Effect of Sample Preparation on Prediction of Fermentation Quality of Maize Silages by Near Infrared Reflectance Spectroscopy*

H. S. Park, J. K. Lee[1], J. H. Fike[2], D. A. Kim[3], M. S. Ko and J. K. Ha[3,]**
National Institute of Subtropical Agriculture, Rural Development Administration, Jeju, 690-150, Korea

ABSTRACT : Near infrared reflectance spectroscopy (NIRS) has become increasingly used as a rapid, accurate method of evaluating some chemical constituents in cereal grains and forages. If samples could be analyzed without drying and grinding, then sample preparation time and costs may be reduced. This study was conducted to develop robust NIRS equations to predict fermentation quality of corn (*Zea mays*) silage and to select acceptable sample preparation methods for prediction of fermentation products in corn silage by NIRS. Prior to analysis, samples (n = 112) were either oven-dried and ground (OD), frozen in liquid nitrogen and ground (LN) and intact fresh (IF). Samples were scanned from 400 to 2,500 nm with an NIRS 6,500 monochromator. The samples were divided into calibration and validation sets. The spectral data were regressed on a range of dry matter (DM), pH and short chain organic acids using modified multivariate partial least squares (MPLS) analysis that used first and second order derivatives. All chemical analyses were conducted with fresh samples. From these treatments, calibration equations were developed successfully for concentrations of all constituents except butyric acid. Prediction accuracy, represented by standard error of prediction (SEP) and R^2_v (variance accounted for in validation set), was slightly better with the LN treatment (R^2 0.75-0.90) than for OD (R^2 0.43-0.81) or IF (R^2 0.62-0.79) treatments. Fermentation characteristics could be successfully predicted by NIRS analysis either with dry or fresh silage. Although statistical results for the OD and IF treatments were the lower than those of LN treatment, intact fresh (IF) treatment may be acceptable when processing is costly or when possible component alterations are expected. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 643-648)***

Key Words : Near Infrared Reflectance Spectroscopy, Fermentation Quality, Corn Silage, Sample Preparation

INTRODUCTION

Corn silage is an important forage in dairy feeding, not only as a winter rations but also as a supplemental feed during the grazing season. Silage is the material produced by the controlled microbial fermentation of crops with high moisture content in a process known as ensilage. Quality of corn silages is commonly evaluated on the basis of pH, NH_3-N, and concentrations of certain short-chain organic acids. However, traditional analytical methods for determining nutritive value of corn silage can be costly and time consuming.

Near-infrared reflectance spectroscopy (NIRS) offers advantages over wet chemistry in terms of simplicity, speed, reduced chemical waste, and a more cost-effective prediction of product functionality. Analysis of feeds and silages by wet chemistry require drying and milling of the sample. Although prediction of composition of dried samples has been carried out for a number of years, the direct analysis of undried silages by NIRS would provide additional gains in reduced time and labor for sample preparation and faster reporting of the results (Shenk and Westerhaus, 1995; De la Roza et al., 1996; Kennedy et al., 1996).

Relatively few studies have been published on the use of NIRS to determine fermentation quality parameters of undried samples, with most results reported for grasses and silages (Daniel et al., 1999; Park et al., 2002). In the case of wet silage samples, heat drying could result in losses of volatile substance, such as short-chain organic acids and alcohols (McDonald et al., 1991). In order to determine the amount of short chain organic acids in wet silage using NIRS, freezing with liquid nitrogen has been adopted in some laboratories to prevent loss of these volatiles from fresh forage samples.

Another problem with using NIRS directly on fresh silages may be increased errors due to differences in sample particle size, temperature and water content. These problems can be overcome by grinding silages in a frozen state with dry ice or liquid nitrogen. However, such procedures are time-consuming and inconvenient due to the cleanup required between samples and the need to thaw the sample for subsequent use.

These issues may be resolved in part by improvements in chemometrics software, spectral data transformation for scatter correction and partial least squares regression. Such advances have minimized some of the interference of particle size variation and water absorption presented by wet silage samples (Baker et al., 1994; Shenk and

* The work was supported by the Brain Korea 21 Project, Korea.
** Corresponding Author: Ha, Jong Kyu. Tel: +82-2-880-4809, Fax: +82-2-857-8710, E-mail: jongha@snu.ac.kr
[1] Hanwoo Experiment Station, National Livestock Research Institute, RDA, Kangwon-do, 232-952, Korea.
[2] Crop and Soil Environmental Science Department, Virginia Tech. Blacksburg, VA 24061, USA.
[3] School of Agricultural Biotechnology, Seoul National University, Seoul, 151-742, Korea.
Received October 17, 2004; Accepted January 19, 2005

Westerhaus, 1995; Gordon et al., 1998). Thus, this experiment was conducted to assess the effect of sample preparation (drying or liquid nitrogen treatment vs. fresh) methods on prediction of fermentation quality of corn silage, and to select an acceptable sample preparation method for wet silage.

MATERIAL AND METHODS

Silage preparation for NIRS scanning

Corn silage samples (n = 112) were collected from dairy farms in Kyunggi-do, Korea. The samples were frozen as soon as they arrived at the laboratory, and stored frozen (-20°C) until analyzed. Prior to NIRS scanning, the silage samples were thawed overnight at 4°C in a refrigerator.

Each sample was subdivided into three sub-samples of ca. 200 g that were either oven-dried (OD), ground under liquid nitrogen (LN), or held intact fresh (IF). Oven-dried sub-samples were dried at 65°C for 24 h and then ground in Wiley mill with a 1 mm screen. Intact fresh sub-samples were measured immediately upon opening the silo with no sample preparation. Liquid nitrogen sub-samples were immersed in liquid nitrogen (-196°C) for 30 min, broken up with a wooden mallet and sub-sequently finely chopped in a kitchen food chopper. The milled samples were thawed at ambient room temperature before being presented for NIRS scanning on a NIRSystem Model 6500 spectrophotometer (Perstorp Analytical Silverstone, USA).

Chemical analysis

All chemical analyses were conducted on the undried samples. Dry matter (DM) was determined by drying for 24 h at 105°C in a forced-draft oven. To determine pH, 10 g of plant tissue were macerated in a blender with 100 ml of distilled water. Silage pH was measured with an electrometric pH meter (HI 9024; HANNA Instrument Inc., UK).

For analysis of short-chain organic acids, 20 g of fresh silage were transferred to 250 ml wide-necked bottle and 100 ml of distilled water were added to each. The bottles were capped and shaken mechanically for 1 h. Resulting solutions were then filtered through Whatman No. 1 filter paper. Five ml of filtrate were combined with 1 ml of a 2.5 g/L solution of pivalic acid (as internal standard) and 2.5 ml of 0.12 M oxalic acid in a 10 ml calibrated flask and diluted to volume. Flasks were centrifuged at 2,600 g for 5 min and the supernatants were collected for injection into a GC.

Short-chain organic acids were determined by gas chromatography (6890N, Agilent Co., USA) on an 80/120-mesh Carbopack B-DA/4% Carbowax (Supelco Inc., Bellefonte, PA, Catalog No. 1-1889) with 20 M column treated with trimesic acid in methanol. Oven conditions included a gas flow-rate of 24 ml/min and oven temperature of 200°C with 1 μl injections of 0.03 M oxalic acid made prior to use.

Analysis of spectral data

Spectra of silage samples were collected in NIRSystems 6500 scanning monochromotor. The OD and LN sub-samples were placed in Quarter cups, and IF sub-samples were placed in Natural Product sample cups which were inserted in a transporting sample device. Absorbance data were collected as log 1/R, from 400 to 2,500 nm. Data analyses were performed using WinISI II-version 1.02a software (Foss NIRSystems/Tecator, Infrasoft International, LLC). In order to determine the best sample preparation of data, the 112 spectra for each of the 3 sample preparation methods were divided into a calibration set (n = 86) and a validation set (n = 36) using the SELECT facility as developed by Shenk and Westerhaus (1991) within WinISI II-version software. This selected a specified number of samples (86), on the basis of their spectral H-distance (equivalent to Mahalanobis distance), which represented the spectral characteristics of the whole population.

Calibrations were developed using modified partial least squares (MPLS) regression with internal cross-validation after scatter correction using standard normal variate (SNV) and detrending. Cross-validation was used to avoid over-fitting of the equations. A 1,4,4,1 curve-smoothing mathematical treatment was applied to the NIRS output. The first number indicates the order of derivative, the second number is the gap in nm over which the derivatives were calculated, the third number is the number of data points used in the first smoothing, and the fourth number refers to the number of nm over which the second smoothing was applied. Calibration statistics included the standard error of calibration (SEC), the coefficient of multi-determination in calibration (R^2), and the standard error of cross-validation (SECV). Optimal calibrations were selected on the basis of minimizing the SECV. An independent validation set (OD = 48, LN = 29 and IF = 27) was used with samples not included in the calibration set. Standard error of prediction (SEP), squared simple correlation coefficient (RSQ), and slope were calculated using WinISI software, version 1.02a.

RESULTS AND DISCUSSION

In developing optimum systems for sample preparation it is important that the spectral data produced, within each method, are fully explored to produce the best prediction relationships. The silage samples selected for this study varied widely in their DM, pH and most short-chain organic acid parameters (Table 1). A number of the corn silage samples contained little or no detectable concentration of butyric acid, which should be undetectable in good silage. A

Table 1. Average±SD and ranges for DM, pH and short-chain organic acids of corn silages according to drying method on DM basis

Sample preparation[1]	Calibration set			Validation set		
	OD	LN	IF	OD	LN	IF
Components						
DM	28.8±5.17	28.5±3.61	28.7±3.81	28.7±2.65	28.1±2.24	28.3±2.24
Range	20.8-49.4	21.8-42.5	21.8-44.4	24.5-38.1	25.0-31.7	25.0-32.0
pH	4.11±0.21	4.07±0.19	4.06±0.19	4.00±0.13	3.99±0.14	4.02±0.16
Range	3.79-4.83	3.79-4.83	3.76-4.83	3.76-4.32	3.76-4.23	3.76-4.46
Short-chain organic acid (%, DM)						
Acetic acid	1.42±0.54	1.46±0.48	1.47±0.48	1.44±0.39	1.41±0.47	1.39±0.43
Range	0.47-2.34	0.52-2.34	0.46-2.34	0.46-2.26	0.46-2.30	0.53-2.24
Propionc acid	0.33±0.20	0.34±0.17	0.35±0.16	0.34±0.12	0.30±0.17	0.30±0.14
Range	0.00-0.81	0.00-0.84	0.00-0.86	0.00-0.64	0.00-0.77	0.00-0.55
Butyric acid	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.004	0.01±0.003	0.01±0.01
Range	0.00-0.05	0.00-0.03	0.00-0.03	0.00-0.02	0.00-0.02	0.00-0.03
Lactic acid	4.85±1.60	4.69±1.49	4.82±1.47	4.84±1.29	5.28±1.43	4.92±1.48
Range	0.36-7.97	0.36-8.46	0.36-7.97	2.06-8.46	2.93-7.97	2.06-8.46
Total acid	6.60±1.58	6.50±1.46	6.63±1.40	6.62±1.16	7.03±1.25	6.61±1.25
Range	2.77-10.17	2.77-10.37	2.77-10.27	4.29-10.16	5.43-10.04	4.29-10.16

[1] OD = Oven-dried ground, LN = Frozen in liquid nitrogen and ground, IF = Intact fresh.

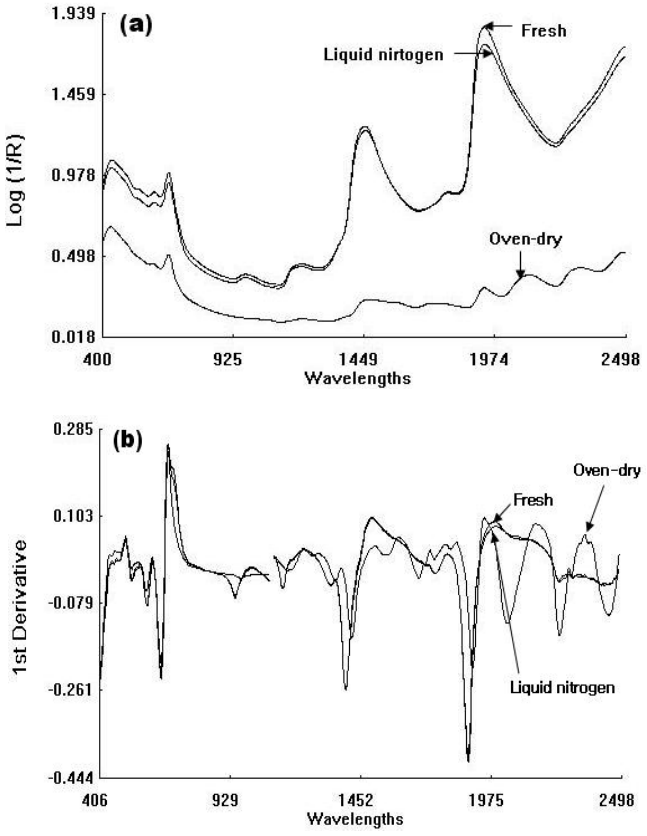

Figure 1. Average NIR spectra of silage either as log1/R (a) or as a first derivative (b) for oven-drying (OD), Liquid nitrogen (LN) and Fresh treatments.

total of 7 parameters were examined in the calibrations. Using MPLS regressions, with the firs (1,4,4,1) derivatization in conjunction with SNV-D, produced a calibrations for each of the parameters are given in Table 1.

Average spectral data from 400 to 2,500 nm for each treatment are shown in Figure 1(a). Lines of OD and LN treatment are obviously not superimposed, which indicates some differences between treatments, although they show similar absorption bands, rendering the curves almost parallel. Baseline shifts may occur due to factors such as differences in sample holder glass, or differences in sample compression, temperature, and particle size (Shenk and Westerhaus, 1995). In order to compensate for baseline shift and to enhance the relevant spectral information, spectra were transformed to their first derivative. The average differentiated spectra for each treatment are presented in Figure 1(b). Although differentiation removed most differences between processing methods, at least two absorption bands could be detected. These differences between treatments suggest that some chemical bonds are present in different concentrations as a result of processing method.

In order to acquire optimum calibration equations, Adesogan et al. (1998) suggested that equations with the largest R^2, smallest standard error of calibration (SEC), and lowest number of spectral terms (to avoid overfitting) should be selected. When developing MPLS equations, cross validation was used to select the optimum number of factors and thus avoid overfitting. For purposes of comparison, the best calibrations were selected on the basis of minimizing the SECV. The standard error of cross validation (SECV) and 1-VR (equivalent to R^2 of cross validation) statistics represent a truer prediction of how the calibration will perform when unknown samples are predict.

For estimates of DM, the NIRS calibration statistics (Table 2) of the LN treatment were slightly better (lower SEC, higher R^2) than those obtained by the OD and IF treatments. The values obtained here provided good

Table 2. NIRS calibration statistics for DM, pH and short-chain organic acids of corn silage

Parameter	Preparation methods[1]	Calibration[2]				
		SEC	R^2	SECV	1-VR	SD/SECV
DM	OD	1.44	0.87	1.65	0.83	3.13
	LN	0.99	0.93	1.30	0.87	2.78
	IF	1.40	0.83	1.55	0.79	2.46
pH	OD	0.08	0.81	0.11	0.63	1.91
	LN	0.06	0.81	0.09	0.63	2.11
	IF	0.08	0.74	0.11	0.54	1.73
Short-chain organic acid (%, DM)						
Acetic acid	OD	0.16	0.91	0.23	0.84	2.35
	LN	0.11	0.94	0.20	0.84	2.40
	IF	0.10	0.95	0.19	0.85	2.53
Propionic acid	OD	0.12	0.66	0.14	0.50	1.43
	LN	0.06	0.83	0.09	0.67	1.89
	IF	0.09	0.63	0.11	0.44	1.46
Butyric acid	OD	0.01	0.27	0.15	0.19	0.07
	LN	0.003	0.26	0.004	0.17	2.50
	IF	0.003	0.19	0.004	0.04	2.50
Lactic acid	OD	0.70	0.79	0.81	0.73	1.98
	LN	0.48	0.89	0.73	0.77	2.04
	IF	0.43	0.90	0.64	0.78	2.23
Total acid	OD	0.67	0.80	0.80	0.71	1.96
	LN	0.52	0.87	0.73	0.76	2.00
	IF	0.58	0.83	0.74	0.73	1.89

[1] OD = Oven-dried ground, LN = Frozen in liquid nitrogen and ground, IF = Intact fresh.
[2] R^2 = Multiple correlation coefficient of determination.
SEC = Standard error of calibration.
SECV = Standard error of cross validation.
1-VR = Multiple correlation coefficient of cross validation.

calibration statistics, in contrast with the findings of Cozzolino et al. (2000). Accurate determination of moisture (or DM) in forages has been hampered both by the lack of a primary reference method that is specific for water and by uncertainties in the change in moisture content during sample preparation. The inability of this calibration to accurately analyze the DM content in our sample set lies in the lack of agreement between the reference method (oven drying) and the NIRS measurement. Many authors (Windham, 1987; Windham et al., 1987) have concluded that NIRS could be used to accurately analyze forages for concentration of DM or moisture when calibrated with laboratory methods that truly define their water content.

The coefficients of determination for estimates of silage pH were slightly greater for the LN and OD treatments than for the IF treatment (0.81 vs. 0.74; Table 2). Lower R^2 for the IF treatment is likely due to differences in particle size and packing characteristics because these samples were not ground. Results of the LN treatment were consistent with reports of Gordon et al. (1998), Park et al. (1998) and Park et al. (2002).

The effects of sample preparation method on the accuracy of short-chain organic acid prediction were similar to those for pH and DM. Performance statistics for the prediction of acetic and lactic acids in the present study were excellent, with R^2 for the LN treatment being slightly greater than for OD and IF treatments These performance statistics are also better than those obtained by Park et al. (1998) and Reeves and Blosser (1989) from NIRS when using liquid nitrogen treatment and dry ice sample preparation methods. Across treatments, NIRS calibration statistics for butyric acid were the worst in all parameters. This is likely due to the low concentrations of butyric acid in the silages used for this study.

The SD/SECV ratio ranged from 0.07 for butyric acid (OD treatment) to 3.13 for DM (OD treatment). It has been suggested that a ratio >2.5 indicates that the calibration is adequate for quality screening purposes and >3.0 indicates that the calibration should perform well for quantitative analyses (Sinnaeve et al., 1994).

In the present study, the LN treatment produced a relatively robust calibration (R^2 = 0.81-0.94, 1-VR = 0.63-0.87) for all parameters except butyric acid. Short-chain organic acids for dried samples cannot be detected directly with NIRS. In the case of wet silage samples, heat drying could result in losses of volatile substances, such as short-chain organic acids and alcohols (McDonald et al., 1991). However, fermentation end-products in the oven-dried

Table 3. Validation statistics[1] for NIRS of DM, pH and short-chain organic acids of corn silage samples

Parameter	Preparation methods[2]	Reference mean (Lab.)	Predicted mean (NIRS)	R^2_V	SEP
DM	OD	28.68	28.92	0.69	1.53
	LN	28.10	27.90	0.81	1.10
	IF	28.23	27.82	0.70	1.34
pH	OD	4.01	4.04	0.68	0.10
	LN	3.99	4.00	0.85	0.06
	IF	4.01	3.99	0.50	0.10
Short chain organic acids (%, DM)					
Acetic acid	OD	1.44	1.45	0.73	0.21
	LN	1.43	1.45	0.88	0.17
	IF	1.36	1.38	0.89	0.13
Propionic acid	OD	0.34	0.37	0.32	0.11
	LN	0.33	0.34	0.65	0.08
	IF	0.30	0.31	0.27	0.12
Lactic acid	OD	4.87	4.97	0.80	0.58
	LN	5.11	4.95	0.66	0.76
	IF	5.13	4.88	0.75	0.75
Total acid	OD	6.66	6.83	0.67	0.67
	LN	6.87	6.79	0.57	0.74
	IF	6.79	6.47	0.73	0.69

[1] R^2_V = Multiple correlation coefficient of validation.
[2] OD = Oven-dried ground, LN = Frozen in liquid nitrogen and ground, IF = Intact fresh.
SEP = Standard error of prediction.

samples may be detected indirectly because their presence in organic complexes affects H bonds (Shenk, 1992; Givens et al., 1997) or the concentrations of organic constituents (Watson et al., 1976).

The results obtained on the independent validation set for the multiple correlation coefficient of validation (R^2_v), standard error of prediction (SEP) and bias are shown in Table 3. The samples in the validation set are normally different to those that are used to develop the prediction equation, and are usually a smaller set than the calibration set. Williams (1987) has provided rules for interpreting values for bias, SEP and correlation between predicted and reference values. He recommended that the SEP should not be more than 3% of the mean reference value. Predictions for all parameters (DM, pH and short-chain organic acids excluding butyric acid) had good validation statistics in present studies. The LN treatment gave the best results among the three sample-preparation methods. Validation of these equations with 26 samples produced SEP of 1.05 and 0.06 and validation coefficients (R^2_v) of 0.81 and 0.85 for DM and pH, respectively. For acetic, propionic, lactic and total acids, the best analytical results were obtained with IF (SEP = 0.13, R^2_v = 0.89), LN (SEP = 0.08, R^2_v = 0.65), OD (SEP = 0.58, R^2_v = 0.80) and IF (SEP = 0.69, R^2_v = 0.73), respectively. Sinnaeve et al. (1994), using fresh silages, have shown that it is possible to derive successful calibrations for pH, acetic acid, and lactic acid (R^2_v = 0.90, 0.85 and 0.86 respectively). Considering the diversity of this population of corn silages the NIRS estimated fermentation end-products such as pH, acetic, lactic and total acids to an acceptable degree.

The results of this study have shown that NIRS analysis of undried silages can provide accurate prediction of a wide range of fermentation products. Sample preparation treatment clearly influenced NIR spectra and accuracy of fermentation products analysis. The time required to carry out the sample preparation procedures is important not only in a commercial laboratory system but also in relation to changes in sample conditions. Although the accuracy of OD and IF treatments were lower than for the LN treatment, NIRS could be used as a screening tool to predict fermentation quality in wet corn silages. However, because little preparation or handling of samples is required, analysis of IF samples is worth consideration given the speed and convenience. Development of NIRS calibrations for silage samples prepared with OD methods will require more research on volatile substances such as short-chain organic acids in dried samples. NIRS offers considerable potential for analysis of wet silage in routine advisory systems using OD and IF preparation methods.

REFERENCES

Adesogan, A. T., E. Owen and D. I. Givens. 1998. Prediction of the *in vivo* digestibility of whole crop wheat from *in vitro* digestibility, chemical composition, in situ rumen degradability, *in vitro* gas production and near infrared reflectance spectroscopy. Anim. Feed Sci. Technol. 74:259-272.

Baker, C. W., D. I. Givens and E. R. Deaville. 1994. Prediction of organic matter digestibility *in vivo* of grass silages by near infrared reflectance spectroscopy: Effect of calibration method, residual moisture and particle size. Anim. Feed Sci. Technol.

50:17-26.

Cozzolino, D. A. Fassio and A. Giminez. 2000. The use of near-infrared reflectance spectroscopy (NIRS) to predict the composition of whole maize plants. J. Sci. Food Agric. 81:142-146.

Daniel Alomar, Rita Fuchslocher and Sergio Stockebrand. 1999. Effects of oven- or freeze-drying on chemical composition and NIR spectra of pasture silage. Anim. Feed Sci. Technol. 80:309-319.

De la Roza, B., A. Martinez, S. Modrono and B. Santos. 1996. Determination of the quality of fresh silage by near infrared reflectance spectroscopy. In (Ed. A. M. C. Davies and P. Williams), Near Infrared Spectroscopy: The Future Waves, Proceedings of the 7th International Conference on Near Infrared Spectroscopy, Montreal, Canada, 6-11 August 1995, NIR Publications, Chichester, UK. pp. 537-541.

Givens, D. I., J. L. De Boever and E. R. Deaville. 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. Nutrition Research Reviews. 10:83-114.

Gordon, F. J., K. M. Cooper, R. S. Park and R. W. J. Steen. 1998. The prediction of intake potential and organic matter digestibility of grass silages by near infrared spectroscopy analysis of undried samples. Anim. Feed Sci. Technol. 70:339-351.

Kennedy, C. A., J. A. Shelford and P. C. Williams. 1996. Near infrared spectroscopic analysis of intact grass silage and fresh grass for dry matter, crude protein and acid detergent fiber. In (Ed. A. M. C. Davies and P. Williams), Near Infrared Spectroscopy: The Future Waves, Proceedings of the 7th International Conference on Near Infrared Spectroscopy, Montreal, Canada, 6-11 August 1995, NIR Publications, Chichester, UK. pp. 524-530.

McDonald, P., A. R. Henderson and S. J. E. Heron. 1991. The biochemistry of silage, second edn. Chalcombe Publications, Marlow. p. 340.

Park, R. S., F. J. Gordon, R. E. Agnew and R. W. J. Steen. 1998. The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. Anim. Feed Sci. Technol. 72:155-167.

Park, R. S., R. E. Agnew and D. J. Kilpatrick. 2002. The effect of freezing and thawing on grass silage quality predictions based on near infrared reflectance spectroscopy. Anim. Feed Sci. Technol. 101:151-167.

Reeves, J. B., III, T. H. Blosser and V. F. Colenbrander. 1989. Near infrared reflectance spectroscopy for analyzing undried silage. J. Dairy Sci. 72:79-88.

Shenk, J. S. and M. O. Westerhaus. 1991. Population definition, sample selection, and calibration procedures for near infrared reflectance spectroscopy. Crop Sci. 31:469-474.

Shenk, J. S. 1992. NIRS analysis of natural agricultural products. In (Ed. K. I. Hildrum, T. Isaaksson, T. Naes and A. Tandberg) Near Infrared Spectroscopy. Bridging the Gap between Data Analysis and NIR Applications. London: Ellis Horwood. pp. 235-240.

Shenk, J. S. and M. O. Westerhaus. 1995. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In (Ed. G. C. Fahey, Jr.) Forage Quality, Evaluation, and Utilization. ASA, Madison. WI. pp. 406-449.

Sinnaeve, G., P. Dardenne, R. Agneessens and R. Biston. 1994. The use of near infrared spectroscopy for the analysis of fresh grass silage. J. Near Infrared Spectrosc. 2:79-84.

Watson, C. A., G. Etchevers and W. C. Shuey. 1976. Relationship between ash and protein contents of flourmill streams determined with the InfraAnalyzer and standard approved methods. Cereal Chem. 53:803-804.

Williams, P. C. 1987. Variables affecting near-infrared reflectance spectroscopic analysis. In (Ed. P. Williams and K. Norris) Near-Infrared Technology in the Agricultural and Food Industries. St. Paul, MN: American Association of Cereal Chemists Inc. pp. 143-167.

Windham, W. R. 1987. Influence of grind and gravimetric technique on dry matter determination of forages intended for analysis by near infrared reflectance spectroscopy. Crop Sci. 27:773-776.

Windham, W. R., J. A. Robertsons and R. G. Leffler. 1987. A comparison of methods for moisture determination of forages for near infrared reflectance spectroscopy calibration and validation. Crop Sci. 27:777-783.