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Technical note

Prediction of the fermentative quality of sunflower silage by near-infrared reflectance spectroscopy (NIRS) on oven-dried samples

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Abstract:

The objective of the present work was to evaluate the predictive ability of calibration equations developed by NIRS (near-infrared reflectance spectroscopy) on dry and ground samples for estimating the fermentative quality of sunflower silage. NIRS spectra of a total of 52 oven-dried and ground silage samples from different laboratory silo tests carried out at the Mabegondo Agricultural Research Center (Centro de Investigacións Agrarias de Mabegondo, CIAM) were registered. The fresh samples were analyzed using reference methods. The pH, lactic acid, acetic acid, ethanol, ammonia nitrogen and soluble nitrogen levels were determined. NIRS calibrations were developed by modified partial least squares regression, performing a regression between spectral and reference data. The predictive capacity of the equations obtained ranged from excellent to good, with cross-validation coefficients of determination (r^2_{cv}) equal to or above 0.88. The RPD index values for all the parameters studied were equal to or above 3.0; therefore, the calibration equations obtained on dry and ground samples can be used satisfactorily to predict the fermentative quality of sunflower silages in routine analyses.

Key words: Forage crops, Fermentation parameters, Reflectance spectroscopy.

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The nutritional evaluation of forages is relevant due to the high variability of their nutritional value and to their high contribution to the total dry matter of cattle rations, compared to concentrate. In addition to the intrinsic characteristics of the forage at the time of cutting, the nutritional value of the silage is fundamentally conditioned by the quality of fermentation developed during storage in the silo[(1)], being highly variable depending on forage ensilability and post-harvest treatment[(2)], and particularly affecting the nitrogen value and the voluntary intake of silage[(3)]. Therefore, for an efficient use of silage, its fermentative quality must be first characterized, for which it is essential to have fast, accurate and reliable methods. Instrumental analyses for determining the fermentative quality parameters of silage are complex, time-consuming and costly.

NIRS (Near-Infrared Reflectance Spectroscopy) technology is widely recognized as a fast, inexpensive and highly accurate analytical technique for characterizing the keeping quality of silage as an alternative to wet analysis[(4)]. Moreover, it is an environmentally clean technology that uses no reagents and generates no waste. NIRS analysis of silage in intact mode, in its fresh state, involves great difficulty, due to the high heterogeneity of the material[(5)]. On the other hand, the presence of water in the intact sample interferes with the

NIRS spectrum, since it absorbs part of the infrared radiation, generating two very significant absorption bands in the spectrum. However, it should be noted that NIRS analysis of dried samples has disadvantages compared to analysis with fresh samples, because the volatile constituents of silage, such as fermentation acids, alcohols and ammonium, are released and lost during the drying process of the sample. In one study, a series of samples before and after the drying process were determined by reference methods, and the prediction of the NIRS equations developed on dry samples were compared with those performed on wet material. As a result, it was observed that the prediction of pH, lactic acid and ammonia nitrogen was more robust on dry material, while the quality of the prediction for acetic acid was better when the NIRS measurement was performed on the wet sample[6]. This is attributed to the fact that the quality of prediction obtained for the different parameters by the two methods is not related to the losses during the drying of the samples, since the reduction in the concentration of lactic acid, acetic acid, ethanol and ammonia nitrogen in the dry matter during drying was 3.5, 57, 53 , and 100 %, respectively, for grass silage, and 3.5, 83, 16, and 100 % for corn silage, in clear correspondence with their volatility (free version)[6].

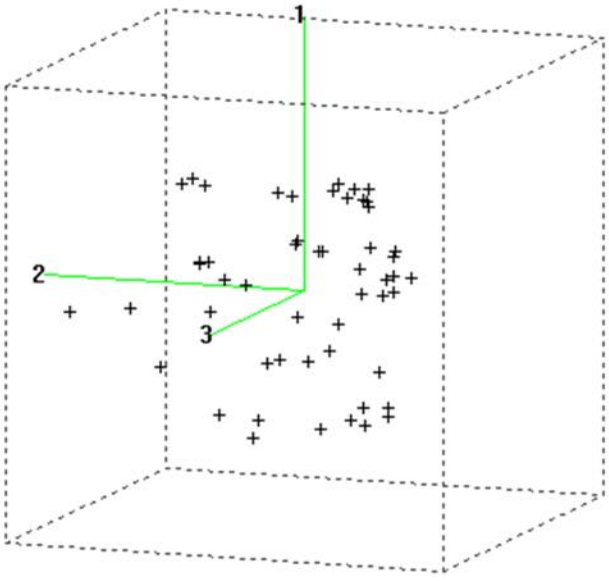
On the other hand, another study evaluated the effect of a corn silage sample preparation (fresh vs. dry and ground) on the estimation of fermentation parameters by NIRS. The results indicate that fresh samples provide a slightly higher predictive ability for acetic acid (r^2_{cv} = 0.85 *vs* 0.82) and lactic acid (r^2_{cv} = 0.78 *vs* 0.73), and a lower predictive ability for pH (r^2_{cv} = 0.54 *vs.* 0.63)[7]. A study carried out at the Mabegondo Agricultural Research Center (Centro de Investigacións Agrarias de Mabegondo, CIAM) in Galicia indicates the convenience of using dry and ground samples instead of intact ones, by obtaining predictive models of the chemical composition and fermentative quality of grass silage with higher accuracy[8]. In another work recently carried out at CIAM, the prediction of fermentation parameters of grass silage was evaluated by means of NIRS calibrations, developed on dry and ground material; the results obtained were satisfactory, with determination coefficients equal to or above 0.80[9]. The knowledge of the fermentative quality of new types of forages in a fast and accurate way requires progress in the development of new NIRS calibrations. In this sense, the objective of the present work was to evaluate the predictive capacity of NIRS calibration equations in dry and ground samples to estimate fermentative quality parameters of sunflower silage.

The work was carried out with a total of 52 sunflower silage samples from different laboratory silo trials conducted at CIAM in 2016 and 2017. The collection of samples covers a high variability in terms of maturity stage, including sunflower samples harvested at different phenological stages according to the Schneiter and Miller scale, from stage R4 (1 wk before flowering) to stage R7 (5 wk after the beginning of flowering)[10]. The forage used for filling the laboratory silos came from the cultivation of two commercial hybrids: a forage variety (Rumbosol 91) and an oil variety (ES Shakira), grown on CIAM's experimental farms

located in two locations in Galicia (Spain): Mabegondo (in the northwestern Atlantic coastal area of Galicia, at 100 masl) and Pobra de Brollón (an inland area of Galicia, at 400 m asl). In addition, the trials included silage without additives and with different additives: formic acid and two commercial inoculants (one based on homofermentative lactic acid bacteria, and the other, on homo- and heterofermentative lactic acid bacteria).

The laboratory silos were opened 60 d after they were filled. The silage samples, after manual homogenization, were divided into two aliquots, one of which was dried in an oven at 80 ºC for 16 h[11], while the other was frozen at -18 ºC; both were vacuum-packed in hermetically sealed plastic containers until fermentative analysis was performed using reference methods. The spectral information of the dried and ground samples at 1 mm was obtained in a Foss NIRSystem 6500 monochromator spectrophotometer (Foss NIRSystem, Silver Spring, Washington, USA), located in a temperature-controlled room (24 ± 1 °C) and equipped with a spin module that performs reflectance (R) measurements in the spectral region between 400 and 2,500 nm, at 2 nm intervals. Absorbance data are expressed as Log (1/R, R= Reflectance). The spectra collection and chemometric analysis of the data was carried out using Win ISI II v.1.5 software (Infrasoft International, Port Matilda, PA, USA)[12]. Using the CENTER algorithm[13], a Principal Component Analysis (PCA) was performed, followed by the calculation of distances between spectra in an n-dimensional space through the Mahalanobis distance, which allowed studying the structure and spectral variability of the population and detecting anomalous samples[13]. The Global Mahalanobis distance (GH) is defined as the distance between a sample and the center of the population in the space defined by the PCA (Figure 1), considering as outlier samples those with GH values above 3 (spectral outlier)[13].

Figure 1: Three-dimensional representation of spectral data of samples according to the global Mahalanobis distance



SNV-Detrend pretreatment was applied to the spectral data[14] in order to correct for the scattered radiation phenomenon and the following eight mathematical treatments were evaluated: 1,5,5,1; 1,6,4,1; 1,10,5,1; 1,10,10,1; 2,5,5,1; 2,6,4,1; 2,10,5,1; 2,10,10,1. The first digit expresses the order of the derivative (1= first derivative, 2= second derivative); the second digit indicates the size of the segment on which the derivative is performed (interval expressed in nanometers); the third and fourth digits indicate the size of the intervals, expressed in nanometers, used for the signal smoothing calculation[15].

The development of the calibration equations was performed by modified partial least squares regression (MPLS)[16] between spectral and reference data, including four cross-validation groups to prevent overfitting, which were used sequentially to perform the validation of the generated equations.

Fermentative analysis of intact silage samples was performed by reference methods, in duplicate[17]. On an extract of 50 g of fresh silage sample, macerated at room temperature for 2 h in 150 ml of distilled water, pH, ammoniacal nitrogen (N-NH_3) was determined with a selective electrode (Orion) and soluble nitrogen (sol-N) by macro Kjeldahl digestion. Fermentation acids (lactic, acetic, and propionic) and ethanol were determined by gas chromatography (Agilent Technologies, USA) with a BR-SwaxAcids high polarity capillary column (30 m x 0.53 mm x 1 µm; Bruker, USA). N-NH_3 and sol-N parameters referred to total nitrogen, and fermentation acids and ethanol, to dry matter.

The statistics used to select the best calibration equations were the standard errors of calibration (SEC) and standard errors of cross-validation (SECV) and the coefficients of determination (r^2_c and r^2_{cv}) obtained in the calibration and cross-validation process, respectively[18]. In addition, other useful statistics were utilized to evaluate the predictive capacity of the calibration equations obtained, such as the RER index, or the ratio between the range of the reference data and the SECV, and the RPD index, or the ratio between the standard deviation of the reference data and the SECV[19].

The descriptive characteristics (range, mean, and standard deviation) of the fermentation parameters of the calibration collective are shown in Table 1; they exhibit a wide range and a high standard deviation for each of the components analyzed using reference methods. This high variability confirms that this group is made up of very diverse silages, a key factor for obtaining robust calibration equations[20]. The mean value (and range of variation) of the dry matter content of the silage population that made up the calibration set was 16.0 % (11.3 to 21.9 %).

Table 1: Range, mean, and standard deviation of fermentative quality parameters of the calibration group (n=52) of sunflower silage

Parameter	Range		Mean	SD
pH	3.55	4.29	3.91	0.21
Lactic acid, %DM	0.00	15.74	7.99	5.51
Acetic acid, %DM	0.52	4.04	2.39	1.10
Ethanol, %DM	0.90	12.50	3.78	3.43
$N\text{-}NH_{3,}$ %TN	2.21	10.37	6.09	2.59
Soluble N, %TN	26.96	52.95	41.41	7.68

DM= dry matter; $N\text{-}NH_{3=}$ ammonia nitrogen; TN= total nitrogen; SD= standard deviation.

Table 2 shows the statistics of the calibration equations obtained for the prediction of fermentation quality parameters. The coefficients of determination in the cross-validation process (r^2_{cv}) provide information on the quality of the calibration, based on which three levels of accuracy of the prediction models have been defined: r^2_{cv} values above 0.90 indicate excellent predictive ability; r^2_{cv} values between 0.89 and 0.70 indicate that the calibration is considered to have good quantitative predictive ability, and calibrations with r^2_{cv} values between 0.69 and 0.50 allow only adequate discrimination between high, medium and low values[21]. Therefore, the r^2_{cv} values for pH (r^2_{cv} =0.98), $N\text{-}NH_3$ (r^2_{cv} =0.96), acetic acid (r^2_{cv} =0.94), lactic acid (r^2_{cv} =0.90), and ethanol (r^2_{cv} =0.90) parameters indicate an excellent predictive ability, while the soluble N content (r^2_{cv} =0.88) exhibits a good accuracy ability[21]. The accuracy of the prediction can be judged according to the values of the RER and RPD indexes[19]; RPD values above 3 and RER values above 10 are taken as indicators of the usefulness of the predictions[19]. The high standard deviation and the wide range of variation of the calibration collective account for the adequate RPD (3.0 - 6.5) and RER (9.0 - 22.8) values obtained.

Table 2: Statistics of the calibration equation developed for the prediction of fermentative quality parameters of sunflower silage

Parameter	MT	SEC	r^2_c	SECV	r^2_{cv}	RER	RPD
pH	(1,5,5,1)	0.02	0.99	0.03	0.98	22.8	6.5
Lactic acid, %DM	(2,10,10,1)	1.63	0.91	1.75	0.90	9.0	3.2
Acetic acid, %DM	(2,10,5,1)	0.17	0.97	0.25	0.94	13.9	4.3
Ethanol , %DM	(2,6,4,1)	0.97	0.92	1.07	0.90	10.9	3.2
$N\text{-}NH_3$, %TN	(2,10,10,1)	0.44	0.97	0.54	0.96	15.1	4.8
Soluble N, %TN	(2,10,10,1)	2.18	0.92	2.58	0.88	10.1	3.0

DM= dry matter; MT= mathematical treatment; $N\text{-}NH_{3=}$ ammonia nitrogen; TN= total nitrogen; SEC= standard error of calibration; SECV= standard error of cross validation; r^2_c and r^2_{cv}: coefficient of determination in calibration and cross validation; RER= Range/SECV; RPD= standard deviation/SECV.

The prediction equations for pH, acetic acid, ethanol, $N\text{-}NH_3$ and sol-N exhibit values of RPD>3 and RER >10, in compliance with those recommended in the literature[19]. Thus, the pH value is the most accurately estimated one (RER=22.8; RDP=6.5), followed by the values for acetic acid (RER=19.5; RDP=4.3), $N\text{-}NH_3$ (RER=19.5; RDP=4.3), ethanol (RER=10.9; RDP=3.2), and sol-N- (RER=10.1; RDP=3.0). In the case of the lactic acid prediction equation, the value of the RER index (9.0) did not reach the recommended value; however, the RPD value (3.2) exceeds the minimum value recommended in the literature[19]. Therefore, the values of the RER and RPD statistics confirm the high accuracy and precision of the equations obtained, ensuring their validity from the point of view of their application in quantitative analysis[19].

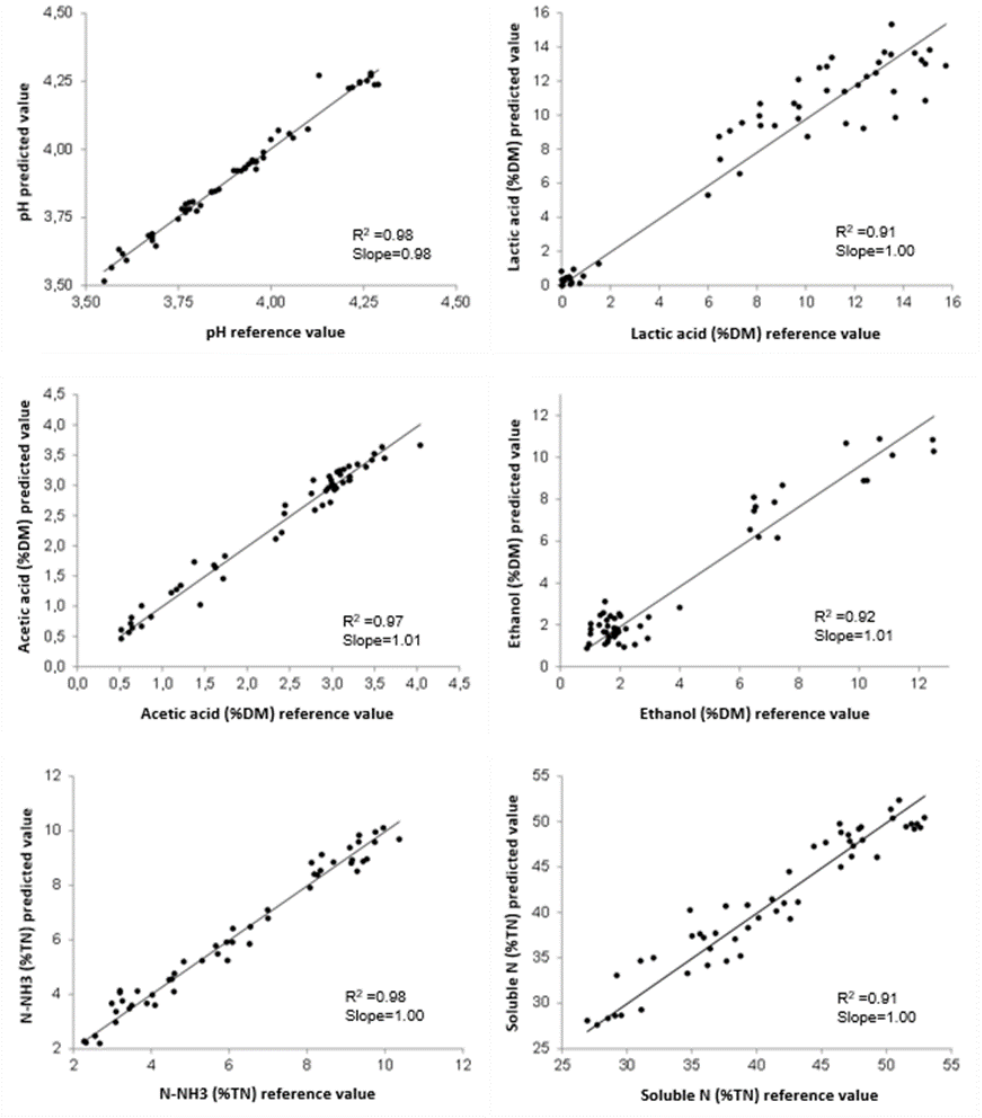
There is little information in the literature on the applicability of the NIRS technique for predicting the pH of forage sunflower silage[22]. A work carried out with a group similar to the present work ―a collection of 50 dry and ground samples of experimental sunflower silage― exhibited a lower predictive capacity for pH estimation than the present work, with lower values of r^2_{cv} (0.86), RER (5.9), and RPD (2.5), and higher values of SECV (0.44)[22]. Other studies, carried out on fresh samples, have obtained a lower predictive capacity for the pH value than the one determined in this work, with r^2_{cv} values of 0.85, 0.72, and 0.78 for grass silage[4], barley silage[23], and ryegrass silage[24], respectively.

The lactic acid, acetic acid, and ethanol content in fresh grass silage samples were determined with a lower precision than that observed in this study, with r^2_{cv} and RPD values of 0.83 and 2.5, 0.73, and 2.0, and 0.77 and 2.8, respectively[25]. Values of r^2_{cv} and RPD of 0.89 and 3.3

for sol-N, and of 0.92 and 4.0 for N-NH_3[25] —similar to those obtained in this study— have been reported for grass silage.

Once the prediction models have been developed, the fit of the data to the model must be evaluated, for which purpose a chart of the values predicted by NIRS versus the reference values is used. Figure 2 shows such a chart for the fermentation quality parameters studied. The results obtained exhibited a high correlation between the values predicted by NIRS and the reference values for all the parameters studied, with values for the coefficient of determination (R^2) of the regression above 0.90, while the values of the slope of the regression ranged between 0.98 and 1.01, confirming the high precision of the equations developed, with values close to 1 in both cases[26].

Figure 2: Reference vs predicted values by NIRS for all fermentation parameters



The reference values of the studied parameters are distributed among all concentration ranges and in a very broad range of variation. In the case of lactic acid concentration, the analytical reference values show a very broad range of variation, but they are not distributed among all concentration ranges (Figure 2), with most of the samples in the range between 6 and 15.7 % DM, and only a small number of samples between 0 and 2 % DM. These low lactic acid contents are related to the application of formic acid to the silages[27].

This work should be considered preliminary as it is based on a limited number of samples, and it is desirable to increase the database in future studies[18]. It is advisable to incorporate new representative samples, with values distributed among the least represented sectors, mainly for the lactic acid content; increasing the number of samples of the calibration group will reinforce and increase the robustness of the developed models[18].

The authors conclude that NIRS technology, applied to dry and ground samples, is a useful and appropriate tool for the prediction of fermentative quality parameters of sunflower silage, and, therefore, an alternative for determining these parameters in relation to conventional analytical methods.

Acknowledgments and conflicts of interest

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