GELLING OF BLUE WHITING MUSCLE UNDER THE COMBINED EFFECTS OF HIGH PRESSURE-TIME-TEMPERATURE

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Abstract

Response Surface Methodology (RSM) was used to determine how the interaction of pressure, time and temperature affects gelling of blue whiting muscle (1.0-2.5% NaCl and 5% starch) as compared with traditional heat processing. Gels were analysed chemically (to ascertain the kind of bonds formed) and physically (rheological parameters, lightness and water holding capacity). High pressure gels were softer, and with superior gel strength and deformability than thermal gels. *Modori* (gel irreversible degradation) was not inhibited in the experimental pressure conditions.

1. INTRODUCTION

A number of studies have been reported in recent years on gelling of muscle of a variety of fish species using high-pressure and mixed pressure-temperature procedures. The authors of these have concluded that the characteristics of the resulting gel differ from those of heat-processed gels: they are glossy, smooth and soft with greater elasticity (1-2).

Other experiments (3-5) have been conducted to demonstrate the effectiveness of high-pressure gelling in fish muscle (*surimi* and mince), but no work has been found on muscle of blue whiting (*Micromesistius poutassou* Risso) which determines bonds formation and relates these to gel texture. Also addressed is the utility from a nutritional standpoint of utilising these new technologies to achieve low salt levels.

In this paper, Response Surface Methodology was used to examine the influence of pressure-time-temperature on the texture of blue whiting muscle gels made with two levels of NaCl, as compared to gels made only by heating at atmospheric pressure. The paper also examines the bonds forming in high-pressure gels, as these exhibit different characteristics from heated gels.

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2. MATERIAL AND METHODS

Blue whiting (*Micromesistius poutassou* Risso) used in this study was caught off the Cantabrian coast and kept about 24 hours at 4°C. Average size was 21.09±1.24 cm and average weight 71.86±17.17 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 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 at -40°C setting (Saabroe SMC, Dinamarca). 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 for the duration of the experiment.

For gel preparation, the washed blue whiting mince was semi-thawed and placed in a refrigerated vacuum homogenizer (Stephan mod. UM5, Stephan u. Söhne GmbH & Co., Germany). The muscle was ground for 1 minute at high speed (rotor angular velocity 3000 rpm). Sodium chloride (PANREAC, Montplet & Esteban S.A., Barcelona, Spain) was added and the mixture homogenized for 3 min. Then 5% starch (CLEARAM CH 20, LAISA, Barcelona, Spain) was added with crushed ice to give the required final gel moisture (80%) and homogenizing continued for 6 min at low speed (1500 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 a pressure-time-temperature combination according to the statistical model described further below, in a high pressure pilot unit (ACB Nº665, GEC ALSTHOM, Nantes, France). For comparison with the conventional gelling procedure, heat gels were made in the conventional way at atmospheric pressure: 37°C 30 min / 90°C 50 min by waterbath immersion. Immediately, the casings were stored in a cold room at 4°C for 24 hours before analysis.

Proximate analysis was performed according to AOAC procedures (6) and crude fat by Bligh and Dyer (7) method as modified by Knudsen *et al.*(8).

Colour was determined on a colorimeter (HunterLab MiniScan MS/S-4000S, Hunter Associates Laboratory Inc., USA) using the CIE Lab scale (D65/10°) where L^* is the parameter that measures luminosity. The result was the average of six measurements taken at ambient temperature at different points on the sample.

Water holding capacity (WHC). This was a modification of the method of Roussel and Cheftel (9). About 1.5 g of sample was placed in a centrifuge tube along with two dried pipette filters (Gilson, Villiers le Bel, France). A centrifuge (Sorvall RT6000B, Du Pont Co., Delaware, USA) was used at 5000 *g* for 15 min at ambient temperature. 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.

For texture analyses samples were removed from their casings, cut (3.5 cm diameter and 3cm height) and tempered at 20°C. Gels were penetrated to breaking-point using a texturometer (Instron mod. 4501, Instron Engineering Corp., Canton, MA, U.S.A.) with a round-ended stainless steel plunger (∅=5 mm). Cross-head speed was 10 mm/min and a 100N load-cell was used. Gel strength

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[N.mm] was determined by multiplying maximum breaking force [N] by breaking deformation [mm]. All determinations were carried out at least in quadruplicate. Also Texture Profile Analysis (TPA) was performed as described by Bourne (10). Samples were placed on the flat plate of the texturometer. Compression was applied by a cylindrical plunger (∅=36 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], elasticity [mm], adhesiveness [g.cm] and cohesiveness. Folding test (FT) was performed on gel slices of 3 mm height and 3.5 mm width (11).

In order to ascertain ionic, hydrogen, hydrophobic bonds and S-S bridges formation, the gels were solubilised in four solutions (12): 0.6 M sodium chloride (S1), 1.5 M urea+ 0.6M sodium chloride (S2), 8 M urea+ 0.6 M sodium chloride (S3) and 0.5 M 2-ß-mercaptoethanol+ 0.6 M sodium chloride+ 8 M urea (S4) at pH=7.0. 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 ambient temperature for one hour then centrifuged for 20 min at 20000*g* in a centrifuge (Beckman J2-MC, Beckman Instruments, Inc., Palo Alto, California, USA). The pellet obtained was homogenized in the S2 by the same process, then again with S3 (in this case, twice) and finally with S4. Protein concentration in the supernatants was determined in triplicate, based on the method of Lowry (13) using a commercial preparation (DC Protein Assay Reagent S nº 500-0116, BIO-RAD Laboratories, California, USA) and in the insoluble protein by the method of Kjeldhal. In order to eliminate interferences in protein determination, solutions were dialysed. Colorimetry was performed in a spectrophotometer (Hitachi 100-40 UV-VIS, Japan) at 750 nm. Results are the average of two determinations and are expressed as percentage of solubilized protein with respect to total protein.

Response Surface Methodology (RSM) was used to study the simultaneous effect of three independent variables (pressure, time, temperature) according to a central composite rotatable design (20 points), using the statistical programme (Statgraphic, STSC Inc., Rockville MC. USA) as shown in table 1. Assessment of error was derived from replication (5x) of one treatment as suggested in the design (14). A 3-D graph was drawn to show interrelationships among these variables; combining pressure-time-temperature (two variables and the third variable keeps constant in the middle level) and response variable: textural properties and water holding capacity. Minimum level of significance was set for $p \leq 0.05$.

One-way analysis of variance was carried out using the Statgraphics computer programme. The difference of means between pairs was resolved by means of confidence intervals using a LSD range test. Level of significance was set for $p \leq 0.05$.

3. RESULTS AND DISCUSSION

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3.1.- Proximate analysis

For muscle: crude protein was 15.50±0.31%, moisture 82.92±0.09%, ash 0.99±0.02% and crude fat 0.50±0.04%; and for mince: crude protein was 12.80±0.30%, moisture 86.59±0.13%, ash 0.43±0.02% and crude fat 0.40±0.06%.

3.2.- Thermal gel

Table 2 shows the results of analysis of thermal gels at atmospheric pressure. The textural parameters of gels made with 1.0% NaCl were superior in gel strength, breaking force and breaking deformation to those of gels made with 2.5% NaCl, as was reported by Ishikawa *et al.* in sardine-*surimi* (15).

3.3.- High pressure gels

In the folding test (r=0.97) on high-pressure gels, the influence of temperature was linear and quadratic ($p \leq 0.01$) on samples with either salt concentration. The highest values occurred at temperatures below 40°C.

The fact that high-pressure blue whiting gels could not be produced at higher temperatures during the times assayed suggests that in the given processing conditions there is no impediment to *modori* (gel irreversible degradation) resulting from the action *alkaline proteases* (11). Ohshima *et al.* (16) reported that much of the sarcoplasmic protein in cod and mackerel white, most of it enzymes, becomes denatured at 400 MPa for 15 min. Shoji *et al.* (17) found that most *transglutaminases* were inhibited at 300 MPa at 0°C for 10 min in crude extract prepared from walleye pollack *surimi*. Gels made by heating at
atmospheric pressure scored maximum in the folding test (Table 2).

Evolution of **gel strength** (GS) of high-pressure gels is shown in figure 1. In gels made with 1.0% NaCl (r=0.99), the significative variables were time, temperature (linear) and pressure-temperatures and pressure-time interactions ($p \leq 0.01$). In gels made with 2.5% NaCl (r=0.99), the influence of time (linear; $p \leq 0.05$), temperature (linear and quadratic; $p \leq 0.01$) and pressure-time and pressure temperature interactions ($p \leq 0.01$) was significant. The highest values of GS occurred at 200-300 MPa pressure and low temperature (0-15°C), or at 400 MPa at 25-30°C for 30 min. Working with silvery pout, Serennes (18) obtained maximum GS at 400 MPa for 35 min at 30°C, reporting that the minimum pressure conditions at which myosin molecules would aggregate was 200 MPa for 15 min at room temperature (19). According to Okazaki *et al.* (20),the optimum pressure-time-temperature combination depends on species, pH and protein concentration. Carlez *et al.*(21) found in threadfin bream *surimi* that the firmest gels were obtained at 300 MPa, 5-10°C for 15 min. Shoji *et al.*(22) reported increasing GS during storage at 5°C after processing at 200 MPa at 0°C for 10 min.

Experimental results show that behaviour of gels processed in the same way differs according to NaCl concentration, higher GS being achieved in gels made with the lower concentration. GS has generally been found to be substantially lower in thermal than in pressure gels. Carlez *et al.*(21) found the same with threadfin bream *surimi*; Serennes (18), however, found that with silvery pout muscle better results were achieved by pressurising muscle previously mixed with 3% NaCl. Then again, in

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sardine muscle high-pressure gels, Ishikawa *et al.* (15) obtained best gel strength with 0% and 2% NaCl.

Breaking force (BF) was significantly influenced by time ($p \leq 0.01$), temperature ($p \leq 0.05$) and pressure-time ($p \leq 0.01$) and pressure-temperature ($p \leq 0.05$) interactions in gels made with 1.0% NaCl ($r=0.99$), the highest values occurring at the lowest working pressures over short times. In gels made with 2.5% NaCl ($r=0.99$), on the other hand, breaking force was influenced by pressure and time (linear; $p \leq 0.05$ in both cases), by temperature (linear and quadratic; $p \leq 0.01$) and by pressure-time and pressure-temperature interactions ($p \leq 0.01$). The highest values were achieved at around 200 MPa pressure at low temperatures. Nagashima *et al.* (23) found no direct relationship between BF and increased pressure in squid gels with EDTA added as enzyme inhibitor. BF in high-pressure gels was generally similar to that of gels made by heating at atmospheric pressure.

Breaking deformation (BD) in figure 2, was influenced by time (linear; $p \leq 0.05$), temperature (linear; $p \leq 0.01$) pressure-temperature interaction ($p \leq 0.05$) in gels made with 1,0% NaCl ($r=0.96$), but only by temperature (linear; $p \leq 0.01$) in gels made with 2.5% NaCl ($r=0.98$). A decreasing of deformation were when time and temperature rise, when more than 300 MPa were applied this fact was not so evident. BD was lower in gels made by heating at atmospheric pressure (Table 2).

Hardness as measured by TPA is shown in figure 3. Only temperature had a clear influence (linear; $p \leq 0.01$) in gels made with 1% NaCl ($r=0.89$) and only pressure (linear; $p \leq 0.05$) on gels made with 2.5% NaCl ($r=0.89$). The highest values in pressure gels occurred in samples with the lower salt concentration and at relatively low temperatures (less than 40°C), particularly at pressures around 200 MPa. Okamoto *et al.* (24), however, found that gels made with egg yolk and white, carp crude actomyosin, and rabbit meat paste tended to be harder as pressure was raised at 25°C for 30 min. In the present experiment, blue whiting muscle gels made by heating at atmospheric pressure were found to be harder than those made by pressurizing (Table 2).

Elasticity diffeered according to salt concentration. In gels made with 1% NaCl ($r=0.83$), elasticity was influenced by pressure (linear; $p \leq 0.05$) and by interaction of pressure and temperature (linear; $p \leq 0.01$), whereas in gels made with 2.5% NaCl ($r=0.92$) only temperature influenced elasticity (linear and quadratic; $p \leq 0.01$). The effect of pressure was greatest at around 300 MPa in gels with 2.5% NaCl and at higher pressures in gels with 1% NaCl. The best temperature was found to be about 30°C for gels with 1% NaCl and about 20°C for gels with 2.5% NaCl. Ishikawa *et al.* (15) reported that elasticity of sardine-*surimi* gels heated at 55°C for 15 min after pressurizing at 100 MPa for 20 min was higher than that obtained by only pressurizing at 400 MPa or heating at 85°C; elasticity was greater in heated-only than in pressurized-only gels. In the present case elasticity was similar in both heated and pressurized gels. As regards the combination of temperature and pressure, behaviour differed according to salt concentration. Elasticity was greater on pressurizing at around 40°C in gels with 1% NaCl, whereas in gels with 2.5% NaCl elasticity was low at temperature above 20°C.

Adhesiveness was only influenced by temperature (linear, $p \leq 0.05$; quadratic, $p \leq 0.01$) in gels with 1% NaCl ($r=0.87$) and gels with 2.5% NaCl ($r=0.91$) (linear and quadratic; $p \leq 0.01$). The highest values of adhesiveness occurred at low pressures and temperatures (200 MPa at ≤15°C) or intermediate pressures and temperatures (300 MPa at 40°C). Gels made by heating at atmospheric pressure were

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much more adhesive than pressure gels. Okamoto *et al.* (24) reported that adhesiveness of gels made from carp crude actomyosin was greater at low spressures (100 MPa, 25°C for 30 min).

Cohesiveness (Fig 4) was influenced by temperature and pressure (linear, $p \leq 0.01$) in gels made with 1% NaCl ($r=0.93$) and by time (linear, $p \leq 0.01$), temperature (linear and quadratic, $p \leq 0.01$), and time-temperature interaction ($p \leq 0.05$) in gels made with 2.5% NaCl ($r=0.99$). The best conditions for obtaining a cohesive gel were found to be low pressure and low temperatures in the case of gels with 1% NaCl, or temperatures around 20°C in gels made with 2.5% NaCl. Cohesiveness of gels made by heating at atmospheric pressure were slightly lower.

Evolution of **water holding capacity** (WHC) in high-pressure gels is shown in figure 5. In gels with 1% NaCl ($r=0.92$), temperature was the significant variable (linear, $p \leq 0.01$), whereas in gels made with 2.5% NaCl ($r=0.90$), these were temperature and time (both linear; $p \leq 0.01$, $p \leq 0.05$). The highest WHC occurred at 200 MPa pressure combined with long time (25 min) or low temperature (10°C) in gels with 1% NaCl, and in similar conditions but for a shorter time (10 min) in gels with 2.5% NaCl. Values were higher in conventional thermal gels than in pressure gels. Okazaki (25) reported that WHC values became lower as gelling pressure increased (200-500 MPa for 20 min).

Lightness (L^*) was positively influenced by pressure and temperature (linear and quadratic, $p \leq 0.01$) irrespective of salt concentration. Values were higher in pressurized than in heated gels. Wada and Ide (26) also found that L^* increased with pressure in gels made from sardine. In gels made with 2.5% NaCl, lightness was also influenced by time (linear, $p \leq 0.01$; quadratic, $p \leq 0.05$), pressure-time interaction ($p \leq 0.05$) and pressure-temperature interaction ($p \leq 0.05$). L^* values fitted the regression model with $r=0.94$ for gels with 1% NaCl and $r=0.95$ for gels with 2.5% NaCl. As was to be expected, the highest values of L^* occurred with temperatures above 50°C.

On the basis of the results from the RSM test, a pressure-time-temperature combination was chosen: 375 MPa for 20 min at 38°C (gel H) in order to identify the kinds of bond forming under pressurization and compare them with those found in conventional heat processing (gel T) elaborated with 1.0% NaCl. **Protein solubility** in various solutions as an indicator of the types of bonds forming in the two gels is shown in figure 6. Protein solubility in S1 and S2 was similar in both gels, with no significant differences between them. According to Carlez *et al.* (21), ionic bonds, and more specifically Ca^{+2} ions, play an important role in gel texture. The highest solubility percentages were found in solution S3. Hydrophobic bonds were more abundant ($p \leq 0.05$) in gel H than in gel T. High pressure has been reported to favour hydrophobic bonds by Ikeuchi *et al.* (27) in actomyosin gels, Mozhaev *et al.*(28) in proteolytic enzymes and Carlez *et al.*(21) in threadfin bream *surimi*. Protein soluble in S4 and insoluble protein differed significantly in both gels ($p \leq 0.05$), which suggests that pressure causes the formation of a smaller number of covalent bonds.

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Table 1
Level of variables in pressure treatment according to RSM experimental design

pressure (MPa)	time (min)	temperature (°C)
200	10	0
245	14	15
310	26	38
375	20	60
420	30	75

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Table 2
Values of analyses performed on thermal gels processed at atmospheric pressure.

Assay	1.0% NaCl		2.5% NaCl	
Folding test	5	a	5	a
Breaking deformation [mm]	10.16±0.48	a	8.51±0.59	b
Breaking force [N]	2.79±0.25	a	1.69±0.10	b
Gel strength [N.mm]	28.37±3.73	a	14.39±1.46	b
Hardness [N]	64.20±7.56	a	59.09±9.07	a
Elasticity [mm]	13.86±1.27	a	13.08±0.99	a
Adhesiveness [g.cm]	2265±324	a	2092±354	a
Cohesiveness	0.61±0.02	a	0.59±0.02	a
Water holding capacity [%]	94.04±0.57	a	92.89±2.14	a
Lightness (L^*)	69.67±0.73	a	68.84±0.26	b

Variables in the same row with different letters are significantly different ($p \leq 0.05$)

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