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Feasibility study on the potential of visible and near infrared reflectance spectroscopy to measure alpaca fibre characteristics

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*Visible (Vis) and near infrared (NIR) reflectance spectroscopy is a rapid and non-destructive technique that has found many applications in assessing the quality of agricultural commodities, including wool. In this study, Vis and NIR spectroscopy combined with multivariate data analysis was investigated regarding its feasibility in predicting a range of fibre characteristics in raw alpaca wool samples. Mid-side samples (*n *= 149) were taken from alpacas from a range of colours and ages at shearing time over 4 years (2000 to 2004) and subsequently analysed for fibre characteristics such as mean fibre diameter (MFD) and standard deviation (and coefficient of variation), spin fineness, curvature degree (and standard deviation), comfort factor, medullation percentage (by weight and number in white samples only) using traditional reference laboratory testing methods. Samples were scanned in a large cuvette using a FOSS NIRSystems 6500 monochromator instrument in reflectance mode in the Vis and NIR regions (400 to 2500 nm). Partial least squares (PLS) regression was used to develop a number of calibration models between the spectral and reference data. Mathematical pre-treatment of the spectra (second derivative) as well as various combinations of wavelength range were used in model development. The best calibration model was found when using the NIR region (1100 to 2500 nm) for the prediction of MFD, which had a coefficient of determination in cross-validation (R^2) of 0.88 with a root mean square standard error of cross validation (RMSECV) of 2.62 μm. The results show the NIR technique to have promise as a semi-quantitative method for screening purposes. The lack of grease in alpaca wool samples suggests that the technique might find ready application as a rapid measurement technique for preliminary classing of shorn fleeces or, if used directly on the animal, the technology might offer an objective tool to assist in the selection of animals in breeding programmes or shows.*

Keywords: alpacas, fibres, near infrared spectroscopy, partial least squares

Introduction

The alpaca (*Lama pacos*) is commercially the most important fibre producer of the South American camelid family (Lupton *et al*., 2006). Alpacas were introduced into Australia during the 1860s but the industry failed to establish and by the 1880s, the farmed alpaca population was all but extinct (McGregor, 2002). With the re-introductions of alpacas to Australia in the late 1980s, members of the alpaca industry have made numerous claims regarding the use of their fibre (McGregor, 2002). If alpaca fibre production is to become a viable alternative industry to sheep wool production, it is essential that easy and cheap methods be available for the alpaca's breeders, wool producers and textile industry.

Near infrared (NIR) spectroscopy coupled with chemometric methods such as principal components analysis (PCA) and partial least squares (PLS) regression have seen a rapid increase in industrial use (Cowe and McNicol, 1985; Monin, 1998; Cleve *et al*., 2000; McClure, 2003). NIR spectroscopy has been used for both at-line and on-line applications in the textile industry, including the measurement of residual wool wax, moisture, synthetic/natural fibre blend ratios and the treatment levels of various chemical processes (Connell and Brown, 1978; Connell, 1983; Coleman *et al*., 1999; Church and O'Neill, 1999; Hammersley and Townsend, 2004; Cozzolino *et al*., 2005). Spectroscopy (both the visible (Vis) and NIR regions) has become a more attractive analytical technique for measuring quality parameters in foods and agricultural products with decreasing instrument prices, simultaneous determination of several quality parameters, the ability to replace expensive and slower reference techniques and the lack of need for sample preparation and minimal data analysis (McCaig, 2002; McClure, 2003). More recent developments have exploited

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the improvements in NIR instrumentation (McClure, 2003). Extensions of the wavelength range into the Vis region enable the measurement of colour in wool (Hammersley, 1992; Hammersley and Townsend, 1994 and 2004; Hammersley *et al*., 1995; Cozzolino *et al*., 2005). Spectroscopy in the NIR region will provide information about the relative proportions of C–H, N–H and O–H bonds that are the primary constituents of the organic molecules (Murray, 1993; Deaville and Flinn, 2000). Many reports are available in the literature related to the use of NIR to predict several parameters in sheep wool samples such as residual grease, moisture content, colour, fibre diameter, medullation and vegetable matter content (Slack-Smith *et al*., 1979; Larsen and Kinnison, 1982; Hammersley, 1992; Hammersley and Townsend, 1994 and 2004; Hammersley *et al*., 1995; Cozzolino *et al*., 2005).

In this study, the potential use of Vis and NIR spectroscopy combined with multivariate data analysis was investigated for its feasibility in predicting a range of fibre characteristics in raw alpaca wool samples.

Material and methods

Wool samples

Mid-side (approx. 100 g) samples ($n = 149$) were taken from alpacas (*L. pacos*) (male and female) of a range of colours and ages at the time of shearing over 4 years (2000 to 2004) and divided for subsequent analysis for fibre characteristics using traditional laboratory testing methods. Fibre characteristics measured included, mean fibre diameter (MFD) with its standard deviation (SDFDM) and MFD coefficient of variation (CVFD) of fibres coarser than 30 μm (%$F > 30$ μm), spin fineness (SPINF), curvature (degree and standard deviation), comfort factor, medullation percentage (by weight and number in white samples only) using the OFDA100 method at commercial fibre-testing service providers (OFDA, IWTO-47-95, 1995) (Sommerville, 2002). In addition, total fleece weight (TFW) (kg) and staple length (SL) (mm) were also measured. SL was estimated on 12 staples measured to the nearest 0.5 cm according to methods described elsewhere (AS 3535-1988, 1988) (Sommerville, 2002).

Visible and near infrared spectroscopy

Samples were scanned over the Vis and NIR regions (400 to 2500 nm) in a large cuvette (260 mm × 55 mm × 33 mm) using a FOSS NIRSystems6500 monochromator instrument (FOSS NIRSystems, Silver Spring, MD, USA) in reflectance mode. Each spectrum was collected at 2 nm intervals (1050 data points) and reflectance (R) data was stored as log (1/R). The spectrum of each sample was the average of 32 successive scans.

Multivariate analysis

PCA was used to study patterns in the NIR raw spectra of the wool samples analysed. Calibration models between reference values and spectral data were developed using PLS with full cross validation. Spectral data was transformed into a NSAS format and exported into The Unscrambler (1996) software for multivariate analysis. PCA was performed before PLS regression models were developed (Martens and Naes, 1989; Naes *et al*., 2002). PCA was used to derive the first principal components (PCs) from the spectral data to examine the possible grouping of samples and to detect possible spectral outliers before using the data set to develop the PLS regression models (Martens and Naes, 1989). No mathematical treatments or spectral transformations were applied when PCA was performed. After PCA analysis, each spectrum was smoothed by a 19-point Savitzky–Golay (SG) second-order filtering operation and transformed using the second derivative (SG) in order to avoid noise and complexity of the spectrum (The Unscrambler, 1996). The optimum number of latent variables/terms in the PLS calibration models were determined by cross validation and defined by the PRESS function (prediction residual error sum of squares) (Naes *et al*., 2002). Due to the limited number of samples available, calibration models were developed and evaluated using full cross validation (Martens and Naes, 1989; Martens and Dardenne, 1998; Naes *et al*., 2002). The resulting calibration equations between the chemical analyses and the Vis and NIR data were evaluated based on the coefficient of determination in calibration (R^2) and the root mean square of the standard error in cross validation (RMSECV). The ratio of standard deviation and RMSECV, namely residual predictive value (RPD) was used to test the accuracy of the calibration models (Williams, 2001).

An RPD value greater than three was considered adequate for analytical purposes in most of NIR applications for agricultural products (Williams, 2001; Fearn, 2002).

Results and discussion

Figure 1 shows the mean and standard deviation spectrum in the NIR region of the alpaca wool samples. The mean spectrum of wool samples showed absorption bands at

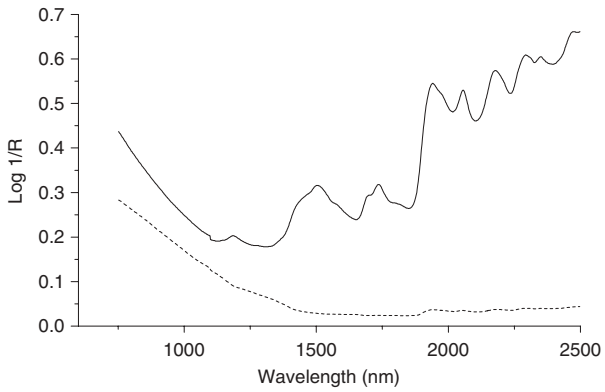

Figure 1 Near infrared mean spectrum (line) and standard deviation (dotted line) of alpaca wool samples analysed (raw spectra, 500 to 2500 nm).

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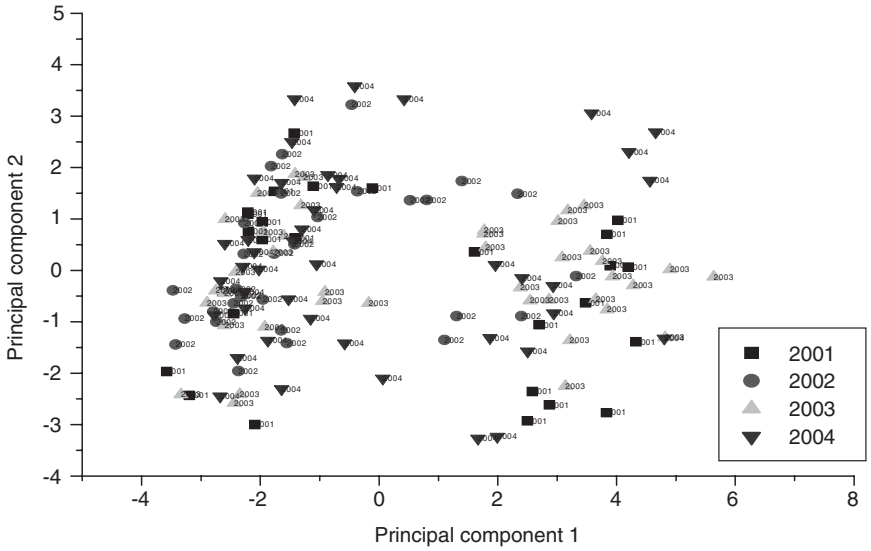

Figure 2 Principal component score plot of alpaca wool samples analysed by visible and near infrared spectroscopy, labelled by year.

1200, 1430 and 1930 nm related with O–H stretch overtones, mainly related with moisture of wool samples (Murray, 1986; Osborne *et al.*, 1993; Cozzolino *et al.*, 2005). Absorption bands around 1700 nm associated with either S–H first overtones or C–H stretch overtones of lipids and fatty acids and at 2120 and 2300 nm they were related to C–H deformation and combination tones associated with amino acids (Murray, 1986; Osborne *et al.*, 1993; Cozzolino *et al.*, 2005). Standard deviation between samples was observed around 1400 and 1900 nm, related with the O–H overtones (water content) (Murray, 1986; Osborne *et al.*, 1993; Cozzolino *et al.*, 2005). PCA is a statistical data analytical technique that has recently seen application in the textile and food industries (Naes *et al.*, 2002) and involves identifying the components accounting for variance within a data set, averaging the spectral set and then comparing this to each spectrum. Additionally, it creates a synthetic spectrum which accounts for the largest part of the variance within the spectral set that produces the first loading vector or principal component (PC). The scaling factor that represents the amount of the loading vector in each of the spectra in the data set is known as the score. Multiplying the loading vector by the score for each spectra and subtracting this from the original spectra produces a new spectral set (Naes *et al.*, 2002). This allowed us to interrogate the spectra in order to investigate for relevant patterns in the set of wool samples analysed. Figure 2 shows the score plot of the first two PCs of the alpaca wool samples analysed by NIR spectroscopy. The first two PC accounted for 76% of the variance of the spectra of the wool samples, PC1 57% and PC2 19%, respectively. The PC score plot shows that no effect of the year was observed in the wool samples analysed, but a separation between wool samples related to fibre colour was evident by the observation of the spectra.

Table 1 *Descriptive statistics for the wool parameters measured in alpaca wool samples*[†]

	n	Mean	Median	s.d.	Min.	Max.
MFD (μm)	149	26.98	26.29	5.36	18.55	41.8
SDFDM (μm)	149	6.67	6.5	1.38	4.1	9.88
CVFD (%)	149	24.95	24.57	3.85	15.24	36.8
SPINF	149	27.22	26.54	5.18	18.89	41.38
CURVEDEG	149	32.5	32.3	6.4	12.8	51
CURVESD	149	20.2	20	3.2	13	27
PMEDNUM (μm)	78	55.8	57	24.6	7.1	94.7
PMEDWT (%w/w)	78	55.2	55.3	28.1	4.1	96.8
TFW (kg)	149	3.1	2.94	0.93	1.47	5.82
SL (mm)	149	132.6	110.4	59.4	64	383
AGEATSHEAR	149	3.8	2.6	3.4	0.2	14.7

[†]Abbreviations are: MFD = mean fibre diameter, SDFDM = standard deviation of fibre diameter, CVFD = coefficient of variation in fibre diameter, SPINF = spin fineness, CURVEDEG = curvature, CURVESD = standard deviation of curvature, PMEDNUM = percent medullation by number, PEMWET = percent medullation by weight, TFW = total fleece weight, SL = staple length, AGEATSHEAR = age of animal at shear, s.d. = standard deviation, Min = minimum, Max = maximum, CV = coefficient of variation = (s.d./mean) × 100, *R* = coefficient of correlation in calibration, RMSECV = root mean square of the standard error of cross validation, RPD = SD/RMSECV, LV = latent variables/number of PLS terms used to develop the calibration models.

Table 1 shows the mean, standard deviation and range of the fibre characteristics analysed in the wool samples. The broad range in chemical composition did not reflect any year effect (ANOVA, data not presented). Table 2 shows the Pearson correlations ($P > 0.05$) between the fibre parameters measured in alpaca wool samples. It was interesting to note that MFD was inversely correlated with SL ($r = -0.10$) and curvature (i.e. crimp) ($r = -0.51$). High and positive correlations were found between SPINF and

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Table 2 *Pearson correlation between fibre characteristics measured in alpaca wool samples*[†]

	TFW	SL	MFD	SDFDM	CURVEDEG	SPINF	CURVESD	PMEDNUM
SL	**0.52**							
MFD	−0.18	−0.1						
SDFDM	−0.10	0.05	**−0.87**					
CURVEDEG	**−0.21**	0.09	**−0.51**	−0.05				
SPINF	**0.17**	−0.1	**0.99**	**0.92**	**0.42**			
CURVESD	**−0.19**	0.14	0.07	0.19	−0.16	−0.10		
PMEDNUM	**−0.40**	0.14	−0.09	-0.09	−0.006	−0.10	−0.07	
PMEDWT	**0.39**	−0.1	0.08	0.09	−0.04	−0.09	0.03	**0.98**

[†]For abbreviations see Table 1 footnote. High correlations are in bold.

Table 3 *Near infrared calibration statistics (cross validation) for the wool parameters measured in alpaca wool samples*[†]

	n	*R*	RMSECV	LV	RPD
MFD (μm)	136	0.88	2.62	8	2.0
SDFDM (μm)	120	0.75	0.86	9	1.6
SPINF	140	0.84	2.81	9	1.8
CURVEDEG	128	0.74	3.39	8	1.9
CURVESD	139	0.46	3.30	10	1.0
PMEDNUM (μm)	74	0.76	15.7	9	1.6
PMEDWT (%w/w)	71	0.86	14.42	10	1.9
TFW (kg)	134	0.70	0.61	6	1.5
SL (mm)	122	0.75	31.9	8	1.9

[†]For abbreviations see Table 1 footnote.

MFD ($r = 0.99$) and SPINF and SDFDM ($r = 0.92$). It was also observed that animal age was correlated positively with MFD, and negatively with SL.

Table 3 shows the NIR calibration statistics for the fibre characteristics measured on the alpaca wool samples. The best calibration model was found when using the NIR region for the prediction of MDF, which had a coefficient of determination in cross-validation (R^2) of 0.88 with a standard error of prediction of 2.62 μm. The value for the RPD was 2, meaning that the technique has promise as a semi-quantitative method for screening purposes. The R^2 and RMSECV for the other parameters measured in alpaca wool were 0.75 (RMSECV: 0.86 μm) for SDFDM; 0.84 (RMSECV: 2.81) for the SPINF; 0.74 (RMSECV: 3.39) for curvature (CURVEDEG); 0.46 (RMSECV: 3.3) for the standard deviation of curvature (CURVESD); 0.76 (RMSECV: 15.7 μm) for the per cent medullation by number (PMEDNUM); 0.86 (RMSECV: 14.42% w/w) for the per cent medullation by weight (PEMWET); 0.70 (RMSECV: 0.61 kg) for the TFW and 0.75 (RMSECV: 31.9 mm) for SL, respectively. The RPD obtained for those calibrations were lower than 3, indicating that they only can be used for rough screening. Other workers have found that fibre diameter in greasy wool samples was poorly predicted with NIR, while clean wool showed good relationships (Hammersley and Townsend, 1994 and 2004; Hammersley *et al*., 1995; Cozzolino *et al*., 2005). The lack of grease in alpaca wool samples suggests that the technique might find ready application as a rapid measurement technique for preliminary classing of shorn fleeces or, if used directly on the animal, the technology might offer an objective tool to assist in the selection of animals in breeding programs or shows.

Figure 3 shows the PLS loadings for the optimal calibrations models developed. It was observed that PLS loadings for MDF were opposite to per cent medullation by number (PMEDNUM). Highest loadings were observed around 1400 and 1900 nm related to O–H tones (mainly water), around 1700 nm related to C–H first overtones, mainly related with fatty acids and grease in the wool samples, and around 2070 and 2200 nm related to C–H combinations tones (Murray, 1986; Miller, 2001; Cozzolino *et al*., 2005).

It is well known that in practice, qualitative or semi-quantitative NIR analyses tend to be less demanding and more straightforward to develop and maintain than quantitative methods, and they can provide information that is very useful in the qualitative assessment of incoming raw material as in the case of wool (Brimmer and Hall, 2001). In the industrial manufacturing environment, these types of simple qualitative checks help ensure that products are kept within specification, which reduces the amount of off-specification material produced or increases the number of samples being analysed. The use of those calibrations might have great interest when the objective is to measure wool fibre diameter for screening purposes, animal selection or for use in breeding programmes, where the accuracy in fiber diameter is not as important as having a rapid and low cost method, when a high throughput sample system is required for on-farm analysis. However, this kind of approach will be used only to determine whether the wool has a low, medium or high fibre diameter.

Conclusion

This preliminary study showed that NIR spectroscopy has potential as a rapid analytical tool for determining MFD of alpaca wool. Thousands of wool samples are generated by animal selection and breeding programmes which commonly look to develop progress in several traits simultaneously. Low-cost, rapid methods are required for

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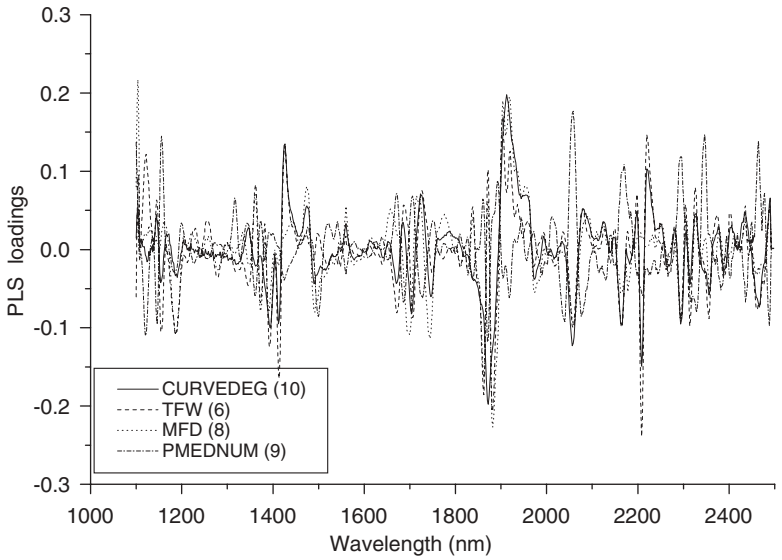

Figure 3 Partial least squares (PLS) loadings of the optimal calibrations for mean fibre diameter (MFD), total fleece weight (TFW), curvature (CURVEDEG) and per cent medullation by number (PMEDNUM) measured by near infrared spectroscopy (1100 to 2500 nm). Optimal number of PLS loadings in brackets.

such programmes. The technique might find ready application as a rapid measurement technique for preliminary classing of shorn fleeces or, if used directly on the animal, as an objective tool to assist in the selection of animals in breeding programmes or at animal shows. Reflectance spectroscopy makes NIR spectroscopy an ideal tool for screening. Further research is required to improve and validate the calibration generated using experimental conditions if the technology is to be used by the industry.

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