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Effect of Combined Heat and High-Pressure Treatments on the Texture of Chicken Breast Muscle (*Pectoralis Fundus*)

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Commercially supplied chicken breast muscle was subjected to simultaneous heat and pressure treatments. Treatment conditions ranged from ambient temperature to 70 °C and from 0.1 to 800 MPa, respectively, in various combinations. Texture profile analysis (TPA) of the treated samples was performed to determine changes in muscle hardness. At treatment temperatures up to and including 50 °C, heat and pressure acted synergistically to increase muscle hardness. However, at 60 and 70 °C, hardness decreased following treatments in excess of 200 MPa. TPA was performed on extracted myofibrillar protein gels that after treatment under similar conditions revealed similar effects of heat and pressure. Differential scanning calorimetry analysis of whole muscle samples revealed that at ambient pressure the unfolding of myosin was completed at 60 °C, unlike actin, which completely denatured only above 70 °C. With simultaneous pressure treatment at >200 MPa, myosin and actin unfolded at 20 °C. Unfolding of myosin and actin could be induced in extracted myofibrillar protein with simultaneous treatment at 200 MPa and 40 °C. Electrophoretic analysis indicated high pressure/temperature regimens induced disulfide bonding between myosin chains.

KEYWORDS: High pressure; heat treatment; chicken breast; protein denaturation; myofibrillar protein; texture profile analysis; differential scanning calorimetry

INTRODUCTION

High-pressure food processing is currently the focus of major research efforts around the world, has potential to offer manufacturers, retailers, and consumers many advantages, and offers huge potential for commercialization (1). In fact, several countries are producing pressurized food products such as fruit jams, fruit jellies, and rice paste (Japan), oyster, ham, chopped onions, and guacamole (United States), and fruit juices (Australia and Europe). Research has also been done on the use of pressure to control the texture of protein goods, including meat and fish (*2*, *3*). Angsupanich et al. (*4*) found that in cod muscle pressures of up to 400 MPa caused increased hardness, and Ashie et al. (*5*) reported similar results with bluefish, except that as the pressure increased beyond 100 MPa the hardness of the muscle decreased. However, Jose et al. (*6*), studying octopus, found that hardness increased when samples were treated at pressures of up to 300 MPa at ambient temperature, but decreased as the pressure increased to 400 MPa. Ma and Ledward (*2*) found that, although increasing pressure on beef muscle at 20 and 40 °C caused increasing hardness, when beef muscle was treated at 60 °C and above, marked tenderization occurred at 200 MPa. No explanation was given for this unexpected decrease in hardness when subjected to pressure of 200 MPa at elevated temperatures (60 and 70 °C), but it was suggested that proteolysis may occur under these conditions, giving rise to the observed decrease in hardness. The present work was undertaken to see if the results found by Ma and Ledward (*2*) in beef were reproduced in chicken and, if so, to attempt to elucidate the role of myofibrillar proteins.

MATERIALS AND METHODS

Sample Preparation and Treatment. Postrigor chicken (*pectoralis fundus*) was obtained from a commercial supplier. The birds had been slaughtered at 47 days of age, and the breast was removed, deboned, and deskinned at the processing plant. The meat was stored in its original packaging for a maximum of 2 days at 4 °C after purchase. Immediately prior to analysis, the chicken breasts were cut parallel along the muscle into pieces (3 × 2.5 × 6 cm), with all visible connective tissue and fat removed, and wrapped in Multivac bags (Bosley International Inc.). The freshness of breast muscle was determined by pH measurement, and only muscle with a pH value in the range of 5.9−6.2 was used in experiments. Due to the size of the breast muscle, samples from different chickens were used for the different treatments. However, within each experiment subsamples from the same muscle were used.

Samples were treated at room temperature and 40, 60, and 70 °C and at pressures from 200 to 800 MPa for 30 min in a Prototype Stansted Food Lab high-pressure rig (Stansted Fluid Power Ltd., Stansted, U.K.) or for 30 min in a water bath for samples treated at ambient pressure (0.1 MPa). Thirty minutes was chosen because this was sufficient time for the core temperature of the samples to reach the desired temperature and remain stable for at least 5 min. The samples were left at room temperature for 1 h prior to further analysis.

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Texture Profile Analysis (TPA). Three hardness determinations were made on subsamples from each of three treated samples (overall, nine determinations). The samples were examined using a Stable Micro Systems Type (version 3.7G) texturometer with a 5 kg load cell. All samples were compressed to 50% of their original height at a speed of 2 mm/s using a cylindrical-shaped piston, 38 mm in diameter. The texture probe was oriented perpendicular to the muscle fibers, and measurements were made at ambient temperature. Hardness was determined as the peak force of the first compression of the sample in the texture profile (a plot of force vs time).

Myofibrillar Protein Extraction. The myofibrillar proteins were extracted using a method adapted from that of Busche et al. (*7*). Fifty grams of the treated or untreated chicken breast was macerated and then homogenized for 15 s using a Braun hand mixer (M_r 400) in 6 volumes of extraction buffer (20 mM Tris-HCl, 100 mM KCl, pH 7.6, and 5 mM EDTA). After centrifugation at 1000*g* for 10 min at 4 °C, the pellets were resuspended in the extraction buffer, and the operation was repeated five times. After the last centrifugation, the pellets were resuspended in 5 volumes of extraction buffer and homogenized using a Braun hand mixer (M_r 400) for 15 s. To remove the connective tissue, the homogenate was filtered through a 20 mesh nylon net, centrifuged at 1000*g* for 10 min, and washed with the buffer. The pellets were resuspended in 100 mM KCl, centrifuged under the same conditions, and finally homogenized in 0.1 M KCl. For texture analysis prior to temperature and pressure treatment, 50 g of extracted myofibrillar protein was sealed into a dialysis tube to obtain a cylindrical shape feasible for the texture analyzer (~2.5 cm in diameter) before being sealed in Multivac bags (Bosley International) and treated under conditions similar to those used for the whole muscle. For texture analysis the cylinders were cut into samples 2 cm thick and analyzed as for the whole muscle.

Myofibrillar Protein Extract Concentration Determination. The concentration of extracted myofibrillar proteins was determined using the Sigma Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich Co. Ltd.), which is in principle similar to the Lowry method (*8*). Bovine serum albumin was used as the protein standard, and the concentration of extracted myofibrillar protein was found to be 748 μg/mL.

Differential Scanning Calorimetry (DSC). DSC was performed on a Perkin-Elmer DSC7 with a TAC/DX Thermal Analyzer Controller. The instrument was calibrated using indium (mp 156.6 °C, ΔH_{fusion} 27.88 kJ/mol). A sample of 10−20 mg was weighed into a standard 40 μL aluminum pan, which was then hermitically sealed and heated from 20 to 95 °C at 10 °C/min. An empty pan was used as reference. A rescan from 20 to 95 °C was carried out on all samples to check for reversibility.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). The SDS-PAGE was carried out using the method described by Laemmli (*9*) with 7.5% polyacrylamide gels containing 1% SDS. Fifty milligrams of finely minced poultry breast muscle or extracted myofibrillar protein was stirred with 10 mL of Tris buffer (pH 7.5) containing 8 M urea, 2% SDS, and 2% 2-mercaptoethanol for 24 h to dissolve the sample. It was filtered using Whatman no. 5 paper, and 1 mL of filtrate was mixed with an equal volume of 0.125 M Tris buffer (pH 6.8) containing 20 mL of 10% SDS, 2 mg of bromophenol blue, and 10 mL of glycerine. The filtrate was heated in boiling water for 2−5 min and then allowed to cool at room temperature for 15 min. Ten microliters of the sample was applied to each well of the gel. Molecular weights were determined using Wide Range molecular weight standards (Sigma-Aldrich, Poole, U.K.) Separation was carried out at a constant current of 60 mA and a maximum voltage of 500 V until the dye reached the bottom of the gel. Fixation of the protein was done using 12% trichloroacetic acid for 1 h, and staining was by Coomassie brilliant blue [80 mL of 0.1% (w/v) in 2% (w/v) phosphoric acid, 10% ammonium sulfate, and 20 mL of methanol adjusted to a final volume of 100 mL (*10*)]. After ~12 h of staining for , the gels were washed with 20% (v/v) methanol in distilled water. For prolonged storage the gels were transferred into 20% (w/v) ammonium sulfate. Another set of samples was treated under similar conditions in the absence of 2-mercaptoethanol.

Statistical Analysis. Statistical analysis of hardness data was carried out using the two-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) (SPSS Inc., Chicago, IL). The mean differences between samples were analyzed at LSD of $p < 0.05$.

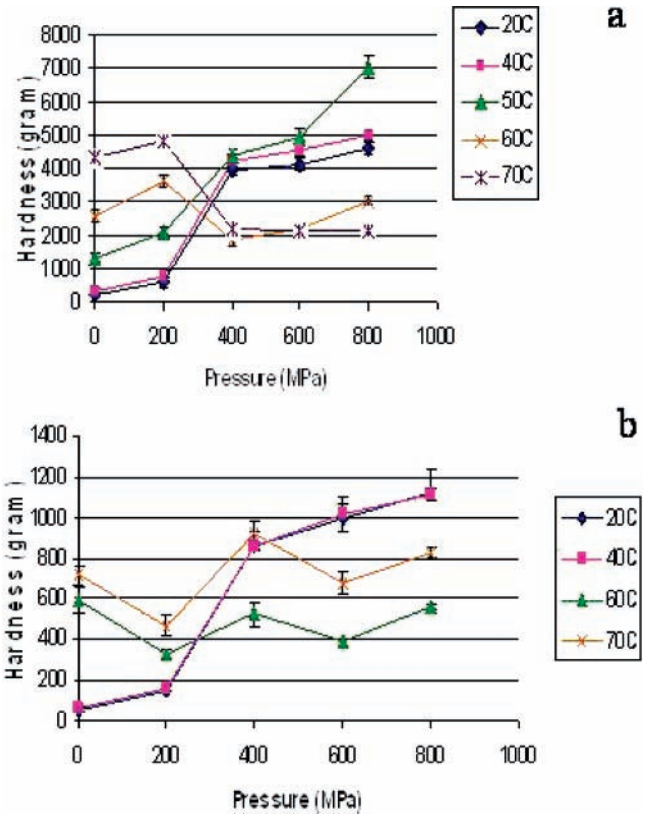

Figure 1. Effect of heat and pressure on the hardness of (**a**) chicken breast muscle and (**b**) extracted myofibrillar protein subjected to different pressure/temperature regimens. All values are the means ± standard deviation of nine determinations.

RESULTS AND DISCUSSION

Effect of Pressure and Heat on Hardness. Heat treatment at ambient pressure produced expected increases in the hardness of chicken breast muscle (**Figure 1**), although significant increases in hardness were observed only at temperatures of ≥50 °C. This suggests that heat-induced denaturation of muscle components was necessary to cause a change in hardness and that the denaturation temperature was between 40 and 50 °C. These results were as expected and are presumably due to denaturation of the myofibrillar and connective tissue proteins (*11*).

Pressure treatments at temperatures ranging from 20 to 50 °C cause an increase in hardness, with the most significant increase being between 200 and 400 MPa. This pattern is similar to that obtained by Angsupanich and Ledward (*3*) for cod muscle and by Ma and Ledward (*2*) for beef muscle. The significant increase at 400 MPa is presumably due to unfolding of the myofibrillar proteins, which may coincide with compression of myosin thick filaments into the Z-line of the muscle that Macfarlane (*12*) and Edwards (*13*) suggested was related to increased toughness or hardness following high-pressure treatments. It can be said that, within the temperature range of 20−50 °C, pressure and temperature are synergistic in their effect on muscle hardness.

Pressure treatment at 60 and 70 °C reveals a very different pressure dependency of the hardness than seen at the lower temperatures. The hardness gradually increased up to 200 MPa,

but then decreased significantly at 400 MPa with little change thereafter, although at 60 °C the increase seen at 800 MPa (**Figure 1**) was significant ($p < 0.05$). Ma and Ledward (*2*) found results similar to those reported here for beef muscle treated at 60 and 70 °C at a range of pressures, although their lowest values for hardness were at 200 MPa and not 400 MPa.

Extracted Myofibrillar Proteins. The effect of combined heat and pressure treatment on the hardness of the myofibrillar protein gels is shown in **Figure 1b**. As expected, at 20 and 40 °C increasing pressure caused increasing hardness, with the most marked increase being at 400 MPa. However, at 60 and 70 °C there is no clear pattern in hardness with different pressure regimens. Hardness fluctuates and shows neither an increasing nor a decreasing trend. It is clear, however, that unlike at lower temperatures, the influence of temperature and pressure is not synergistic at these higher temperatures, which agrees with the findings for whole muscle.

DSC. Muscle proteins normally give three endothermic transitions which represent unfolding of myosin (~59 °C), a mixture of collagen/sarcoplasmic protein (~66 °C), and actin (~78 °C), although these may differ due to differences in pH and ionic strength (*14*, *15*). At ambient pressure (0.1 MPa), changes in the DSC thermograms of chicken breast muscle were observed only at 60 and 70 °C, which agrees with the hardness data (**Figure 1**). At 60 °C the myosin peak had totally disappeared, which suggests that denaturation of myosin has occurred, and this may relate to the significant increase in hardness seen.

Observation of the thermograms of chicken muscle treated at different pressures (**Figure 2a**) at ambient temperature shows that at 200 MPa, the myosin peak disappears, which correlated to a moderate increase in hardness (**Figure 1**). In the thermograms of the samples treated at 400, 600, and 800 MPa, the actin and myosin peaks completely disappeared and TPA showed the hardness of the samples correspondingly increased as the actin and myosin denatured (**Figure 1**).

At 60 °C (**Figure 2c**) myosin is completely denatured by temperature alone; actin, however, was not affected. Compared with the other thermograms, actin denatures with pressures >200 MPa. At 70 °C (**Figure 2d**) both actin and myosin denature even without the application of pressure. It is interesting to note that in several of the high-pressure-treated samples thermal transitions are seen at temperatures of ≥80 °C, perhaps indicating the presence of some new relatively stable protein structures.

Extracted Myofibrillar Proteins. Figure 3a shows the thermograms of the myofibrillar proteins treated at different temperatures at 0.1 MPa. After treatment at 20 and 40 °C both the myosin and actin peaks are clearly visible, but at 60 °C all of the peaks were completely lost. Thus, in the extracted myofibrillar proteins the actin denatures at a lower temperature compared to the whole muscle. According to Wu et al. (*16*), this is because purification or removal of sarcoplasmic protein decreases the heat stability of the protein. At 70 °C both the actin and myosin peaks are absent even after treatment at 0.1 MPa.

When treated at 20 and 40 °C (**Figure 3b**) and pressures ranging from 0.1 to 800 MPa, most of the peaks are lost. However, at ≥200 MPa there appeared a new or modified structure (N) with a lower melting transition (51 °C). This is similar to results on cod muscle and turkey (*3*, *4*). At 800 MPa all of the peaks are lost with no other new peaks emerging. After treatment at 60 and 70 °C, all peaks were lost, even at 0.1 MPa. The DSC results do not give rise to any obvious reason

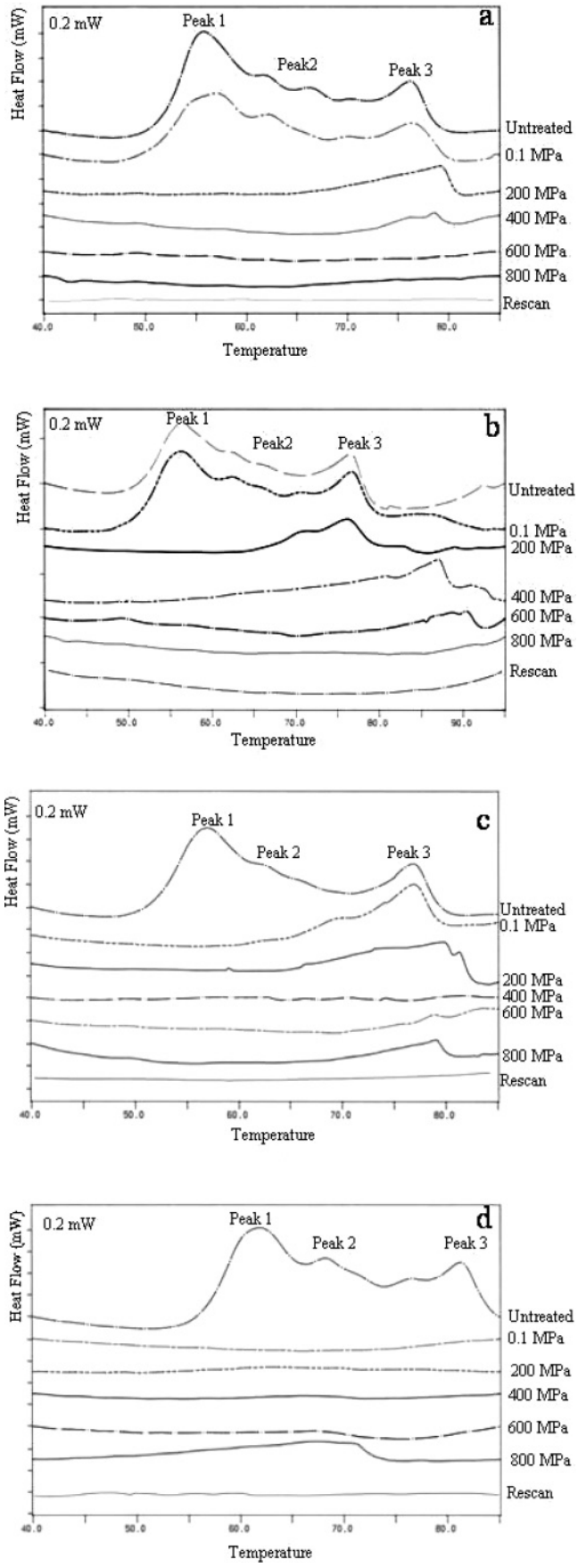

Figure 2. DSC thermograms of whole chicken breast muscle after treatment at different pressures at (**a**) 20, (**b**) 40, (**c**) 60, and (**d**) 70 °C.

for the tenderization of whole muscle seen at 60 and 70 °C and relatively low pressure (400 MPa). However, it is interesting

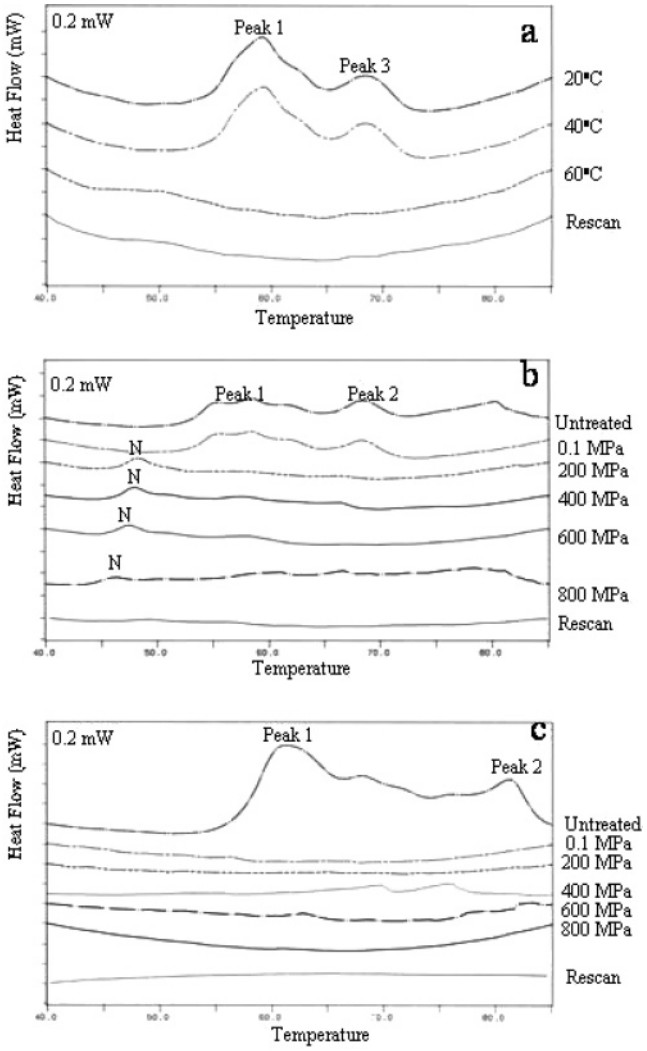

Figure 3. DSC thermograms of extracted myofibrillar proteins after treatment (**a**) at a range of different temperatures at ambient pressure and at a range of different pressures at (**b**) 40 and (**c**) 70 °C.

to note that in beef and cod a new or modified hydrogen-bonded structure is seen at 200 MPa (*2*, *4*), and this may correspond to the new peak (N) that appears in the thermograms of chicken myofibrillar protein at this pressure. Interestingly, the loss in hardness seen in the extracted myofibrillar gels is seen at 200 MPa at 60 and 70 °C (**Figure 3c**), the pressure at which the new structure is seen (**Figure 3b**). In whole muscle there is evidence for the new/modified structure only at 400 and 600 MPa at 40 °C (**Figure 2b**); however, this peak is not well-defined in chicken, although it appears to be in cod, turkey, and beef (*2*−*4*).

SDS-PAGE. In the presence of SDS, urea, and 2-mercaptoethanol (a disulfide bond cleaving agent) all of the samples were solubilized prior to electrophoresis. Thermograms show that the bands of myosin (205 kDa) and actin (45 kDa) are not reduced or lost under any of the temperature−pressure regimens used, indicating no non-disulfide covalent bonds are formed.

Samples treated in the absence of 2-mercaptoethanol gave a small fraction of insoluble residue, which was removed by filtration. This was especially noticed in the more severely treated samples. Without 2-mercaptoethanol, any disulfide bonds produced during the temperature−pressure treatment will remain intact and presumably lead to loss of solubility, and this causes the concentration of the heavy myosin chain (202 kDa) to decrease as the pressure increases. This occurred at or above

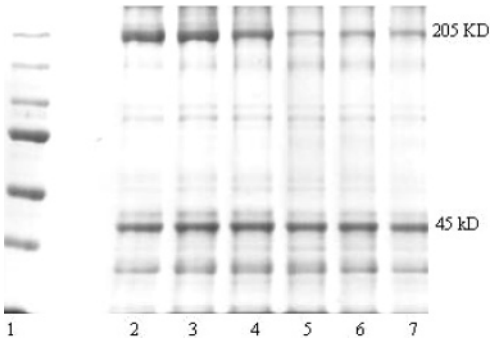

Figure 4. SDS-PAGE electrophoregram of whole poultry muscle treated without 2-mercaptoethanol at 60 °C: (lane 1) reference; (lane 2) untreated; (lane 3) 0.1 MPa; (lane 4) 200 MPa; (lane 5) 400 MPa; (lane 6) 600 MPa; (lane 7) 800 MPa.

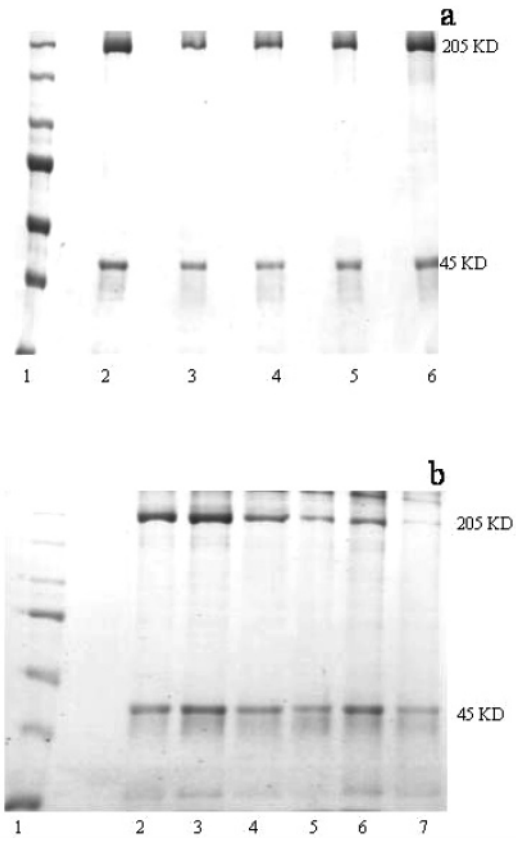

Figure 5. SDS-PAGE electrophoregram of extracted myofibrillar protein in the absence of 2-mercaptoethanol treated at different pressures at (**a**) 20 °C [(lane 1) reference; (lane 2) untreated; (lane 3) 200 MPa; (lane 4) 400 MPa; (lane 5) 600 MPa; (lane 6) 800 MPa] and (**b**) 70 °C [(lane 1) reference; (lane 2) untreated; (lane 3) 0.1 MPa; (lane 4) 200 MPa; (lane 5) 400 MPa; (lane 6) 600 MPa; (lane 7) 800 MPa].

40 °C/400 MPa and 60 °C/200 MPa (**Figure 4**) and above 70 °C/200 MPa. As stated earlier, in the presence of 2-mercaptoethanol, complete solubilization of the sample was achieved, and there were no differences in the electrophoretic patterns. This indicates that disulfide bonding involving the myosin heavy chains takes places under the more extreme conditions, as might be expected from previous studies on the effects of pressure on proteins (*17*). The results found for the whole muscle were confirmed using the extracted myofibrillar proteins, where in the absence of β-mercaptoethanol, loss of myosin was observed at higher temperatures (**Figure 5b**) if compared to samples treated at 20 °C (**Figure 5a**).

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