Assessment of high hydrostatic pressure effects on sarcoplasmic beef proteins using the 'lab on a chip' technology

B. Marcos[1*], J. Kerry[2], N. Grèbol[3] & A.M. Mullen[1]
[1]Teagasc, Ashtown Food Research Centre, Dublin 15, Ireland.
[2]Department of Food and Nutritional Sciences, University College, Cork, Ireland.
[3]CENTA, Finca Camps i Armet, 17121 Monellls, Spain.
*Email: begonya.marcos@teagasc.ie

Abstract

High pressure processing (HPP) induces conformational changes in proteins leading to protein denaturation, aggregation or gelation, which have a major impact on meat quality. The aim of this work was to evaluate the effects of HPP on bovine sarcoplasmic proteins. *M. longissimus dorsi* samples were pressurized at 200-600 MPa and 10-30°C. HPP resulted in a reduction of protein solubility. The most important loss of protein solubility was observed at 400 and 600 MPa, suggesting a more pronounced denaturation of sarcoplasmic proteins at higher pressure levels. Protein profiles of sarcoplasmic proteins were obtained using chip based capillary electrophoresis. A band of 88 KDa, only present in non-treated (NT) meat, proved to be the most pressure labile protein band. Another temperature-pressure sensitive band was that of 92 KDa, showing higher concentration in NT meat than in any pressurized sample. At 400 MPa, pressure and temperature proved to have an additive effect on the banding pattern. Finally, pressurization at 600 MPa was the treatment that most markedly affected the protein profile. The 'lab on a chip' technology proved to be effective in identifying differences in sarcoplasmic protein profiles among pressure treatments. Relating these profile changes with quality attributes may aid the optimisation of HPP of muscle foods.

Introduction

High pressure processing (HPP) is a non-thermal preservation technology efficient to inactivate the vegetative microorganisms. Various biochemical studies indicate that pressures above 100-200 MPa, at room temperature, can cause: the dissociation of oligomeric structures into their subunits, partial unfolding and denaturation of monomeric structures, protein aggregation, and protein gelation, whenever pressure and protein concentration are high enough (Cheftel, 1997).

Nowadays, there are some companies all over the world (Japan, USA, Italy, Spain, Germany and Australia) using this technology to process meat products (Aymerich et al., 2008). Thus, rapid methods to monitor the effects of HPP on meat constituents are very valuable for the food industry.

Capillary electrophoresis (CE) principles have been transferred to a chip format that integrates separation, staining, virtual distaining, and detection steps (Vasilyeva et al., 2004). The 'lab on a chip' technology offers advantages of speed and minimal requirements for technical expertise on the part of the operator (Uthayakumaran et al 2005). To the best of our knowledge the application of chip based CE to meat protein analysis is very limited (Corcoran et al 2006). The aim of this study was to evaluate the effect of HPP on beef sarcoplasmic protein profile using the 'lab on a chip' technology.

Materials and Methods

Sample preparation: Three beef *M. Longissimus dorsi* from crossbred heifers slaughtered at 24 months of age were obtained from a local Irish distributor. After slaughter, carcasses were suspended by the hip for 7 days before dissection. After trimming, the muscles were sampled and vacuum packed in polyamide polyethylene bags.

HP treatment: HPP was carried out using a 120l industrial pressurization unit (Hyperbaric, Burgos, Spain). Samples were treated for 20 min with a combination of 3 pressure levels (200, 400 and 600MP) and 3 temperature levels (10, 20 and 30°C). Non-treated (NT) meat was kept as a control. After HPP the samples were let to cool down and were immediately frozen at -80°C for further analysis. Triplicates of each treatment were obtained.

Extraction of sarcoplasmic proteins: Samples were ground in a cryogenic freezer mill (SPEX CertiPrep, Inc., Metuchen, NJ, USA). Two grams of pulverized meat were homogenized in 6ml of 20 mM TRIS, 2mM EDTA, 4mM $MgCl_2$ and 10µl/ml protease inhibitor mix (GE Healthcare) buffer, pH 7.6. Homogenate was centrifuged at 14,000 rpm for 20 min at 4°C. Supernatant containing sarcoplasmic proteins was removed and frozen at -20°C. Protein concentration was determined by Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) based on coomassie blue dye binding method (Bradford, 1976).

Bovine serum albumin was used as the standard. Extractability of sarcoplasmic proteins was expressed as µg /g meat.

Chip-based CE: Sarcoplasmic extracts were analysed on an Agilent 2100 Bioanalyser using a Protein 200 Plus Assay (Agilent Technologies, Waldbronn, Germany). Two replicates of every extract were run on different chips. The Bioanalyser software automatically provides protein profiling, calculating band sizes (KDa) and relative concentration (µg/ml) of each separated peak.

Statistical analysis: Data were analysed using the GLM procedure from of SAS (version 9.1, SAS Institute, Cary, NC, USA). The model included temperature, pressure, temperature×pressure interaction, and treatment as fixed effects. Differences among fixed effects in the banding pattern were assessed independently for each band and weighted by band size. Non significant interactions ($p>0.05$) were dropped from the model. Differences were assessed by the Tukey test ($p < 0.05$).

Results and Discussion

The effect of pressure on the extractability of sarcoplasmic proteins was dependent of the temperature of treatment, as indicated by significant interaction ($p<0.05$) between both effects. Table 1 shows the effect of combination of pressure and temperature treatments on protein extractability. HPP resulted in a reduction of protein solubility. The most important loss of protein solubility was observed at 400 and 600 MPa for all temperatures studied (Table 1), suggesting a more pronounced denaturation of sarcoplasmic proteins at higher pressure levels. The effect of temperature was more evident at 600 MPa, with a reduction of protein solubility with increasing temperature.

Table 1. Sarcoplasmic protein extractability (µg/g meat)

NT	200 MPa			400 MPa			600 MPa			Sign.	SE
	10ºC	20ºC	30ºC	10ºC	20ºC	30ºC	10ºC	20ºC	30ºC		
78.06^{a}	64.94^{b}	71.82^{ab}	60.76^{b}	42.63^{c}	43.06^{c}	34.41^{cd}	36.67^{cd}	27.69^{de}	19.2^{e}	<0.001	2.59

NT: non-treated, SE: standard error. Different letters indicate differences among values

Protein 200 Plus Assay produced a pattern of well-resolved bands in a range of 16.52±0.27 to 214.36±1.63 KDa. The statistical analysis showed no interaction ($p<0.05$) between temperature and pressure effects on the relative concentration of each protein band. These results suggest that pressure effect on sarcoplasmic protein profiling was independent from the temperature of the treatment and vice versa.

The effects of both parameters on the relative concentration of protein bands are shown in Tables 2 and 3. An increase of the relative concentrations of bands 16.52±0.27 and 22.98±0.63 KDa was observed when pressurizing at 400 and 600 MPa, compared with 200 MPa (Table 2). On the contrary, lower concentrations ($p<0.05$) of bands of 32.71±0.93, 35.8±0.78, 44.33±1.10, and 52.66±0.48 KDa were observed when samples were treated at 400 and 600 MPa than at 200 MPa. On the other hand, Table 3 shows the effect of processing temperature on protein bands. These results show that even at mild temperatures, small differences in temperature may have a significant effect on meat proteins under pressure conditions.

Table 2. Pressure effect on sarcoplasmic protein band profiles

Band size (KDa)	Sign.	Pressure (MPa)		
		200	400	600
16.52±0.27	***	$474.91±38.86^{b}$	$705.36±38.74^{a}$	$757.04±38.78^{a}$
22.98±0.63	***	$68.29±28.89^{b}$	$305.30±28.36^{a}$	$347.88±28.37^{a}$
32.71±0.93	***	$273.43±21.71^{a}$	$122.15±21.18^{b}$	$77. 80±22.89^{b}$
35.8±0.78	***	$590.54±58.91^{a}$	$95.03±72.23^{b}$	$122.97±67.49^{b}$
41.59±0.99	***	$592.24±105.11^{b}$	$1581.87±97.27^{a}$	$599.78±99.49^{b}$
44.33±1.10	**	$1473.09±142.92^{a}$	ne	$677.74±188.27^{b}$
52.66±0.48	***	$65.71±5.31^{a}$	$21.04±5.75^{b}$	ne
66.82±1.51	***	$74.72±12.56^{b}$	$158.12±12.73^{a}$	$44.20±12.72^{b}$
76.00±0.55	*	Ne	$11.92±1.12^{a}$	$5.85±1.13^{b}$

Values are means of relative concentration (µg/ml) of the band±standard error. ne: non-estimated. Significance levels: *** $p<0.001$;** $p<0.01$; * $p<0.05$; NS no significant. Different letters within a row indicate differences among values. Note: only bands were significant effects were observed are shown.

Table 3. Temperature effect on sarcoplasmic protein band profiles

Band size (KDa)	Sign.	Temperature (°C)		
		10	20	30
16.52±0.27	***	451.01±38.92^{b}	733.92±38.85^{a}	752.38±38.61^{a}
22.98±0.63	**	177.94±28.57^{b}	335.79±28.44^{a}	207.74±28.61^{b}
27.11±0.52	*	143.82±32.15^{b}	244.18±32.16^{ab}	267.70±24.79^{a}
32.71±0.93	*	105.72±23.14^{b}	205.27±21.35^{a}	161.67±21.28^{ab}
41.59±0.99	*	710.12±98.15^{b}	1122.95±98.32^{a}	940.82±105.38^{ab}

Values are means of relative concentration (μg/ml) of the band±standard error. ne: non-estimated. Significance levels: *** $p<0.001$;** $p<0.01$; * $p<0.05$; NS no significant. Different letters within a row indicate differences among values. Note: only bands were significant effects were observed are shown.

Comparisons of all pressure treatments with control (NT), showed that a band of 88.07±0.72 KDa, only present in NT meat, was the most pressure labile protein band. Another temperature-pressure sensitive band was that of 92.85±0.42 KDa, showing higher concentration in NT meat (166.36±8.06 μg/g) than in any pressurized sample ($p<0.001$). At 400 MPa, pressure and temperature proved to have and additive effect on the banding pattern. Finally, pressurization at 600 MPa was the treatment that affected more markedly the protein profile.

Conclusions

The results obtained with the chip based CE showed a strong influence of pressure and mild temperatures on beef sarcoplasmic protein profiling. Thus, the 'lab on a chip' technology proved to be effective in monitoring the effect of HPP of meat on sarcoplasmic protein profiles. Relating these profile changes with quality attributes may aid the optimisation of HPP of muscle foods. Further analysis is needed to identify the proteins affected by temperature-pressure treatments.

References

Aymerich, T, Picouet, P.A., Monfort, J.M. (2008). Decontamination technologies for meat products. Meat Science, 78, 114-129.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Cheftel, J. C. & Culioli, J. (1997). Effects of high pressure on meat: A review. Meat Science, 46, 211-236.

Corcoran, D., Mee, B., Sullivan, P., O'Doherty, E., O'Reilly, K., Reynolds, A., O'Doherty, J. & Mullen, A. M. (2006). Dietary manipulation of porcine intramuscular fat levels and subsequent proteomic analysis. In 52nd International Congress of Meat Science and Technology, Dublin.

Dooley, J. J., Sage, H. D., Brown, H. M. & Garrett, S. D. (2005). Improved fish species identification by use of lab-on-a-chip technology. Food Control, 16, 601-607.

Uthayakumaran, S., Batey, I. L. & Wrigley, C. W. (2005). On-the-spot identification of grain variety and wheat-quality type by Lab-on-a-chip capillary electrophoresis. Journal of Cereal Science, 41, 371-374.

Vasilyeva et al. (2004). Development of a chip-based capillary gel electrophoresis method for quantification of a half-antibody in immunoglobulin G_4 samples. Electrophoresis, 25, 3890-3896.