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Influence of Ultimate pH on Bovine Meat Tenderness During Ageing

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ABSTRACT

The aim of this work was to evaluate the influence of ultimate pH and ageing at $2\pm 2^{\circ}\text{C}$ on the tenderness of beef. The longissimus thoracis et lumborum from 23 young bulls excised at 28 hours post mortem were grouped into : Normal (pH 5.5 to 5.8) moderate DFD ($5.8 < \text{pH} < 6.2$) and DFD (pH 6.2 to 6.7).

The toughness measured by Warner-Bratzler shear force (WBSF) and by sensory evaluation, were significantly ($P < 0.05$) lower in the DFD group than in the Normal one and a significant ($P < 0.001$) linear relationship was found between the ultimate pH and the tenderness evaluated by both methods. The myofibrillar protein solubility (MPS) extracted at pH 7.0 was significantly higher in the DFD group at all post mortem times whereas for myofibrillar

fragmentation index (MFI) higher values ($P<0.05$) were only found at day 1, particularly in the DFD group.

In all 3 groups toughness decreased ($P<0.05$) from 1 to 6 days, with no further decrease at 13 days. This decrease in toughness was concomitant with the increase of MFI observed in all pH groups. Collagen solubility in all 3 groups was not affected by ageing. No significant ($P>0.05$) differences in soluble collagen and myofibrillar protein solubility (MPS) extracted at pH 5.5 were found between the pH groups on any of the storage days.

The tenderness evaluated by both methods was significantly ($P<0.05$) related with MFI, MPS at pH 7.0, cooking loss and juiciness. Total and soluble collagen, sarcomere length, intramuscular fat and MPS at pH 5.5 were not significantly ($P>0.05$) related with the tenderness of the meat.

INTRODUCTION

The high ultimate pH of meat, as a consequence of depleted muscular glycogen reserves prior to slaughter, greatly affects meat quality. Various stress factors have been mentioned as responsible for glycogen depletion: time and manner of transportation of animals from the farm to the abattoir; diet restrictions; mixing animals of different lots; lairage time; climatic factors; pathological conditions and genetic factors. In practice, any situation which provokes a substantial depletion of muscle glycogen reserves will origin meat with a high ultimate pH, if the animal is slaughtered before its energetic reserves are restored (Tarrant, 1981).

Meat with high ultimate pH is dark, is more susceptible to bacterial spoilage and has reduced flavour. Nevertheless, this meat is also associated with a higher tenderisation rate (Watanabe *et al.*, 1996) or with a better ultimate tenderness (Bouton *et al.*, 1973).

The mechanism for the improvement of meat tenderness during *post mortem* storage at refrigeration temperatures remains controversial. Proteolysis of myofibrillar proteins may play 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 (Koochmaraie, 1996). Besides enzymatic mechanisms, a high ionic strength of *post mortem* muscle will contribute to meat tenderisation (Wu and Smith, 1987).

The relationship between ultimate pH and tenderness is, however, controversial; some authors (Bouton *et al.*, 1973; Guignot *et al.*, 1994) have found a linear relationship between these two parameters whereas others (Purchas, 1990; Jeremiah *et al.*, 1991; Purchas and Aungsupakorn, 1993), found a curvilinear relationship, with a minimum of tenderness between 5.8-6.2 pH values. Some hypotheses have been proposed to explain this curvilinear relationship. Yu and Lee (1986) suggested a differential proteolytic activity to justify the toughness of meat with pH values of 5.8-6.3. According to these authors, in meat with high ultimate pH (>6.3) the activity of neutral proteases, i.e. calpains, is favoured and at low pH (<5.8) they suggest an enhanced cathepsins activity. However, at intermediate pH values the pH is neither optimal for calpains nor for cathepsins activity, resulting in the lowest degree of meat tenderisation during ageing. Also non enzymatic mechanisms have been proposed. Takahashi (1996) states that many meat tenderness changes can

be explained by the direct effect of calcium on myofibrillar proteins and that this effect is pH-dependent, with a minimum level at pH 6.5. Purchas (1990) and Purchas and Aungsupakorn (1983) have suggested that the decrease in sarcomere length as the pH increases to 6.2 may partially explain the higher level of toughness of intermediate pH meat. The cooking temperature of meat also seems to influence the relationship between ultimate pH and tenderness. In raw meat tenderness is not related with pH (Dransfield, 1981); at medium cooking temperatures (65°C) the relationship is curvilinear and at high temperatures (90°C) it is linear (Bouton *et al.*, 1971). This effect of temperature on meat tenderness may result from the effect of cooking on water holding capacity (Purchas, 1990) or on the connective tissue (Dransfield, 1994).

The aim of this work was to evaluate the influence of ultimate pH on tenderness during ageing of bovine meat.

MATERIALS AND METHODS

Animals and muscle samples

Twenty-three Portuguese autochthonous Maronesa bulls aged 8 to 11 months were slaughtered commercially giving carcass weights between 100-164 kg. Carcasses were chilled for 1 hr at 0°C, 4 m/s and then kept at 1°C until 24 hr *post mortem*. At about 28 hr *post mortem* *longissimus thoracis et lumborum* was excised (between the 8th rib and the 2nd lumbar vertebra) and divided into three parts (about 600 g). One part of the muscle was used for meat characterisation at day 1 *post mortem* and the other two were vacuum packed and aged at 2±2°C until 6 or 13 days *post mortem*. At the end of each period

samples were cut and frozen at -18° C for determination of myofibrillar fragmentation index and sensorial tenderness. For determination of myofibrillar protein solubility, collagen and intramuscular fat the samples were minced before frozen.

pH of raw meat

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 hr *post mortem*) the muscles were segregated into three groups: Normal (pH 5.5 to 5.8) moderate (mod) DFD (5.8<pH<6.2) and DFD (pH 6.2 to 6.7).

Sarcomere length

About 200 mg of muscle were fixed in a solution of 2% glutaraldehyde and 0.2 M sucrose in 0.2 M phosphate buffer pH 7.1 (Honikel *et al.*, 1981). Bundles of 3-4 fibres were removed from the fixed muscle tissue and the length of 10 consecutive sarcomeres was measured (30 groups of 10 sarcomeres for each sample) by phase contrast (x 1000).

Myofibrillar fragmentation index

Myofibrillar fragmentation index (MFI) was determined as described by Culler *et al.* (1978). The frozen samples were minced in a cutter, after any visible fat and connective tissue had been removed. 4 g (in duplicate) of minced meat were homogenised for 30 s in 40 ml 0.02M potassium phosphate buffer (pH 7.0) containing 100 mM KCl, 1 mM EDTA, 1 mM MgCl₂ and 1 mM NaN₃ at about 2°C using a Waring Blendor mixer. After centrifugation at 1000g for 15

min the supernatant was discarded. The sediment was resuspended in 40 ml of buffer, using a stir rod, centrifuged again and the supernatant discarded. This sediment was resuspended in 10 ml buffer and filtrated through a polyethylene strainer to remove connective and fat tissue. An additional 10 ml buffer was used to facilitate the passage of myofibrils through the strainer. After determination of the protein concentration of the suspension by the biuret method (Gornall *et al.*, 1949), the suspension was diluted with buffer to 1.0 mg/ml protein concentration. The exact protein concentration was determined using the micro-biuret method (see Clark, 1984) and the suspension of myofibrils diluted to 0.5 ± 0.05 mg/ml. MFI is the value of absorbance of myofibrillar suspension, measured at 540 nm and multiplied by 200.

Myofibrillar protein solubility

The myofibrillar protein solubility (MPS) was determined, essentially, according to Claeys *et al.* (1994). Minced muscle (2.00 g in duplicate) was thawed overnight in a refrigerator and homogenised in 20 ml 0.02 M potassium phosphate buffer (pH 7.0) containing 100 mM KCl, 1 mM EDTA, 1 mM MgCl_2 and 1 mM NaN_3 using an Ultra Turrax. After centrifugation at 1500g for 10 min the supernatant was discarded and the sediment resuspended with a stir rod in 20 ml of buffer and centrifuged (1500g for 10 min). The sediment of isolated myofibrils was resuspended with a stir rod in 30 ml of 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 pH 5.5. Tubes were placed in a water bath at 20°C and

shaker manually every hour. After 4 h the tubes were centrifuged at 3000g for 30 min. The protein concentration was determined in the supernatant by the biuret method (Gornall *et al.*, 1949) and the results expressed as mg solubilized protein / g of muscle.

Soluble and total collagen

For soluble and total collagen determination, 20 ml of 0.1M sodium phosphate buffer pH 7.0 was added to 4.00 g (in duplicate) of finely minced meat (thawed overnight in the refrigerator) and homogenised using a stir rod in a centrifuge tube. Each sample was heated at 70°C in a water bath for 30 min with occasional stirring (5 times). Tubes were cooled in an ice bath for 10 min and centrifuged at 10 000g for 15 min (Culler *et al.*, 1978). The supernatant and the sediment were hydrolysed in 7N sulphuric acid. Hydroxyproline was measured at 558 nm (AOAC, 1990). 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 (Cross *et al.*, 1973). The collagen was expressed as the total (soluble + insoluble) and as soluble collagen as a percentage of the total.

Intramuscular fat

Intramuscular fat was determined at day 1 with 20.000 g (in duplicate) of minced meat in a Soxhlet apparatus using petroleum ether (ISO, 1973).

Cooking loss

Cooking loss was determined immediately in meat samples (about 150 g; 3 cm thick) with similar geometry, individually placed inside polyethylene bags in a

water bath at 75°C. Samples were heated to 70°C (monitored with thermocouples introduced in the core) and cooled for 40 min under running tap water. After that they were taken from the bags, dried with filter paper and weighed. The cooking loss was expressed as the percentage of loss by heating based on the initial weight.

Warner-Bratzler shear force

After measurement of cooking loss the samples were stored overnight in a refrigerator and used for objective determination of tenderness (after equilibration at ambient temperature). The Warner-Bratzler shear force (WBSF) was measured in 10-12 sub-samples with 1 cm² cross section and 4-5 cm length with fibres perpendicular to the direction of the blade attached to a Stevens QTS 25 apparatus.

Sensory evaluation

For sensory evaluation 1.5 cm thick steaks were thawed overnight at 2±2°C and heated in a double side contact grill (230°C), covered with aluminium foil, to an internal temperature of 70°C. Thermocouples were used to monitor endpoint temperature. Three samples with about 1.5 x 1.5 x 3 cm (one for each time *post mortem*) were presented to a semi-trained panel of 6 assessors. They were asked to rank the meat on a 9 point scale for tenderness (1 = extremely tough, 9 = extremely tender) and for juiciness (1 = extremely dry, 9 = extremely juicy).

Statistical analysis

The data were analysed using the “General Linear Model” procedure of Systat 5.0. The model used accounted for the effects of ultimate pH group, time of ageing and interaction. The LSD test was used to locate differences between means at 5% level of probability. The relationship between variables was assessed by a Pearson simple correlation.

RESULTS AND DISCUSSION

Influence of ultimate pH and ageing

The ultimate pH of *longissimus* varied between 5.56 and 6.70. Table 1 shows the results for the three pH groups. Ageing the meat until 6 days *post mortem* results in a decrease of WBSF in all pH groups. A longer period of ageing does not result in significant ($P>0.05$) decrease in meat toughness. Meat with Normal pH was significantly ($P<0.05$) tougher than modDFD and DFD groups in all days *post mortem*. At day 1, DFD meat was already more tender than the Normal at day 13 *post mortem*, and at day 6 the meat with Normal pH was twice as tough as the DFD meat. It was observed that the tenderness assessed sensorially and by WBSF increases linearly with pHu (Fig 1) so, the curvilinear relationship founded by some authors, with a minimum of tenderness between 5.8-6.2 ultimate pH values (Purchas, 1990; Jeremiah *et al.*, 1991; Purchas and Aungsupakorn, 1993) is not substantiated by the present work. Dransfield (1996) refers that initially the meat with high ultimate pH is

more tender than normal pH meat as a result of a higher tenderisation rate, but with prolonged ageing the ultimate tenderness became similar. Nevertheless, in this work, with the longer period of ageing (13 days) the meat with higher pHu was still more tender, thus, the ultimate tenderness was influenced by pHu.

DFD group tended to have larger sarcomere lengths than the two others groups, however this difference was significant ($P < 0.05$) only at day 1 *post mortem*. In the Normal group a significant ($P < 0.05$) decrease of sarcomere length occurred between day 1 and day 6. This suggests that, in this group, at day 1, *rigor mortis* was not yet complete and with muscle excision it may shorten naturally. This decrease in sarcomere length in the Normal group might explain the increase in cooking loss found only in this group from day 1 to day 6 *post mortem*.

MFI shows a significant increase between day 1 and day 6 in all groups. Various reports show that MFI increases with the ageing of meat (Olson *et al.*, 1976; Watanabe *et al.*, 1996). 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 (Olson *et al.*, 1976). The comparison of different groups for each day *post mortem* shows that at 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 thoracicus 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, the higher increase being found in the meat with lower pHu (< 5.8). The results of the present work differ from those of these authors, because at day 1 *post mortem* significant differences ($P < 0.05$) were found between the groups of pH. However from day

1 to day 6 a larger increase in MFI was also observed in the lower pH groups. These results suggest that in meat with higher pH there occurs a higher proteolytic activity responsible for the rupture of myofibrils early in day 1 *post mortem* while in meat with a normal or intermediate pH the proteolysis is more gradual.

In all groups, MPS pH 7.0 increased with ageing; however, this increase is only significant ($P < 0.05$) in the DFD group. At all three days *post mortem* the DFD group had the highest SPM pH 7.0. With respect to MPS pH 5.5 no significant differences ($P > 0.05$) were found between pHu groups. The higher solubility at pH 7.0 of proteins found on DFD meat might be explained by a higher proteolysis. Claeys *et al.* (1994), report that at pH 7.0 are preferentially solubilized titin, filamin, nebulin and myosin heavy chain. Except for the last all are reported as being degraded preferentially by calpains (Goll *et al.*, 1983) that, as is known, have an optimum activity at pH values near neutrality. The results of Watanabe and Devine (1996) in sheep support this view, since 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 throughout the ageing period was higher in the DFD group (the only one that showed significant differences), thus, more favourable to calpain activity. Beltran *et al.* (1997) found that the activity of m-calpain measured at 7th day post mortem was increased in meat with high ultimate pH, suggesting that this could enhance the tenderisation of meat. At pH 5.5 the most important proteins that are solubilized are tropomyosin and troponins (Claeys *et al.*, 1994). As these proteins might be degraded by calpains and by cathepsins (Goll *et al.*, 1983; Yu and Lee, 1986) that might partially explain the

absence of significant differences in the MPS pH 5.5 between the three pH groups.

MFI was more dependent on the period of ageing than on the pHu, since the differences in MFI between pH groups were only significant at day 1 *post mortem*. The increase in this parameter was high in all pH groups during ageing. However MPS pH 7.0 was significantly different among the pH groups in each ageing period, indicating an eventual higher dependence on the pHu. These results suggest that MFI and MPS are based on different phenomena of degradation of myofibrillar proteins.

With respect to collagen solubility no significant differences ($P>0.05$) were found with the ageing of meat, in accordance with the work of Pierson and Fox (1976). Between pHu groups no significant ($P>0.05$) differences were found but higher values of soluble collagen were observed in the Normal and modDFD groups. Although muscle does not contain collagenase in detectable amounts, the lysosomal cathepsins B and L might have a collagenolytic activity releasing the cross-links attachments between collagen molecules and so increasing the solubility of collagen (Etherington, 1984). Assuming that cathepsins had an effective collagenolytic activity, this action should be more evident in the meat with low pHu and less evident in the one with higher pHu.

Relationships with tenderness

Table 2 shows the simple correlation coefficients of various parameters with WBSF and sensorial tenderness.

The results of this work show that sarcomere length is not related with tenderness except at day 6 *post mortem* and only with WBSF.

At day 1 *post mortem* the MFI is strongly correlated with WBSF and with panel tenderness, (-0.68 and 0.67 respectively) but, at days 6 and 13 *post mortem* the relationship became weaker. Culler *et al.* (1978) showed in beef aged 14 days at 2°C that MFI might explain about 50 % of tenderness variation. This value is slightly higher than that obtained by us at day 1 (about 45 %) but, at day 13 the contribution of MFI on tenderness is substantially lower (26 % and 19 % for WBSF and sensorial tenderness, respectively).

In this study, MPS at pH 7.0 was correlated ($P < 0.001$ at 1 and 6 days *post mortem*, $P < 0.01$ at 13 days *post mortem*) with the tenderness assessed by both methods. However, no significant ($P > 0.05$) correlation was found for the MPS at pH 5.5. It should be noted that, at day 1 the MFI and MPS pH 7.0 were strongly correlated ($P < 0.001$) with WBSF and with sensorial tenderness, but at day 6 the MPS pH 7.0 showed a better relationship with the WBSF (-0.83) and with the sensorial tenderness (0.76) than MFI (-0.52 and 0.35 for the WBSF and sensorial tenderness, respectively). This suggests that at day 6 *post mortem* MPS pH 7.0 is a better predictor of tenderness than MFI.

Cooking loss and juiciness were significantly ($P < 0.001$) correlated with tenderness assessed by both methods. Bouton *et al.* (1973) considered that the higher water holding capacity of meat with high pH_u contributes to their high tenderness.

Neither total collagen nor soluble collagen were significantly ($P > 0.05$) correlated with tenderness assessed by both methods. The lack of correlation found in this work may be due to the fact that the soluble collagen contribution for tenderness is more important among animals with different ages, while the

contribution of total collagen for tenderness is more important between muscles with different amount of collagen (Dransfield, 1994). Besides, when meat is cooked at 70°C and especially from young animals as in this work, the myofibrillar component is the main determinant of tenderness (Bouton *et al.*, 1981).

Intramuscular fat was low (1.9%) and was not correlated with tenderness.

CONCLUSIONS

In beef, tenderness at 1, 6 and 13 days increased linearly with increasing ultimate pH (5.5 to 6.7). The myofibrillar protein solubilized at pH 7.0 seemed more dependent on ultimate pH whereas MFI seemed more dependent on the ageing period. This suggests that MFI and MPS are based on different phenomena of degradation of myofibrillar proteins.

Fig. 1. Relationship between WBSF (Kg/cm^2) and sensorial tenderness with pHu for the day 1 (● ———) day 6 (□ — —) and day 13 *post mortem* (+.....)

TABLE 1

Mean $\pm$ Standard Deviation for the Three Quality Groups.

Measurement	Day pm	Normal (n=7)	DFDmod (n=8)	DFD (n=8)
pHu		5.7 ± 0.1	6.1 ± 0.1	6.5 ± 0.2
WBSF (Kg/cm ²)	1	15.2 ^a ± 2	12.8 ^c ± 1	9.5 ^{bd} ± 2
	6	11.6 ^{bc} ± 3	9.2 ^d ± 2	5.9 ^e ± 3
	13	12.3 ^c ± 3	8.2 ^d ± 1	6.0 ^e ± 1
Sensorial tenderness	1	3.8 ^a ± 0.6	5.6 ^c ± 0.8	6.3 ^{cd} ± 0.9
	6	4.8 ^{bc} ± 1.0	6.7 ^d ± 0.7	7.0 ^d ± 0.8
	13	4.6 ^{ab} ± 1.1	6.6 ^d ± 0.4	7.0 ^d ± 0.7
Sarcomere length (µm)	1	1.77 ^a ± 0.14	1.56 ^b ± 0.14	1.67 ^{ab} ± 0.27
	6	1.57 ^b ± 0.09	1.53 ^b ± 0.14	1.67 ^{ab} ± 0.20
	13	1.56 ^b ± 0.16	1.55 ^b ± 0.11	1.59 ^b ± 0.14
MFI	1	68 ^a ± 9	80 ^{ad} ± 18	89 ^d ± 15
	6	113 ^b ± 23	123 ^{bc} ± 9	120 ^{bc} ± 12
	13	119 ^{bc} ± 16	126 ^c ± 8	128 ^c ± 12
MPS pH 7.0 (mg/g)	1	21 ^a ± 3	26 ^{ad} ± 9	33 ^{cde} ± 14
	6	25 ^{ab} ± 6	32 ^{bde} ± 5	39 ^{ef} ± 7
	13	26 ^{abc} ± 2	32 ^{bde} ± 5	42 ^f ± 8
MPS pH 5.5 (mg/g)	1	18 ^a ± 3	21 ^{abc} ± 4	19 ^{ab} ± 4
	6	21 ^{abc} ± 1	24 ^c ± 3	21 ^{abc} ± 4
	13	22 ^{bc} ± 2	23 ^{cd} ± 3	20 ^{abd} ± 3
Collagen solubility (% total)	1	9.2 ^{ab} ± 2.6	9.2 ^{ab} ± 5.2	7.6 ^{ab} ± 1.8
	6	10.0 ^a ± 3.1	9.0 ^{ab} ± 4.3	7.0 ^{ab} ± 2.0
	13	8.9 ^{ab} ± 2.5	8.8 ^{ab} ± 2.5	6.6 ^b ± 2.8
Cooking loss (%)	1	14.5 ^a ± 1.8	14.4 ^c ± 2.6	11.6 ^d ± 2.4
	6	20.0 ^b ± 1.2	14.4 ^c ± 3.0	10.4 ^d ± 1.8
	13	20.5 ^b ± 1.0	15.3 ^{ac} ± 2.1	12.0 ^d ± 2.1

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

TABLE 2

Simple Correlation of Various Parameters with WBSF (above) and Sensorial
Tenderness (below) at 1, 6 and 13 Days *Post Mortem*.

	Day 1	Day 6	Day 13
Sarcomere length	-0.11 -0.00	-0.55** 0.28	-0.23 0.20
MFI	-0.68*** 0.67***	-0.52* 0.35	-0.51* 0.44*
MPS pH 7.0	-0.66*** 0.70***	-0.83*** 0.76***	-0.60** 0.55**
MPS pH 5.5	-0.22 0.47*	0.21 -0.04	0.29 -0.19
Cooking loss	0.79*** -0.84***	0.67*** -0.64***	0.74*** -0.76***
Juiciness	-0.78*** 0.88***	-0.74*** 0.80***	-0.75*** 0.87***
Total collagen	-0.26 0.27	-0.13 0.06	0.30 -0.23
Soluble collagen	0.09 -0.07	0.40 -0.26	0.34 -0.20
Intramuscular fat	0.06 0.03	0.10 -0.21	0.13 -0.23
Sensorial tenderness	-0.85***	-0.80***	-0.88***

* P < 0.05; ** P < 0.01; *** P < 0.001; others non-significant

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