THE ABILITY OF NEAR INFRARED SPECTROSCOPY (NIR) TO DISCRIMINATE SEASONAL AND DIETARY INFLUENCES ON LAMB MEAT QUALITY

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Abstract - The aim of this study was to investigate the ability of NIR to discriminate the effects of seasonal influences and finishing diet on the fatty acid profiles of lean meat and adipose tissue in lamb. A total of 67 lambs were sampled at an abattoir in April, June and July to represent finishing regimes of predominantly concentrate (C), grass plus concentrate (GC) and fresh grass (G). Near infrared spectra (NIR) were measured on the cut surface of the *longissimus dorsi* (LD) muscle at the anterior end of the loin and also on the subcutaneous fat cover. Fatty acid analysis of the LD showed that lamb slaughtered in July (G) had ratios of n-6:n-3 fatty acids typical of grass fed animals whereas there was little difference between the other two slaughter times. Discriminant analysis on the NIR spectra of the LD resulted in an overall correct classification of the 3 slaughter groups of 68%. Use of the fat spectra showed a more accurate prediction with an overall classification of 88%.

Key Words – Diet, Health, Season

I. INTRODUCTION

The perception amongst consumers and health professionals that red meat, high in saturated fatty acids (SFA), is a risk factor for coronary heart disease [1] has stimulated research to manipulate the fatty acid composition of red meat. Nutritionists have recommended a higher intake of polyunsaturated fatty acids (PUFA), specifically to increase the ratio of PUFA to SFA (P:S) and decrease the ratio of n-6: n-3 PUFA [1, 2]. Genetic and nutritional approaches to accomplish these nutritional goals have been widely studied, the latter proving to be more effective. Human intervention studies have shown the health benefit of nutritionally enhanced red meat from grass-fed animals [3].

Standard gas-chromatographic (GC) analysis of fatty acids is time consuming and to differentiate meat with enhanced nutritional qualities, a rapid, online method is required. Near infrared spectroscopy (NIR) meets the criteria for online installation and has been reported to predict fatty acids [4].

The aim of this study was to investigate the ability of NIR to discriminate the effects of seasonal influences and finishing diet on the fatty acid profiles of lean meat and adipose tissue of lamb.

II. MATERIALS AND METHODS

Sample material

A total of 67 lambs were obtained through a commercial abattoir in Northern Ireland, at three times of the year namely, April, June and July. Information obtained from the meat plant indicated that samples chosen represented commercial feeding practices of animals finished on a diet with a high proportion of concentrate (April), a mixture of grass and concentrate (June) and grazed grass (July).

Lambs were slaughtered under standard commercial practice and chilled according to the abattoir's normal chilling regime. At 2 days post-slaughter, the loin muscle was removed from each carcass & vacuum packed samples were transported to the laboratory.

NIR analysis

At 7 days post-slaughter, the reflectance spectrum of each sample was measured. Scans were taken of the freshly cut surface of the *longissimus dorsi* (LD), at the anterior end of each loin.

A portable NIR spectrometer (LabSpec 5000, ASD Inc, USA) wavelength range 350-2500 nm, with attached high intensity contact probe, was used to scan the samples. Once scanning was complete, a 1 cm sub-sample was taken from the LD of each loin and placed in frozen storage (-80^{o}C) for fatty acid analysis. The remainder of each loin was blast frozen and held at -20^{o}C until required.
Due to problems with the NIR equipment, scans of the samples associated with grass feeding (July) could not be performed on the fresh meat.

After a period of 8 months, samples were thawed and further NIR scans taken of both the lean meat and the outside surface of subcutaneous fat cover.

Fatty acid analysis:

Sub-samples were removed from frozen storage and allowed to thaw. Fatty acid analysis was determined using capillary column GC following preparation of the fatty acids as methyl esters (FAME) as described by Dawson *et al.*[5].

Statistical analysis:

Principal Component Analysis (PCA)

Spectral data was imported into Genstat. Prior to performing PCA, the wavelength range to be used was reduced from 350-2500nm to 400-2350nm in order to prevent noise at the start and end of the spectrum affecting the accuracy of results. PCA was performed on the NIR spectral data (reflectance) associated with frozen/ thawed scans of the lean meat and subcutaneous fat cover. Hotelling T^2 statistic was incorporated within the procedure to assist in the identification of outliers.

Stepwise Discriminant Analysis

SPSS was used to conduct discriminant analysis. The NIR spectral data (400-2350nm) associated with the lean and subcutaneous fat was subjected to a number of spectral transformations and pre-treatments. This resulted in a total of 10 transformed spectra for each raw spectrum acquired. Multivariate stepwise discriminant analysis was performed on each batch of results to thus determine the ability of NIR to correctly classify samples according to dietary treatment/ authentication. Full cross validation was performed to test the accuracy of the discriminant analysis model.

III. RESULTS AND DISCUSSION

Reflectance spectra (350-2500nm)

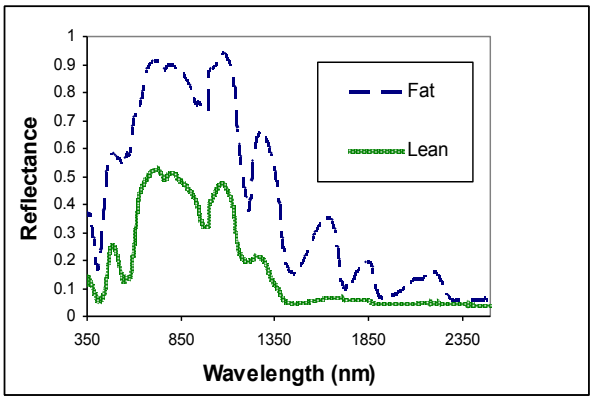

Figure 1. Typical spectra of raw, lean meat and subcutaneous fat

In the visible region, the spectrum of the subcutaneous fat cover shows spectral characteristics typical of the myoglobin pigments associated with lean meat. [6]. Thus in the visible region (380-780nm) the subcutaneous fat spectra is influenced by the underlying muscle tissue, but this appears to have less influence in the NIR region (1400-2300nm). Differences in fat thickness may have an influence on the spectra [4]. The spectral data associated with the fat cover has more distinct peaks when compared with the lean meat and may be related to the lower water content of the adipose tissue. The water content of red meat varies between 70-90% and is a strong absorber in the infrared region dominating the lean meat spectra [7]. Clear peaks associated with scans of the fat can therefore be observed as its water content is significantly lower. Therefore, peaks relating to the hydrocarbon bonds of the fatty acids [8] can be analysed.

Fatty Acid Analysis

The lower IMF content in lamb, finished in July is in agreement with Daley *et al* [9], who reported that ruminants finished on a diet with a high proportion of grass have lower IMF levels

compared with those finished on grain [9].The mean SFA and PUFA contents of meat from all three finishing periods were very similar and hence had little impact on the P:S ratio. According to Raes *et al* [10], dietary n-3 sources do not affect the P:S ratio as it is mainly influenced by the genetics of the animal and much less by nutrition [10].

Table 1 Summary of seasonal influence (and diet) on major fatty acid groups in lamb.

Fatty Acid	April (concentrate) (n=25)		June (grass/concentrate) (n=25)		July (grass) (n=17)	
	Mean	SD	Mean	SD	Mean	SD
IMF [1]	3.2	0.760	3.0	0.767	2.9	0.869
SFA [2]	12.6	3.248	12.3	3.531	12.4	3.832
PUFA[2]	2.7	0.595	2.6	0.460	2.0	0.401
P:S	0.23	0.057	0.23	0.082	0.17	0.045
Total n6[2]	2.40	0.536	2.17	0.402	1.07	0.206
Total n3[2]	0.32	0.080	0.40	0.146	0.88	0.199
n6/n3	7.68	1.561	6.07	2.285	1.22	0.098
CLA[2]	0.18	0.054	0.19	0.058	0.47	0.214

IMF [1] Intramuscular fat (g/100g muscle)
SFA[2]: Total saturated fat; PUFA[2]; total polyunsaturated fatty acids ; CLA[2]: total conjugated linoleic acid- ClAc9,t11 + CLA t10,c12 ([2] all expressed as mg/g muscle)
P:S: polyunsaturated to saturated fatty acid ratio; Total n-6/ Omega 6; sum of n-6 fatty acids- C18:2c + C18:3c 6,9,12 +C20:4c; Total n-3/ Omega-3; sum of n-3 fatty acids- C18:3c 9,12,15 + C20:5cn3 + C22:5c + C22:6c

Lower levels of n-6 PUFA, higher levels of n-3 PUFA and CLA are typical of animals finished on an omega-3 rich diet such as grass. The subsequent lowering of the n:6-n:3 ratio was due to changes in these fatty acid proportions that were in keeping with current dietary recommendations [3].

Principal Component Analysis (PCA)

PCA was performed on the NIR spectral data associated with scans of the lean meat and subcutaneous fat cover respectively. The PCA plot associated with the lean meat is shown in figure 2.
For the reflectance data of the lean spectra, the first 3 principal components accounted for 98 % of the total variance. No distinct clusters or groups of samples relating to dietary category were identifiable in the PCA. The same was true of the spectral data associated with the subcutaneous fat cover (not shown). Therefore, further statistical analysis was performed.

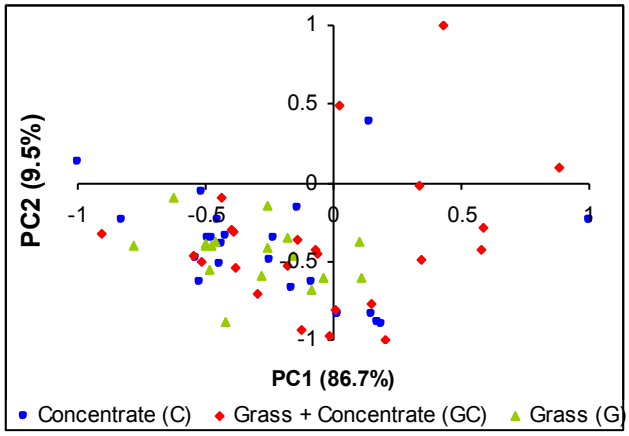

Figure 2: PCA plot of NIR spectral data associated with the lean meat

Stepwise Discriminant Analysis

Spectral data was subjected to a number of mathematical transformations and pre-treatments. Stepwise Discriminant Analysis was performed on each spectral transformation to determine the mathematical treatment that demonstrated the greatest ability to correctly classify samples into their dietary categories.
The discriminant analysis showed that in general, for all spectral transformations the number of correctly classified samples was greater when the fat spectra were used rather than the corresponding lean spectra. For the lean spectra, the best model with 68% correct classification was obtained using multiplicative scatter correction (MSC). Using the first derivative function of the reflex attenuance (RA= $-\log_{10}[R]$, where R is the reflectance) on the fat spectra, a correct classification of 96% was obtained. Full cross validation confirmed these models.

Table 2: Full cross validation of discriminant models

Tissue/ Model	Actual	Predicted		
		April (C)	June (GC)	July (G)
Lean/ MSC	April	15	7	3
	June	7	15	3
	July	0	3	14
	% correctly classified: 68%			
Fat/ RA1	April	23	1	1
	June	5	20	0
	July	0	1	16
	% correctly classified: 88%			

The large number of variables used in the latter model (12 wavelengths) is relatively large for the number of samples (67). This may represent over fitting and provide a less robust model for future prediction. The next best model for the fat spectra, based on reflectance values with 4 point smoothing, used 6 variables and resulted in an overall correct classification of 79%. This model may provide a more robust, although less accurate, prediction. Further evaluation and interpretation of the wavelengths included in these models is therefore required.

The NIR discriminant analysis of the LD showed that although some July (G) samples were misclassified as June (GC), no July samples were misclassified as April (C). The NIR discrimination showed misclassification of the April and June samples across all 3 seasonal sample times however, the major misclassification was between April and June. This is in keeping with the major fatty acid groups which showed clear differentiation between July and the other 2 months (table 1).

NIR was able to accurately discriminate between seasonal diet when spectral data of the fat was utilised rather than the lean. These findings are in line with previous results which showed that dietary authentication could be identified using perirenal fat [8].

IV. CONCLUSION

The results associated with this investigation demonstrate the considerable potential of NIR in determining the diet authentication of lamb. However, this procedure needs to be developed further and tested in preparation for online application.

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