High-pressure-induced gel of sardine (*Sardina pilchardus*) washed mince as affected by pressure-time-temperature

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ABSTRACT

Response surface methodology was used to determine how interactions of pressure (150-500 MPa), time (10-30 min) and temperature (0-75 °C) affected gelling of sardine washed mince (2.5% NaCl) as compared with conventional heat processing at atmospheric pressure. The best conditions for optimum rheological parameters were pressure around 300 MPa, chilled temperature (<10 °C) and short processing times (10 min). Pressurizing considerably improved the rheological values a mince which exhibited low gel-forming ability under conventional heat treatment.

Key words: high pressure, gel, sardine, ultrastructure, rheology

INTRODUCTION

Few studies have been published on using high-pressure technology to improve the gel-forming ability of sardine muscle washed mince, for developing new textures and producing high-quality value added products (Hayashi, 1989; Cheftel, 1995; Serennes *et al.*, 1996; Pérez-Mateos *et al.*, 1997a). Some studies have demonstrated the effectiveness of high pressure gelling in fish washed mince, and some characteristics in heat-treated gels were improved by pressure (Ko *et al.*, 1990; Yamamoto *et al.*, 1990; Chung *et al.*, 1994; Pérez-Mateos *et al.*, 1997b). No work on washed mince of sardine has been reported which determined the most suitable pressure conditions for that species. The behavior pattern of the gelling process differs according to species (Ko *et al.*, 1990), state of protein and gel-forming ability of washed mince (Lanier and Lee, 1992), and on the pressure-time-temperature combination (Yamamoto *et al.*, 1990; Okazaki, 1991). Our objective was to examine the effects of pressure, time and temperature on texture of sardine washed mince gels and to determine conditions that would maximize rheological parameters compared to gels made by heating at atmospheric pressure.

MATERIAL AND METHODS

Materials

Sardines (*Sardina pilchardus* Risso) were caught off the Mediterranean coast. Four lots of washed mince were used: M1- caught in May, average size 14.4 ± 0.9 cm and average weight 26.7 ± 6.4 g; M2- M1 after 7 mo storage at -80 °C; M3 - caught in May, average size 13.9 ± 0.8 cm and average weight 25.9 ± 7.5 g and M4 - caught in October, average size 13.1 ± 1.0 cm and average weight 31.7 ± 8.3 g.

Fish were headed, gutted and washed 24-48 h after being caught. Skin and bones were removed with a deboning machine (Baader, model 694, Lübeck, Germany). Minced muscle at 1 part mince by weight to 3 parts of an aqueous solution of 0.5% sodium bicarbonate by volume was stirred for 10 min and then held without stirring another 10 min at $0-3$ °C. After draining, excess water was removed using a screw press (Baader 523). Sorbitol (4%) and sodium tripolyphosphate (0.2%) were added as cryoprotectants. The washed mince was immediately vacuum-packed in plastic bags (Cryovac BB-1, Grace, Barcelona, Spain) and frozen in a plate-freezer (Saabroe SMC, Denmark) at -30 °C. The bags were stored at -80 °C in a vertical freezer cabinet (Revco ULT, Giralt, Revco Scientific Inc., Asheville NC) until gel preparation.

Proximate analysis

Crude protein, ash and moisture were determined according to AOAC (1984) procedures and crude fat as reported by Bligh and Dyer (1959). None of the analyses shows cryoprotectants added.

Protein functionality

Apparent viscosity (cP) based upon the method described by Borderías *et al.* (1985), was determined by the measurement of a homogenate at 3-5 °C in a viscosimeter (Brookfield-LV, Brookfield Engineering NABS, Stoughton, MA). For each measurement the rotor was run 3 min at 12 rpm. Measurements were made in triplicate.

Protein solubility in 5% NaCl was determined using the method of Ironside and Love (1958). Washed minces were solubilized in 5% NaCl using a homogenizer for 1 min (Omnimixer, Ivan Sorvall Inc., Norwalk, CT) then centrifuged at 3000 x *g* for 30 min at 0-5 °C (Sorvall RT6000B, DuPont Co., Wilmington, DE). Soluble protein was precipitated by addition of 60% TCA then centrifuged at 3000 x *g* for 15 min following the method of Kjeldhal to determine protein in the pellet.

Color

Color was determined on a colorimeter (HunterLab MiniScan MS/S-4000S, Hunter Associates Laboratory Inc., Reston, VA) using the CIE Lab scale (D65/10°). Measurements were standardized with a white calibrating tile. Results were averages of 6 measurements, taken at ambient temperature at different points on the sample, of L* the parameter that measured

lightness.

Gel preparation

- 5 Washed sardine mince was tempered at about -5/ -1 °C and placed in a refrigerated vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany). The washed minced muscle was ground for 1 min at high speed (start II). Sodium chloride (2.5% w/w) in gel (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added and the mixture homogenized for 3 min at slow speed (start I) with crushed ice to give the required final gel moisture (78%).
- 10 Homogenizing was beaten slowly (start I) for 6 min at under vacuum. Batters were stuffed into flexible plastic casings (Krehalon Soplaril, Barcelona, Spain) of 40 µm thickness and 3.5 cm dia. The filled casings were subjected to pressure-time-temperature combinations according to the described statistical model, in a high pressure pilot unit (ACB 665, Gec Alsthom, Nantes, France). For comparison with the conventional gelling procedure, heat-induced gels were made
- 15 way by waterbath immersion at atmospheric pressure: 37 °C, 30 min followed by 90 °C, 50 min. Samples were immediately cooled with running water then stored overnight in a cold room (4 °C) before analysis.

Water holding capacity

Water holding capacity (WHC) was determined by the method of Montero *et al.* (1996). About 1.5 g sample was placed in a centrifuge tube, along with two dried pipets (Gilson Pipetman, Villiers le Bel, France). WHC was expressed as water retained 100 g water present in the sample prior to centrifuging (Sorvall RT6000B, DuPont Co., Wilmington, DE) at 4000 x *g* for 15 min at ambient temperature. All determinations were made in triplicate.

Gel-forming ability

Samples were removed from their casings, cut (3.5 cm dia and 3 cm height) and tempered at 20 °C for about 2 h. *Folding test* was determined according to Suzuki (1981) on slices of 3 mm height and 3.5 cm width in accordance with a 5-point grade system as follows: 5 = no crack when folded into quadrants; 4 = no crack when folded in half; 3 = crack develops gradually when folded in half; 2 = crack develops immediately when folded in half; 1 = crumbles when pressed by fingers. In *puncture test* (breaking force, breaking deformation and work of penetration), gels were penetrated to breaking-point using a texturometer (Instron 4501, Instron Eng. Corp., Canton, MA) with a round-ended stainless steel plunger (Ø=5 mm). Cross-head speed was 10 mm/min and a 100 N load-cell was used. All determinations were carried out at least in quadruplicate. For Texture Profile Analysis (TPA), samples were placed on the flat plate of the texturometer. Compression was applied by a cylindrical plunger (Ø=58 mm) adapted to a 5 kN load cell at a deformation rate of 50 mm/min. Based on previous trials to establish a compression limit that would ensure no cracking and recoverability of most samples, we compressed samples to 50% of height. In the test, each sample was compressed twice. Parameters determined were: hardness, elasticity, adhesiveness and cohesiveness. All determinations were performed at least in quadruplicate.

Scanning electron microscopy (SEM)

Small (2-3 mm) cubes of gel were taken from the center of the cylindrical gel specimen for microscopic examination. Samples were examined following the procedure described by Gómez-Guillén *et al.* (1997).

Statistical analysis

Response surface methodology (RSM) was used to study the simultaneous effects of 3 independent variables in the first experiment (pressure: 200, 245, 310, 375, 420 MPa; time: 10, 14, 20, 26, 30 min; temperature: 0, 15, 38, 60, 75 °C) and two variables in the second experiment (pressure: 150, 200, 325, 450, 500 MPa; temperature: 0, 8, 20, 33, 38 °C) according to a central composite rotatable design, using a statistical program (Statgraphic, STSC Inc., Rockville, MD). Assessment of error was derived from replication of one treatment as suggested in the design (Cochran and Cox, 1957). Each parameter was represented by a second-order polynomial expression which predicted the effects of the combination of independent variables with a multiple correlation coefficient (r):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i,j=1}^k \beta_{ij} X_i X_j$$

Here the estimated response is Y ; equation parameter estimates (β_0 for constant, β_i for linear terms, β_{ii} for quadratic terms, β_{ij} for interaction terms); levels of factors are x_i , x_j and the number of factors is k . The significance of the equation parameters for each response variable was assessed by F test in order to check the goodness of the model as a whole. Water holding capacity was plotted to show visual interrelationships among these variables in the first experiment, combining pressure-time-temperature (two variables, the third variable remaining constant at its

mean level) versus each response variable. In the second experiment, a 3-D graph was plotted for each response variable versus the independent variables pressure-temperature.

Analysis of variance was conducted by F test and the mean of differences between pairs was determined by confidence intervals using the *Duncan test*, taking the temperature as an independent variable and pressure and time as covariables. The level of significance was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate analysis of washed minces

The first washed mince (M1) contained: crude protein $12.7 \pm 0.2\%$, moisture $78.7 \pm 0.2\%$, ash $0.8 \pm 0.02\%$ and crude fat $4.6 \pm 0.2\%$, and the second (M2) was a part of the first washed mince (M1) which was kept for 7 mo in storage at $-80\text{ }^{\circ}\text{C}$ and therefore had the same composition. The other washed minces (M3 and M4) showed similar values (crude protein: $12.6 \pm 0.3\%$ and $12.7 \pm 0.4\%$, moisture: $78.9 \pm 0.3\%$ and $78.8 \pm 0.1\%$, ash: $0.8 \pm 0.02\%$ and $0.2 \pm 0.01\%$ and crude fat: $4.4 \pm 0.2\%$ and $4.1 \pm 0.3\%$, respectively).

Heat-induced gel (T-M1)

Washed mince M1 exhibited high apparent viscosity ($3003 \pm 47\text{ cP}$) and considerable protein solubility in 5% NaCl ($53.2 \pm 1.5\%$), indicating that protein functionality was adequate. Gómez-Guillén *et al.* (1996) reported similar apparent viscosity but higher protein solubility in washed mince with high functional quality. The heat-induced gel ($37\text{ }^{\circ}\text{C}$, 30 min/ $90\text{ }^{\circ}\text{C}$, 50 min

at atmospheric pressure) had $81.7 \pm 1.9\%$ WHC and scored the maximum in the folding test (5), indicating good gel-forming ability. The values of rheological parameters in the penetration test (breaking force: 13.2 ± 0.5 ; breaking force: 3.4 ± 0.1 ; work of penetration: 45.1 ± 2.5) and compression tests (hardness: 58.3 ± 5.2 ; elasticity: 91.3 ± 6 ; adhesiveness: 1650 ± 164 ; cohesiveness: 68 ± 1) were higher than those reported with high functional capacity protein from the same species (Gómez-Guillén *et al.*, 1996; Montero *et al.*, 1996). The lightness of the heat-induced gel ($L^* = 67.3 \pm 0.8$) was significantly greater than that of the washed mince ($L^* = 61.6 \pm 0.6$) due to the high temperature (90 °C).

High-pressure-induced gels (pressure-time-temperature)

Regression coefficients for the folding test and the puncture test parameters (Table 1) showed they were affected by temperature, being very much lower at temperatures $> 40\text{ }^{\circ}\text{C}$. Only gels
 5 pressurized at temperatures under $40\text{ }^{\circ}\text{C}$ achieved the maximum score in the folding test. The same pattern has been reported for blue whiting (*Micromesistius poutassou*) washed mince gels subjected to the same pressure-time-temperature combinations (Pérez-Mateos *et al.*, 1997a). This indicated that under these experimental conditions *modori* (irreversible gel degradation) was not inhibited. On the other hand, Ko (1996) reported inhibition of *modori* in milkfish (*Chano*
 10 *chanos*) *suwari* gel pressurized at 300 MPa, 60 min. Pressure may either activate or deactivate enzymes depending on processing conditions (Hoover, 1993; Seyderhelm *et al.*, 1996).

No differences were found in the predicted values of breaking deformation using the polynomial equations for high-pressure-induced gels made at $< 40\text{ }^{\circ}\text{C}$ (Table 1) and heat-induced gels made at
 15 atmospheric pressure (T-M1 as above). However, Pérez-Mateos *et al.* (1997b) reported that deformation values were higher in high-pressure-induced gels made from washed mince of blue whiting (*Micromesistius poutassou*).

Estimated values of breaking force and work of penetration were higher in high-pressure-induced
 20 gels made at $< 10\text{ }^{\circ}\text{C}$ (Table 1) than in heat-induced gels made at atmospheric pressure (T-M1 as stated above). The same effect has been reported in gels from washed mince of blue whiting (*Micromesistius poutassou*) (Pérez-Mateos *et al.*, 1997b) or with *surimi* from Alaska pollack (*Theragra chalcogramma*) (Shoji *et al.*, 1991).

25 Chilled or moderately warm temperatures ($< 40\text{ }^{\circ}\text{C}$) were therefore, better than high temperatures ($90\text{ }^{\circ}\text{C}$) in high-pressure treatment. In gels made from washed mince of blue

whiting (*Micromesistius poutassou*), maximum breaking force values were found at 200 MPa, 0-10 °C, 10 min or 400 MPa, 37 °C, 20 min (Pérez-Mateos *et al.*, 1997b); in silvery pout (*Pollachius virens*) at 400 MPa, 30 °C, 35 min (Serennes *et al.*, 1996); and in *surimi* from bream (*Nemipterus tambuloides*) at 300 MPa, 5-10 °C, 15 min (Carlez *et al.*, 1995). The mechanics of gel formation at high pressure are not fully understood. A few studies, using differential calorimetry, have determined that part of this mechanism was somewhat to be similar to heat treatment at atmospheric pressure (Iso *et al.*, 1994).

The compression parameters in texture profile analysis (Table 2) showed that when heated under pressure the gel softened and lost adhesiveness, and in long processes resulting gels were less cohesive. Gels also tended to be softer at higher pressures. Hardness values estimated using the polynomial equations for the high-pressure-induced gels were lower than in the heat-induced gel made at atmospheric pressure, except in samples pressurized at < 10 °C. Also Pérez-Mateos *et al.* (1997b) in gels from washed mince of blue whiting (*Micromesistius poutassou*) and Carlez *et al.* (1995) with *surimi* of bream (*Nemipterus tambuloides*) reported less hardness in the high-pressure-induced gels. Okamoto *et al.* (1990), however, found that hardness of carp actomyosin gels increased with pressure and was greater than in heat-induced gels made at atmospheric pressure. Adhesiveness was much lower in high-pressure-induced gels (Table 2) than in heat-induced gels made at atmospheric pressure (T-M1 as stated above). This difference has likewise been found in gels from carp actomyosin (Okamoto *et al.*, 1990) and from washed mince of blue whiting (*Micromesistius poutassou*) (Pérez-Mateos *et al.*, 1997b). Where high-pressure-induced gels estimated using polynomial equations (Table 2) were made at low temperatures, cohesiveness values were generally higher than in heat-induced gels (T-M1 as stated above). The same pattern was also reported in gels made from blue whiting gel with 2.5 % NaCl (Pérez-Mateos *et al.*, 1997a).

Elasticity values did not fit the model well ($r = 0.75$). Data revealed a slight downward tendency in elasticity where high-temperature pressurized processes were prolonged. Values were high in all cases and did not differ markedly with respect to temperature under high pressure, also there was no difference between high-pressure and atmospheric-pressure induced gels. Ishikawa *et al.* (1991) reported that the viscoelastic properties of heat-induced sardine *surimi* gels (85 °C, 15 min) were greater than in high-pressure-induced gels made at 500-400 MPa, 20 min and lower than in gels made in a double pressure/temperature process. Pérez-Mateos *et al.* (1997b), however, found elasticity greater in high-pressure-induced gels made from washed mince of blue whiting (*Micromesistius poutassou*).

There was a significant influence of temperature on lightness (Table 2), as estimated values of L^* increased with temperature. Some studies reported lightness values were greater when higher pressure levels were used (Shoji *et al.*, 1990; Wada and Ide, 1991; Ohshima *et al.*, 1993).

Maximum estimated values of water holding capacity ($r = 0.93$) and the quadratic effect of temperature tended to be attained with pressurization at around 40 °C (Fig. 1) and were higher than the maxima of the heat-induced gel made at atmospheric pressure. According to some studies, high-pressure processing reduced water loss (Deuchi and Hayashi, 1991; Murakami *et al.*, 1994). Okazaki (1991) reported variations in WHC of soluble proteins according to species, with a clear decrease in sardine sarcoplasmic proteins from 500 MPa at 20 min or longer. However, Pérez-Mateos *et al.* (1997b) found that WHC was higher in heat-induced gels from washed mince of blue whiting (*Micromesistius poutassou*) containing 5% starch, which may have been due to gelatinization of the starch caused by the high temperature (90 °C). In high-pressure induced gels, gelatinization did not appear to take place, so WHC was not high. According to Douzlas *et al.* (1996), 15-88% gelatinization was achieved at pressures of 300-500 MPa, while in heat processing at atmospheric pressure, gelatinization was generally complete, depending on the

amount of water available (Muhr and Blanshard, 1982).

Temperature had a very marked effect on most of the parameters. The pattern was very different depending on whether low or high temperatures were used. Thus we decided to carry out analysis of variance to determine whether temperature canceled the effects of pressure over a broad temperature range (0-75 °C), since according to the experimental data the pattern was not constant. The largest number of significant differences in the tests (Table 3) fell within the upper temperature range. A second experiment was therefore carried out with 38 °C as the bottom of the temperature range and a slightly wider range of pressures (150 - 500 MPa) in order to further focus the effect. The optimum processing time was taken as 10 min, as the target rheological parameters were either better with short times or did not vary greatly. Gilleland *et al.* (1995) used longer pressurizing times (30-120 min) but found no time-dependent differences in gelling of *surimi* of Alaska Pollack (*Theragra chalcogramma*) at 300 MPa.

Heat-induced gel (T-M2)

When washed mince M1 had been 7 mo in storage at -80 °C, a further gel assay was performed (T-M2) at atmospheric pressure (processing at 37 °C, 30 min/90 °C, 50 min) for comparison with high-pressure induced gels from washed mince. The apparent viscosity of this washed mince was 3550 ± 63 cP and its protein solubility in 5% NaCl was 48.9 ± 6.8%. We compared this gel (breaking force: 15.2 ± 0.4; breaking force: 4.5 ± 0.3; work of penetration: 68.2 ± 6.1) and compression tests (hardness: 47.4 ± 2.5; elasticity: 97.7 ± 0.3; adhesiveness: 1061 ± 116; cohesiveness: 76 ± 2) with the previous heat-induced gel (T-M1 as stated above), which scored maximum in the folding test. There was an improvement in gel-forming ability when the washed minced muscle had been kept in frozen storage. The reason for this may be that gelling was favored by denaturation and aggregation, or that enzymes which would normally degrade the gel

during heating were deactivated (Montero *et al.*, 1996). A similar tendency was reported in gels from washed mince of sardine (*Sardina pilchardus*) which had been kept chilled three days (Huidobro *et al.*, 1992).

- 5 The lightness of the washed mince ($L^* = 60.7 \pm 0.9$) did not change as a result of frozen storage and hence did not alter the lightness in the heat-induced gel ($L^* = 67.6\% \pm 0.8$). Montero *et al.* (1996) reported that lightness of washed sardine mince remained stable during frozen storage regardless of packing conditions.

10 High-pressure-induced gel (pressure-temperature)

- Maximum folding test scores (5) were attained by gels made under all pressure-temperature combinations used in the second experiment (150-500 MPa/0-38 °C), including atmospheric pressure. Rheological parameters in the puncture test were compared (Fig. 2): breaking
- 15 deformation ($r = 0.89$), breaking force ($r = 0.93$) and work of penetration ($r = 0.93$). The graphs show the quadratic effects of pressure and temperature and the linear effects of temperature ($p \leq 0.05$). These parameters were highest at intermediate pressures in the range 300-400 MPa and in processes carried out at chilled temperatures (0-10 °C). In the atmospheric-pressure heat-induced gel, there was little variance from estimated values. However, Pérez-Mateos *et al.* (1997a,b),
 - 20 working with washed blue whiting mince of low gel-forming ability, found much higher values for breaking deformation and work of penetration in pressure-induced gels than in heat-induced gels made at atmospheric pressure. Experiments on pressure-induced gels made from Alaska pollack (*Theragra chalcogramma*) surimi have likewise shown breaking deformation values were
 - 25 highest at 200-300 MPa, 0 °C, 10 min and higher than those found in a heat-induced gel (Shoji *et al.*, 1991). Again, higher breaking force values have been found in gels from washed minced muscle of sardine (*Sardinops melanostictus*) and Alaska pollack (*Theragra chalcogramma*) upon

increasing the pressure (Ko *et al.*, 1990).

The parameters considered in the texture profile assay (Fig. 3) showed the linear effect of temperature ($p \leq 0.05$) on: hardness ($r = 0.87$), adhesiveness ($r = 0.86$) and cohesiveness ($r = 0.81$).

5 The response surface graphs showed a tendency for these values to decrease in high-pressure processing at temperatures $> 40^\circ\text{C}$. High-pressure-induced gels proved harder, and more adhesive and cohesive, than heat-induced gels at atmospheric pressure, except under very high pressure (500 MPa) at 40°C . A decrease in adhesiveness was reported in carp actomyosin gels with increasing pressure (Okamoto *et al.*, 1990). The experimental data for elasticity did not
10 quite fit the model ($r = 0.76$), but the experimental data indicated that maximum elasticity occurred at ≈ 300 MPa. Pérez-Mateos *et al.* (1997a) likewise reported greater elasticity in blue whiting gels made at around 300 MPa.

There was a significant effect ($p \leq 0.05$) of pressure-temperature interaction (Fig.4) on lightness ($r = 0.80$).
15 Lightness of high-pressure-induced gels was generally similar to that of heat-induced gels made at atmospheric pressure. L^* values tended to be highest at 400 MPa and moderate temperature (40°C), or at relatively low pressure (200 MPa) and lower temperatures ($0-10^\circ\text{C}$). There have been reports of higher values in high pressure induced gels than in heat-induced gels: e.g., in blue whiting gels (Pérez-Mateos *et al.*, 1997b), in carp actomyosin gels (Okamoto *et al.*,
20 1990), and in carp and mackerel *surimi* gels (Yoshioka *et al.*, 1992). Hence optimum processing conditions for maximizing certain rheological parameters were in the region of 300 MPa at chilled temperature ($<10^\circ\text{C}$) for 10 min.

Heat and high-pressure-induced gels with different gel-forming ability (M3 and M4)

25 Differences ($p \leq 0.05$) were detected in the rheological parameters of the heat-induced gels

depending on washed mince quality (T-M1 and T-M2 as seen above). Therefore, we analyzed the effects of pressure treatment where rheological values were highest, on washed minces with different gel-forming abilities (Table 4).

Washed mince M3 exhibited high apparent viscosity (4017 ± 74 cP) and low protein solubility in 5% NaCl ($37.8 \pm 0.9\%$), indicating a high degree of protein aggregation. The resulting gels scored the maximum in the folding test. High-pressure processing (P-M3) produced better rheological characteristics than in heat-induced gels (T-M3) made at atmospheric pressure (Table 4). The parameters considered were higher ($p \leq 0.05$) with the exception of breaking deformation, which was similar. Lightness values were higher ($p \leq 0.05$) in the heat-induced gel ($L^* = 64.3 \pm 1.3$) than in the high-pressure-induced gel ($L^* = 61.4 \pm 1.7$), and both exceeded the lightness of the washed mince ($L^* = 56.52 \pm 1.7$).

Washed mince M4 was of high functional quality with 2747 ± 193 cP apparent viscosity and high protein solubility in 5% NaCl ($60.7 \pm 1.0\%$) (Gómez-Guillén *et al.*, 1996). Both gels again scored maximum in the folding test. The heat-induced gel exhibited high gel-forming ability comparable to that in washed sardine mince gels with egg white (Gomez-Guillén *et al.*, 1997). The target rheological parameters were not improved by high-pressure processing (P-M4) and in some cases were lower ($p \leq 0.05$). However, in most cases values were higher than those obtained with other washed mince quality (Table 4). As with washed mince M3, lightness values were higher ($p \leq 0.05$) in the heat-induced gel ($L^* = 66.1 \pm 1.6$) than in the high-pressure-induced gel ($L^* = 64.1 \pm 0.4$), although all values were higher than in the original washed mince ($L^* = 53.7 \pm 1.7$).

SEM images at 3,000x magnifications were made of gels from washed minces M3 and M4 (Fig. 5) using either heat treatment (T: 37 °C, 30 min/ 90 °C, 50 min) or high pressure treatment (P: 300 MPa, <10 °C, 10 min). The heat-induced gel from washed mince M3 exhibited an oriented

fibrous structure; that from washed mince M4 also had a fibrous network, but with a more open structure and more zones of globule aggregation. Álvarez *et al.* (1993) reported that a combination of the two structures resulted in high work of penetration values.

5 The high-pressure-induced gel from M3 exhibited a structure with more compact zones than the heat-induced gel, and consequently the rheological parameters were higher, although WHC was lower ($73.0 \pm 2.2\%$) than in the heat-induced gel ($WHC = 77.1 \pm 2.2\%$). This may be due to the fact that there were adjacent cavities which coalesced to form larger, elongated cavities from which retained water was more readily released. The high-pressure-induced gel from M4 had a
 10 spongy structure (highest elasticity) with rounded individual cavities. Much compacting was evident, as was the loss of globular structure, resulting in lower work of penetration (Gómez-Guillén *et al.*, 1997). Water holding capacity ($78.7 \pm 2.2\%$) was higher ($p \leq 0.05$) than in the heat-induced gel ($71.45 \pm 1.2\%$), possibly due to greater compactness. Both high-pressure-induced gels exhibited considerable compactness which was reflected as hardness.

15 CONCLUSIONS

High-pressure treatment at $> 40^\circ\text{C}$ did not prevent onset of *modori* or irreversible destruction of the gel. The high-pressure-induced gels generally exhibited a more even, glossy, bubble-free
 20 surface than did heat-induced gels made at atmospheric pressure. The high-pressure process yields advantages over conventional heat treatment in that gelling could be achieved in a short time (10 min) and without heating. High-pressure treatment improved the rheological properties of sardine washed mince with low gel-forming ability but did not so improve those with high gel-forming ability.

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Figure 1.- Response surface for water holding capacity of sardine gels from washed mince M1 by pressure-time-temperature treatment

Figure 2.- Response surface for breaking deformation, breaking force and work of penetration of sardine gels from washed mince M2 from pressure-temperature treatment

Figure 3.- Response surface for hardness, adhesiveness and cohesiveness of sardine gels from washed mince M2 for pressure-temperature treatment

Figure 4.- Lightness of sardine gels from washed mince M2 for pressure-temperature treatment

Figure 5.- SEM (3000x) of gels from two washed minces by heat treatment or high pressure treatment (bar = 10 μ m)

TM3: gel made at 37 °C, 30 min/ 90 °C, 50 min from washed mince M3;

PM3: gel made at 300 MPa, <10 °C, 10 min from washed mince M3;

TM4: gel made at 37 °C, 30 min/ 90 °C, 50 min from washed mince M4;

PM4: gel made at 300 MPa, <10 °C, 10 min from washed mince M4.

Table 1.- Regression model fitted for folding test and puncture test of sardine gels from washed mince M1 subject to pressure ($i=1$), time ($i=2$) and temperature ($i=3$) parameters

	Folding test		Breaking deformation		Breaking force		Work of penetration	
	RC	SE	RC	SE	RC	SE	RC	SE
b_0	5.04	0.28	12.08	0.56	1.10	0.23	13.24	3.53
b_1	0	0.19	-0.59	0.37	-0.11	0.15	-2.57	2.34
b_2	0	0.19	-0.77	0.37	-0.14	0.15	-2.48	2.34
b_3	-1.79**	0.19	-2.64**	0.37	-1.13**	0.15	-17.72**	2.34
b_{11}	-0.24	0.19	0.14	0.36	0.23	0.15	4.37	2.28
b_{22}	-0.24	0.19	0.15	0.36	0.21	0.15	3.62	2.28
b_{33}	-1.12**	0.19	-1.03*	0.36	0.36*	0.15	3.59	2.28
b_{12}	0	0.25	0.14	0.48	-0.04	0.20	-0.81	3.06
b_{13}	0	0.25	-0.12	0.18	-0.16	0.20	-2.58	3.06
b_{23}	0	0.25	-0.68	0.48	-0.17	0.20	-1.96	3.06
r	0.96		0.94		0.93		0.93	

RC = regression coefficient; SE = standard error; * significant at $p \leq 0.05$

** significant at $p \leq 0.01$; r = multiple correlation coefficient

Table 2.- Regression model fitted for compression and lightness of sardine gels from washed mince M1 subject to pressure ($\dot{i}=1$), time ($\dot{i}=2$) and temperature ($\dot{i}=3$) parameters

	Hardness		Adhesiveness		Cohesiveness		Lightness	
	CR	ES	CR	ES	CR	ES	CR	ES
b_0	22.47	1.02	609.30	37.48	0.65	0.03	66.14	1.02
b_1	-1.58*	0.68	-18.75	24.87	-0.01	0.02	0.28	0.68
b_2	-1.36	0.68	7.23	24.87	-0.05*	0.02	-0.33	0.68
b_3	-18.95**	0.68	-274.44**	24.87	-0.21**	0.02	2.81**	0.68
b_{11}	0.97	0.66	-33.70	24.20	0.01	0.02	0.41	0.66
b_{22}	-0.08	0.66	-48.93	24.20	0.01	0.02	0.60	0.66
b_{33}	5.21**	0.66	-13.91	24.20	-0.05*	0.02	0.70	0.66
b_{12}	1.00	0.88	18.43	32.49	0	0.03	-0.49	0.89
b_{13}	1.14	0.88	27.15	32.49	0	0.03	-1.12	0.89
b_{23}	-1.78	0.88	38.01	32.49	-0.09**	0.03	-1.50	0.89
r	0.99		0.96		0.97		0.84	

RC = regression coefficient; SE = standard error; * significant at $p \leq 0.05$

** significant at $p \leq 0.01$; r = multiple correlation coefficient

Table 3.- Analysis of variance of rheological parameters of sardine gels from washed mince M1 under pressure-time-temperature conditions

	FT	BD	BF	WP	Ha	El	Ad	Co
0 °C	a	a	a	a	a	a	a	a
15 °C	a	a	a	a	b	ab	b	a
38 °C	a	a	b	b	c	ab	b	a
60 °C	b	b	b	b	d	ab	c	b
75 °C	c	b	b	b	d	b	c	b

Folding test (FT), breaking deformation (BD), breaking force (BF), work of penetration (WP), hardness (Ha), elasticity (El), adhesiveness (Ad) and cohesiveness (Co). Different letters in the same column indicate significant differences ($p \leq 0.05$) between treatment temperature

Table 4.- Puncture test and compression test of sardine gels from two washed minces

	Breaking deformation [mm]	Breaking force [N]	Work of penetration [N.mm]	Hardness [N]	Elasticity [%]	Adhesiveness [g.cm]	Cohesiveness [%]
T-M3	13.4a	3.5a	46.4a	51.4a	88.7a	1162a	76a
P-M3	13.8ab	5.0ab	73.6b	84.4b	93.3b	1344a	84b
T-M4	17.34ac	6.9b	117.6c	52.4a	90.7a	1258a	73a
P-M4	16.0c	5.0a	81.3b	76.4b	96.5b	1349a	82b

TM3: gel made at 37 °C, 30 min/ 90 °C, 50 min from washed mince M3.

PM3: gel made at 300 MPa, <10 °C, 10 min from washed mince M3.

TM4: gel made at 37 °C, 30 min/ 90 °C, 50 min from washed mince M4.

PM4: gel made at 300 MPa, <10 °C, 10 min from washed mince M4.

Different letters (a, b, c) in the same column indicate significant differences ($p \leq 0.05$) among gels