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Transglutaminase activity in pressure-induced gelation assisted by prior setting

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Abstract

Gels from horse mackerel (*Trachurus trachurus*) mince were induced by high pressure treatment at 300 MPa, 25 °C, 15 min. The effect of a prior setting at 25 °C, during 2 h at atmospheric pressure, on microstructural and rheological properties of gels was examined. Homogenates from minced muscle and ground salted paste in a saline medium containing 2-β-mercaptoethanol were prepared and subjected to high pressure treatment. Protein solubility, SDS–PAGE and TGase activity in the supernatants were determined. Residual TGase activity was significantly reduced after pressurization. Gels were found to be stronger and exhibited a denser and more homogeneous network when a prior setting preceded high pressure.
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1. Introduction

High pressure technology can induce gelation of proteins, to create new products from fish muscle or analogues of existing products, in which colour, flavour and nutritional value are only minimally affected (Messens, Van Camp, & Huyghebaert, 1997). Pressure-induced protein gelation was initially used as an alternative to the more aggressive thermal treatments, the latter causing more severe denaturation (Cheftel & Culioli, 1997). When pressure is released, the refolding process of proteins starts, establishing new interactions to produce the protein aggregation necessary for the formation of a gel network. This aggregation may even begin during the partial unfolding of myofibrillar proteins (Carlez, Borderías, Dumay, & Cheftel, 1995). As shown by studies conducted largely on fish muscle proteins, this entire process can take place at less than 10 °C in several minutes (Carlez et al., 1995; Oshima,

Ushio, & Koizumi, 1993; Peréz-Mateos & Montero, 1997; Shoji, Saeki, Wakameda, Nakamura, & Nonaka, 1990). The effect of pressure is highly dependent on a number of factors, such as the concentration and characteristics of the protein system, pressure level, temperature and treatment duration, salt concentration and pH (Messens et al., 1997; Yamamoto, Hayashi, & Yasui, 1993). A number of studies describe pressure-induced fish gels as softer and more elastic than thermally induced gels (Chung, Gebrehiwot, Farkas, & Morrissey, 1994; Okamoto, Kawamura, & Hayashi, 1990; Peréz-Mateos & Montero, 2000). Scanning electron microscopy has shown pressure-induced sardine mince gels to present a more compacted, less fibrous-oriented structure than conventional thermal gels (Montero, Peréz-Mateos, & Solas, 1997).

Pressure induces changes which disturb the balance of the non-covalent interactions that stabilize the native conformation of the proteins, particularly myosin and actin (Okamoto et al., 1990). When pressure is released, the protein will be restructured initially by hydrogen bonding, and later any hydrophobic or electrostatic forces will be superimposed on that structure. A significant

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contribution of hydrogen and ionic bonds has been reported in low temperature pressure-induced gels from threadfin bream (Carlez et al., 1995) and blue whiting (Peréz-Mateos, Lorenço, Montero, & Borderías, 1997). Also, hydrophobic interactions have been reported to play a predominant role in Alaska pollack gels (Gilleland, Lanier, & Hamann, 1997). On the other hand, the contribution of covalent disulphide bonds to polymerization of myosin heavy chain in pressure-induced gels has been reported to occur at relatively high pressure levels, above 300 MPa (Angsupanich, Edde, & Ledward, 1999; Carlez et al., 1995; Gilleland et al., 1997).

A great number of studies have focussed on protein aggregation via formation of covalent non-disulphide cross-links, catalyzed by an endogenous calcium-dependent transglutaminase (TGase) (Kamath, Lanier, Foegeding, & Hamann, 1992; Kimura et al., 1991), largely responsible for the "setting" phenomenon at temperatures around 25 or 40 °C. However, information about the contribution of TGase activity to pressure-induced gels is scarce. Endogenous muscle TGase in walleye pollack surimi has been reported to be relatively pressure-sensitive, and therefore could not be involved in high pressure gelation, nor in gelation following high pressure treatment (Shoji, Saeki, Wakameda, & Nonaka, 1994). Later studies have demonstrated that TGase activity was not hindered by pressures between 100 and 300 MPa, and remains active enough to continue working during later setting (Ashie & Lanier, 1999; Gilleland et al., 1997). On the other hand, (Pérez-Mateos, Gómez-Guillén, Hurtado, Solas, & Montero, 2002) found that breaking force was considerably increased in Atlantic mackerel pressure-induced gels, when a setting at 25 °C for 2 h before pressure treatment (300 MPa, 25 °C) was included.

In the present work it was intended to favour the formation of TGase-mediated covalent bonds before the application of a high-pressure gelling treatment at 300 MPa. For this purpose, microstructural and rheological properties of the resulting gels, with and without prior setting at 25 °C during 2 h, were evaluated. Remaining TGase activity and SDS–PAGE profile, in both treated muscle and ground salted paste homogenates, were also determined.

2. Materials and methods

2.1. Material

Horse mackerel (*Trachurus trachurus*) used in this study was caught off the Cantabrian coast and kept at 4 °C for about 24–48 h. 75 kg of fish were headed, gutted and washed. Skin and bones were removed with a deboning machine (Baader 694, Lübeck, Germany). The resulting mince was washed in a solution of NaCl (0.2%)

at 0–3 °C, proportion 3:1 (solution:minced muscle). After draining, excess water was removed using a screw press (Baader 523, Lübeck, Germany). Sorbitol (4%) and tripoly-phosphate (0.5%) were added as cryoprotectants. The mince was immediately vacuum-packed in bags (Cryovac BB-1, Grace, Spain) and frozen in a plate-freezer (Sabroe SMC, Denmark). The bags were stored at −80 °C in a freezer cabinet (Revco ULT, Giralt, Revco Scientific, Inc., Asheville, N.C., USA) in order to minimize alteration during frozen storage up to gel preparation.

2.2. Gel preparation

Frozen mince was tempered in a chilled room and placed in a refrigerated vacuum homogeniser (Stephan UM5, Stephan u. Söhne GmbH & Co., Germany). It was ground for 1 min at high speed. Sodium chloride (2% w/w, with respect to final gel weight) (Panreac, Montplet & Esteban S.A., Barcelona, Spain) was added and homogenised for 3 min at slow speed, keeping the sample temperature below 10 °C.

Salt ground paste (batter) was stuffed into flexible plastic casings (Krehalon Soplaril, Barcelona, Spain) of 40 μm thickness and 3.5 cm diameter. High-pressure treatment was performed in a high pressure pilot unit (ACB 665, Gec Alsthom, Nantes, France) where the temperature of the immersion medium was controlled via a thermocouple. The pressure was increased by 2.5 MPa/s. The following treatments were applied: 300 MPa, 25 °C, 15 min (P) and the same treatment with a prior setting at atmospheric pressure 25 °C, 2 h (SP). All the casings were immediately cooled with water at 0 °C and stored in a cold room at 4 °C for 18 h before analysis.

2.3. Rheological measurements

Formed gels were removed from their casings, cut (3.5 cm diameter, 3 cm height) and tempered at 22 °C. Folding test resistance of a slice (3.5 cm diameter and 3 mm high) folded over twice, score was 1–5.

Puncture test was performed with a round-ended stainless steel plunger ($\emptyset = 5$ mm) at 10 mm/min using a load-cell of 100 N. Breaking deformation (mm), breaking force (N) and work of penetration ($N \times$ mm) were measured.

Elasticity (%) and hardness (N) were determined by a stress-relaxation test, with a compression to 40% and 1 min relaxation, at a deformation rate of 50 mm/min, using a cylindrical plunger ($\emptyset = 58$ mm) adapted to a load-cell of 5 kN. Per cent relaxation was calculated as $YT = 100 \times (F_0 - F_1)/F_0$, where F_0 is the force registered at the onset of relaxation immediately after sample compression (hardness [N]) and F_1 is the force registered after 1 min relaxation. Thus, 100-YT is taken as an in-

dex of elasticity and is expressed as per cent elasticity of the gel. At least four replications of all determinations were performed.

2.4. Scanning electron microscopy

Cubes of 2–3 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 CO_2 as transition fluid in a dryer (Balzer CPD030, Liechtenstein) and mounted on copper sample holders, followed by sputter-coating with gold in a metallizer (Balzer SCD004). Samples were kept in a dryer prior to examination by scanning microscope (Jeol, JSM 6400, Japan) at 20 kV. Micrographs of each gel were taken at 1000 magnifications.

2.5. Preparation and treatment of homogenates

Homogenates from raw muscle (M) and batter (B) were prepared as follows: 10 g of minced muscle or batter were homogenized with 20 ml of a solution composed of 20 mM Tris-hydroxymethyl-aminomethane, 5 mM EDTA and 10 mM 2-β-mercaptoethanol (pH 7.5), in an Omni-mixer (model 17106; Omni International, Waterbury, CT, USA) cooled with crushed ice. As TGase activator, $CaCl_2$ (70 mM) was added to some of the studied samples. The resulting homogenates were subjected to the above-described high pressure treatment, with and without the previous setting. After treatment, they were centrifuged at 4 °C for 30 min, at 43,700 g in a Beckman Centrifuge model J2-MC (Beckman Instruments Inc., Palo Alto, CA, USA). The protein concentration in the supernatant was determined in a protein/nitrogen analyzer LECO model FP-2000 (LECO Corp., St. Joseph, MI, USA) calibrated with EDTA, following the technique 992.15 (A.O.A.C., 2000). The extraction was done in duplicate and results, averages of 3 determinations, were expressed as percent protein remaining in the supernatant with respect to total protein in the homogenate prior to centrifuging, which was previously determined. The soluble protein of the untreated muscle was considered as the reference for the 100% solubility.

2.6. Electrophoretic study (SDS–PAGE)

The supernatant (soluble protein) was treated with a solution consisting of 2.5% SDS, 5% mercaptoethanol, 10 mM Tris HCl, 1 mM EDTA, and 0.002% bromophenol blue to obtain a protein concentration of 2 mg/ml, and then heated for 5 min in a boiling water bath. Samples were analyzed by SDS–PAGE in a SE 250 Mighty Small II Vertical Unit (Pharmacia LKB Bio-

technology, Uppsala, Sweden) using 5% polyacrylamide gels for stacking and 10% polyacrylamide gels for resolving. Electrophoresis conditions were 20 mA/gel and 250 V. The protein bands were stained with Coomassie Brilliant Blue R-350 (Phast Gel™ Blue R tablets, Pharmacia LKB Biotechnology). As reference for molecular weights, two standard high-molecular-weight reference kits (Pharmacia LKB Biotechnology) were used. One was composed of thyroglobulin 330 kDa, ferritin half unit 220 kDa, albumin 67 kDa, catalase subunit 60 kDa, lactate dehydrogenase subunit 36 kDa, and ferritin subunit 18.5 kDa. The other was composed of myosin 212 kDa, α_2-macroglobulin 170 kDa, β-galactosidase 116 kDa, transferrin 76 kDa, and glutamic dehydrogenase 53 kDa.

2.7. Transglutaminase activity

The supernatant (soluble protein) was used to determine residual TGase activity, following the colorimetric hydroxamate assay method (Folk & Cole, 1965). The final reaction mixture contained 500 μl of the supernatant, together with 200 μl of 1.0 M Tris-acetate buffer (pH 6.0), 150 μl of 0.1 M CBZ–L-glutaminylglycine, 50 μl of 0.1 M $CaCl_2$, 50 μl of 2 M hydroxylamine and 50 μl of 0.02 M Na_2EDTA. After 10 min of incubation at 37 °C, ferric chloride–trichloroacetic acid reagent was added to stop the reaction. The mixture was centrifuged at 4000g during 15 min, and the red colour of the supernatant was measured at 525 nm in a spectrophotometer UV-1601 (Model CPS-240, Shimadzu, Japan). L-Glutamic acid–γ-monohydroxamic acid was used as a standard for calibration. One unit of TGase activity was defined as the amount of enzyme needed to produce 1 μmol of hydroxamic acid/min.

2.8. Statistical analysis

One-way analysis of variance was carried out using the SPSS computer program (SPSS Statistical Software, Inc., Chicago, III). The difference of means between pairs was resolved by means of confidence intervals using a Tukey test. Level of significance was set for $p \leqslant 0.05$.

3. Results and discussion

Fig. 1 shows SEM micrographs of horse mackerel mince gels induced by high pressure (300 MPa, 25 °C, 15 min), with prior setting at 25 °C for 2 h (SP) and without setting (P). In both gels, we observed areas having a predominantly reticular structure and areas presenting a more aggregated or compact morphology. The SP gel presented a denser, more homogeneous network than the P gel, with smaller and more evenly distributed
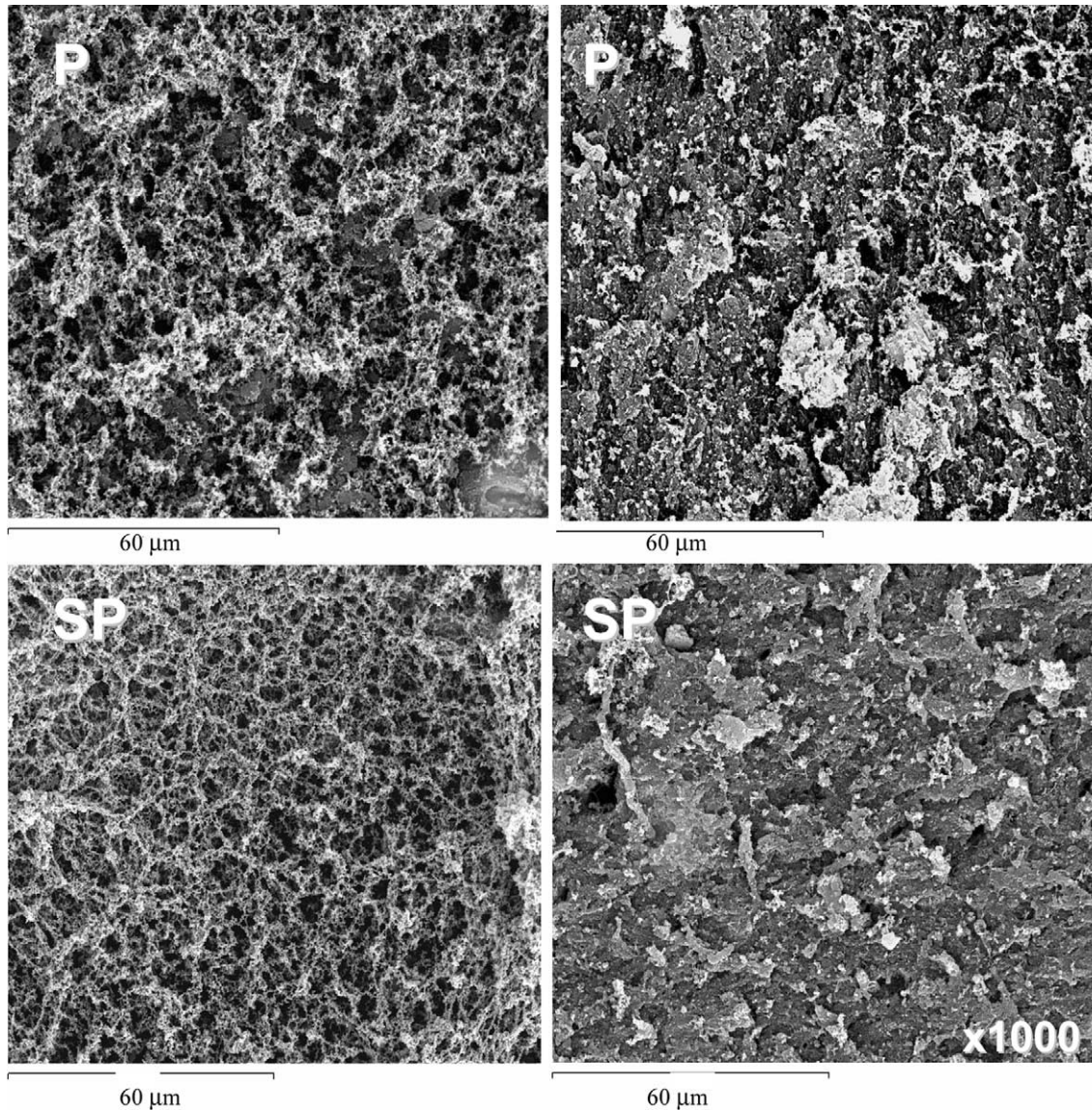
Fig. 1. SEM micrographs (1000×) of pressure-induced gels (300 MPa, 25 °C, 15 min) with previous setting at 25 °C for 2 h (SP), or without setting (P).

holes. The non-reticular areas, likewise, appeared denser, with fewer and smaller cavities, whereas, in the P gel, these areas presented cracking.

In both cases, gels scored the maximum (5) in the folding test. This means that the pressure treatment applied was appropriate for induction of protein gelation and, moreover, prior setting did not inhibit subsequent gelling. According to the puncture test, the strength of the gel made under pressure with prior setting at 25 °C (SP) was significantly ($p \leqslant 0.05$) greater than that of the unset gel (P); the former scored higher for breaking force, even although breaking deformation values were similar (Table 1). There was no difference between the two gels in terms of hardness and elasticity, as determined by compression–relaxation test (Table 1). The apparent lack of correlation between the two types

Table 1
Rheological properties of pressure-induced gels (300 MPa, 25 °C, 15 min) with prior setting at 25 °C for 2 h (SP), and without setting (P)

	P	SP
Breaking force (N)	2.11 ± 0.23^a	2.85 ± 0.27^b
Breaking deformation (mm)	13.91 ± 0.93^a	15.00 ± 0.45^a
Work of penetration ($N \times$ mm)	27.70 ± 2.60^a	44.30 ± 4.00^b
Hardness (N)	24.59 ± 2.10^a	24.08 ± 1.92^a
Elasticity (%)	43.00 ± 0.74^a	42.00 ± 1.39^a

Different letters a, b indicate significant differences ($P \leqslant 0.05$).

of rheological measurement has been extensively reviewed by Lee and Chung (1989). These authors suggested that, while the penetration test measures the degree of compactness or density of actomyosin, the compression test reveals the overall binding property of

the gel material. Assuming that is the case, the higher degree of compactness in the gel network as a consequence of setting should be largely related to the nature of the bonds involved in protein aggregation. In this connection, increased cross-linking of myosin heavy chains at temperatures around 25 °C has been largely attributed to TGase activity catalyzing covalent epsilon-amino-(γ-glutamyl) lysine type bonds in many fish species (Kamath et al., 1992; Takeda & Seki, 1996; Tsukamasa et al., 1993). Nowsad, Katoh, Kanoh, and Niwa (1996) found TGase activity in horse mackerel (*Trachurus japonicus*) to be maximum at 25–30 °C and related it to increased breaking strength of heat-set gels, where formation of covalent bonds (catalyzed by TGase) was important. Similarly, a relationship between MHC polymerization catalyzed by TGase and an increase in breaking strength or shear stress, without affecting shear strain, has been reported in gels from other fish species, such as Pacific whiting (Lee & Park, 1998) or carp (Tsukamasa, Miyake, Ando, & Makinodan, 2000).

In pressure-induced gels, an increase in breaking force, together with increased gel matrix density induced by setting prior to pressurization, has been reported in mackerel (*Scomber scombrus*) gels (Pérez-Mateos et al., 2002) under identical pressure/time/temperature conditions to the present study; this coincided with a significant increase in non-disulphide covalent bonding with respect to the pressure-induced gel without prior setting. These findings indicate that moderate pressurization (300 MPa) after setting does not disrupt the structure, which has presumably been reinforced as a result of previous TGase activity. However, it still remains to be seen whether there is any further formation of TGase-mediated covalent bonds during pressurization at 300 MPa/25 °C, or whether TGase activity is activated or inactivated during such high pressure treatment.

A number of studies in which setting was induced immediately after pressurization (300 MPa, 4 °C) have shown that the enzyme remains active enough to continue working during later setting at 25 or 40 °C, and its activity is actually enhanced by the fact that the pressure facilitates the denaturation and breakdown of myosin, the principal substrate of TGase (Gilleland et al., 1997). However, these authors considered it improbable that the enzyme was involved in cold pressure-induced gelation. Later studies on Alaska pollack have also demonstrated that endogenous TGase activity is not affected by pressures between 100 and 300 MPa (Ashie & Lanier, 1999). These studies conflict with the findings of Shoji et al. (1994), who reported that endogenous TGase was relatively sensitive to high pressure in walleye pollock surimi.

There is persistent disagreement as to whether high pressure really activates or inactivates muscle enzymes. Leaving aside possible differences due to the type of enzyme and other factors, such as pressure level, time/temperature, pH and so forth, the pattern of enzymatic activity appears to differ, depending on whether studies are conducted on crude extract or on muscle. In crude extracts, the enzyme is unprotected, and therefore intramolecular changes in enzyme structure/conformation are the main cause of pressure-induced inactivation. In muscle, on the other hand, pressurization entails changes in the conformation of the myofibrillar proteins that influence the specificity of the enzyme/substrate interaction and cause disruption of cell membranes – these last releasing a number of enzymes (e.g., lysosomal proteases) which may cause an increase of proteolytic activity, depending on the pressure level used (Chung et al., 1994).

To determine the effect of high-pressure treatment on TGase activity outside the rigid and highly-organized environment of the muscle structure, samples of the muscle and of the batter used to produce the corresponding gels were homogenized in a saline medium containing 2-β-mercaptoethanol. These were subjected to the experimental high-pressure treatments, with or without prior setting and with or without added $CaCl_2$ as an enzyme activator. The homogenates were then centrifuged prior to determination of protein concentration and composition and the TGase activity remaining in the resulting crude extract.

Fig. 2 shows the percentage of insolubilization of muscle and batter homogenates subjected to the different treatments, with and without added $CaCl_2$. The reference (100%) was the soluble protein (expressed as per cent protein remaining in the supernatant with respect to the total amount of protein in the homogenate prior to centrifuging) in non-pressurized muscle.
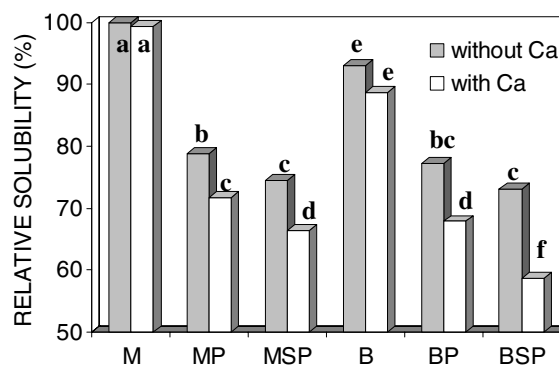

Fig. 2. Relative protein solubility of homogenates from muscle (M) and batter (B) before treatment, and after high pressure treatment with prior setting (MSP, BSP) and without setting (MP, BP), with and without added $CaCl_2$, expressed as percent protein remaining in the supernatant with respect to total protein prior to centrifuging. The soluble protein of the untreated muscle was considered as the reference for 100% solubility. Different letters a,b,c, etc. indicate significant ($P \leqslant 0.05$) differences.

Pressurization produced approximately 20% insolubilization of the muscle homogenate protein; insolubilization was slightly but significantly ($p \leqslant 0.05$) higher in samples with prior setting at 25 °C. The presence of mercaptoethanol indicated no significant contribution of S–S bonds to pressure-induced protein aggregation. When calcium was added, the percentage of remaining soluble protein decreased even further, especially in samples with prior setting. This effect could be due to aggregation facilitated by direct interaction of the calcium ion with proteins, but it could also be due to the calcium ion favouring the formation of non-disulphide covalent bonds by TGase.

The procedure for rendering a batter suitable for gelling entails fibrillar breakdown as a result of grinding with salt and hence considerable alteration of the original muscle structure. This favours denaturation of the myofibrillar proteins and massive rupturing of lysosomes and other cell membranes, causing a number of proteases and other enzymes (the latter possibly including TGase) to be released into the medium.

The batter homogenate in Fig. 2 appears slightly less soluble that the whole muscle homogenate, indicating some aggregation following grinding and mixing with salt. Such early protein aggregation during this step has been reported elsewhere for Alaska pollack surimi (Nowsad, Kanoh, & Niwa, 1993). One possible explanation for this is that when the electrostatic bonds sustaining the native configuration of the myofibrillar proteins were disrupted, these proteins quickly began to interact with one another, forming new bonds whose nature depends very much on the temperature of the medium. Pressurization also induced protein aggregation in the batter homogenate. This effect was more pronounced in samples subjected to prior setting, particularly where calcium was present. It was similar to the effect noted above in whole muscle, but slightly more intense.

It is generally agreed that high pressure denaturation involves low energy levels and is therefore largely ineffective in the disruption of strong covalent bonds. Setting prior to pressurization produced a number of bonds which augmented protein aggregation and which persisted in the gel network after pressurization; this is consistent with our earlier observations at the ultrastructural level.

Fig. 3 shows the electrophoretic profiles of the corresponding muscle and batter extracts with and without added CaCl$_2$, after pressurization with and without prior setting. In whole muscle sample (Fig. 3(a)), high pressure caused the disappearance of high-MW protein bands in an approximate range of 60–200 kDa, some of these corresponding to products of MHC proteolysis (Niwa, Suzuki, Nowsad, & Kanoh, 1993). There are two possible reasons for this: (i) that they were excluded from the soluble protein fraction by aggregation; and (ii) that they underwent further breakdown and were included in the lower-MW fractions. Both effects have been reported, in some cases occurring simultaneously (Jiménez-Colmenero, Cofrades, Carballo, Fernández, & Fernández-Martín, 1998). In the interface zone between gel stacking and gel resolving, all the profiles presented a band associated with the presence of myosin (MHC) which persisted in spite of pressurization. The band associated with actin, which is very pronounced in the muscle profile, is fainter and more dissociated in the
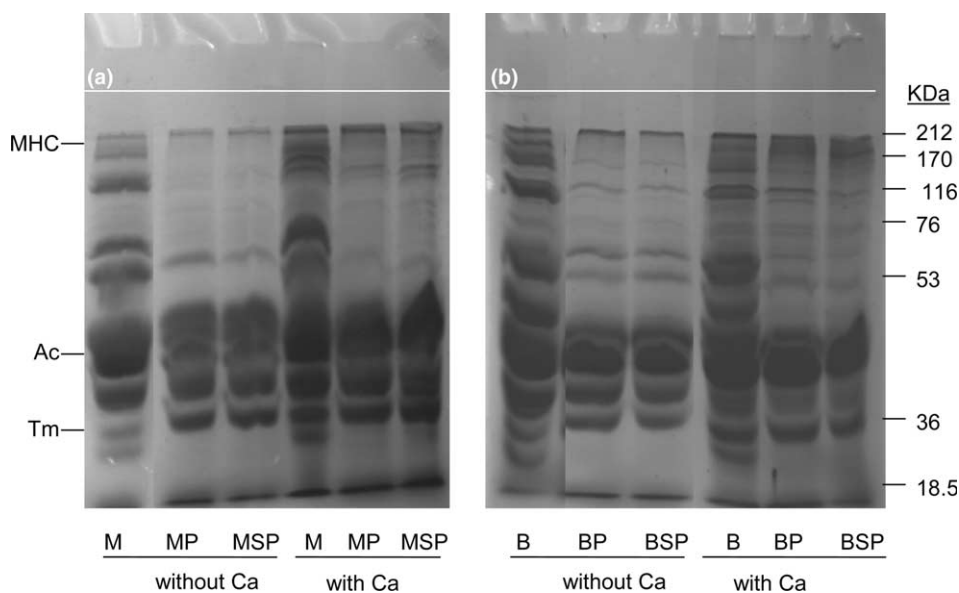

Fig. 3. SDS–PAGE of soluble protein of homogenates from muscle (a) and batter (b) before treatment, and after high pressure treatment with prior setting (MSP, BSP) and without setting (MP, BP), with and without added CaCl$_2$. MHC: myosin heavy chains; Ac: actin; Tm: tropomyosin.

profiles of pressurized samples. The opposite is true of the band associated with tropomyosin; this is more intense in pressurized samples, as is the band at the bottom, corresponding to low-MW peptides accumulated in the polyacrylamide gel. This supports the hypothesis that higher-MW peptides were broken down into smaller peptides. From the electrophoretic profiles, the composition of the soluble fraction does not appear to have been affected by prior setting.

The profiles of the samples with added calcium are similar to the above, with the following differences: (i) some bands (e.g., MHC and actin) are slightly more intense in all the muscle samples; and (ii) the bands corresponding to tropomyosin and a peptide of slightly lower molecular weight than tropomyosin are slightly less intense in the untreated muscle sample.

More bands were detected in the electrophoretic profiles of soluble protein for the batter than for the whole muscle, especially when $CaCl_2$ was added (Fig. 3(b)). With and without prior setting, the pressure effect was similar to that described above for whole muscle, i.e., some bands tended to disappear, while the low-MW peptide band at the bottom tended to become more intense.

In pressurized batter samples with added calcium, the prevalence of some bands was considerably greater than in the case of whole muscle. Moreover, the band at the interface between gel stacking and gel resolving was less intense in the pressurized samples, especially those with prior setting; this was accompanied by intensificsation of the band of approximately 180 kDa, which is associated with a peptide produced by proteolysis of the MHC (Niwa et al., 1993).

According to these findings, grinding of whole muscle with salt (batter) produces a greater variety of soluble peptides, possibly the result of rupture of muscle fibres; this, combined with the denaturation of the main myofibrillar proteins, facilitates interaction between proteases and their substrates. The mechanics of the aggregation/bond rupture effect of high pressure are similar to those in whole muscle; however, in the presence of calcium, the proteolytic effect predominates, especially after setting at 25 °C. This can be accompanied by augmented activity of certain proteases – for example calpains, which are most active at around 30 °C with neutral pH and become active in the presence of the calcium ion (Kolodziejska & Sikorski, 1996).

We evaluated the residual TGase activity in muscle and batter homogenates after high pressure treatment (without and with added $CaCl_2$). Fig. 4 shows specific TGase activity, i.e., units of enzyme activity, as a function of protein concentration in the supernatants. The object was to take into account the protein aggregation occurring as a result of the treatment and to be better able to compare the different samples.
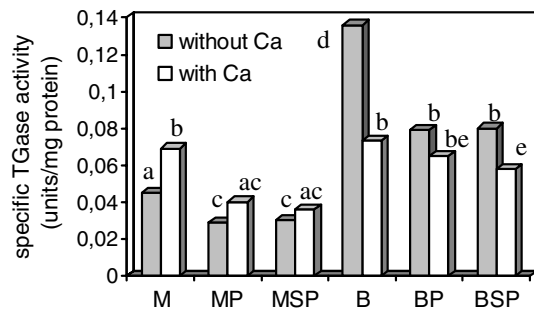

Fig. 4. Specific TGase activity, expressed as units of enzyme activity per mg soluble protein, in homogenates from muscle (M) and batter (B) before treatment, and after high pressure treatment with prior setting (MSP, BSP) and without setting (MP, BP), with and without added $CaCl_2$. Different letters a,b,c, etc. indicate significant ($P \leqslant 0.05$) differences.

Pressurizing of the muscle homogenates reduced TGase activity by 35%, with no significant differences ($P \leqslant 0.05$) between samples with or without prior setting. Residual TGase activity was greater in samples with added calcium, which was to be expected given that the calcium ion is an enzyme activator. However, the effect of the treatments was similar to that found in calcium-free samples: TGase activity decreased, in this case by slightly more (42% reduction of activity). These findings confirm that the enzyme can survive high pressure treatment, as also reported by Gilleland et al. (1997) and Ashie and Lanier (1999) in Alaska pollack. Moreover, it can survive even after prior setting at 25 °C. However, its activity was reduced to some extent as a consequence of high pressure.

Specific residual TGase activity in batter homogenates was considerably higher than in the muscle homogenates in most cases. This is attributed to the effect of grinding with salt, which can facilitate interaction between enzyme and substrates. Here again, pressurization, with or without prior setting, reduced the activity of the enzyme (by approximately 42%), which in this case was slightly greater than in the whole muscle. Unlike the case of muscle homogenates, TGase activity was reduced by addition of calcium. The most plausible explanation is that the calcium ion activates a number of proteases, among them calpains, which are released from the muscle fibres and which are also favoured by setting at 25 °C. This explanation is consistent with the electrophoretic profiles referred to above.

4. Conclusion

Gel matrices are denser and stronger when gels are subjected to setting at 25 °C prior to pressurization (300 MPa, 25 °C) than when they are not subjected to such setting. Pressurization significantly reduces residual

TGase activity, but the structures favoured by prior setting are not weakened by pressure-induced gelation.

Acknowledgements

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