Chilled storage of high-pressure and heat-induced gels of blue whiting (*Micromesistius poutassou*) muscle

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ABSTRACT

Microbiological, rheological and chemical characteristics were examined in gels made from muscle of blue whiting (*Micromesistius poutassou*) subjected to three different combinations of pressure, temperature and time: 200 MPa 3°C 10 min (lot L), 375 MPa 38°C 20 min (lot H) and atmospheric pressure 37°C 30 min/ 90°C 50 min (lot T), and kept in chilled storage for 20 days.

Microorganism content dropped at the outset as pressurizing took effect, the highest microbial content being found in lot L; however as the effect was not lethal, the total load increased over the following days. Microbial load was quite lower in lot T. During chilled storage, the values of breaking deformation, breaking force and cohesiveness of lot L were higher than those of the other lots, although this did decrease over storage. The heat-induced gel was much harder, had greater water holding capacity and was considerably more stable than the high-pressure-induced gels. The lightness value was higher in lot H than in the other two lots. In general, changes in protein solubility tended towards the cleavage of strong bonds as a result of microbial action. The electrophoretic profiles evolved differently in each of the lots over the chilled storage period. However, all of them exhibited large numbers of bands of lower molecular weight which could be the results of degradation. This was particularly evident in lot H. The heat-induced gels exhibited a highly porous ultrastructure, quite different from the high-pressure-induced gels; these had a more compact matrix which expanded as storage progressed.

INTRODUCTION

There have been a number of articles in recent years on gelling of *surimi* and minced fish using high-pressure technology at different temperatures, to obtain glossier, smoother, softer and more deformable gels than are possible by heating (6,7,13,14). Pérez-Mateos *et al.* (14) found that with blue whiting mince, gels made by applying 200 MPa at 3°C for 10 min or 375 MPa at 38°C for 20 min were stronger and more deformable than heat-induced gels. The object of the present experiment was to compare these two gels with the heat-induced gel in terms of the changes occurring in the rheological pattern and the total viable microorganism count, and chemical and microstructural alterations during chilled storage.

Few references were found in the literature to storage of high-pressure-induced gels made from fish muscle. Wada (21), working with sardine muscle gels stored at 5°C for 4 days, found that the total number of microorganisms, the K-value index and free fatty acids were all lower in samples subjected to high pressure than in those not, and hence their storage life was considerably prolonged. Shoji *et al.* (17) found that in high-pressure-induced Alaska pollack *surimi* gels, breaking force increased considerably in the course of storage at 5°C for 120 hours, owing to polymerization of the myosin heavy chains. Shoji *et al.* (19) reported that this polymerization appeared to involve not only cross-linkages but also hydrophobic interactions. Shoji *et al.* (18) suggested that because of this, it would be possible to control the quality of these gels through both the high-pressure process and storage conditions.

MATERIALS AND METHODS

Blue whiting (*Micromesistius poutassou* Risso) used in this study was caught off the

Cantabrian coast and kept at 4°C for about 24 hours. Average size was 23.4 ± 1.2 cm and average weight 77.8 ± 12.3 g. Fish were headed, gutted and washed. Skin and bones were removed with a deboning machine (Baader, model 694, Lübeck, Germany). Muscle was minced and washed in a solution of 0.2% NaCl at 0-3°C, proportion 3:1 (solution: minced muscle), first with constant stirring for 10 min then without stirring for another 10 min. After draining, excess water was removed using a screw press (Baader, model 523, Lübeck, Germany). Sorbitol (4%) and tripolyphosphate (0.2%) were added as cryoprotectants. The mince was immediately vacuum-packed in bags (Cryovac BB-1, Grace, Barcelona, Spain) and frozen in a plate-freezer (Saabroe SMC, Denmark) to a temperature of -30°C. The bags were stored at -80°C in a freezer cabinet (Revco ULT, Giralt, Revco Scientific Inc., Asheville, NC, USA) in order to minimize alteration during frozen storage up to gel preparation.

Proximate analysis was performed according to AOAC procedures (1) and crude fat by Bligh and Dyer (2). For mince the composition was: crude protein $12.3\% \pm 0.3$, moisture $82.9\% \pm 1.2$, crude fat $0.5\% \pm 0.04$ and ash $0.6\% \pm 0.01$. Analyses do not show the cryoprotectants added.

Gel preparation. Washed blue whiting mince was tempered at about -4 °C and placed in a refrigerated vacuum homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany). The muscle was chopped for 1 minute at high speed (start II). 1% sodium chloride (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added and the mixture homogenized for 3 min at slow speed (start I). It has been reported that the highest values of work of penetration are obtained with a concentration of 1% NaCl in the gel (14). Next 5% modified waxy corn starch (Clearam CH 20, Laisa, Barcelona, Spain) was added with crushed ice to give the required final gel moisture (78%) and homogenizing continued for 6 min at 1,500 rpm in vacuum conditions. The resulting batters were stuffed into cases (Krehalon

Soplaril, Barcelona, Spain) of 40 μm thickness and 3.5 cm diameter. The filled casings were subjected to either of two pressure-time-temperature combinations: 200 MPa for 10 min at 3°C (lot L) and 375 MPa for 20 min at 38°C (lot H) in a high-pressure pilot unit (ACB 665, Gec Alsthom, Nantes, France). For comparison with the traditional gelling procedure, heat-induced gels were made at atmospheric pressure: 37°C for 30 min/90°C for 50 min by waterbath immersion (lot T). All the casings were immediately stored in a cold room at 4°C analyzed at 1, 5, 10 and 20 days.

Microbiological analysis. Total viable microorganism counts were carried out on PCA incubated at 30°C for 72 hours. This was expressed in log ufc/g of gel.

Folding test. A slice 3.5 cm in diameter and 3 mm high was folded over twice, scoring from 1 to 5 according to Suzuki (20).

Rheological analysis. For texture analyses, samples were removed from their casings, cut (3.5 cm diameter, 3 cm height) and tempered at 20°C. Puncture test (8): Gels were penetrated to breaking-point using a texturometer (Instron 4501, Instron Engineering Corp., Canton M.A., USA) with a round-ended stainless steel plunger ($\varnothing = 5 \text{ mm}$). Cross-head speed was 10 mm/min and a 100 N load-cell was used. Breaking force [N] and breaking deformation [%] were determined as the product of both parameters. All determinations were carried out at least in quadruplicate. Texture Profile Analysis (3). Samples were placed on the flat plate of the texturometer. Compression was applied by a cylindrical plunger ($\varnothing = 58 \text{ mm}$) adapted to a 5 kN load cell at a deformation rate of 50 mm/min. On the basis of previous trials to establish a compression limit that would ensure no cracking and recoverability of most samples, it was decided to compress samples to 50% of height. In the test, each sample was compressed twice running. The parameters determined were hardness [N] and cohesiveness. All

determinations were performed at least in triplicate.

Water holding capacity. This was performed according to the method of Montero *et al.* (11).

Water holding capacity (WHC) was expressed as water retained per 100 g of water present in the sample prior to centrifuging. All determinations were carried out in triplicate.

Color of mince and gels was determined on a colorimeter (HunterLab MiniScan MS/S-4000S, Hunter Associates Laboratory Inc., Reston, VA, USA) using the CIELab scale (D65/10°) where L* is the parameter that measures lightness. The result was the average of six measurements taken at ambient temperature at different points on the sample.

Protein solubility of gel. This was determined in different solutions as a measurement of the types of bonds present in the three gels and their evolution over the period of chilled storage. The gels were solubilized in four solutions at pH = 7.0 (10,15): 0.6 M sodium chloride (S1), 1.5 M urea + 0.6 M sodium chloride (S2), 8 M urea + 0.6 M sodium chloride (S3) and 0.5 M 2-β-mercaptoethanol + 8 M urea + 0.6 M sodium chloride (S4). Two grams of chopped gel was homogenized with 10 mL of S1 (Ultraturrax TP18/10, Janke & Kunkel, Ika-Werk, Staufen, Germany) for 2 min at maximum speed. The resulting homogenate was stirred at 4°C for 1 h and then centrifuged for 20 min at 20,000g (Beckman J2-MC centrifuge, Beckman Instruments Inc., Palo Alto, California, USA). The pellet obtained was homogenized in S2 by the same process, then again in S3 (in this case, twice) and finally in S4. Protein concentration was determined in triplicate: in the supernatants, based on the method of Lowry (9) using a commercial preparation (DC Protein Assay Reagent S n° 500-0116, Bio-Rad Laboratories, Hercules, CA, USA), and in the insoluble protein by the method of Kjeldhal. Solutions were dialysed in order to prevent interference in protein determination, Colorimetry was performed in a spectrophotometer (UV-1203, Shimadzu, Kyoto, Japan) at 750 nm. Results are the

average of two determinations and are expressed as percentage of solubilized protein with respect to total protein.

Electrophoresis. Soluble proteins were adjusted to a final average concentration of 2 mg/mL.

5 Electrophoresis was carried out on a Phast-System apparatus (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) using 12.5% polyacrylamide gels (SDS-PAGE, PhastGel, Pharmacia Biotechnology AB, Uppsala, Sweden). Electrophoresis conditions were 10 mA, 250 V and 3 W, at 15°C. The protein bands were stained with coomassie brilliant blue (PhastGel Blue R, Pharmacia, Uppsala, Sweden). As reference for molecular weights, a standard high molecular
10 weight protein mix (HMW, Pharmacia Biotech., Piscataways, NJ, USA) was used: ferritin half-unit (220 kDa), albumin (67 kDa), catalase subunit (60 kDa), lactate dehydrogenase subunit (36 kDa) and ferritin subunit (18.5 kDa). The bands were analysed using an Image Analyzer (3CX, Bio Image and Visage, Millipore Co., Ann Arbor, Michigan, USA) and scanner (3CX, Microtek Scanners, K&M Co., USA).

15 Scanning electron microcopy (SEM). Cubes of 2 to 3 mm were cut from inside the gels for microscopic examination. Samples were fixed in 2% glutaraldehyde in phosphate buffer (pH = 7.3) and dehydrated in increasing series of acetone (from 40 to 100%). They were then critical-point dried with CO₂ as transition fluid in a dryer (Balzer CPD030, Liechtenstein) and
20 mounted on copper sample holders, followed by sputter-coating with gold in a metallizer (Balzer SCD004). Samples were kept in a dryer until examination by scanning microscope (Jeol Scanning Microscope, JSM 6400, Japan) at 20 kV. Micrographs were taken of each gel at x500 magnifications.

25 Statistical analysis. Two-way analysis of variance was carried out using the Statgraphics statistical computer program (STSC Inc., Rockville MD, USA). Differences between the means

for data pairs were computed using confidence intervals according to the LSD range test [significance level: ($p \leq 0.05$)].

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RESULTS AND DISCUSSION

Total microorganism counts (Table 1). After gelling and overnight storage at 4°C, it was initially found that the total viable count was significantly lower than in the batter prior to the gelling process (6.4 log ufc/g). The count was much lower ($p \leq 0.05$) in the lot T than in the high-pressure-induced gels, due largely to the high temperature in heat induction (90°C for 50 min). The decrease in the total viable count was smaller in lot L because the pressure-time-temperature conditions applied were milder than for the lot H. The counts increased during storage in all the gels ($p \leq 0.05$), although the patterns of increase differed. Pressurization affected the microorganisms (7,6,16), and the total viable count had fallen further by the 5th day of chilled storage ($p \leq 0.05$); it should be said that the magnitude of pressure cannot account for the lower value found in lot L, since the pressure used was lower. However, since the effect of pressure was not lethal, the total loads increased considerably over the following days of storage, and by day 20 they had exceeded the maximum acceptable level (6 log cfu/g) in L lot.

The gels of all the lots scored 5 in the folding test after preparation and chilling for one night at 4°C. The folding test values for the gels in lots T and L held steady at maximum throughout storage; however, the scores for the gels in lot H had fallen to 2 by the fifth day. This decrease would not appear to be connected with the increase in the microorganism count, since this was greatest in lot L.

The breaking deformation values (Fig. 1) for the gels in lot L remained much higher ($p \leq 0.05$) than for the gels in the other two lots, as was previously reported by Pérez-Mateos *et al.* (15). These values decreased ($p \leq 0.05$) over the storage period in the high-pressure-induced gels (L and H lots), a development apparently related to the increase in microorganisms, but

increased slightly ($p \leq 0.05$) in the heat-induced gels (lot T) up to day 5 and then held steady at that level. However, Shoji *et al.* (17,18) have described an increase in breaking strength of high-pressure-induced gels over 5 days' chilled storage, due to an increase in myosin heavy chain polymers as new cross-linkages formed.

The breaking force values (Fig. 1) show that the lot T was the most stable ($p \leq 0.05$) over the storage period, whereas the values for the lot H decreased ($p \leq 0.05$) in the first 5 days, stabilizing thereafter. The highest breaking force values ($p \leq 0.05$) were recorded for the lot L on day 5, although these had fallen to the same level as in the heat-induced lot by the end of storage. This trend would appear to be related to the microbial load. Shoji *et al.* (17) reported that breaking force values for high-pressure-induced (at 0°C) gels increased with storage time at 5°C and peaked on day 5, although the authors cited did not store gels for any longer. The breaking force values in lot L were consistent with those described by Shoji *et al.* (17); in both cases, the gels were made at low temperature.

Hardness values (Fig. 1) underwent changes in the early days of chilled storage and then levelled off. This may be connected with the changes in covalent bonds linking the myosin heavy chains (17). The lot T was much harder ($p \leq 0.05$) at all times, while the lot H was the softest ($p \leq 0.05$). Hardness declined in both lots during the first 5 days of chilled storage.

Cohesiveness values (Fig. 1) generally remained fairly stable, varying ($p \leq 0.05$) only within a very narrow margin. Lot L exhibited the highest cohesiveness at all times ($p \leq 0.05$). Loss of cohesiveness was more evident, especially at the end of storage (day 20), in the lot H, which exhibited the lowest cohesiveness values of all the lots.

Water holding capacity (Fig. 2) values were higher in the heat-induced gel than in the high-

pressure-induced gels throughout storage ($p \leq 0.05$). According to Murakami *et al.* (12), however, high-pressure treatment could reduce the volume of exudate. Pérez-Mateos *et al.* (14) reported that in blue whiting mince gels containing 5% starch, the significant factor affecting WHC was temperature rather than pressure. Then again, Douzlas *et al.* (5) reported that using pressures of 300-500 MPa, 15-88% gelatinization was achieved as compared to full gelatinization around the gelatinizing temperature in heat-induced gel at atmospheric pressure, which suggests that WHC is improved by heating to gelatinization temperatures. The differences ($p \leq 0.05$) in WHC of lots L and H could be due to the greater weakness of the lot H as indicated by the rheological parameters considered.

With respect to variation ($p \leq 0.05$) of lightness values (Fig. 2), these were highest in lot H, lowest in lot L and intermediate in lot T (15). Each lot varied only within a very narrow margin over the storage period. Okamoto *et al.* (13) also found that lightness increased the higher the pressure. The greater lightness of lot H was probably due to the combination of pressure and moderate temperature (15).

Protein solubility (Fig. 3): solubility in S1 increased ($p \leq 0.05$) in pressurized lots over chilled storage. The higher proportion of S1-soluble protein would indicate that the number of protein molecules either unbonded or linked by weak ionic bonds was greater; this may be connected with the increase in microorganisms noted earlier. In heat-induced lot, solubility in S3, used as a measurement of cleavage of hydrophobic interactions over storage, increased ($p \leq 0.05$), which may be connected with a slight increase in the rheological parameters considered. In lot L, on the other hand, solubility in S3 declined significantly in favour of the insoluble fraction (In) between the first and the fifth day; this changes were reflected rheologically in an increase in most of the parameters. Again in lot L, solubility in S3 increased and the insoluble fraction declined after the fifth day, probably due to the microorganisms, which likewise increased and

could hydrolyse such aggregates to produce more soluble forms (S1). In lot H, solubility in S3, solubility in S4 and the insoluble fraction (In) all decreased in the course of storage. This would indicate the breaking of hydrophobic interactions and strong bonds, which would favour solubility in S1: that is, formation of weak bonds. This could account for the changes in rheological properties observed in this lot as the microorganism count rose. The proportion of covalent bonds in lot H decreased from the outset of storage; this could be connected with deactivation of *transglutaminases* which takes place in treatment at pressures over 300 MPa, thus preventing any formation of covalent bonds (19).

SDS-PAGE patterns of the fractions soluble in the different solutions (Fig. 4) exhibited differing profiles for the various lots over the storage period. In lot T, there was no great difference between the profile at day 1 and in the final control, whereas in the case of the pressurized lots, this difference was considerable. In lot H, both the aggregates and the protein fractions of molecular weight 150-200 kDa in the fraction containing hydrophobic bonds (S3) practically disappeared, while the low-molecular-weight bands increased. Again in lot H, in the fraction containing disulfide bonds (S4), aggregates at the point of application and two groups of bands (150-200 and 10-60 kDa), which were apparent at the outset of storage, had disappeared almost entirely by the end of the period. In lot L, bands at 30-40 kDa were more evident in the soluble fractions containing weak bonding or unbonded proteins (S1 and S2) at the outset of storage than at the end. Moreover in lot L, the aggregates at the point of application in the solutions containing hydrophobic interactions (S3) disappeared; disulfide bonds (S4) were less apparent, but in both profiles there was an increase in the bands in the range 60-150 kDa. This proteolysis could be due to microbial activity.

Microscopy (Fig.5). Throughout storage, gel T exhibited cavities which were much larger than

those found in the pressurized gels, making for a very open, uniform mesh. As in the other determinations described heretofore for lot T, there was little change by the end of storage. The pressurized lots presented a very similar appearance between them at the outset; however, by the end of storage, their ultrastructures were very different. Gel L exhibited a network structure on a compact matrix with fewer cavities and gel H exhibited a much looser, open matrix, reflecting the loss of rheological properties and water holding capacity. As regards the starch, gelatinization in cavities was greater in heat-induced than high-pressure-induced gels. In the latter, starch granules were quite evident, a fact consistent with the lower values of water holding capacity (22). Because the cavities were larger and more numerous, the protein matrix in the heat-induced gel exhibited matting and protein aggregation, which made the gel harder (4).

CONCLUSIONS

Heat-induced gels are more stable to chilled storage than high-pressure-induced gels. From the standpoint of total viable counts, the gels would remain fit for consumption for the first 10 days of storage. However, lot H had lost its sensory and rheological properties by the fifth day of storage.

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Table 1.- Log counts of total viable microorganisms of gels T, L and H (T: 37°C 30 min/90°C 50 min; L: 200 MPa 3°C 10 min; H: 375 MPa 38°C 20 min). Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) in different controls for each lot; different letters (x, y, z) indicate significant differences ($p \leq 0.05$) among lots for each control

gel	Storage time (days)			
	1	5	10	20
T	<1 a/x	<1 a/x	1,45±0,21 b/x	2,96±0,01 c/x
L	5,53±0,02 a/y	3,51±0,02 b/y	5,01±0,10 c/y	7,55±0,01 d/y
H	4,47±0,03 a/z	4,13±0,04 b/z	4,60±0,01 c/z	5,51±0,09 d/z

T	S1	a/x	b/x	c/x	b/x	L	S1	a/y	b/y	a/y	c/y	H	S1	a/z	b/z	c/z	d/z
	S2	a/x	b/x	b/x	b/x		S2	a/y	bc/y	b/y	d/y		S2	a/x	b/y	c/y	bc/z
	S3	a/x	bc/x	c/x	d/x		S3	a/x	b/y	c/y	d/y		S3	a/y	b/z	a/z	c/y
	S4	a/x	b/x	c/x	b/x		S4	a/y	b/y	c/y	d/y		S4	a/z	b/z	b/z	c/z
	In	a/x	b/x	c/x	c/x		In	a/y	b/y	c/y	d/y		In	a/z	b/z	c/z	c/z

Figure 1.- Breaking deformation, breaking force, hardness and cohesiveness of gels during chilled storage of lots T, L and H (T: 37 °C, 30 min/ 90 °C, 50 min; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 38 °C, 20 min). Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between controls for each lot, and different letters (x, y, z) indicate significant differences ($p \leq 0.05$) among lots for each control

Figure 2.- Water holding capacity and lightness of gels during chilled storage of lots T, L and H (T: 37 °C, 30 min/ 90 °C, 50 min; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 38 °C, 20 min). Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between controls for each lot, and different letters (x, y, z) indicate significant differences ($p \leq 0.05$) among lots for each control

Figure 3.- Protein fraction (%) in different solutions: 0.6 M sodium chloride (S1), 1.5 M urea + 0.6 M sodium chloride (S2), 8 M urea + 0.6 M sodium chloride (S3) and 0.5 M 2- β -mercaptoethanol + 0.6 M sodium chloride + 8 M urea (S4) at pH = 7.0 and insoluble fraction (In) of gels during chilled storage of lots T, L and H (T: 37 °C, 30 min/ 90 °C, 50 min; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 38 °C, 20 min). Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between controls for each lot, and different letters (x, y, z) indicate significant differences ($p \leq 0.05$) among lots for each control

Figure 4.- SDS-PAGE (12.5%) patterns of the soluble fractions (1) 0.6 M sodium chloride, (2) 1.5 M urea + 0.6 M sodium chloride, (3) 8 M urea + 0.6 M sodium chloride and (4) 0.5 M 2- β -mercaptoethanol + 0.6 M sodium chloride + 8 M urea of gels during chilled storage of lots T, L and H (T: 37 °C, 30 min/ 90 °C, 50 min; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 38 °C, 20 min). Different letters (a, b, c) indicate significant differences ($p \leq 0.05$) between controls for each lot, and different letters (x, y, z) indicate significant differences ($p \leq 0.05$) among lots for each control. Where Ag = aggregated, MHC = myosin heavy chain, Ac = actin, p = pattern

Figure 5.- Scanning electron microscope images at x500 magnifications of gels at the outset (A, B, C) and the end of chilled storage (D, E, F) from lots T, L and H respectively of lots T, L and H (T: 37 °C, 30 min/ 90 °C, 50 min; L: 200 MPa, <10 °C, 10 min; H: 375 MPa, 38 °C, 20 min)

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