YIELDS OF EASILY RELEASABLE MYOFILAMENTS DURING THE POST-MORTEM STORAGE OF BEEF, PORK AND CHICKEN AT 4°C.

Motherway, M.M.[1], Reville, W.J.[2], Shinnick, S.[2], Murray, B.E.[3] and Zeece M.G.[4].
[1]Dept. Biochemistry, Trinity College, Dublin, Ireland.
[2]Dept. Biochemistry, University College, Cork, Ireland.
[3]Teagasc, The National Food Centre, Dublin, Ireland.
[4]Dept. Food Technology, University of Nebraska, Lincoln, Nebraska, USA.

Background

Meat is tender if cooked and eaten at slaughter, toughens with the onset of rigor-mortis and gradually grows more tender again during subsequent post-mortem storage – conditioning (1). The improvement in tenderness during conditioning is mainly and widely ascribed to limited proteolysis of certain myofibrillar proteins by the enzyme calpain (2).

It has been postulated that calpain initiates myofibrillar turnover in skeletal muscle *in-vivo* by effecting the release of intact myofilaments (easily releasable myofilaments – ERM) from the periphery of the myofibril (3, 4) and considerable evidence has been gathered to support this hypothesis (e.g. 5). Calpain activity persists post-mortem and yields of ERM have been measured to increase steadily over the 14 day post-mortem storage of bovine muscle at 4°C (6). Yields of ERM post-mortem may be a sensitive index of the progress of tenderisation during conditioning.

Objective

The objective was to test the hypothesis that ERM yields post-mortem sensitively reflect the progress of conditioning by measuring these yields during the post-mortem storage of 3 species (beef, pork and chicken) that display distinctly different rates of improvement in tenderness during conditioning.

Methods

Myofibrils and ERM were prepared as previously described (6), at-death and after 1, 7 and 14 days post-mortem storage of bovine, porcine and chicken muscle at 4°C. Ten 18 month old Friesan heifers were used in the study and the muscle sampled was the sternomandibularis. Six Large White x Landrace crossed female pigs, 3 to 4 months old, were used in the study and the muscle sampled was the psoas major. Six Cobb T500 female 6 week old chickens were used and the breast (pectoralis major) and leg muscle (mixed) were sampled.

The bovine and porcine muscles were excised from the animals immediately post-mortem and tied on sticks at rest length during storage. The chicken muscle was aged on the bone. Protein concentration was measured by the Lowry (7) and the Bradford (8) methods. ERM yields are expressed as the percentage of total myofibrillar protein released from myofibrils when they are gently agitated under standard relaxing conditions in the presence of 3mM Mg-ATP and in the absence of Ca^{++}. As a control, protein release in the absence of Mg-ATP was also measured (6). Chicken ERM were examined in the transmission electron microscope after negative staining with 1% uranyl acetate

Results and Discussion

Figure 1 compares the pattern of ERM release during the post-mortem storage of beef, pork and chicken (breast) muscle over a 14 day post-mortem period. Protein release figures in the absence of ATP were very low at all times (e.g. at-death beef – 0.10%, and day 14 beef – 0.22% total myofibrillar protein; at-death chicken breast – 0.22%, and day 14 chicken breast – 0.25% total myofibrillar protein) and are omitted from Figure 1 for clarity.

ERM release shows distinctly different patterns for each species. ERM yields are lowest for beef, higher for pork and highest for chicken. Using ERM as an index of the progress of conditioning and assuming it is underpinned by proteolysis, Figure 1 shows that proteolysis in beef steadily increases over the course of 14 days post-mortem storage. It falls off in pork after 7 days post-mortem and it is essentially complete in chicken after a couple of days post-mortem storage. The patterns revealed by ERM yields confirms the well known conditioning profiles of these species, i.e. chicken conditions faster than pork which conditions faster than beef (9).

Chicken leg muscle ERM yields were also measured during conditioning. The results showed the same pattern as revealed by chicken breast muscle in Figure 1, but the yields from the mixed leg muscle tended to be lower than from breast muscle. (Percentage yields at-death: breast 3.62±0.78, leg 2.24±0.60; day 1: breast 8.60±0.72, leg 7.34±1.47; day 14: breast 18.40±4.39, leg 13.15±2.44 – figures are means ± SD of 6 animals).

The protein subunit composition of myofibrils and ERM fractions was analysed after separation by SDS-PAGE electrophoresis (results not shown). The protein subunit patterns showed that conditioning proceeds fastest in chicken and slowest in beef, with pork intermediate in rate, as evidenced by (a) the rate of appearance of 24-30kDa components, (b) the increasing ratio of actin to myosin, and (c) proteolysis of protein with subunit molecular weights in excess of 200,000kDa. The increasing yields of ERM during conditioning reflect the progressive weakening of the links, at the Z line and elsewhere, that hold the peripheral myofilaments in the myofibril.

ERM fractions were also examined after negative staining in the transmission electron microscope. The ratio of thin to thick myofilaments in ERM preparations increased over the conditioning period in each of the 3 species. This was particularly evident in the case of chicken where, after 14 days conditioning, one had to search diligently to find a thick myofilament such was the relative abundance of thin myofilaments. Typical figures for the ratio of thin to thick myofilaments in chicken breast ERM preparations are: at-death (1.2:1); day 1 (4:1); day 7 (9:1). Figures 2 and 3 illustrate typical fields of view in the electron microscope of ERM prepared from chicken breast muscle after 1 and 7 days conditioning respectively.

Conclusion

This study has shown that ERM yields are a sensitive index of the progress of conditioning in 3 principal meat animals.

Pertinent Literature

(1) Lawrie, R.A. Meat Science (Pergamon Press, 1991).
(2) Koohmaraie, M. Meat Science, 36, 39 (1994).
(3) Dayton, W.R., Goll, D.E., Stromer, M.H., Reville, W.J., Zeece, M.G., Robson, R.M. In: Proteins and Biological Control, 551-577, Cold Spring Harbour Laboratory, (1975).
(4) Van der Westhuyzen, D., Matsumoto, K., Etlinger, J. J. Biol. Chem., 256(22), 11791, (1981).

(5) Murray, B.E., Reville, W.J., O'Sullivan, S., Tighe, A., Kennedy, A., Zeece, M.G. Proc. 23rd FEBS Mtng. Basel, P14.8, August 1995.
(6) Reville, W.J., Ahern, S., Murray, B.E., Zeece, M.G. Sciences des Aliments, 14, 431, (1994).
(7) Lowry, G.H., Rosenbough, N., Farr, A.L., Randall, R. J. Biol. Chem., 193, 265, (1951).
(8) Bradford, M.M. Anal. Biochem. 72, 248, (1976).
(9) Takahashi, K. Proc. 45th Int. Congr. Meat Sci. Technol., Yokohama, Japan, pp. 230-235, (1999).

Acknowledgements

Ms. Mary Heapes and Ms. Aisling Ní Mhurchú, Electron Microscopy Unit, University College, Cork, assisted with the preparation of the figures.

This research was funded through the Irish Department of Agriculture Food and Forestry non-commissioned research programme.

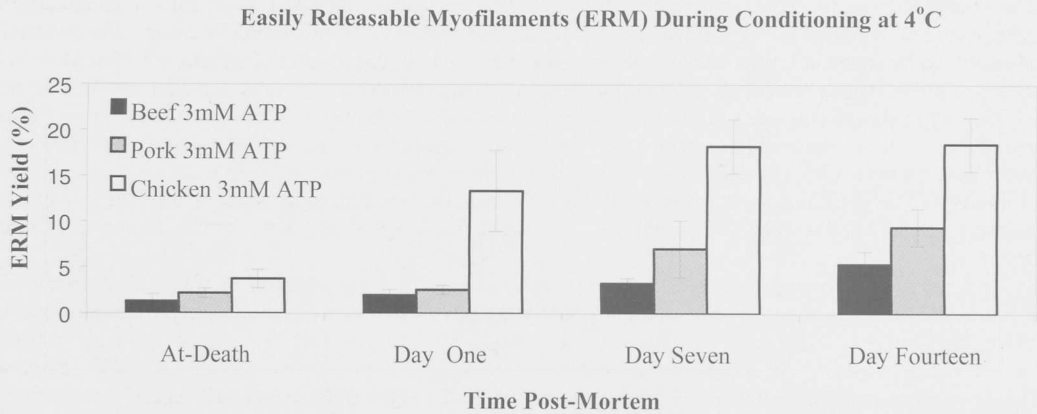

Figure 1. Yields of ERM measured at-death and after 1, 7 and 14 days post-mortem storage at 4°C of bovine sternomandibularis muscle, porcine psoas major muscle and chicken pectoralis major muscle. Yields are means ± SD expressed as a percentage of total myofibrillar protein. 10 cattle, 6 pigs and 6 chickens were used in the study.

Figure 2. Transmission electron micrograph of negatively stained (1% uranyl acetate) chicken breast muscle ERM prepared after 1 day post-mortem storage of muscle at 4°C. Thick and thin myofillaments are seen in roughly equal abundance. Magnification 40,000x.

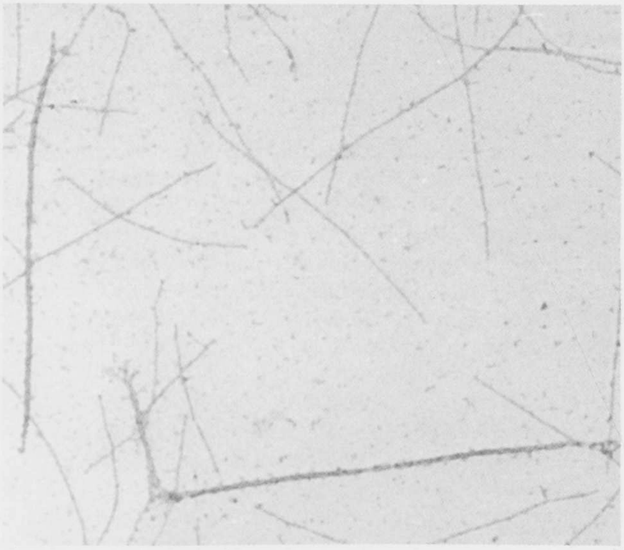

Figure 3. Transmission electron micrograph of negatively stained (1% uranyl acetate) chicken breast muscle ERM prepared after 7 days post-mortem storage of muscle at 4°C. Thin myofilaments are now seen in greater abundance than thick myofilaments. Magnification 40,000x.