NIR determination of quality parameters in fresh alfalfa and dehydrated derived products

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Introduction

Looking at the agro-feeding world, one can easily realise that near infrared (NIR) spectroscopy has become a very important spectroscopic technique in the last years. Traditionally, the near infrared zone was the most used for quantitative analyses. This technique began to be used in forage analysis during the 70's decade (Norris *et al*., 1976)[1] when NIR was suggested as a quick, precise, accurate and non-destructive method for forage quality evaluation. Some of the parameters analysed using these methodologies were crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin and *in vitro* digestibility.

The area dedicated to the production of forage in the Autonomous Community of Catalonia, located in the north-east of Spain (41º39′N, 0º51′E), is at present of about $7 \cdot 10^5$ ha. The main forage in this area, from the economic as well as surface extension points of view, is alfalfa, with $5 \cdot 10^5$ ha obtaining a production of 2.35 MT of fresh matter.

Alfalfa is an appreciated forage for its protein content and the quality of its fibre, as well as for its environmentally safe character. The current management of alfalfa in dehydration plants has increased even more the need for the development of fast methodologies for quality control of fresh and dehydrated derived products.

Fourteen transforming industries of forages are located in Lleida, being one of the main Spanish province producer of alfalfa. There are few studies developed at the moment using directly fresh alfalfa although it should be noticed the extensive work of Pierre Dardenne *et al*.[2] The high moisture and fibre content of fresh samples which prevent milling processes, together with the heterogeneity of the sample, the influence of the cut and different environmental conditions are the main factors that make difficult the work with fresh material. The chemistry department of the University of Lleida (UdL), in connection with the company TransAlfals & La Vispesa SCL, have undertaken the present work which aims at the developing of a methodology for the quality control of the different products involved in the production process of dehydration plants using NIR spectrometers. Calibration models were developed for fresh alfalfa as well as for products derived of dehydrated material presented in different formats (pellets, cubes and bales) for different uses related with animal feeding.

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Material and methods

Sample description

Fresh alfalfa samples were collected between the years 1998 and 2003 in the Lleida area, just at the arrival at the industrial plant. Alfalfa arrives mainly cut in pieces of about 8 cm. As sampling is the main error source in the process of quality prediction, a mixing procedure of a sample of about 10kg is conducted by a picking device which allows to select a subsample of about 100 g, enough to fill different times the sample cell for recording the spectra in NIR devices. No other manipulation is performed until the cell is filled and only the manual cutting of the exceeding size is done to be able to close the cell.

Typical management of the field in the area include six harvests per season, although in some areas only five cuts can be performed. All the different cuts, seasons, areas, fertilizer managements and vegetative stages are represented in the sample calibration set.

Dehydrated alfalfa products were collected during the above mentioned years. Include: (i) *pellets* of cylindrical form with 5mm diameter of pressed material, specially recommended for bovine cattle; (ii) *bales*, a big dehydrated alfalfa package of around 750 kg. with stem length between 30 and 35 cm of length, optimum product for bovine cattle feeding; and (iii) *cubes* of pressed material, another presentation of long fibre forage. Each side of the cube has a length of 3.5 cm, and this product is mainly used for the feeding of cows, bulls and horses.

No grounding of the samples was performed: the sampling cell was filled with intact pellets; with intact alfalfa obtained from the bales (the procedure for collect the samples from bales is the same than that is described above for fresh alfalfa) or from unfold cubes.

NIR equipment

The sample spectra have been recorded with a Bran+Luebbe's InfraAlyzer 2000 Spectrometer, an instrument with a 19-filter wheel which allows the reading of absorbances at the following wavelengths: 1445, 1680, 1722, 1734, 1759, 1778, 1818, 1940, 1982, 2100, 2139, 2180, 2190, 2208, 2230, 2270, 2310, 2336 and 2348 nm. This instrument has a rotating cup module for working with heterogeneous samples. The spectrum of one sample is the average of a pre-defined number of spectra registered while this sample is turning. In this study, the average of three successive registered spectra has been calculated for each sample of the calibration set.

The NIR instrument is controlled through a PC with the software Sesame (version 3.01), from Bran+Luebbe. This program allows the management of the spectra recording procedure in the hard disc of the computer (to use them for calibration or prediction purposes), the definition of the calibration parameters and some more activities related with the instrument management.

Reference methods

Material coming from the filling of the cells for NIR spectrum recording is used in the reference analysis. Reference values were obtained using the traditional procedures: Moisture was determined as a percent value obtained from the relative difference of weights before and after sample drying in an oven 24 hours at 103ºC. CP was estimated from the percent of N calculated with the Kjeldahl method (multiplying this for 6.25). Crude fibre (CF) was determined using the Weende scheme. ADF and NDF were calculated following the Van Soest respective schemes.[3,4]

Calibration methods

Calibration models were developed using multivariate projection methods. First of all, a principal component analysis (PCA) of the NIR spectra was performed as a preliminary data study, to see

patterns, groups of samples, strange samples and differences. After that, Partial Least Squares (PLS) was used to correlate the reference values and the spectral data.[5] The software used for the multivariate calibration was Unscrambler (version 6.11b).[6]
No data pre-treatment was used before calibration. The validation method used was full cross validation.

Results and discussion

NIR calibration is a time-consuming process that implies a lot of model tests using new samples for prediction and successive re-calibrations with the inclusion of new (different) samples in the calibration set in order to increase the property's numerical range and to cover all levels of factors that influence the spectra in order to yield a robust regression model. The high number of factors influencing the spectra in this case leads to manage large calibration sets, increasing the cost of the process.
Quality parameters considered for fresh alfalfa are moisture and crude protein. Concerning dehydrated derived products (pellets, bales or cubes), it seems necessary to include in the study some additional properties relevant for animal feeding. Calibrations of moisture, CP, CF, ADF and NDF were included in the study for alfalfa packed in bales, the main dehydrated derived product.

Calibrations of moisture and crude protein

Table 1 presents the main characteristics of the sample set as well as the main statistics of the PLS-models obtained for moisture and CP properties for all the products of this study.

Table 1. Statistical description of moisture and protein PLS-models for all alfalfa products.

Product	Fresh alfalfa		Dehydrated pellets		Dehydrated bales		Dehydrated cubes	
Properties	Moist	CP	Moist	CP	Moist	CP	Moist	CP
# of samples	845	845	690	690	288	288	127	127
Max value	73.40	27.16	11.93	20.74	17.78	20.85	17.82	18.92
Min value	10.86	8.01	3.46	10.98	6.12	11.03	7.51	14.88
Mean value	39.65	18.58	7.93	16.43	11.35	15.83	10.72	16.90
Standard deviation	13.17	3.12	1.45	1.54	2.01	1.69	2.31	0.98
# of X-variables	19	19	18	19	19	18	19	19
Optimum # of PC's	4	7	4	8	2	7	2	5
Correlation coefficient	0.98	0.92	0.91	0.93	0.95	0.81	0.98	0.79
RMSEP	2.84	1.25	0.60	0.56	0.65	0.99	0.51	0.60

It is worth noticing for fresh alfalfa, the raw vegetal material, the big moisture range of the samples. The comparison of the property range with the root mean square error of prediction (*RMSEP*) of the model indicates that a useful model has been obtained. In the predicted versus measured plot (Figure 1) a small residual non-linearity is seen, which is not surprising given the large range considered. Under these conditions, local calibration models[2] could improve the prediction given the relatively large data set available, although the sticking to the software licensed with the instrument prevents this use from an industrial point of view. CP calibration in fresh samples is also interesting in order to promote good management practices among farmers and in order to optimise and homogenise the products of the plant. The *RMSEP* obtained is higher than that reported for dehydrated samples measured as bales in the present work (Table 1) but looking at the present results, the impact of the high water content in the *RMSEP* of protein seems moderate, without invalidating the use of this

model for an improved management of the stocks in the plant. Results obtained for pellets seem very interesting due to the good statistics of the model and the convenience of the procedure which uses intact pellets to fill the sample cub.

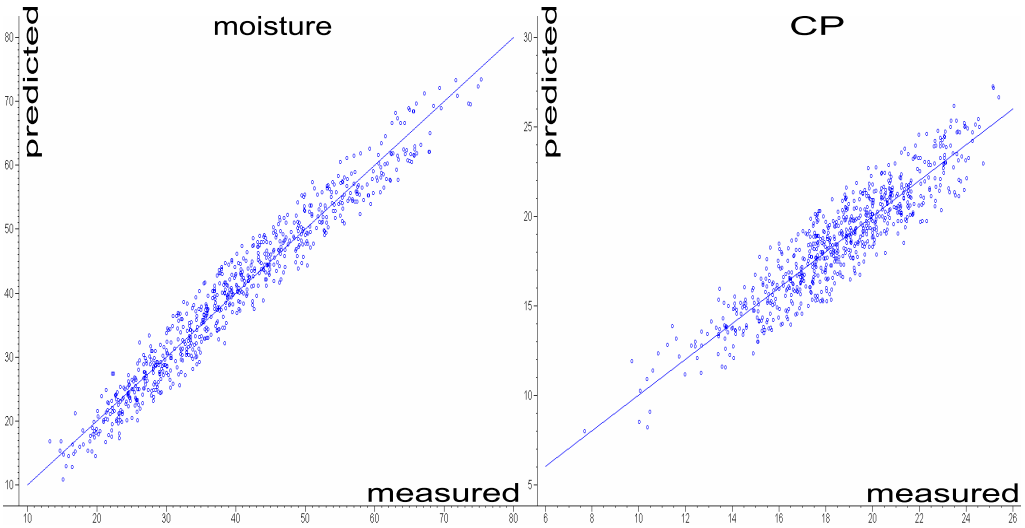

Figure 1. Predicted vs measured diagrams corresponding to moisture and CP models.

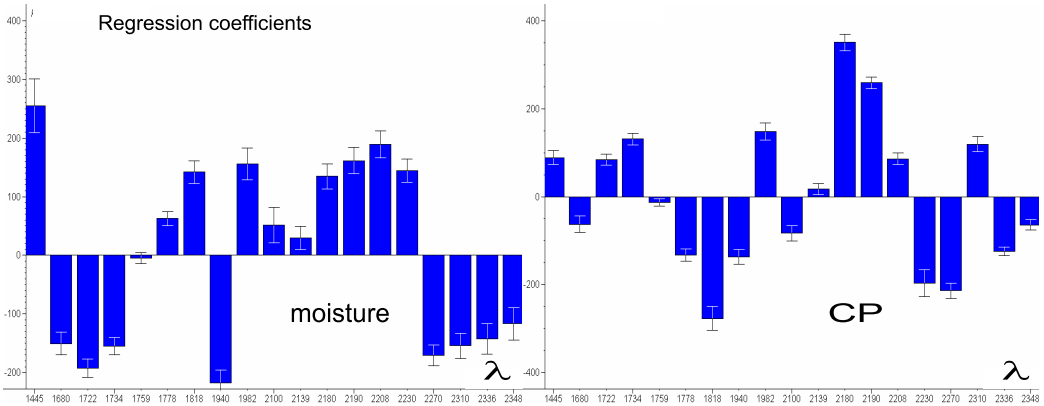

Figure 2. Regression coefficient diagrams corresponding to moisture and CP.

Finally, Figure 2 shows the regression coefficients of the models of moisture and CP for fresh alfalfa. The stability of these coefficients, calculated by means of the Martens' Uncertainty Test is also plotted in the figure and allow to conclude that very stable models for both properties have been obtained.

Results obtained in the present work can be also be compared with those reported by Dardenne *et al.*[2] for fresh alfalfa. The comparison should be taken with caution due to the very different

conditions of the data: NIR equipment (Foss NIRSystem 6500, a NIR spectrometer that reads the full range of absorbances from 400 to 2500 nm with a step of 2 nm), calibration method (modified-7PLS), sample management (dried and ground samples), mathematical pre-treatment of the spectra (SNV-detrending and a second derivative) and validation method (segmented cross validation with four groups). Although the aforementioned differences, total RMSEP values obtained in both works are quite comparable.

Calibrations of fibre

Dehydrated alfalfa in bales is currently the main product of dehydration plants. The presentation as big cubes of 2 × 1 × 0.6 m leads to an easy manipulation and transport and its long fibre content makes this product very appreciated for bovine cattle feeding.

Table 2. Calibration PLS-models obtained for fibre properties calibrated from samples of dehydrated alfalfa in bales.

Fibre property	CF	ADF	NDF
# of samples	211	211	211
Max value	48.49	52.42	64.49
Min value	18.55	21.94	31.49
Mean value	32.17	36.49	46.96
Standard deviation	5.86	6.36	6.29
Optimum # of PC's	7	7	7
Correlation coefficient	0.89	0.91	0.89
RMSEP	2.65	2.60	2.82

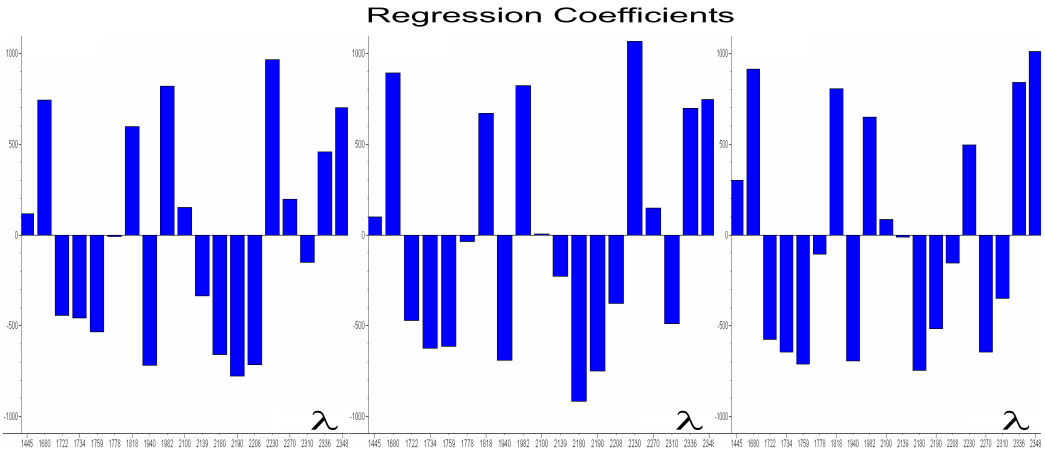

Figure 3. Regression coefficients obtained from the PLS-models of CF, ADF and NDF.

For this reason, PLS regression models were developed for the calibration of fibre parameters as more interesting properties from a nutritional point of view. Table 2 shows the characteristics of the models obtained for these properties whose regression coefficients are plotted in Figure 3. Statistics reported indicate the utility of the NIR methodology for the prediction of fibre in intact dehydrated alfalfa.

Conclusions

NIR spectroscopy is a rapid and useful method for the determination of quality properties of fresh alfalfa and dehydrated products as results reported in this work indicate. The *RMSEP* of moisture and CP reported for fresh material are higher than those corresponding to dehydrated samples, but the values of Table 1 indicate a clear utility of NIR spectroscopy for the management of fresh material in the dehydration industrial plants as well as for the improvement of the management procedures of the farmers. Rotating cup accessory allows to work directly with intact fresh material, without drying and grounding. This fact helps NIR integration into the normal industrial process.
Fresh material (due to its own characteristics) need large sample sets in order to obtain robust models given the large number of factors that influence the samples and the spectra. However, 19 filter NIR instruments can provide good predictions of the main properties of fresh and dehydrated material. These instruments, although its decreasing use in laboratory, can be of high interest for in situ work in the industrial plants, given its robust behaviour out of laboratory conditions.

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