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PRELIMINARY INVESTIGATION ON THE USE OF RAMAN SPECTROSCOPY TO CHARACTERISE ADIPOSE TISSUE.

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Background:

In general, human nutritional guidelines recommend a reduction in the intake of both total fat and saturated fat in the diet. Breeding programmes and dietary modifications have been used in attempts to modify animal fats to benefit the consumer. Characterisation of lipids by fatty acid composition, including *cis/trans* isomers *etc.* involves time-consuming extractions and preparation of methyl esters, followed by gas chromatography. For optimal separation of *cis/trans* isomers long capillary columns are required.

Infrared absorption spectroscopy is well-established in the analysis of oils and fats but also requires solvent extraction. Raman spectroscopic studies have shown that bulk properties, such as degree of unsaturation (Bell, 1998; Sadeghi-Jorabchi, 1990), adulteration (Baeten, 1996) and *cis-trans* ratio (Bell, 1998; Bailey, 1972) can be determined. Fluorescence due to carotenoids was found to be a problem in early dispersive Raman measurements. More recently, FT Raman methods have been shown to solve this problem at a high capital cost. However, low cost dispersive instruments are now being produced and they can be used to obtain spectra from food samples (e.g. butter (Bell, 1998)) with negligible interference from fluorescence. The performance of this low cost equipment is adequate to allow studies of *cis/trans* and total unsaturation in commercial oils and fats, and has the potential to be extended to on-line measurements.

Objectives:

The objectives of this study were three-fold :-

1) To investigate the experimental conditions needed to obtain interpretable Raman spectra of neat adipose tissue.

2) To identify variations in Raman spectra that may arise due to sampling.

3) To compare the Raman spectra of beef and sheep subcutaneous adipose tissue.

Methods:

Samples of lamb and beef adipose tissue were obtained from carcasses slaughtered under commercial conditions. A 20 cm^2 sample was dissected from the mid-back region at 2 days *post-mortem* for lambs and 7 days *post-mortem* for the beef. All samples were stored at 2°C prior to dissection. Raman spectra were obtained within 24 hrs of dissection for beef, and 48 hrs for lamb.

The spectra were obtained using 40 s accumulations with a conventional dispersive Raman spectrometer (250 mW, 785 nm excitation, liquid-nitrogen cooled CCD detector). Initially, several repetitions (5 for spot focus and 7 for line focus) were carried out on one sample, the individual spectra being recorded on sample areas separated by at least 1 cm. Line focus was used thereafter.

Results and Discussion:

Raman spectra obtained without any sample preparation show clearly identifiable peaks. Of particular interest are the peaks at 1440 and 1660 cm^{-1}, whose heights and/or areas directly relate to the number of CH_2 groups and C=C groups, respectively, in the sample. The height and area ratios of these two peaks at different sites on the same sample have smaller coefficients of variation when line focus is used. This suggests that there are local variations in composition which are averaged out to some extent when a larger area of the sample is probed by line, as opposed to point, focus. However, point focus would be useful for studying highly localised (μm) compositional changes. Larger-scale compositional variation is also observed. Fig.1 shows noticeable differences between the front and the back of the samples, especially in the 1660, 1270 (both *cis* unsaturation) 1050-1150 and 850-900 cm^{-1} (C-C backbone conformation) regions. A higher 1660/1440 cm^{-1} ratio indicates greater unsaturation (Sadeghi-Jorabchi, 1990) and hence Fig.1 shows the inside surface to be more unsaturated. This was characteristic of all the samples.

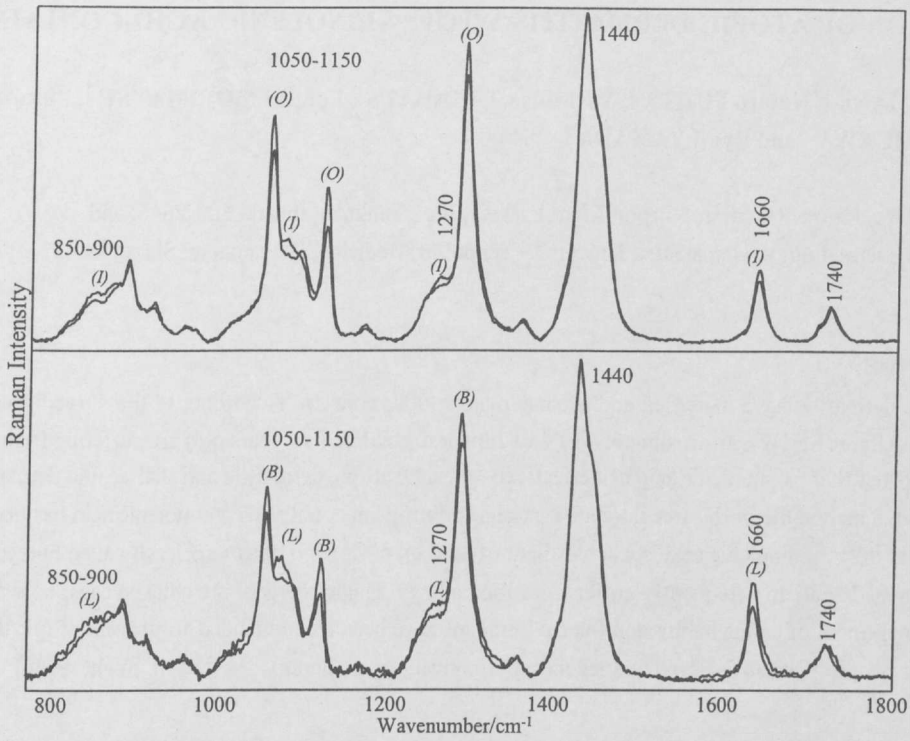

Fig. 1. Comparison of the Raman spectra of the inside *(I)* and outside *(O)* of lamb adipose tissue. Identity of highest peaks are marked appropriately. Spectra are the sum of the 7 repetitions using line focus and are unsmoothed. A standard 17 point baseline correction has been used to remove the broad fluorescence background.

Fig. 2. Comparison of the Raman spectra of the inside surface of beef *(B)* and lamb *(L)* adipose tissue. Spectra are the sum of three repetitions for one sample of each. All other conditions as in Fig. 1.

Table 1. Comparison of height and area ratios (1660/1440 cm^{-1}) of adipose tissue samples.

	Outside				Inside					Outside		Inside	
	Height	CV	Area	CV	Height	CV	Area	CV		Area	CV	Area	CV
Point	0.211	8.6	0.091	10.1	0.184	9.2	0.106	8.0	Beef	0.128	10.4	0.146	13.9
Line	0.181	5.7	0.094	7.4	0.194	5.0	0.098	5.8	Lamb	0.116	19.1	0.151	20.4

The lamb and beef spectra indicate that the lamb samples were more unsaturated on the inside than the beef (Fig 2), for the outside the converse was found (Table 1). It is known that there are differences in the fatty acid composition of beef and lamb fat (Enser, 1996) but overall bulk properties of IV, chain length *etc.* are quite similar, although markedly different from pork. Moreover, breed and sex differences have been reported for fatty acid distribution and these should also be taken into account when comparing species (Solomon, 1990). The beef samples were from bullocks mainly of the Charolais breed, however, our small data set for lamb contained four of each of the three sex types, but was confounded by dietary treatment. The larger CV values for lamb may reflect this fact. The four silage fed entire males had higher ratios (0.170) than the silage fed females (0.165) and concentrate fed castrates (0.124)

Conclusion:

These preliminary investigations show that repeatable spectra of sufficient quality to characterise animal adipose tissue can be obtained quickly and with no sample preparation. Raman spectroscopy may provide a suitable technique for on-line analysis after further calibration with chemical analysis.

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