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Effect of combining high-pressure processing and frozen storage on the functional and sensory properties of horse mackerel (*Trachurus trachurus*)

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ABSTRACT

Frozen storage of horse mackerel (*Trachurus trachurus*) is limited by lipid damage causing sensory quality losses. This work deals with changes in functional and sensory properties during frozen storage of horse mackerel treated by high hydrostatic pressure processing (HPP) prior to freezing. Three levels of pressure (150, 300, and 450 MPa), pressure holding time (0.0, 2.5, and 5.0 min), and frozen storage time (0, 1, and 3 months) were studied. Expressible water, colour parameters, mechanical texture parameters and sensory parameters were evaluated in raw and cooked samples. The texture profile analysis of raw and cooked HPP samples suggested that a product texture similar or close to fresh muscle is possible. The sensory analysis showed that a 150 MPa treatment yielded high acceptability values. Although acceptability decreased during frozen storage, values remained close to those of fresh samples.

1. Introduction

Horse mackerel (*Trachurus trachurus*, L.) is one of the most important stocks of pelagic fisheries in southern Europe including Spain and Portugal. This fatty fish species is captured in amounts larger than consumption levels and thus a large portion of the catch is underutilised and transformed into animal feed. Frozen preservation of fatty fish species for consumption beyond their catching season is limited because its shelf life is shortened by a rapid deterioration of sensory quality (Aubourg, Rodriguez & Gallardo, 2005, Aubourg, Torres, Saraiva, Guerra-Rodríguez & Vázquez, 2013). This is due to the presence of highly unsaturated fatty acid and pro-oxidant molecules causing substantial enzymatic and non-enzymatic rancidity strongly decreasing product quality (Ramalhosa, Paiga, Morais, Rui Alves, Delerue-Matos & Prior Pinto Oliveira, 2012, Richards & Hultin, 2002). On the other hand, fatty fish species have high nutritional value due to their omega-3 polyunsaturated fatty acids levels (Farvin, Grejsen & Jacobsen, 2012). Therefore, new technologies to improve the quality of horse mackerel stored frozen are of industry interest. Several methodologies have been studied to inhibit oxidation during horse mackerel frozen storage, but the results have revealed limited beneficial effects (Farvin, Grejsen & Jacobsen, 2012).

High hydrostatic pressure processing (HPP) can pasteurize foods and food ingredients. In general, HPP treatments help in retaining and improving sensory properties of preserved foods, leading to greater shelf-life and safety (Alvarez-Virrueta, Garcia-Lopez, Montalvo-Gonzalez, Ramirez, Mata-Montes-de-Oca & Tovar-Gomez, 2012, Cortez-Vega, Fonseca, Feisther, Silva & Prentice, 2013, Escobedo-Avellaneda, Pateiro-Moure, Chotyakul, Torres, Welti-Chanes & Perez-Lamela, 2011, Mujica-Paz, Valdez-Fragoso, Tonello Samson, Welti-Chanes & Torres, 2011, Rios-Romero, Tabilo-

Munizaga, Morales-Castro, Reyes, Perez-Won & Araceli Ochoa-Martinez, 2012, Téllez-Luis, Ramírez, Pérez-Lamela, Vázquez & Simal-Gándara, 2001).

Research on HPP applications in the seafood industry include processing of surimi and kamaboko (Uresti, Velazquez, Ramirez, Vazquez & Torres, 2004, Uresti, Velazquez, Vazquez, Ramirez & Torres, 2005b, Uresti, Velazquez, Vazquez, Ramirez & Torres, 2006, Wang et al., 2013, Wang et al., 2013), pressure-assisted thermal processing (Ramirez, Saraiva, Perez Lamela & Torres, 2009), and pressure-assisted freezing (Alizadeh, Chapleau, de lamballerie & Le-Bail, 2007) and thawing (Rouille, Lebail, Ramaswamy & Leclerc, 2002). HPP modifies the structure in both pressurized muscles and extracted myofibrils, affecting the texture and colour in meat and fish products (Buckow, Sikes & Tume, 2013). It was also reported that HPP affects protein substrates making them more accessible to enzymes such as microbial transglutaminase (Gomez-Guillen, Montero, Solas & Perez-Mateos, 2005).

The shelf life of fatty fish species during frozen storage could be extended by HPP treatments applied as a pre-treatment before freezing. Oxidative endogenous enzymes can be inactivated before further storage and processing (Murchie et al., 2005). Previous works have demonstrated an inhibition of endogenous enzymes in Atlantic mackerel (*S. scombrus*) and horse mackerel subjected to an HPP pre-treatment prior to freezing and subsequent frozen storage (Fidalgo, Saraiva, Aubourg, Vázquez & Torres, 2013). The inhibition of lipid hydrolysis was also observed in Atlantic mackerel (Vázquez, Torres, Gallardo, Saraiva & Aubourg, 2013) and horse mackerel (Torres, Vázquez, Saraiva, Gallardo & Aubourg, 2013) under the process conditions here studied.

To further evaluate the possible use of HPP pre-treatments as a means to improve the quality of horse mackerel stored frozen, it is necessary to investigate its effects on the sensory and functional properties of horse mackerel during frozen storage. For example, some pressure levels induce modifications of myofibrillar proteins reflected in visual appearance, protein functionality and mechanical properties changes (Uresti, Velazquez, Vazquez, Ramirez & Torres, 2005a). Therefore, this study focused on the changes in the functional and sensory properties of frozen horse mackerel subjected to HPP pre-treatments before freezing and stored for up to three months at -10ºC, a temperature chosen to represent accelerated frozen storage.

2. Materials and methods

2.1. Raw fish, processing, storage and sampling

Horse mackerel (180 kg) obtained at the Ondarroa harbour (Bizkaia, Northern Spain) was transported under refrigeration to the AZTI Tecnalia (Derio, Spain) pilot plant for HPP treatment within 6 hours after being caught close to the Bask coast. Samples were packed in polyethylene bags (three whole horse mackerels per bag) and vacuum sealed (0.04 MPa). The length and weight of the specimens was in the range of 0.25-0.3 m and 0.2-0.25 kg, respectively.

HPP treatments were performed in a 55-L high pressure unit (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain) at room temperature (20ºC). Conditions of pressure level (150, 300 and 450 MPa) and pressure holding time (0, 2,5 and 5 min) followed the experimental statistical design described below. In all cases, water at 10-12ºC was employed as the pressurising medium applied at a 3 MPa/s rate yielding come up times of 50, 100 and 150 s for 150, 300 and 450 MPa treatments, respectively. Decompression time was less than 3 s. After HPP processing, horse mackerel individuals were kept frozen at –20ºC for 48 h before storage at –10ºC and sampling after 0, 1 and 3 months of storage. A relatively high temperature (-10ºC) was chosen as an accelerated storage condition to determine in less time the effect of the different HPP pre-treatments. For each treatment, three batches or replicates (n=3) were analysed.

Fish samples were first thawed at 4ºC for 24 h, eviscerated, bones removed manually and then filleted before analysis. Samples with no HPP treatment were subjected to the same freezing and frozen storage conditions and considered as frozen controls. Fresh fish with no HPP treatment (fresh controls) were also analysed. The analytical procedures described below were carried out on raw or cooked samples of white muscle. Cooked fish was prepared in an oven at 200 ºC for 10 min reaching at least 68ºC at the centre point.

2.2. Expressible water content and colour

The expressible water content was determined for raw and cooked samples following the procedures previously described (Uresti, Lopez-Arias, Ramirez & Vazquez, 2003). Colour of raw samples was determined following the procedures described by the same authors using an X-Rite Spectrophotometer model 968 (X-Rite, Grand Rapids, MI, USA) calibrated against black and white tiles. Values of L, a* and b* were calculated based on illuminant C and the 2° standard observer. Six samples were evaluated for each treatment and replicated 3 times.

2.4. Texture profile analysis (TPA)

Texture profile of raw samples cut into small cubes (2 x 2 x 1.5 cm) was determined at room temperature using a TA-XTplus texturometer (Stable Micro System, Viena Court, UK) equipped with a 50-mm diameter cylindrical aluminium probe (P/50). Samples were compressed to 75% of the original height at a 60 mm/min compression speed to estimate hardness, adhesiveness, springiness, cohesiveness and chewiness values (Anton & Luciano, 2007, Castro-Briones, Calderon, Velazquez, Salud-Rubio, Vazquez & Ramirez, 2009, Sun, 2009). Six samples were analysed for each treatment and replicated 3 times.

2.5. Sensory analysis

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

2.6. Statistical analysis

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

$$y^j = b_0^i + b_1^i x_1 + b_2^i x_2 + b_3^i x_3 + b_4^i x_1 x_2 + b_5^i x_1 x_3 + b_6^i x_2 x_3 + b_7^i x_1^2 + b_8^i x_2^2 + b_9^i x_3^2$$

where x_i (i = 1–3) are the code variables for pressure level, holding pressure time, and storage time; y^j (j = 1–14) are the dependent variables (raw expressible water, cooked expressible water, L, a*, b*, hardness, adhesiveness, springiness, cohesiveness, chewiness, sensory odour, sensory taste, sensory texture, and sensory acceptability); and, b_0^i, $b_0^i \ldots b_9^i$ are regression coefficients estimated from the experimental data by multiple linear regression. Model terms were selected or rejected based on p-values at 95% confidence level determined by analysis of variance (ANOVA). Partial models of the quadratic model were also obtained and analysed by ANOVA.

3. Results and discussion

3.1. Expressible water

The expressible water of fresh horse mackerel muscle was 24.6 % before cooking and 32.9 % after cooking. This parameter is related to the water holding capacity. Fish processing should have no more than a minimum effect on this parameter to retain an acceptable product sensory quality. After frozen storage for 3 months, expressible water for horse mackerel muscle with no HPP treatment increased to 41.6 % and 42.9 % in raw and cooked muscle, respectively. This result clearly shows that the frozen storage has a negative effect on the juiciness of the fish flesh.

HPP treatments yielded expressible water values higher than those for fresh horse mackerel muscle for any frozen time considered (Table 1). However, values for some HPP-treated raw samples were lower than 40 %. The three independent variables (pressure level, holding time and frozen time) showed an effect on the expressible water of raw samples. Therefore a multifactor ANOVA was carried out to assess their relative influence yielding a significant model ($p < 0.0001$). The evaluation of the F-values of the three variables confirmed that expressible water was highly affected by the HPP treatment (term of pressure level, quadratic term of pressure level and holding time of pressure). The correlation coefficient r^2 of the model was 0.67. The prediction of the model obtained for the effect of the HPP treatment variables (pressure level and holding time) on expressible water of samples stored for 3 months is shown in Figure 1a. The model shows that any holding time at 150 MPa applied before freezing and frozen storage yielded expressible water values lower than 38% reflecting a water holding capacity sufficient for a desirable juiciness and thus an improved frozen muscle quality. For example, a 38.7% expressible water value was considered optimal for low-salt restructured fish products obtained from Atlantic mackerel (Martelo-Vidal, Mesas & Vazquez, 2012).

The effect of HPP pre-treatment and frozen storage on expressible water of cooked fishes was evaluated by multifactor ANOVA and the model obtained was significant with a F-value of 4.14 and a moderate correlation coefficient (r^2 = 0.62). The results obtained indicate that the frozen storage effect exerted on the expressible water of cooked muscle (F-value = 18.80) was higher than that of the pressure level (linear and quadratic term had F-values of 0.08 and 5.99, respectively) and pressure holding time (F-value = 0.53). These statistical parameters confirm the effect of frozen storage time on expressible water of cooked muscle and the negligible effect of the HPP treatment on the expressible water of the cooked fish muscle. Figure 1b shows that for any pressure level the expressible water increases with frozen storage time. After 3 months of frozen storage, the value of expressible water for cooked samples were similar than those of non-HPP treated frozen samples. HPP-treated samples showed a lower water holding capacity but differences between HPP-pretreated and conventional freezing disappeared after cooking. These results are in agreement with those of a previous study where the same treatments were applied to Atlantic mackerel (Aubourg, Torres, Saraiva, Guerra-Rodríguez & Vázquez, 2013).

3.2. Fish muscle colour

Frozen storage affected the fish muscle colour (Table 1). In raw, fresh-fish muscle, the mean L, a*, and b* values were 42.6, 0.39, and 6.23, respectively. L values increased during frozen storage of controls, with values reaching 47.2 after 3 months of frozen storage. The a* values increased to 1.47 and the b* values increased considerably reaching 9.66 after 3 months of frozen storage indicating a shift in the flesh colour towards yellow.

The effect of HPP pre-treatment and frozen storage on raw fish L value was evaluated by multifactor ANOVA yielding an F-value of 34.89 implying that the model was significant with an r^2 value considered good (0.90). The pressure effect exerted on the raw muscle L-value (F-values of 165.94 and 31.55 for the linear and quadratic terms, respectively) was higher than that of the frozen storage time (F-value = 21.09) and pressure holding time (F-value = 3.27). Figure 2 shows that the pressure level increased L value considerably reaching values close to 78. Storage time showed an important negative effect implying that muscle lightness decreased for long storage times. Similar effects of high-pressure treatments on colour were observed in the muscle of Atlantic mackerel after applying a similar treatment (Aubourg, Torres, Saraiva, Guerra-Rodríguez & Vázquez, 2013). Other studies have confirmed that HHP increases the *L* * values of horse mackerel (Erkan, Uretener, Alpas, Selcuk, Ozden & Buzrul, 2011). Using a pressure level around 150 MPa, a lightness value similar to that of fresh fish muscle can be obtained after 3 months of frozen storage.

The effect of HPP pre-treatment and frozen storage time on a* and b* parameters of raw fish was also evaluated by multifactor ANOVA. For a* values, the F-value (6.57) implied that the model was significant while the r^2 was 0.68. The analysis of the ANOVA parameters obtained indicate that a* value changes for raw muscle were due to the first (F-value = 18.08) and second order storage time terms (F-value = 8.54) while the HPP effect was negligible. The multifactor ANOVA for b* parameters showed a F-value of 4.56 implying that the model was significant while the r^2 was 0.60. The ANOVA parameters obtained indicate that b* value changes in raw muscle were due to interaction of the HPP parameters (F-value =12.36) followed by the storage time term (F-value = 10.40). The parameter b* has been directly related to lipid oxidation development with an important relationship between its value and the formation of polymerised Schiff bases and fluorescent compounds (Undeland, Hultin & Richards, 2003).

3.3. Texture profile analysis of raw samples

All texture parameters for fresh muscle of controls and HPP-treated samples were affected by freezing and frozen storage. Hardness of fresh muscle was 7849 g and increased to 9610 g after 3 months of frozen storage. Table 2 summarizes the HPP pre-treatment effects the on texture of raw frozen horse mackerel muscle. The HPP pre-treatment and frozen storage effects on the hardness of raw fish were evaluated by multifactor ANOVA. A significant model was obtained although the r^2 was low (0.47). F-values confirmed that hardness was highly affected by pressure level (F-value = 13.40), pressure holding time (F-value = 10.19) and frozen storage (F-value = 7.50).

The adhesiveness after 3 months of frozen storage (-132.15 g·s) was higher than that of fresh samples (-77.9 g·s). The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage on adhesiveness of raw muscle produced a significant model ($p < 0.0001$). F-values confirmed that adhesiveness was highly affected by the linear (F-value = 98.01) and quadratic pressure level (F-value = 98.42) terms. The frozen storage time (F-value = 2.10) and pressure holding time (F-value = 2.97) effect were less important implying that in HPP pre-treated samples, the effect of pressure holding time and frozen storage time on the adhesiveness of muscle is negligible. The prediction of the model ($r^2 = 0.87$) for the effect of pressure level and frozen storage on adhesiveness is shown in Figure 3. For any storage time, high pressure levels pre-treatments caused a significant adhesiveness increase. However, low pressure levels (150-160 MPa) yielded adhesiveness values close to 70 g·s, i.e., similar to that of fresh muscle. This result is in accordance with the negative effect on adhesiveness found during HPP treatment before freezing of Atlantic mackerel (Aubourg, Torres, Saraiva, Guerra-Rodríguez & Vázquez, 2013) and during freezing of salmon before smoking (Martinez, Salmeron, Guillen & Casas, 2010).

Springiness of fresh and frozen muscles was less affected, ranging 0.25-0.28. Table 2 shows the springiness values of HPP treated samples, ranging 0.242-0.361. They were in the range found for other fish products such as restructured fish products (0.20-0.60) from gilthead sea bream (*Sparus aurata*) (Andres-Bello, Garcia-Segovia, Ramirez & Martinez-Monzo, 2011). The multifactor ANOVA led to an F-value 1.79, and a model that was not significant. This implies that springiness was not affect by the variations of the pressure treatment and frozen storage time.

Cohesiveness of fresh and frozen muscles ranged 0.25-0.28 while values of HPP treated samples ranged 0.204-0.331 (Table 2). The multifactor ANOVA confirmed that cohesiveness was highly affected by frozen storage time (F-value = 7.52). However, the model r^2 was low 0.27. The cohesiveness obtained at low pressure (150 MPa) was close to 0.27, i.e., an intermediate value to that of fresh muscle without pre-treatment (0.23) and that after 3 months of frozen storage (0.29).

Fresh muscle chewiness increased from 527 to 781 g after 3 months of frozen storage. The multifactor ANOVA of chewiness values covering a wide range (422-1586 g) led to an F-value 3.72 while the model r^2 was 0.26 suggesting that the conditions of HPP pre-treatment can be selected to obtain a chewiness similar to that of fresh muscle.

3.4. Texture profile analysis of cooked samples

All texture parameters for cooked HPP-treated samples were affected by freezing and frozen storage (Table 3). Hardness of cooked muscle without treatment (control) was 9798 g (fresh) increasing after 3 months of frozen storage to 15229 g. The effect of HPP pre-treatment and frozen storage on the hardness of cooked muscle was evaluated by multifactor ANOVA. A significant model was obtained with a good correlation coefficient ($r^2 = 0.61$) considering the heterogeneity of this kind of samples. F-values confirmed that hardness was highly affected by frozen storage (F-value = 9.26) followed by pressure holding time (F-value = 2.41) and pressure level (F-value = 2.13). The response surface for the model obtained showed that HPP treatments decreased the hardness (Figure 4) while the opposite effect was observed for the frozen storage time. After freezing (0 months of frozen time), a hardness similar to fresh muscle (12250 g) was predicted for samples pre-treated at 375 MPa, a value that compares very well with the hardness of the control frozen muscle, i.e., 15229 g after 3 months of frozen storage.

Freezing increased the adhesiveness of the cooked muscles of fresh samples from -167 to -397 g·s, and then decreased during frozen storage reaching -149 g·s after 3 months. The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage on the adhesiveness of cooked muscle yielded a significant model ($p < 0.0029$).

F-values confirmed that the adhesiveness was highly affected by the quadratic terms of pressure holding time (F-value = 9.71) and pressure level (F-value = 8.98) while frozen storage time was less important. At high pressure, the predicting model ($r^2 = 0.57$) for the pressure level and frozen storage effect on adhesiveness showed that HPP pre-treatments caused a significant adhesiveness increase when high pressure levels for any storage time. However, low pressure levels (150 MPa) yielded values close to -167 g·s, i.e., an adhesiveness similar to that of fresh muscle.

The springiness of the cooked muscle of fresh samples was 0.326 increasing to 0.437 after 3 months of frozen storage. The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage on springiness of cooked muscle produced a significant model ($p < 0.0018$). Springiness was highly affected by the quadratic terms of pressure level (F-value = 24.30) and frozen storage time (F-value = 4.24). The model obtained ($r^2 = 0.57$) showed that intermediate pressure levels (225 MPa) yielded values close to 326, i.e., a springiness similar to that of fresh muscle. However, this value increased with frozen storage time.

The cohesiveness of fresh and frozen muscles was less affected, ranging 0.477-0.485. The multifactor ANOVA of the effect of HPP pre-treatment and frozen storage on cohesiveness of cooked muscle produced a significant model ($p < 0.0001$). The evaluation of the F-values confirmed that cohesiveness was highly affected by the interaction pressure level- pressure holding time (F-value = 22.71) and the frozen storage time (F-value = 20.76). At high pressure holding times, the prediction model ($r^2 = 0.72$) for the pressure level and frozen storage effect on cohesiveness values showed that HPP pre-treatments caused a significant cohesiveness increase for any storage time. However, low pressure levels (150 MPa) applied for 5 min yielded cohesiveness values close to those of the cohesiveness of fresh muscle.

The chewiness of cooked fresh muscle was 1604 g, increasing to 3349 g after 3 months of frozen storage. The multifactor ANOVA of chewiness values led to an F-value 5.37 and the model r^2 was 0.68. F-values confirmed that chewiness was highly affected by storage time (F-value = 10.22). The results suggest that the effect of HPP pre-treatments prior to freezing can delay the increase in chewiness values caused by the subsequent frozen storage. This suggests the possibility of maintaining values close to those of fresh samples (1600) for up to 2 months of storage time. Samples stored for 3 months showed chewiness values lower than those for frozen controls. Finally, all textural changes observed can be related to the observation that the HPP can induce various structural changes on muscle proteins which are dependent on the pressure level and the duration of the treatment (Buckow, Sikes & Tume, 2013).

3.5. Sensory analysis

The evaluation of sensory odour, taste and texture using a 1 to 6 scale corresponding to freshness to putridness, respectively, are shown in Table 4. For the parameter flesh odour, the multifactor ANOVA of flesh odour values led to an F-value 12.79 and the model r^2 was 0.81. The evaluation of F-values showed that flesh odour was highly affected by the pressure level-storage time interaction (F-value = 48.27), and the linear (F-value = 11.99) and quadratic storage time terms (F-value = 20.82).

Figure 5 shows the model for the effect of pressure and frozen storage time. During frozen storage time, flesh odour reached putridness levels in samples treated at 450 MPa. However, flesh odour for 150 MPa-treated samples remained in the fresh value range (1-1.2) during storage. This result is not in agreement with that obtained for a similar pre-treatment applied on Atlantic mackerel where no effect was detected on flesh odour (Aubourg, Torres, Saraiva, Guerra-Rodríguez & Vázquez, 2013). This could imply an effect of species on the sensory values of frozen HPP pre-treated fishes, suggesting that more studies are needed for other fish species.

Regarding flesh taste, the multifactor ANOVA yielded an F-value of 5.51 implying that the model was significant. F-values indicate that taste was affected mainly by the pressure level-frozen storage time interaction (F-value = 16.04) and frozen storage time (F-value = 12.88). The model r^2 was 0.53.

The multifactor ANOVA of the sensory parameter texture led to an F-value of 17.42 implying that the model was significant. F-values showed that the sensory texture was affected mainly by pressure level (F-value = 39.71 and frozen storage time (F-value = 12.42). No quadratic effects were observed. The model r^2 was 0.62. Low pressure treatments (150 MPa) yielded mean texture values below 2 and lower than those observed for frozen controls (3.4).

The consumer acceptability scale ranged from 1 (low) to 5 (high). The multifactor ANOVA analysis led to an F-value of 23.92 implying that the model was significant (p-value probability > 0.0001). F-values showed that acceptability was affected mainly by pressure level (F-value = 105.53) followed by frozen storage time (F-value = 17.31) and the quadratic effect of pressure level (F-value = 8.41). These results suggest a strong influence of pressure level on acceptability. The model r^2 was 0.83. The model predictions (Figure 6) suggest that pre-treatments at low pressure levels yield cooked fish with high acceptability. HPP treatments at 150 MPa yielded acceptability values around 5-4 (decreasing with frozen storage). Although acceptability decreased with frozen storage time, values remained close to those of fresh samples (5) and higher than those for frozen samples (3).

4. Conclusions

HPP-treated samples showed a lower water holding capacity but differences between HPP and conventional freezing methods disappeared after cooking. The pressure treatment increased lightness considerably but the storage time showed an important negative effect implying that the muscle lightness decreased with long storage time. The texture profile analysis of raw and cooked HPP samples suggested that a product texture similar or close to fresh muscle is possible. The sensory analysis showed that a 150 MPa treatment yielded high acceptability values. Although acceptability decreased with frozen storage time, values remained close to those for fresh samples. HPP pre-treatments applied before freezing and frozen storage improve some functional and sensory properties in horse mackerel muscle indicating that this new technology can be a useful alternative for fish processors.

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Table 1

Effects on expressible water and colour of high hydrostatic pressure processing (HPP) as a pre-treatment to freezing and subsequent frozen storage of horse mackerel (*Trachurus trachurus*). Experimental treatment codes use P, H and F for pressure (MPa), holding time (min), and frozen storage time (months), respectively.

	Expressible water, % w/w		L	a*	b*
Treatment	Raw	Cooked	Raw	Raw	Raw
1 (P450H0F0)	41.25	37.78	73.03	1.22	14.23
2 (P450H2.5F0)	45.73	37.97	80.33	-0.35	12.63
3 (P450H5F0)	43.83	40.56	73.42	1.02	11.50
4 (P300H0F0)	46.74	38.97	75.10	0.44	13.61
5 (P300H2.5F0)	43.28	38.17	75.55	0.77	12.19
6 (P300H2.5F0)	45.91	42.35	75.76	-0.33	14.01
7 (P300H2.5F0)	47.10	42.70	70.65	1.10	14.37
8 (P300H5F0)	43.30	38.66	74.51	2.19	14.35
9 (P150H0F0)	34.41	38.77	47.27	1.99	8.68
10 (P150H2.5F0)	42.72	38.13	54.98	0.97	11.13
11(P150H2.5F0)	35.88	41.50	58.56	-0.27	11.49
12(P150H5F0)	38.44	39.07	59.59	1.87	13.32
13 (P450H0F1)	42.62	38.06	74.22	0.91	13.07
14 (P450H2.5F1)	46.68	39.80	74.23	1.01	13.18
15 (P450H5F1)	48.77	40.42	77.39	-0.12	10.65
16 (P300H0F1)	40.58	42.03	68.79	-4.09	6.11
17 (P300H2.5F1)	49.04	40.67	70.02	-0.13	9.93
18 (P300H2.5F1)	45.61	39.90	72.51	0.48	12.99
19 (P300H2.5F1)	50.05	37.22	75.84	-0.88	10.63
20 (P300H5F1)	46.20	41.54	76.17	0.17	14.63
21 (P150H0F1)	39.85	39.59	56.27	0.21	11.22
22 (P150H2.5F1)	35.42	38.57	48.86	1.33	8.23
23 (P150H2.5F1)	39.08	43.62	57.01	-0.17	10.53
24 (P150H5F1)	41.42	37.61	57.69	-0.90	8.94
25 (P450H0F3)	48.95	36.81	67.42	2.07	12.26
26 (P450H2.5F3)	44.87	41.12	75.61	-1.35	8.46
27 (P450H5F3)	43.27	40.39	72.62	3.03	13.54
28 (P300H0F3)	43.96	46.11	59.87	2.36	12.76
29 (P300H2.5F3)	41.69	44.84	72.75	0.36	12.15
30 (P300H2.5F3)	44.90	47.64	66.05	1.89	13.42
31 (P300H2.5F3)	50.00	48.01	68.68	0.19	10.79
32 (P300H5F3)	43.08	42.43	65.56	3.02	9.75
33 (P150H0F3)	38.71	40.66	49.54	1.83	10.52
34 (P150H2.5F3)	38.17	42.49	44.74	3.33	9.11
35 (P150H2.5F3)	40.35	40.61	56.90	2.20	12.17
36 (P150H5F3)	41.50	44.48	49.82	4.11	12.17

Table 2

Effect on the raw muscle texture of high hydrostatic pressure processing (HPP) as a pre-treatment as a pre-treatment to freezing and subsequent frozen storage of horse mackerel (*Trachurus trachurus*). Experimental treatment codes use P, H and F for pressure, holding time, and frozen storage time, respectively.

Experiments	Hardness (g)	Adhesiveness (g·s)	Springiness	Cohesiveness	Chewiness (g)
1 (P450H0F0)	7340	-325.8	0.371	0.297	900
2 (P450H2.5F0)	10379	-318.9	0.358	0.437	1649
3 (P450H5F0)	10327	-405.6	0.369	0.374	1767
4 (P300H0F0)	11412	-398.6	0.372	0.439	1843
5 (P300H2.5F0)	10349	-462.0	0.287	0.387	1207
6 (P300H2.5F0)	7153	-261.2	0.224	0.373	647
7 (P300H2.5F0)	11768	-313.3	0.348	0.405	1910
8 (P300H5F0)	7840	-352.9	0.296	0.333	807
9 (P150H0F0)	13474	-262.0	0.383	0.506	2630
10 (P150H2.5F0)	15809	-492.9	0.406	0.527	3459
11(P150H2.5F0)	12964	-419.6	0.407	0.491	3074
12(P150H5F0)	11828	-340.2	0.363	0.456	2063
13 (P450H0F1)	9147	-201.4	0.298	0.379	1118
14 (P450H2.5F1)	17368	-355.1	0.492	0.557	4798
15 (P450H5F1)	16396	-190.8	0.423	0.554	4029
16 (P300H0F1)	11180	-402.0	0.344	0.392	1468
17 (P300H2.5F1)	13219	-383.2	0.321	0.483	2046
18 (P300H2.5F1)	10334	-347.3	0.345	0.379	1461
19 (P300H2.5F1)	12188	-457.0	0.332	0.429	1780
20 (P300H5F1)	11755	-237.6	0.293	0.431	1487
21 (P150H0F1)	11753	-234.5	0.337	0.490	1960
22 (P150H2.5F1)	10494	-168.8	0.320	0.429	1451
23 (P150H2.5F1)	13924	-299.3	0.413	0.523	3065
24 (P150H5F1)	9791	-93.3	0.329	0.422	1389
25 (P450H0F3)	9030	-129.9	0.372	0.426	1496
26 (P450H2.5F3)	14640	-339.9	0.422	0.513	3310
27 (P450H5F3)	10470	-192.3	0.368	0.450	1884
28 (P300H0F3)	10091	-345.1	0.308	0.410	1312
29 (P300H2.5F3)	13931	-382.7	0.378	0.515	2875
30 (P300H2.5F3)	12313	-479.2	0.312	0.493	1960
31 (P300H2.5F3)	10711	-439.2	0.284	0.466	1458
32 (P300H5F3)	13090	-277.3	0.367	0.523	2586
33 (P150H0F3)	12352	-133.3	0.369	0.555	2579
34 (P150H2.5F3)	16968	-385.0	0.434	0.536	3980
35 (P150H2.5F3)	12816	-172.5	0.462	0.483	2835
36 (P150H5F3)	12680	-206.4	0.423	0.495	2737

Table 3

Effect on the cooked muscle texture profile analysis of high hydrostatic pressure processing (HPP) as a pre-treatment to freezing and subsequent frozen storage of horse mackerel (*Trachurus trachurus*). Experimental treatment codes use P, H and F for pressure, holding time, and frozen storage time, respectively.

Experiments	Hardness (g)	Adhesiveness (g·s)	Springiness	Cohesiveness	Chewiness (g)
1 (P450H0F0)	7340	-325.8	0.371	0.297	900
2 (P450H2.5F0)	10379	-318.9	0.358	0.437	1649
3 (P450H5F0)	10327	-405.6	0.369	0.374	1767
4 (P300H0F0)	11412	-398.6	0.372	0.439	1843
5 (P300H2.5F0)	10349	-462.0	0.287	0.387	1207
6 (P300H2.5F0)	7153	-261.2	0.224	0.373	647
7 (P300H2.5F0)	11768	-313.3	0.348	0.405	1910
8 (P300H5F0)	7840	-352.9	0.296	0.333	807
9 (P150H0F0)	13474	-262.0	0.383	0.506	2630
10 (P150H2.5F0)	15809	-492.9	0.406	0.527	3459
11(P150H2.5F0)	12964	-419.6	0.407	0.491	3074
12(P150H5F0)	11828	-340.2	0.363	0.456	2063
13 (P450H0F1)	9147	-201.4	0.298	0.379	1118
14 (P450H2.5F1)	17368	-355.1	0.492	0.557	4798
15 (P450H5F1)	16396	-190.8	0.423	0.554	4029
16 (P300H0F1)	11180	-402.0	0.344	0.392	1468
17 (P300H2.5F1)	13219	-383.2	0.321	0.483	2046
18 (P300H2.5F1)	10334	-347.3	0.345	0.379	1461
19 (P300H2.5F1)	12188	-457.0	0.332	0.429	1780
20 (P300H5F1)	11755	-237.6	0.293	0.431	1487
21 (P150H0F1)	11753	-234.5	0.337	0.490	1960
22 (P150H2.5F1)	10494	-168.8	0.320	0.429	1451
23 (P150H2.5F1)	13924	-299.3	0.413	0.523	3065
24 (P150H5F1)	9791	-93.3	0.329	0.422	1389
25 (H450H0F3)	9030	-129.9	0.372	0.426	1496
26 (H450H2.5F3)	14640	-339.9	0.422	0.513	3310
27 (H450H5F3)	10470	-192.3	0.368	0.450	1884
28 (P300H0F3)	10091	-345.1	0.308	0.410	1312
29 (P300H2.5F3)	13931	-382.7	0.378	0.515	2875
30 (P300H2.5F3)	12313	-479.2	0.312	0.493	1960
31 (P300H2.5F3)	10711	-439.2	0.284	0.466	1458
32 (P300H5F3)	13090	-277.3	0.367	0.523	2586
33 (P150H0F3)	12352	-133.3	0.369	0.555	2579
34 (P150H2.5F3)	16968	-385.0	0.434	0.536	3980
35 (P150H2.5F3)	12816	-172.5	0.462	0.483	2835
36 (P150H5F3)	12680	-206.4	0.423	0.495	2737

Table 4

Effects on the cooked muscle sensory analysis of high hydrostatic pressure processing (HPP) as a pre-treatment to freezing and subsequent frozen storage of horse mackerel (*Trachurus trachurus*). Experimental treatment codes use P, H and F for pressure, holding time, and frozen storage time, respectively.

Experiments	Sensory odour	Sensory taste	Sensory texture	Sensory acceptability
1 (P450H0F0)	1	1	4	2
2 (P450H2.5F0)	1	1	2	4
3 (P450H5F0)	2	2	3	1
4 (P300H0F0)	1	1	1	4
5 (P300H2.5F0)	1	1	2	3
6 (P300H2.5F0)	1	2	1	2
7 (P300H2.5F0)	2	3	2	2
8 (P300H5F0)	1	1	1	4
9 (P150H0F0)	1	2	1	4
10 (P150H2.5F0)	2	2	2	3
11(P150H2.5F0)	3	3	2	3
12(P150H5F0)	1	2	1	3
13 (P450H0F1)	2	2	4	1
14 (P450H2.5F1)	2	2	4	2
15 (P450H5F1)	2	2	4	1
16 (P300H0F1)	2	2	3	2
17 (P300H2.5F1)	2	2	2	3
18 (P300H2.5F1)	2	2	3	2
19 (P300H2.5F1)	2	2	3	2
20 (P300H5F1)	2	2	2	3
21 (P150H0F1)	2	2	2	3
22 (P150H2.5F1)	2	3	2	3
23 (P150H2.5F1)	2	2	1	4
24 (P150H5F1)	1	1	2	5
25 (P450H0F3)	2	2	2	4
26 (P450H2.5F3)	2	3	4	1
27 (P450H5F3)	1	2	3	2
28 (P300H0F3)	1	2	3	1
29 (P300H2.5F3)	1	3	3	1
30 (P300H2.5F3)	2	2	4	1
31 (P300H2.5F3)	2	3	2	2
32 (P300H5F3)	2	3	3	1
33 (P150H0F3)	1	1	1	5
34 (P150H2.5F3)	1	2	2	4
35 (P150H2.5F3)	1	2	2	4
36 (P150H5F3)	1	2	2	3

FIGURE LEGENDS

Fig 1. Model prediction for the effect of HPP treatment and frozen storage time on expressible water of raw muscles (A) and cooked muscles (B) of horse mackerel (*Trachurus trachurus*). Frozen storage time was fixed at 3 months (A) and holding time was fixed at 2.5 min (B). Values for fresh controls were 24.6 % and 32.9 % for raw and cooked samples, respectively, while those for frozen controls (3 month) were 41.6 % and 42.9 % for raw and cooked samples, respectively.

Fig 2. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on lightness parameter (L) of raw muscle of horse mackerel (*Trachurus trachurus*). Holding time was fixed at 2.5 min. Values for controls were 42.6 % and 47.2 % for fresh and 3 month frozen samples, respectively.

Fig. 3. Model prediction for the effect of pressure level (MPa) and frozen storage time (months) on adhesiveness of raw muscle of horse mackerel (*Trachurus trachurus*). Holding time was fixed at 2.5 min. Values for controls were -77.9 g·s and -132.15 g·s for fresh and 3 month frozen samples, respectively.

Fig. 4. Model prediction for the effect of pressure level (MPa) and frozen storage time (months) on hardness of cooked muscle of horse mackerel (*Trachurus trachurus*). Holding time was fixed at 2.5 min. Values for controls were 9798 g and 15529 g for fresh and 3 month frozen samples, respectively.

Fig. 5. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on odour of cooked fillets of horse mackerel (*Trachurus trachurus*). Pressure holding time was fixed at 5 min. Values for controls were 1 and 1.2 for fresh and 3 month frozen samples, respectively.

Fig. 6. Model prediction for the effect of pressure level (MPa) and frozen storage time (month) on sensory acceptance of cooked fillets of horse mackerel (*Trachurus trachurus*). Holding time was fixed at 0 min. Values for controls were 5 and 3 for fresh and 3 month frozen samples, respectively.

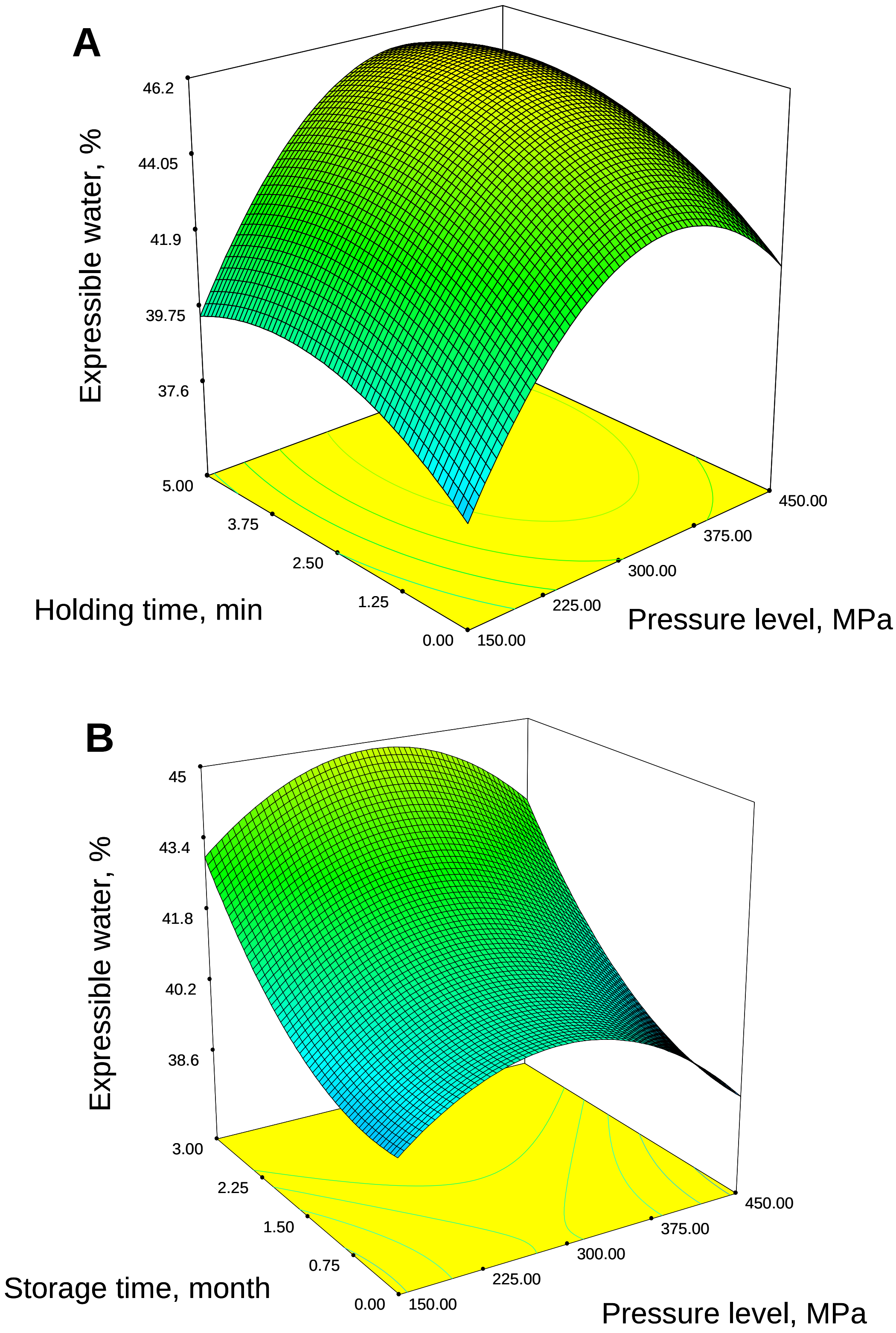
A
Expressible water, %
46.2
44.05
41.9
39.75
37.6
5.00
3.75
2.50
1.25
0.00
Holding time, min
150.00
225.00
300.00
375.00
450.00
Pressure level, MPa
B
Expressible water, %
45
43.4
41.8
40.2
38.6
3.00
2.25
1.50
0.75
0.00
Storage time, month
150.00
225.00
300.00
375.00
450.00
Pressure level, MPa

Figure 1

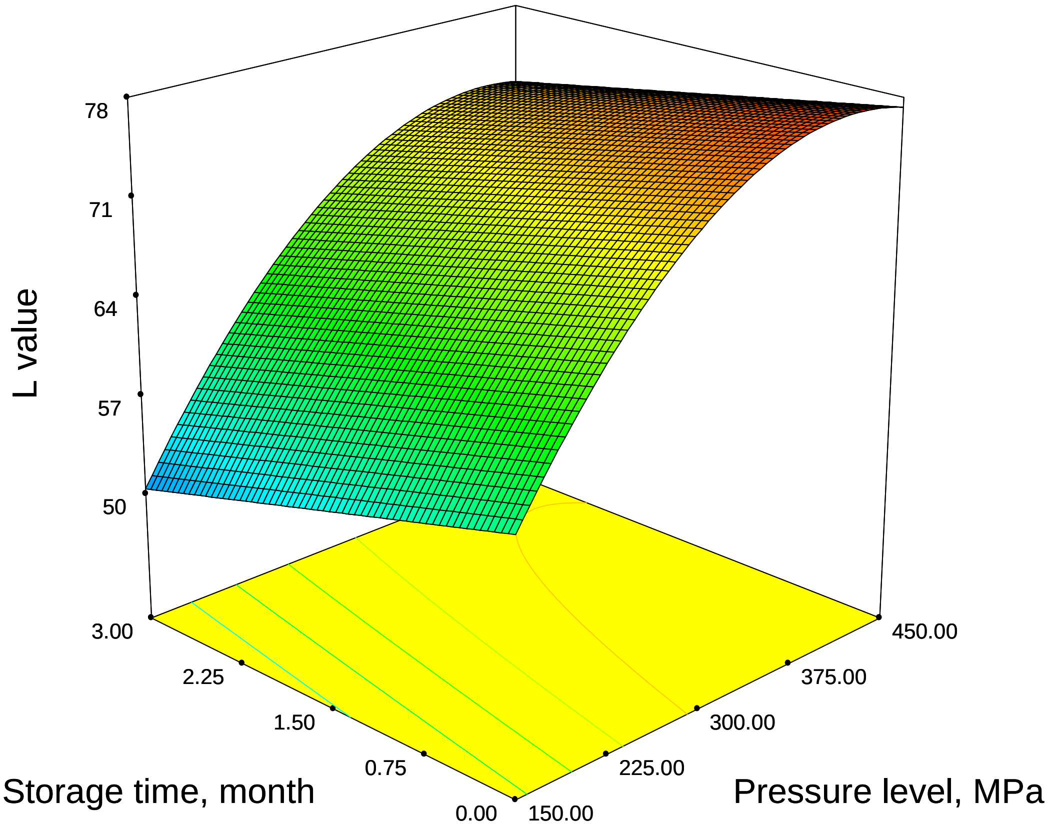
L value
78
71
64
57
50
3.00
2.25
1.50
0.75
0.00
Storage time, month
150.00
225.00
300.00
375.00
450.00
Pressure level, MPa

Figure 2

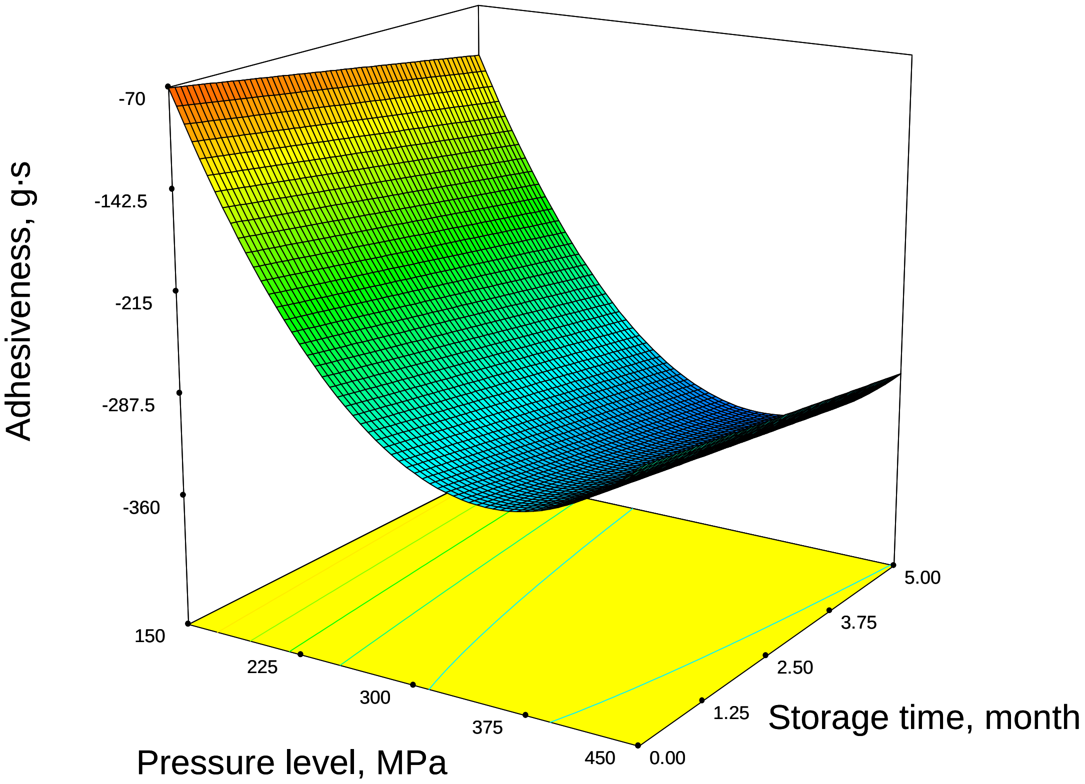
Adhesiveness, g·s
-70
-142.5
-215
-287.5
-360
150
225
300
375
450
Pressure level, MPa
0.00
1.25
2.50
3.75
5.00
Storage time, month

Figure 3

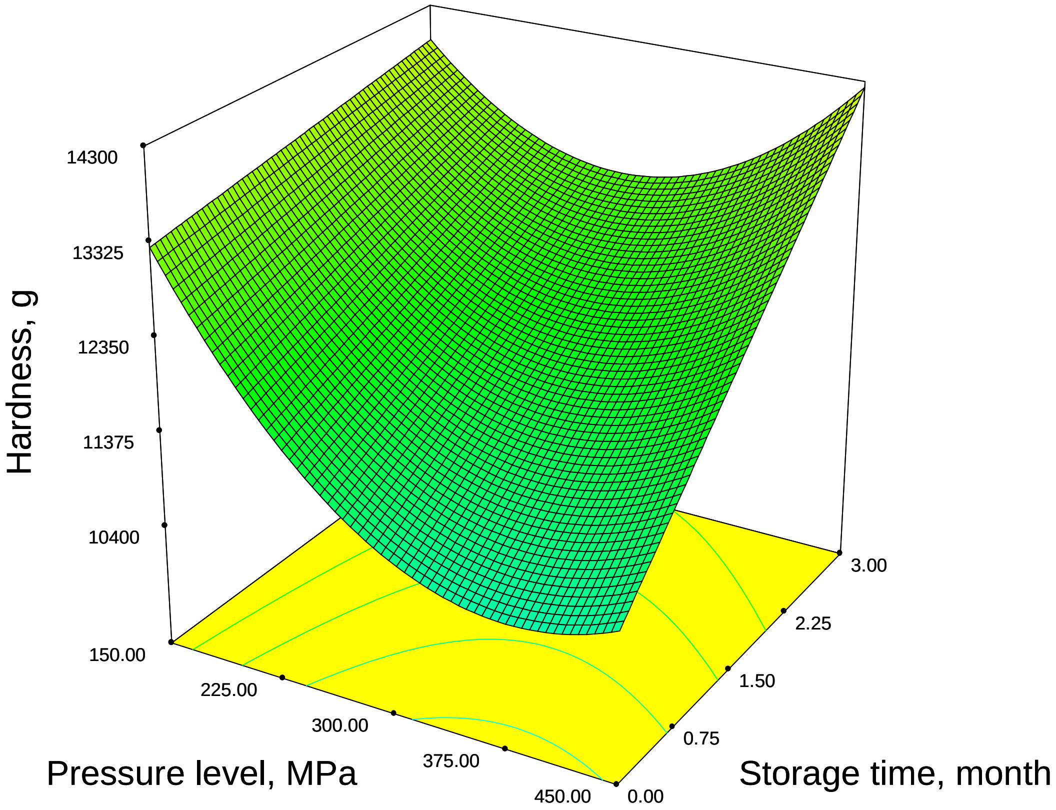
Hardness, g
14300
13325
12350
11375
10400
150.00
225.00
300.00
375.00
450.00
Pressure level, MPa
0.00
0.75
1.50
2.25
3.00
Storage time, month

Figure 4

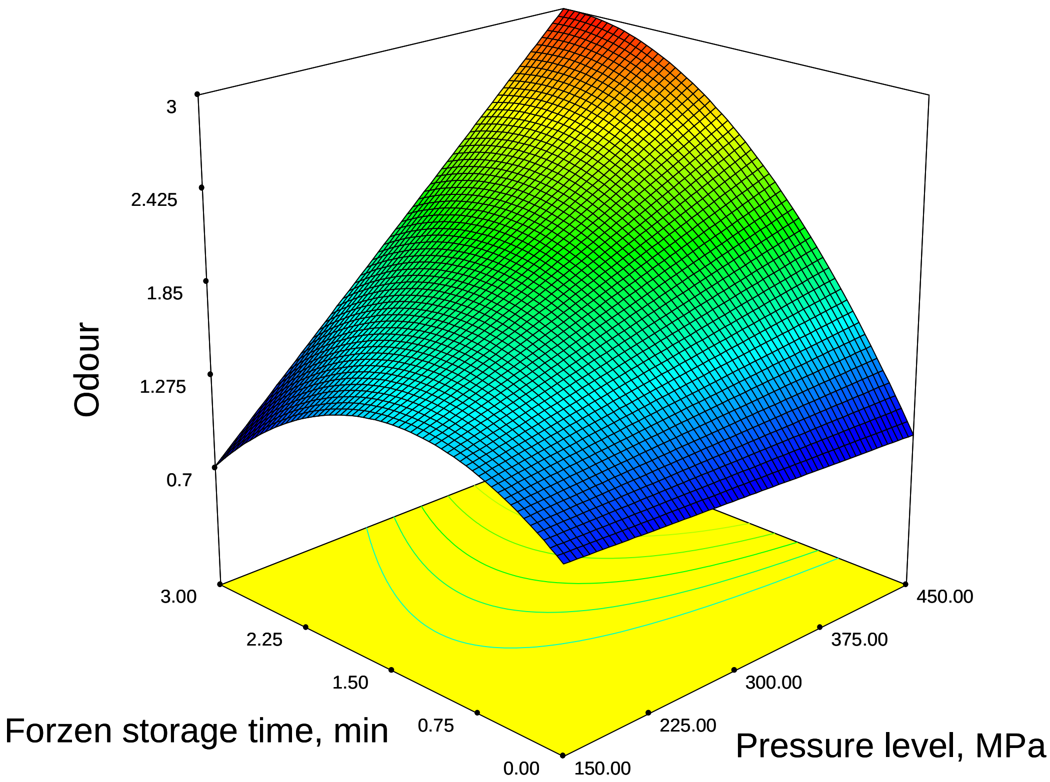
3
2.425
1.85
1.275
0.7
Odour
3.00
2.25
1.50
0.75
0.00
Forzen storage time, min
150.00
225.00
300.00
375.00
450.00
Pressure level, MPa

Figure 5

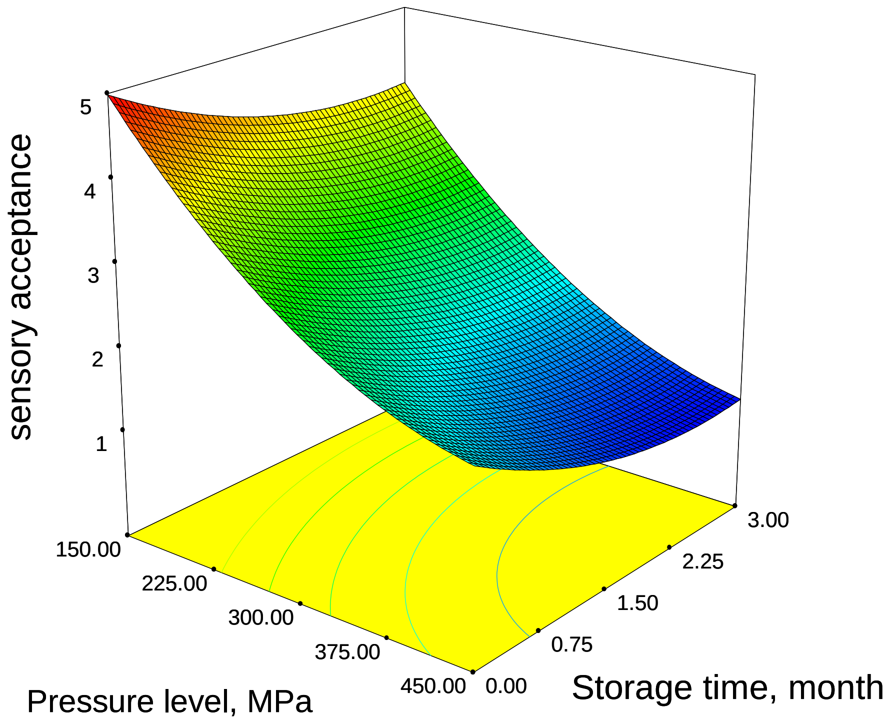
sensory acceptance
5
4
3
2
1
150.00
225.00
300.00
375.00
450.00
Pressure level, MPa
0.00
0.75
1.50
2.25
3.00
Storage time, month

Figure 6