Muscle protein electrophoretic pattern affected by heating treatment

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Electrophoretic pattern changes in muscle proteins following heat treatment

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Abstract— **The aim of this study was to characterize the heat treatment-induced changes in electrophoretic patterns of myofibrillar proteins. The study was carried out on *longissimus thoracis* (LT) muscle from two pig lines (Galia and Redone). The following *post mortem* metabolic and meat quality parameters were determined: pH_{24h}, lactate, residual glycogen, glycolytic potential and drip loss. Heat treatment at 100°C was applied for either 10 min or 30 min. SDS-PAGE electrophoresis was performed before and after cooking. The results showed that Galia-breed LT muscles had lower pH_{24h} and higher residual glycogen, glycolytic potential and drip loss than Redone-breed LT muscle. We found different electrophoretic patterns between raw and cooked meat for each breed. Electrophoretic pattern profiles after heating showed a decrease in myosin (up to 50%) and actin (up to 40%) together with new bands of lower molecular weight (mainly at 80 kDa, 60 kDa and 25 kDa). The appearance of these new bands could be explained either by heating-induced myosin and actin band breakdown or by changes in protein solubility and extractability. Research is currently being led to identify these proteins and thus confirm these hypotheses.**

Keywords—**glycogen, heating, electrophoresis**

I. INTRODUCTION

Most populations worldwide generally cook meat before eating it. Heat treatments promote the production of free radicals and consequently can negatively impact on the sensory and nutritional properties of meats and meat products, particularly through oxidation of the protein and lipid components. Furthermore, heat treatments swiftly trigger protein denaturation, which causes conformational changes [1] and an increase in surface hydrophobicity [2]. The formation of carbonyls and disulphide bridges triggers protein-protein interactions [3]; [4]; [5] that change the electrophoresis profile patterns, particularly the intensity of the myosin band [6]. However, previous studies have demonstrated that protein fragmentation occurs during heat treatment on beef [7] and that poultry meat shows practically no changes in electrophoretic patterns, probably due to a change in protein solubility [8]. The aim of this study was to characterize the electrophoretic patterns of heat-treated pig *Longissimus thoracis* muscle presenting variable sugar contents.

II. MATERIALS AND METHODS

The experiment was led on meat samples from 46 sows of the Galia (n=24) and Redone (n=22) breed lines slaughtered at about 110 kg live weight in a commercial slaughterplant. At 24 h *post mortem*, the *Longissimus thoracis* muscle was removed from each carcass, frozen in liquid nitrogen, and stored at -80°C. The following *post mortem* metabolic and meat quality parameters were evaluated: pH_{24h}, lactate, residual glycogen, glycolytic potential, and drip loss. The meat (5 g) was placed in a polypropylene tube and heated at 100°C for 10 and 30 min in a digital temperature-controlled dry bath. Myofibrillar proteins were extracted according to [9]. Protein concentration was determined by the RC-DC Protein Assay (BioRad). Electrophoretic patterns were profiled before and after cooking. Ten µg of myofibrillar proteins were loaded onto 8% gels for sodium dodecyl sulfate—polyacrilamide gel electrophoresis (SDS-PAGE) at 80 V for 20 min and 120 V for 2 hours. After migration, the gels were stained with Coomassie blue (colloidal R 250), colour-resolved, and scanned with a GS-800 Calibrated Densitometer (UMAX). Image analysis and quantification were performed on Quantity One software (BioRad). Band intensity was expressed as optical density (DO/mm^2).

Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure of the SAS system. All values are reported as means ± SEM. Any significant effects found were -t-tested by comparison of means.

III. RESULTS AND DISCUSSION

A. Meat quality traits

Ultimate pH was lower in the LT muscle from Galia breed than in LT muscle from Redone (Table 1). Lactate, residual glycogen and glycolytic potential were higher in Galia muscle. Residual glycogen was three time higher than Redone LT muscle ($p<0.05$). Moreover LT muscle from Galia exhibited 50% more drip loss after 2 days of storage. These results are in agreement with several studies where muscles with a high level of glycogen at slaughter leaded to low ultimate pH and high drip loss [10]; [11].

Table 1: Least squares means ± SEM of pH_{24h}, lactate, residual glycogen, glycolytic potential and drip loss determination from *Longissimus thoracis* muscle of Redone and Galia pigs.

	Galia	Redone	p
	Mean ± sem	Mean ± sem	
pH 24h	5.52 ± 0.02	5.65 ± 0.02	0.0001
Lactate (µmol/g muscle)	104.8 ± 1.58	90.32 ± 2.55	0.0001
Residual glycogen (µmol/g muscle)	9.10 ± 1.12	3.17 ± 0.63	0.0001
Glycolytic potential (µmol eq lactate/g muscle)	123.03 ± 2.78	96.66 ± 3.34	0.0001
Drip loss (%)	3.76 ± 0.55	2.45 ± 0.22	0.0379

B. Electrophoresis pattern

The electrophoretic profile patterns of raw and cooked LT muscle (Redone & Galia lines) are reported in Figure 1. Muscle from both pig lines showed pattern differences between raw muscle and muscle cooked for 10 and 30 minutes. We quantified 22 protein bands found on the electrophoresis gels (B1 to B22) (Table 2). The results show a significant decrease in myosin band intensity (B1) after 10 min of heat treatment at 100°C (33%), reaching up to a 50% decrease after 30 min. Actin band intensity (B13) followed a similar pattern, decreasing by 30 to 40% as heat treatment continued. Bands B2, B3 and B4, with a molecular weight in the range 92 to 150 kDa, also showed a heating-related decrease in band intensity. In total, 6 bands were not visible on the electrophoretic profiles from raw muscle, i.e. bands B6, B9, B11, B12 and B14 with a molecular weight in the range 37 to 75 kDa plus band B20 with a molecular weight in the range 20 to 25 kDa. The appearance of these new bands of a lower molecular weight that myosin (B6 ~ 70 kDa and B9 ~ 60 kDa) could be due to myosin breakdown. [7] used myosin-specific antibodies to show that heating sparked the appearance of 70 kDa and 58 kDa bands corresponding to myosin protein breakdown. The intensity of bands B5, B8 and B18 showed a heat treatment effect and breed x treatment interaction. This interaction is explained by the fact that the intensity of these bands decreases after 10 min of heat treatment but then increases after longer heat treatment. These proteins appear to undergo breakdown at short heating times, and their subsequent increase in band intensity may be evidence of a change in protein solubility overlapping the breakdown of higher-molecular weight proteins. [8] highlighted that myofibrillar proteins lose solubility as heating temperature increases. The breed effect remained moderate under our experimental conditions. Although the differences were significant, little variation was observed. Band B9 gave a lower intensity in Galia-breed muscle, which has a 3-fold higher glycogen content that Redone-breed muscle.

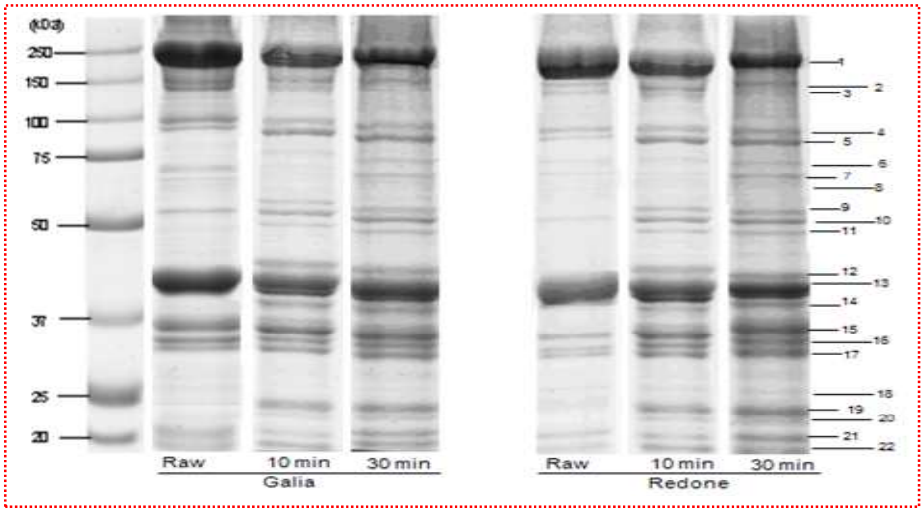

Figure 1: Electrophoresis profile pattern of Galia and Redone pig breed line LT muscles, raw and after heat treatment at 100°C for 10 and 30 minutes.

Table 2: Quantification of gel electrophoresis bands for proteins in Galia and Redone pig breed line LT muscles, raw and after heat treatment at 100°C for 10 and 30 minutes

Bands	Breed		Treatment			Breed	Treatment	Interaction
	Galia	Redone	Raw	Cooked 10 min	Cooked 30 min	p	p	p
B1	20.67^{a} ± 1.22	19.33^{b} ± 1.27	27.64^{a} ± 0.65	18.40^{b} ± 0.44	13.96^{c} ± 0.36	0.023	0.0001	NS
B2	5.91 ± 0.15	5.64 ± 0.12	6.41^{a} ± 0.16	5.69^{b} ± 0.12	5.23^{c} ± 0.08	NS	0.0001	NS
B3	4.67 ± 0.22	4.47 ± 0.21	5.64^{a} ± 0.24	4.41^{b} ± 0.15	3.67^{c} ± 0.09	NS	0.0001	NS
B4	4.06 ± 0.12	3.86 ± 0.12	4.55^{a} ± 0.08	3.69^{b} ± 0.16	3.65^{b} ± 0.06	NS	0.0001	NS
B5	4.68 ± 0.18	4.50 ± 0.12	3.84^{a} ± 0.13	5.31^{b} ± 0.16	4.62^{c} ± 0.05	NS	0.0001	0,004
B6	2.12 ± 0.07	2.13 ± 0.09	ND	1.84^{a} ± 0.05	2.41^{b} ± 0.03	NS	0.0001	NS
B7	2.09^{b} ± 0.10	2.28^{a} ± 0.12	2.56^{a} ±0.08	1.49^{b} ± 0.04	2.52^{a} ± 0.06	0.011	0.0001	NS
B8	1.84^{b} ± 0.07	2.02^{a} ± 0.09	2.13^{a} ± 0.11	1.50^{b} ± 0.04	2.16^{a} ± 0.05	0.035	0.0001	0.027
B9	2.45^{b} ± 0.06	2.75^{a} ± 0.07	ND	2.43^{a} ± 0.06	2.77^{b} ± 0.07	0.0007	0.0002	NS
B10	3.14^{b} ± 0.13	3.48^{a} ± 0.14	2.52^{a} ± 0.10	3.70^{b} ± 0.14	3.71^{b} ± 0.05	0.004	0.0001	NS
B11	2.09^{b} ± 0.06	2.27^{a} ± 0.05	ND	2.11± 0.06	2.26 ± 0.05	0.031	NS	NS
B12	3.63 ± 0.13	3.86 ± 0.12	ND	3.88 ± 0.12	3.61 ± 0.13	NS	NS	NS
B13	13.94^{b} ±0.75	15.01^{a} ± 0.81	19.20^{a} ± 0.54	12.97^{b} ± 0.43	11.25^{c} ± 0.26	0.026	0.0001	NS
B14	4.51 ± 0.16	4.78 ± 0.16	ND	4.17^{a} ± 0.13	5.13^{b} ± 0.08	NS	0.0001	NS
B15	7.43 ± 0.23	7.28 ± 0.27	6.28^{a} ± 0.29	8.34^{b} ± 0.21	7.45^{c} ± 0.15	NS	0.0001	NS
B16	5.99 ± 0.15	5.88 ± 0.18	5.17^{a} ± 0.22	6.38^{b} ± 0.12	6.26^{b} ± 0.10	NS	0.0001	NS
B17	4.88 ± 0.16	5.05 ± 0.12	4.41^{a} ± 0.20	5.11^{b} ± 0.14	5.37^{b} ± 0.09	NS	0.0002	NS
B18	1.41^{b} ± 0.05	1.74^{a} ± 0.08	1.84^{a} ± 0.09	1.34^{b} ± 0.05	1.55^{b} ± 0.06	0.0001	0.0001	0.033
B19	3.52 ± 0.29	3.55 ± 0.25	1.89^{a} ± 0.07	4.28^{b} ± 0.23	4.43^{b} ± 0.14	NS	0.0001	NS
B20	2.03 ± 0.14	1.92 ± 0.07	ND	1.71^{a} ± 0.05	2.24^{b} ± 0.09	NS	0.0007	NS
B21	2.86 ± 0.14	2.84 ± 0.12	3.30^{a} ± 0.09	2.14^{b} ± 0.06	3.11^{a} ± 0.12	NS	0.0001	NS
B22	3.03 ± 0.13	3.21 ± 0.15	3.03 ± 0.15	2.85 ± 0.17	3.47 ± 0.16	NS	NS	NS

^{a,b,c} Means in the same row with different superscript letters are significantly different at P<0.05

IV. CONCLUSIONS

Heated meat samples yield different electrophoretic profile patterns to the raw meat samples. Applying heat treatment has effects on the meat proteins, not just by forming oxidized proteins as we had previously reported [6] but also by triggering protein hydrolysis reactions or changes in protein solubility. However, breed effects remained moderate. This research is set to be extended by using mass spectrometry to identify the neoformed bands.

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