RESEARCH ON PROTEIN CONTENT AND TOTAL NITROGEN FOR *MEDICAGO SATIVA* USING FT-NIR SPECTROMETRY

CERCETARI PRVIND CONTINUTUL DE PROTEINA ŞI AZOTUL TOTAL DIN MEDICAGO SATIVA FOLOSIND SPECTROMETRIA FT-NIR

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***Abstract**: Using NIRS technique it becomes more frequently used for determining physico-chemical properties of feed. This technique is elegant and very precise. The protein content of Medicago sativa is rich in essential amino acids, giving it a high biological value. This paper aims to highlight a way of direct analysis method undestructive protein and total nitrogen using near infrared spectrometry in conjunction with reflected attenuated total. The total nitrogen content was determinate with Kjeldahl method and using mathematical calculation was determinate the protein content. All this results we use for calibration of PerkinElmer FT-NIR Spectrometer Spectrum 100N. We have built mathematical models for Medicago sativa, based on these techniques and multivariated analysis allows the determination of an error prediction for the best protein and the total nitrogen of 0.00%.*

***Rezumat:** Folosirea tehnicii NIRS a devenit în ultima perioadă cea mai frecvent utilizată pentru determinarea proprietăţilor fizico-chimice ale furajelor. Această tehnică este o tehnică elegantă şi foarte precisă. Conţinutul de proteină din Medicago sativa este bogat în amino acizii esenţial, conferindu-i o valoare biologică ridicată. Prezenta lucrare îşi propune să evidenţieze o modalitate de analiză directă prin metoda nedestructivă a proteinei şi azotului total folosind tehnica spectrometrie în infrarosu apropiat coroborată cu reflectanţa totală atenuată. Azotul total a fost determinat cu ajutorul metodei Kjeldahl şi folosind calculul matematic am determinat proteina. Toate aceste rezultate le-am folosit pentru determinarea curbei de calibrare a apartatului PerkinElmer FT-NIR Spectrometer Spectrum 100N. S-a construit modelul matematice pentru Medicago sativa, acestea având la bază tehnici de analiză multivariabile care permit determinarea cu o eroare de predicţie mai bună pentru proteină şi pentru azotul total de 0.00 %.*

***Keywords:** NIR, protein, total nitrogen, non-destructive methods, feed, Medicago sativa.*
***Cuvinte cheies:** NIR, proteină, azot total, metodă nedestructivă , furaj, Medicago sativa.*

INTRODUCTION

NIR spectroscopy has been applied in the food industry and agriculture for determination of water, protein, oil, fat, and carbohydrate contents.

In 1973, P. Williams reported the use of a commercial NIR grain analyzer for analyses of cereal products following the pioneer work of Norris and others. Later Williams and Karl Norris would edit a comprehensive text on the subject of NIR analysis for commercially important biological materials.

Forage analysis using NIR measurement has been a major application of the technique largely due to the work of J.S. Shenk, M. Westerhaus, W. Barton, G. Marten, N. Martin, and a host of others who improved upon the technique and worked toward it□s widespread use and acceptance among scientists as a valid analytical technique.

The energy band is defined for convenience as the near infrared (0.78 to 2.50 microns); the infrared (or mid-infrared) 2.50 to 40.0 microns; and the far infrared (40.0 to 1000

microns). However, even though official standards, textbooks, and the scientific literature generally state that the NIR spectral region extends from 780-2500 nanometers (12821 - 4000 cm^{-1}), a simple set of liquid phase hydrocarbon spectra demonstrates that the vibrational information characterized by the harmonic vibrations of the C-H stretch fundamental and their corresponding combination bands occurs from approximately 690 to 3000 nm.

A spectrum may, or may not, contain information related to the sample chemistry measured using any specific reference method. Spectra-structure correlation provides a basis for the establishment of a known cause and effect relationship between instrument response and reference (analyte) data, in order to provide a more scientific basis for multivariate-based near infrared spectroscopy. When performing multivariate calibrations, analytically valid calibration models requires a relationship between X (the instrument response data or spectrum), and Y (the reference data). The use of probability alone tells us only if X and Y 'appear' to be related. If no cause-effect relationship exists between spectra-structure correlation and reference values the model will have no true predictive importance. Thus, knowledge of cause and effect creates a basis for scientific decision-making.

MATERIAL AND METHOD

This study was conducted at the University of Agricultural Sciences and Veterinary Medicine, located on Cluj Napoca in 2009, at ICAR Laboratory for destructive method: Kjeldahl method for determinating the nitrogen and the mathematical calculation for protein, and then the samples were collected with NIR spectrum to build a calibration model at Laboratory of Grassland and Forages Plants Cultures.

Samples of *Medicago sativa* were obtained from The Research – Agricultural Development Jucu from during the period from 2009. The samples from 2009 were used solely for calibration. The samples from 2009 were all from The Research – Agricultural Development Jucu and were randomly split up into a calibration set and a validation set. NIR measurements were carried out using a FT-NIR spectrometer (PerkinElmer Spectrum One, PerkinElmer) with an NIRA detector. The samples were directly measured, i.e. through the bottom of the intact glass vials by diffuse reflectance without any extra preparation.

All spectra were recorded on a PerkinElmer FT-NIR Spectrometer Spectrum 100N fitted with a „plug-and-play" sampling system accessory for reflectance measurement (NIRA). In the same time each sample was measured using a Kjeldahl extractor for nitrogen determination and mathematical calculation for protein. Using these values for spectra we build a mathematical model for direct determination of these two chemical properties of the samples. For this calculation Spectrum Quant+ v4.60 is used.

RESULTS AND DISCUSSIONS

Forty-tow samples of *Medicago sativa* were determinate with Kjeldahl method and then we collect the spectra with a PerkinElmer FT-NIR Spectrometer Spectrum 100N. Spectra were recorded by filling a standard sample cup with the sample and scanning in interleaved mode.

Sixteen replicate measurements of each of the calibration samples were collected, and the mean spectrum used for the generation of the calibration equations. Data was collected over the range 10000 to 4000 cm^{-1} at 16 cm^{-1} resolution with 2 cm^{-1} step, and then data was collected over the whole range of the NIR spectrum since this data set may be used to determine a number of other properties in *Medicago sativa* from these spectra.

A typical spectrum representative of the samples is shown in Figure 1.

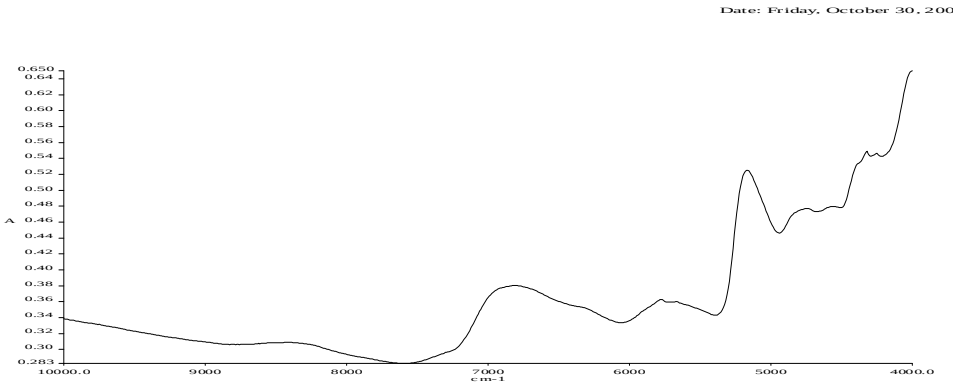

Figure 1 – Typical spectrum of sample

A partial least squares analysis (PLS) was performed on the data (42 spectra). It is possible to predict values for protein in sample in the independent validation set.

Various mathematical pretreatments were tested and a second derivative function chosen to provide Standard Error of Prediction (SEP) value of 0.29 for protein and 0.04 for nitrogen using 18 PLS factors and full cross validation. Figure 2 show the variation of component number 1 (PC 1) with wavelength.

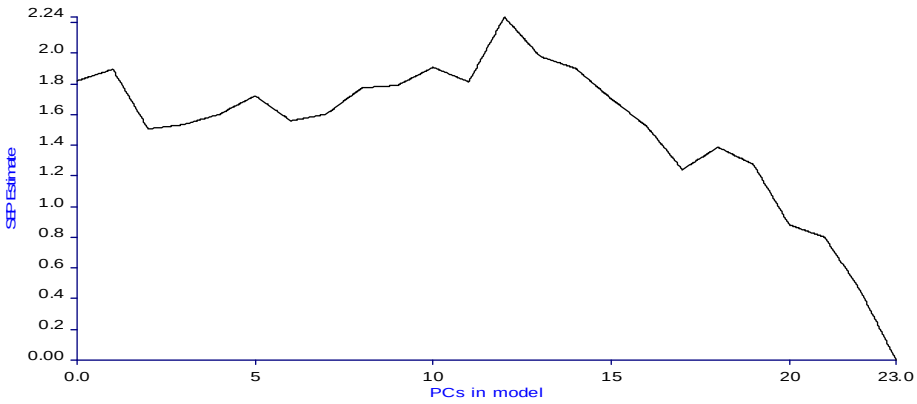

Figure 2 – Evolution of PC 1 with wavelength

Full cross validation excludes each standard in turn from the calibration set, performs the calibration and then predicts the excluded standard using that calibration. Smaller prediction errors may be obtained using a larger number of PLS factors.

However, it was decided to optimize the calibration for robustness which is better achieved by performing independent validation over time.

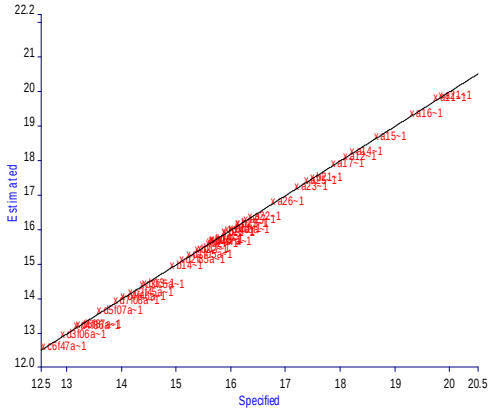

Figure 3 – Estimated vs. Specified plot for protein

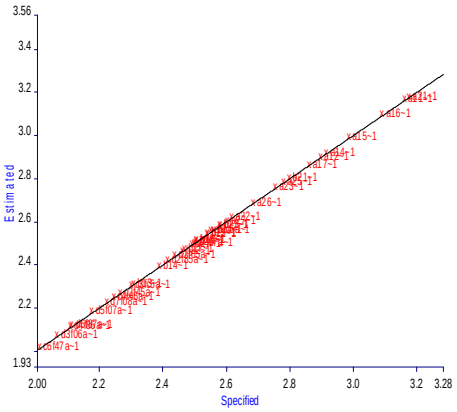

Figure 3a – Estimated vs. Specified plot for total nitrogen

Figures 3 and 3a are the illustrated plots of Estimated versus Specified values, first for protein and second for nitrogen. This provides an adequate starting point for the calibration model.

The regression model summaries for the full cross validation model are shown in Table 1.

Table 1

Regression model summaries

Method Name: proteinm Ident: Spectrum QUANT+ v4.51
No. of properties: 2 No. of standards: 39 Calibrated: Yes
Calculation Parameters: Algorithm: PLS2 Range: 10000 to 4000 cm-1 Interval: 2 cm-1
Analysis Type: Absorbance Scaling (Spectra): Mean Scaling (Property): Mean Smooth: None Baseline correction: Derivative - Order: 1 Width: 9 Normalization: None
Ordinate threshold: Upper threshold: 1.5 A Lower threshold: None
Number of factors: Minimum: 1 Maximum: 100 Blank regions: None

To support validation, a series of samples were run a week later and both the protein and total nitrogen content predicted using the calibrated model. Table 2 shows the results along with the reference values supplied. Additional statistics in terms of the total M-distance and residual ratio give an indication of how well the model covers these samples.

Table 2

Spectrum Quant+ v4.60 PREDICTION RESULTS PROTEINM

Sample	A11	A21	B27
Normalization	None	None	None
RMS Error	1,065e-004	9,119e-005	3,432e-005
Peak to Peak Error	8,984e-004	9,95e-004	03,255e-0,004
Total M-Distance	0,56	0,7037	0,9514
Residual Ratio	0,9697	0,7106	0,1007
PROTEIN			
Calculated Value	19,71	19,8	15,54
Reference Value			
R-error	1,425e-006	1,49e-006	1,594e-006
M-Distance	0,56	0,7037	0,9514
TOTAL NITROGEN			
Calculated Value	3,154	3,167	2,487
Reference Value			
R-error	3,332e-007	3,482e-007	3,727e-007
M-Distance	0,56	0,7037	0,9514

CONCLUSION

The results obtained in this paper work demonstrate that several compositional fractions of forage from different types *Medicago sativa* can be accurately predicted by NIRS on fresh plant material.

The example detailed here illustrates that it is possible to determine a number of properties present in *Medicago sativa* samples with accuracy which is of a similar order to that of the reference method using FT-NIR spectroscopy.

Based on the samples supplied, it has been shown that FT-NIR and partial least squares can be used to determine protein of *Medicago sativa* with very good standard error of prediction (SEP). This proves that FT-NIR spectroscopy is an extremely reliable, non-destructive and rapid technique for the quantity of many chemical and physical properties.

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