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High pressure induced changes in beef muscle proteome: Correlation with quality parameters

Begonya Marcos [1], Anne Maria Mullen *

Teagasc Food Research Centre Ashtown, Ashtown, Dublin 15, Ireland

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ABSTRACT

The relationship between pressure induced changes on individual proteins and selected quality parameters in bovine *longissimus thoracis et lumborum* (LTL) muscle was studied. Pressures ranging from 200 to 600 MPa at 20 °C were used. High pressure processing (HPP) at pressures above 200 MPa induced strong modifications of protein solubility, meat colour and water holding capacity (WHC). The protein profiles of non-treated and pressure treated meat were observed using two dimensional electrophoresis. Proteins showing significant differences in abundance among treatments were identified by mass spectrometry. Pressure levels above 200 MPa strongly modified bovine LTL proteome with main effects being insolubilisation of sarcoplasmic proteins and solubilisation of myofibrillar proteins. Sarcoplasmic proteins were more susceptible to HPP effects than myofibrillar. Individual protein changes were significantly correlated with protein solubility, L*, b* and WHC, providing further insights into the mechanistic processes underlying HPP influence on quality and providing the basis for the future development of protein markers to assess the quality of processed meats.

1. Introduction

High pressure processing (HPP) is a non-thermal technology used in the food industry because of its capacity to diminish the microbial load while preserving most of the sensory, nutritional and functional properties of the processed food products (Rivalain, Roquain, & Demazeau, 2010). Indeed, the meat industry is increasingly adopting this technology as a post-processing technology to extend the shelf life and to improve the safety of ready-to-eat meat products (Cheftel, 1995; Marcos, Aymerich, & Garriga, 2005; Realini, Guàrdia, Garriga, Pérez-Juan, & Arnau, 2011). Application of HPP to raw meat has shown to induce colour and texture alterations and thus has not been considered appropriate as an industrial practice (Carlez, Veciana-Nogues, & Cheftel, 1995; Cheftel & Culioli, 1997; McArdle, Marcos, Kerry, & Mullen, 2011). However, HPP has been proposed as a possible way of improving the functional properties of muscle proteins in processed meat (Jimenez Colmenero, 2002; Macfarlane & McKenzie, 1976; Messens, Van Camp, & Huyghebaert, 1997). High pressure can affect protein conformation and can lead to protein denaturation, aggregation or gelation (Cheftel & Culioli, 1997; Gross & Jaenicke, 1994; Picouet et al., 2012). The outcome is dependent upon protein susceptibility, the applied pressure and temperature, and the duration of the pressure treatment (Sun & Holley, 2010).

The effects of HPP on proteins are mainly related to the rupture of non-covalent interactions within protein molecules (Galazka, Sumner, & Ledward, 1996). Covalent bonds and primary structures of the proteins are thought not to be affected by high pressure (Heremans & Smeller, 1998). Pressure induced denaturation of proteins is likely to occur because of the destabilisation of non-covalent interactions in the tertiary structure (Chapleau, Mangavel, Compoint, & Lamballerie-Anton, 2004). Even if pressurised proteins retain most of their secondary structure, a small degree of unfolding occurs, which exposes hydrophobic regions of the protein and can lead to protein aggregation (Cheftel & Culioli, 1997; Sikes, Tornberg, & Tume, 2010). Sikes et al. (2010) have proposed that in complex, composite structures like meat, consisting of water, lipids and numerous individual soluble and fibrous proteins, dissociation of protein aggregates on high pressure treatment can lead to the solubilisation of proteins. That can, in turn, be initiated by the breakage of salt bonds and/or hydrophobic bonding.

Considering the effect of HPP on meat proteins, it is important to further investigate these effects to better understand its impact on meat quality. Previous observations by the authors suggested the relationship between changes in sarcoplasmic proteins and alteration of meat quality in beef *longissimus thoracis et lumborum* (LTL) muscle pressurised in a range of 200–600 MPa (Marcos, Kerry, & Mullen, 2010). The authors reported the relationship between modification of the sarcoplasmic protein fraction and alterations of water holding capacity and colour in pressurised beef. Many studies have dealt with the effects of HPP on specific proteins or protein fractions (Chapleau & Lamballerie, 2003;

* Corresponding author. Tel.: +353 1 805 95 00.
E-mail addresses: begonya.marcos@irta.cat (B. Marcos), anne.mullen@teagasc.ie (A.M. Mullen).
[1] Author B. Marcos is presently with: IRTA, Food Technology, Finca Camps i Armet, E-17121 Monells, Girona, Spain.

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Lee, Kim, Lee, Hong, & Yamamoto, 2007; Macfarlane, McKenzie, & Turner, 1986; Sikes et al., 2010). However, to our knowledge, no reports characterising the effects of high pressure processing on both sarcoplasmic and myofibrillar extracts with further identification of the proteins affected by HPP are available. Therefore, the aim of this study was to identify pressure induced changes in the LTL proteome and to analyse their relationship with alterations in selected meat quality parameters in order to better understand the effect of HPP on the proteome and gain further insights into how this impacts on quality.

2. Materials and methods

2.1. Sample preparation and high pressure processing (HPP)

Beef LTL muscles were obtained from a local Irish distributor. Briefly, carcasses from 3 crossbred heifers slaughtered at 24 months of age were hip hung within 1 h of slaughter for 3 days. Muscles were excised, individually vacuum packed and stored at 4 °C until sampling. At 7 days post-mortem muscles were cut into 2.5 × 2.5 × 3 cm pieces. From each muscle a 300 g portion of meat pieces was vacuum packed in polyamide polyethylene bags and randomly assigned to each treatment. Meat samples were treated in an industrial pressurisation unit Model Wave 6000 (Hyperbaric, Burgos, Spain), with a vessel volume of 120 l. HPP was performed at 200, 400 and 600 MPa for 20 min at 20 °C. A 300 g portion was also taken from each muscle for a non-treated (NT) control. Each treatment was carried out in triplicate (i.e. meat from an individual animal equates to one replicate). Meat portions were allowed to cool down at room temperature for 30 min after high pressure processing, cut into individual pieces for each analysis, vacuum packed and frozen at −80 °C. Samples were transported on dry ice to Teagasc Ashtown (Dublin, Ireland) for subsequent analysis. Samples for proteomic analysis were used directly from frozen and samples for quality analysis were thawed at 4 °C for 12 h before analysis.

2.2. Colour measurement

The internal colour of non-treated and pressurised samples was measured on a transversal section of the meat using a HunterLab spectrophotometer (Ultrascan XE, Hunter Associates Laboratory, Inc., Reston, VA), with a D65 illuminant, 10° standard observer angle and 25 mm port size. Freshly cut samples were allowed to bloom for 20 min prior to analysis. Colour coordinates were determined using the 1976 CIELAB system and the results were expressed as L* (lightness), a* (redness) and b* (yellowness). The instrument was calibrated before each series of measurements using white (L* = 100) and black (L* = 0) standard tiles. Colour measurements were taken at three locations on each sample and averaged. The total colour difference (ΔE) was determined as an estimate of colour changes. ΔE was calculated as suggested by Jung, Ghoul, and de Lamballerie-Anton (2003):

$$\Delta E = \left[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2\right]^{1/2} = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}.$$

The colour values of non-treated samples (L_0^*, a_0^*, b_0^*) were used as reference values for ΔE calculation.

2.3. Expressible moisture

Expressible moisture (EM) was determined with a centrifugal method according to Pietrasik and Shand (2004) with some modifications. Meat samples (1.5 × 1.5 × 2.5 cm) of known weight (3.5 ± 0.2 g) were placed in 50 ml centrifuge tubes lined with a thimble consisting of Whatman No. 3 filter paper folded around Whatman No. 50 filter paper. Samples were centrifuged at 5000 rpm for 20 min at 4 °C. EM was expressed as the percentage of moisture loss after centrifugation in relation to the initial sample weight.

2.4. Extraction of muscle proteins

Meat pieces of approximately 5 g were ground in a cryogenic freezer mill (SPEX CertiPrep, Inc., Metuchen, NJ, USA). Sarcoplasmic proteins were extracted from 2 g of pulverised muscle homogenised in 6 ml of extraction buffer (pH 7.6) containing 20 mM TRIS, 2 mM EDTA, 4 mM $MgCl_2$ and 10 μl/ml protease inhibitor mix (GE Healthcare, Uppsala, Sweden). Homogenates were centrifuged at 14,000 rpm for 20 min at 4 °C. Supernatants containing sarcoplasmic proteins were removed and frozen at −80 °C until further analysis. The pellet was washed three times with distilled water and re-suspended in a denaturing solution (7 M urea, 2 M thiourea 2% CHAPS, 0.8% Pharmalyte broad range pHs 3–10 (GE Healthcare), and 1% DTT). Homogenates were centrifuged at 7000 rpm for 45 min at 20 °C. Supernatants containing myofibrillar proteins were removed after centrifugation and frozen at −80 °C until further analysis.

Protein concentrations were determined using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford method (Bradford, 1976). Bovine serum albumin was used as the standard. Sarcoplasmic and myofibrillar protein solubility were expressed as μg protein/g meat. Total protein solubility was calculated as the sum of sarcoplasmic and myofibrillar protein solubility of each sample.

2.5. Two dimensional electrophoresis (2DE)

Muscle proteins were separated by 2D-electrophoresis (2DE). Nine hundred μg of either sarcoplasmic or myofibrillar proteins was included in a buffer containing 7 M urea, 2 M thiourea, 2% CHAPS, 0.3% DTT, 1% Pharmalyte pH 3–10, and traces of bromophenol blue. Samples were loaded onto immobilised pH gradient strips (pHs 4–7, 24 cm, GE Healthcare) that were rehydrated for 16 h. Isoelectric focusing (IEF) was performed using an Ettan IPHphor 3 IEF Unit (GE Healthcare). The voltage was applied as follows: step 1, 500 V for 1 h; step 2, 3500 V until 75,000 Vh were reached; step 3, 8000 V for 1 h. After IEF, the strips were equilibrated for 15 min at room temperature in 10 ml of equilibration buffer (50 mM Tris–HCl pH 8.8, 6 M urea, 30% Glycerol, 2% SDS, and traces of bromophenol blue) with 1% DTT (w/v) added immediately before use. After removing the DTT solution, the strips were equilibrated for 15 min at room temperature in 10 ml of equilibration buffer with 2.5% of iodoacetamide added immediately before use. In the second dimension, proteins were resolved on 12% SDS-PAGE gels using a Protean plus Dodeca Cell system (Bio-Rad). The gels were fixed with 50% ethanol and 2% phosphoric acid for 3 h with gentle shaking. After removing the fixing solution, the gels were washed three times in distilled water for 20 min. The gels were stained with 660 mg/l of colloidal Coomassie Brilliant Blue G-250 (Sigma-Aldrich, St. Louis, MO, USA) in 3% phosphoric acid, 17 ammonium persulfate, and 34% methanol for 5 days with gentle shaking. Two technical replicates were obtained for each sample.

2.6. Image analysis

Gel images were acquired using a GS-800 densitometer (Bio-Rad) and analysed using Progenesis Samespots version 3.2 software (Nonlinear Dynamics, Durham, NC, USA). Briefly, the gel image with the most spots detected was assigned as a reference, and all remaining gel images were aligned to this reference gel. Gel alignment was verified manually after automatic alignment. Landmark spots were used to confirm spot matching across all gels and manual verification was used to screen out any dust artefacts or incorrectly identified spots. The normalised volume for each spot on each gel was calculated with the software. Log transformation of the spot volumes was used to generate normally distributed data. Log normalised volume was used to compare spot