FACTORS AFFECTING THE WATER CHANGES IN MEAT DURING TENDERISATION

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Background

Kristensen and Purslow (2001) showed that the water-holding of pork gradually decreased immediately post *rigor mortis* during ageing followed by an increase as ageing continued. They interpreted this as being due to degradation of the cytoskeletal proteins. They suggested that when the cytoskeletal proteins degrade, free water appears to be lost more readily during the first part of post mortem storage after which the water holding capacity increases to levels one day post mortem. It is not clear whether the water is directly associated with the cytoskeletal proteins or degradation of the protein assists with water appearance over time.

It is possible that the increase in water arises from connective tissue squeezing water as the structure of the meat is degraded through the breakdown of the cytoskeletal proteins. However an alternative view is that the water involved in the tertiary protein structures is released and this is the source of water rather than that from other compartments within the muscle. The degradative changes in the cytoskeletal proteins over time (ageing) results in increased drip and such changes are also likely to be those associated with meat tenderisation. In this study we explore the changes in drip and bound water associated with shear force changes as the meat ages.

It is now generally regarded that changes in the proteins holding the meat together termed structural or cytoskeletal proteins are responsible for shear force changes as the meat ages or tenderises. These proteins, desmin, titin, nebulin and others are affected by the action of endogenous proteases (calpain) in such a way to affect the tertiary structure altering water binding.

Objectives

To determine the relationships between centrifuge-expressed water, tightly-bound water, ultimate pH_u and meat tenderisation.

Materials and Methods

Animals

Lambs (n=24, approximately 12 months old), were processed in a commercial abattoir. The animals were electrically stunned followed by throat cut to sever carotid arteries and jugular veins and the *m. longissimus thoracis* (LT) from one side between 5th rib (T5) and 6th lumbar vertebra (L6) was removed immediately after stimulation and tightly wrapped by rolling in 4 layers of polythene cling film (11mm, GLAD wrap Clorox, New Zealand Ltd., Auckland New Zealand) and placed in a polythene bag (Devine et al, 2002). The wrapped muscle was chilled initially at 15°C until *rigor mortis* was complete.

Meat ageing

Following *rigor mortis* muscles were cut into four equal pieces along the length of the muscle, beginning at the lumbar end, placed in polythethene bags and held in air at 15°C and aged for 0, 5, 24 and 72hours as described previously (Lowe *et al.*, 2002). The meat samples were frozen at the specified times to stop further ageing. Thermocron iButton, Dallas Semiconductor Corp., Dallas Texas, USA) were placed both in the muscles to ensure the required temperatures were maintained. The ultimate pH (pH_u,) was measured with a Mettler Toledo pH meter with a combination puncture electrode (Mettler Toledo GmbH Process Switzerland) at approximately 24 hours post *rigor mortis*.

Cooking

Meat samples obtained for shear force measurement were stored at -20°C and cooked from the frozen state in an 85°C water bath to an internal temperatures of 75°C and then cooled rapidly in ice. The shear force was determined from six 1 cm x 1 cm samples sheared perpendicular to the orientation of the muscle fibres using an Instron with a tooth-shaped head to conform to the specification of a MIRINZ tenderometer (Graafhuis, *et al*., 1991).

Water binding measurements

At *rigor mortis* (0 ageing) a sample from the middle of the LT sample was weighed (approximately 2 g) and was cut so that the muscle fibres were vertical. The piece was transferred to a plastic centrifugation tube with the same orientation into which were placed 5 mm diameter polycarbonate beads and centrifuged for 15 min at 1800 G (Hettich Zentrifugen Tuttlingen, Germany). The duration of centrifugation was based on the extensive data from Kristensen and Purslow (2001) for pork. The same speed and duration of centrifugation was used throughout all experiments.

The centrifugation loss (free water) was the difference in weight before and after centrifugation. The remaining meat post-centrifugation, was then dried in a 105°C oven for 24 hours and the water loss determined. The water remaining was termed "bound water" for our purposes. The total water lost was the difference from original weight and final dried weight.

The procedure was undertaken at ageing times 0, 5, 24 and 72 hours. This gave shear force values, centrifuge loss (free water), a bound water compartment and total water changes over time.

Curves were fitted using the statistics function in Sigma Plot.

Results and Discussion

There was a large scatter in the results that appeared to be unrelated to measurement procedures and is in line with the observations of Honikel and Hamm (1984) showing that water binding of meat from different animals is highly variable. The pH_u over the range 5.4-6.16 was plotted against centrifuge-expressed water (analogous to free water or drip) at 72 hours (Fig 1 a) indicating that as the pH_u increased the centrifuge-expressed water decreased and this was in part responsible for the variability. The data was sorted into a low pH_u values (5.43-5.55) and normal to intermediate pH_u values (5.56-6.16) giving two sets of curves for each attribute (Fig 1b-1d). The two samples with pH_u values of 5.86 and 6.16 were left in the calculations, and form part of the discussion, although they were at the extreme edge of the relationships (Fig. 1 a) – with these included the significance between the low pH_u and normal pH_u attributes was not altered.

The centrifugation loss (Fig 1b) and total water loss (not shown) increased exponentially, whereas bound water (Fig. 1 d) and shear (Fig. 1 c) decreased exponentially. As the duration of ageing increased, the centrifugation loss increased (Fig 1 b) but changes with regard to the water content past this period were not considered in relation to factors such as re-uptake (Kristensen and Purslow, 2001). Final shear force values were achieved at 72 hours at 15°C (Fig 1 c).

There was a significantly ($p< 0.05$) greater (1.5 times) increase in free water 24 hours for meat of a low pH_u than for the normal to intermediate pH_u meat and a significant two-fold significant increase at 72 hours ($p< 0.05$).

For the shear force the curves for low pH_u meat were significantly more tender than for normal to intermediate pH_u meat at 24 hours and 72 hours ($p< 0.05$) (Fig. 1 c). The absence of a difference at 0 hours is expected as shear values start from the same point whatever the pH_u and in the case of sheep age to similar values but intermediate pH_u meat ages more slowly (Watanabe *et al*., 1995: Watanabe & Devine, 1996).

The bound water retained upon heating to 105°C also decreased over the ageing duration and there was significantly less water bound at 72 hours for the low pH_u group (Fig 1 d) than for the normal to intermediate pH_u group.

The total water (bound plus free) (range 75-90%) released also increased with ageing, with more being released for the low pH_u group.

When the two samples with pH_u values of 5.86 and 6.16 were removed from the calculations the relationships and significance between the low pH_u and normal pH_u attributes did not change.

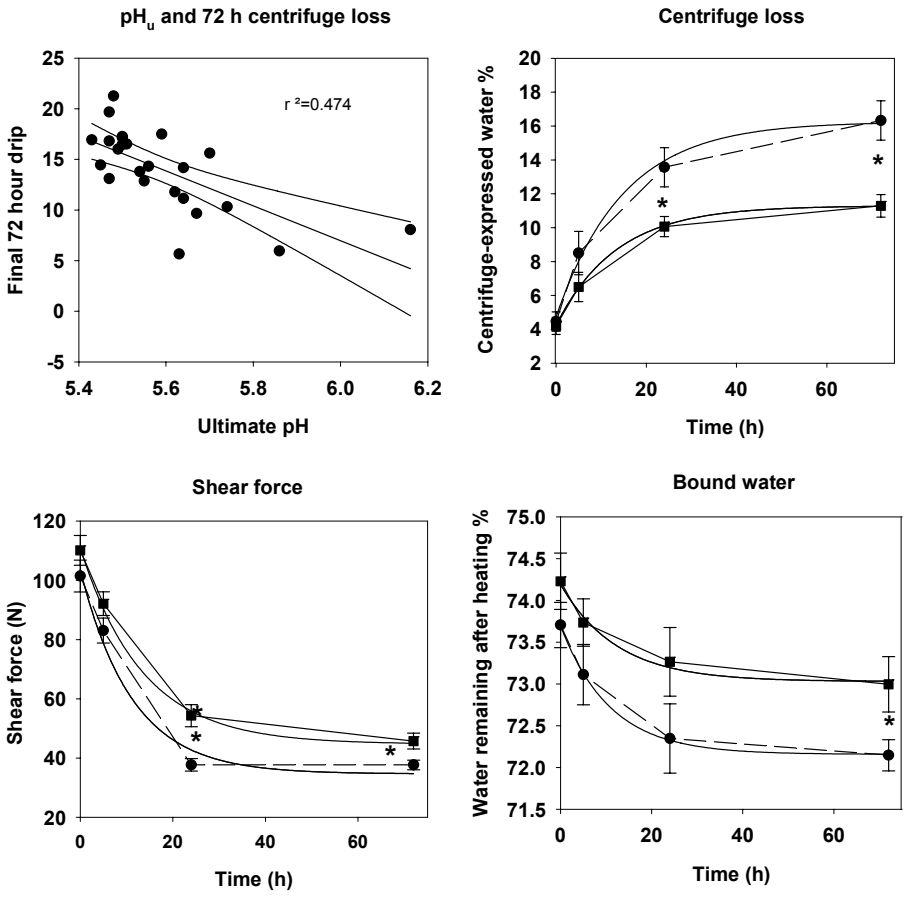

Figure 1. (**a**) The relationship between pH_u.and centrifuge-loss at 72 h ageing. The subsequent graphs are obtained from attributes sorted on the basis of low pH_u (5.43-5.55) (filled circles dashed line) and normal to intermediate pH_u values (5.56-6.16) (filled squares solid lines). (**b**) The increase in centrifuge-loss (free water) over time as the meat ages. (**c**) The reduction in shear force over time. (**d**) The decrease in the water retained in the meat after drying over time as the meat ages (bound water). The * indicates significant difference between the points on the curves at $p< 0.05$). The error bars are ± se and the fitted curves are derived from exponential functions.

The changes over time for free water, bound water and the shear force, are described by fitted exponential curves. It is difficult to establish causative interrelationships because such relationships could be fortuitous. However, the present experiments are consistent with changes in the water components being affected by degradation of cytoskeletal proteins. Free water expressed as drip, naturally increases over time, but the amount produced is also sensitive to various *prerigor* temperature effects (Offer *et al*., 1991) and as the temperatures were similar in this study, the relationship between centrifuge-expressible water and shear force is not unexpected.

The contractile proteins, actin and myosin, are components that are intimately bound to water in their tertiary structure and release of this water is a consequence of changes in interfilament spacing and protein denaturation related to the *prerigor* temperatures, sarcomere length and pH conditions (Honikel *et al.*, 1986; Offer *et al*., 1991). After *rigor mortis* is achieved, the myosin is protected to some extent through the formation of actomyosin (Offer *et al*., 1991), but the consequences persist while the meat ages. The characteristics of water binding by myosin, affected by temperature, pH_u and processing conditions, not only provides the initial drip (analogous but not identical to the centrifuge-expressed water) from meat, but this drip increases over time supplemented from the water associated with the breakdown of cytoskeletal

proteins. The bound water changes are still smaller that the changes that occur in free water. Previous studies by others have only been concerned with the water that comes out as drip rather than water associated with cytoskeletal proteins.

The water released over time includes the contribution from centrifuge-expressed water and bound water and suggests that the water content of meat known as drip is significantly affected by ageing duration. All sources of water are not known at this stage, while some is likely to be water located in the muscle fibres other water is likely to come from components of the extracellular matrix such as hyaluronan (Fraser & Laurent, 1996) that also bind water.

Conclusions

The increase of free water over time is an inevitable consequence of meat tenderisation, with low pH_u meat having more free water, less bound water, greater tenderisation than meat with an increasingly elevated pH_u. The water release during tenderising may be directly related to breakdown of the cytoskeletal proteins.

References

Devine, C. E., Payne, S. R. & Wells, R. W. (2002). Effect of restraint on sheep meat tenderness with *rigor mortis* at 18°C. Meat Science 60 155-159

Fraser, J. R. E. & Laurent, T. C. (1996). Hyaluronan, Chapter 5. *In* Extracellular Matrix ed. W. D. Comper, Vol 2 Molecular Components and Interactions, OPA (overseas Publishers Association) Amsterdam B.V. Published in Netherlands by Harwood Academic Publications GmbH.

Graafhuis, A.E., Honikel, K.O., Devine, C.E. & Chrystall, B.B. (1991). Meat tenderness of different muscles cooked to different temperatures and assessed by different methods. Proceedings 37th International Congress of Meat Science and Technology, Kulmbach, pp 365-368

Honikel K. O. & Ham R. (1984). Measurement of water holding capacity and juiciness. *In* A. M. Pearson, & T. R. Dutson. Quality attributes and their measurement in meat, poultry and fish products pp 125-161, Glasgow, UK: Blackie Academic and Professional.

Honikel, K. O. (1987). The water binding of meat. Fleischwirtschaft 67 1098-1987.

Honikel, K. O. Kim, C. J. Hamm, R. & Roncales, P. (1986). Sarcomere shortening of prerigor muscles and its influence on drip loss in meat. Meat Science 16 267-282.

Kristensen L. & Purslow, P. (2001). The effect of ageing on the water-holding capacity of pork: the role of cytoskeletal proteins. Meat Science 58 17-23.

Lowe, T. E., Peachey, B. M. & Devine, C. E. (2002). The effect of nutritional supplements on growth rate, stress responsiveness, muscle glycogen and meat tenderness in pastoral lambs. Meat Science 62 391-397.

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis Meat Science 30 157-184

Offer, G. & Cousins, T. (1992). The mechanism of drip production: formation of two compartments of extracellular space in muscle post mortem. Journal of the Science of Food and Agriculture 58 107-116.

Watanabe, A., Daly, C.C. & Devine, C.E. (1995). The effects of ultimate pH of meat on the tenderness changes during ageing. Meat Science 42 67-78.

Watanabe, A. & Devine, C.E. (1996). The effect of meat ultimate pH on rate of Titin and Nebulin degradation. Meat Science 42 407-413