PE4.90 High pressure processing of beef: correlation between meat quality and proteomic profile 319.00

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***Abstract*— The relationship between changes in myofibrillar protein profile and meat quality indicators (myofibrillar and total protein solubility, colour and water holding capacity) as affected by HPP were investigated. Beef M. *longissimus dorsi* were pressurized at 200, 400 and 600 MPa. Total protein concentration, L* and b* values and WHC were the quality traits most affected by the pressure level applied. Proteome analysis was used to investigate changes in myofibrillar protein profiles caused by HPP. The results revealed that sarcoplasmic proteins present in the myofibrillar extracts, were the most important protein fraction affected by high pressure processing. There was good agreement between individual protein concentrations and quality assessments. Overall, the concentration of total proteins, colour measurements and WHC showed strong correlation with changes in spot concentrations.**

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***Index Terms*—High pressure processing, myofibrillar protein profile, 2DE, meat quality assessment.**

I. INTRODUCTION

HIGH pressure processing (HPP) is a non-thermal preservation technology efficient to inactivate the vegetative microorganisms. High hydrostatic pressure induces conformational changes in proteins leading to protein denaturation, aggregation or gelation, which have a major impact on meat quality [1]. Several authors have studied pressure-induced structural changes on myofibrillar proteins [2, 3, 4]. Depolymerisation was the main effect observed [5]. As a consequence of depolymerisation, high hydrostatic pressure induces solubilisation of myofibrillar proteins [1].

The aim of this work was to investigate and identify changes in myofibrillar protein profile as affected by high pressure processing and relate those changes with meat quality indicators.

II. MATERIALS AND METHODS

A. Sampling and high pressure processing (HPP)

Pressurization of beef M. *Longissimus dorsi* was carried out using a pressurization unit Wave 6000 (Hyperbaric). Samples were pressurized at 200, 400 and 600 MPa (20 min, 20°C). Non-treated (NT) meat was used as a control. Triplicates of each treatment were obtained.

B. Colour measurement

Meat colour was measured with a HunterLab spectrophotometer (Ultrascan XE, Hunter Lab.), with a D65 illuminant and 10° standard observer angle. Colour coordinates were determined using the 1976 CIELAB system and the results were expressed as L* (lightness), a* (redness) and b* (yellowness). The colorimeter was calibrated before each series of measurements. Three measurements were taken for each sample.

C. Water holding Capacity (WHC)

Water holding capacity was calculated as expressible moisture (EM). EM was determined with a centrifugal method according to Pietrasik & Shand [6]. EM was expressed as the percentage of moisture loss after centrifugation in relation to the initial sample weight.

D. Proteome analysis

Two grams of muscle tissue were pulverized in a freezer mill and homogenized with 6 ml of extraction buffer (20 mM TRIS, 2mM EDTA, 4mM $MgCl_2$, 10µl/ml protease inhibitor mix, pH 7.6). Homogenates were centrifuged at 14,000 rpm for 20 min at 4°C. Supernatant containing sarcoplasmic proteins were removed. The pellet was washed three times with distilled water and re-suspended in a denaturing solution (7 M Urea, 2M thiourea 2% CHAPS, 0.8% pharmalyte,1% DTT). After centrifugation, myofibrillar proteins were recovered from the supernatant. Protein solubility, expressed as µg/g meat, was determined using the Bio-Rad Protein Assay Kit (Bio-Rad). Total protein solubility was calculated from the solubility of both fractions.

Myofibrillar proteins were separated by 2D-electrophoresis (2DE) using IPG 4-7 and 12.5% SDS PAGE. Spots of interest were excised from the corresponding gels and digested with trypsin. Protein identification was performed with a LTQ Linear Ion Trap mass spectrometer (Thermo Finnegan). The search was done using the Sequest search algorithm

with Bioworks Browser (v.3.2) against the UniProt/ SwissProt Bovine database.

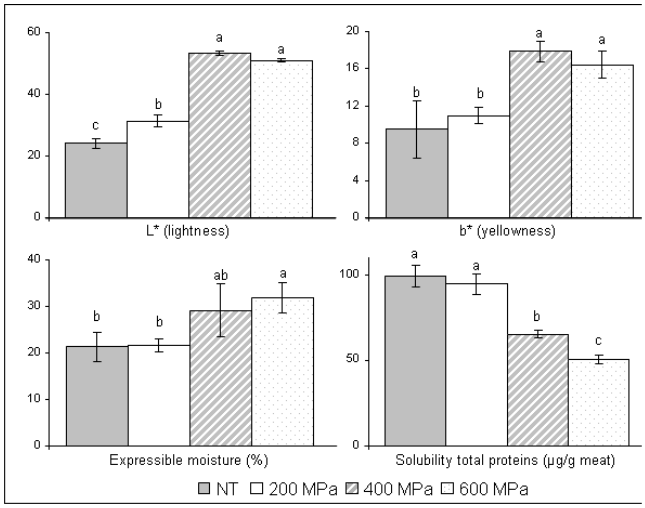
E. Statistical analysis

Data were analysed using the GLM procedure from SAS 9.1. Differences among treatments were assessed by the Tukey test ($p < 0.05$). Pearson correlation coefficients were calculated with SAS. Principal Component Analysis (PCA) was carried out with the PROC FACTOR procedure (method= PRINCIPAL).

III. RESULTS AND DISCUSSION

Quality assessment of pressurized beef showed that total protein solubility, water holding capacity (expressed as expressible moisture), L* and b* values were significantly affected by HPP. The results reflected minimal alteration in meat pressurized at 200 MPa, while HPP (high pressure processing) at higher pressure levels induced more pronounced changes in quality parameters (Fig. 1). These observations are in accordance with other research reporting the effects of high hydrostatic pressure on meat [1, 7, 8].

Figure 1. Quality measurements of beef samples. Results are means of triplicates, bars are standard deviations. Different letters indicate differences between treatments. NT: non-treated.



Proteome analysis was performed in an attempt to understand the relationship between changes in myofibrillar protein profile and meat quality. Proteins contained in the myofibrillar extract were separated with 2DE. Figures 2 and 3 show representative 2DE patterns of the proteins extracted from non-treated and pressurized muscles. A total of 1,363 spots were included in the statistical analysis. The evaluation of pressure effects on protein intensity, showed 54 protein spots affected by HPP ($p<0.01$, fold increase≥ 2). From those, 46 proteins were identified by mass spectrometry. The results revealed that most of the spots showing different intensities among treatments ($p<0.01$) were identified as sarcoplasmic proteins. Those findings suggest increased precipitation of sarcoplasmic proteins onto myofibrils with increasing pressure levels. On the other hand, spot proteins more abundant in non-treated samples ($p<0.01$) were myofibrillar proteins, suggesting solubilisation of myofibrillar proteins as a consequence of HPP.

Figure 2. 2DE image of myofibrillar extracts obtained from a non-treated muscle. Protein spots that significantly changed in abundance between treatments are numbered.

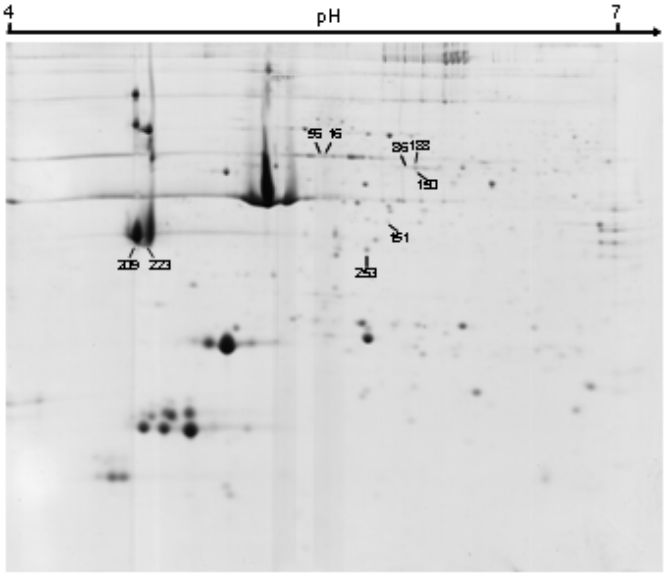
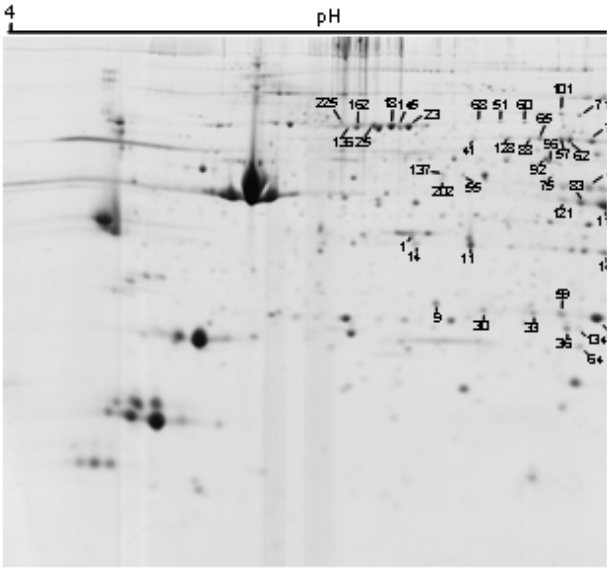
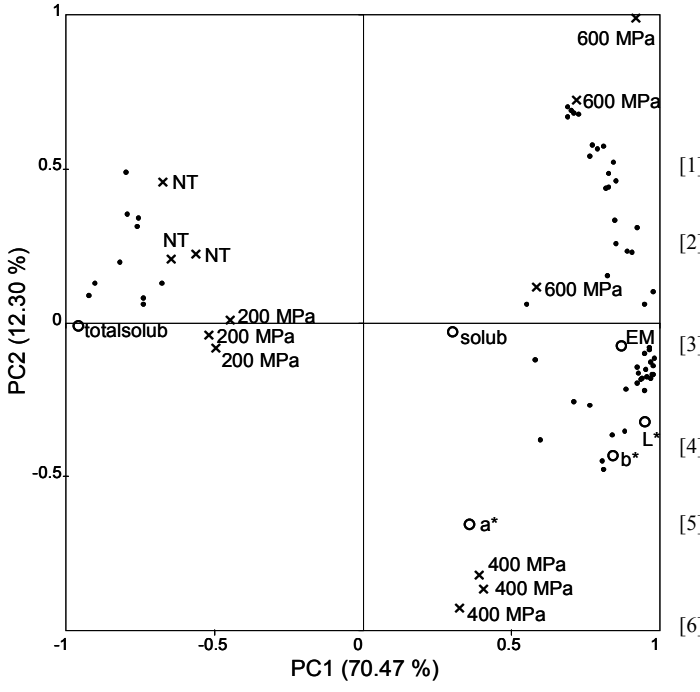


Figure 3. 2DE image of myofibrillar extracts obtained from a muscle sample pressurized at 600 MPa. Protein spots that significantly changed in abundance between treatments are numbered.



Correlation analysis showed good agreement between individual spot concentrations and quality assessments. About 60% of spots that significantly changed in abundance due to HPP were highly correlated ($p<0.01$, r values in a range of 0.70-0.97) with changes in quality traits, except for a* values.

Figure 4. Principal Component Analysis of beef samples (×) and variables (• protein spot intensity; O, quality variables). NT: non-treated, EM: expressible moisture, totalsolub: solubility of total proteins, solub: solubility of myofibrillar proteins.



The relationships among pressure treatments and meat quality parameters were studied with the principal component analysis (PCA). Figure 4 shows the results of the two first components which accounted for the 82.8% of the variance. Samples could be separated into 3 different clusters according to the pressure level applied. The protein spots placed on the left hand side of the first factor (Fig. 4), more abundant in NT and 200 MPa treated samples, correspond to myofibrillar proteins. On the right hand side of the first factor the protein spots correspond to sarcoplasmic proteins present in the myofibrillar extract, more abundant in 400 and 600 MPa treated samples. Abundance of sarcoplasmic proteins present in the myofibrillar extracts was correlated with expressible moisture, L* and b* values, while were negatively correlated with total protein solubility. Those results suggest the relationship between pressure induced sarcoplasmic protein denaturation and both water holding capacity and colour of beef.

IV. CONCLUSION

Evaluation of the changes on the myofibrillar extract evidenced changes in the whole proteome of beef muscle, which could be related with quality parameters. Investigation of those proteins as possible quality markers for processed meats may be further studied.

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