Evaluation of Pretreatment Strategies for Near-Infrared Spectroscopy Calibration Development of Unground and Ground Compound Feedingstuffs

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Chemometric procedures are usually applied to near-infrared (NIR) spectra in order to obtain prediction models. These procedures include the application of different combinations of spectral mathematical pretreatments for the improvement of calibrations and the selection of the best model on the basis of validation results. In this work, we used an automatic routine to obtain calibrations for unground and ground compound feedingstuffs ($N = 354$ samples), including 49 combinations of pretreatments (first and second derivatives, an auto scaling procedure, detrending and two versions of multiplicative scatter correction). Calibrations for crude fiber and crude protein were developed without elimination of outliers and with 2 or 9 maximum passes of elimination of outliers. Validation statistics were highly influenced by the pretreatments used, as a combined result of their ability to improve the detection of outliers and the model adjustment. The standard error of prediction (SEP) values ranged from 0.61 to 1.27 for crude protein (CP) and from 0.74 to 1.33 for crude fiber (CF). In spite of the fact that validation statistics did not show a clear distribution pattern, some combinations of pretreatments provided consistently better results.

Index Headings: Near-infrared reflectance spectroscopy; NIRS; Spectral signal pretreatments; Compound feedingstuffs; Unground analysis.

INTRODUCTION

Near-infrared reflectance spectroscopy (NIRS) has undergone an exciting evolutionary process during the last thirty years, these days exhibiting an excellent reputation as an accurate and reproducible technique for qualitative and quantitative analysis in many fields, such as agro-food, the pharmaceutical and chemical industries, human health, environmental studies, etc.

Currently, NIRS analysis is conceived as a synthesis of spectroscopy, mathematics, statistics, and instrumentation, with the progress in this technology being closely related to the advances in these fields, particularly in the development of chemometric algorithms. Chemometric procedures[1,2] are usually applied to NIR spectra for extracting relevant information, since the spectra are influenced by features such as temperature, moisture content, particle size, texture, light scattering, sample manipulation, and other sources of error.[3,4] Precision and accuracy in NIRS analysis are subject to the availability of algorithms or spectral data pretreatments that reduce the above mentioned effects, particularly the scatter effect. This is one of the most relevant factors influencing the spectra of agro-food products.[5,6] However, there is not a standard procedure for determining which pretreatment will give the best results when developing NIRS calibrations.

The selection of a suitable spectral pretreatment method is not easy since, in many cases, the software packages operating with NIRS instruments offer several different mathematical transformations, including derivatives, multiplicative scatter correction (MSC), standard normal variate (SNV), detrending (DT), and orthogonal signal correction (OSC). Sometimes, however, there is neither a clear definition of the chemometric algorithms used, nor clear recommendations for their correct application. This circumstance could cause a chaotic situation for beginners in NIRS calibration development, who could obtain uncertain results with the prediction models used.

Furthermore, the conclusions obtained by different authors regarding the use of certain spectral pretreatments such as derivatives, MSC, etc., cannot be generalized to other data sets, since some of them have been performed with artificial data sets[7,8] or with synthetic spectra or spectra modified by the inclusion of intended variations.[9–12] Other studies have been devoted to the comparison of pretreatments on calibration development for drugs or chemical products,[13–15] whose compositions are relatively simple in contrast to agro-food materials and, particularly, compound feedingstuffs.

While pretreatments seem to be of importance in calibration development, the statistical significance of the improvements obtained with their use in the prediction ability of NIRS equations is often not established. Moreover, most of the published works recommend the use of a specific pretreatment on the basis of the minimum values obtained for the standard error of cross-validation (SECV) or the standard error of prediction (SEP). This method of obtaining conclusions about the optimum pretreatment of the spectral data is a risk, mainly when other factors affecting the final SECV or SEP values may have influenced the results (i.e., outlier samples removed from the calibration set).

Recently, several authors[16–19] used statistical tests to compare the SEP or bias-corrected SEP (SEP(c)) values obtained after the application of different pretreatments to NIR data. The statistical tests used are mostly based on a procedure published by Fearn.[20] Delwiche and Reeves[19] concluded that, even with statistical testing, for the development of a NIRS calibration, the selection of the appropriate pretreatment must ultimately be based on the modeler's judgment.

Fine and homogeneous milling has traditionally been recommended to reduce the effect caused by particle size differences, which affects the scatter properties of the irradiated surface. NIRS instruments underwent considerable modifications during the 1990s. Versatile NIRS analyzers, with different sample presentation attachments and large scanning windows, allowing the analysis of unground material, are now

commercially available.[21] However, the majority of the mathematical pretreatments usually used with low moisture materials, such as forages and feeds, have been developed to correct additive and multiplicative scatter errors caused by the interaction of NIR radiation with ground material.

The main goal of this paper is to evaluate the statistical significance of prediction errors obtained after the application of different spectral treatments to ground and unground compound feeds.

MATERIALS AND METHODS

Samples and Reference Data. A total of 354 commercial compound feedingstuffs, destined for different production animals (poultry, cattle, pig, ovine, and equine), were used in this study. The reference data for crude protein (CP) and crude fiber (CF) were determined by AOAC methods 976.06 and 978.10.22.

The global set was randomly split into a calibration set ($n = 324$) and a validation set ($n = 30$) in order to estimate prediction errors for all the calibrations obtained. The chemical composition for both sets is displayed in Table I.

Near-Infrared Reflectance Spectroscopy Hardware. All the samples were scanned in two different forms: unground and ground to 1 mm particle size, using a cyclonic mill (Cyclotec 1093 sample mill, Foss-Tecator, Höganäs, Sweden).

Reflectance spectra for ground samples were performed with a Foss NIRSystems 6500 SY-I monochromator, equipped with a spinning module. The instrument works in reflectance mode in the spectral range of 400–2500 nm, taking readings every 2 nm (spectral bandpass 10 ± 1 nm). Samples were analyzed using standard circular cups (3.75 cm diameter).

Unground samples were scanned using a Foss NIRSystems 6500 SY-II monochromator, from 400 to 2500 nm, every 2 nm (spectral bandpass 10 ± 1 nm). Analysis was performed using a transport module and a rectangular "coarse" cell (16.4×3.3 cm).

Near-Infrared Reflectance Spectroscopy Software. All spectra were manipulated and processed, and all calibration equations were obtained using the ISI softwares NIRS3 ver. 4.0 and WINISI ver. 1.5 (Infrasoft International, Port Matilda, PA).

Statistical tests for SEP(c) comparisons were computed with Microsoft Excel.

Mathematical Pretreatments. A total of 49 combinations of pretreatments (Table II) were applied to obtain modified partial least squares (MPLS) calibration equations.[23,24] The mathematical pretreatments used were spectral auto scaling (AS),[25] detrending (DT),[25] a combination of AS followed by DT (ADT), a combination of DT followed by AS (DTA), two versions of multiplicative scatter correction (MSC)[26] included

in WINISI software (Standard MSC and Weighted MSC), and four different derivative math treatments.

Auto Scaling Based on Standard Normal Variate.[25] The SNV centers each spectrum and then scales it by its own standard deviation (σ), correcting shifts on the log(1/R) axis. The transformation is applied to each spectrum individually, and the resulting spectra have mean zero and unit variance.

$$X_{\text{SNV},i} = \frac{X_{\log(1/R),i} - \overline{X}_{\log(1/R)}}{\sigma_{\log(1/R)}} \tag{1}$$

where $X_{\text{SNV},i}$ is the SNV spectrum value at wavelength i, $X_{\log(1/R),i}$ is the log(1/R) spectrum value (raw data) at wavelength i, $\overline{X}_{\log(1/R)}$ is the mean value of log(1/R) spectrum (for all wavelengths), $\sigma_{\log(1/R)}$ is the standard deviation of the log(1/R) spectrum (for all wavelengths), and i is the wavelength.

The AS algorithm (wrongly called SNV) as included in WINISI software has been simplified excluding spectral centering, so transformed spectra have non-zero mean and unity variance.

$$X_{\text{AS},i} = \frac{X_{\log(1/R),i}}{\sigma_{\log(1/R)}} \tag{2}$$

Detrending.[25] Detrending is performed using a second-order polynomial in a regression analysis, where the spectral value of a sample is the response or dependent variable and the independent variable is given by the wavelengths, correcting effects due to baseline curvature. DT correction is also applied to individual spectra and gives a spectrum with zero mean and non-unity variance.

$$\hat{X}_{\log(1/R),i} = A + B \cdot W + C \cdot W^2 + e_i \tag{3}$$

$$X_{\text{DT},i} = X_{\log(1/R),i} - \hat{X}_{\log(1/R),i} \tag{4}$$

where $X_{\text{DT},i}$ is the DT spectrum value at wavelength i, $\hat{X}_{\log(1/R)}$, i is the estimated value of $X_{\log(1/R),i}$ obtained from the quadratic function, and W is the wavelength value.

Combinations ADT and DTA. The ADT and DTA methods are calculated by applying both AS and DT sequentially in differing order. ADT is calculated directly by the WINISI software, while DTA is obtained by separately applying both transformations. ADT transformation results in a spectrum that has mean zero and non-zero variance, while DTA correction gives a spectrum with non-zero mean and variance of one. Both ADT and DTA were found to be related by a scaling factor.[27]

Multiplicative Scatter Correction.[26] Standard MSC (SMSC) is performed by calculating a least squares regression between each spectrum and the mean spectrum of the whole data set; thus, it is considered a set-dependent transformation. Afterwards, the regression coefficients are used to correct the spectra, as stated below:

$$X_{\log(1/R),i} = a_i + b_i \overline{X}_{\log(1/R),i} + e_i \tag{5}$$

$$X_{\text{MSC},i} = \frac{(X_{\log(1/R),i} - a_i)}{b_i} \tag{6}$$

where $X_{\text{MSC},i}$ is the MSC spectrum value at wavelength i, $X_{\log(1/R),i}$ is the log(1/R) spectrum value (raw data) at

TABLE I. Composition of calibration and validation sets (%).[a]

	Global set		Calibration		Validation	
	CP	CF	CP	CF	CP	CF
n	354	351	324	321	30	30
Mean	17.2	7.6	17.2	7.5	17.1	8.0
Minimum	11.9	1.4	11.9	1.4	12.7	3.2
Maximum	32.5	25.3	32.5	25.3	21.1	18.1
SD	2.28	4.37	2.30	4.33	2.09	4.89

[a] n: number of samples; SD: standard deviation; CP: crude protein; CF: crude fiber.

TABLE II. Combinations of pretreatments used in calibration.[a]

Equation	Scatter correction	Derivative	Equation	Scatter correction	Derivative
1	None	None	26	ADT	1,5,5,1
2	AS	None	27	ADT	1,10,5,1
3	DT	None	28	ADT	1,10,10,1
4	ADT	None	29	ADT	2,5,5,1
5	DTA	None	30	ADT	2,10,5,1
6	Standard MSC	None	31	ADT	2,10,10,1
7	Weighted MSC	None	32	DTA	1,5,5,1
8	None	1,5,5,1	33	DTA	1,10,5,1
9	None	1,10,5,1	34	DTA	1,10,10,1
10	None	1,10,10,1	35	DTA	2,5,5,1
11	None	2,5,5,1	36	DTA	2,10,5,1
12	None	2,10,5,1	37	DTA	2,10,10,1
13	None	2,10,10,1	38	Standard MSC	1,5,5,1
14	AS	1,5,5,1	39	Standard MSC	1,10,5,1
15	AS	1,10,5,1	40	Standard MSC	1,10,10,1
16	AS	1,10,10,1	41	Standard MSC	2,5,5,1
17	AS	2,5,5,1	42	Standard MSC	2,10,5,1
18	AS	2,10,5,1	43	Standard MSC	2,10,10,1
19	AS	2,10,10,1	44	Weighted MSC	1,5,5,1
20	DT	1,5,5,1	45	Weighted MSC	1,10,5,1
21	DT	1,10,5,1	46	Weighted MSC	1,10,10,1
22	DT	1,10,10,1	47	Weighted MSC	2,5,5,1
23	DT	2,5,5,1	48	Weighted MSC	2,10,5,1
24	DT	2,10,5,1	49	Weighted MSC	2,10,10,1
25	DT	2,10,10,1			

[a] AS: auto scaling; DT: detrending; ADT: auto scaling + detrending; DTA: detrending + auto scaling; MSC: multiplicative scatter correction.

wavelength i, $\overline{X}_{\log(1/R)}$ is the mean value of all $\log(1/R)$ spectra of the data set at wavelength i, and i is the wavelength.

Weighted MSC (WMSC)[28] is defined as a modification of SMSC that uses a correction for the spectral mean and standardizes at each wavelength. In this transformation, to compute the simple linear regression, the absorbances are weighted according to their standard deviation.

Dhanoa et al.[29] found that MSC and SNV were lineally related, while Helland et al.[30] confirmed the existence of connections among other pretreatments developed for scatter correction.

Spectral Derivatives Spectral differentiation or derivatives are frequently used in NIR spectroscopy. As the main methods used to calculate derivatives, Hopkins[31] cited the convolution function developed by Savitzky and Golay,[32] the point-difference method used in Grams software (Thermo Galactic, Salem, NH), and the Norris derivative or "segment-gap" method used in Vision and NSAS software (FOSSNIRSystems, Silver Spring, MD) and in ISI software (Infrasoft International, Port Matilda, PA).

The ISI derivative math treatments are defined by a four-digit notation (a, b, c, d), where a is the derivative order, b is the derivative gap, c is the smoothing segment, and d is the second smoothing segment.[28]

Calibration Development and Management of Outlier Samples. Modified partial least squares (MPLS) calibrations were obtained using an automatic routine implemented with the option "Teach automatic sequence" included in the ISI software.[28] The NIRS prediction models were obtained using the spectral range 1100–2498 nm, for each chemical parameter and milling status, and none, 2, or 9 maximum number of outlier elimination passes. Limits for outlier detection were 2.5 and 3 for T and H statistics, respectively. T outliers are defined as samples with significant differences between their laboratory and predicted values, while H outliers are defined as samples whose spectra show excessive distance ($H > 3$) to the spectral center of the calibration set.[28]

Statistical Analysis. The performance of NIRS equations was evaluated by examining the statistical values obtained for calibration and validation with test set procedures. The main calibration statistics used were 1-VR (determination coefficient for cross-validation) and SECV (standard error of cross-validation), while validation was evaluated by SEP(c) (standard error of prediction bias corrected) and r^2 (determination coefficient for validation).

The SEP(c) values were compared according to the procedure published by Fearn,[20] based on pair-wise comparisons of the standard deviation of the residual vectors coming from two models and on the r^2 between these two vectors. First, the correlation coefficient between the two sets of prediction errors (r) was found and then it was calculated:

$$K = \frac{2(1 - r^2)t_{n-2,0.025}^2}{n - 2} \tag{7}$$

where $t_{n-2,0.025}^2$ is the upper 2.5% point of a t distribution for $n - 2$ degrees of freedom.

Then, we found

$$L = \sqrt{K + \sqrt{(k^2 - 1)}} \tag{8}$$

The next two functions define the lower and the upper limits of a 95% confidence interval for the ratio of the true standard deviations. If the interval includes 1, errors are not significantly different at the 5% level:

$$\frac{\text{SEP(C)}_1}{\text{SEP(C)}_2} x \frac{1}{L} \quad \text{and} \quad \frac{\text{SEP(C)}_1}{\text{SEP(C)}_2} xL \tag{9}$$

The previous test was compared with the procedure used by Roggo et al.[17] This method, based on a Fisher test, defines a

confidence interval for errors with non-significant differences with minimum error obtained (Error$_{min}$):

$$(\text{Error}_{min}, \text{Error}_{min}\sqrt{F_{1-\alpha,n-1,n-1}}) \qquad (10)$$

where α is the significance level (5% in this study) and $(n-1)$ is the degrees of freedom.

RESULTS AND DISCUSSION

The effect of data pretreatments is easily appreciated by visual observation of the transformed spectra. Thus, Fig. 1 shows that the degree of spread observed in raw data (log(1/R)) for ground compound feeds along the Y-axis is reduced by the use of any of the pretreatments selected. The mentioned effect is more remarkable with unground product (Fig. 2), with the differences in particle size in this case becoming the main source of spectral variation. AS transformation (note, not SNV) applied by ISI software[28] does not produce significant effects in reducing the spread observed in the original log(1/R) data, as centering, by subtraction of the mean absorbance value for each spectrum, is not applied. This simplification is a consequence of the common use of AS together with other transformations, such as DT or derivatives, which implement corrections in the absorbance axis. DT and MSC reduce spectral variation, maintaining peak position in relation to the log(1/R) raw data. In addition, DT removes the ascending baseline effect. Application of both derivatives drastically
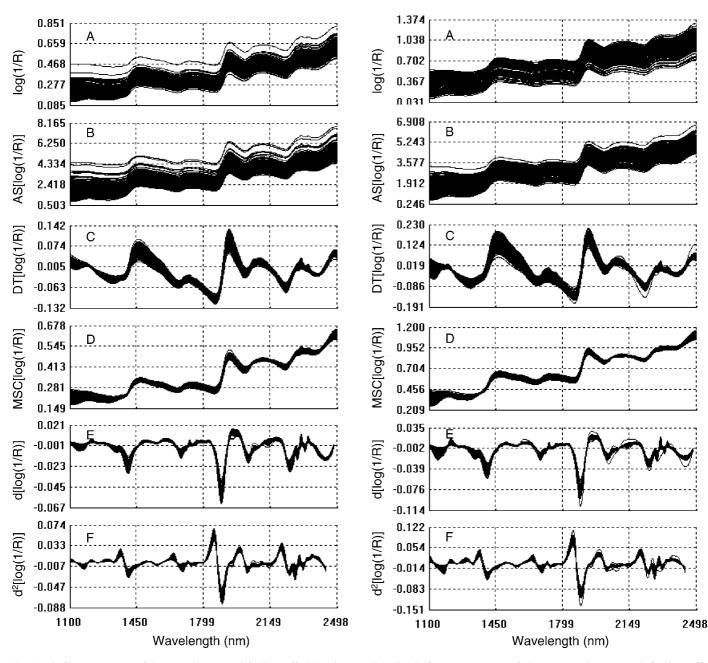

FIG. 1. Reflectance spectra of the ground compound feedingstuffs belonging to the calibration set. (**A**) Raw spectra; (**B**) AS transformed spectra; (**C**) DT transformed spectra; (**D**) standard MSC transformed spectra; (**E**) derivative 1,5,5,1; and (**F**) derivative 2,10,10,1.

FIG. 2. Reflectance spectra of the unground compound feedingstuffs belonging to the calibration set. (**A**) Raw spectra; (**B**) AS transformed spectra; (**C**) DT transformed spectra; (**D**) standard MSC transformed spectra; (**E**) derivative 1,5,5,1; and (**F**) derivative 2,10,10,1.

changes spectral shapes, duplicating original peaks into positive and negative, and introducing new peaks.

As was stated above, it is impossible to know beforehand which pretreatment will lead to the most accurate model with a certain calibration set. Thus, we used the option "teach automatic sequence" included in the ISI software to create a macro command that allows the calculation of 49 regression models for each constituent (analyte) with just one order. This option has an invaluable utility, as for this paper it was necessary to develop and validate 588 equations with more than 300 calibration samples. Although actual computers are able to perform calculations in a reasonable period of time, each outlier elimination pass implies a new calibration computation and, consequently, an increment in the final computation extent.

Although we are aware of the importance of a detailed discussion about the causes and management rules of the outliers detected during calibrations development for complex products, such as compound feedingstuffs, in the present paper this phase was omitted due to the huge number of calibrations to test. Nevertheless, using an automatic deletion procedure, it was possible to obtain regression models with 0 to 9 outlier elimination passes. This fact allowed us to test the effect of spectral pretreatments on the number of samples detected as T or H outliers.

The influence of the data pretreatments used in calibration, for 0, 2, and 9 maximum number of outlier elimination passes with a selected number of MPLS factors that varied between 2 and 16, is displayed in Table III. It can be observed that remarkable differences appear between the minimum and maximum SECV values. It can be also observed that SECV values for protein are lower for ground product calibrations

than for unground product calibrations for all levels of automatic outlier elimination passes, although these differences are reduced when outliers are eliminated. SECV values for fiber only show important differences between unground and ground samples without automatic outlier elimination, indicating that the presence of outliers affects the performance of calibrations to a greater extent than particle size.

Regarding the ability of the pretreatments to detect real outliers, Table IV shows the mean, minimum, and maximum number of samples deleted during calibration development for 2 or 9 maximum number of automatic outlier elimination passes. The selection of 9 passes as maximum for the elimination of outliers was done for comparison purposes only, and it is not recommended. However this option does not suppose the mandatory reaching of this limit in the development of calibrations, as a lower number of passes is generally selected. The minimum number of samples deleted was obtained with CP for ground product without any pretreatment, while the maximum value corresponded to CP for unground product using DT transformation. It can be observed that the number of outliers detected during the first two passes is quite consistent for both parameters and milling status. This fact could indicate that these samples present real anomalies in their spectra or lab value. With a higher number of elimination passes, a higher variation in outliers detected with different pretreatments is revealed. Nevertheless, if we compare the minimum number of outliers detected with 9 and 2 maximum number of elimination passes, we find that the differences are slight. This tells us that some pretreatments show a better ability to detect real outliers during calibration development, while other transformations lead to the detection of false anomalies, such as samples with extreme laboratory values.

TABLE III. Maximum and minimum SECV values for CP and CF calibrations, with 0, 2, or 9 maximum number of outlier elimination passes for all pretreatments.[a]

Calibrations			No elimination passes				Max of 2 elimination passes				Max of 9 elimination passes			
			SECV	eqa	terms	n	SECV	eqa	terms	n	SECV	eqa	terms	n
Unground samples	CP	Minimum	1.22	43	14	324	0.88	18	13	308	0.61	31	16	281
		Maximum	1.82	1	4	324	0.98	7	12	314	0.92	36	13	311
	CF	Minimum	1.57	35	3	321	0.60	29	11	304	0.47	35	10	279
		Maximum	1.95	13	2	321	0.85	2	13	305	0.68	7	16	297
Ground samples	CP	Minimum	0.91	16	10	324	0.61	48	10	305	0.52	43	14	295
		Maximum	0.98	13	7	324	0.70	1	12	314	0.70	1	14	317
	CF	Minimum	0.94	18	9	321	0.60	26	14	308	0.51	19	11	289
		Maximum	1.10	1	13	321	0.80	1	13	307	0.71	24	9	313

[a] CP: crude protein; CF: crude fiber; SECV: standard error of cross-validation; eqa: equation; terms: number of MPLS factors; n: size of the calibration set.

TABLE IV. Mean, minimum, and maximum number of samples deleted during calibration development for CP and CF with 2 or 9 maximum number of outlier elimination passes.[a]

	Max of 2 passes								Max of 9 passes							
	CP				CF				CP				CF			
	Unground		Ground		Unground		Ground		Unground		Ground		Unground		Ground	
	n	eqa	n	eqa	n	eqa	n	eqa	n	eqa	n	eqa	n	eqa	n	eqa
Mean	13	⋯	13	⋯	16	⋯	11	⋯	32	⋯	21	⋯	28	⋯	23	⋯
Minimum	10	7	10	1	12	27	8	12	13	18	7	1	19	44	8	24
Maximum	17	17	19	48	19	5	16	14	45	3	32	5	42	35	34	31

[a] CP: crude protein; CF: crude fiber; n: number of samples deleted; eqa: equation.

TABLE V. SEP(c) maximum, minimum (lower confidence limit), and upper confidence limit for CP and CF calibrations with 0, 2, or 9 maximum outlier elimination passes.[a]

		Unground samples						Ground samples					
		0 passes		2 passes		9 passes		0 passes		2 passes		9 passes	
		SEP(c)	eqa	SEP(c)	eqa	SEP(c)	eqa	SEP(c)	eqa	SEP(c)	eqa	SEP(c)	eqa
CP	Minimum	0.78	44	0.64	1	0.61	8	0.75	48	0.69	35	0.68	35
	Maximum	1.27*	1	0.90*	36	0.86*	11	0.86	3	0.82	6	0.82	8
	Upper CL	1.06		0.87		0.83		1.02		0.94		0.92	
CF	Minimum	0.85	23	0.78	20	0.74	10	0.83	47	0.74	35	0.75	47
	Maximum	1.33*	20	0.95	16	1.02*	2	1.14*	3	1.09*	3	1.07*	13
	Upper CL	1.16		1.06		1.01		1.13		1.01		1.02	

[a] CP: crude protein; CF: crude fiber; CL: confidence limit; SEP(c): standard error of prediction bias corrected; eqa: equation; * indicates significant differences with minimum error at $=0.05$.

The importance of the use of an appropriate mathematical transformation is emphasized, since elimination of false outliers during calibration could cause a reduction in the prediction ability of calibrations.

The influence of pretreatments on the predictive ability of calibrations was determined with paired comparisons of the minimum SEP(c) value and the rest of the SEP(c) values using the statistical tests proposed by Fearn[20] and Roggo et al.[17] The results obtained with both statistical tests matched up. The information is summarized in Table V, including the maximum and minimum SEP(c) values for both analytical parameters and for different numbers of outlier elimination passes, and also the SEP(c) confidence limit value, calculated as $\mathrm{SEP(c)_{min}} x \sqrt{F_{0.95,29,29}}$. According to Roggo et al.,[17] the models that have SEP(c) values between SEP(c)min and SEP(c) confidence limit were not significantly different. Table V shows that the prediction errors were also affected by the pretreatment used, since differences between the maximum and minimum are evident for both parameters.

Comparison of prediction errors for CP showed that there were not significant differences among calibrations developed with ground samples, while for unground samples the predictive ability of the calibrations was significantly lower, indicating that the selection of the best combination of pretreatments is essential when the particle size is one of the main sources of spectral variations. For unground samples, minimum errors were found for combinations of pretreatments WMSC+1,5,5,1 and DT+1,5,5,1; while significant maximum errors were obtained for DT, WMSC and none; DT+2,5,5,1, 2,5,5,1 and DTA+2,10,5,1; and ADT+2,10,5,1, SMSC+2,10,5,1, and AS+2,10,5,1 and 2,5,5,1; for 0, 2, and 9 maximum number of outlier elimination passes, respectively. In spite of the non-significant differences found for ground products, minimum errors were obtained for pretreatments WMSC+2,10,5,1 and DTA+2,5,5,1; while maximum errors were found for DT, SMSC, and 1,5,5,1 for 0, 2, and 9 maximum number of outlier elimination passes, respectively. Other validation statistics such as bias, slope, and determination coefficient fulfilled the control limits suggested by Shenk et al.,[33] except for a few calibrations developed for ground product with 9 maximum outlier elimination passes, where SEP(c) values were superior to the calculated limit. Nevertheless, this fact can be explained, as the applied control limits seem to be more restrictive for models with better calibration statistics.

For errors for CF, the differences among minimum errors

were significant for the criteria used, except for unground product calibrations developed with a maximum of two outlier elimination passes. Table V shows that the confidence limits were very close to the maximum error values for the majority of cases, indicating that only a few of the pretreatments produced errors significantly higher than the minimum error. Minimum errors for unground product were found for DT+2,5,5,1, DT+1,5,5,1, and 1,10,10,1, while maximum errors were obtained for WMSC alone or combined with second derivatives, AS+1,10,10,1 and AS, for 0, 2, and 9 maximum number of outlier elimination passes, respectively. For ground products, minimum errors appeared with WMSC+2,5,5,1 and DTA+2,5,5,1; and maximum errors were obtained for DT; SMSC, 1,10,10,1, 1,5,5,1, ADT, DT+1,10,5,1, none, DT+1,10,10,1, DT+2,10,5,1, 2,10,10,1, DT+2,10,10,1 for 0 outlier elimination passes, DT and DT+2,10,10,1, none, DT+2,10,5,1 for 2 outlier elimination passes, and 2,10,10,1 for 9 outlier elimination passes. Most of the calibrations presented validation statistics within the recommended values when elimination of outliers was not used. Setting 2 outlier elimination passes, we observed an increment in the non-conformities with the recommended values for validation, especially for ground products, since their calibration statistics were better. Most of acceptable validation tests for ground products were obtained for models that used a second derivative in their pretreatments. For the highest number of outlier elimination passes, only the calibration developed with WMSC+2,5,5,1 satisfied the control limits for ground products, while five calibrations (four of them including first derivatives) passed the control limits for unground products.

It resulted that it was impossible to find a clear pattern for the combination of pretreatments that provided the best and worse results in validation tests, as was concluded for other agro-food products.[17,18] However, the use of derivatives alone or in combination with other pretreatments showed an important effect on validation results.

In this study, it was not observed that SEP(c) followed SECV values, as in the work published by Delwiche and Reeves,[19] although a full cross-validation model (leave-one-out) may have reduced the differences between both statistics.

CONCLUSION

The effect of spectral pretreatments on the predictive ability of NIRS calibration models can be defined by their ability to improve the detection of real outliers during calibration and the

The best pretreatments in calibration and in prediction phases usually differ. Therefore, when evaluating pretreatments it would be desirable to use some automatic software routines that permit efficient testing of different validation sets and/or the execution of a large number of cross-validation passes.

degree of adjustment among the constituent values and the transformed spectral data.

We did not find a clear distribution pattern for results with the different data pretreatment methods evaluated, but since scatter is the main source of spectral variability in the studied products, most of the scatter-correction algorithms tested improved the predictive ability of the equations developed.

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