INFLUENCE OF ULTIMATE pH ON BOVINE MEAT TENDERNESS DURING AGEING

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Introduction

The high ultimate pH of meat, as consequence of depleted muscular glycogen reserves prior to slaughter by stress factors has an important reflex in meat quality. Meat with high ultimate pH is dark in colour with a reduced capacity of myoglobin oxygenation, more susceptible to bacterial spoilage and has a reduced flavour. Nevertheless, this meat is also associated with a higher tenderisation rate (Watanabe *et al.*, 1996) or with a better ultimate tenderness possibly associated to a higher water holding capacity of meat (Dransfield, 1992). The mechanism of improvement of meat tenderness during *post-mortem* storage at refrigeration temperatures remains controversial. However, proteolysis of myofibrillar proteins may have an important role. In contrast to lysosomal cathepsins and the multicatalytic proteinase complex, substantial evidence suggests that calpains (mainly μ-calpain) are the primary system responsible for *post mortem* proteolysis (Koohmaraie, 1996). The aim of this work was to assess the influence of ultimate pH during ageing on tenderness of bovine meat, myofibrillar fragmentation and myofibrillar and collagen solubility.

Materials and Methods

In this work, males (n=23) of a Portuguese autochthonous breed (Maronesa) with 8-11 months and carcasses weight of 100-164 Kg were used. Carcasses were chilled 1 h at 0°C, 4 m/s and kept at 1°C until 24 h *post mortem*. At 28 h *post mortem longissimus* was excised (between 8th rib and 2nd lumbar vertebra) and cut in three parts (± 600 g). One part of the muscle was used for meat characterisation at day 1 *post mortem* and the two other were vacuum packed and aged at 2±2°C until 6 and 13 days *post mortem*.

The pH was measured directly in the muscle using a combined glass electrode with a pH-meter Crison 2002. Based on ultimate pH (pHu, measured at 28 h *post mortem*) the muscles were segregated in three quality groups: Normal (pH≤5.8) moderated (mod) DFD (5.8<pH< 6.2) and DFD (pH≥6.2).

Warner-Bratzler shear force (WBSF) was determined using a Warner-Bratzler blade attached to a Stevens QTS 25 apparatus. Samples were heated in plastic bags by immersion in a water bath at 75°C to an internal temperature of 70°C. After cooling under running tap water 10-12 samples (±1 cm^2 cross section and 4-5 length) with fibres perpendicular to the direction of the blade were taken. For sensory evaluation of tenderness ±1.5 cm thick steaks were covered with an aluminium foil and heated in a double side contact grill (230°C) to an internal temperature of 70°C. To a semi-trained panel of 6 members it was asked to rank the meat on a 9-point scale (1 = extremely tough, 9 = extremely tender).

Myofibrillar fragmentation index (MFI) was determined in frozen samples as described by Culler *et al.* (1978). After determination of protein concentration of the suspension by the biuret method (Gornall *et al.*, 1949), the suspension was diluted with 0.02 M potassium phosphate buffer (pH 7.0) to 1.0 mg/ml protein concentration. The exact protein concentration was determined using the micro-biuret method (Itzhaki and Gill, 1964 as cited by Clark, 1984) and the suspension of myofibrils was diluted to 0.5 ± 0.05 mg/ml. MFI is the value of absorbance of myofibrillar suspension, measured at 540 nm multiplied by 200.

Myofibrillar protein solubility (MPS) was determined in a high ionic strength buffer at pH 7.0 (0.4 M NaCl, 1 mM EDTA, 19 mM KH_2PO_4, 31 mM Na_2HPO_4 and 1 mM NaN_3) for the determination of MPS pH7.0 or at pH 5.5 (0.4 M NaCl, 1 mM EDTA, 100 mM citric acid and 1 mM NaN_3) for determination of MPS pH5.5 according to Claeys *et al.* (1994). The protein concentration was determined in the supernatant by the biuret method (Gornall *et al.*, 1949) and results expressed as mg solubilized protein / g muscle.

For soluble collagen determination, 20 ml of 0.1M sodium phosphate buffer pH 7.0 was added to 4.000 g of finely minced meat. Each sample was heated at 70°C in a water bath for 30 min with occasional stirring. Tubes were cooled in an ice bath for 10 min and centrifuged at 10 000 g for 15 min (Culler *et al.*, 1978). The supernatant was used for determination of soluble collagen and the sediment for determination of insoluble collagen, both after hydrolysis with sulphuric acid 7N (AOAC, 1990). The absorbance was measured at 558 nm. The amount of hydroxyproline in the supernatant and in the sediment was converted to soluble and insoluble collagen using a factor 7.25 and 7.52 respectively. The soluble collagen was expressed as percentage of total (soluble + insoluble).

Two-way analysis of variance: pHu and day *post mortem* was performed with Systat 5.0, LSD test was used to locate differences between means.

Results and Discussion

Table 1 shows the means and standard deviations of the parameters studied for the three pH groups. Ageing the meat until 6 days *post mortem* results in a decrease of WBSF in all pH groups. Meat with Normal pH was significantly (P<0.05) tougher than modDFD and DFD groups in all days *post mortem*. Thus, the ultimate tenderness was influenced by pHu.

MFI shows a significantly increase between day 1 and day 6 in all groups. Various works showed that MFI increases with ageing of meat (Olsson *et al.*, 1976; Heinz *et al.*, 1994). This increase of MFI with ageing seems to be related to the phenomenon of myofibrils breaking into shorter segments at or near the Z-disk during *post mortem* storage of meat (Olsson *et al.* 1976). The comparison of different groups for each day *post mortem* shows that in day 1 the DFD group had the highest MFI but at days 6 and 13 the differences were not significant (P>0.05). Watanabe *et al.* (1996), in sheep *longissimus thoracicum et lumborum* showed that MFI, assessed by microscopy, at one day *post mortem* varies only slightly with the ultimate pH, but, with the ageing of meat until the 6th day the values of MFI became different, being the higher increase found in the meat with low pHu (<5.8). The results of present work differ from those of these authors, once in the day 1 *post mortem* it was found significant differences (P<0.05) between the groups of pH. However from day 1 to day 6 it was also observed a higher increase on MFI in the group of lower pH. These results

suggest that in meat with higher pH occurs a higher proteolytic activity responsible for the rupture of myofibrils early in the day 1 *post mortem* while in the meat with a normal or intermediate pH the proteolysis is more gradual.

TABLE 1 – Means and standard deviation for the three quality groups.

Parameters	Day *pm*	Normal (n=7)	DFDmod (n=8)	DFD (n=8)
WBSF (Kg/cm^2)	1	15,22 ^{a} ± 2,05	12,84 ^{c} ± 1,15	9,47 ^{bd} ± 2,31
	6	11,56 ^{bc} ± 2,71	9,20 ^{d} ± 1,59	5,86 ^{e} ± 2,67
	13	12,30 ^{c} ± 3,31	8,17 ^{d} ± 1,17	6,00 ^{e} ± 1,38
Panel tenderness	1	3,80 ^{a} ±0,64	5,56 ^{c} ± 0,83	6,30 ^{cd} ± 0,90
	6	4,75 ^{bc} ± 1,03	6,69 ^{d} ± 0,73	7,02 ^{d} ± 0,79
	13	4,62 ^{ab} ± 1,08	6,57 ^{d} ± 0,38	6,98 ^{d} ± 0,70
MFI	1	68,04 ^{a} ± 9,06	79,68 ^{ad} ±17,99	88,71 ^{d} ± 15,32
	6	112,79 ^{b} ± 22,87	122,83 ^{bc} ± 9,46	119,84 ^{bc} ± 12,22
	13	118,79 ^{bc} ± 15,77	126,38 ^{c} ± 8,15	128,10 ^{c} ± 12,03
MPS pH 7,0 (mg/g)	1	20,65 ^{a} ± 2,95	26,37 ^{ad} ±8,63	32,78 ^{cde} ± 13,65
	6	25,08 ^{ab} ± 5,74	32,43 ^{bde} ± 5,28	38,79 ^{ef} ± 6,74
	13	26,13 ^{abc} ± 2,44	32,43 ^{bde} ± 5,47	41,62 ^{f} ± 8,02
MPS pH 5,5 (mg/g)	1	17,97 ^{a} ± 2,56	21,06 ^{abc} ± 4,38	18,89 ^{ab} ± 3,61
	6	21,31 ^{abc} ± 0,88	23,64 ^{c} ± 3,26	20,68 ^{abc} ± 3,98
	13	21,53 ^{bc} ± 1,93	23,00 ^{cd} ± 3,10	20,00 ^{abd} ± 2,90
Collagen solubility (% total)	1	9,16 ^{ab} ± 2,55	9,23 ^{ab} ± 5,22	7,60 ^{ab} ± 1,75
	6	10,00 ^{a} ± 3,13	9,03 ^{ab} ± 4,33	7,01 ^{ab} ± 1,96
	13	8,93 ^{ab} ± 2,46	8,78 ^{ab} ± 2,46	6,56 ^{b} ± 2,75

Means without same letters are significantly different (P<0.05).

In all groups MPS pH 7.0 shows a tendency for increase with ageing, however this increase is only significant (P<0.05) in DFD group. In all three days *post mortem* the DFD group have the highest SPM pH 7.0. With respect to MPS pH 5,5 it was not found significant differences (P>0.05) between pHu groups. The higher solubility at pH 7.0 of DFD meat might be explained by a higher proteolytic activity by calpains. Claeys *et al.* (1994), reports that at pH 7.0 are preferentially solubilized titin, filamin, nebulin and myosin heavy chain. Except the last one, all are reported as being degraded preferentially by calpains (Goll *et al.*, 1983), that as known, have an optimum activity at pH values near neutrality. The results of Watanabe & Devine (1996) in sheep support that, once they verified that the degradation of titin and nebulin was higher in the meat with high ultimate pH (>6.3). In the present experiment, it was observed that the increase of MPS pH 7.0 along the ageing period was higher in the DFD group (the only one that showed significant differences), thus, the more favourable to calpain activity. At pH 5.5 the most important proteins that are solubilized are tropomyosin and troponins (Claeys *et al.*, 1994). These proteins might be degraded as by calpains as by cathepsins (Goll *et al.*, 1983; Yu & Lee, 1996) that might partially explain the absence of significant differences in the MPS pH 5.5 between the three pH groups.

No significant difference (P>0.05) were found in the collagen solubility with ageing. Pierson & Fox (1976) found similar relations. Between pHu groups no significant (P>0.05) differences were found but, the high values of soluble collagen were observed in the normal and modDFD groups. Although the muscle do not contain collagenase in detectable amounts, the lysosomal cathepsins B and L might have a collagenolytic activity, release the cross-links attachments between collagen molecules so, it might increase the solubility of collagen (Etherington, 1984). Assuming that cathepsins had an effective collagenolytic activity, this action showed be more evident in the meat with low pHu and lower in the one with high pHu.

Conclusions

The results show that ultimate pH affects the ultimate tenderness of bovine meat. As observed DFD meat is more tender than Normal since the day 1 *post mortem*. The higher values of MPS pH 7.0 found in DFD meat in all days *post mortem* might be a reflex of a higher proteolytic activity conduced by calpains. Otherwise, values of MFI are significantly different between the groups of pHu only at day 1 *post mortem*. This suggest that in aged meat the myofibrillar fragmentation is independent of ultimate pH.

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