TENDERNESS CHANGES DURING AGING OF MEAT AT VARIOUS pH VALUES

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SUMMARY

A range of pH_u values from 5.4-6.7 in sheep loins was produced by injection of adrenaline. When aging was blocked with $ZnCl_2$, the pH_u did not influence the shear force. For muscles without $ZnCl_2$ injection, the tenderness at one day post-mortem was greatest for meat of high pH_u (about 6.3), was less for low pH_u (below 5.8) and least for intermediate pH_u (5.8-6.3) meat. Differences related to pH_u disappeared after 6 days post-mortem. This least tenderness at intermediate pH_u could not be explained by the conversion from titin 1 to titin 2 on raw meat, but the least degradation of nebulin was observed at this pH_u region. MFI at one day post-mortem was higher for meat with pH_u values of 5.4 and 6.7 than for other pH_u values. During storage, the changes in MFI at pH_u values from 6.4 to 6.7 were less pronounced than for other pH_u values, with the greatest changes being observed at pH_u 5.4. The least fragmentation of raw meat was observed at pH_u values around 6.4. Tenderness after 6 days aging, measured on the cooked product, appears similar irrespective of pH_u of all meat, while the MFI of raw meats shows differences related to pH_u. The differences in tenderness related to pH_u suggest that pH_u affects rate of aging, but not the extent of aging.

Introduction

Stress affects the tenderness of cooked meat through its effects on the ultimate pH (pH_u) of the raw meat (Bouton *et al.*, 1971; Purchas, 1990; Purchas and Aungsupakorn, 1993; Devine *et al.*, 1993). As the pH_u increases from 5.5 to 6.0, the tenderness decreases, but above pH_u 6.0, the effect is reversed. The reasons for this curvilinear relationship have not been adequately explained. Purchas (1990) showed that sarcomere length was one of the reasons for this phenomenon. Yu and Lee (1986) have suggested that this phenomenon is the result of reduced degradation of muscle proteins at the intermediate pH_u values (pH 5.8-6.3) via differential activity by two enzyme systems. The effects of calcium without enzyme involvement (Hattoria and Takahashi, 1979; 1982; Takahashi *et al.* 1987) also have been used to explain the increase in tenderness.

These studies on effect of pH_u on the further aging processes do not consider the effects of aging on the properties of raw as well as cooked meat, whether the toughness at rigor is modified by pH_u, or whether meat of a moderate pH_u will evenutally age to the same level of tenderness as meat of high and low pH_u. The objective of the present study was to establish the effects of pH_u on tenderness by using $ZnCl_2$, an inhibitor of aging (Koohmaraie, 1990), and to reveal the differences between raw meat and cooked meat for assessment of aging.

Material and Methods

Treatment of Animals

Eight sheep (Romney-Suffolk cross, 12 months old) were used in this study; two sheep were used as controls and the remaining six were injected with various levels of adrenaline at 24 h, 16 h and 5 h before slaughter (total doses; 0.2mg/kg - 0.5mg/kg), and two of them were exercised by being chased for 7 min. at one hour before slaughter. Dressed carcasses were moved to a room at 10 within 50 minutes of slaughter. When muscle had entered rigor (high pH animals) or when pH had fallen below 6.5, the left-side loin muscles were injected with $ZnCl_2$ solution (50 mM $ZnCl_2$, 0.9% NaCl, 0.05% aniline blue as a marker dye), as described previously (Devine & Graafhuis, 1994). The loins on the right side were injected with a mixture of 0.9% saline and 0.05% aniline blue. The ultimate pH was determined 24 h after slaughter according to the method of Bendall (1975). The loin muscles were excised from the carcass at 20 h after slaughter. Each loin was divided into

three portions. One portion was used as the first day post-mortem sample and other potions were dipped in 1 mM sodium azide, vacuum-packed and stored at 10°C for 3 and 6 days before being examined.

Measurements of Tenderness and Myofibrillar Fragmentation Index (MFI)

Approximately 100 g and 40 mm thick samples of meat were individually cooked in a water bath at 80°C until the internal meat temperature reached 75°C. The portions were then immediately removed and chilled on ice for 30 min. Samples 10 mm x 10 mm cross section were sheared using a MIRINZ tenderometer. Only samples penetrated by the aniline blue dye were used for tenderness assessment. For MFI measurements, according to procedures of Takahashi *et al.* (1967), one gram of fresh muscle was blended for 2 min with 10 ml of buffer. The MFI was determined by phase contrast light microscopy as the percentage of myofibrils that were 1-4 sarcomeres long (F) in relation to the total number of fibrils ($\sum$): MFI (%)=F x 100/$\sum$.

Analysis of titin and nebulin

Loin muscles from another seven sheep treated as above to raise pH_u, were analyzed by SDS-polyacrylamide gel electrophoresis, as described by Frits *et al.* (1992).

Results and Discussion

Changes in Shear Force

The adrenaline administration/exercise treatment resulted in a large range of pH_u values, varying from pH_u 5.4 to 6.7. Figure 1 shows that of $ZnCl_2$ treatment completely inhibited aging in the loin, even after 6 days of meat storage. As the toughness was the same, irrespective of pH_u, this suggests that pH_u does not affect the basal toughness of rigor meat. The shear force value of high pH_u (e.g. 6.7) muscle is already low at 1 day post-mortem. In an initial appraisal, the optimum conditions for rapid aging appear to be better at low pH_u values (e.g. 5.4) than at high pH_u values (e.g. 6.7). High pH meat going into rigor earlier than low pH meat, therefore appears to age faster over 1 day, but this is due to a longer period of time being available post mortem for aging to take place. When these time lags of commenceing of aging were considered, and assuming that tenderisation starts after rigor (Devine and Graafhuis; 1994), figure 3 shows that the aging rate after rigor for the low pH_u group (pH_u <5.8; mean pH_u 5.4) was in fact very rapid, and the rate of aging for the intermediate pH_u group (pH_u 5.8-6.3; mean pH_u 6.1) was still the slowest.

Curvilinear relationship

The present study showed a curvilinear relationship between pH_u and shear force at 1 and 3 days post-mortem, but such a relationship has not always been observed (Bouton *et al.*, 1972; Guignot *et al.*, 1992). The difference between these and our results could be explained by cold shortening in the low pH_u meat. Curvilinear relationship was also observed for both MFI related to pH_u (Fig. 2), similar to that described by Takahashi *et al.* (1987). The minimum tenderness and minimum MFI have different values and this could arise from differences when evaluating cooked meat using a tenderometer and raw meat by MFI (Fig. 1 and 2). It is difficult to determine a reason for this difference from the present experiments, but there are significant effects on other aspects of meat properties at these pH_u values as there is an apparent decrease of cooking loss that takes place above pH_u 6.0 (Purchas & Aungsupakorn, 1993), and Bouton *et al.* (1982)

The present study showed that the curvilinear relationship between shear force and pH_u disappeared after storage of 6 days at 10°C (Fig. 1), but equal shear force values do not mean that the same degree of aging has been reached when comparing meat tenderness with MFI differences at 6 days post-mortem (Fig. 2). Marked differences in shear force were also observed at one day post-mortem but there were only small differences observed with MFI (Fig. 1 and 2). Such differences would have important implications when using only MFI to determine aging rates.

Degradation of Titin and Nebulin

Titin and nebulin play an important role for meat toughness. As aging proceeds, conversion from titin 1 to 2 and degradation of nebulin has been observed. Figure 4 shows that conversion of titin was fastest for meat of pH_u 6.2 and 6.3, and titin 2 of the meat with pH 6.9 was broken into other products. Least degradation of nebulin was observed on the meat of pH_u 5.9 and 6.0. These results indicated nebulin degradation seemed to be related to the formation of a curvilinear relationship between pH_u and toughness.

References

Bendall, J.R. (1975). Cold contracture and ATP-turnover in red and white musculature of the pig, post-mortem. J. Sci. Food Agric., 26, 55-71.

Bouton, P.E., Harris, P.V. & Shorthose, W.R. (1971). The effect of ultimate pH upon the water-holding capacity and tenderness of mutton. J. Food Sci., 36, 435-439.

Bouton, P.E., Harris, P.V. & Shorthose, W.R. (1972). The effects of ultimate pH on ovine muscle; mechanical properties. J. Food Sci., 37, 356-360.

Devine, C.E. & Graafhuis, A.E. (1994). The basal tenderness of unaged lamb. Meat Sci. Accepted for publication.

Devine, C.E., Graafhuis, A.E., Muir, P.D. & Chrystall, B.B. (1993). The effect of growth rate and ultimate pH on meat quality in lambs. Meat Sci., 35, 63-77.

Fritz, J.D., Mitchell, M.C., Marsh, B.B. & Greaser, M.L. (1992) Titin content of beef in relation to tenderness. Meat Sci., 33, 41-50.

Guignot, F., Touraille, C., Renerre, M. & Monin, G. (1992). Relationships between ultimate pH and meat quality in veal. 38th International Congress of Meat Science and Technology, Clermont-Ferrand, 367-370.

Hattori, A. & Takahashi, K. (1979). Studies on the post-mortem fragmentation of myofibrils. J. Biochem. 85, 47-56.

Koohmaraie, M. (1990) Inhibition of post mortem tenderisation in ovine carcasses through infusion of zince. J. Anim. Sci., 68, 1476-1483.

Marsh, B.B. (1954). Rigor mortis in beef. J. Sci. Food. Agric. 5, 70-75.

Purchas, R.W. (1990). An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. Meat Sci., 27, 129-140.

Purchas, R.W. & Aungsupakorn, R. (1993). Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. Meat Sci., 34, 163-178.

Takahashi, K., Fukazawa, T. & Yasui, T. (1967). Formation of myofibrillar fragments and reversible contraction or sarcomeres in chicken pectoral muscle. J. Food Sci., 32, 409-413.

Takahashi, K., Kim, O.H. & Yano, K. (1987). Calcium-induced weakening of Z-disks in post-mortem skeletal muscle. J. Biochem. 101, 767-773.

Yu, L.P. & Lee, Y.B. (1986). Effects of post-mortem pH and temperature on bovine muscle structure and tenderness. J. Food Sci. 51, 774-780.

Figure 1. Post-mortem changes in shear force with respect to different ultimate pH vlaues. The loin muscles were stored for 1 day ( ) 3 days ( ) and 6 days ( ) at 10°C. For $ZnCl_2$ treated meat, only the shear force values after 6 days holding post mortem (●) are shown.

Figure 2. Post-mortem changes in myofibrillar fragmentation index. (Symbols are the same as in Figure 1.)

Figure 3. Toughness disappearance for three different pH_u groups stored at 10°C for 24, 72 and 144 hours after slaughter. The disappearance of toughness was expressed as a ratio (%) of the shear force values measured at each aging period with the shear force values obtained from the meat treated with $ZnCl_2$ at the first day (initial toughness - no aging). Zero time is the time of rigor onset.

Figure 4. Changes of titin and nebulin with respect to different ultimate pH values at 2 days post-mortem.