USING DENSITY TO MEASURE MYOFIBRILLAR DENATURATION

P. M. Dobbie, S. J. Falconer, N. J. Simmons, C. C. Daly
AgResearch, Ruakura Agricultural Research Centre, Private Bag 3123, Hamilton, New Zealand

Background

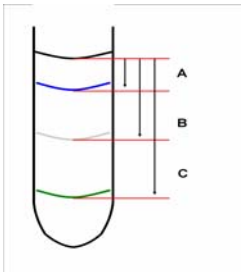
Excessive denaturation of myofibrillar proteins during the post mortem period can have important implications for meat quality. Denaturation of myofibrillar proteins by conditions of high temperature and low pH during, particularly during the pre-rigor period, causes exaggerated shrinkage of the myofibrillar lattice due to reduced electrostatic repulsions between the thick filaments (Offer, et al, 1983. Offer & Knight, 1988). Lattice shrinkage contributes to a paler meat colour, due to increased reflectance, and increased drip loss due to expulsion of fluid from the intra-lattice space. Although the implications of myofibrillar denaturation are well recognised in pork as the PSE condition (Offer & Knight, 1989), similar consequences are reported in beef (Simmons et al, 2000).
Current methods for quantifying myofibrillar denaturation have limitations: direct measurement of the myofibrillar lattice is complex and slow (Offer, et al 1989; Smulders et al., 1990.); reduced myofibrillar solubility is, in our experience, relatively insensitive inconsistent; and myosin ATPase activity is indirect and influenced by unrelated events such as proteolysis (Ouali, 1990). We describe here a simple method to measure denaturation through changes in myofibrillar density.

Objectives

Current methods for measuring directly the changes in myofibrils post mortem are slow and difficult. We set out to develop a simple method of measuring myofibrillar denaturation through changes in myofibrillar density.

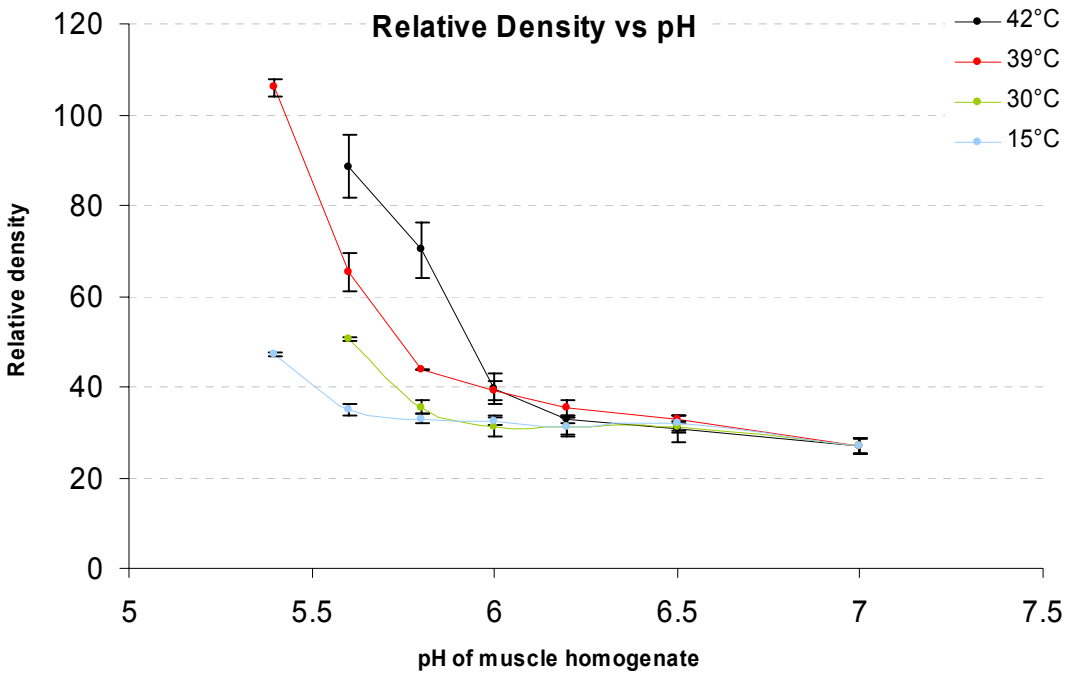
Materials and methods

Beef longissimus samples were held at constant pre-rigor temperatures of 15, 30, 39 and 42°C.
When the pH of the meat reached 7.0, 6.8, 6.5, 6.2, 6.0, 5.8, 5.6 and 5.4; 5 gram samples were taken. These 5 gram samples were Ultra-Turrax homogenised for 20 second at 13500rpm in 30ml of pH 5.3 homogenisation solution, (250mM.Sucrose; 10mM.KCl; 1mM.EDTA, pHed to 5.3 with KOH).
0.5 ml of the homogenate was centrifuged @ 6000g for 2 min and the supernatant discarded. The remaining pellet was washed with 1.0 ml of homogenisation solution, centrifuged and the resulting washed pellet re-suspended in 0.5 ml homogenisation solution.
15 µl of washed, re-suspended homogenate was then thoroughly mixed with 1.5 ml of a 30% Percoll:Sucrose:KCl:Triton (Ratio 29:23:47:1) gradient solution containing 10 µl of Amersham Biosciences density marker beads (mixture of blue (1.042 g/ml) and green (1.063 g/ml)) in the same tube. This mixture was centrifuged at 18,000g for 30 min.
The tube was then carefully removed from the centrifuge and distance from the bottom of the meniscus to "A" Blue marker bead, "B" myofibrils and "C" Green marker bead layers was measured.



Results were expressed as a Ratio of $\frac{B-A \times 100}{C-A}$

Results and discussion



Increasing the pH of the Percoll gradient decreased the density of the myofibrils. A higher pH increases the electrostatic charge on the filaments and, consequently, the lattice spacing (Offer & Knight). The measured myofibrillar density therefore probably reflects the extent to which denaturation events has modified the amount of charge on the myofibrillar filaments.

Varying denaturing conditions were produced by maintaining beef muscle samples in different constant temperatures during the pre-rigor temperature. Because the rate of glycolysis, and hence pH decline, is temperature-dependent, the changes in myofibrillar density during the pre-rigor period were measured at equivalent pH values. The results clearly demonstrate that denaturation depends on the meat pH reaching values below pH 6. Furthermore, a very extreme temperature dependency was evident: although the density at ultimate pH in the 30C samples was significantly greater (?) than the 15C samples, a further 9C increased produced a very dramatic increase in density.[This next sentence assumes that the density at pH 6 is significantly greater at 39 than 42 – exclude otherwise: The significantly greater density at 39C compared with 42C when measured at pH 6 can probably be attributed to the slower rate of pH decline at the lower temperature, thereby allowing more time for denaturation to occur event though the kinetics will be slower at the lower temperature.]

Conclusions

A simple methodology is described to measure changes in myofibrillar density that can be attributed to denaturation events. The procedure can be carried out at any stage in the pre-rigor period, is quantitative and is not affected by proteolytic events.

References

Offer, et.al.1983 Offer, G., Restall, D., Trinick, J. Water-Holding in Meat. In; *Recent Advances in the Chemistry of Meat. Bailey, A.J Ed. Royal Soceity of Chemistry*

Offer and Knight, 1988. G. Offer and P. Knight, The structural basis of water-holding in meat. *R.A. Lawrie, Editor, Developments in Meat Science—4, Elsevier Applied Science, London (1988), pp. 63–243.*

Offer and Knight, 1989. Offer, G.; Knight, P.; Jeacocke, R.; Almond, R.; Cousins, T.; Elsey, J.; Parsons, N.; Sharp, A.; Starr, R.; Purslow, P. The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstructure , 1989 , 8 (1) 151-170*

Ouali 1990. Ouali, A. *Journal of Muscle Foods 1 (1990) pp. 129-165*

Simmons et al, 2000. Simmons, N.J., Daly, C.C., Gilbert, K.V.2000 *Proceedings of the New Zealand Society of Animal Production, 60, 39-43*

Smulders et al., 1990. Smulders, F.J.M. ; Marsh, B.B.; Swartz, D.R.; Russell, R.L.; Hoenecke, M.E. Beef tenderness and sarcomere length *Meat science. 1990. v. 28 (4) , p. 349-363.*