THE INFLUENCE OF ULTIMATE pH ON THE TENDERISATION OF MEAT.

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SUMMARY

The influence of ultimate pH (pHu) on the tenderisation process in bovine *m.longissimus dorsi* (LD) and *m.semimembranosus* (SM) during storage at 4°C was examined. Muscles were divided into normal, intermediate, and high groups based on their pHu and were subjected to biochemical, sensory and mechanical tests. A rapid increase in tenderisation (by 48 hours *post mortem*) was clearly evident in high pHu meat compared with the other groups. Results suggest that this effect is brought about by the increased proteolytic activity of endogenous proteases.

INTRODUCTION

Despite many studies the precise mechanism by which beef carcasses improves tenderness during *post mortem* storage at refrigerated temperatures still remains unresolved. Tenderness is regarded as the principle determinant of the eating quality of meat (Szczesniak 1971). Consequently a clearer understanding of the mechanisms of the *post mortem* tenderisation process is important if control of the process is to be realised at industrial level.

A number of broad mechanisms have been cited in the literature (see Ouali, 1990). Among these, *post mortem* proteolytic degradation of myofibrillar proteins is often considered the primary mechanism of meat tenderisation. Two distinct protease systems have been proposed. These include the neutral calpains and the acidic lysosomal proteases. Because of their pH dependency it would be expected that the ultimate pH (as measured at 48 h post mortem) should influence the rate and extent of the tenderisation process especially in relation to proteolysis of the myofibril component of toughness.

The relationship between ultimate pH (pH_u) and meat tenderisation has been previously studied (Bouton et al. 1973a; Yu and Lee, 1986; Purchas 1990; Guignot et al., 1994). It has been cited that high pH_u meat is more tender than meat of normal pH_u while meat of intermediate pH_u is toughest. However explanations for these variations in meat texture have not been fully given. Purchas (1990) suggested that a decrease in sarcomere length as the pH_u increases from 5.5 to 6.2 may partly explain the increased toughness of intermediate pH muscle. Varying degrees of tenderisation due to differences in muscle pH was attributed to different levels of proteolysis by Yu and Lee (1986). They specified that removal of z-lines by neutral proteases in high pHu meat resulted in more tender meat compared with normal and intermediate pHu meat. M-calpain activity measured at 7 days *post mortem* in beef *longissimus dorsi* increased significantly in high pHu meat compared with controls (Beltran et al. 1993) with a concommitant increase in tenderness supporting the view of increased proteolysis. However, others (Bouton et al. 1973b; Purchas, 1990) have suggested that the increased water-holding capacity of high pH_u meat partly accounts for the increase in tenderness. Finally, it has been recently suggested that animals treated with androgenic compounds may produce more tender meat by way of a treatment effect (Shackleford et al. 1994).

The present work was carried out to assess the influence of pH_u on the biochemical, mechanical and sensory properties of beef *m.longissimus dorsi* (LD) and *m.semimembranosus* (SM) during ageing.

MATERIALS AND METHODS

Hereford cross heifers (n=12) aged 18-24 months and weight 355-470kg were used in this study. Sub-cutaneous injections of adrenaline were administered to eight animals at selected times pre-slaughter, in order to deplete muscle glycogen stores, thereby producing muscles with elevated pHu. Muscles having a pHu range of 5.4-5.7 were used as controls (n=4). Varying doses of adrenaline were administered in order to obtain muscles with a medium pHu range of 5.8-6.2 (n=4) and high pHu range of 6.3-6.9 (n=4) . The animals were slaughtered conventionally, held at 15°C for 5 hours, then at 4°C for 48 hours *post mortem*. LD and SM were excised at 48 hours *post mortem*.

pH values were taken at 3, 24, 48 hours and at 7 and 14 days *post mortem* on muscle homogenates in 5mM sodium iodoacetate and 150mM potassium chloride at pH 7.0 (Bendall 1973). Steaks (2.5cm thick) were cut at 2,7 and 14 days *post mortem* following ageing at 4°C. Sensory analysis was performed by an in-house eight-membered trained taste panel on steaks grilled to an internal temperature of 70°C, according to American Meat Science Association Guidelines (1978). Panelists were asked to rank, on an eight point hedonic scale, the steaks for tenderness (1 = extremely tough, 8 = extremely tender), juiciness and flavour. Myofibrils were extracted and SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed according to a procedure by Greaser (1993). Sarcomere lengths were measured by diffraction of a laser beam according to the procedure described by Cross et al. (1980). Warner Bratzler Shear force values were taken on 1.25cm diameter cores cut from 2.5cm steaks cooked to an internal temperature of 70°C, using an Instron Universal testing machine, Model 1140 (Shackelford et al. 1991). A trained panel of five used a graphic scale to assess tenderness of steaks cooked to 60°C and 80°C in the waterbath and cut to 1cm^2 parallel to the fibres. . The percentage cook loss was calculated from the difference in the weight of the uncooked and cooked steak at 60, 70 and 80°C. The results were statistically analysed using a one way classification design (Genstat. Numerical Alogorithms Group Ltd. Oxford.)

RESULTS AND DISCUSSION

LD and SM were divided into three different pH groups according to their pHu ; normal 5.4-5.8, intermediate 5.9-6.2 and high >6.3. Biochemical, mechanical and sensory parameters were measured in the excised muscles at 2, 7 and 14 days *post mortem*.

Shear force values decreased during the ageing period of each muscle and pHu group (Table 1 and 2). High pHu LD shear force values were substantially lower than the other two groups at each storage time, particularly after two days storage (Table 1). Shear force values for the intermediate pHu group of SM were higher (Table 2).

High pHu muscles scored the highest in tenderness evaluation. No significant difference was detected in juiciness or flavour for either muscle between the groups (results not shown). High pHu muscle also scored the highest in tenderness for texture assessment at 60°C and 80°C.

SDS gel electrophoresis profiles indicated a higher degree of proteolysis in high pHu muscle as shown by the presence or absence of the 30kDa band. This fragment has been suggested previously as an indicator of *post mortem* proteolysis (Olson et al. 1977). The 30kDa band was clearly evident in SDS gels of extracted myofibrils even after two days in high pHu muscle indicating rapid proteolytic activity by this time. Previously changes in beef myofibrils were detected wthin 4 to 6 hours of slaughter depending on muscle pH (Troy et al. 1986)

Based on the foregoing results, high pHu meat undergoes a more rapid tenderisation process than normal and intermediate pHu meat. In fact shear force, sensory and biochemical analyses indicate that by 48 hours *post mortem*, high pHu meat is as tender as normal pHu meat after 14 days storage. These results suggest that proteolysis is the major cause of the increase in tenderness within 48 hours *post mortem*. The principal proteases and substrates involved in this tenderisation process have not been clearly identified but the neutral protease system, calpains, has been widely proposed as being one of the principal components (Ouali 1990). However, the myofibrillar proteins involved in calpain proteolysis have not been elucidated to date. The 30kDa fragment used in this work, as an indicator of proteolysis, has been previously shown to be a product of calpain action on myofibrillar proteins (Ouali et al. 1983). Recently, tryptic digestion of this fragment yielded polypeptides which have a similar amino acid sequence to part of the troponin-T protein (Haritos et al. 1994).

However the structural role which troponin-T plays in the tenderisation of meat through a proteolytic pathway is unknown.

The suggestion that tenderness differences between the pHu groups is related to sarcomere length is not substantiated in this work. Sarcomere lengths generally decreased with increasing pHu at 48 hours *post mortem* (Tables 1 and 2). Although shorter sarcomere lengths have been associated with increased toughness (Bouton et al. 1973b), results from this work indicate that the degree of proteolysis is much more significant in inducing the tenderisation effect. There are a number of reports where tenderness and sarcomere length did not correlate well (Culler et al. 1978) and according to Locher (1985), sarcomere length cannot be taken in isolation as a measure of expected tenderness.

Cook loss decreased with increasing pHu at each temperature tested. However in agreement with the juiciness scores obtained by sensory analysis in the work of Bouton et al. (1973a), panelists in the present work did not detect any significant difference in the amount of moisture squeezed out in the chewing process. Whether the increased water holding capacity (WHC) influences the tenderness ratings of the panelists without affecting the juiciness ratings is unknown. However cook loss did decrease substantially in the intermediate pHu group compared with normal pHu group without a concomittant increase in tenderness. Therefore if increased WHC does influence the tenderness of meat with regard to pH effects, it does so to a lesser extent than proteolysis.

It is unlikely that the adrenaline treatment itself influenced the biochemical, sensory and mechanical results as intermediate pHu muscles were excised from animals administered varying adrenaline doses. Yet these muscles in general were no more tender than non-treated controls.

CONCLUSION

It is concluded that the rapid increase in meat tenderisation (within 48 hours) of high pHu meat is brought about by the proteolytic activity of endogenous proteases. This suggests that the pH environment within the muscle during the early *post mortem* period greatly influences meat tenderness.

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