IMAGING OF INTACT OVINE *M. SEMIMEMBRANOSUS* BY CONFOCAL RAMAN MICROSCOPY

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Abstract – Chemical imaging of intact lamb using Raman microscopy is a useful technique which can provide insight about the differences in chemical composition and spatial orientation of the myofibril in its native state. This paper explores the potential for Raman microscopy to further understand the variation in tenderness of lamb semimembranosus (n = 26). Preliminary investigation demonstrated that differences in vibrations pertaining to tryptophan, tyrosine, α-helix, C- H deformation and the amide I and III bands can be distinguished between tough (53 – 74N) and tender (26 - 36N) lamb using spectral mapping. Furthermore, a new method of chemical image analysis is proposed to facilitate the comparison between multiple chemical images of complex samples and to determine the heterogeneity of the chemical vibrations within a single chemical image.

Keywords- Raman microscopy, shear force, sheep muscle

I. INTRODUCTION

It is well established that tenderness is a critical factor in determining meat eating quality and consumer acceptance of meat products. As tenderness is determined by the interactions between myofibrils and the connective tissue matrix as well as the extent of myofibrillar degradation during ageing, much research has focused on the ability of technologies to objectively measure tenderness [1]. Of these technologies, Raman spectroscopy has been highlighted as having potential, as it is rapid, non-destructive and not sensitive to varying water content [2]. Meat science research has not over looked these advantages and recently studies have applied Raman spectroscopy to investigate the effects of ageing and cooking on pork loin [3], predict sensory quality of beef silverside [4], shear force of frozen and thawed lamb loin [5] and fresh intact lamb semimembranosus [6]. However, as meat is complex, changes in Raman spectra that reflect the variation in tenderness have not yet been fully characterised. Confocal Raman microscopy is an ideal tool for elucidating the composition and structure of cells in their native state [7]. This paper proposes a new approach for using Raman confocal microscopy and chemical imaging to determine the chemical and spatial differences in Raman spectra of ovine *m. semimembranosus* (SM) and the potential for spectral mapping and chemical imaging to further understand variations in tenderness of ovine topsides.

II. MATERIALS AND METHODS

At 1 day post slaughter, a SM was removed from each of 80 carcases over 4 consecutive days (20 per day) from the same abattoir. Carcases were randomly selected and were from different consignments, thus were of different backgrounds, ages and gender to represent animals typically processed by the abattoir to obtain a spread of shear force values. A section of 1-2g also was removed at 1 day post mortem, fixed in gluteraldehyde and paraformaldehyde as previously described [8]. After further sections were removed to determine pHu [9] and sarcomere length [10], SMs were vacuum packed then aged for 4 days at -1ºC.

At 5 days post mortem, sections were excised (mean 65g) for shear force and cooking loss measurement [11]. Sections were also removed to determine collagen content [12].
Samples were ranked using the shear force values at 5 days post mortem, and of these 13

of the most tender (26.3 - 36.0N) and 13 of the toughest (53.2 - 74.3N) were cut and set, as previously described [8] onto gold slides. Raman microscopic measurements were conducted on these set samples using a WiTec Raman microscope with 80mW of laser power and a 3.45 sec integration time. Spectral wavenumber values and intensities were mapped over a 40µm area (20µm x 20 µm) of the myofibril using WiTec project software and a 50x microscopic image of the scan area was taken [13]. Band assignments for individual spectra were completed using Opus software [14].

III. RESULTS AND DISCUSSION

Chemical images of intact ovine SM generated using the total integrated intensity of Raman bands between 500- 2100cm^{-1} (Fig. 1), which represent chemical bonds found in meat [3] gives a good overview of the morphological differences between the tough (Fig 1; A) and tender (Fig 1; B) samples. Based on these example chemical images, spatial changes of chemical bond intensities between tender and tough samples can be elicited using Raman microscopy. Spectral changes observed in the tender sample (Fig 1; B) suggests that the ultra-structural degradation of myofibrillar proteins which results in myofibrillar detachment and leads to changes to the topography of the sample. However, the same is not observed in the tough sample (Fig 1; A). Changes to the spatial intensities of the tough sample may be attributed to the connective tissue matrix which does not degrade during the ageing period and is responsible for background toughness [15].

Extracting underlying spectra (Fig 2), suggests that the orientation of collagen could be responsible for these spectral changes of the tough sample, as the angle of polarisation affects the ratio of intensity between the amide I and III bands at approximately 1300 and 1650cm^{-1} [16]. Furthermore, a shift in the C-H deformation vibration of the tough sample denotes a change in the number of C-H bonds which are stabilised by C-N bonds rather than C-O bonds [17]. This may reduce the ability of proteolysis to cleave these bonds, resulting in less myofibrillar degradation.

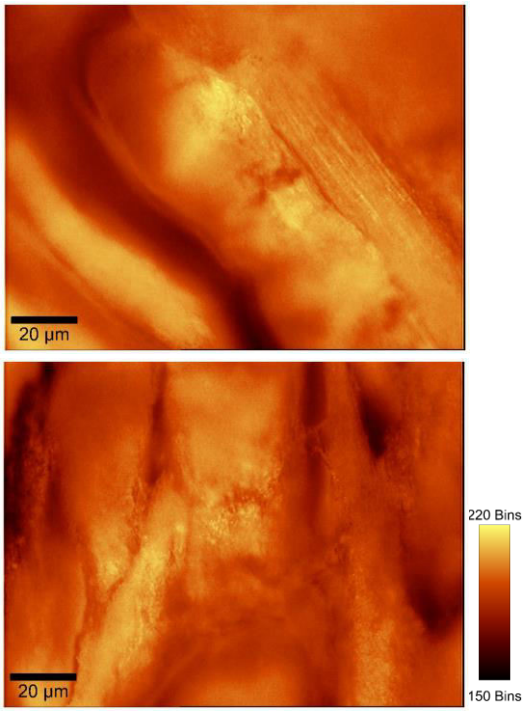

Figure 1 An example of a chemical image of tough (74N; A) and tender (26 N; B) ovine *semimembranosus* showing the morphological differences.

Shifts in amino acid side chain vibrations at approx. 750cm^{-1} as well as 820 and 853cm^{-1} that characterise the vibrations of tryptophan and tyrosine respectively [18], suggests that the ability of these chemical bonds to destabilise during ageing is also reduced in the tough sample.

While this information provides some indication of the spatial and chemical changes between the two examples presented in this paper, it is difficult to ascertain whether the proposed spatial changes and tentative band assignments will be valid across the chemical images for the entire data set.

A limitation in generating single chemical maps that there is no direct way to compare between chemical images of different samples of lamb SM. As intact lamb muscle is complex, with many amino acid vibrations from a variety of proteins in concentrations and orientations, which are unique to each sample causing some spectral features to overlap it is difficult to determine changes in Raman spectra compared to a set

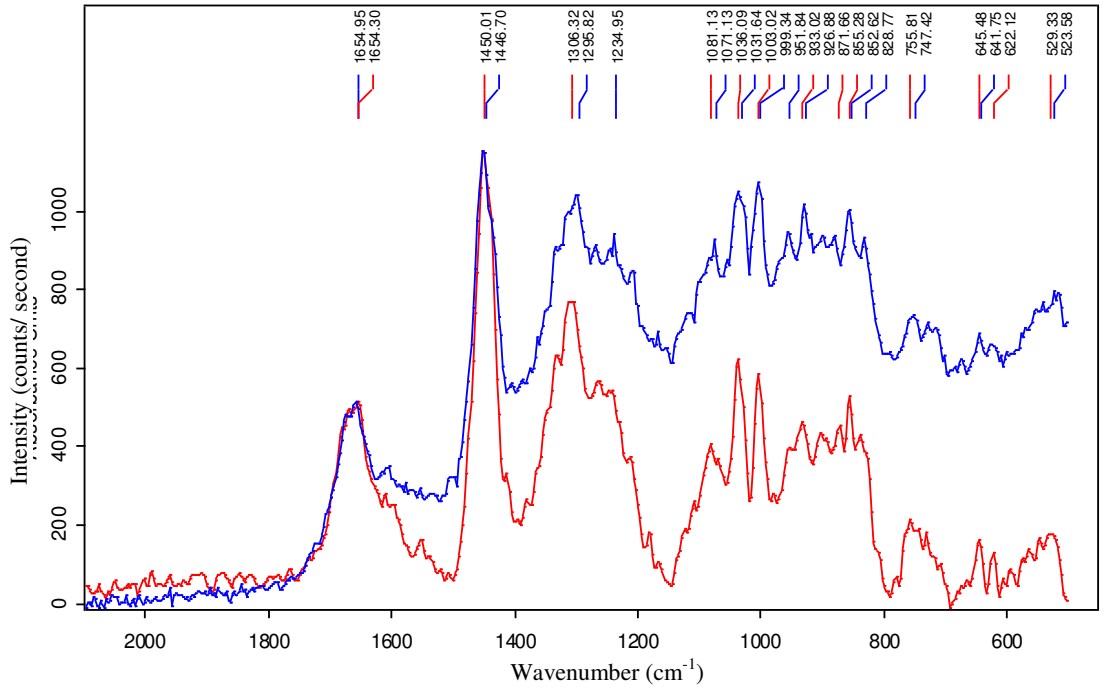

Figure 2. Raman spectra for tender (blue; 26N) and tough (red, 74N) lamb *m. semimembranosus* illustrating changes in key amino side chain vibrations (750, 826, 853cm^{-1}), α- helix (930cm^{-1}) and amide I (1300cm^{-1}) and III bands (1650cm^{-1}).

reference spectra. However, this information on the changes of spectra between samples holds the information needed to determine what chemical characteristics are linked to variation in tenderness.

One way of overcoming this limitation is to generate chemical images with restricted wavenumbers isolating the specific bands which have previously been identified as being important to the prediction of tenderness and shear force by previous Raman studies on meat. Such band wavenumbers would include the amino acid side chain vibrations of tryptophan (750 cm^{-1}), the tyrosine doublet (826 & 853 cm^{-1}) and the α- helix (930 cm^{-1}) as well as the peptide backbone vibrations of and the amide I and III bands (1245, 1268, 1300 and 1650 cm^{-1}) [3-6], illustrated in Fig 2.

These chemical images could then be analysed using image analysis software [19] to distinguish the proportion of the spectral maps which have a high and low intensity at these wavenumbers. By breaking down the Raman map through classifying areas with similar intensity range into a specific class (Fig 3), the total proportion of the area with the same intensity can be compared within and across samples. Proportions of the area across the myofibril associated with high and low intensities for the amino acid vibrations of tryptophan (750cm^{-1}) and the tyrosine doublet (826 & 853cm^{-1}) will modelled against shear force values, particle size analysis (measure of proteolysis) and sarcomere length, as previous studies have linked the intensity of these peaks with the concentration and structure of these amino acids in tough and tender meat [3,5,6].

Collagen concentrations could be related to the ratio of the amide I and III bands as these peptide backbone vibrations are characteristic of hydroxyproline, which is a typical constituent of connective tissue [5].

By conducting an analysis on the intensity ranges at the specific wavenumber targeting these amino acids, and peptide backbone structures, which have been linked to tenderness, the heterogeneity of these specific chemical vibrations across myofibrils at a micrometer level may be ascertained. This information could provide further explanation of the differences between the Raman spectra of tough and tender samples of lamb and why proteolysis is able to degrade some myofibrils and improve tenderness, more than others.

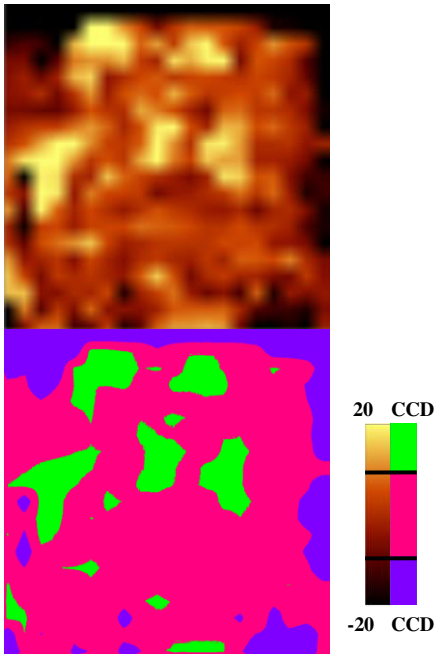

Figure 3. An example of a Raman chemical map for the Tryosine peak (826cm^{-1}) illustrating the classification of areas with high (green), moderate (pink) and low (purple) intensity.

IV. CONCLUSION

Chemical imaging using Raman microscopy is a tool which could be useful in determining what differences in composition and spatial arrangement of amino acids may contribute to the discrimination of tough and tender meat samples. Although current limitations exist in the application of Raman spectroscopy to complex samples of lamb using a combination of confocal Raman microscopy and image analysis, these limitations may be reduced and further insight into the causes of variation of meat quality could be provided.

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