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The determination of sulphur in wool using near infrared reflectance spectrometry

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ABSTRACT

A method and calibration for estimating sulphur (S) concentration in wool using near infrared reflectance spectrometry (NIRS) is described. The NIRS measurements were calibrated against wet chemistry determinations of wool S ($R^2 = 0.81$, relative standard error $\pm 3.7\%$, $n=339$). The first derivative spectra with 14 partial least squares terms gave the lowest cross-validation error for the calibration using an independent set of wool samples ($R^2 = 0.78$, cross-validation error ± 0.11 , $n=66$, mean 2.88 g S/100g wool). The correlation spectrum gave a series of approximately equally weighted peaks from about 1300 - 2400 nm, corresponding to -NH stretching and bending modes (protein). The -SH absorption between 1736 and 1746 nm could also be identified. Although scouring differences contributed to marked spectral differences in the visible region (yellowness was related to the first 3 principal component scores) these did not affect the overall S calibration. Uses for the calibration include wool S as an indicator of lifetime wool production, changes in wool composition in response to supplemented cysteine or energy intake, and the relationship between wool S and wool characteristics.

Keywords: NIRS; plasma emission spectrometry; Romney sheep; wool scouring.

INTRODUCTION

Wool S is present mainly as cysteine residues in the high and ultra-high S proteins. Wool S concentration is inversely related to wool growth (Harris *et al.*, 1997), and research into the metabolic pathways involved may lead to ways to modify wool quality by altering its chemical composition, or ways to produce wool more efficiently (Reis *et al.*, 1990). Recent research has identified wool S as a useful predictor of wool production in sheep (Antram *et al.*, 1991; Sun *et al.*, 1991; Blair and Lee, 1997).

Wet chemistry methods for determining S concentration in wool by acid digestion and plasma emission spectrometry are typically slow and expensive procedures. A faster and more cost effective method was sought to screen a large number of samples for animal selection purposes, and so a NIRS calibration was developed. NIRS has previously been used for measuring moisture and fibre characteristics of cotton (Taylor, 1995), and for measuring moisture and colour in wool (Hammersley and Townsend, 1995), but this is the first time that a NIRS method has been used to measure wool S concentration.

MATERIALS AND METHODS

A scanning spectrometer (Model 6500, NIRSystems Inc., Silver Spring, MD, USA) and PC software by Infrasoft International (version 3.1) were used. Samples of scoured bulk wool were scanned at 2 nm increments across the spectral range of 400 to 2500 nm using a small ring cup in a transport cell holder according to the instrument manufacturers instructions. A total of 339 samples were scanned and a calibration set was developed using a first derivative modified partial least squares (PLS) mathematics treatment (Shenk and Westerhaus, 1994). A sub-set of 66

samples were used for cross-validation. The calibration was based on wool S concentrations determined by wet chemistry of sub-samples of the scanned wool.

Wool samples were collected over a period of five years from mid-side patches of male and female Romney sheep of various ages and from several environments. The samples were of varying staple lengths. Several different wool scouring methods were employed, but the main method made use of aqueous detergent solutions (Lee and Grace, 1988; Antram *et al.*, 1991). Wool samples of 150 mg were weighed out under conditions of constant temperature ($20 \pm 2^\circ\text{C}$) and relative humidity (65%). The samples were then prepared by either microwave acid digestion in a closed system, or, in the case of the earlier samples, by wet digestion with $\text{CCl}_4/\text{Br}_2/\text{HNO}_3$ in a refluxing open flask (Antram *et al.*, 1991). For the microwave method, wool samples was digested in 1 ml 30% H_2O_2 and 3 ml 70% HNO_3 by microwave heating in sealed Teflon containers for 3 min at 240 watts, then removed from the sealed containers and heated for 1 min at 240 watts. Cooled digests were resuspended in 15 ml de-ionised water. Sulphur was determined in appropriate dilutions of these extracts by inductively coupled plasma emission spectrometry (ICP-ES) under operating conditions described by Pritchard and Lee (1984). An in-house QC reference wool sample was analysed in parallel with each batch of unknowns. This standard was taken from a single mid-side patch of bulk wool from a Romney sheep, and prepared by scouring the staples and trimming to 1 cm lengths (Antram *et al.*, 1991).

RESULTS AND DISCUSSION

Results of determining S concentration of the in-house QC reference wool by acid digestions and ICP-ES analysis are shown in Table 1. While there was no signifi-

cant difference in percent S between the two digestion methods, the microwave method had twice the variation (RSD 6%) of the open flask method (RSD 3%). Health and safety issues have prompted our laboratory to adopt the microwave method, and recent work using selenium internal standard corrections has since reduced that within-batch variation (RSD <1%; Martin, unpublished data).

FIGURE 1: NIRS spectrum for wool: wavelength vs. $\log(1/R)$ (–) and first derivative (...).

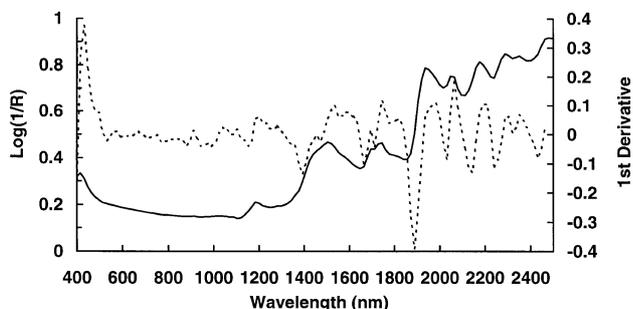


TABLE 1: Sulphur content of a scoured Romney wool staple (in-house QC wool reference) as determined by wet chemistry methods compared to the NIRS prediction.

	Concentration of sulphur (%)		
	mean	SE	RSD ¹
Wet digestion			
CCl ₄ /Br ₂ /HNO ₃ (n=21)	3.03	0.018	2.73
HNO ₃ /microwave (n=17)	3.12	0.043	5.80
NIR prediction (n=11)	2.932	0.006	0.684 ²

¹ relative standard deviation

² within-batch repeatability

Absorbance readings over the scanned wavelength region and their first derivative spectrum are shown in Figure 1. The correlation between absorbance at different wavelengths and wool S concentration gave a series of approximately equally weighted peaks from about 1300 - 2400 nm, corresponding to -NH stretching and bending modes (protein) (Figure 2). The -SH absorption between 1736 and 1746 nm could also be identified. Results of the NIRS calibration developed using the first derivative PLS mathematics treatment in conjunction with laboratory wet chemistry determinations of wool S are presented in Table 2.

FIGURE 2: Correlation between sulphur concentration in wool with the first derivative of the NIRS spectrum.

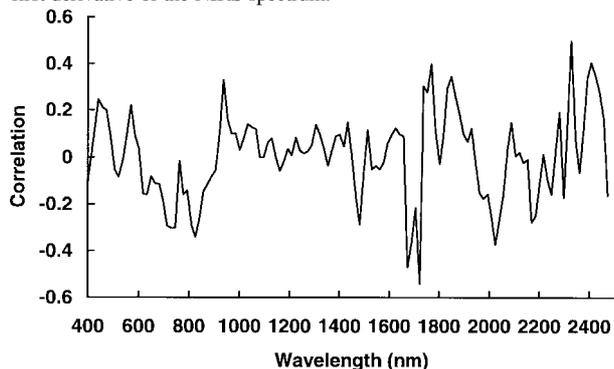


TABLE 2: Calibration and cross-validation analysis for the prediction of sulphur in scoured wool by NIRS.

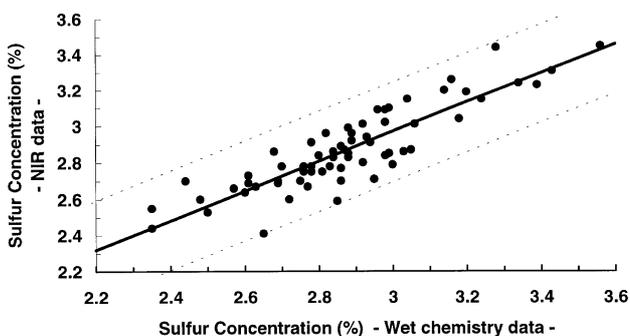
Calibration (n=339)	mean	SEC¹	R²
sulphur (%)	2.93	0.11	0.81
Cross-validation (n=66)			
	Laboratory wet chemistry sulphur (%)	NIRS prediction sulphur (%)	
mean	2.88		2.87
SEP ²		0.11	
bias		0.01	
standard deviation	0.24		0.22
slope		0.96	
R ²		0.78	

¹ standard error of calibration

² standard error of performance

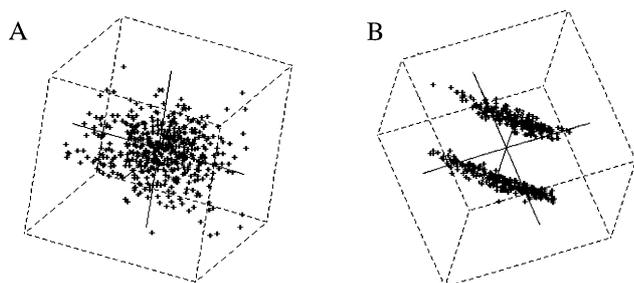
Several other mathematic approaches were evaluated, including multiple regression analysis and PLS using the second derivative of the absorbance spectrum, but the first derivative spectra with 14 PLS terms gave the lowest cross-validation error for the NIRS calibration, based on an independent set of 66 wool samples (R²=0.78, cross-validation error 0.11, mean 2.88 g S/100g wool). The validation regression using the wet chemistry data from this data set plotted against the NIRS calibration prediction is shown in Figure 3.

FIGURE 3: Prediction of sulphur concentration in wool from the NIR calibration compared with laboratory wet chemistry determinations using ICP-ES.



Although scouring differences contributed to marked spectral differences in the visible region (yellowness was related to the first 3 PC scores) these did not affect the overall S calibration. Figure 4A illustrates the three principal components (10th, 7th and 6th) that relate to the S concentration in wool, while Figure 4B shows the effect of the first three principle components (1st, 2nd and 3rd), which relate to differences in scouring. When calibrating solely on scouring technique, higher SEP and lower R² statistics resulted. It was therefore decided that the calibration should include all scoured wool samples from a range of techniques. This resulted in a robust calibration with

FIGURE 4: Principal component scores for (A) wool S and (B) scouring method - the latter indicative of changes in wool colour - for all samples.



lower standard error for the prediction and greater R^2 values (Table 2).

NIRS prediction for the concentration of S in the QC reference wool was 2.93 ± 0.006 g S/100 g wool, which compared favourably with the mean value obtained using the wet chemistry methods over a 2 -3 year period (Table 1).

CONCLUSIONS

We have described a NIRS method for estimating Romney wool S concentrations with excellent precision. The robustness of the calibration could be improved by expanding the calibration database to include other wool types, such as the fine wool of the Merino. The method is non-destructive, uses very small samples, and once the sample has been scanned, its spectral information can be correlated with a wide range of other wool characteristics.

We have recently applied this NIRS calibration to the analysis of wool S in several research studies, including wool S as an indicator of lifetime wool production (Blair and Lee, 1997), changes in wool composition in response to supplemented cysteine or energy intake (Sherlock, Lee and Harris, unpublished data), and the relationship between wool S and wool characteristics, e.g. intrinsic strength, medulation, lustre, bulk and crimp with season

and physiological state (Kendall, Pearson, Harris, Lee, unpublished work).

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