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

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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.

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 & 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; 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.

Materials and Methods

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 hour 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.

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 & de Lamballerie-Anton (2003):

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

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

Expressible moisture

Expressible moisture (EM) was determined with a centrifugal method according to Pietrasik & 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 5,000 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.

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, 2mM EDTA, 4mM MgCl₂ and 10µl/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, 2M thiourea 2% CHAPS, 0.8% Pharmalyte broad range pH 3-10 (GE Healthcare), and 1% DTT). Homogenates were centrifuged at 7,000 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.

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 7M urea, 2M thiourea, 2% CHAPS, 0.3% DTT, 1% Pharmalyte pH 3-10, and traces of bromophenol blue. Samples were loaded onto

immobilised pH gradient strips (pH 4-7, 24 cm, GE Healthcare) that were rehydrated for 16h. 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, 3,500 V until 75,000 Vh were reached; step 3, 8,000 V for 1h. After IEF, the strips were equilibrated for 15min at room temperature in 10ml of equilibration buffer (50 mM Tris-HCl pH 8.8, 6M 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 10ml 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 (BioRad). The gels were fixed with 50% ethanol and 2% phosphoric acid for 3h with gentle shaking. After removing the fixing solution, the gels were washed three times in distilled water for 20min. 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.

Image analysis

Gel images were acquired using a GS-800 densitometer (BioRad) 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 abundance. Significant differences among treatments were evaluated by summarising the number of spots in the comparative proteomic studies with $p < 0.05$, 2-fold increase, and $q < 0.01$.

Spot identification

Proteins were subjected to in-gel protein digestion. Excised gel spots were destained with 70µl of 200mM NH_4HCO_3 with shaking for 10 min at 37°C. After centrifugation, the solution was removed. Then, 70µl 200mM NH_4HCO_3 /acetonitrile (2:3) were added. After shaking for 10 minutes at 37°C and centrifugation, the solution was removed. Addition of 70µl of 50mM NH_4HCO_3 was followed by shaking for 10 min at 37°C. After centrifugation, the solution was removed. Finally, 70µl of acetonitrile was added to each tube and shaken for 10 minutes at 37°C. After removal of the

solution, gel spots were incubated with 200 mM ammonium bicarbonate for 20 min; this step was repeated until they become colourless. The gel pieces were rehydrated with 30 µl of 50 mM ammonium bicarbonate containing 0.2µg of trypsin (Promega, Madison, WI, USA) and digestion was performed overnight with shaking at 37°C. The supernatant was then dried down in a vacuum centrifuge (Eppendorf Concentrator 5301, Hamburg, Germany) at 45°C.

Thermo Scientific LTQ ORBITRAP XL mass spectrometer connected to an Exigent NANO LC.1DPLUS chromatography system incorporating an auto-sampler was used for analysis. Tryptic peptides were resuspended in 0.1% formic acid. Each sample was loaded onto a Biobasic C18 Picofrit™ column (100 mm length, 75 mm ID) and was separated by an increasing acetonitrile gradient, using a 25 min reverse phase gradient (0-50% acetonitrile for 11 min) at a flow rate of 30 nl/min. The mass spectrometer was operated in positive ion mode with a capillary temperature of 200°C, a capillary voltage of 9V, a tube lens voltage of 100V and with a potential of 1800V applied to the frit. All data was acquired with the mass spectrometer operating in automatic data dependent switching mode. A high resolution MS scan (300-2000 Dalton) was performed using the Orbitrap to select the five most intense ions prior to MS/ MS analysis using the Ion trap. The search was done using TurboSEQUENT (Bioworks Browser 3.3.1 SP1; Thermo Finnigan, UK) searching the bovine subset of the Uniprot /Swissprot fasta database for fully and partially tryptic peptides. Each peptide used for protein identification met specific SEQUEST parameters, i.e. cross-correlation values ≥ 1.9 , ≥ 2.5 , ≥ 3.2 and ≥ 3.2 for single-, double-, triple- and quadruple-charged peptides, respectively.

Statistical analysis

Data obtained from the quality analysis were analysed using the General Linear Model from SAS (version 9.2, SAS Institute, Cary, NC, USA). The treatment (NT, 200 MPa, 400 MPa, and 600 MPa) was included in the model as a fixed effect, with animal as a random effect. Differences between means were assessed by the Tukey test ($p < 0.05$). Pearson correlation analysis was used to investigate the relationship among quality indicators and spot intensities. Correlation coefficients were calculated with the CORR procedure of SAS 9.2. A multiple testing correction consisting of a modification of the effective number (Cheverud, 2001) was performed as suggested by Li and Ji (2005).

Results and discussion

Impact of HPP on meat quality

Table 1 shows the effect of high pressure processing on protein solubility of bovine LTL. Pressurisation of beef samples induced a reduction of total protein solubility which seemed to be explained by changes in the sarcoplasmic fraction ($p < 0.001$), with the exception of HPP at 200 MPa (Table 1). Processing above this pressure level induced an increasing loss of protein solubility as the

pressure level increased. HPP had no effect ($p>0.05$) on myofibrillar protein solubility. This fact was confirmed by the correlation analysis which showed a strong positive correlation between total and sarcoplasmic protein solubility (Table 2). A reduction of muscle protein solubility is an indicator of protein denaturation as solubility decreases due to the formation of insoluble protein aggregates that can no longer be extracted (Fischer, Hamm, & Honikel, 1979; Laakkonen, Sherbon, & Wellington, 1970). Thus, the decreased protein solubility observed suggests certain sarcoplasmic protein denaturation induced by exposure to pressures above 200 MPa. Pressure induced denaturation would lead to the formation of aggregates, most probably generated through intermolecular disulfide bridges (Galazka, Dickinson, & Ledward, 2000). These results agree with previous observations reporting reduction of sarcoplasmic protein solubility in pressurised beef (Kim, Lee, Lee, Kim, & Yamamoto, 2007; Marcos et al., 2010). Differing effects of HPP on the solubility of myofibrillar proteins, either solubilisation or insolubilisation, have been observed depending on the protein under study (Chan, Omana, & Betti, 2011; Chapleau & Lamballerie, 2003; Iwasaki, Noshiroya, Saitoh, Okano, & Yamamoto, 2006; Sikes, Tobin, & Tume, 2009).

The water holding capacity (WHC) of beef muscle was calculated by means of the expressible moisture (EM), which measures the percentage of moisture loss of the meat under specific conditions. Pressurisation at 200 MPa had no impact on EM of beef, while samples at 400 and 600 MPa showed increased EM compared to NT beef (Table 1). This result indicates a reduction of WHC of beef at the higher pressure levels. Kim et al. (2007) reported a similar reduction in WHC of bovine *semitendinosus* muscle treated at 400–500 MPa, although they also reported decreased WHC at 200 MPa. Fernández et al. (2007) also reported higher EM in beef pressurised at 650 MPa. Myofibrillar proteins, myosin and actin, and to some extent tropomyosin are known as the main water-binding components in muscle tissue (Zayas, 1997). Some authors have highlighted the important role of sarcoplasmic proteins in determining WHC of meat (Joo, Kauffman, Kim, & Park, 1999; Monin & Laborde, 1985) and its presence in the centrifugal drip (Di Luca, Mullen, Elia, Davey, & Hamill, 2011). Moreover, precipitation of sarcoplasmic proteins on the myofibrils has been suggested as a possible cause of the loss of water binding capacity in meats with altered water retention properties (Lopez-Bote & Warriss, 1988; Monin & Laborde, 1985). In this sense, a negative correlation ($p < 0.01$) between sarcoplasmic protein solubility and expressible moisture was observed (Table 2), confirming the relationship between pressure induced denaturation of sarcoplasmic proteins and the loss of WHC in pressurised meat.

Pressurised meat experienced a significant increase of L^* (lightness) compared to non-treated meat (Table 1). Samples pressurised at 400 MPa and 600 MPa showed the highest L^* values. Increased lightness of meat is a well documented result of pressurisation of red muscles (Carlez et al., 1995; Goutefongea, Rampon, Nicolas, & Dumont, 1995; McArdle, Marcos, Mullen, & Kerry, 2013; Serra et

al., 2007; Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991). The whitening effect induced by pressure has been related to protein coagulation, which would affect sample structure and surface properties (Goutefongea, Rampon, Nicolas, & Dumont, 1995), and to globin denaturation and heme group displacement or release (Carlez et al., 1995). HPP did not alter the redness (a^*) of the meat ($p>0.05$). An increase in yellowness (b^*) compared to NT samples was observed at pressures above 200 MPa. Higher b^* values obtained at 400 and 600 MPa agree with the brownish colouration of meat observed at these pressures, and could be related to the pressure induced oxidation of ferrous myoglobin to ferric metmyoglobin (Carlez et al., 1995). The total colour difference (ΔE) was determined as an estimate of colour changes in pressurised meat. HPP at 400 and 600 MPa induced more pronounced colour changes compared to 200 MPa (Table 1). It has been suggested that a change of 10 units in ΔE would be considered to significantly modify meat appearance (Jung et al., 2003). According to that, it can be concluded that HPP at 200 MPa, with ΔE lower than 10, slightly modified meat appearance. On the contrary, more severe treatments (400 & 600 MPa) strongly modified meat appearance. This result agrees with previous observations by Marcos et al. (2010). It should be noted that stronger modifications observed at higher pressure levels might be also influenced by a higher adiabatic heating with increasing pressure levels.

Correlations of colour coordinates with other quality parameters showed that pressure induced changes in protein solubility and EM were both associated with changes in L^* and b^* coordinates (Table 2). Several authors have suggested a relationship between increased meat lightness and precipitation of sarcoplasmic proteins (Goutefongea et al., 1995; Joo et al., 1999; McLoughlin & Goldspink, 1963; van Laack et al., 1994).

Influence of HPP on bovine protein profiles

The protein profiles of non-treated (NT) and pressurised (200, 400 & 600 MPa) beef LTL were obtained with 2-DE. Figure 1 shows two representative 2-DE gels showing the effects of HPP on sarcoplasmic protein profiles. After removal of false positives and saturated or poorly reducible zones, 988 spots were successfully matched across the whole set of gel images. In order to assess in detail the differences among treatments, variations in protein expression were quantified by statistically comparing relative spot intensities. A total of 44 spots were differentially represented among the studied treatments ($p<0.01$ and 2-fold increase). From these, 37 spots were successfully identified with MS (Table 3). The protein spots showing differences in abundance among treatments were mainly sarcoplasmic proteins, although 11 protein spots corresponded to myofibrillar proteins. The effect of HPP on protein abundance was different depending on the type of protein. In the sarcoplasmic fraction HPP increased the amount of myofibrillar proteins present while decreasing the amount of sarcoplasmic proteins (Table 3). HPP at the lowest pressure level (200 MPa) had little

effect on the sarcoplasmic protein profile. Among sarcoplasmic proteins, the most pressure labile were cytosol aminopeptidase, glycerol-3-phosphate dehydrogenase [NAD⁺], and glycogen phosphorylase, which experienced losses at 200 MPa. On the contrary, the most pressure resistant sarcoplasmic proteins were heat shock 70 kDa protein 1A, malate dehydrogenase, retinal dehydrogenase 1, and serotransferrin, which decreased only after HPP at 600 MPa ($p < 0.01$). On the other hand, the presence of myofibrillar protein fragments present in the soluble fraction (myosin light chain 1, tropomyosin α -1 chain, tropomyosin β -chain, troponin T, fast and slow skeletal muscle) increased due to HPP at pressures above 200 MPa (Table 3).

Figure 2 shows two representative 2-DE gels of the myofibrillar protein fraction extracted from non-pressurised and pressurised beef. A total of 954 protein spots were included in the statistical analysis. Fifty six spots showed differences among treatments ($p < 0.01$ and 2-fold increase). From these, 52 spots were successfully identified by MS (Table 4). As previously reported, the effect of HPP was dependent on the type of protein. HPP induced a decrease of the abundance of myofibrillar proteins, while increasing the abundance of sarcoplasmic proteins in the myofibrillar fraction (Table 4). HPP at 200 MPa had little effect on the protein profile. The proteins in the myofibrillar fraction showing differences in abundance among treatments were mainly sarcoplasmic proteins. Only six myofibrillar protein spots were affected by HPP and were identified as actin, F-actin-capping protein, tropomyosin α -1 and β chain, and tubulin β -2B chain (in two separate spots). Among the sarcoplasmic proteins in the myofibrillar extract, flavin reductase, glycerol-3-phosphate dehydrogenase [NAD⁺], heat shock 70KDa protein 1A, actin and tropomyosin β chain were the only proteins altered at 200 MPa. The highest pressure level applied (600 MPa) was necessary to modify the presence of α -enolase, malate dehydrogenase, peptide methionine sulfoxide reductase, serotrasferrin, and serum albumin content in the myofibrillar protein fraction (Table 4).

In agreement with our results several authors have reported pressure induced changes in the sarcoplasmic protein fraction of meat due to HPP, with a stronger effect as the pressure of the treatment increased, mainly above 200MPa (Angsupanich & Ledward, 1998; Cheah & Ledward, 1996; Cheret, Hernandez-Andres, Delbarre-Ladrat, Lamballerie, & Verrez-Bagnis, 2006). Cheah and Ledward (1996) attributed the decreased sarcoplasmic band intensities to protein degradation or insolubilisation due to denaturation and aggregation of proteins. However, Ohshima, Ushio, and Koizumi (1993) suggested that rather than being degraded by HPP, certain fish sarcoplasmic proteins become covalently linked together. Pressure induces the disruption of hydrophobic and electrostatic interactions while enhancing hydrogen bonding as well as promoting formation of disulphide bonds (Mozhaev, Heremans, Frank, Masson, & Balny, 1994). Our results would confirm the insolubilisation of sarcoplasmic proteins which were extracted together with the myofibrillar protein fraction after HPP. To our knowledge, this is the first study reporting the effects of HPP on individual sarcoplasmic

proteins. Most investigations have focused on pressure induced changes in the myofibrillar protein fraction. Several authors have observed the solubilisation of myofibrillar proteins due to HPP (Cheah & Ledward, 1996; Jung, de Lamballerie-Anton, & Ghoul, 2000; Sikes et al., 2009). Increased solubility of myofibrillar proteins due to HPP has been related to protein depolymerisation and subsequent increased interactions between protein constituents and water (Cheftel & Culioli, 1997). Similarly to our results, other authors have reported pressure induced solubilisation of myosin light chains, tropomyosin, troponin T, and actin (Angsupanich, Edde, & Ledward, 1999; Iwasaki et al., 2006; Jung et al., 2000; Ma, Zhou, Ledward, Yu, & Pan, 2011; McArthur & Wilding, 1996). Solubilisation of α -actinin, troponin C and M-protein and C-protein has also been reported (Iwasaki et al., 2006; Lee et al., 2007; Ma et al., 2011).

Relationship between protein abundance and meat quality traits

Tables 5 and 6 show the significant correlations between meat quality traits and the spot volume of the proteins that were affected by HPP.

In the sarcoplasmic protein fraction a negative correlation between sarcoplasmic protein abundance and L^* , b^* , ΔE and EM was observed, while myofibrillar proteins were positively correlated ($p < 0.006$). For the myofibrillar extracts, the correlations were inversed, a positive correlation between sarcoplasmic protein abundance and L^* , b^* , ΔE and EM was observed, while myofibrillar proteins presented a negative correlation with the studied quality parameters ($p < 0.005$). On the contrary, no correlations were found between protein spot volumes and meat redness (a^* , $p > 0.05$). These data indicate that pressure induced solubilisation of myofibrillar proteins and insolubilisation of sarcoplasmic proteins resulted in paler meat with decreased WHC. These results reflect the impact of pressure induced changes on proteome profiles, mainly on sarcoplasmic proteins, and on meat quality parameters. These results would confirm the strong role of sarcoplasmic protein fraction on meat quality previously suggested by the authors (Marcos et al., 2010). Similarly, other authors have highlighted the role of sarcoplasmic proteins such as aldehyde dehydrogenase, glycerol-3-P-dehydrogenase, protein DJ-1, serotransferrin, β -enolase, creatine kinase M-type and heat shock protein 70 kDa on meat L^* and drip loss (Hwang, Park, Cho, & Lee, 2004; Kwasiborski et al., 2008; Sayd et al., 2006; van de Wiel & Zhang, 2007). As expected, total protein solubility was strongly correlated with protein abundance. However, looking into each protein fraction, no correlation with myofibrillar protein solubility was observed ($p > 0.05$).

Conclusions

Data from this study indicate that HPP at pressure levels above 200 MPa strongly modified the protein profiles and the quality parameters of bovine *longissimus thoracis et lumborum* muscle. HPP

induced strong changes in protein solubility suggesting that monitoring of protein solubility in processed meats can provide an indication of meat quality.

The proteins that were more affected by HPP were the sarcoplasmic proteins in the range of molecular weights and isoelectric points covered by this study. The relationship between changes at the proteome levels and changes in meat quality provide further insights into the mechanistic processes underlying HPP influence on quality and may provide the basis for the development of protein markers to assess the quality of processed meats.

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Figure 1. Representative 2-DE gels of sarcoplasmic extracts obtained from bovine *longissimus thoracis et lumborum* muscle: a) non-treated and b) treated at 600 MPa. Muscle proteins with significantly different abundance among treatments are marked and numbered. See table 3 for detailed list of muscle proteins from sarcoplasmic fraction that changed in abundance due to high pressure processing.

Figure 2. Representative 2-DE gels of myofibrillar extracts obtained from bovine *longissimus thoracis et lumborum* muscle: a) non-treated and b) treated at 600 MPa. Muscle proteins with significantly different abundance among treatments are marked and numbered. See table 4 for detailed list of muscle proteins from myofibrillar fraction that changed in abundance due to high pressure processing.

Table 1. Effect of high pressure processing on the quality parameters of *longissimus thoracis et lumborum* muscle.

	Protein solubility ($\mu\text{g/g}$)			EM	L^*	a^*	b^*	ΔE
	total	myofibrillar	sarcoplasmic					
NT	99.24 ^a	21.18	78.06 ^a	21.80 ^c	26.07 ^c	8.03	9.40 ^b	-
200 MPa	94.59 ^a	22.77	71.82 ^a	22.40 ^{bc}	33.20 ^b	9.23	10.87 ^b	7.64 ^b
400 MPa	65.36 ^b	22.30	43.06 ^b	30.01 ^{ab}	55.49 ^a	11.19	17.55 ^a	31.35 ^a
600 MPa	50.56 ^c	22.87	27.69 ^c	32.72 ^a	53.01 ^a	8.96	16.41 ^a	28.09 ^a
SE	2.73	0.74	2.82	1.74	0.77	0.81	1.05	0.79
p	<0.001	NS	<0.001	<0.01	<0.001	NS	<0.01	<0.001

Results are means of three replicates. Different letters within a column indicate differences among treatments. Total, sum of sarcoplasmic and myofibrillar protein solubility; EM, expressible moisture (% of moisture loss); L^* , lightness; a^* , redness; b^* , yellowness; ΔE , total colour difference; NT, non-treated; SE, standard error; NS, non-significant, $p>0.05$.

Table 2. Correlation coefficients between quality parameters.

	<i>solubility</i>			L*	a*	b*	ΔE	EM
	total	myofibrillar	sarcoplasmic					
<i>solubility:</i>								
total	1	-0.304	0.998***	-0.904***	-0.303	-0.851***	-0.864**	-0.856**
myofibrillar		1	-0.358	0.321	0.495	0.380	-0.078	0.129
sarcoplasmic			1	-0.906***	-0.320	-0.856***	-0.859**	-0.847**
L*				1	0.525	0.903***	0.999***	0.841**
a*					1	0.692*	0.478	0.430
b*						1	0.957***	0.767**
ΔE							1	0.804**
EM								1

L*: lightness; a*: redness; b*: yellowness; ΔE : total colour difference; EM: expressible moisture.
 Numbers marked in bold show significant correlation; *p<0.05; **p<0.01; ***p<0.001.

Table 3. Identification of proteins contained in the sarcoplasmic fraction which were significantly altered due to HPP.

Spot	Protein name	Accession number ^x	Protein probability	Cov. ^y	M _W theoretical (KDa)	pI theoretical	matching peptides ^z	Normalised spot volume (x10 ³)					p
								NT	200 MPa	400 MPa	600 MPa	SE	
Sarcoplasmic proteins													
15	Beta-enolase	Q3ZC09	2.52E-10	26.5	47.07	7.6	9	2153 ^a	2620 ^a	220 ^b	268 ^b	171	<0.001
18	Beta-enolase	Q3ZC09	2.76E-10	18.4	47.07	7.6	7	1994 ^a	2530 ^a	223 ^b	322 ^b	169	<0.001
83	Creatine kinase M-type	Q9XSC6	1.59E-11	25.2	42.96	6.7	7	9150 ^a	11153 ^a	2062 ^b	2296 ^b	692	<0.001
93	Creatine kinase M-type	Q9XSC6	4.02E-10	27.3	42.96	6.7	8	3804 ^a	4405 ^a	750 ^b	891 ^b	313	<0.001
112	Creatine kinase M-type	Q9XSC6	1.92E-10	22.6	42.96	6.7	6	1839 ^a	2118 ^a	431 ^b	628 ^b	290	<0.01
78	Cytosol aminopeptidase	P00727	2.76E-09	45.9	56.25	6.1	20	465 ^a	479 ^b	559 ^b	94 ^b	41	<0.001
69	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	Q5EA88	7.88E-14	27.5	37.62	6.4	9	2307 ^a	547 ^b	366 ^b	397 ^b	74	<0.001
172	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	Q5EA88	2.78E-13	22.9	37.62	6.4	7	1475 ^a	542 ^b	475 ^b	399 ^b	69	<0.001
289	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	Q5EA88	5.77E-14	42.1	37.62	6.4	13	1695 ^a	753 ^{bc}	1000 ^b	710 ^c	59	<0.001
322	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	Q5EA88	1.89E-14	42.1	37.62	6.4	12	1343 ^a	670 ^b	702 ^b	617 ^b	65	<0.001
43	Glycogen phosphorylase, muscle form	P79334	2.25E-11	21.9	97.23	6.6	14	438 ^a	92 ^b	61 ^b	54 ^b	24	<0.001
353	Heat shock 70 kDa protein 1A	Q27975	2.28E-12	30.6	70.22	5.6	21	1913 ^a	2056 ^a	1705 ^{ab}	1061 ^b	155	<0.01
360	Heat shock 70 kDa protein 1A	Q27975	5.72E-13	30.4	70.22	5.6	15	1106 ^a	1096 ^a	983 ^a	733 ^b	167	<0.01
249	Malate dehydrogenase, cytoplasmic	Q3T145	2.33E-12	32.6	36.42	6.2	8	5013 ^a	5218 ^a	5228 ^a	1898 ^b	383	<0.001
302	Malate dehydrogenase, cytoplasmic	Q3T145	1.33E-14	30.8	36.42	6.2	8	1200 ^a	1131 ^{ab}	874 ^b	523 ^c	70	<0.001
74	Peroxiredoxin-6	O77834	3.89E-08	37.9	25.05	6.0	9	1581 ^a	1877 ^a	307 ^b	450 ^b	155	<0.001
2	Phosphoglucomutase-1	Q08DP0	3.42E-13	25.8	61.55	6.4	12	1884 ^a	2418 ^a	109 ^b	180 ^b	105	<0.001
17	Phosphoglucomutase-1	Q08DP0	4.89E-12	30.2	61.55	6.4	11	978 ^a	1136 ^a	100 ^b	143 ^b	96	<0.001
32	Phosphoglucomutase-1	Q08DP0	5.52E-11	27.4	61.55	6.4	10	1310 ^a	1556 ^a	173 ^b	203 ^b	84	<0.001
40	Phosphoglucomutase-1	Q08DP0	1.78E-14	42.3	61.55	6.4	16	3006 ^a	2465 ^{ab}	444 ^b	361 ^b	507	<0.01
119	Phosphoglucomutase-1	Q08DP0	2.44E-14	29.4	61.55	6.4	10	392 ^a	438 ^a	92 ^b	145 ^b	47	<0.01
151	Phospholysine phosphohistidine inorg. pyrophosphate phosphatase	Q0VD18	4.13E-12	32.2	29.16	5.3	5	166 ^a	150 ^{ab}	70 ^{bc}	41 ^c	19	<0.01
65	Retinal dehydrogenase 1	P48644	2.44E-14	32.3	54.77	6.2	13	1334 ^a	1424 ^a	1354 ^a	221 ^b	153	<0.01
48	Serotransferrin	Q29443	2.37E-11	30.4	77.70	6.7	17	732 ^a	733 ^a	882 ^a	118 ^b	41	<0.001
168	Serotransferrin	Q29443	1.91E-11	35.6	77.70	6.7	18	763 ^a	798 ^a	951 ^a	252 ^b	77	<0.01
394	Serum albumin	P02769	1.39E-12	34.9	69.25	5.8	19	4846 ^a	4794 ^a	4338 ^{ab}	2844 ^b	279	<0.01

Myofibrillar proteins

23	Myosin light chain 1, skeletal muscle isoform	A0JNJ5	6.32E-11	37.5	20.92	5.0	6	157 ^c	133 ^c	918 ^b	1318 ^a	85	<0.001
61	Myosin light chain 1, skeletal muscle isoform	A0JNJ5	1.79E-12	56.8	20.92	5.0	11	2878 ^b	3598 ^b	16841 ^a	18993 ^a	1220	<0.001
91	Myosin light chain 1, skeletal muscle isoform	A0JNJ5	3.33E-15	26.6	20.92	5.0	6	391 ^b	532 ^b	1317 ^{ab}	2170 ^a	237	<0.01
104	Myosin light chain 1, skeletal muscle isoform	A0JNJ5	3.33E-16	51.6	20.92	5.0	9	541 ^{bc}	468 ^c	2387 ^a	1721 ^{ab}	272	<0.01
11	Tropomyosin alpha-1 chain	Q5KR49	1.11E-14	50.7	32.67	4.5	17	1010 ^b	853 ^b	18356 ^a	27034 ^a	2953	<0.01
9	Tropomyosin beta chain	Q5KR48	8.39E-10	30.3	32.82	4.5	8	2387 ^b	3227 ^b	30130 ^a	35136 ^a	3139	<0.001
5	Troponin T, fast skeletal muscle	Q8MKI3	6.77E-10	23.2	32.11	6.0	7	106 ^b	165 ^b	1224 ^{ab}	2166 ^a	426	<0.01
41	Troponin T, fast skeletal muscle	Q8MKI3	2.78E-09	28.0	32.11	6.0	6	134 ^b	139 ^b	710 ^{ab}	1104 ^a	194	<0.01
174	Troponin T, fast skeletal muscle	Q8MKI3	1.17E-09	28.4	32.11	6.0	7	468 ^b	518 ^b	1347 ^{ab}	1721 ^a	300	<0.05
85	Troponin T, slow skeletal muscle	Q8MKH6	1.73E-07	22.1	31.27	5.7	6	305 ^b	390 ^b	1251 ^{ab}	1767 ^a	292	<0.01
162	Troponin T, slow skeletal muscle	Q8MKH6	1.65E-06	21.7	31.27	5.7	6	349 ^b	325 ^b	1115 ^{ab}	1263 ^a	190	<0.01

^x According to UniProt/Swiss-Prot database. All proteins corresponded to proteins sequenced in *Bos taurus*. ^y Percentage of coverage of the entire amino acid sequence. ^z Number of matching peptides. M_w: molecular weight; pI: isoelectric point; NT: non-treated; SE: standard error; p: significance. abc: different superscripts within a row indicates differences between treatments.

Table 4. Identification of proteins contained in the myofibrillar fraction which were significantly altered due to HPP.

Spot	Protein name	Accession number ^x	Protein probability	Cov. ^y	M _W theoretical (KDa)	pI _{theoretical}	matching peptides ^z	Normalised spot volume (x10 ³)					p
								NT	200 MPa	400 MPa	600 MPa	SE	
Myofibrillar proteins													
151	Actin, aortic smooth muscle	P62739	2.40E-09	16.7	41.98	5.1	5	176 ^a	105 ^b	74 ^b	74 ^b	11	<0.001
253	F-actin-capping protein subunit alpha-2	Q5E997	1.53E-12	46.9	32.96	5.5	8	710 ^a	644 ^{ab}	569 ^{bc}	525 ^c	19	<0.001
223	Tropomyosin alpha-1	Q5KR49	3.90E-11	49.3	32.67	4.5	18	25472 ^a	23523 ^a	16376 ^b	15452 ^b	1420	<0.01
209	Tropomyosin beta chain	Q5KR48	1.96E-08	39.4	32.82	4.5	13	13735 ^a	9257 ^b	9241 ^b	7541 ^b	493	<0.001
150	Tubulin beta-2B chain	Q6B856	6.28E-09	30.1	49.92	4.6	10	343 ^a	249 ^{ab}	143 ^b	162 ^b	29	<0.01
188	Tubulin beta-2B chain	Q6B856	6.11E-10	29.7	49.92	4.6	9	493 ^a	411 ^{ab}	248 ^c	307 ^{bc}	24	<0.001
Sarcoplasmic proteins													
96	Aldehyde dehydrogenase, mitochondrial	P20000	2.22E-15	33.1	56.62	7.5	13	132 ^c	145 ^c	317 ^b	455 ^a	16	<0.001
178	Aldehyde dehydrogenase, mitochondrial	P20000	1.93E-13	35.6	56.62	7.5	14	54 ^c	69 ^{bc}	98 ^{ab}	117 ^a	6	<0.001
75	Alpha-enolase	Q9XSJ4	1.09E-11	30.40	47.30	6.4	9	48 ^b	33 ^b	58 ^b	127 ^a	6	<0.001
137	Alpha-enolase	Q9XSJ4	1.19E-11	23.7	47.30	6.4	7	336 ^a	152 ^b	393 ^a	358 ^a	18	<0.001
49	Beta-enolase	Q3ZC09	1.05E-09	18.4	47.07	7.6	7	436 ^b	597 ^b	2035 ^a	1633 ^a	175	<0.001
70	Beta-enolase	Q3ZC09	2.05E-11	16.1	47.07	7.6	6	400 ^b	315 ^b	1264 ^a	1169 ^a	105	<0.001
73	Beta-enolase	Q3ZC09	2.30E-09	12.7	47.07	7.6	5	86 ^b	118 ^b	329 ^a	305 ^a	30	<0.001
90	Beta-enolase	Q3ZC09	1.52E-09	18.43	47.07	7.6	7	69 ^b	65 ^b	230 ^a	223 ^a	18	<0.001
54	Creatine kinase M-type	Q9XSC6	8.44E-14	31.2	42.96	6.7	10	620 ^b	1239 ^b	2798 ^a	2160 ^a	182	<0.001
83	Creatine kinase M-type	Q9XSC6	2.78E-13	31.8	42.96	6.7	11	623 ^b	832 ^b	2251 ^a	2261 ^a	154	<0.001
117	Creatine kinase M-type	Q9XSC6	4.05E-09	25.5	42.96	6.7	7	1829 ^b	2379 ^b	5384 ^a	5357 ^a	248	<0.001
121	Creatine kinase M-type	Q9XSC6	8.23E-10	23.4	42.96	6.7	6	322 ^b	385 ^b	851 ^a	912 ^a	35	<0.001
125	Creatine kinase M-type	Q9XSC6	3.46E-09	30.4	42.96	6.7	9	48 ^b	57 ^b	127 ^a	132 ^a	13	<0.001
134	Flavin reductase	P52556	2.62E-12	36.9	22.12	6.7	5	99 ^b	228 ^a	259 ^a	259 ^a	18	<0.001
144	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	Q5EA88	6.44E-14	47.3	37.62	6.4	17	855 ^b	1867 ^a	2164 ^a	1914 ^a	185	<0.01
145	Heat shock 70 kDa protein 1A	Q27975	4.73E-13	32.1	70.22	5.6	18	397 ^c	693 ^b	727 ^b	997 ^a	49	<0.001
138	Heat shock protein beta-1	Q3T149	1.37E-09	31.7	22.38	6.0	6	148 ^a	114 ^{ab}	58 ^b	57 ^b	15	<0.01
1	Malate dehydrogenase, cytoplasmic	Q3T145	1.58E-12	22.5	36.42	6.2	5	31 ^b	24 ^b	65 ^b	343 ^a	24	<0.001
11	Malate dehydrogenase, cytoplasmic	Q3T145	5.11E-14	28.7	36.42	6.2	7	206 ^b	207 ^b	303 ^b	1520 ^a	67	<0.001

14	Malate dehydrogenase, cytoplasmic	Q3T145	6.14E-07	17.7	36.42	6.2	5	79 ^b	75 ^b	148 ^b	509 ^a	18	<0.001
36	Peptide methionine sulfoxide reductase	P54149	3.97E-13	48.5	25.80	7.4	8	142 ^b	134 ^b	300 ^b	683 ^a	66	<0.01
9	Peroxiredoxin-6	O77834	9.76E-09	29.0	25.05	6.0	7	118 ^b	168 ^b	925 ^a	892 ^a	76	<0.001
28	Phosphoglucomutase-1	Q08DP0	3.08E-10	14.6	61.55	6.4	6	45 ^b	84 ^b	206 ^a	223 ^a	19	<0.001
41	Phosphoglucomutase-1	Q08DP0	8.33E-13	33.1	61.55	6.4	15	208 ^b	256 ^b	830 ^a	913 ^a	47	<0.001
57	Phosphoglucomutase-1	Q08DP0	1.61E-10	27.9	61.55	6.4	11	181 ^b	186 ^b	690 ^a	752 ^a	52	<0.001
62	Phosphoglucomutase-1	Q08DP0	1.88E-08	18.3	61.55	6.4	8	113 ^b	106 ^b	364 ^a	378 ^a	29	<0.001
65	Phosphoglucomutase-1	Q08DP0	5.04E-10	25.3	61.55	6.4	10	134 ^b	122 ^b	639 ^a	669 ^a	34	<0.001
76	Phosphoglucomutase-1	Q08DP0	2.31E-12	34.9	61.55	6.4	16	374 ^b	382 ^b	1370 ^a	1622 ^a	110	<0.001
88	Phosphoglucomutase-1	Q08DP0	1.71E-11	34.9	61.55	6.4	15	454 ^b	557 ^b	1694 ^a	1595 ^a	124	<0.001
64	Protein DJ-1	Q5E946	3.64E-10	50.8	20.02	7.2	7	134 ^b	122 ^b	438 ^a	506 ^a	32	<0.001
202	Rab GDP dissociation inhibitor beta	P50397	6.66E-15	40.9	50.46	5.9	15	108 ^b	94 ^b	128 ^{ab}	174 ^a	13	<0.01
92	Retinal dehydrogenase 1	P48644	8.02E-13	45.7	54.77	6.2	19	259 ^{bc}	233 ^c	481 ^b	823 ^a	53	<0.001
51	Serotransferrin	Q29443	5.83E-11	28	77.70	6.7	15	55 ^b	47 ^b	53 ^b	217 ^a	22	<0.01
60	Serotransferrin	Q29443	2.08E-10	33.7	77.70	6.7	18	92 ^b	64 ^b	74 ^b	280 ^a	20	<0.001
68	Serotransferrin	Q29443	3.46E-11	26	77.70	6.7	14	18 ^b	17 ^b	18 ^b	67 ^a	8	<0.01
71	Serotransferrin	Q29443	4.26E-13	36.6	77.70	6.7	21	87 ^b	79 ^b	83 ^b	315 ^a	22	<0.001
101	Serotransferrin	Q29443	1.69E-12	35.5	77.70	6.7	21	104 ^b	98 ^b	95 ^b	316 ^a	30	<0.01
18	Serum albumin	P02769	7.98E-12	50.7	69.25	5.8	25	863 ^c	1017 ^{bc}	1299 ^b	1971 ^a	67	<0.001
23	Serum albumin	P02769	9.73E-12	58	69.25	5.8	32	374 ^b	445 ^b	941 ^b	2471 ^a	186	<0.001
25	Serum albumin	P02769	4.00E-14	59.5	69.25	5.8	32	345 ^b	290 ^b	527 ^b	1712 ^a	202	<0.001
136	Serum albumin	P02769	7.55E-14	55.7	69.25	5.8	29	245 ^b	370 ^b	644 ^b	1418 ^a	113	<0.001
162	Serum albumin	P02769	8.46E-10	30.3	69.25	5.8	15	91 ^c	136 ^b	162 ^b	238 ^a	9	<0.001
225	Stress-70 protein, mitochondrial	Q3ZCH0	1.71E-12	35.6	73.70	5.9	19	278 ^b	254 ^b	306 ^b	413 ^a	21	<0.01
8	Triosephosphate isomerase	Q5E956	1.00E-30	67.9	26.67	6.5	13	601 ^b	567 ^b	4273 ^b	4547 ^a	103	<0.001
27	Triosephosphate isomerase	Q5E956	2.32E-12	57.4	26.67	6.5	11	72 ^b	53 ^b	293 ^a	217 ^a	24	<0.001
30	Triosephosphate isomerase	Q5E956	6.44E-14	52.6	26.67	6.5	10	116 ^b	129 ^b	576 ^a	631 ^a	19	<0.001
33	Triosephosphate isomerase	Q5E956	5.46E-13	57.4	26.67	6.5	11	135 ^b	206 ^b	658 ^a	708 ^a	34	<0.001
59	Triosephosphate isomerase	Q5E956	2.44E-14	50.6	26.67	6.5	11	260 ^b	262 ^b	1010 ^a	1143 ^a	57	<0.001

^x According to UniProt/Swiss-Prot database. All proteins corresponded to proteins sequenced in *Bos taurus*. ^y Percentage of coverage of the entire amino acid sequence. ^z Number of matching peptides. M_w: molecular weight; pI: isoelectric point; NT: non-treated; SE: standard error; p: significance. abc: different superscripts within a row indicates differences between treatments.

Table 5. Significant correlation coefficients between quality parameters and spot volume of the proteins of the sarcoplasmic fraction affected by high pressure processing.

Spot	Protein name	Protein solubility		L*	b*	AE	EM
		total	sarcoplasmic				
Sarcoplasmic proteins							
15	Beta-enolase	0.893	0.887	-0.911	-0.919	-0.991	-0.833
18	Beta-enolase	0.868	0.863	-0.901	-0.903	-0.990	-0.809
83	Creatine kinase M-type	0.850	0.842	-0.921	-0.864	-0.979	-0.843
93	Creatine kinase M-type	0.863	0.857	-0.906	-0.898	-0.984	-0.831
112	Creatine kinase M-type			-0.842	-0.759	-0.905	-0.823
353	Heat shock 70 kDa protein 1A						-0.786
360	Heat shock 70 kDa protein 1A						-0.752
74	Peroxiredoxin-6	0.812	0.806	-0.906	-0.863	-0.979	-0.842
2	Phosphoglucomutase-1	0.880	0.869	-0.919	-0.838	-0.99	-0.853
17	Phosphoglucomutase-1	0.846	0.835	-0.917	-0.827	-0.972	-0.865
32	Phosphoglucomutase-1	0.852	0.838	-0.883	-0.788	-0.989	-0.826
40	Phosphoglucomutase-1	0.740		-0.874		-0.946	-0.798
119	Phosphoglucomutase-1	0.765	0.754	-0.875	-0.811	-0.927	-0.806
151	Phospholysine phosphohistidine inorg. pyrophosphate phosphatase	0.834	0.840	-0.863	-0.787	-0.828	-0.775
394	Serum albumin	0.778	0.768				-0.781
Myofibrillar proteins							
23	Myosin light chain 1, skeletal muscle isoform	-0.944	-0.941	0.886	0.831	0.862	0.899
61	Myosin light chain 1, skeletal muscle isoform	-0.934	-0.935	0.950	0.826	0.935	0.844
91	Myosin light chain 1, skeletal muscle isoform	-0.854	-0.856	0.795			
104	Myosin light chain 1, skeletal muscle isoform			0.854	0.760	0.844	
11	Tropomyosin alpha-1 chain	-0.921	-0.933	0.914	0.826	0.918	0.817
9	Tropomyosin beta chain	-0.917	-0.921	0.931	0.816	0.911	0.804
5	Troponin T, fast skeletal muscle	-0.788	-0.785				
41	Troponin T, fast skeletal muscle	-0.820	-0.811	0.748			0.791
174	Troponin T, fast skeletal muscle	-0.785	-0.784				
85	Troponin T, slow skeletal muscle	-0.814	-0.805	0.767			0.787
162	Troponin T, slow skeletal muscle	-0.818	-0.807	0.820			0.827

Only quality variables and protein peaks showing significant correlations are shown ($p < 0.006$, considering multiple testing correction as suggested by Cheverud 2001). L*: lightness; a*: redness; b*: yellowness; ΔE : total colour difference; EM: expressible moisture.

Table 6. Significant correlation coefficients between proteins contained in the myofibrillar fraction presenting differences among treatments and quality parameters.

Spot	Protein name	Protein solubility (µg/g)		L*	b*	AE	EM
		total	sarcoplasmic				
Myofibrillar proteins							
151	Actin, aortic smooth muscle	0.751	0.760	-0.827	-0.821		
253	F-actin-capping protein subunit alpha-2	0.848	0.854	-0.895	-0.816		-0.892
223	Tropomyosin alpha-1	0.845	0.834	-0.907	-0.756	-0.845	-0.793
209	Tropomyosin beta chain	0.747	0.769				
150	Tubulin beta-2B chain	0.848	0.856	-0.831	-0.892		
188	Tubulin beta-2B chain	0.806	0.799	-0.919	-0.861	-0.870	-0.767
Sarcoplasmic proteins							
96	Aldehyde dehydrogenase, mitochondrial	-0.959	-0.960	0.872	0.799		0.879
178	Aldehyde dehydrogenase, mitochondrial	-0.925	-0.930	0.872	0.809		0.802
75	Alpha-enolase	-0.827	-0.823				0.786
49	Beta-enolase	-0.794	-0.789	0.93	0.818	0.908	0.837
70	Beta-enolase	-0.846	-0.843	0.913	0.824	0.934	0.883
73	Beta-enolase	-0.878	-0.871	0.913	0.914	0.875	0.813
90	Beta-enolase	-0.910	-0.902	0.912	0.800	0.918	0.778
54	Creatine kinase M-type	0.785	-0.780	0.937	0.82	0.901	
83	Creatine kinase M-type	-0.911	-0.905	0.952	0.826	0.923	0.883
117	Creatine kinase M-type	-0.911	-0.905	0.972	0.850	0.950	0.887
121	Creatine kinase M-type	-0.946	-0.944	0.962	0.871	0.946	0.906
125	Creatine kinase M-type	-0.872	-0.867	0.886	0.882	0.852	0.754
145	Heat shock 70 kDa protein 1A	-0.803	-0.819	0.768			
1	Malate dehydrogenase, cytoplasmic	-0.799	-0.795				
11	Malate dehydrogenase, cytoplasmic	-0.787	-0.785		0.438		
14	Malate dehydrogenase, cytoplasmic	-0.834	-0.834		0.507		
36	Peptide methionine sulfoxide reductase	-0.838	-0.841		0.515		
9	Peroxiredoxin-6	-0.898	-0.893	0.953	0.822		0.86
28	Phosphoglucomutase-1	-0.931	-0.932	0.949	0.875	0.956	0.831
41	Phosphoglucomutase-1	-0.877	-0.880	0.941	0.859	0.889	0.764
57	Phosphoglucomutase-1	-0.961	-0.959	0.948	0.868	0.929	0.862
62	Phosphoglucomutase-1	-0.942	-0.942	0.913	0.844	0.893	0.831
65	Phosphoglucomutase-1	-0.939	-0.939	0.923	0.870	0.909	0.824

76	Phosphoglucomutase-1	-0.889	-0.890	0.949	0.826	0.930	0.780
88	Phosphoglucomutase-1	-0.917	-0.906	0.926	0.799	0.921	0.851
64	Protein DJ-1	-0.942	-0.935	0.920	0.878	0.923	0.881
202	Rab GDP dissociation inhibitor beta	-0.792	-0.784				
92	Retinal dehydrogenase 1	-0.901	-0.894	0.750			0.838
18	Serum albumin	-0.855	-0.854				0.750
23	Serum albumin	-0.753	-0.755				
25	Serum albumin	-0.851	-0.849				0.776
136	Serum albumin	-0.904	-0.913	0.786			
162	Serum albumin	-0.921	-0.923	0.761			0.822
225	Stress-70 protein, mitochondrial	-0.772	-0.765				
8	Triosephosphate isomerase	-0.957	-0.952	0.964	0.897	0.976	0.870
27	Triosephosphate isomerase	-0.792	-0.782	0.891	0.870	0.916	
30	Triosephosphate isomerase	-0.950	-0.947	0.967	0.891	0.972	0.885
33	Triosephosphate isomerase	-0.952	-0.950	0.962	0.8903	0.946	0.881
59	Triosephosphate isomerase	-0.949	-0.947	0.942	0.887	0.941	0.863

Only quality variables and protein peaks showing significant correlations are shown ($p < 0.005$, considering multiple testing correction as suggested by Cheverud 2001). L*: lightness; a*: redness; b*: yellowness; ΔE : total colour difference; EM: expressible moisture.