3:13 Variable response of beef myofibrils to salt solutions

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Introduction

Meat at slaughter is about 75% water, but this value can change considerably in subsequent handling. Losses of water occur as evaporation, drip and during cooking. Gains of up to 40% occur as a result of the treatment of meat with saline solutions during the processing of meat to sausages, burgers and hams, and much of this added water is retained on cooking. Polyphosphates are often also added because they are found to act synergistically with sodium chloride in causing water uptake and in addition they promote the formation of the sticky exudate that binds meat pieces together, especially on cooking. It is believed that pyrophosphate is the active component of polyphosphates (Hamm and Neraal, 1977).

As a result of their study of the behaviour of isolated myofibrils, Offer and Trinick (1983) proposed the hypothesis that changes in the water content of meat originate from changes in the volume of myofibrils. They had observed by phase contrast light microscopy that myofibrils isolated from rabbit psoas muscle take up water by lateral expansion in the presence of salt, and that if pyrophosphate is added, swelling occurs at a lower salt concentration, with a greater extraction of protein from the A-band. They also observed considerable variability in the amount of swelling and A-band extraction, particularly between preparations of myofibrils, suggesting that myofibrils contribute to the variability that is found in industry in the behaviour of meat during processing.

We have extended the work of Offer and Trinick by addressing the following questions: (1) do the results from rabbit apply to other species such as beef? (2) is the amount of swelling found in beef myofibrils adequate to account for the water uptake by processed beef? (3) does pyrophosphate affect the maximum water uptake by myofibrils achieved at high salt concentrations? (4) what is the cause of the variability in swelling and in A-band extraction?

Materials and Methods

Preparation of myofibrils

5 mm diameter strips of beef sternomandibularis muscle were obtained immediately post mortem from a 30 month Friesian bull and an 18 month Friesian heifer, tied onto plastic sticks at a sarcomere length of about 2.7 um, vacuum packed and held at 10°C for 24 h. Myofibrils were prepared by homogenizing at top speed in an MSE homogenizer 0.5 g of chopped muscle in 5 ml 0.10 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM dithiothreitol, 10 mM K phosphate, pH 7.0 at 5°C. To ensure the production of a representative mixture of myofibrils from this tough muscle, the myofibrils released by the first 5 minutes of homogenization were separated from the larger material by centrifugation and this material rehomogenized for one minute to liberate more myofibrils. Five cycles of homogenization and centrifugation were used to break down all the fibres to myofibrils. Myofibrils were washed with the same buffer and stored in ice.

Light microscopy

Offer and Trinick (1983) observed myofibrils that had adhered to the glass coverslip. We wanted to be sure that the myofibrils we studied were freely bathed on all sides, without the impediment to swelling that might arise from binding to the coverslip, because this could cause spurious variability in the results. Therefore myofibrils were usually observed suspended between the bars of a gold electron microscope grid attached to the underside of the glass coverslip. Salt solutions were drawn between the slide and coverslip from a pool on one side of the coverslip by a piece of filter paper touching the opposite side. The myofibrils were viewed with a 100 x phase contrast objective, and recorded photographically at a magnification of 430 x. Many individual myofibrils were irrigated with a series of increasing salt concentrations from 0.1 M to 1.0 M NaCl, with or without 10 mM pyrophosphate, in 1 mM $MgCl_2$, 10 mM Na acetate pH 5.5 at about 20°C.

Results and Discussion

(a) Qualitative behaviour of beef myofibrils

When treated with salt, many beef myofibrils behave like those from rabbit: swelling occurs mainly between 0.5 M and 1.0 M NaCl, and there is extraction of A-band material especially from the central H-zone (Fig. 1).

The Z-line is sometimes also lost. The addition of pyrophosphate to the salt solutions has effects on beef myofibrils similar to its effects on rabbit myofibrils: swelling typically begins below 0.5 M NaCl and a greater fraction of the A-band is extracted than in salt alone especially at its edges (Fig. 2).

Thus it is clear that myofibrils can be the sites of water uptake in beef muscle during processing, and the A-band material could furnish the sticky exudate.

(b) Quantitative behaviour of beef myofibrils

To study the swelling of beef myofibrils in more detail, we have estimated the change in volume of myofibrils following salt treatment. Because the sarcomere length does not change on salt treatment, we are able to do this by measuring myofibril diameter. We corrected our measurements for the errors arising from viewing such small objects in the light microscope by constructing a calibration curve using glass fibres of similar, known width. The fractional change in volume was computed as (final diameter^2 - initial diameter^2)/initial diameter^2. The precision of the method was judged by comparing measurements from pairs of micrographs for which there had been no change of irrigation solution. Apparent changes in volume were small and clustered around zero, with a standard deviation of 0.085.

Our initial results for the swelling of the A-band (Fig. 3) show that a broad range of swellings is observed both in salt alone and with salt plus pyrophosphate. In contrast to the observations of Offer and Trinick (1983) on rabbit myofibrils where a two fold increase in volume was usual, many beef myofibrils did not swell at all, even in 1.0 M NaCl, and the maximum overall swelling was a fourfold increase in volume. The overall average increase in volume on irrigation to 1.0 M NaCl was about 40% whether pyrophosphate was present or absent. This is sufficient swelling for myofibrils to be the sole sites of water uptake in salt-treated beef.

More detailed inspection of Fig. 3 shows the effect of pyrophosphate on swelling. At 0.5 M NaCl, although many fibrils do not swell (Fig. 3a), there are significantly more that swell appreciably when pyrophosphate is present (Fig. 3c). Thus pyrophosphate acts to promote A-band swelling at this salt concentration. It is apparent from comparing Fig. 3 (b) and (c) that on raising the salt concentration still further, to 1.0 M, pyrophosphate no longer promotes swelling; instead there are greater volume increases in myofibrils treated with salt alone. The result is that the overall swelling from 0.1 M to 1.0 M NaCl is unaffected by pyrophosphate. The action of pyrophosphate on A-band swelling thus appears to be to promote swelling of some myofibrils, but it does not increase the maximal extent of swelling (in 1.0 M NaCl), or induce a greater fraction of the myofibrils to swell than would swell in high concentrations of salt alone.

(c) Variable behaviour of beef myofibrils

It is clear in Figs 1 to 3 that beef myofibrils do not all behave identically when treated with salt. Within a single field of view, some myofibrils can be seen to double in volume while others do not swell at all (e.g. arrows in Fig. 1 and the spread of data in Fig. 3); whilst the A-bands are largely removed from some myofibrils, in others they appear resistant to extraction (arrows on Fig. 2). The Z-discs sometimes expand as much as the A-bands, but sometimes only the A-bands swell producing a corrugated outline. The extent of Z-disc removal also varies. It is important to note that the sarcomeres of a single myofibril all behave in the same way, the variability being between myofibrils. Our data stand in contrast with those of Offer and Trinick (1983) who reported variability between preparations rather than within preparations. Because variability is found in fresh preparations, conditioning is unlikely to be its cause. Sternomandibularis contains a mixture of different fibre types and the myofibril preparation will therefore also be a mixture. If myofibrils from different fibre types differ in their response to salt treatment, this could explain the variability that we

observe, and would suggest a cause for the variability in the suitability of meat for processing that is observed in the meat industry.

Acknowledgements

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References

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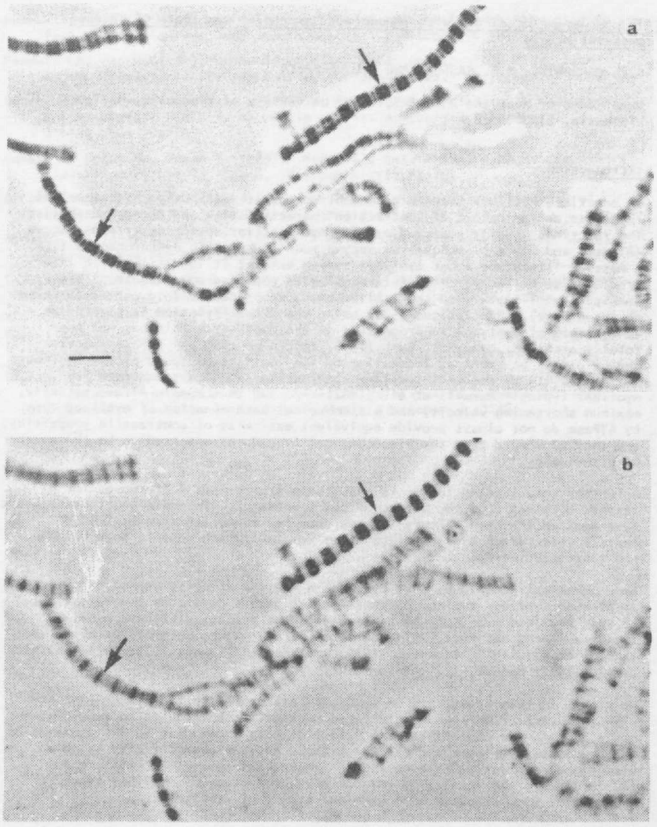

Figure 2. Effect of salt + pyrophosphate on beef myofibrils. Irrigation was with solutions containing 10 mM Na pyrophosphate and (a) 0.1 M NaCl followed by (b) 0.5 M NaCl. Compare the appearance of the myofibrils labelled by arrows. Scale bar in (a) indicates 5 μm.

Figure 1. Effect of salt on beef myofibrils. Irrigation with solutions containing (a) 0.1 M NaCl followed by (b) 1.0 M NaCl. Compare the appearance of the myofibrils labelled by arrows. Scale bar in (a) indicates 5 μm.

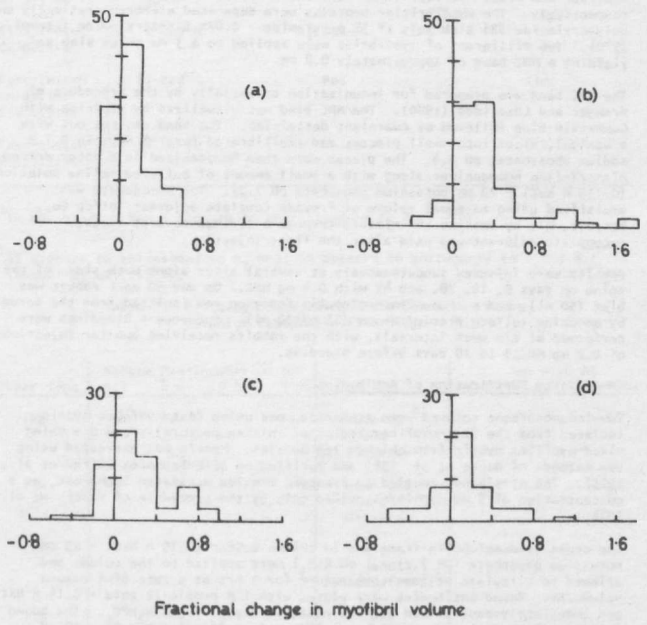

Figure 3. Histograms to show the fractional change in volume of beef myofibrils on changing irrigation solution from (a) 0.1 M to 0.5 M NaCl or (b) 0.5 M to 1.0 M NaCl, both without pyrophosphate; (c) 0.1 M to 0.5 M NaCl or (d) 0.5 M to 1.0 M NaCl both in the presence of 10 mM pyrophosphate. All at pH 5.5 and 20°C.