Near infrared analysis of the fodder shrub tagasaste (*Chamaecytisus proliferus*) for nutritive value and anti-nutritive factors

P.C. Flinn

Agriculture Victoria, Pastoral and Veterinary Institute, Private Bag 105, Hamilton, Vic. 3300, Australia.

N.J. Edwards and C.M. Oldham

Faculty of Agriculture, University of Western Australia, Nedlands, WA 6907, Australia.

D.M. McNeill

Department of Agriculture, University of Queensland, St Lucia, Qld 4067, Australia.

Introduction

Tagasaste (*Chamaecytisus proliferus*), commonly known as tree lucerne, is a perennial leguminous shrub native to the Canary Islands. In Western Australia, tagasaste is grown on deep sands for grazing by sheep as a means of filling the "autumn feed gap" instead of the traditional approach of hand-feeding with grain. Cattle, in contrast, can be used to graze the shrub year-round.[1] Because of their deep roots, the shrubs also have environmental benefits such as reducing wind erosion and ground water tables. However, liveweight gains from grazed tagasaste are lower than nutritive value measurements suggest. This is thought to be due to the presence of phenolic compounds, which act as a chemical defence against herbivory and insect attack.[2] In this paper, we report the use of near infrared (NIR) spectroscopy to measure nutritive value, total phenolics and a range of minerals in some 2000 tagasaste samples from various research trials. NIR spectra were also used to help understand the chemistry of the phenolic compounds.

Materials and methods

Four different sample populations of tagasaste were studied: (1) 260 separated leaf and stem fractions; (2) 169 unseparated samples spectrally selected from a total of 474; (3) 140 samples similar to (2) but forming a separate independent population and (4) 18 samples totally different in respect of source and seasonal conditions. All samples where oven-dried at 60°C and ground to pass a 1 mm screen.

Sample populations 1 and 2 were analysed for crude protein (CP) and ash by standard AOAC procedures; acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin by the methods of Goering and Van Soest;[3] total phenolics (expressed as tannic acid equivalents) using a

modification of the method of Price and Butler[4] and the minerals Ca, Mg, P, K, S, Na, Zn, Mn, Fe, Cu and Se by inductively coupled plasma emission spectroscopy (ICP).

Spectra were collected on all samples as log 1/*R*, from 1100 to 2500 nm, using either a model 6500 or 6250 monochromator (NIRSystems Inc., Silver Spring, MD) which had previously been spectrally matched.[5] Spectral data were transformed using "standard normal variate" and Detrend, then calibrations were developed for each constituent in populations 1 and 2 using modified PLS and first derivative math treatment (1,4,4,1). This was accomplished by the use of ISI software (Infrasoft International, Port Matilda, PA). Cross-validation was carried out by splitting the data into four segments. The calibration equations obtained were then tested on populations 3 and 4. Equations were also derived from a combination of population 2 and the leaf samples from population 1, then tested on population 4.

Results and discussion

Calibration equations based on the leaf/stem samples (population l) did not work satisfactorily on the test set of unseparated samples (population 3) due to the asymmetric nature of the calibration population [Figure 1(a)]. Equations based on the spectrally selected samples (population 2) worked well on most samples in population 3, as the two populations were similar and the calibration set more symmetric [Figure 1(b)]. However, equations based on population 2 were

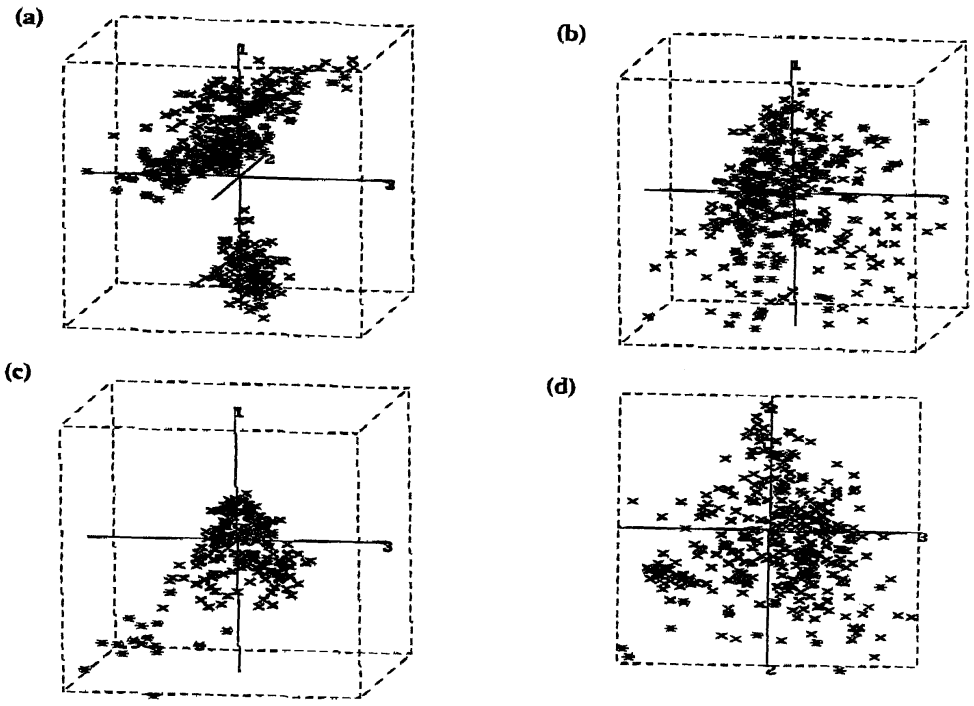

Figure 1. Symmetry plots of PCA scores for tagasaste samples: (a) leaf/stem (x) vs unseparated test set (*); (b) unseparated calibration set (x) vs unseparated test set (*); (c) unseparated calibration set (x) vs different test set (*) and (d) unseparated calibration set plus leaf samples from (a) (x) vs different test set (*) (top view).

unsuitable for 16 of the 18 samples in population 4, which were clearly very different [Figure 1(c)]. This was thought to be due to differences in sample source, seasonal conditions and drying treatment. Population 4 was better accounted for when calibration equations were based on a combination of population 2 and the 138 leaf samples from population 1, although several of the 18 test samples were still outliers [(Figure 1(d)].

The calibration statistics for each constituent in the combined sample population, comprising population 2 (169 unseparated samples) plus the 138 leaf samples from population 1, are shown in Table 1.

High calibration accuracy (high R^2, low *SECV*, *SECV*/*SD* ratio ≤0.3) was obtained for CP, ash, ADF, NDF, lignin and total phenolics. Satisfactory calibration accuracy was obtained for the major minerals (Ca, Mg, P, K and S). Calibrations for Na, Zn, Mn and Fe were less accurate but possibly good enough to correctly classify 70–80% of samples into high and low groups. Cu and Se could not be determined in tagasaste using NIR.

These results were in general agreement with the findings of Clarke *et al.*,[6] who concluded that coefficients of variation should be less than 20% for NIR mineral analysis to be meaningful.

Figure 2 shows the 2nd derivative NIR spectra of three tagasaste samples with high levels (210–250 g kg^{-1}) of total phenolics, compared with three samples with low levels (10–20 g kg^{-1}). The "high" samples exhibited stronger absorptions (troughs in 2nd derivative) at 1672 nm which could be due to an aromatic C–H stretch first overtone band, similar to that from flavan-3-ols and flavan-3,4-diols found in condensed tannins.[7]

Total phenolics higher than 170 g kg^{-1} DM were associated with total rejection of tagasaste by sheep. Conversely, it appeared more palatable when phenolic levels were at or below 70 g kg^{-1} DM.[1]

Table 1. Mean, range and standard deviation in chemical composition (expressed as either g or mg kg^{-1} DM) of a population of tagasaste samples including 169 unseparated samples plus 138 leaf samples, together with NIR calibration statistics.

Variable	*N*	Mean	Range	*SD*	*SECV*	R^2	*SECV*/*SD*
CP (g)	287	207	113–353	51.6	5.59	0.99	0.11
Ash (g)	290	38.5	22.8–71.3	10.8	2.76	0.94	0.26
ADF (g)	285	229	157–318	34.5	10.3	0.91	0.30
NDF (g)	265	330	225–463	51.3	15.9	0.90	0.31
Lignin (g)	281	65.9	28.5–102	15.9	4.91	0.91	0.31
Phenolics (g)	227	93.7	14.0–254	58.0	12.4	0.96	0.21
Ca (g)	279	4.96	1.10–14.2	2.56	0.90	0.88	0.35
Mg (g)	283	2.96	0.90–5.90	0.78	0.39	0.76	0.50
P (g)	267	1.84	0.40–5.60	1.04	0.46	0.81	0.44
K (g)	284	9.06	2.10–23.4	4.78	1.85	0.85	0.39
S (g)	272	1.42	0.50–4.10	0.72	0.23	0.89	0.32

Table 1 (continued). Mean, range and standard deviation in chemical composition (expressed as either g or mg kg^{-1} DM) of a population of tagasaste samples including 169 unseparated samples plus 138 leaf samples, together with NIR calibration statistics.

Variable	*N*	Mean	Range	*SD*	*SECV*	R^2	*SECV/SD*
Na (mg)	152	387	60–1071	247	154	0.62	0.62
Zn (mg)	275	24.7	6.3–57.8	10.7	6.44	0.65	0.60
Mn (mg)	268	19.6	0.3–74.9	15.5	8.44	0.70	0.54
Fe (mg)	148	80.6	9.0–181	37.5	20.4	0.70	0.54
Cu (mg)	45	1.94	0.4–5.3	1.06	0.97	0.21	0.92
Se (mg)	127	1.67	0.5–3.3	0.67	0.70	0.00	1.04

N = number of samples.
SD = standard deviation of values across population.
SECV = standard error of cross-validation.
R^2 = coefficient of determination.
CP = crude protein.
ADF = acid detergent fibre.
NDF = neutral detergent fibre.

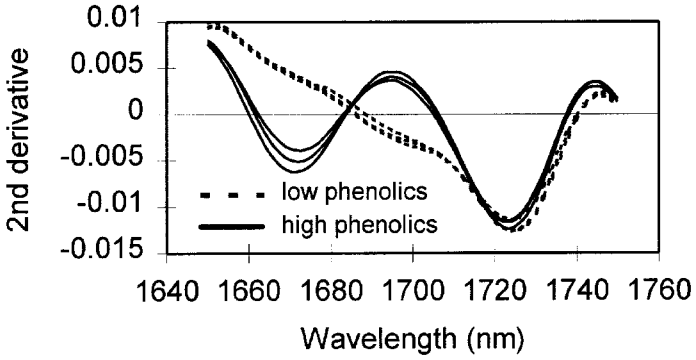

Figure 2. Second derivative spectra of tagasaste samples with high and low phenolics.

Recent research has identified luteolin and apigenin as the major phenolics in tagasaste, Work is in progress to clarify the role of these compounds in the plant and their effect on animal production. NIR calibrations are also being developed for luteolin and apigenin, instead of relying on the less satisfactory total phenolics assay. Another research objective is to improve animal performance by using grazing management to manipulate phenolic levels.

Acknowledgements

Work reported in this study was funded in part by Martindale Pty Ltd, the Meat Research Corporation and the Rural Industries Research and Development Corporation. The skilled technical assistance of Glenys Downes and Caroline MacDonald is gratefully acknowledged.

References

1. C.M. Oldham, "Cattle Production from Tagasaste—A New Industry for the Sandplain?", in *Advances in Research on Tagasaste, No. 3 May 1993*, Ed by C.M. Oldham and G. Allen. Martindale Research Project, University of Western Australia, Perth, pp. 23–36 (1993).
2. J.B. Harborne, "The Chemical Basis of Plant Defences" in *In Plant Defences Against Mammalian Herbivory*, Ed by R.T Palo and C.T. Robbins. CRC Press, London, pp. 45–49 (1991).
3. H.K. Goering and P.J. Van Soest, *Forage Fibre Analyses (Apparatus, Reagents, Procedures and Some Applications).* USDA-ARS, Agric. Handbook No. 379, Washington, DC (1970).
4. M.L. Price and L.G. Butler, *J. Agric. Food Chem.* **25,** 1268 (1977).
5. P.C. Flinn and L.D. Saunders, "Making the Old a Slave to the New: Using Existing Calibrations on a New NIR Instrument" in *Leaping Ahead with Near Infrared Spectoscopy,* Ed by G.D. Batten, P.C. Flinn, L.A. Walsh and A.B. Blakeney. RACI NIR Spectroscopy Group, North Melbourne, pp. 129–132 (1995).
6. D.H. Clark, H.F. Mayland and R.C. Lamb, *Agronomy J.* **79,** 485 (1987).
7. W.R. Windham, S.L. Fales and C.S. Hoveland, *Crop Sci.* **28,** 705 (1988).