

## DETERMINATION OF CHEMICAL COMPOSITION OF MEDICAGO SATIVA PLANT BY DESTRUCTIVE AND NON-DESTRUCTIVE METHOD

Laura Monica DALE<sup>1</sup>, Ioan ROTAR<sup>1</sup>, Roxana VIDICAN<sup>1</sup>, Vasile FLORIAN<sup>1</sup>,  
Alin MOGOS<sup>2</sup>

<sup>1</sup>USAMV Cluj Napoca, Faculty of Agriculture

<sup>2</sup>SC CROMATEC PLUS SRL –Perkin Elmer

Calea Mănăştur, nr. 3-5, cod 400372, Cluj Napoca, Cluj

Corresponding author: [dale\\_lm@yahoo.com](mailto:dale_lm@yahoo.com)

**Abstract:** Using NIRS technique it becomes more frequently used for determining physico-chemical properties of feed, especially chemical composition (dry matter, protein, ash, fiber, fat, non-nitrogenous extractive substances. This technique is an elegant and very precise. The contents of dry matter, crude protein, crude ash, crude fiber, crude fat, non – nitrogenous extractive substances of biological material *Medicago sativa* are determined by destructive methods and then using them was build the calibration curve for NIRS with which we will determine all the chemical properties of the material using prediction. For each property we have used these method assessment: for the crude protein was used Kjeldahl method, for crude fat was used Soxhlet method, for crude ash performed Hennenberg – Stohmann method, for crude fiber was used Hennenberg - Stohmann method and for non – nitrogenous extractive substances was used mathematical calculation. This paper aims to highlight a way of direct analysis nondestructive method of crude protein, crude fat, crude fiber, crude ash, non-nitrogenous extractive substances using near infrared spectrometry technique in conjunction with reflected attenuated total. All these results determined by the classical method, the destructive method, were used to build the calibration curve for device FT-NIR Spectrometer Perkin Elmer Spectrum 100 N with accessory N NIRA. Mathematical model was built using the near infrared spectroscopy technique in conjunction with multivariable calibration techniques using the Perkin Elmer program Spectrum Quant + 4.60. These techniques based on multivariate analysis allow the determination of an error prediction for the best crude protein content is 0.00 %, for crude fat the content ranges from 0.015 to 0.074 %, the crude ash from 0.00 to 1.17 %, for crude cellulose the content ranges from 0.00 to 0.30 % and for non – nitrogenous extractive substances from 0.26 to 1.73 %. The method provides a rapid and reliable alternative to traditional quantitative methods for determining which usually requires several hours to complete.

**Key words:** NIRS, protein, fat, ash, cellulose, non – nitrogenous extractive substances, *Medicago sativa*

### INTRODUCTION

Commercial grains are commonly analysed by using NIR spectroscopy. The major constituents of grains are water, protein, oil, fibre, minerals and carbohydrates and it is commercially important to quantities the composition. The NIR spectra of such materials are, thus, dominated by the overtones and combination bands of C-H, N-H, O-H and C=O bonds.

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.

Traditionally, dispersive instruments, available since the 1940s, were used to obtain infrared spectra. In recent decades, a very different method of obtaining an infrared spectrum has superseded the dispersive instrument. Fourier-transform infrared spectrometers are now predominantly used and have improved the acquisition of infrared spectra dramatically. Infrared spectroscopic techniques in combination with chemo-metrics enable the analysis of raw materials without time-consuming sample preparation methods. Fourier transform infrared (FTIR) spectroscopy has been shown to be a promising tool for the analysis of specific sugars, casein and urea. The use of Fourier transform technology in the NIR region has increased spectral reproducibility and wave number precision in comparison to results from other instruments.

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.

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 nano-meters (12821 - 4000  $\text{cm}^{-1}$ ), 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.

The advantages of NIR spectroscopy include speed, simultaneity, non-destructive sample measurement and especially a great potential for on-line analysis. In the case of determination of components in the samples, it is nevertheless necessary to perform an accurate calibration of the NIR spectrometer using an appropriate file of calibration standards of the known composition, using appropriate analytical methods known as reference methods. The main disadvantage of the method is its dependence on reference methods, low sensitivity to minor components, limited transmission of calibrations between various devices in some types of spectrometers and a complicated interpretation of spectral data.

## **MATERIAL AND METHODS**

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 determining 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 Cojocna Farm of USAMV Cluj Napoca from during the period from 2009. The samples from 2009 were used solely for calibration. The samples from 2009 were all from Cojocna Farm of USAMV Cluj Napoca 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-two samples of *Medicago sativa* were determined 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  $\text{cm}^{-1}$  at 16  $\text{cm}^{-1}$  resolution with 2  $\text{cm}^{-1}$  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.

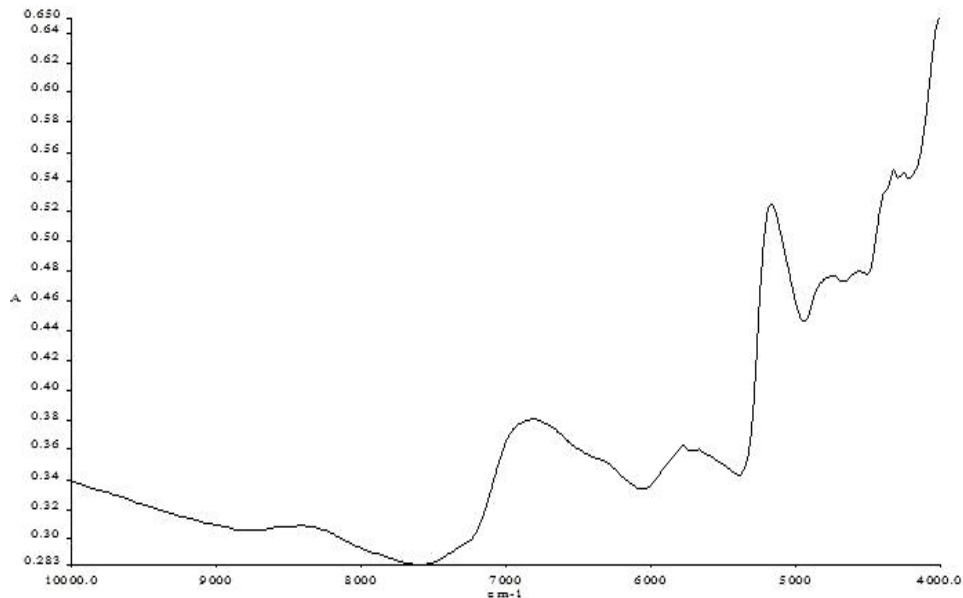


Figure 1 – Typical spectrum of sample

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.

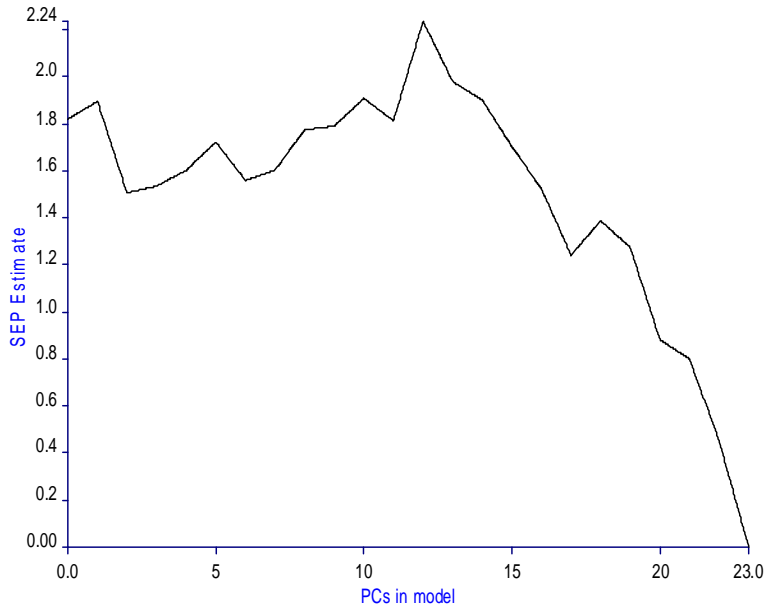


Figure 2 – Evolution of PC 1 with wavelength

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.

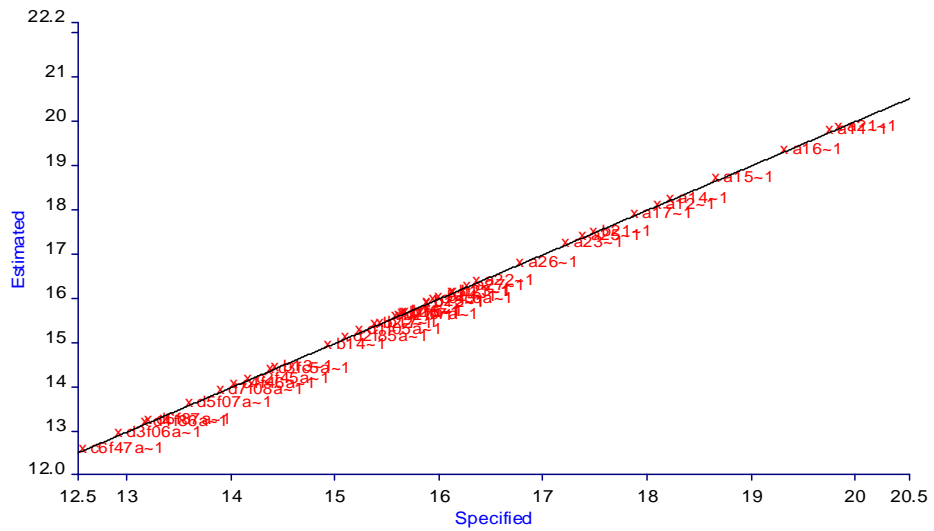


Figure 3 – Estimated vs. Specified plot for crude protein

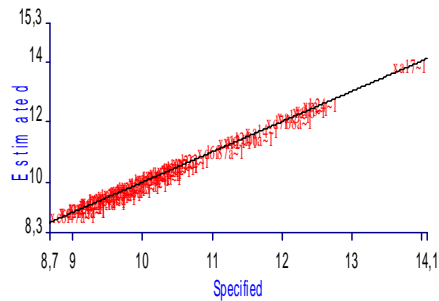


Figure 4 – Estimated vs. Specified plot for crude ash

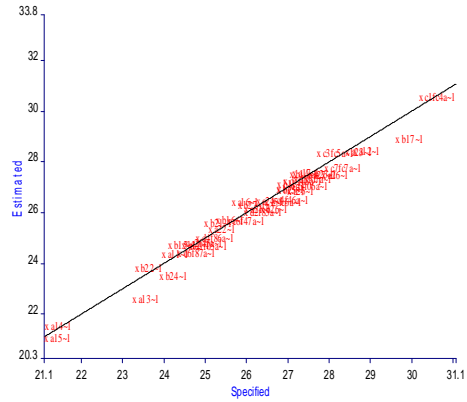


Figure 5 – Estimated vs. Specified plot for crude fat

Figures 3, 4 and 5 are the illustrated plots of Estimated versus Specified values, first for crude protein, second for crude ash and third crude fat.

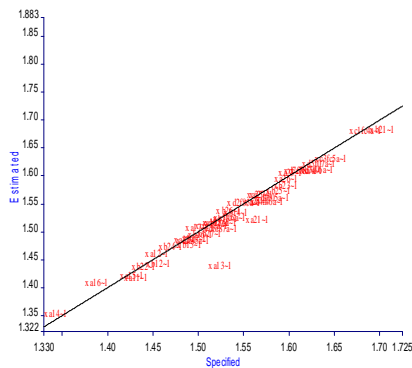


Figure 6 – Estimated vs. Specified plot for crude celluloses

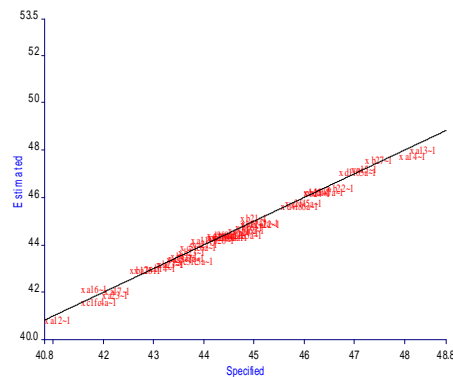


Figure 7 – Estimated vs. Specified plot for non-nitrogenous extractive substances

Figures 6 and 7 are the illustrated plots of Estimated versus Specified values, first for crude celluloses and second for crude non-nitrogenous extractive substances.

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.

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.

## Regression model summaries

Method Name:	NIRS
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

### CONCLUSIONS

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.

The crude protein content determinate both destructive method and non-destructive method was between 12,74 – 16,16%, the crude fat was between 1,41 – 1,59%, the crude ash was between 8,67 – 11,95%, crude celluloses was between 24,07 – 30,19% and the crude non-nitrogenous extractive substances was between 30,72 – 45,67%.

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.

### Acknowledgments

This work is a part of a research project supported by a grant from POSDRU/6/1.5/S/20, România. We thank for ICAR laboratory because a lot of analyses we made there. Thanks are also due to Mogos Alin (SC CROMATEC Plus SRL – Spectroscopy Product Manager – Perkin Elmer) for his help to elucidate the spectral data.

Spectrum Quant+ v4.60 PREDICTION RESULTS PROTEINM

Sample	A11	A21	B27
Normalization	None	None	None
RMS Error	1,876e-004	3,376e-004	5,623e-004
Peak to Peak Error	2,321e-003	3,084e-003	6,024e-003
Total M-Distance	0,122	0,1187	0,1154
Residual Ratio	2,632	11,26	27,79
CRUDE PROTEIN			
Calculated Value	19,71	19,8	15,54
Reference Value	19,70988	19,79659	15,54084
R-error	1,425e-006	1,49e-006	1,594e-006
M-Distance	0,56	0,7037	0,9514
CRUDE ASH			
Calculated Value	9,91	9,133	10,43
Reference Value	11,1730	8,9394	9,9448
R-error	7,246e-007	7,152e-007	7,801e-007
M-Distance	0,2344	0,2023	0,4306
CRUDE FAT			
Calculated Value	1,418	1,471	1,429
Reference Value	1,4184	1,469	1,431
R-error	34,77e-003	35,45e-003	35,07e-003
M-Distance	0,08773	0,1371	0,1195
CRUDE CELLULOSES			
Calculated Value	23,99	26,08	25,84
Reference Value	23,9483	26,0791	25,8391
R-error	0,1266	0,1264	0,1262
M-Distance	0,122	0,1187	0,1154
CRUDE NON-NITROGENOUS EXTRACTIVE SUBSTANCES			
Calculated Value	44,24	43,06	42,37
Reference Value	43,7504	43,0596	41,4401
R-error	0,9192	0,9371	0,9271
M-Distance	0,08773	0,1371	0,1095

### BIBLIOGRAFY

- BALDONI R. și GIARDINI L., (2002), *Coltivazioni Erbacee: Foraggere E Tappeti Erbosi*, Scienza E Tecniche Delle Produz. Vegetali, vol.3.
- BÜNING-PFAUE H. (2003): Analysis of water in food by near infrared spectroscopy. *Food Chem.*, 82, 107–115.
- CARRIER, L., PUJA I., ROTAR, I. (1998). For a better grass production. Ed. Risoprint, Cluj Napoca, p90:149.
- COTTRILL B. R., D.E. BEEVER, A. R.AUSTIN AND D. F.OSBOURN (1982). The effect of protein- and non-protein-nitrogen supplements to maize silage on total amino acid supply in young cattle, The Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 SLR, *Br. J. Nutr.* (1982), 48, 527-541.
- HAALAND, D. M., AND E. V. THOMAS, (1988). Partial least-squares methods for spectral analyses: Relation to other quantitative calibration methods and the extraction of qualitative information. *J. Anal. Chem.* 60:1193–1202.
- HANSEN, P. W. (1998). Urea determination in milk using Fourier transforms infrared spectroscopy and multivariate calibration. *Milk Sci. Intr.* 53:251–255.
- HANSEN, P. W., A. S. VAN BRAKEL, J. GARMAN, AND L. NORGAARD. (1999). Detection of specific sugars in dairy process samples using multivariate curve resolution. *J. Dairy Sci.* 82:1351–1360.
- HEWAVITHARANA, A. K., AND B. VAN BRAKEL. (1997). Fourier transform infrared spectrometric method for the rapid determination of casein in raw milk. *Analyst* 122:701–704.

- LAURA MONICA DALE, IOAN ROTAR, ROXANA VIDICAN, ALIN MOGOS. (2009). Research on protein content of a cob and stalk of maize by ft-nir spectrometry, *Analele Universitatii din Oradea, Fascicula Protectia Mediului*, vol XIV, anul 14, pag:111-119.
- FLORIAN C.V., 2009, Studiul influenței principalelor boli ale lucernei asupra recoltei și calității furajului, Teză de doctorat, USAMV Cluj-Napoca
- MCCLURE, W. F. AND STANFIELD, D. L., 2002, 'Near-infrared Spectroscopy of Biomaterials', in *Handbook of Vibrational Spectroscopy*, Vol. 1, Chalmers, J. M. and Griffiths, P. R. (Eds), Wiley, Chichester, UK, pp. 212-225.
- WILLIAMS, P., 2002, 'Near-infrared Spectroscopy of Cereals', in *Handbook of Vibrational Spectroscopy*, Vol. 5, Chalmers, J. M. and Griffiths, P. R. (Eds), Wiley, Chichester, UK, pp. 3693-3719.
- ROTAR, I. AND L. CARLIER (2005). *Cultura Pajiștilor*. Editura Risoprint, Cluj-Napoca, 321p.
- ROTAR I., LAURA DALE, ROXANA VIDICAN, A. MOGOS, O. CECLAN, 2009, Research on protein content and toral nitrogen and a cob of maize strains by FT-NIR spectrometry, *Bulletin of UASVM Cluj-Napoca, seria Agricultura*, nr. 66(1), pp. 465-467, Print ISSN 1843-5246, Electronic ISSN 1843-5386
- SPECTRUM QUANT+ Tutorial, PerkinElmer Co., (2006).
- VINTU V., A. MOISUC, GH. MOTCĂ, I. ROTAR, 2004 - *Cultura pajiștilor și plantelor furajere*, Ed. Ion Ionescu de la Brad, Iași
- WILLIAMS, P. C., AND K. H. NORRIS. (1987). *Qualitative applications of near-infrared reflectance spectroscopy in Near Infrared Technology in Agricultural and Food Industries*. P. Williams and K. Norris, ed. American Association of Cereal Chemists: St. Paul, USA.