for the production of surimi and kamaboko (Uresti, Velazquez, Ramirez,
62 Vazquez, & Torres, 2004; Uresti, Velazquez, Vazquez, Ramirez, & Torres, 2005;
63 Uresti, Velazquez, Vazquez, Ramirez, & Torres, 2006), cold-smoked fish (Lakshmanan,
64 Parkinson, & Piggott, 2007), thermal processing (Ramirez, Saraiva, Perez Lamela, &

65 Torres, 2009), and for pressure-assisted freezing (Alizadeh, Chapleau, de Lamballerie,
66 & Le-Bail, 2007) and thawing (Rouille, Lebail, Ramaswamy, & Leclerc, 2002).

67 An additional positive effect of HPP treatment is that oxidative endogenous
68 enzymes can be inactivated before further storage and processing of fish products
69 (Murchie et al., 2005). For example, recent previous work demonstrated an inhibition of
70 lipid hydrolysis in Atlantic mackerel (*S. scombrus*) samples subjected to an HPP pre-
71 treatment before freezing and frozen storage (Vázquez, Torres, Gallardo, Saraiva, &
72 Aubourg, 2012). The same effect was observed for Atlantic horse mackerel (*Trachurus*
73 *trachurus*) samples (Torres, Vázquez, Saraiva, Gallardo, & Aubourg, 2012). However,
74 this beneficial effect should be assessed also by determining the HPP effect on sensory
75 and functional properties. Therefore, this study focuses on changes after freezing and
76 frozen storage of the functional and sensory properties of Atlantic mackerel (*S.*
77 *scombrus*) subjected to HPP pre-treatments throughout their frozen storage for up to
78 three months.

79

80 **2. Materials and methods**

81

82 *2.1. Raw fish, processing, storage and sampling*

83

84 Atlantic mackerel (180 kg) caught close to the Bask coast was obtained at the
85 Ondarroa harbour (Bizkaia, Northern Spain) and immediately transported to the AZTI
86 Tecnalia (Derio, Spain) pilot plant for HPP treatment. Samples were packed in
87 polyethylene bags (three whole mackerels per bag) and vacuum sealed at 400 mbar. The
88 length and weight of the specimens was in the 28-33 cm and 230-280 g range.

89 HPP treatments were performed in a 55-L high pressure unit (WAVE
90 6000/55HT; NC HYPERBARIC, Burgos, Spain). The following HHP treatments were
91 applied (pressure value and pressure holding time, respectively): T-1 (450 MPa, 0.0
92 min), T-2 (450 MPa, 2.5 min), T-3 (450 MPa, 5.0 min), T-4 (300 MPa, 0.0 min), T-5
93 (300 MPa, 2.5 min), T-6 (300 MPa, 2.5 min), T-7 (300 MPa, 2.5 min), T-8 (300 MPa,
94 5.0 min), T-9 (150 MPa, 0.0 min), T-10 (150 MPa, 2.5 min), T-11 (150 MPa, 2.5 min),
95 T-12 (150 MPa, 5.0 min).

96 In all cases, water was employed as the pressurising medium applied at a 3
97 MPa/s rate. Come up times for 150, 300 and 450 MPa treatments were 50, 100 and 150
98 s, respectively, while decompression time was less than 3 s. Inlet water was adjusted to
99 keep temperature conditions during HPP treatment at room temperature (20°C). After
100 HPP processing, mackerel individuals were kept frozen at -20°C for 48 h before storage
101 at -10°C and sampling after 0, 1 and 3 months of storage. A relatively higher
102 temperature (-10°C) than that employed for commercial frozen purposes (-18°C) was
103 chosen so that lipid damage (the different damage pathways encountered) could be
104 speeded up (accelerated storage test) and the effect of previous HPP treatment analyzed
105 in a shorter duration study.

106 For analysis, fish samples were thawed at 4°C for 24 h, eviscerated, bones
107 removed manually and then filleted. Samples with no HPP treatment (frozen controls)
108 were subjected to the same freezing and frozen storage conditions. Fresh fish with no
109 HPP treatment (fresh controls) were also analysed. For each treatment, three batches or
110 replicates (n=3) were analysed independently. The analytical procedures described
111 below were carried out on the white muscle, raw or cooked. Cooked fish was prepared
112 in an oven at 200 °C for 10 min reaching at least 68°C at the centre point.

113

114 2.2. *Expressible water content*

115

116 The expressible water content was determined for raw and cooked samples
117 following the procedures described by Uresti, Lopez-Arias, Ramirez, & Vazquez
118 (2003).

119

120 2.3. *Colour*

121

122 Colour was determined only for raw samples following the procedures described
123 by Uresti, Lopez-Arias, Gonzalez-Cabriaes, Ramirez, & Vazquez (2003) and using a
124 X-Rite Spectrophotometer model 968 (X-Rite, Grand Rapids, MI, USA) calibrated
125 against black and white tiles. Values of L, a*, and b* were calculated based on
126 illuminant C and the 2° standard observer. Six samples were evaluated for each
127 treatment.

128

129 2.4. *Texture profile analysis (TPA)*

130

131 The texture profile was determined in raw samples using a TA-XTplus
132 texturometer (Stable Micro System, Viena Court, UK). Samples of raw beef patties
133 were cut into small cubes (2 x 2 x 1.5 cm) and analyzed at room temperature. TPA was
134 carried out using a 50-mm diameter cylindrical aluminium probe (P/50). Samples were
135 compressed to 75% of the original height using a 60 mm/min compression speed to
136 estimate hardness, adhesiveness, springiness, cohesiveness and chewiness values
137 (Anton & Luciano, 2007; Castro-Briones, Calderon, Velazquez, Salud-Rubio, Vazquez,
138 & Ramirez, 2009; Sun, 2009). Six samples were analyzed for each treatment.

139

140 2.5. *Sensory analysis*

141

142 Sensory evaluation of mackerel fillets was undertaken by 10 trained panellists
143 (mean age 32 yrs, 21-45 yrs range) and were all volunteers from the University of
144 Santiago de Compostela (Spain) exhibiting no known illness at the time of examination.
145 Evaluations were performed in a sensory panel room at 21 ± 1 °C. Cooked fish samples
146 were presented to panellists on individual plates. Four training sessions were organized
147 to make sure that sensory descriptors were understood (ISO, 1993). Panellists were first
148 asked to score the overall odour, taste and texture intensity using a six-point scale from
149 0 (fresh fish) to 6 (strong putrid fish). For the hedonic rating the panellists were asked to
150 rate fish sample acceptability using a scale from 1 (dislike extremely) to 5 (like
151 extremely).

152

153 2.6. *Statistical analysis*

154 The experimental design was statistically analysed using the Design Expert®
155 7.1.1 software (Stat-Ease, Inc., Minneapolis, MN). The set of experiments followed the
156 Box-Behnken design (Box & Behnken, 1960), formed by combining two-level factorial
157 designs with incomplete block designs. This procedure creates designs with desirable
158 statistical properties but with only a fraction of the experiments required for a three-
159 level factorial design. Error assessment was based on a replication of the central point
160 for each storage time (0, 1, and 3 months) as suggested in the Box-Behnken design. The
161 mathematical model used as a first approach to analyse the experimental data was a
162 second order polynomial described as follows:

163 $y_i = b0_i + b1_i x_1 + b2_i x_2 + b3_i x_3 + b4_i x_1 x_2 + b5_i x_1 x_3 + b6_i x_2 x_3 + b7_i x_1^2 + b8_i x_2^2 + b9_i x_3^2$

164 In the above equation, x_1 , x_2 and x_3 are the code variables for pressure level,
165 holding pressure time and storage time, respectively; y_i ($i=1-14$) are the dependent
166 variables (raw expressible water, cooked expressible water, L, a^* , b^* , hardness,
167 adhesiveness, springiness, cohesiveness, chewiness, sensory odour, sensory taste,
168 sensory texture, and sensory acceptability), and $b_{0_i} \dots b_{9_i}$ are regression coefficients
169 estimated from the experimental data by multiple linear regression. The results were
170 analyzed using analysis of variance (ANOVA). Model terms were selected or rejected
171 based on P-values at 95% confidence level. Partial models of the quadratic model were
172 also obtained and analyzed by ANOVA.

173

174 **3. Results and discussion**

175

176 *3.1. Expressible water*

177

178 The expressible water of fresh mackerel muscle was $26.6 \pm 2.4\%$ before cooking
179 and $34.6 \pm 3.5\%$ after cooking. This parameter is related to the fish meat water holding
180 capacity and affects the product juiciness. Fish processing should have no more than a
181 minimum effect on this parameter to retain an acceptable product sensory quality. After
182 frozen storage for 3 months, expressible water for Atlantic mackerel muscle with no
183 HPP treatment increased to 38.2% and 48.3% in raw and cooked muscle, respectively.
184 HPP treatments yielded expressible water values higher than those for fresh mackerel
185 muscle for any frozen time considered (Table 1). However, values for some HPP-treated
186 samples were lower than those for frozen controls with no HPP treatment. Since the
187 three independent variables (pressure level, holding time and frozen time) showed an
188 effect on the expressible water of raw samples, a multifactor ANOVA was carried out to

189 assess their relative influence. Thus, a significant ($p < 0.0001$) model was attained. The
190 evaluation of the F-values of the three variables confirmed that expressible water was
191 highly affected by the pressure level although an important effect of frozen storage
192 could also be concluded. The correlation value r^2 of the model was 0.78. The prediction
193 of the model obtained for the effect of the two variables that exerted a higher influence
194 on expressible water (pressure level and frozen storage time) is shown in Figure 1. The
195 employment of the HPP as a pre-treatment to freezing and frozen storage can lead to a
196 significant expressible water increase if high levels of pressure are selected. However,
197 HPP at low levels (150 MPa) yielded expressible water values lower than 40%,
198 improving the quality of frozen muscle, implying a water holding capacity sufficient for
199 a desirable juiciness. An expressible water of 38.7% was considered optimal for low-
200 salt restructured fish products from Atlantic mackerel (Martelo-Vidal et al., 2012).

201 The effect of HPP pre-treatment and frozen storage on expressible water of
202 cooked fishes was evaluated by multifactor ANOVA. Although an F-value of 6.17
203 implied that the model was significant, the correlation value r^2 was very low (0.37).

204 The results obtained indicate that the effect exerted on expressible water of
205 cooked muscle by frozen storage (F-value = 17.52) was higher than that of the pressure
206 level (F-value = 0.47) and pressure holding time (F-value = 0.53). All these statistical
207 parameters confirm the effect of frozen storage time on expressible water of cooked
208 muscle and the negligible effect of the HPP treatment on the expressible water of the
209 cooked fish muscle. These results are in agreement with those of a study of the effects
210 of pressure-shift freezing and pressure-assisted thawing on the quality of sea bass
211 muscle (*Dicentrarchus labrax*) where high-pressure-treated samples showed a water
212 holding capacity decrease but differences between high-pressure and conventional
213 freezing methods disappeared after cooking (Tironi, Lebail, & De Ilamballerie, 2007).

214

215 3.2. *Flesh colour*

216

217 Frozen storage affected the muscle colour (Table 1). In the raw fresh muscle the
218 mean colour parameters were: L, 44.8; a*, 5.66 and b*, 7.94. It was observed an
219 increase of L parameter during frozen storage of controls, with values up to 63.3 at 3
220 months of frozen storage. The a* values decreased to 1.04 and the b* values
221 considerably increase up to 15.25 after 3 months of frozen storage indicating flesh
222 colour towards yellow. The effect of HPP pre-treatment and frozen storage on raw fish
223 L value was evaluated by multifactor ANOVA. The F-value of 14.81 implied that the
224 model was significant. The correlation value ($r^2 = 0.81$) can be considered good. The
225 pressure effect exerted on the raw muscle L-value (F-value = 66.22) was higher than
226 that of the frozen storage time (F-value = 22.34) and pressure holding time (F-value =
227 2.90). Figure 2 shows that the pressure level increases considerably the L value,
228 reaching values close to 78. The storage time showed an important negative quadratic
229 effect (F-value = 12.51) implying that the muscle lightness decreased with long storage
230 time. Similar effects of high-pressure treatments on colour were observed in the muscle
231 of sea bass (*Dicentrarchus labrax*) where an increase on the value of L was observed
232 with the pressure (Tironi et al., 2007). The model obtained for L can be used to select a
233 desirable lightness. For instance, using a pressure level around 150 MPa, lightness
234 similar to that of fresh muscle can be obtained after 3 months of frozen storage.

235 The effect of HPP pre-treatment and frozen storage time on a* and b*
236 parameters of raw fishes was also evaluated by multifactor ANOVA. For a* values,
237 although the F-value (3.50) implied that the model was significant, the correlation value
238 ($r^2 = 0.56$) was low. The results obtained indicate that changes in the a* values for raw

239 muscle was due to the first (F-value = 17.01) and second order storage time terms (F-
240 value = 12.30) while the HPP effect was negligible. The multifactor ANOVA for b*
241 parameters showed also a low F-value (1.37), which implied that the model was not
242 significant and an effect of HPP was not observed.

243

244 *3.3. Textural profile analysis*

245

246 The changes on textural parameters during frozen storage of controls compared
247 with the values of fresh muscle were evaluated. All parameters were affected by
248 freezing and frozen storage. Hardness of fresh mackerel muscle was 33.30 N increasing
249 to 87.09 N after freezing and decreased slightly after 3 month of frozen storage (65.08
250 N). Adhesiveness of the frozen muscles (around -60 g·s) was lower than that of the fresh
251 samples (-98.8 g·s). Springiness and cohesiveness were less affected. Both fresh and
252 frozen muscles were in the narrow range, 0.20-0.30 for springiness and 0.17-0.22 for
253 cohesiveness. Chewiness of fresh muscle was 1.33 N increasing to 6.12 N after freezing
254 and frozen storage for 1 month decreasing after 3 months to only 2.84 N.

255 Table 2 shows the results of HPP as pre-treatment on frozen mackerel texture
256 profile analysis of raw muscle. The effect of the HPP pre-treatment and frozen storage
257 on the hardness of raw fish was evaluated by multifactor ANOVA. A significant ($p <$
258 0.0001) model was obtained. The evaluation of the F-values of the three variables
259 confirmed that hardness was highly affected by the pressure level (F-value = 18.46),
260 although an important effect of pressure holding time was also observed (F-value =
261 8.34). A significant interaction pressure level-pressure holding time was observed,
262 according to their F-value score (21.83). This analysis implies that when a HPP pre-

263 treatment is applied, the effect of frozen storage time on the hardness of muscle can be
264 negligible.

265 The correlation value r^2 of the model was 0.67. The prediction of the model
266 obtained for the effect of the two variables that exerted a higher influence on hardness
267 (pressure level and pressure holding time) is shown in Figure 3. Pre-treatments at high
268 pressure levels caused a significant increase in hardness. However, HPP at low levels
269 (150 MPa) yielded hardness values below 78 N, maintaining hardness levels similar to
270 frozen muscle without HPP pre-treatment but with the beneficial effect of lipid
271 oxidation inhibition observed in other studies (Vazquez et al., 2012). The HPP influence
272 on hardness has been observed also in other fish species like cod (*Gadus morhua*). An
273 increase in hardness was observed due to pressure while only minor changes in hardness
274 were observed during frozen storage (Matser, Stegeman, Kals, & Bartels, 2000).

275 The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage
276 on adhesiveness of raw muscle produced a significant model ($p < 0.0001$). The
277 evaluation of the F-values for the three variables confirmed that adhesiveness was
278 highly affected by the pressure level (F-value = 140.78), frozen storage time (F-value =
279 27.78) and the interaction pressure level-frozen storage time (F-value score = 22.04).
280 This analysis implies that when a HPP pre-treatment is applied, the effect of pressure
281 holding time on the adhesiveness of muscle is negligible. The correlation value r^2 of the
282 model was 0.83. The prediction of the model obtained for the effect of pressure level
283 and frozen storage on adhesiveness is shown in Figure 4. HPP pre-treatments caused a
284 significant adhesiveness increase when high pressure levels and long storage time were
285 selected. However, low pressure levels (150-175 MPa) yielded values close to 100 g·s,
286 i.e., an adhesiveness similar to that of fresh muscle. This result is in accordance with the

287 negative effect on adhesiveness found during freezing of salmon before smoking
288 (Martinez, Salmeron, Guillen, & Casas, 2010).

289 Springiness values, 0.189-0.346 (Table 2), are in the range found for other fish
290 products like restructured fish products (0.20-0.60) from gilthead sea bream (*Sparus*
291 *aurata*) obtained by Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo
292 (2011). The multifactor ANOVA led to an F-value 5.66, which implied that the model
293 was significant. The evaluation of the F-values showed that springiness was affected
294 mainly by frozen storage (F-value = 8.44) and less by pressure level (F-value = 7.05)
295 and pressure holding time (F-value = 1.51). The correlation value r^2 of the model was
296 0.34, suggesting that the model cannot be use for predictions and can only be use to
297 identify trends.

298 The multifactor ANOVA confirmed that cohesiveness was highly affected by
299 the pressure level (F-value = 49.57), pressure holding time (F-value = 25.82), frozen
300 storage time (F-value = 21.67) and the interaction pressure level-pressure holding time
301 (F-value score = 8.40). The correlation value r^2 of the model was 0.81. The HPP pre-
302 treatment to freezing and frozen storage caused a significant increase on cohesiveness
303 when high pressure and long storage time were selected. These results are in accordance
304 to the effect on cohesiveness found for freezing of salmon before smoking (Martinez et
305 al., 2010).

306 The cohesiveness obtained at high pressure level (0.34) is in the range observed
307 for other fish products such as restructured fish products from gilthead sea bream
308 (*Sparus aurata*) when values of 0.30-0.40 were obtained (Andres-Bello et al., 2011).
309 Moreover, low pressure levels (150 MPa) yielded cohesiveness values close to 0.20-
310 0.24, i.e., values similar to those of frozen muscle without pre-treatment.

311 Chewiness values were found in a wide range (3.72-29.87 N). The multifactor
312 ANOVA led to an F-value 27.94. Chewiness was mainly affected by the pressure level-
313 pressure holding time interaction (F-value = 50.23), followed by pressure level (F-value
314 = 22.57), quadratic pressure level effect (F-value = 16.94), and pressure holding time
315 (F-value = 8.05). The results suggest that the effect of frozen storage time is negligible
316 when a HPP pre-treatment is used previous to freezing.

317 The correlation value r^2 of the model was 0.79. HPP pre-treatment led to a
318 significant increase on chewiness when high levels of pressure and long pressure
319 holding times were selected. However, low pressure levels (150 MPa) yielded
320 chewiness values around 400-600 g, i.e., similar to those for frozen muscle without HPP
321 pre-treatment. These chewiness values are in the range observed for restructured fish
322 products from gilthead sea bream (Andres-Bello et al., 2011).

323

324 *3.4. Sensory analysis*

325

326 The evaluation of sensory odour, sensory taste and sensory texture using a scale
327 from 1 to 6 corresponding to a sense from freshness to putridness, respectively, are
328 shown in Table 3. The multifactor ANOVA of the parameter sensory odour led to a low
329 F-value (0.90), showing that the HPP pre-treatment did not affect the odour of the flesh.

330 The multifactor ANOVA analysis of the parameter sensory taste led to an F-
331 value 4.15, which implied that the model was significant. The evaluation of the F-values
332 showed that sensory taste was mainly affected by pressure level (F-value = 12.09).
333 However, the correlation value r^2 of the model was very low (0.28). Low pressure levels
334 (150 MPa) yielded taste values similar to that of frozen fish (around 2). This result

335 suggests that pressure treatments break membranes releasing compounds affecting the
336 taste, a hypothesis to be studied in the future.

337 The multifactor ANOVA of the parameter sensory texture led to an F-value of
338 33.94 implying that the model was significant. The evaluation of the F-values for the
339 showed that sensory texture was affected mainly by frozen storage time (F-value =
340 70.46), pressure level (F-value = 66.03) and the quadratic effect of frozen storage time
341 (F-value = 18.21). The correlation value r^2 of the model was 0.86. The use of HPP at
342 low levels (150 MPa) yielded mean texture values of 2.2 that are lower than those for
343 frozen controls (3.1).

344 The scale of acceptability for consumers was from 1 to 5, being 5 the highest
345 acceptability and 1 the worst. The multifactor ANOVA analysis led to an F-value
346 105.91, which implied that the model was significant (p-value probability > 0.0001).
347 The evaluation of the F-values for the different independent variables showed that
348 acceptability was affected mainly by pressure level (F-value = 480.87) followed by
349 frozen storage time (F-value = 54.25) and the quadratic effect of pressure level (F-value
350 = 42.47). These results suggest a very strong pressure level effect. The correlation value
351 r^2 of the model was 0.97. The prediction of the model is shown in Figure 5 suggesting
352 that pre-treatments at low pressure levels yield a high acceptability of cooked fish. HPP
353 treatments at 150 MPa yielded acceptability values around 4.3-3.45 (decreasing with
354 frozen storage), which were similar to those of fresh mackerel. Although acceptability
355 decreased with frozen storage time, values remained above the intermediate value.

356

357 **4. Conclusions**

358

359 HPP pre-treatments applied before freezing and frozen storage improve some
360 functional and sensory properties in Atlantic mackerel muscle indicating that they can
361 be a useful alternative for fish processors seeking to better utilize this resource often
362 used for low market value products such as a non human feed ingredient.

363

364

365 **Acknowledgements**

366 The authors thank Dr. María Lavilla from AZTI Tecnalia (Derio, Spain) for
367 carrying out all HPP treatments. This work was supported by the Secretaría Xeral de
368 I+D from the Xunta de Galicia (Spain) through Research Project 10TAL402001PR.

369

370

371 **References**

372 Alizadeh, E., Chapleau, N., de Lamballerie, M., & Le Bail, A. (2007). Effect of
373 different freezing processes on the microstructure of Atlantic salmon (*Salmo salar*)
374 fillets. *Innovative Food Science & Emerging Technologies*, 8(4), 493-499.

375 Alvarez-Virrueta, D. R., Garcia-Lopez, E. G., Montalvo-Gonzalez, E., Ramirez, J. A.,
376 Mata-Montes-de-Oca, M., & Tovar-Gomez, B. (2012). Effect of high hydrostatic
377 pressure on postharvest physiology of the "Ataulfo" mango. *CyTA-Journal of Food*,
378 10(3), 173-181.

379 Andres-Bello, A., Garcia-Segovia, P., Ramirez, J. A., & Martinez-Monzo, J. (2011).
380 Production of cold-setting restructured fish products from gilthead sea bream
381 (*Sparus aurata*) using microbial transglutaminase and regular and low-salt level.
382 *CyTA-Journal of Food*, 9(2), 121-125.

383 Anton, A. A., & Luciano, F. B. (2007). Instrumental texture evaluation of extruded
384 snack foods: A review. *Ciencia y Tecnología Alimentaria*, 5(4), 245-251.

385 Aubourg, S. R., Rodriguez, A., & Gallardo, J. M. (2005). Rancidity development during
386 frozen storage of mackerel (*Scomber scombrus*): effect of catching season and
387 commercial presentation. *European Journal of Lipid Science and Technology*,
388 107(5), 316-323.

- 389 Box, G., & Behnken, D. (1960). Some new three level designs for the study of
390 quantitative variables. *Technometrics*, 2, 455-475.
- 391 Castro-Briones, M., Calderon, G. N., Velazquez, G., Salud-Rubio, M., Vazquez, M., &
392 Ramirez, J. A. (2009). Effect of Setting Conditions using Microbial
393 Transglutaminase during Obtention of Beef Gels. *Journal of Food Process*
394 *Engineering*, 32(2).
- 395 Erickson, M. (1997). Flavor and Nutritional Quality Deterioration in Frozen Foods. In
396 M. Erickson & Y. C. Hung, *Quality in Frozen Food* (pp. 141-173). New York,
397 USA: Chapman & Hall.
- 398 Escobedo-Avellaneda, Z., Pateiro-Moure, M., Chotyakul, N., Antonio Torres, J., Weltri-
399 Chanes, J., & Perez-Lamela, C. (2011). Benefits and limitations of food processing
400 by high-pressure technologies: effects on functional compounds and abiotic
401 contaminants. *CyTA-Journal of Food*, 9(4), 351-364.
- 402 ISO (1993). ISO 8586-1993. Sensory analysis. General guidance for the selection,
403 training and monitoring of assessors. *International Organization for*
404 *Standardisation. Geneva: Switzerland.*
- 405 Lakshmanan, R., Parkinson, J. A., & Piggott, J. R. (2007). High-pressure processing and
406 water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *Lwt-Food*
407 *Science and Technology*, 40(3), 544-551.
- 408 Martelo-Vidal, M. J., Mesas, J. M., & Vazquez, M. (2012). Low-salt restructured fish
409 products from Atlantic mackerel (*Scomber scombrus*) with texture resembling
410 turkey breast. *Food Science and Technology International*, 18(3), 251-259.
- 411 Martinez, O., Salmeron, J., Guillen, M. D., & Casas, C. (2010). Effect of freezing on the
412 physicochemical, textural and sensorial characteristics of salmon (*Salmo salar*)
413 smoked with a liquid smoke flavouring. *LWT-Food Science and Technology*, 43(6),
414 910-918.
- 415 Matser, A. M., Stegeman, D., Kals, J., & Bartels, P. V. (2000). Effects of high pressure
416 on colour and texture of fish. *High Pressure Research*, 19(1-6), 109-115.
- 417 Mujica-Paz, H., Valdez-Fragoso, A., Tonello Samson, C., Weltri-Chanes, J., & Antonio
418 Torres, J. (2011). High-Pressure Processing Technologies for the Pasteurization and
419 Sterilization of Foods. *Food and Bioprocess Technology*, 4(6), 969-985.
- 420 Murchie, L. W., Cruz-Romero, M., Kerry, J. P., Linton, M., Patterson, M. F., Smiddy,
421 M., & Kelly, A. L. (2005). High pressure processing of shellfish: A review of
422 microbiological and other quality aspects. *Innovative Food Science & Emerging*
423 *Technologies*, 6(3), 257-270.
- 424 Ramirez, R., Saraiva, J., Perez Lamela, C., & Torres, J. A. (2009). Reaction Kinetics
425 Analysis of Chemical Changes in Pressure-Assisted Thermal Processing. *Food*
426 *Engineering Reviews*, 1(1), 16-30.

- 427 Richards, M. P., & Hultin, H. O. (2002). Contributions of blood and blood components
428 to lipid oxidation in fish muscle. *Journal of Agricultural and Food Chemistry*,
429 50(3).
- 430 Rios-Romero, E., Tabilo-Munizaga, G., Morales-Castro, J., Reyes, J. E., Perez-Won,
431 M., & Araceli Ochoa-Martinez, L. (2012). Effect of high hydrostatic pressure
432 processing on microbial inactivation and physicochemical properties of
433 pomegranate arils. *Cyta-Journal of Food*, 10(2), 152-159.
- 434 Rouille, J., Lebail, A., Ramaswamy, H. S., & Leclerc, L. (2002). High pressure thawing
435 of fish and shellfish. *Journal of Food Engineering*, 53(1), 83-88.
- 436 Sun, X. D. (2009). Utilization of restructuring technology in the production of meat
437 products: a review. *CyTA-Journal of Food*, 7(2), 153-162.
- 438 Téllez-Luis, S. J., Ramírez, J. A., Pérez-Lamela, C., Vázquez, M., & Simal-Gándara, J.
439 (2001). Application of high hydrostatic pressure in the food preservation. *Ciencia y*
440 *Tecnología Alimentaria*, 3(2), 66-80.
- 441 Tironi, V., Lebail, A., & De Lamballerie, M. (2007). Effects of pressure-shift freezing
442 and pressure-assisted thawing on sea bass (*Dicentrarchus labrax*) quality. *Journal of*
443 *Food Science*, 72(7), C381-C387.
- 444 Torres, J. A., Vázquez, M., Saraiva, J., Gallardo, J. M., & Aubourg, S. P. (2012). Effect
445 of previous high hydrostatic pressure conditions on lipid damage development in
446 frozen Atlantic horse mackerel (*Trachurus trachurus*). *Food and Bioprocess*
447 *Technology*, *In press*.
- 448 Uresti, R. M., Lopez-Arias, N., Gonzalez-Cabriales, J. J., Ramirez, J. A., & Vazquez,
449 M. (2003). Use of amidated low methoxyl pectin to produce fish restructured
450 products. *Food Hydrocolloids*, 17(2), 171-176.
- 451 Uresti, R. M., Lopez-Arias, N., Ramirez, J. A., & Vazquez, M. (2003). Effect of
452 amidated low methoxyl pectin on the mechanical properties and colour attributes of
453 fish mince. *Food Technology and Biotechnology*, 41(2), 131-136.
- 454 Uresti, R. M., Velazquez, G., Ramirez, J. A., Vazquez, M., & Torres, J. A. (2004).
455 Effect of high-pressure treatments on mechanical and functional properties of
456 restructured products from arrowtooth flounder (*Atheresthes stomias*). *Journal of*
457 *the Science of Food and Agriculture*, 84(13), 1741-1749.
- 458 Uresti, R. M., Velazquez, G., Vazquez, M., Ramirez, J. A., & Torres, J. A. (2006).
459 Effects of combining microbial transglutaminase and high pressure processing
460 treatments on the mechanical properties of heat-induced gels prepared from
461 arrowtooth flounder (*Atheresthes stomias*). *Food Chemistry*, 94(2), 202-209.
- 462 Uresti, R. M., Velazquez, G., Vazquez, M., Ramirez, J. A., & Torres, J. A. (2005).
463 Effect of sugars and polyols on the functional and mechanical properties of
464 pressure-treated arrowtooth flounder (*Atheresthes stomias*) proteins. *Food*
465 *Hydrocolloids*, 19(6), 964-973.

466 Vázquez, M., Torres, J. A., Gallardo, J. M., Saraiva, J., & Aubourg, S. P. (2012). Lipid
467 hydrolysis and oxidation development in frozen mackerel (*Scomber scombrus*):
468 Effect of a high hydrostatic pressure pre-treatment. *Innovative Food Science and*
469 *Emerging Technologies, in press.*

470

471

472 Table 1

473 Effects on expressible water and colour of high hydrostatic pressure processing (HPP)
 474 as a pre-treatment for frozen Atlantic mackerel (*Scomber scombrus*). Experimental
 475 treatment codes use P, H and F for pressure, holding time, and frozen storage time,
 476 respectively.

Experiments	Expressible water % w/w Raw	Expressible water % w/w cooked	L raw	a* raw	b* raw
1 (P450H0F0)	40.37	40.97	57.47	6.77	13.72
2 (P450H2.5F0)	20.00	38.17	66.15	3.98	15.31
3 (P450H5F0)	42.35	38.92	69.21	1.84	10.72
4 (P300H0F0)	42.72	38.61	64.87	2.31	15.12
5 (P300H2.5F0)	33.86	47.60	52.30	4.79	13.06
6 (P300H2.5F0)	45.97	41.40	72.08	-0.09	13.75
7 (P300H2.5F0)	38.79	38.06	65.91	3.48	15.01
8 (P300H5F0)	42.69	42.10	59.73	6.40	15.13
9 (P150H0F0)	41.42	36.29	51.33	2.96	12.41
10 (P150H2.5F0)	32.24	35.97	50.58	4.74	13.38
11(P150H2.5F0)	26.21	39.41	45.87	5.63	11.47
12(H150H5F0)	36.75	37.08	45.99	1.63	7.91
13 (P450H0F1)	44.85	44.81	70.79	0.61	12.61
14 (P450H2.5F1)	54.39	41.86	71.63	2.86	15.14
15 (P450H5F1)	48.70	39.38	76.34	-0.41	10.06
16 (P300H0F1)	41.33	45.26	63.16	1.18	14.23
17 (P300H2.5F1)	47.02	43.61	71.67	1.76	15.96
18 (P300H2.5F1)	46.98	40.23	73.57	0.53	14.29
19 (P300H2.5F1)	45.28	46.47	73.44	-0.72	11.26
20 (P300H5F1)	48.15	42.62	65.82	2.07	15.41
21 (P150H0F1)	39.79	44.75	60.68	-0.44	13.90
22 (P150H2.5F1)	40.68	45.27	59.89	1.84	14.78
23 (P150H2.5F1)	33.05	46.77	61.77	1.83	15.16
24 (P150H5F1)	36.14	42.05	54.43	1.68	13.05
25 (H450H0F3)	48.94	44.00	73.06	1.93	15.20
26 (H450H2.5F3)	50.43	47.04	76.55	0.18	10.32
27 (H450H5F3)	46.63	46.07	74.19	0.44	11.52
28 (P300H0F3)	45.71	49.35	62.37	0.89	12.10
29 (P300H2.5F3)	47.94	45.38	72.90	1.36	14.25
30 (P300H2.5F3)	49.67	44.20	71.51	-0.59	11.42
31 (P300H2.5F3)	45.15	43.16	65.48	3.37	14.61
32 (P300H5F3)	48.38	50.08	77.89	-0.96	13.38
33 (P150H0F3)	35.82	44.99	50.25	0.60	11.77
34 (P150H2.5F3)	37.85	46.33	58.57	1.70	14.54
35 (P150H2.5F3)	40.15	39.82	62.72	2.84	15.80
36 (P150H5F3)	37.80	41.25	58.43	2.64	14.02

477 Table 2

478 Effect on the raw muscle texture profile analysis of high hydrostatic pressure processing

479 (HPP) as a pre-treatment for frozen Atlantic mackerel (*Scomber scombrus*).

480 Experimental treatment codes use P, H and F for pressure, holding time, and frozen

481 storage time, respectively.

Experiments	Hardness (N)	Adhesiveness (g·s)	Springiness	Cohesiveness	Chewiness (N)
1 (P450H0F0)	98.27	-250	0.307	0.242	7.40
2 (P450H2.5F0)	118.59	-237	0.286	0.331	11.20
3 (P450H5F0)	170.53	-299	0.384	0.316	20.70
4 (P300H0F0)	101.27	-143	0.295	0.199	6.91
5 (P300H2.5F0)	61.44	-59	0.238	0.247	3.75
6 (P300H2.5F0)	142.56	-248	0.372	0.253	13.41
7 (P300H2.5F0)	90.36	-197	0.284	0.239	6.23
8 (P300H5F0)	120.53	-226	0.282	0.264	9.17
9 (P150H0F0)	75.26	-58	0.223	0.212	3.74
10 (P150H2.5F0)	90.54	-109	0.295	0.209	6.91
11(P150H2.5F0)	135.19	-69	0.346	0.242	11.99
12(H150H5F0)	92.44	-69	0.249	0.215	5.23
13 (P450H0F1)	104.01	-237	0.256	0.281	7.59
14 (P450H2.5F1)	89.37	-252	0.321	0.427	13.98
15 (P450H5F1)	201.28	-310	0.355	0.395	29.87
16 (P300H0F1)	95.54	-118	0.243	0.191	4.92
17 (P300H2.5F1)	145.06	-184	0.343	0.299	15.03
18 (P300H2.5F1)	108.74	-266	0.260	0.274	8.21
19 (P300H2.5F1)	94.37	-190	0.241	0.289	6.54
20 (P300H5F1)	135.68	-213	0.282	0.274	10.61
21 (P150H0F1)	127.50	-79	0.360	0.302	14.22
22 (P150H2.5F1)	82.62	-90	0.262	0.211	5.14
23 (P150H2.5F1)	96.31	-68	0.303	0.219	6.21
24 (P150H5F1)	72.49	-58	0.244	0.239	4.64
25 (H450H0F3)	102.36	-344	0.283	0.293	8.44
26 (H450H2.5F3)	133.21	-488	0.310	0.320	13.34
27 (H450H5F3)	199.74	-279	0.356	0.375	26.70
28 (P300H0F3)	69.89	-267	0.189	0.267	3.45
29 (P300H2.5F3)	93.50	-385	0.216	0.281	6.14
30 (P300H2.5F3)	117.41	-336	0.268	0.276	8.89
31 (P300H2.5F3)	125.83	-285	0.240	0.300	9.09
32 (P300H5F3)	103.64	-141	0.230	0.337	8.08
33 (P150H0F3)	82.91	-34	0.216	0.259	5.03
34 (P150H2.5F3)	80.27	-49	0.199	0.269	11.70
35 (P150H2.5F3)	129.15	-51	0.250	0.310	10.59
36 (P150H5F3)	72.30	-40	0.217	0.227	3.72

482

483 Table 3

484 Effects on the cooked muscle sensory analysis of high hydrostatic pressure processing
485 (HPP) as a pre-treatment for frozen Atlantic mackerel (*Scomber scombrus*).
486 Experimental treatment codes use P, H and F for pressure, holding time, and frozen
487 storage time, respectively.

Experiments	Sensory odour	Sensory taste	Sensory texture	Sensory acceptability
1 (P450H0F0)	3	4	3	1
2 (P450H2.5F0)	1	3	3	1
3 (P450H5F0)	1	2	2	1.5
4 (P300H0F0)	1.5	1.5	1	2
5 (P300H2.5F0)	2	2	1.3	2.5
6 (P300H2.5F0)	2	2	1	2.5
7 (P300H2.5F0)	3	4	1.5	2
8 (P300H5F0)	3	4	2	2
9 (P150H0F0)	2.5	2.5	1	3
10 (P150H2.5F0)	2	1	1.2	4
11(P150H2.5F0)	3.5	3	1	4.5
12(H150H5F0)	4	3.5	1.5	4.5
13 (P450H0F1)	3	4	3	1.5
14 (P450H2.5F1)	4	4	3.5	1.5
15 (P450H5F1)	2	3	4	1
16 (P300H0F1)	4	5	2.8	2
17 (P300H2.5F1)	4	4	3.2	2
18 (P300H2.5F1)	3	5	3.5	2.3
19 (P300H2.5F1)	3	4	3	2
20 (P300H5F1)	3	4	2	2.5
21 (P150H0F1)	1	2	1.5	4
22 (P150H2.5F1)	2	2	1.5	4
23 (P150H2.5F1)	1	2	1.2	4
24 (P150H5F1)	2	2	2	4
25 (H450H0F3)	3	4	3	1
26 (H450H2.5F3)	3	4	4	1
27 (H450H5F3)	2	5	4.2	1
28 (P300H0F3)	3	4	2.8	2
29 (P300H2.5F3)	2	2	3	1.5
30 (P300H2.5F3)	3	4	3.5	1
31 (P300H2.5F3)	2	2	3	1.5
32 (P300H5F3)	3	4	3	1
33 (P150H0F3)	3	2	2	2
34 (P150H2.5F3)	3	3	2	3
35 (P150H2.5F3)	2	2	2	3
36 (P150H5F3)	2	1	1	4

488

489 FIGURE LEGENDS

490

491 **Fig 1.** Model prediction for the effect of pressure level (MPa) and frozen storage time
492 (months) on expressible water of raw muscles of Atlantic mackerel (*Scomber*
493 *scombrus*). Holding time was fixed at 2.5 min.

494

495 **Fig 2.** Model prediction for the effect of pressure level (MPa) and frozen storage time
496 (month) on lightness parameter (L) of raw muscle of Atlantic mackerel (*Scomber*
497 *scombrus*). Holding time was fixed at 2.5 min.

498

499 **Fig. 3.** Model prediction for the effect of pressure level (MPa) and pressure holding
500 time (min) on hardness of raw muscle of Atlantic mackerel (*Scomber scombrus*). Frozen
501 storage time was fixed at 1.5 month.

502

503 **Fig. 4.** Model prediction for the effect of pressure level (MPa) and frozen storage time
504 (month) on adhesiveness of raw muscle of Atlantic mackerel (*Scomber scombrus*).
505 Holding time was fixed at 2.5 min.

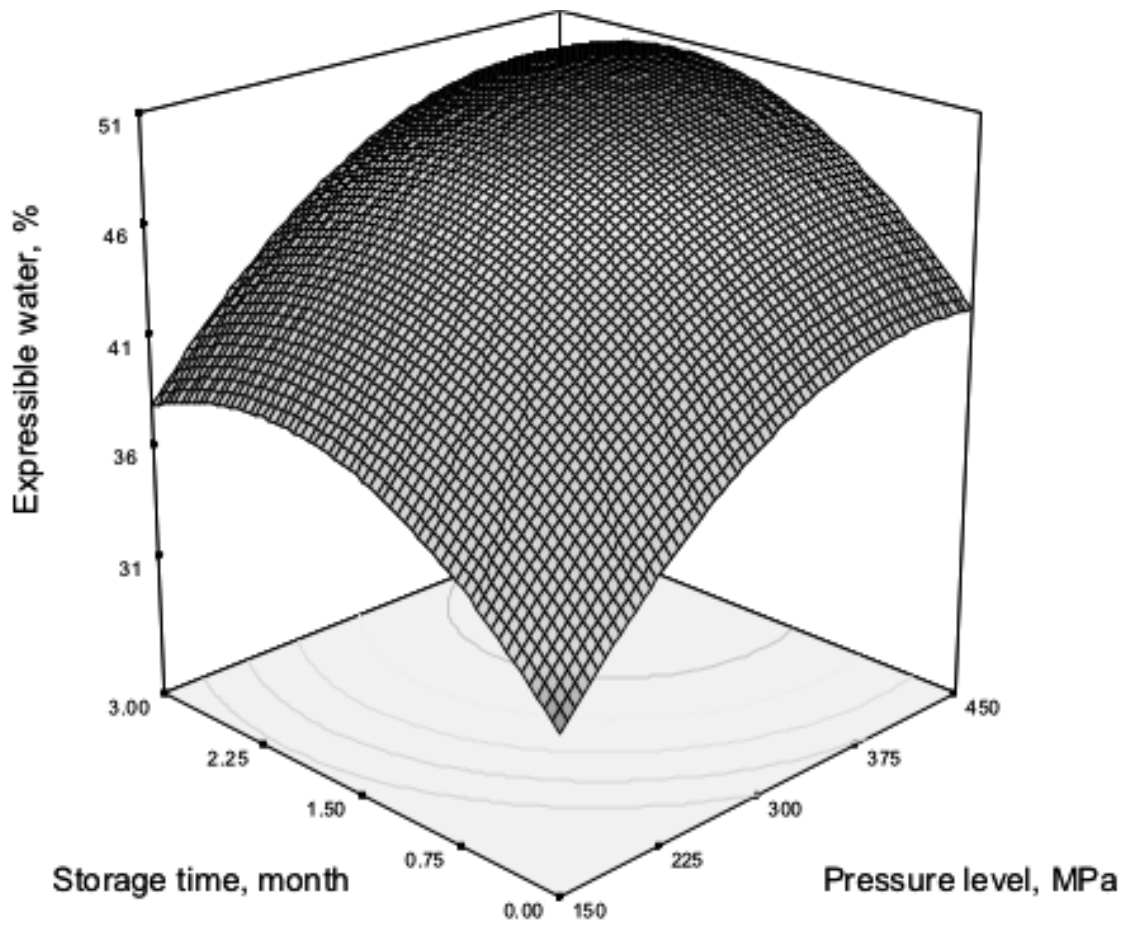
506

507 **Fig. 5.** Model prediction for the effect of pressure level (MPa) and frozen storage time
508 (month) on sensory acceptance of cooked fillets of Atlantic mackerel (*Scomber*
509 *scombrus*). Holding time was fixed at 2.5 min.

510

511

512



513

514

515

516

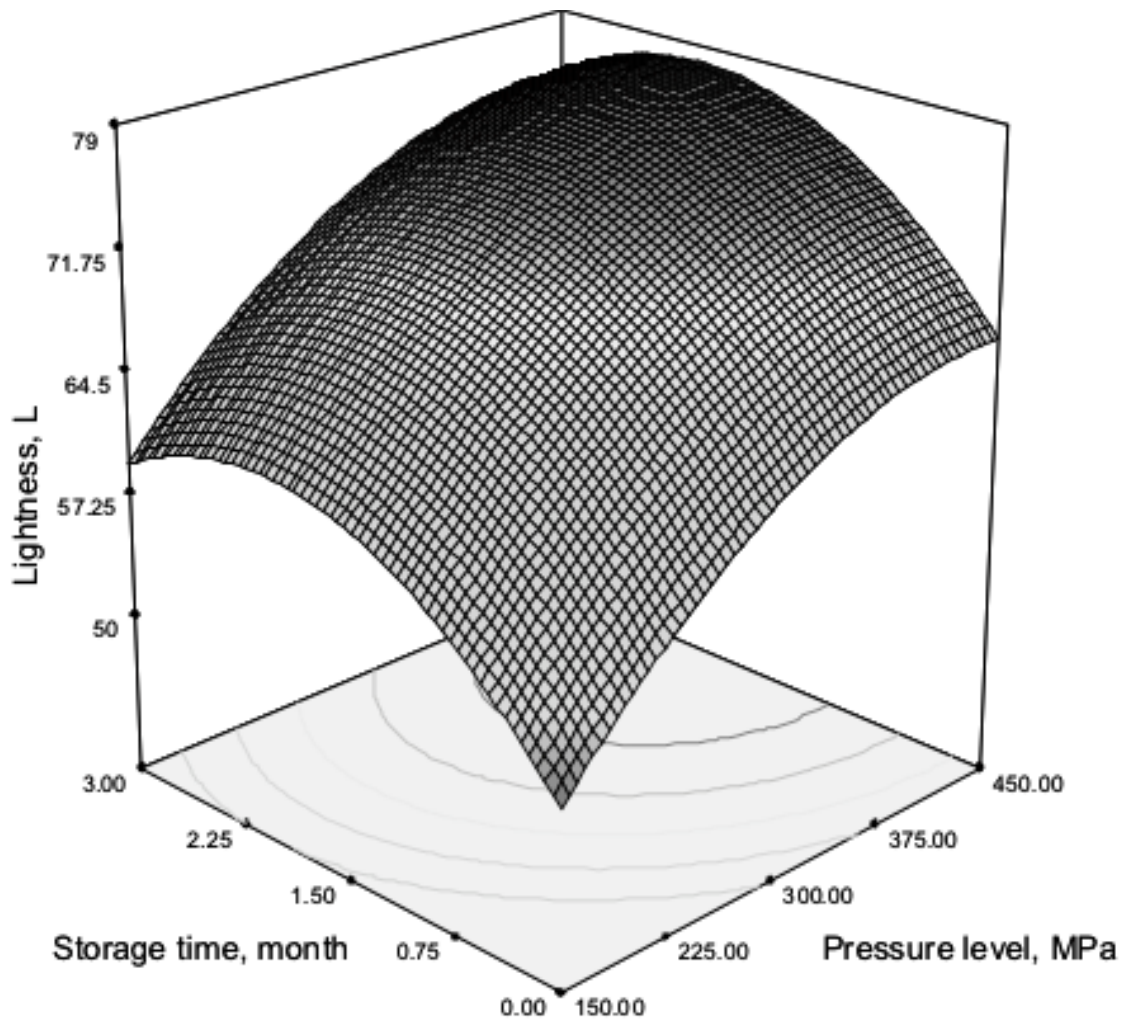
517

518

519

520

521 Figure 1



522

523

524

525

526

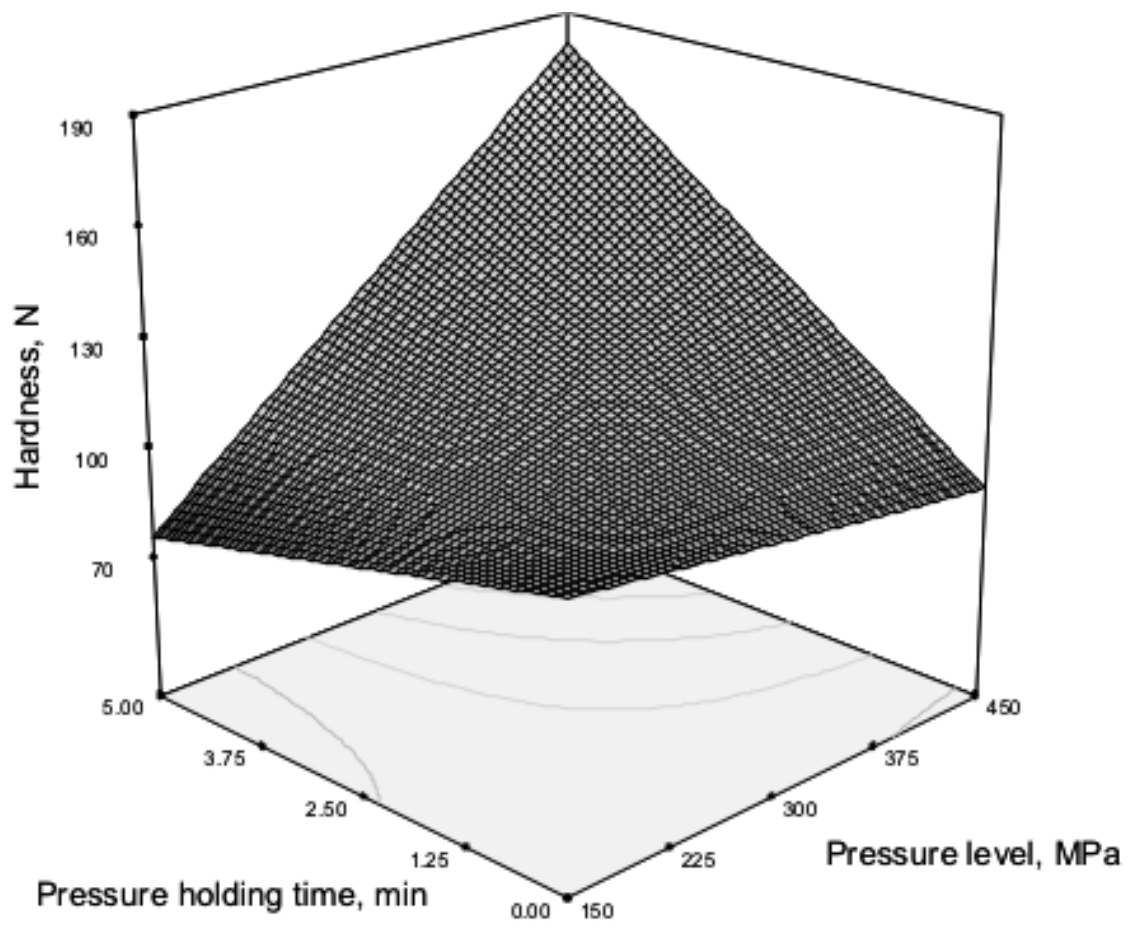
527

528

529 Figure 2

530

531



532

533

534

535

536

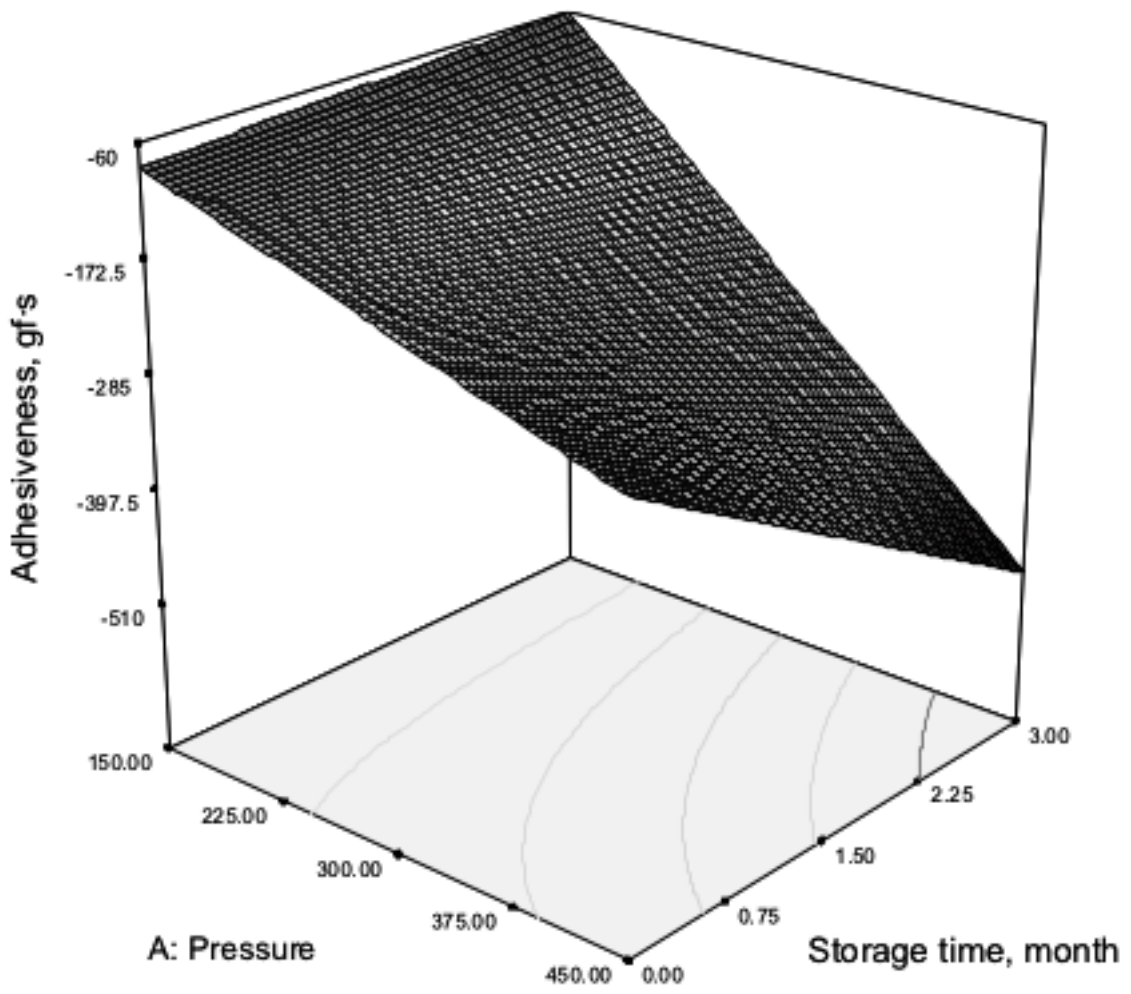
537

538

539 Figure 3

540

541



542

543

544

545

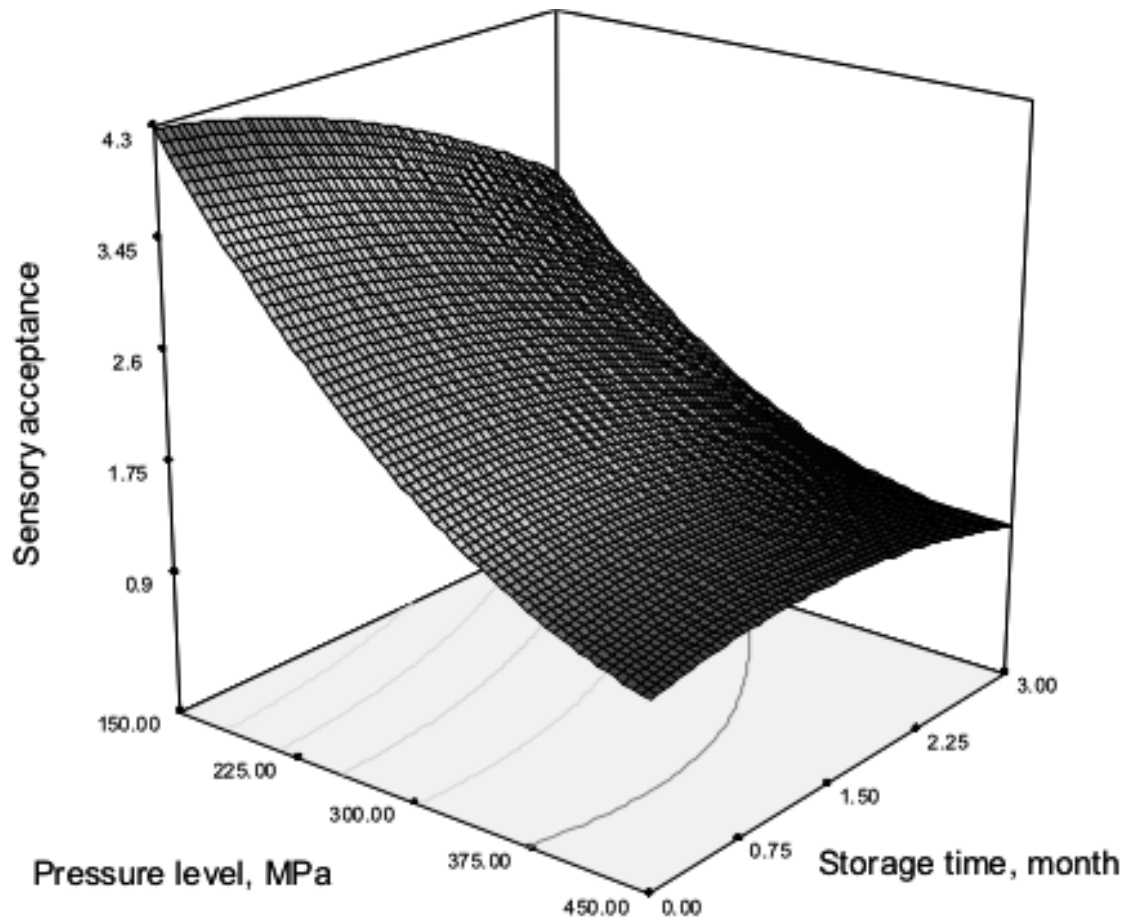
546

547

548

549 Figure 4

550



551

552

553

554

555

556

557

558

559 Figure 5

560

561

562