Ann Sci For (1992) 49, 481-488

Original article

The use of near-infrared reflectance spectroscopy in litter decomposition studies

R Joffre [1], D Gillon [1], P Dardenne [2], R Agneessens [2], R Biston [2]

[1] *Centre d'Écologie Fonctionnelle et Évolutive, CNRS, BP 5051, 34033 Montpellier Cedex, France;*
[2] *CRA Gembloux, Station de Haute Belgique, 100 rue de Serpont, 6800 Libramont-Chevigny, Belgium*

(Received 12 March 1992; accepted 2 July 1992)

Summary — The biochemical nature of leaf litter is a key factor in regulation of its decomposition. Conventional wet chemical analysis of samples is destructive, time-consuming and expensive. The objective of this study was to evaluate the potentiality of near infrared reflectance spectroscopy (NIRS) for determining litter chemistry during the decomposition process using a wide range of species and decomposition stages. The litter of 8 species of evergreen and deciduous broad-leaved trees, conifers and shrubs were used in both laboratory and field experiments. Near-infrared reflectance measurements were made with an NIRS Systems 5000 spectrophotometer over the range 1100–2500 nm. Calibration samples were analysed for ash, carbon and nitrogen. Acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined using Van Soest procedures. Stepwise regression (SR) calibrations and partial least squares (PLSR) calibrations were developed and compared as well as the effect of scatter correction. The PLS algorithm was used to create the predictive models using all the information in the spectrum to determine the chemical concentration. Using scatter correction always gave better results. Both regression methods provided acceptable validation statistics for C, N and ash. The PLSR had better prediction accuracy for ADF and ADL. For these two constituents, the improvement of SECV was 34 and 25% respectively. Our results showed that NIRS is an effective tool to predict nitrogen, ash and proximate carbon fractions in decomposition studies and that PSLR method improves calibration compared with SR method.

decomposition / leaf chemistry / litter / NIRS

Résumé — Utilisation de la spectroscopie proche infrarouge dans les études de décomposition de litières. *La composition biochimique des litières est un des facteurs clés de la régulation de leur décomposition. Les méthodes d'analyse chimique par voie humide sont destructives, longues et coûteuses et ces contraintes sont rapidement limitantes dans les études en milieux hétérogènes et plurispécifiques, comme le sont les milieux forestiers spontanés méditerranéens. L'objectif de cette étude est d'évaluer les potentialités de la spectrométrie de réflexion dans le proche infrarouge (SPIR) pour l'étude et le suivi de la décomposition des litières forestières. Les échantillons utilisés proviennent d'expériences menées sur le terrain et en laboratoire sur 8 espèces méditerranéennes : feuillus caducifoliés et sempervirents, et résineux. Les spectres des litières, obtenues à différents stades de décomposition, ont été enregistrés entre 1100 et 2500 nm avec un spectrophotomètre NIRS 5000. Un tiers des échantillons a été analysé par voie humide : cendres totales, carbone, azote, ligno-cellulose et lignine (ADF et ADL méthode Van Soest). À partir de ces analyses, des modèles prédictifs de concentration de chaque composé chimique ont été établis, avec et sans correc-*

tion de tendance, par deux méthodes de régression : i) régression multiple pas à pas (stepwise) et ii) au moyen d'un algorithme d'ajustement par la méthode des moindres carrés PLS (partial least squares). *À la différence des méthodes de régression multiples basées sur un petit nombre de longueurs d'ondes, cette méthode utilise l'ensemble de l'information spectrale. La correction de tendance améliore toujours les résultats de calibration. Les deux méthodes de régression donnent des résultats comparables pour le carbone, l'azote et les cendres. Pour la ligno-cellulose et la lignine, les erreurs standards de validation obtenues par la méthode de calibration PLS sont inférieures de 25 et 34% à celles obtenues par la méthode de régression multiple. Ces résultats montrent que la SPIR peut être utilisée dans les études de décomposition et que la méthode de calibration basée sur l'ensemble du spectre (PLS) est plus performante pour la prédiction des fractions carbonées complexes. Par sa rapidité et sa fiabilité, cette méthode réduit les contraintes analytiques et permet d'aborder les études de décomposition en milieu hétérogène.*

décomposition / litière / chimie du feuillage / SPIR

INTRODUCTION

Within a climatic area, the biochemical nature of leaf litter is certainly the most important factor in the regulation of its decomposition (O'Connell, 1988; Berg and McClaugherty, 1989; Taylor *et al,* 1989). The rate of decay varies with nitrogen and phosphorus concentration and also with carbon chemistry (Swift *et al,* 1979; McClaugherty and Berg, 1987). The carbon chemistry of the litter substrate is usually divided into 3 fractions: extractives (lipids, sugars, phenolics), polymer carbohydrates (cellulose, hemicellulose) and acid-insoluble compounds (AIC = lignins). In classical forage fiber analysis, the last 2 fractions constitute the ADF (acid detergent fiber) and the last fraction the ADL (acid detergent lignin). Each of these fractions represents a mixture of constituents extracted at the same time using the Van Soest analytical technique (1963); however, they are very useful to understand litter decay. Indeed, because lignins can operate both as a carbon and energy source and as a modifier of the activity of decaying organisms, they are as important as nutrient content for resource quality.

Conventional wet chemical analysis of samples is destructive, time-consuming and expensive when a large number of samples is required. Moreover, for some constituent, such as proximate carbon fractions, no standard method has been established (Ryan *et al,* 1990). Although near infrared reflectance spectroscopy (NIRS) has become widely used as a nondestructive method for quality analysis of grain (Williams, 1975) and forage (Norris *et al,* 1976), few ecological studies have used this technique. Dalal and Henry (1986), Krishnan *et al* (1980) and Morra *et al* (1991), used NIRS to predict C and N concentrations in soils. Card *et al* (1988), Wessman *et al* (1988) and McLellan *et al* (1991a, b) showed that NIRS may be useful for the determination of leaf chemistry.

Using a wide range of species and decomposition stages, our objectives were i) to determine the changes in spectra during decomposition process, ii) to evaluate the potential of NIRS for determining litter chemistry during decomposition, and iii) to compare the stepwise regression (SR) and partial least squares regression (PLSR) calibration methods.

MATERIALS AND METHODS

Litter decomposition experiment

Two data set collected from 2 experiments were used. The first experiment was conducted in the

laboratory and concerned 8 species (*Quercus pubescens* L, *Quercus ilex* L, *Quercus coccifera* L, *Castanea sativa* Miller, *Pinus halepensis* Miller, *Fagus sylvatica* L, *Cistus monspeliensis* L, *Cistus albidus* L). Leaf litter of these species were collected at the leaf-fall period near Montpellier. In the laboratory, a microcosm system, as described by Taylor and Parkinson (1988), was used. Air dried samples of 7.00 ± 0.01 g were remoistened in water for 24 h and were placed over a 2 mm nylon mesh on the soil surface of the microcosms. Microscosms were maintained at 22 °C and watered once a week maintaining soil moisture at 80% of field capacity. Five replicates of each litter were removed after 0.5, 1, 2, 4, 6, 10 and 14 months. The second experiment was conducted in the field, in a *Q pubescens* forest (50 km NE of Marseille, southern France), and concerned the 2 species *Q pubescens* and *P halepensis*. In this experiment, 5 mm mesh bags containing 10 g of air-dried litter, collected near this forest, were placed on the soil surface. Five replicates were removed after 5, 12, 19 and 26 months. All samples were dried in a ventilated oven at 60 °C until constant weight, weighed and then ground in a cyclone mill through a 1-mm mesh.

NIRS analysis

A total of 330 samples were scanned with a near-infrared reflectance spectrophotometer (NIRSystems 5000). Each sample was packed into a sample cell having a quartz-glass sample. Two reflectance measurements of monochromatic light were made from 1100 to 2500 nm to produce an average spectrum with 700 data points at 2 nm intervals over this range. The band-pass used 10 nm and the wavelength accuracy 0.5 nm. Reflectance *(R)* is converted to absorbance *(A)* using the following equation:

$$A = \log (1/R)$$

Data analysis was conducted using ISI software system (Shenk and Westerhaus, 1991b).

Sample selection and chemical methods

Approximately one-third of the samples were selected for providing the calibration sample set analysed by wet chemical techniques. On the basis of the standardized H distances from the average spectrum in the space of the principal components, we first eliminated 4 samples (on the total population of 330 samples) with H > 3.0 (Shenk and Westerhaus, 1991a). The second algorithm used standardized H distance among pairs of samples to define neighbourhoods. The average distance between pairs of closest samples was 0.068, and using an H = 0.125, 91 samples were selected.

These samples were analysed for ash (550 °C for 3 h) and moisture (105 °C for 24 h). Carbon and nitrogen content were determined with a Perkin Elmer elemental analyser (PE 2400 CHN) and acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined using Van Soest procedures (1963, 1965) adjusted for Fibertec (Van Soest and Robertson, 1985). Considering the important weight loss of litter after several months of incubation, analyses could not be achieved on all samples because of the lack of material.

Statistical methods

Stepwise regression (SR) calibrations and partial least squares (PLSR) calibrations were developed and compared for C, N, ADF, ADL, and ash with each calibration using 6 math treatments corresponding to first and second derivative and a gap of 5, 10, and 15 data points or 10, 20, and 30 nm. For all these previous math treatments, results obtained with and without the detrending method (Barnes *et al*, 1989) were compared.

Stepwise is performed by selecting the wavelength that is the most highly correlated with the reference values and adding it to the equation. The second wavelength is added by calculating all partial correlations with all other wavelengths and selecting the wavelength with the highest partial correlation. The process continues until the addition of a wavelength makes no additional improvement in explaining the variation in the reference value (*F* value significant at 0.01). After each wavelength is added to the equation, the program re-evaluates all wavelength in the equation before continuing (Windham *et al*, 1989; Shenk and Westerhaus, 1991b).

Partial least squares (PLS) algorithm was used to create predictive models (Martens and Jensen, 1982). PLS differs from wavelength searches in that it uses all the information in the

spectrum to determine the analyte concentration, a fundamental advantage over single wavelength applications. Because the entire spectrum is used, each wavelength is averaged into the answer. PLS is the marriage of principal component analysis (PCA) and multiple linear regression (MLR). PCA reduces the spectral data to a few combinations of the absorptions that account for most of the spectral information but also relates to the sample reference values (Shenk and Westerhaus, 1991b). The first vector (called a loading) used by the PLS algorithm is the result of the cross multiplication of the spectral variance of the data and the correlation spectrum. The first loading is used to fit the training spectra based on a least square is then correlated with the chemical value. This results in an overall correlation coefficient and a preliminary estimate of the chemical values. The residual errors between the actual and predicted chemical values are calculated, as are the residual spectra from the curve fitting process. Both of these residuals are plugged back into the start of the program. The same calculations are performed on the residuals to obtain the second loading and scores. This stepwise addition of loadings continues until sufficient terms have been added to explain the chemical data. Cross validation is used to estimate the optimal number of terms in the calibration to avoid overfitting. It consists of selecting, for instance, 1 quarter of the samples for the prediction and 3 quarters to develop the model. The algorithm is repeated 4 times and all the residuals of the 4 predictions are pooled to provide a standard error of cross validation (SECV) on independent samples. The minimum SECV determines the number of terms to be used. The final model is then recalculated with all the samples to obtain the standard error of calibration (SEC).

In order to compare the 2 calibration methods, only the math treatment that provided the most accurate prediction of each constituent has been taken into account.

RESULTS AND DISCUSSION

Changes in spectra during decomposition process

The modification of the litter chemical components during decomposition was related to a progressive and important distortion of the spectra. The example of *Quercus pubescens* litter shows that this alteration is far more rapid in the laboratory than in the field (fig 1a, b). As decomposition progresses, absorbance in the region between 1100 and 1400 nm increases as emphasized by Mc Lellan *et al* (1991a). This baseline shift can be related to the modification of the mineral matter/organic matter ratio of the samples as decomposition progresses. Ash concentration increases with time and decay state: from 82, 98, 128, 180 to 216 g kg^{-1} dry matter at 0, 5, 12, 19 and 26 months of decomposition in field experiments whilst this concentration varies from 55, 78, 180, 248 to 441 at 0, 0.5, 2, 4 and 6 months in laboratory experiments. The increased reflectance in the 1100-1400 region caused by

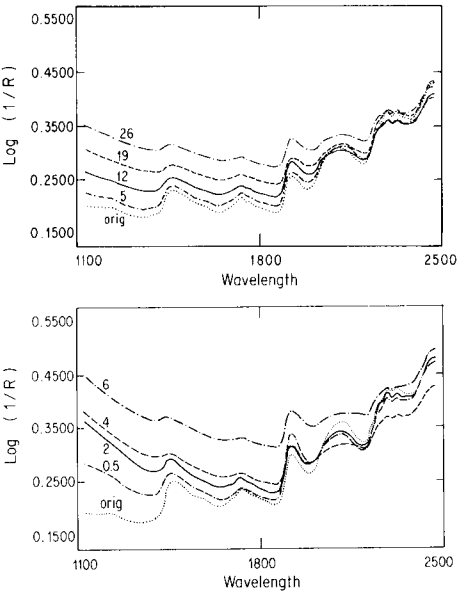

Fig 1. a Spectra of litter samples of *Quercus pubescens* at the beginning and after 5, 12, 19 and 26 months during the field decomposition experiment. **b** Spectra of litter samples of *Quercus pubescens* at the beginning and after 0.5, 2, 4 and 6 months during the laboratory decomposition experiment.

the increase of mineral component agrees with data from Paul (1988) for soil contamination in silage and Windham *et al* (1991) for increasing ash concentration in forage, esophageal and fecal samples.

Calibration equations

The calibration equations were carried out on samples characterized by a wide range of chemical components concentration (table I). Furthermore, as emphasized by McLellan *et al* (1991a), the chemical nature of decomposing plant materials was more heterogeneous than that of green foliage. This sample heterogeneity could have led to low determination coefficients and high SEC and SECV. Yet, on the whole, SEC and SECV were weak except for ADF (tables II, III). The use of scatter correction gave similar or better result in all cases. Determination coefficients ranged from 0.87–0.99 (except 0.82 and 0.78 for ADF by SR with and without scatter correction). The 2 methods of regression gave similar good prediction results for C, N and Ash. The PLSR had better prediction accuracy for ADF and ADL. For these 2 constituents, the improvement of SECV was 34 and 25% respectively.

Among all the analysed constituents, ADF is the most complex, as it is made up of all lignins and celluloses which probably have different decomposition rates. ADF thus results from several different components in variable proportions, registered by chemical analysis as a single entity, but probably related to different spectra. The SEC value indicates that different chemical components are not expressed by the ADF global value measured here.

Graphic comparisons between values predicted with NIR calibration equations and those obtained by chemical analyses are displayed in figure 2. The prediction equation is all the more effective as the

Table I. Wet chemical range (%) for each constituent within the calibration sample set.

Variable	*n*	*Mean*	*Range*
C	51	45.3	27.3 – 58.6
N	47	1.09	0.45 – 1.94
ADF	73	53.4	33.4 – 70.4
ADL	70	33.1	17.7 – 51.6
Ash	68	15.9	3.4 – 44.1

Table II. Modified partial least squares regression. Equation calibration statistics for C, N, ADF, ADL and Ash.

		With scatter correction			*Without scatter correction*			
Variable	*n*	*SEC*	R^2	*SECV*	*SEC*	R^2	*SECV*	*Math treatment**
C	51	0.68	0.98	1.11	1.29	0.95	2.29	2 5 5
N	47	0.04	0.99	0.09	0.05	0.98	0.09	2 10 5
ADF	73	2.64	0.90	3.60	2.99	0.88	5.25	2 5 5
ADL	70	1.93	0.96	2.58	2.03	0.96	3.04	2 5 5
Ash	68	0.87	0.99	1.52	1.74	0.98	2.42	2 5 5

* Math treatment indicates the mathematical transformation of spectral data: the first number is the order of the derivative function, the second is the segment length in data points over which the derivative was taken, and the third the segment length over which the function was smoothed.

Table III. Stepwise regression. Equation calibration statistics for C, N, ADF, ADL and Ash.

		With scatter correction			*Without scatter correction*			
Variable	*n*	*SEC*	R^2	*SECV*	*SEC*	R^2	*SECV*	*Math treatment**
C	51	0.76	0.97	0.82	1.23	0.95	1.40	2 5 5
N	47	0.06	0.96	0.08	0.07	0.96	0.09	2 10 5
ADF	73	3.61	0.82	3.96	4.05	0.78	4.29	2 5 5
ADL	70	3.30	0.91	3.42	2.81	0.87	2.94	2 5 5
Ash	68	1.58	0.98	1.73	2.51	0.95	2.63	2 5 5

* Math treatment indicates the mathematical transformation of spectral data: the first number is the order of the derivative function, the second is the segment length in data points over which the derivative was taken, and the third the segment length over which the function was smoothed.

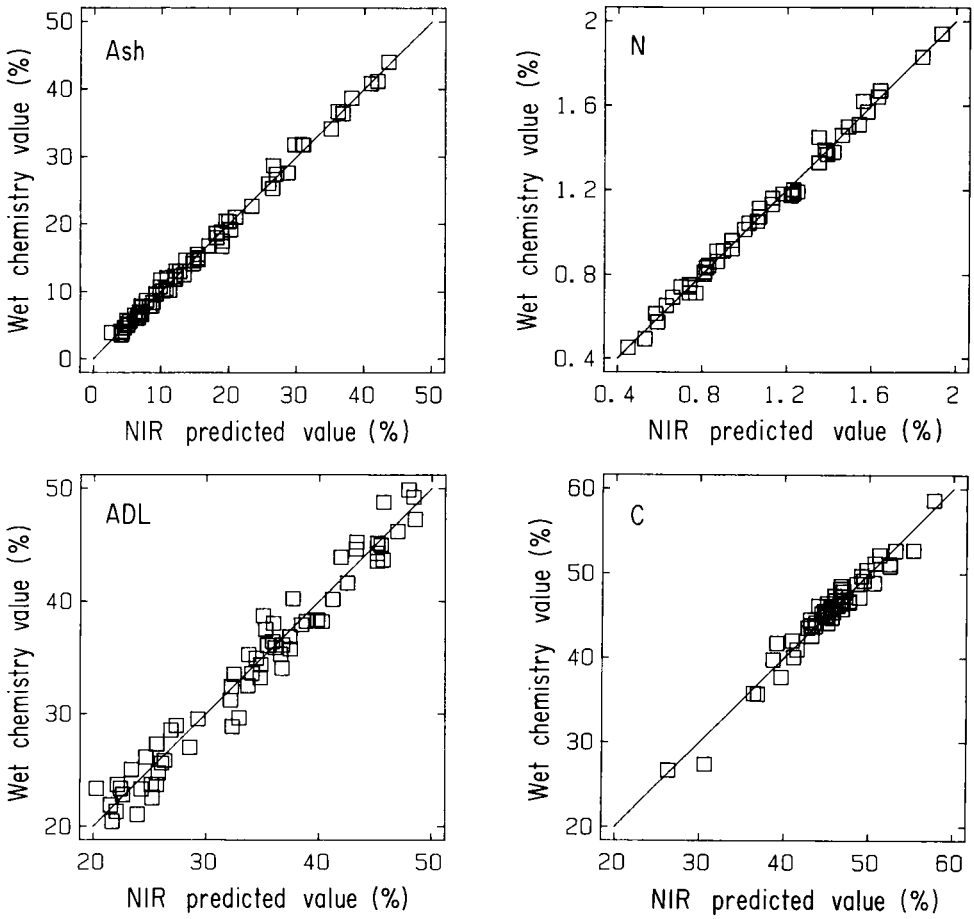

Fig 2. Relationship between NIR predicted values and wet chemistry values (% of dry matter).

points are near the theoretical correspondence 1:1 (diagonal line).

The results obtained show that NIRS is an effective tool to predict nitrogen, ash, and proximate carbon fractions from forage fiber techniques in the study of decomposition of leaf litter from a variety of evergreen and deciduous broad-leaved species, conifers and shrubs. However, the interpretation of forage fiber analysis in decomposing leaf material remains difficult. Ryan *et al* (1990) emphasized that "forage fiber lignin analysis may be less sensitive than the forest products lignin analysis to changes that occur during decomposition". Complementary studies are now in progress using the same plant material to test NIRS efficiency in order to determine carbon chemistry according to forest product techniques.

ACKNOWLEDGMENTS

This research was supported by the Programme Interdisciplinaire de Recherches sur l'Environnement (PIREN) of the Centre National de la Recherche Scientifique (CNRS).

REFERENCES

Barnes RJ, Dhanoa MS, Lister SJ (1989) Standard normal variate transformation and detrending of NIR spectra. *Appl Spectrosc* 43, 772-777

Berg B, McClaugherty C (1989) Nitrogen and phosphorous release from decomposing litter in relation to the disappearance of lignin. *Can J Bot* 67, 1148-1156

Card DH, Peterson DL, Matson PA, Aber JD (1988) Prediction of leaf chemistry by the use of visible and near-infrared reflectance streptoscopy. *Remote Sens Environment* 26, 123-147

Dalal RC, Henry RJ (1986) Simultaneous determination of moisture, organic carbon, and total nitrogen by near-infrared spectrophotometry. *Soil Sci Soc Am J* 50, 120-123

Krishnan P, Alexander JD, Butler BJ, Hummel JW (1980) Reflectance technique for predicting soil organic matter. *Soil Sci Soc Am J* 44, 1282-1285

Martens H, Jensen SA (1982) Partial least squares regression: a new two-stage NIR calibration method. *In: Proc 7th World Cereal Bread Cong* (Holas J, Kratchovil R, eds) Elsevier, Amsterdam, 607-647

McClaugherty C, Berg B (1987) Holocellulose, lignin and nitrogen levels as rate-regulating factors in late stages of forest litter decomposition. *Pedobiologia* 30, 101-112

McLellan TM, Aber JD, Martin ME, Melillo JM, Nadelhoffer KJ (1991a) Determination of nitrogen, lignin and cellulose content of decomposing leaf material by near infrared reflectance spectroscopy. *Can J For Res* 21, 1684-1689

McLellan TM, Martin ME, Aber JD, Melillo JM, Nadelhoffer KJ, Deway B (1991b) Comparison of wet chemistry and near infrared reflectance measurements of carbon fraction chemistry and nitrogen concentration of forest foliage. *Can J For Res* 21, 1689-1694

Morra MJ, Hall MH, Freeborn LL (1991) Carbon and nitrogen analysis of soil fractions using near-infrared reflectance spectroscopy. *Soil Sci Soc Am J* 55, 288-291

Norris KH, Barnes RF, Moore JE, Shenk JS (1976) Predicting forage quality by infrared reflectance spectroscopy. *J Anim Sci* 43, 889-897

O'Connell AM (1988) Nutrients dynamics in decomposing litter in karri (*Eucalyptus diversicolor* F Muell) forests of south-western Australia. *J Ecol* 76, 1186-1203

Paul C (1988) Effect of soil contamination on NIRS analysis of grass silage. *In: Analytical Applications of Spectroscopy* (Creaser CS, Davies AMC, eds) Royal Soc of Chemistry, Burlington House, London, 84-90

Ryan GM, Melillo JM, Ricca A (1990) A comparison of methods for determining proximate carbon fractions of forest litter. *Can J For Res* 20, 166-171

Shenk JS, Westerhaus MO (1991a) Population definition, sample selection, and calibration procedures for near-infrared reflectance spectroscopy. *Crop Sci* 31, 469-474

Shenk JS, Westerhaus MO (1991b) *ISI NIRS-2. Software for near-infrared instruments Infrasoft international*

Swift MJ, Heal OW, Anderson JM (1979) *Decomposition in Terrestrial Ecosystems.* Blackwell Sci, Oxford

Taylor B, Parkinson D (1988) A new microcosm approach to litter decomposition studies. *Can J Bot* 66, 1933-1939

Taylor B, Parkinson D, WFJ Parsons (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* 70, 97-104

Van Soest PJ (1963) Use of detergent in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J Assoc Off Anal Chem* 46, 829-835

Van Soest PJ (1965) Use of detergent in the analysis of fibrous feeds. III. Study of effects of heating and drying on yeild of fiber and lignin in forages. *J Assoc Off Anal Chem* 48, 785-790

Van Soest PJ, Robertson JB (1985) *Analysis of forages and fibrous foods: a laboratory manual for animal science.* Cornell Univ Pub

Wessman CA, Aber JD, Peterson DL, Melillo JM (1988) Foliar analysis using near-infrared reflectance spectroscopy. *Can J For Res* 18, 6-11

Williams PC (1975) Applications of near-infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. *Cereal Chem* 52, 561-576

Windham WR, Mertens DR, Barton II FE (1989) Protocol for NIRS calibration: Sample selection and equation development and validation. *In: Near Infrared Reflectance Spectroscopy: Analysis of Forage Quality* (GC Marten, JS Shenk, FE Barton II, eds). Agric. Han book 643. USDA-ARS, Washington, 96-103

Windham WR, Hill NS, Stuedemann JA (1991) Ash in forage, esophageal, and fecal samples analysed using near-infrared reflectance spectroscopy. *Crop Sci* 31, 1345-1349