High pressure induced changes on sarcoplasmic protein fraction and quality indicators

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Abstract

The combined effect of pressure and mild temperature treatments on bovine sarcoplasmic proteins and quality parameters was assessed. M. *longissimus dorsi* samples were pressurised in a range of 200-600 MPa and 10-30°C. High Pressure Processing (HPP) induced a reduction of protein solubility ($p<0.001$) compared to non-treated controls (NT), more pronounced above 200 MPa. HPP at pressures higher than 200 MPa induced a strong modification ($p<0.001$) of meat colour and a reduction of water holding capacity (WHC). SDS-PAGE analysis demonstrated that HPP significantly modified the composition of the sarcoplasmic protein fraction. The pressurisation temperature mainly affected protein solubility and colour; a smaller effect was observed on protein profiles. Significant correlations ($p<0.001$) between sarcoplasmic protein solubility and both expressible moisture ($r= -0.78$) and colour parameters ($r= -0.81$ to -0.91) suggest that pressure induced denaturation of sarcoplasmic proteins could influence to some extent WHC and colour modifications of beef. Changes in protein band intensities were also significantly correlated with protein solubility, meat lightness and expressible moisture. These results describe the changes induced by HPP on sarcoplasmic proteins and confirm a relationship between modification of the sarcoplasmic protein fraction and alteration of meat quality characteristics.

Introduction

High pressure processing (HPP) is being increasingly used by the meat industry as a post-processing technology to extend the shelf life and to improve the safety of ready-to-eat meat products. Application of HPP to raw meat, has not been considered appropriate as an industrial practice because of colour and texture alterations derived from pressurisation (Carlez, Veciana-Nogues & Cheftel, 1995; Cheftel & Culioli, 1997). However, high pressure processing has been proposed as a possible way of improving the functional properties of muscle proteins (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, depending on the protein system, the applied pressure, the temperature and the duration of the pressure treatment (Cheftel et al., 1997; Gross & Jaenicke, 1994). It is important to further investigate these effects to better understand the relationships between HPP of raw meat and the resultant effects on quality and protein characteristics.

By far the most labile proteins of post-mortem muscle are those of the sarcoplasm (Lawrie, 1998). The sarcoplasmic proteins are the soluble proteins of the sarcoplasm, to which belong most of the enzymes of the glycolytic pathway, creatine kinase and myoglobin. A mixture of several hundred globular proteins of relatively low molecular weight is known to be present in the sarcoplasmic fraction (Bendixen, 2005; Tornberg, 2005). Denaturation of sarcoplasmic proteins has proved to have an impact on meat quality parameters such as colour and water holding capacity (Bendall & Wismer-Pedersen, 1962; Lawrie, 1998; Sayd et al., 2006). Moreover, sarcoplasmic proteins have a role in the quality of processed meats, as they participate in the consistency of cooked meat (Farouk, Wieliczko, Lim, Turnwald & MacDonald, 2002; Tornberg, 2005).

In spite of accounting for about 30% of total muscle protein, the role of sarcoplasmic proteins on the functional properties of meat has received less attention compared to myofibrillar proteins (Miyaguchi, Nagayama & Tsutsumi, 2000). We hypothesise that HPP might induce changes on the sarcoplasmic protein profile and this could have an impact on meat quality. Thus, this work is focused on the monitoring of the effects of high pressure processing on sarcoplasmic fraction of bovine *longissimus* muscle and its relationship with pressure induced changes in meat quality. Colour measurement, water holding capacity and protein solubility are parameters that can be used as simple indicators for monitoring meat quality. To our knowledge, no similar studies comparing pressure effects at different mild temperatures have been reported. Therefore, this study was designed to evaluate the impact of temperature on sarcoplasmic proteins and quality parameters when high pressure processing is performed at mild temperatures.

Materials and Methods

Sample preparation and High Pressure Processing (HPP)

Beef M. *longissimus dorsi* 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 300g portion of meat pieces was assigned to each treatment. These meat portions were randomly assigned and vacuum packed in polyamide polyethylene bags. Vacuum packed samples were treated in an industrial pressurisation unit Model Wave 6000 (Hyperbaric, Burgos, Spain), with a vessel volume of 120 l. HPP for 20 min with a combination of 3 pressure levels (200, 400 and 600MPa) and 3 temperature levels (10, 20 and 30°C) was performed. A 300g 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). After high pressure treatment, these samples were allowed to cool down and were immediately frozen at -80°C for further analysis. Samples were thawed at 4 °C for 12 h before analysis.

Colour measurement

The internal colour of non-treated and pressurised samples was measured on the freshly cut transversal section of the meat using a HunterLab spectrophotometer (Ultrascan XE, Hunter Associates Laboratory, Inc., Reston, VA), 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 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 weigh.

Extraction of sarcoplasmic proteins

Meat samples were ground in a cryogenic freezer mill (SPEX CertiPrep, Inc., Metuchen, NJ, USA). Sarcoplasmic proteins were extracted from 2 g of pulverized muscle homogenized in 6 ml of extraction buffer (pH 7.6) containing 20 mM TRIS, 2mM EDTA, 4mM $MgCl_2$ 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. Protein concentration was determined using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) based on the Bradford method. Bovine serum albumin was used as the standard. Protein solubility of sarcoplasmic proteins was expressed as µg protein/g meat.

SDS-PAGE electrophoresis

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970). SDS-PAGE was resolved in 12.5% polyacrilamide resolving gel with a 4% stacking gel. Protein samples were denatured by mixing with sample buffer (2% SDS, 10% glycerol, 0.1M Tris-HCL at pH 6.8, 1% β-mercaptoethanol, traces bromophenol blue) and heated at 95ºC for 5 min. Fifteen µg of protein were loaded per lane. High and low molecular weight standards were run on each gel to determine protein band molecular weights. Gels were run in a Mini-PROTEAN Tetra Cell system (Bio-Rad). The gels were run at 100 V for approximately 2h 45 min. Gels were stained for 1h in Bio-safe coomassie stain (Bio-Rad) and destained over night in water. Stained gel images were captured using a G-800 Densitometer (Bio-Rad). The densities of the bands were quantified using Quantity One software (Bio-Rad). To account for slight variation in protein loading, the density of protein bands was expressed as relative intensity. The sum of all bands in a profile

was considered as the total and the relative intensity of each band to the total was calculated as a percentage (Ryu, Choi & Kim, 2005).

Statistical analysis

Data were analysed using the General Linear Model from SAS (version 9.1, SAS Institute, Cary, NC, USA). Temperature, pressure, temperature × pressure interaction, and treatment (NT and all HP treatments) were included in the model as fixed effects with animal as random effect. Differences among fixed effects in the banding pattern were assessed independently for each band. Band size was included in the model as a weight variable. Only bands consistent across all replicates were included in the analysis. Non significant interactions ($p>0.05$) were dropped from the model. Differences were assessed by the Tukey test ($p<0.05$). Pearson correlation coefficients were evaluated to characterize the relationship among quality indicators and band intensities.

Results and Discussion

Sarcoplasmic protein solubility

The effect of high pressure on the solubility of sarcoplasmic proteins was found to be dependent on the temperature of treatment, as indicated by a significant interaction between both effects (Table 1). Figure 1 shows the effect of combined pressure and temperature treatments on sarcoplasmic protein solubility of bovine M. *longissimus dorsi*. High pressure processing (HPP) induced a reduction of protein solubility ($p<0.001$) compared to NT (non-treated) samples. Pressurisation at 200 MPa resulted in protein concentrations of 83, 92, and 78% of the original value, for treatments performed at 10, 20 and 30°C, respectively, showing a small loss of sarcoplasmic proteins. A more pronounced decrease of protein concentration was registered when processing at higher pressure levels (Figure 1). Protein concentrations of

about 44-55% and 25-47% of the original values were observed after processing at 400 and 600 MPa, respectively. Samples pressurised at 600 MPa showed lower solubility than those treated at 400 MPa for treatments applied at 20°C and 30°C (Figure 1). Goutefongea, Rampon, Nicolas & Dumont (1995) also reported decreased sarcoplasmic protein extractability in minced beef and pork treated at 600 MPa (30 min at 20°C), they observed solubility losses of sarcoplasmic proteins of about 10 and 15%, respectively, expressed as % of total proteins.

Solubility of proteins is of primary importance in meat processing as it is closely related to many other functional properties (Zayas, 1997). Moreover, sarcoplasmic protein solubility has proved to be a good indicator for muscle quality (Joo, Kauffman, Kim & Park, 1999; Lopez-Bote, Warriss & Brown, 1989; Sayre & Briskey, 1963). Changes in muscle protein solubility represent a measure of protein denaturation, as the solubility is decreased 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 denaturation of sarcoplasmic proteins induced by high pressure processing, which was more pronounced processing above 200 MPa. Pressure induced denaturation would lead to the formation of aggregates, most probably generated through intermolecular disulfide bridges (Galazka, Dickinson & Ledward, 2000).

No effect of the temperature of pressurisation ($p>0.05$) on protein solubility was detected at 200 and 400 MPa (Figure 1). At 600 MPa, though, lower protein solubility was recorded, pressurising at 30°C compared to 10°C. Sarcoplasmic proteins have been reported to coagulate when bovine muscle reaches 40-60°C (Miyaguchi et al., 2000). As a consequence of the adiabatic heating inherent to HPP, which could be of about 3°C/100 MPa depending on food composition, pressurisation at higher pressures would result in not only pressure induced

but also temperature induced protein denaturation. These results reflect the importance of temperature control during HPP even when pressurising at mild temperature.

Expressible moisture (EM)

The water holding capacity (WHC) of beef muscle was calculated by means of the expressible moisture (EM). HPP at 200MPa did not alter the EM value, thus having no impact on WHC. However, at both 400MPa and 600MPa the EM was increased indicating a reduction in WHC at these pressure levels (Table 2). No effect of the pressurisation temperature ($p>0.05$) was observed on the studied samples. Other authors have reported a decrease on WHC of meat after HPP. A similar reduction (8-12%) in WHC of bovine *semitendinosus muscle* treated at 400-500 MPa was observed by Kim, Lee, Lee, Kim & Yamamoto (2007), although they also reported decreased WHC at 200 MPa. Fernández, Sanz, Molina-García, Otero, Guignon & Vaudagna (2007) also reported increase of EM in beef treated at 650 MPa.

Myofibrillar proteins, myosin and actin, and to some extent tropomyosin are the main water-binding components in muscular tissue (Zayas, 1997). However, several authors have reported that sarcoplasmic proteins play an important role in determining WHC of meat (Joo et al., 1999; Monin & Laborde, 1985). Moreover, precipitation of sarcoplasmic proteins on the myofibrils has been suggested as the possible cause of WHC loss in meats with altered water retention properties (Lopez-Bote & Warriss, 1988; Monin et al., 1985). In our experiment, a significant ($p<0.001$) negative correlation between sarcoplasmic protein solubility and expressible moisture was observed (Table 4). This information would suggest that pressure induced denaturation of sarcoplasmic proteins could influence to some extent the loss of WHC in pressurised meats.

Colour measurements

Instrumental analysis of colour parameters showed no interaction ($p>0.05$) between pressure and temperature (Table 1). Those results indicate that pressure and temperature had an independent effect on the colour coordinates. Pressurised meat experienced a significant increase of L* values compared to non-treated meat (Table 2). Samples pressurised at 400 MPa showed great effect i.e. highest L* (Table 3). Increased lightness of meat is a well documented result of application of HPP on red muscles (Carlez et al., 1995; Goutefongea et al., 1995; Shigehisa, Ohmori, Saito, Taji & Hayashi, 1991). This whitening effect had been related either to protein coagulation, which would affect sample structure and surface properties (Goutefongea et al., 1995), or to globin denaturation and heme group displacement or release (Carlez et al., 1995). No significant differences of a* values among individual treatments and NT meat were found (data not shown). Comparing the pressure levels, meat treated at 600 MPa showed lower a* values than meat treated at 400MPa (Table 3). Other authors have observed a reduction of a* values at pressures above 350-400 MPa (Carlez et al., 1995; Jung et al., 2003). The reduction of a* values at higher pressures has been related to the oxidation of ferrous myoglobin to ferric metmyoglobin and it would result in the brown coloration of meat observed at those pressures (Carlez et al., 1995). This postulation would also be consistent with the increase of yellowness (b*) at 400 and 600 MPa (Table 3). Pressurisation at 200 MPa caused no changes ($p > 0.05$) in b* values compared to non-treated meat (Table 2). The temperature of pressurisation had no significant effect ($p>0.05$) on a* and b* values of meat, while higher L* values were observed in samples pressurised at 30°C than at 10°C (Table 3).

The total colour difference (ΔE) was determined as an estimate of colour changes. A significant effect of pressure and temperature on ΔE was recorded (Table 3). Pressurisation at 400 and 600 MPa, and pressurisation at 30°C, were the treatments which induced more pronounced colour changes of beef. Jung et al. (2003) suggested that a change of 10 units was

considered to modify significantly the appearance of meat colour. According to this consideration, it could be extracted that pressure treatments at 200 MPa, which caused ΔE close to 10, would slightly modify meat appearance, while more sever treatments would strongly modify meat appearance. Although sensory panels were not used visual inspection of the meat by operator corroborates this suggestion.

Among colour parameters, a strong correlation between both L* and b* with ΔE was observed (Table 4), indicating that variations in lightness and yellowness account for most of the total colour changes observed in pressurised meat. Correlations of colour coordinates with other quality parameters showed that pressure induced changes in protein solubility and EM were largely associated with changes in L* and b* (Table 4). The relationship between increase in meat lightness and precipitation of sarcoplasmic proteins has been suggested by several authors (Joo et al., 1999; McLoughlin & Goldspink, 1963; van Laack, Kauffman, Sybesma, Smulders, Eikelenboom & Pinheiro, 1994). Moreover, Goutefongea et al. (1995) reported inverse relationship between variations in L* values and solubility of sarcoplasmic proteins after processing beef at 600 MPa.

SDS-PAGE electrophoresis

Figure 2 is a representative SDS-PAGE gel showing the effects of combined pressure and temperature treatments on bovine sarcoplasmic protein profiles. In order to assess in detail the observed differences, variations in gel patterns of sarcoplasmic protein fractions were quantified by statistically comparing relative band intensities among treatments. A total of 45 bands were detected in a range of 14.8-120.6 KDa, from those, 22 major bands present in the electrophoretic profiles showed significant differences among treatments. The protein patterns showed that sarcoplasmic proteins were modified according to both the pressure level and the temperature of treatment. Overall, the pressure level applied had a greater effect on sarcoplasmic protein profile than the temperature.

The most pressure labile bands were bands 2 and 18 (88.7±1 and 28.9±1 KDa, respectively), which were found to have higher intensity in control samples than in any pressurised samples. The gel patterns of sarcoplasmic protein fractions varied among different treatments. HPP at higher pressure levels exhibited significantly lower intensity ($p<0.001$) in bands 5 (62.4±1.5 KDa), 6 (59.5±0.9 KDa), 8 (47.5±1.5 KDa) and 10 (41.7±0.5 KDa) than NT and 200 MPa samples. Similarly, Kim et al.(2007) reported decreased amounts of 60 and 46 KDa sarcoplasmic proteins in beef pressurised above 400 MPa, however, no band quantification was reported. Band 19 (25.9±0.9 KDa) showed lower band intensity ($p<0.001$) in samples pressurised at 600 MPa than at 200 MPa. Cheah & Ledward (1996) previously observed 300-400 MPa to induce marked changes in soluble protein patterns of minced pork. Decreased band intensities could be related to protein degradation or insolubilization of sarcoplasmic proteins due to protein denaturation. Ohshima, Ushio & Koizumi (1993) suggested that rather than being degraded by high pressure, certain fish sarcoplasmic proteins become covalently linked together and are thus resistant to extraction with SDS.

On the contrary, other protein bands were increased with increasing pressure levels. Bands 14 (34.4 ±0.3 KDa), 16 (31.6±0.25 KDa) and 21 (22.7±0.2 KDa) were not detected clearly in NT and 200 MPa samples, while their relative intensity increased with increasing pressure levels ($p<0.01$). Figure 2 shows clearly that protein band 12 (38.45 ±1.2 KDa) was more abundant ($p<0.01$) in samples pressurised at 400MPa than in any other treatment. Increased band intensities could be either due to solubilisation of myofibrillar proteins or accumulation of degradation products. Increased solubility of certain myofibrillar proteins due to HPP has been reported as a consequence of protein depolymerisation and subsequent increased interactions between protein constituents and water (Cheftel et al., 1997; Okamoto & Suzuki, 2002).

The majority of the protein bands were not affected by the temperature at which HPP was applied. Only the band corresponding to 41.7±0.5 KDa, showed reduced intensity when pressure treatment was applied at 30°C, compared to lower temperatures. No other bands showed changes in band intensity ($p>0.05$) due to the temperature of treatment.

In order to relate changes induced by HPP in sarcoplasmic protein profile with changes on quality indicators, correlation analysis was performed. Thirteen protein bands showed significant correlations with sarcoplasmic protein solubility. From those, bands 1, 5, 6, 8 and 24 showed strong positive correlations ($r= 0.61$ to 0.80), while bands 14, 16 and 17 showed strong negative correlations ($r= -0.60$ to -0.79). Correlation of band intensities with meat lightness revealed inversed correlations than those observed with protein solubility ($p<0.001$). That is bands 5, 6 & 8 were negatively correlated with lightness ($r= -0.66$ to -0.83), while bands 14, 16 were positively correlated ($r= 0.69$ and 0.63, respectively). These inverse relationships are to be expected as these quality parameters were inversely related. The strongest correlations ($p<0.001$) of band intensities with EM were for bands 5 ($r= -0.62$), 8 ($r= -0.73$) and 14 ($r= 0.68$). The correlations confirm a relationship between changes on sarcoplasmic protein profile and changes in meat quality characteristics.

Conclusions

Data from this study indicate that HPP at pressure levels above 200 MPa strongly modified the sarcoplasmic protein fraction and the quality parameters of bovine M. *longissimus dorsi*. The pressurisation temperature mainly affected protein solubility and colour, and to a less extend protein profiles, indicating the importance of the temperature control during HPP, even at mild pressurisation temperatures (10-30°C). The reported correlations suggest colour and protein solubility may be a simple way to monitor changes brought about in sarcoplasmic proteins as a result of meat processing such as high pressure processing. Identification of

affected proteins will be further analysed with 2D-electrophoresis and mass spectrometry to fully understand changes undergone by meat after HPP.

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Table 1 – Table of significances.

	P	T	P×T	treatment
solubility	<0.001	<0.001	<0.05	<0.001
L*	<0.001	<0.01	NS	<0.001
a*	<0.01	NS	NS	NS
b*	<0.001	NS	NS	<0.001
Δ*E*	<0.001	<0.001	NS	<0.001
EM	<0.001	NS	NS	<0.001

P: pressure; T: temperature; treatment: pressure/temperature and non-treated control; L*: lightness; a*: redness; b*: yellowness; Δ*E*: total colour difference EM: expressible moisture; NS: p>0.05

385 **Table 2 -** Effect of high pressure processing at mild temperatures on colour parameters and
386 expressible moisture of M. *longissimus dorsi*.

Treatment		L*	b*	Δ*E*	EM
	NT	24.04^{d}	9.50^{c}	-	21.75^{d}
	200 MPa	30.74^{c}	13.18^{bc}	7.76^{b}	23.90^{bcd}
10°C	400 MPa	51.78^{ab}	18.03^{a}	29.18^{a}	30.58^{abc}
	600 MPa	50.33^{b}	16.80^{a}	27.34^{a}	33.35^{a}
	200 MPa	31.30^{c}	10.98^{c}	7.54^{b}	22.24^{cd}
20°C	400 MPa	53.49^{ab}	17.90^{a}	30.8^{a}	29.90^{abc}
	600 MPa	51.00^{b}	16.45^{ab}	27.88^{a}	32.74^{a}
	200 MPa	34.73^{c}	11.01^{c}	10.96^{b}	25.66^{abcd}
30°C	400 MPa	55.34^{a}	18.10^{a}	31.62^{a}	31.22^{ab}
	600 MPa	52.55^{ab}	16.65^{ab}	29.41^{a}	30.89^{ab}
	p	<0.001	<0.001	<0.001	<0.001
	SE	0.84	0.87	0.93	1.76

387 Results are means of three replicates. Different letters within a column indicate significant
388 differences among values. L*: lightness; b*: yellowness; Δ*E*: total colour difference; EM:
389 expressible moisture; SE: standard error. NT: non-treated. p and SE values for treatment effect
390 include NT and all pressure treatments.
391
392

393 **Table 3 -** Pressure and temperature effect on colour parameters and expressible

394 moisture of M. *longissimus dorsi*.

	Pressure effect				
	200 MPa	400 MPa	600 MPa	p	SE
L*	32.26^{c}	53.54^{a}	51.30^{b}	<0.001	0.49
a*	9.57^{ab}	10.75^{a}	8.84^{b}	<0.01	0.35
b*	11.73^{b}	18.01^{a}	16.63^{a}	<0.001	0.39
Δ*E*	8.75^{b}	30.53^{a}	28.21^{a}	<0.001	0.74
EM	23.93^{b}	30.56^{a}	32.33^{a}	<0.001	0.99
	Temperature effect				
	10°C	20°C	30°C	p	SE
L*	44.28^{b}	45.26^{b}	47.13^{a}	<0.01	0.48
Δ*E*	21.42^{b}	22.07^{ab}	24.00^{a}	<0.001	0.54

395 Results are means of nine replicates. Different letters within a row indicate significant differences
396 among values. L*: lightness; a*: redness; b*: yellowness; Δ*E*: total colour difference EM:
397 expressible moisture; SE: standard error.
398

399 **Table 4 -**Correlation coefficients between quality parameters.

	solubility	L*	a*	b*	Δ*E*	EM
solubility	1	**-0.907****	-0.094	**-0.807****	**-0.871****	**-0.782****
L*		1	0.321	**0.899****	**0.998****	**0.802****
a*			1	**0.526***	0.216	0.318
b*				1	**0.922****	**0.714****
Δ*E*					1	**0.751****
EM						1

400 L*: lightness; a*: redness; b*: yellowness; Δ*E*: total colour difference; EM: expressible moisture.
401 Numbers marked in bold show significant correlation; *p<0.05; **p<0.001.
402
403

Figure 1 - Solubility of sarcoplasmic proteins in NT (non-treated) and pressurised bovine M. *longissimus dorsi* samples. Different letters indicate significant differences ($p<0.001$) among treatments.

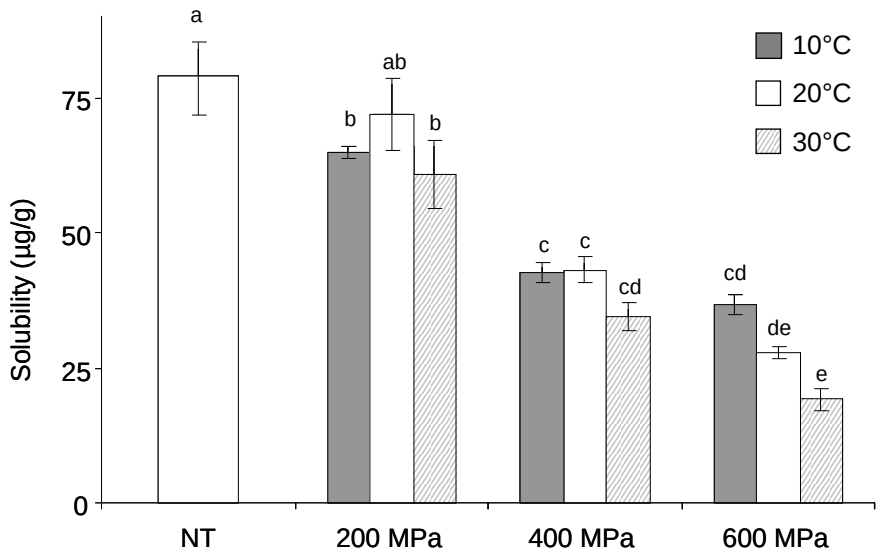

Figure 2 –Synthetic SDS-PAGE gel of sarcoplasmic extracts of non-treated (NT) and pressurised samples (HMW: high molecular weight marker, LMW: low molecular weigh marker). Numbers represent band number. Only bands significantly affected by HPP are marked.