PRELIMINARY RAMAN SPECTROSCOPIC INVESTIGATION OF THE CHANGES IN BEEF STERNOMANDIBULARIS MUSCLE DURING THE THAWING OF MUSCLE STRIPS FROZEN PRE-RIGOR.

J. Rene Beattie[a], Steven J. Bell[a], Bruce W. Moss[b,c]

[a] School of Chemistry, Queen's University Belfast, Northern Ireland BT9 5AG
[b] School of Agriculture and Food Science, Queen's University Belfast, Newforge Lane, Northern Ireland BT9 5PX
[c] Department of Agriculture and Rural Development for Northern Ireland, Agriculture and Food Science Centre, Newforge Lane, Belfast , Northern Ireland BT9 5PX

Background

The potential of Raman spectroscopy in the analysis of foods has long been known, mainly due to the high information content (which can be understood in terms of chemical and physical parameters) which can be obtained without sample destruction and on any physical state, even in the presence of water. Very few investigations have been carried out on the application of Raman spectroscopy to the study or analysis of meat since Asher *et al* (Asher, et al 1976) first published a short account of the Raman spectrum of whole muscle. A number of studies in the mid 1980s investigated contraction of single fibres and various purified myofibrillar protein (Pézolet et al 1988). However, much of the work has concentrated on simplified systems and only recently has any work been attempted on bulk meat tissue (Beattie et al, 2004;Brondum et al 2000;Pedersen et al 2003).

The background understanding of the factors governing the Raman spectrum of meat is very limited so that it is important to extend the investigations of intact samples to conditions which are somewhat distant from conventionally processed consumer beef but where the data are easier to interpret. During the rigor process there are biochemical changes due to post mortem glycolysis (eg ATP depletion, lactate accumulation) and also changes in protein conformation due to the formation of actomyosin. Muscles frozen pre rigor when thawed go into rigor at a much faster rate and with a greater degree of contraction than normal rigor (Lawrie 1998). Davey & Graafhuis (1981) used thaw rigor as a technique for the early prediction of ultimate pH, since this was achieved within 10 to 20 minutes of thawing the samples. Thaw rigor provides a potential technique for studying boith the biochemical changes and contraction together by allowing samples to contract whilst thawing and the biochemical changes independent of contraction by securing the muscle to prevent contraction during thawing. The latter procedure should be similar to the normal rigor process where little contraction is observed aas the muscle goes into rigor.

Objectives

To study the changes in Raman spectra as strips of beef *sternomandibularis* muscles frozen pre rigor go into rigor on thawing in conditions which allow and prevent physical contraction occurring.

Materials and methods

Experimental

Samples of *m. sternomandibularis* were dissected from the neck at 30 min *post mortem* from 6 animals. From each muscle sample a number of strips 5 mm thick, 10 mm wide and 60 mm long were cut, wrapped in aluminium foil and placed in liquid nitrogen within 10 minutes of dissection.

Raman Spectroscopy

Raman spectra were recorded using the 785 nm excitation wavelength Raman spectrometer previously described (Beattie et al., 2004). Removal of the 'fluorescence' background was effected in SpectraCalc ™ where the multiple point baseline correction program was modified to allow the positions used for baseline points to be fixed. The initial and final spectra were averaged then subtracted to elucidate the variation between spectra recorded pre-thaw *rigor* and post thaw *rigor*.

The samples rapidly dissected upon removal from the freezer to give a fresh cut surface. Typically recording commenced *ca.* 8-10 min after removing from the freezer, while defrosting was complete *ca.* 12-15 min after

removal. Spectra were recorded for 1 min with a 20 second delay before recording the subsequent spectrum. The precise start time of each spectrum was recorded and every three spectra the length of the muscle was measured. For the thaw *rigor* studies the sample was not moved from its location. Half of the sample strips were loosely clipped on the rotating stage to allow contraction to occur, while half of the strips were firmly clamped at each end to prevent significant amounts of contraction.

The presence of collagen in the beef samples was determined by the band intensities at positions which are known to correspond to various modes of proline and hydroxyproline (Beattie 2002). Any samples showing exceptionally large changes in collagen within the spectra upon contraction were eliminated, to allow clearer interpretation of the changes affecting the myofibrillar proteins. Subtracted Raman spectra were generated to elucidate the changes in the Raman spectrum during thaw *rigor*. For the unclamped sample set this subtracted spectrum was produced by averaging all the samples prior to the onset of contraction, those after contraction had completed and performing a spectral subtraction normalised on the phenylalanine band at 1003 cm^{-1}, which is widely recognised as being insensitive to environmental factors. A residual band was evident at a slightly higher wavenumber positions, but was sufficiently weak to not interfere with the normalisation. For the clamped data set, the spectra recorded at the same times when contraction started and had completed in the unclamped samples were selected for averaging and subtracted as before.

A sample of silverside aged for 21 days (Beattie,et al., 2004) was used to compare the effects of sample orientation relative to the incident beam by mounting the sample with the myofibrillar long axis parallel and perpendicular to the incident beam.

Results and discussion

Figure 1 shows the average Raman spectrum of the pre-thaw *rigor* samples, with some bands of interest marked with a number referring the table of band assignments (Table 1).

The subtracted Raman spectrum from the unclamped data set is shown in Figure 2a. There are large changes in the regions known to be sensitive to the secondary and tertiary structure of proteins, the amide I (1640-1685 cm^{-1}), amide III (1225-1305 cm^{-1}) and ν(C-C) region (880-1020 cm^{-1}). One possible explanation of changes in these regions is typically that there is widespread conformational change within the proteins of the sample. However, there is another explanation which might apply in this particular case due the very high degree of orientation involved in the myofibrillar proteins and that is that the Raman spectra might have changed due to reorientation. It is well known that the Raman spectrum of highly oriented molecules can look different depending on their orientation relative to the incident radiation. Figure 2b shows the subtraction Raman spectrum obtained by subtracting the Raman spectrum of aged beef acquired with the myofibrillar long axis parallel to the beam away from the Raman spectrum acquired with a perpendicular orientation. Comparing Figure 2 a and b shows that there is a striking similarity in many areas, which would suggest that much of the changes occurring in the three regions highlighted above may be arising from reorientation of the myofibrillar proteins.

The average contraction observed was 60 % of the original muscle length, the level of contraction at which the myosin pushes against the Z-disk that hold the actin in place. Since both major proteins (actin and myosin) overlap fully and the anchor proteins are being perturbed, it is reasonable to assume much of the protein will be distorted, causing widespread reorientation with respect to the myofibrillar long axis. Thus it seems reasonable to assign the changes in the amide and ν(C-C) regions to reorientation of the myofibrillar proteins rather than large scale conformational changes.

Figure 2c shows the subtracted Raman spectrum of the clamped samples, and it is readily noticeable that the effect of thaw *rigor* on the amide and ν(C-C) regions is significantly different from the unclamped samples. The amide I and ν(C-C) regions are noticeably weaker for the clamped samples, suggesting a much slighter change in orientation. This is not surprising as only a small amount of overlap occurs between the actin and myosin so only a few myosin S2 heads are reorienting, along with the various other minor proteins involved in the binding of myosin to actin. If the predominant effect had been conformational change, rather than orientation, it would be expected that the spectra of the clamped and unclamped data set would be much more similar. The fit between the orientation subtraction spectrum and the unclamped thaw rigor is expected since the effect of extreme contraction would be that the myofibrillar axes would be buckled and instead of recording the Raman spectrum using a beam which truly perpendicular to the fibre axis we are recording some of the fibres at an angle to that axis.

While there are clear differences between the amide and ν(C-C) regions of the Raman spectra of the clamped and unclamped data sets, there are also some bands which show similar changes between the two data sets. These bands are marked in Figure 2 by the dashed vertical lines. These bands are all ones that are known to be affected the polarity of the environment of the protein. In the clamped data set the $CH_{2\ sc}$ band at 1450 cm^{-1}, which is negatively correlated with hydrophilicity, shows a strong decrease, which indicates a strong increase in the polarity of the protein environment. This result is expected as the massive contraction observed in thaw *rigor* is caused the release of large amounts of calcium salts, which will significantly increase the ionic strength of the myofibrillar matrix, thus the polarity of the proteins' environment. The same band at 1450 cm^{-1} is observed to increase, contrary to the expectation of the environmental changes, but in line with the changes to the orientation of the myofibrils, showing that the reorientation has a stronger effect on that band than the environmental effects.

Conclusions

We have demonstrated that the Raman spectrum of meat is affected by the process of thaw *rigor*. When the sample is unrestrained and able to contract freely, these changes can primarily be attributed to the reorientation of the myofibrillar proteins. When the sample is restrained so that significant amounts of contraction are not possible, it was found that the effect of reorientation was reduced and that the increase in ionic strength during thaw *rigor* affected the environmentally sensitive bands.

References

Asher, I.M., Carew, E.B., & Stanely, H.E. (1976). Laser Raman spectroscopy: A new probe of the molecular conformations of intact muscle and its components. In *Physiology of smooth muscle*, E. Bulbring & M.F. Shuba, eds. New York: Raven, pp. 229-238.

Beattie, J.R. (2002). Application of Raman spectroscopy to meat composition and quality. PhD thesis, The Queen's University of Belfast, Belfast.

Beattie, R.J., Bell, S.E.J., Farmer, L.J., Moss, B.W., & Desmond, P.D. (2004). Preliminary investigation of the application of Raman spectroscopy to the prediction of the sensory quality of beef silverside. *Meat Science 66*, 903-913.

Brondum, J., Byrne, D.V., Bak, L.S., Bertelsen, G., & Engelsen, S.B. (2000). Warmed-over flavour in porcine meat - a combined spectroscopic, sensory and chemometric study. *Meat Science 54*, 83-95.

Davey & Graafhuis (1981) Early indication of the DFD condition in pre rigor beef carcasses. in Problem of Dark Cutting Beef. Ed D.E. Hood & P.V. Tarrant 1981 Martinus Nijhoff Publishers

Lawrie, R .A. (1998) Meat Science , Woodhead Publishing Ltd , Cambridge , England

Pedersen, D.K., Morel, S., Andersen, H.J., & Engelsen, S.B. (2003). Early prediction of water-holding capacity in meat by multivariate vibrational spectroscopy. *Meat Science 65*, 581-592.

Pézolet, M., Pigeon, M., Ménard, D., & Caillé, J.P. (1988). Raman spectroscopy of cytoplasmic muscle fiber proteins. Orientational order. *Biophysical Journal 53*, 319-325.

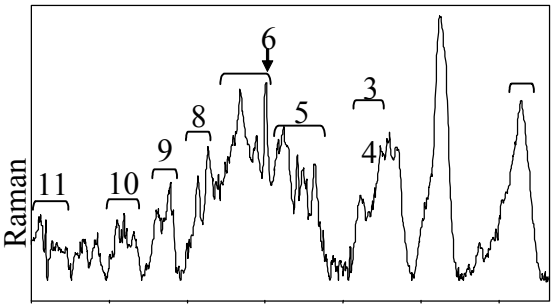

Figure 1 Average Raman spectrum of pre-thaw rigor *m. sternomandibulasris*. Peaks of interest are number as for

No	Band Position/ cm^{-1}	Comments
1	1640-1685	Amide I – COONH.
2	1450	CH_2 scissor.
3	1225-1305	Amide III
4	1260	His, tautomer II.
5	1020-1130	C-C, N stretch.
6	1003	Phe ring stretch.
7	880-1020	C-C stretching bands
8	820-860	Tyr
9	600-800	Cys and Met
10	500-570	Cys-Cys and skeletal
11	410-480	Pro and Hyp bands

Table 1 Assignments of the main peaks of interest in the Raman spectra of meat and muscle. The band numbers refer to the average spectrum of aged pork meat in Figure 1.

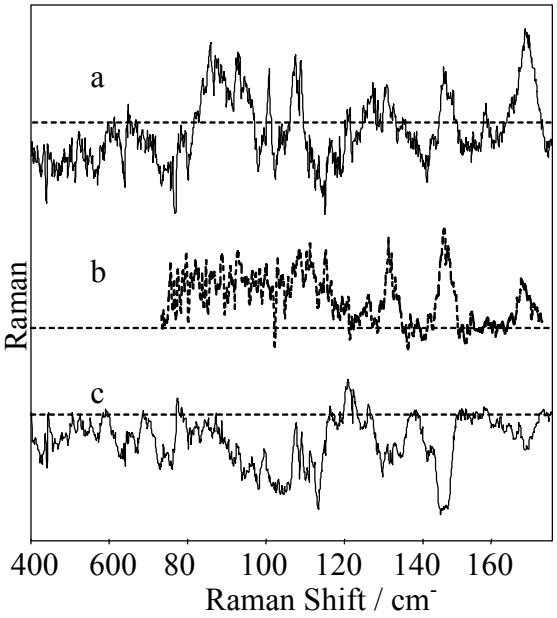

Figure 2 Effect of the development of thaw rigor on the Raman spectrum of beef which has a) not been clamped and c) been clamped. b) subtraction spectrum of parallel and perpendicular spectra of aged beef.