Determination of chemical composition of fresh corn plant by near infrared reflectance spectroscopy

A. Fassio[a] and D. Cozzolino[b,c]

[a]*Summer Crop Program, INIA La Estanzuela, Colonia, Uruguay. E-mail: fassio@inia.org.uy*

[b]*Animal Nutrition and NIRS Lab, INIA La Estanzuela*

[c]*Actual address: The Australian Wine Research Institute, PO Box 197, Glen Osmond,. South Australia 5064, Australia. E-mail: Daniel.Cozzolino@awri.com.au*

Introduction

The characterisation of forages to feeding animals is becoming important for several reasons. The forages are inherently variable in nutritive value, depends on many factors such as climate, degree of maturity, type of soil, etc. Given the importance and variability of forages, it is vital that methods exist that can reliably assess their nutritional attributes.[1,3] Near infrared (NIR) reflectance spectroscopy offers advantages of simplicity, speed, reduced chemical waste, and more cost-effective prediction of product functionality.[1,2] NIR spectroscopy represents a radical departure from conventional analytical methods, in that the entire sample of forage is characterised in terms of its absorption properties in the near infrared region, rather than separate sub-samples being treated with various chemicals to isolate specific components.[3] This forces the analyst to abandon his traditional narrow focus on the sample (one analyte at a time) and to take a broader view of the relationship between components within the sample and between the sample and the population from which it comes [3]. Forage is usually analysed by NIRS in dry and ground presentation. Measuring fresh material could reduce preparation costs and possible compositional alterations [4, 5]. NIRS has gained widespread acceptance for the analysis of forage quality constituents on dry material, however little attention has been given to the use of NIRS for chemical determinations on undried and unground forages. The objectives of this experiment was to evaluate the potential use of NIRS for assess dry matter (DM), acid detergent fibre (ADF), nitrogen and ash on undried and unground whole corn as forage.

Materials and methods

One hundred and twenty whole fresh corn (*Zea mays* L) samples (n = 120) from the Summer Crop Program and Variety Research Program at INIA La Estanzuela, Colonia, Uruguay, were used. Samples (400 g fresh material) were cut to approximately 40 to 50 mm heights and chopped (10 to 20 mm length) from experimental plots (5 m × 1.5 m). Samples were taken during summer 1999 and 2000. Whole corn samples were dried out at 65°C in a forced—air oven to constant weight (approximately 48 hours), and milled using a Wiley forage mill fitted with a 1 mm grid (Arthur H. Thomas, Philadelphia, PA, USA), before chemical analysis. Nitrogen was determined using a semi – micro automated Kjeldahl method (Tecator, Sweden).[6] Ash was determined by calcination at 500°C.[6] Acid detergent fibre was determined according standard procedures.[7] All samples were analysed in duplicate. Coarse and fresh samples were scanned in the visible and near infrared region

Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

in reflectance (400–2500 nm) using a scanning monochromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA), wrapped in PVC bags (Part number) and placed in a coarse sample cell (200 × 30 × 20 mm) (Part number IH-0395 or NR 7080, NIRSystems, USA). Dry samples were scanned in a small circular cup cell (Part number IH – 0307 NIRSystems, USA). Reflectance data was stored as log (1/*R*) at 2 nm intervals (where *R* = reflectance) to give a total 1050 data points. Samples were scanned once (no repeated spectral measurements were made) and were not rotated when spectra collection was made. Two pairs of lead sulphide detectors collected the reflectance spectra. Reflectance energy readings were referenced to corresponding readings from a ceramic reference disk. The spectrum of each sample was the average of 32 successive scans. Predictive equations were developed using modified partial least squares (MPLS)[8] regression with internal cross-validation and scatter correction using standard normal variate and detrend (*SNV-D*).[9] Cross-validation was used to avoid overfitting of the equations. The mathematical treatment applied was 1,4,4,1. The first number indicates the order of derivative (one is first derivative of log 1/*R*), the second number is the gap in data points over which the derivative is calculated; the third number is the number of data points used in the first smoothing and the fourth number refers to the number of data points over which the second smoothing is applied. Calibration statistics calculated include the standard error of calibration (*SEC*), the coefficient of determination in calibration (R^2_{CAL}), the standard error of cross validation (*SECV*) and the coefficient of determination in cross validation (R^2_{VAL}) [8]. The optimum calibrations were selected on the basis of minimising the standard error of cross validation (*SECV*). This error was calculated by an internal validation of 33 per cent of samples randomly taken by the software routine, which was predicted by an equation based on a calibration with the remaining 66 per cent of all samples. The outlier elimination pass was set to allow the computer program to remove outliers twice before completing the final calibration. The SECV/SD (standard error of cross validation /standard deviation of the constituent data) ratio was also calculated to evaluate the performance of the calibrations. Before calibration and validation CENTER and SELECT algorithms were applied.[8] The Center program ranks spectra in a file according to their Mahalanobis distance (*H* statistic) from the average spectra of the file using PC scores. Two detection outlier passes were used to avoid samples with $H > 3$ and $t > 2.5$. H is the global *H* for the samples when calculated the PCA file. It is associated with spectral characteristics of the sample. If more than 20 per cent of the samples (analyses) are displayed with *H* values greater than 3 during routine analysis, the calibration may need to be updated.[8] Spectra and data were manipulated using International Software, ISI version 3.00 (Port Matilda, PA, USA).

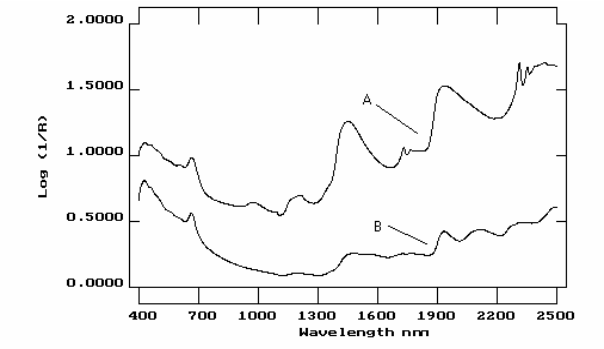

Figure 1. VIS and NIR mean spectrum of wet corn (A) and dry corn samples (B).

Results and discussion

Figure 1 shows the mean spectrum of both wet and dry whole corn samples in the visible and near infrared region. The mean spectrum of the wet presentation has absorption bands in the visible region at 434 nm related with Soret absorption band and at 670 nm related with plant pigments, chlorophyll *a* and *b* respectively.[10] In the near infrared region the mean spectrum show bands at 978 nm, at 1210 nm and 1450 nm related with O–H stretch first and second overtone respectively, and at 1934 nm with water.[11] At 2312 nm, 2352 nm and at 2438 nm related with CH combination bands, respectively. The SD of the mean spectrum at 1940 nm shows a saturation peak related with water content (not shown). Dry sample presentation had in the visible region of the spectra shows two absorption bands at 608 and 666 nm respectively. These absorption bands are related with the absorption of chlorophyll *a* and *b*, respectively.[10] In the near infrared region the spectra shows absorption bands at 1204 nm related with CH stretch second overtone, at 1472 and 1932 nm related with OH stretch second and first overtones, respectively. At 1726 nm with CH stretch first overtone and 2112 and 2344 nm with CH combination bands, respectively.[11] Table 1 shows the NIR

Table 1. Near infrared calibration statistics for dry matter, nitrogen, ash and ADF in fresh whole corn plant (g/kg DM).

	n	*SEC*	R^2_{CAL}	*SECV*	R^2_{VAL}	T
DM	93	28.1	0.96	49.9	0.86	11
Ash	108	7.2	0.94	11.6	084	11
ADF	109	41.7	0.83	46.2	0.80	4
N	110	1.3	0.36	1.5	0.12	4

DM: dry matter; ADF: acid detergent fibre; N: nitrogen; *n*: number of samples after outlier passes; *SEC*: standard error in calibration; R^2_{CAL}: coefficient of determination in calibration; *SECV*: standard error in cross validation; R^2_{VAL}: coefficient of determination in cross-calibration; T: number of PLS terms used to perform the calibration models

calibration statistics for dry matter (DM), acid detergent fibre (ADF), nitrogen and ash in wet presentation to the instrument. The coefficient of determination in calibration (R^2) and *SECV* for wet presentation were for DM 0.96 (*SECV*: 49.9); for nitrogen 0.36 (*SECV*: 1.5); for ADF 0.83 (*SECV*: 46.2) and for ash 0.94 (*SECV*: 11.5) in g kg^{-1} dry weight, respectively. DM and ash showed excellent NIRS calibration statistics meanwhile nitrogen was the poorest parameter predicted by the NIRS calibration models. Nitrogen determination is very sensitive to sample handling in the field and transport to the laboratory. Such conditions could change protein solubility, losses of volatile compounds such as ammonia. Heat damage protein could affect the nitrogen of the plant before both scanned and chemical analysis in the field. Maillard products are formed at higher drying temperatures especially in moist products. They cause lower contents of sugars and true protein than those present in fresh samples.[4] Higher temperatures were verified in the field in Uruguay summer (> 40°C). Consequently all these changes produced losses of total nitrogen in the sample. The errors showed no clear trend related to the nitrogen concentration. The inability of this calibration to accurately analyse the nitrogen content in our sample set lies in the lack of agreement between the reference method and the NIRS measurement. These results could indicated that in wet presentation factors such as sub-sampling, particle size and residual moisture on the sample surface could affect calibration performance. For DM it appeared that NIRS behaved better in the range between 150 to 250 g kg^{-1}, although some outlier samples were observed. These outliers could be produced by an excessive time in the field previous dry matter determination. Many authors concluded that NIRS could be used to accurately analyse forages for concentration of DM or moisture when calibrated with laboratory methods that more truly define their water content (for example,. Karl Fischer

titration). In fresh samples, the time between field sampling–scanning and scanning–reference moisture determination could be critical. Another critical factor in moisture determination centres on the need to scan the samples and immediately weigh samples for reference analysis by oven drying, thus avoiding evaporative losses. NIRS gave a good estimate of the cell wall content (ADF). DM reference method (oven drying) produces changes in the nature of the cell walls due to high temperature. These changes and losses could substantially influence the accuracy of predicting parameters such as nitrogen compounds and fibre fractions. Whole corn plant consists of an assemblage of plant parts and tissues whose physical structure, chemical composition and digestibility differ. These differences are caused by differences in the proportions and digestibility of the various tissues, such as parenchyma and sclerenchyma.[12] The very different function of the botanical components in the whole - plant results in quite different composition of ears, stems and leaves, so whole plant maize shows a strongly bimodal distribution in composition as is illustrated for both ADF and ash (two sub-populations) (see Figures 2 and 3). In NIR calibrations, such

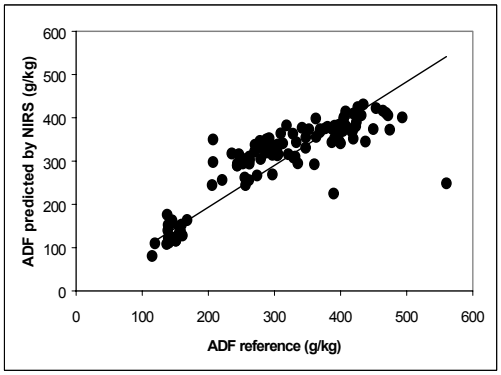

Figure 2. NIRS predicted data against data measured by reference method for ADF on wet corn samples (g kg^{-1}).

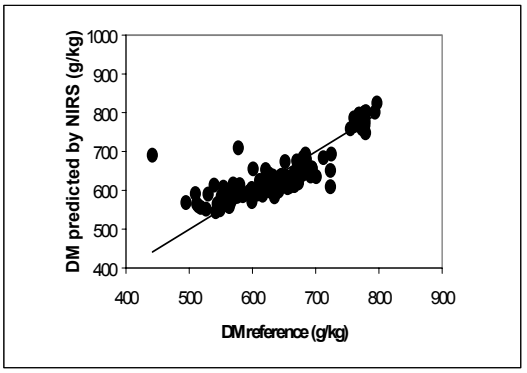

Figure 3. NIRS predicted data against data measured by reference method for DM on wet corn samples (g kg^{-1}).

bimodal distributions raise questions as to whether the increased variability arising from bimodality should be taken as an advantage favouring, better calibrations. The present study suggested that the NIRS method might be used to determine ADF in forage maize breeding programmes involving large germplasm evaluation in mass screening programmes. The *SECV*/*SD* for NIRS calibration statistics for the parameters evaluated demonstrate how well the calibration models performed in predicting the reference data.[13] If a product shows a narrow range in composition, or if the error in estimation is large compared with the spread (as SD) in composition, then regression finds increasing difficulty in finding stable NIR calibrations. Where the error exceeds one-third of the SD of the population, regression can be misleading. In all the treatments applied DM presents the best prediction accuracy while nitrogen did not fit the requirements for a good calibration. The relationship for the parameters analysed in this work were 0.38, 0.40, 0.45 and 0.94 for DM, ash, ADF and N respectively. These figures could imply that for practical analysis of a large volume of fresh forages from the field, either the accuracy of the chemical results and the presentation to the instrument needed to be discussed. Fresh samples present logistical advantages analysed by NIR because of the need to minimise delays between sampling and laboratory scanning that might lead to changes in sample composition. On the other hand the analysis of fresh coarse samples by NIRS

provides greater flexibility, but present problems with sample surface wetness or the interference of the plastic bags. Also some factor that could influence the presentation to the instrument needs to be analysed such the particle size and particle size distribution, could influence near infrared readings. This is the particularly importance in materials like whole corn plant with such no - homogeneous physical composition.

Conclusions

It is concluded that useful NIR prediction models for quality parameters of fresh whole corn were obtained. Using NIRS technique the accuracy of prediction of dry matter, acid detergent fibre and ash on undried corn is relatively similar to that reported elsewhere using dried samples. Wet presentation offers considerable potential for using simpler methods of forage analysis in routine advisory systems avoiding sample preparation (grinding or drying) and as well as can become an alternative technique to be use in plant breeding programs in which a large number of samples must be analysed. However, re-calibration may be necessary over the next years for either nitrogen or dry matter. Especially because these parameters are susceptible to change with climatic or agronomic conditions. Further work has been done to analyse organic matter digestibility in fresh forages.

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