

## Research Article

# Prediction of Chemical Composition and Fermentation Parameters in Forage Sorghum and Sudangrass Silage using Near Infrared Spectroscopy

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## ABSTRACT

This study was conducted to assess the potential of using NIRS to accurately determine the chemical composition and fermentation parameters in fresh coarse sorghum and sudangrass silage. Near Infrared Spectroscopy (NIRS) has been increasingly used as a rapid and accurate method to analyze the quality of cereals and dried animal forage. However, silage analysis by NIRS has a limitation in analyzing dried and ground samples in farm-scale applications because the fermentative products are lost during the drying process. Fresh coarse silage samples were scanned at 1 nm intervals over the wavelength range of 680–2500 nm, and the optical data were obtained as  $\log 1/\text{Reflectance}$  ( $\log 1/R$ ). The spectral data were regressed, using partial least squares (PLS) multivariate analysis in conjunction with first and second order derivatization, with a scatter correction procedure (standard normal variate and detrend (SNV&D)) to reduce the effect of extraneous noise. The optimum calibrations were selected on the basis of minimizing the standard error of cross validation (SECV). The results of this study showed that NIRS predicted the chemical constituents with a high degree of accuracy (i.e. the correlation coefficient of cross validation ( $R^2_{cv}$ ) ranged from 0.86–0.96), except for crude ash which had an  $R^2_{cv}$  of 0.68. Comparison of the mathematical treatments for raw spectra showed that the second-order derivatization procedure produced the best result for all the treatments, except for neutral detergent fiber (NDF). The best mathematical treatment for moisture, acid detergent fiber (ADF), crude protein (CP) and pH was 2,16,16 respectively while the best mathematical treatment for crude ash, lactic acid and total acid was 2,8,8 respectively. The calibrations of fermentation products produced poorer calibrations ( $RPD < 2.5$ ) with acetic and butyric acid. The pH, lactic acid and total acids were predicted with considerable accuracy at  $R^2_{cv}$  0.72–0.77. This study indicated that NIRS calibrations based on fresh coarse sorghum and sudangrass silage spectra have the capability of assessing the forage quality control

(**Key words** : Forage sorghum, Sudangrass, Corase silage, Chemical composition, Fermentation, NIRS)

## I . INTRODUCTION

Forage sorghum and sudangrass were one of summer forage grass species that are widely cultivated in South Korea. It widely grows in the all areas of South Korea in summer season and has been used as an important component in winter forage-livestock systems. Annually, Forage sorghum and sudangrass grow more than thirty-five thousand hectares in South Korea and is used to make an ensilage (MAFRA, 2014).

The quality control of forage is an important field of interest in utilization research and marketing for forage. Wet chemistry is a traditional method to analyze forages for nutrient contents. However, this technique is often destructive, expensive, and time consuming and is not really adapted to real-time feedstuff analysis. Near Infrared

Spectroscopy (NIRS) has been used to accurately and quickly measure the nutrient contents. A major advantage of NIRS is to analyze samples without chemical treatments, resulting in reduction of cost and chemical wastes (Barber et al., 1990). In addition, NIRS has shown a huge potential for assessing the physic chemical properties of many substances and materials, and little sample preparation before analysis is required (Roggo et al., 2007; Siesler et al., 2002).

Analysis of fresh silages by NIRS traditionally includes the process of drying and milling of samples. This process leads to the loss of pH and volatile acids that are important components of the silage. The predictive accuracy of NIRS depends on obtaining a calibration set that represents the variation in the main population, accurate laboratory analyses and the application of the best

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mathematical transformation for spectral data. As spectra can be affected by drying and grinding methods, particle size, and completion and temperature of sample among other variables, it is recommended that the consistent sample preparation method needs to be used for calibration and routine analysis. Sample preparation and measurement conditions of the calibration set and the predicted samples should match each other to obtain good results (Stuth et al., 2003). Relatively few studies on comparison of the effect of sample preparation and storage conditions of forages on NIRS to determine quality parameters have been published in recent years. Results about fermentation parameters obtained by calibration on wet material have been reported for grass silage (Snyman and Joubert, 1992; Sinnaeve et al., 1994) and for alfalfa and corn silages (Reeves et al., 1989). NIRS measurements conducted on wet material compared to dried material permit very rapid analyses in general and enable to analyze volatile and especially non-ionic volatile compounds. However, practical experience has shown that it may be difficult to obtain the same prediction accuracy on the main constituents.

The objective of this study was to assess the usefulness of NIRS in determining the nutritional composition and fermentative parameters on fresh coarse samples of forage sorghum and sudangrass silages. In this study, we assessed the predictive value of NIRS calibration models and also investigated cost-effective and timesaving methods for assessment of forage quality in field populations.

## II. MATERIALS AND METHODS

### 1. Sample collection and preparation

Forage sorghum and sudangrass silage samples (n=309) were collected from cattle farms and total mixed ration companies in South Korea during the period from 2013 to 2014. During sample collection, the following data were recorded: 1) the date and stage of maturity at harvest, 2) the type of structure in which the forage was ensiled, and 3) the use of additives. All the silages had a particle size between 15 and 20 cm. The samples were stored at  $-20^{\circ}\text{C}$  immediately after collection. Prior to NIRS scanning of the fresh samples, the silage samples were thawed overnight at

$4^{\circ}\text{C}$  and cut in pieces of 3 to 5 cm for easy packing in the sample cup. A subsample of each silage was dried in a forced-air oven at  $65^{\circ}\text{C}$  for 78 h and then milled to pass a 1 mm screen for subsequent chemical analysis.

### 2. Reference analysis

All reference analyses, except fermentation parameters were performed on dried sample material. A subsample of each silage was dried at  $68^{\circ}\text{C}$  and milled for the determination of ash, neutral detergent fiber and acid detergent fiber according to the methods of Association of Official Analytical Chemists (AOAC, 1990). Crude protein N was determined by the Dumas principle (Sweeny, 1989) using a Leco CN-2000 instrument (Leco Corporation, St. Joseph, MI). The dry matter (DM) was determined by drying at  $68^{\circ}\text{C}$  for 72h. Aqueous extracts of the haylages were used to analyze volatile fatty acids as described previously (Porter, 1992). Total acids were calculated as the sum of the individual acid concentrations in the silage. The pH of the silages was estimated from the extract as described in the Association of Official Analytical Chemists (AOAC, 1990).

### 3. NIR spectra collection

Reflectance spectra were obtained using the SpectraStar 2500 (Unity Scientific, Brookfield, USA) that can scan the wavelength range of 680~2500 nm. The coarse fresh silage samples (particle size 3~5 cm) were packed in a rotating circular quartz sample cup of 150 mm diameter. The data were recorded at 1 nm intervals as  $\log 1/R$  (where  $R$  = reflectance), resulting in 1,820 points per sample. Each sample was scanned twice by manually rotating the sample cup approximately  $360^{\circ}$  relative to the previous scan. Hence, two spectra were collected per sample.

### 4. NIR Calibration

The spectral data processing and statistical analyses were performed using the U-Cal (V.2.04) software package. Before developing calibration models, the original spectra were subjected to different pre-treatment methods for

improving signal-to-noise ratio and, therefore, maximizing the signal intensity for the analytes of interest (Heise and Winzen, 2002). Spectral correction algorithm (standard normal variate (SNV) and detrend (SNV&D)) was used to decrease the effects of light scattering due to particle size.

Mathematical transformations described by four digits were tested (e.g., 1, 4, 4, 1). The 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> digits indicate the subtraction order, the gap or subtraction interval, the first smoothing segment, and the second smoothing segment, respectively. These treatments were applied for extracting relevant spectral information and reducing the quantity of data by means of subtracting smoothed segments of the spectra.

The calibration equations were obtained by partial least square (PLS) regression (Martens and Martens, 2001). Cross-validation was conducted to select the optimal number of terms in the equation in order to avoid over-fitting. In this process, the standard error of cross-validation (SECV) (uncertainty estimator) and the proportion of observed variance ( $R^2_{cv}$ , certainty estimator) were determined (Shenk and Westerhaus, 1994).

### III. RESULTS AND DISCUSSION

#### 1. Reference data

The silage samples selected for this study varied widely in their chemical composition and fermentation parameters.

The mean, minimum, maximum and standard deviation for each of the chemical and fermentation parameters examined were given in Table 1. The ranges of variation of the content of moisture was very large. The variation of fermentation products was very narrow except for lactic and total acids. A number of used forage sorghum and sudangrass silage samples in this study contained non-detectable (or nearly non-detectable) propionic and butyric acids. The amount of mean ash content was slightly more abundant than that of ash content found in other studies on extensive grassland (Richter et al., 2010). For the other constituents, the values were continuously dispersed.

#### 2. NIR spectra of fresh coarse silages

Raw spectra data and its second derivative from 680 nm to 2500 nm for each silage sample were shown in Fig. 1 (a, b). There were two apparent peaks at about 1440 and 1940 nm, which may result from the absorption by water in fresh coarse silage samples. The stronger absorption peaks indicated much higher moisture content in fresh silage and its masking effect on other spectral features. It was concluded that the physical state affected the NIR spectra of silage.

#### 3. NIRS calibration of chemical components

A wide range of regression and data transformation

Table 1. The range in chemical composition and fermentation parameters of the 309 forage sorghum and sudangrass silages

Constituent	n	Mean	Min.	Max.	S.D.
<i>Chemical composition</i>					
Moisturer (%)	309	66.44	26.70	85.80	11.44
Acid detergent fiber (% DM)	269	43.15	34.60	68.70	4.26
Neutral detergent fiber (% DM)	269	63.51	45.80	76.40	4.01
Crude protein (% DM)	269	8.37	3.20	14.20	2.29
Crude ash (% DM)	171	9.89	6.20	15.70	2.95
<i>Fermentation parameters</i>					
pH(1:5)	262	4.96	3.70	9.60	1.08
Acetic acid (% DM)	259	1.60	0.02	7.39	1.25
Butyric acid (% DM)	259	0.92	0.02	5.40	1.12
Lactic acid (% DM)	259	3.12	0.04	12.66	2.65
Total acid (% DM)	259	5.27	0.05	16.60	3.14

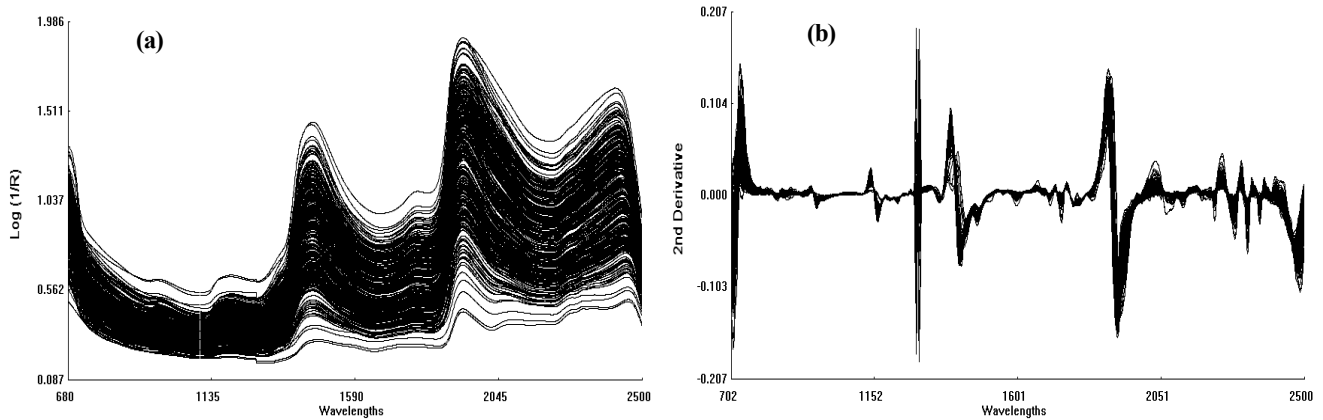


Fig. 1. Original (a) and second derivative (b) of NIRS spectra of the fresh forage sorghum and sudangrass silage samples (n=309).

techniques have been studied by others for the prediction of biological parameters of silage, using undried samples for chemical composition as described previously (Park et al., 1998). However, a comprehensive examination has not been conducted using data from wet coarse fresh silage. Therefore, in the present study, different permutations of a range of mathematical treatment techniques were explored within each of the parameters.

The statistics developed from different mathematical treatments were presented in Table 2. The best spectral mathematical treatments were selected from a total of 20 calibrations developed for each chemical parameter based on the  $R^2_{cv}$ , SECV and RPD statistics. Comparison of the mathematical treatments for raw spectra showed that second-order derivatization procedure consistently performed best. The best mathematical treatment for moisture, ADF and CP was 2.16.16., while that for NDF and CA was 2.8.8.

Liu et al. (2008) indicated that good calibration models were obtained by first-order derivative treatment for prediction of DM and NDF digestibility in fresh silages. However, Loren and Alomar et al. (2009) reported that better calibration models were obtained by second-order derivative mathematical treatment for DM, NDF and ADF in fresh pasture silages. The results obtained by the mathematical treatments varied in their predictive quality based on the parameter involved.

Comparison among the  $R^2_{cv}$  showed that all selected calibrations were robust except for the calibration for the total ash ( $R^2_{cv} = 0.68$ ). Among the models obtained, is worth

mentioning the low predictive value of the crude ash calibration, which was expected given that it corresponds to inorganic and mineral substances that do not have absorption in the near infrared region, although these compounds, which can associated with organic structures through oxides, chelates or other compounds, which can allow for their indirect estimation using NIRS (Shenk and Westerhaus, 1994; Cozzolino et al., 2000).

The RPD provides comparison of the performance of all NIRS calibrations irrespective of the different units of the chemical parameters. RPD less than 2.4 are equations with low reliability for predictive purpose (Williams, 2001). In RPD between 2.4 and 3.0, the predictive quality increases and the equation can be applied for the aim of approximation or classification in ranges. When RPD is higher than 3.0, the quality of the equation is acceptable for prediction.

The RPD ratio ranged from 2.12 for total ash to 6.41 for DM content. The  $R^2_{cv}$  statistic showed that total ash with RPD ratio of 2.12 is not a robust calibration. However, previous studies using coarsely ground hay (Reddersen et al., 2014) and using undried ground pasture silage (Park et al., 1998) successfully developed a calibration to predict total ash with RPD 4.8 and 3.07, respectively.

#### 4. NIRS calibration of fermentation parameter

The pH and volatile components are an indicator of the fermentation quality of the haylage, but it is costly to measure them in routine silage analysis. As these volatile

Table 2. Mathematical treatments and statistical indicators of the calibrations developed for the chemical composition parameters predicted

Constituents	Math treatment	Calibration			Validation		RPD <sup>d</sup>
		N	SEC <sup>a</sup>	R <sup>2</sup>	SECV <sup>b</sup>	R <sup>2</sup> <sub>cv</sub> <sup>c</sup>	
Moisture (%)	1,4,4	222	1.77	0.97	1.89	0.96	6.05
	1,8,8	220	1.74	0.97	1.84	0.96	6.23
	2,8,8	230	1.82	0.97	2.03	0.96	5.64
	2,16,16	222	1.53	0.98	1.78	0.96	6.41
Acid detergent fiber (% DM)	1,4,4	264	1.59	0.83	2.88	0.74	1.48
	1,8,8	252	1.66	0.81	2.46	0.77	1.73
	2,8,8	247	1.68	0.79	2.48	0.74	1.72
	2,16,16	235	1.65	0.80	2.10	0.87	2.03
Neutral detergent fiber (% DM)	1,4,4	232	1.57	0.76	2.30	0.66	1.74
	1,8,8	206	1.49	0.78	1.79	0.75	2.24
	2,8,8	182	1.63	0.69	1.73	0.86	2.31
	2,16,16	220	1.79	0.73	2.08	0.79	1.93
Crude protein (% DM)	1,4,4	261	0.85	0.86	1.44	0.70	1.59
	1,8,8	247	0.79	0.88	1.15	0.83	1.99
	2,8,8	238	0.92	0.83	1.27	0.76	1.81
	2,16,16	230	0.74	0.89	0.98	0.88	2.35
Crude ash (% DM)	1,4,4	153	1.25	0.82	1.78	0.59	1.65
	1,8,8	150	1.10	0.87	1.54	0.65	1.92
	2,8,8	152	0.62	0.96	1.39	0.68	2.12
	2,16,16	154	0.86	0.91	1.72	0.64	1.71

<sup>a</sup> SEC : standard error of calibration,<sup>b</sup> SECV : standard error of cross validation,<sup>c</sup> R<sup>2</sup><sub>cv</sub> : coefficient of determination of cross validation,<sup>d</sup> RPD : ratio of performance deviation (SD/SECV).

components are lost when silage are dried, NIRS analysis based on dried samples provides a poor accuracy of these parameters.

The statistics developed from different mathematical treatments for fermentation parameters of silage were presented in Table 3. The present study using fresh coarse silage showed good calibrations for pH, lactic and total acid, except acetic and butyric acid. This was also obtained by the PRD ratios which ranged from 2.32~4.17. Better results were obtained from second-order derivative treatment than from the first-order derivative. The best mathematical treatment was 2,16,16 for pH and acetic acid, while that was 2,8,8 for butyric, lactic acid and total acid. However, Reeves et al. (1989), which used undried alfalfa silages, obtained successful calibration for acetic, propionic and butyric acid (R<sup>2</sup> 0.84, 0.86 and 0.80 and SEC 5.1, 0.6 and

2.2 (g/kg DM), respectively). Park et al. (1998), which used undried ground grass silages, also obtained successful calibrations for acetic, butyric and lactic acids acid (R<sup>2</sup><sub>cv</sub> 0.73, 0.83 and 0.83 and SECV 2.0, 2.6 and 2.5 (g/kg DM)).

The calibration result (R<sup>2</sup>=0.92, Mathematical treatment 1,4,4) for butyric acid had a very strong accuracy. However, the cross-validation result for butyric acid did not have a strong accuracy in a range of 0.02~5.40% (DM) and a mean of 0.92% (DM) because a range of butyric acid in the population of silages was too narrow or because butyric acid concentrations were too low to represent the population of silages. Therefore, the calibration developed from this data set did not predict higher concentrations of acetic, butyric and propionic acids with good accuracy.

Considering the diversity of this population of forage sorghum and sudangrass silages, the NIRS predictions of

Table 3. Mathematical treatments and statistical indicators of the calibrations developed for the fermentation parameters predicted

Constituents	Math treatment	Calibration			Validation		RPD <sup>d</sup>
		N	SEC <sup>a</sup>	R <sup>2</sup>	SECV <sup>b</sup>	R <sup>2</sup> <sub>cv</sub> <sup>c</sup>	
pH (1:5)	1,4,4	229	0.25	0.85	0.33	0.71	3.24
	1,8,8	219	0.24	0.85	0.30	0.74	3.59
	2,8,8	217	0.22	0.87	0.28	0.76	3.86
	2,16,16	209	0.23	0.82	0.26	0.77	4.17
Acetic acid (% DM)	1,4,4	171	0.46	0.39	0.46	0.27	2.69
	1,8,8	215	0.52	0.63	0.66	0.40	1.89
	2,8,8	170	0.48	0.32	0.50	0.24	2.53
	2,16,16	189	0.40	0.65	0.48	0.44	2.60
Butyric acid (% DM)	1,4,4	187	0.20	0.92	0.56	0.37	1.99
	1,8,8	149	0.31	0.14	0.32	0.00	3.53
	2,8,8	193	0.34	0.85	0.66	0.45	1.71
	2,16,16	163	0.26	0.68	0.35	0.39	3.21
Lactic acid (% DM)	1,4,4	235	0.76	0.91	1.47	0.66	1.80
	1,8,8	212	0.89	0.85	1.26	0.68	2.10
	2,8,8	207	0.86	0.85	1.14	0.72	2.32
	2,16,16	209	0.96	0.79	1.18	0.63	2.25
Total acid (% DM)	1,4,4	250	1.10	0.85	1.82	0.60	1.72
	1,8,8	238	1.04	0.85	1.46	0.68	2.14
	2,8,8	206	0.95	0.86	1.15	0.78	2.73
	2,16,16	203	1.06	0.82	1.29	0.71	2.44

<sup>a</sup> SEC : standard error of calibration,<sup>b</sup> SECV : standard error of cross validation,<sup>c</sup> R<sup>2</sup><sub>cv</sub> : coefficient of determination of cross validation,<sup>d</sup> RPD : ratio of performance deviation (SD/SECV).

fermentation parameters were good for pH, lactic and total acids.

#### IV. CONCLUSION

The present study showed that analysis of fresh coarse silage by NIRS can provide accurate predictions of a wide range of chemical components and fermentation parameters in forage sorghum and sudangrass. The ability of calibration prediction increased with sample preparation and measurement standardization. In comparison with scatter correction, mathematical treatment for spectral data could produce a better prediction. This might offer considerable potential for using NIRS for coarse silage analysis in routine adversary systems.

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