PLS "FTIR - CRUDE FIBER" MODEL FOR FORAGES FROM HILL PERMANENT GRASSLAND

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Abstract

In this study was obtain a FTIR calibration model to predict the crude fiber content of forages harvested in period October 2007 - August 2009 from hill permanent grassland (Grădinari, Caraş-Severin District). The forages samples were purchased in different vegetation stages, considering also that experimental field was organized in ten experimental trials fertilized organic, mineral, and organo-mineral. The floristic composition of forages was determined gravimetrically. From *Poaceae* were present *Festuca rupicola* and *Calamagrostis epigejos*. *Fabaceae* family was represented by *Trifolium repens* and *Lathyrus pratensis*. From other botanical family: *Rosa canina*, *Filipendula vulgaris*, *Galium verum* and *Inula britanica*.
To obtain the calibration model "FTIR-CF" was used the results for this parameter by chemical method and the reflectance values from FTIR spectra, only for the 4 selected ranges. Partial last square (PLS) regression was used to obtain the calibration model, implemented in Panorama program (version 3, LabCognition, 2009). The statistical parameters R^2=0.8167 and RMSEC=2.5315, and the differences between chemical results and predicted values suggest that it is promising to develop FTIR models to predict the crude fiber contents of forages from grassland.

Key words: crude fiber, FTIR, forages, grassland.

The spectrum corresponding of classic infrared domain (MIR – Medium Infrared), in the range from 400 to 4000 cm^{-1}, is considered as representing the spectral fingerprint of any compound (Gergen, 2009). MIR spectroscopy has important applications particularly in determination of molecular structure. More recently are the studies referring to the possibilities of analysis of certain components, especially water (De Leo & Nollet, 2000), organic acids, sugars (Bureau et al., 2009) and polyphenols (Gergen, 2009). When the spectrum is recorded by a spectrometer with an interferometer using Fourier transformation, the spectrometric method is called FTIR (Fourier Transformation of Infrared Spectrum).

The compounds which are predominantly found in plants that are forages consisting will present characteristic absorption in IR spectra in some specified spectral ranges. For the correct identification of these spectral ranges, which determine also the number of principal components of calibration, will depend the model quality.

The spectral domain specific for characteristic bounds of crude fiber are: 1000 - 1300 cm^{-1} (specific for the CO ether bonds vibrations), 1220-1440 cm^{-1} (specific for deformations of alkyl-OH bond vibrations), 3300-3400 cm^{-1} (specific for symmetric and asymmetric stretcher of alkyl- OH bond vibrations); 2840-3000 cm^{-1} (specific for symmetric and asymmetric deformation of C-H bond vibrations). Over these molecular vibration fields may overlay the spectral ranges 1000-1300 cm^{-1} and 2800-3000 cm^{-1}, specific for C-H and CH_2 bonds vibrations from cycloalkyl skeleton of cellulose molecules (Skoog & Leary, 1996). These spectral overlays influence the prediction model, which will be less accurate, suggest for the values of statistical parameters.

Each bond of chemical compounds absorbs typically in infrared, the intensity and frequency of this absorption depending both on the molecular structure, and its concentration in a mixed sample [Brian, 1996]. The characteristic groups for crude fiber are hydroxyl and alkyl ether bonds from cellulose structure (*figure 1*).

Our research regarding the applications of this method aimed to obtain a calibration model for the determination of crude fiber content (%) of harvested forages from the permanent pasture (Grădinari, Caraş-Severin).

MATERIAL AND METHOD

Experimental field

The permanent grassland from Grădinari (Caraş - Severin District) was divided in ten fertilized trials: one unfertilized, three fertilized with organic sheep manure, three exclusive mineral and three organic-mineral.

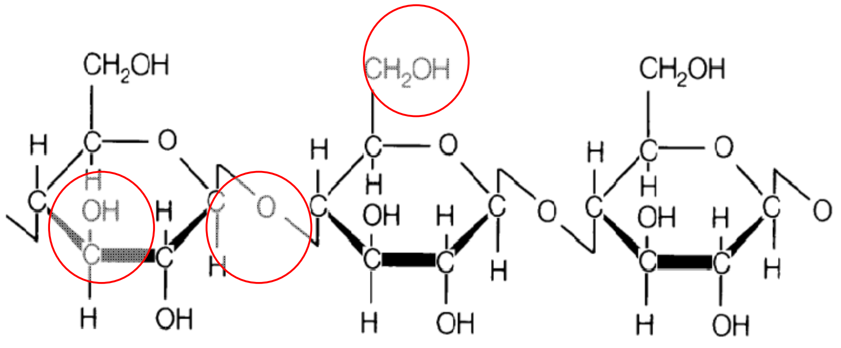

Figure 1 **Structure of cellulose and the characteristic groups active in the IR spectrum**

The trials were organized in randomized plots, in multiple stage blocks with five replications: V1-unfertilized trial, V2-20 t/ha sheep manure, V3-40 t/ha sheep manure, V4-60t/ha sheep manure, V5-20 t/ha sheep manure + $50P_2O_5$(Kg/ha), V6-20 t/ha sheep manure + $50P_2O_5$ (Kg/ha) + 50 K_2O (Kg/ha), V7-20 t/ha sheep manure + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha) + 50N(Kg/ha), V8-100 N (Kg/ha) + 50 P_2O_5 (Kg/ha) + 50 K_2O (Kg/ha), V9-150 N (Kg/ha) + $50P_2O_5$(Kg/ha) + 50 K_2O (Kg/ha), V10 - (100+100)N (Kg/ha) + 50 P_2O_5 (Kg/ha) + $50K_2O$(Kg/ha).

The annual average temperature in this region was around 10.4^{o}C and the soil of permanent grassland was Calcic Luvisol.

Samples

The "FTIR-CF" (FTIR–crude fiber) calibration model was performed with the samples harvested in period October 2007 – August 2009, in different vegetal stages.

The floristic composition of forages samples was determined gravimetrically. The forages from the trials fertilized with fermented sheep manure were characterized by high percents of *Trifolium repens* (dominant) and *Lathyrus pratensis*. In samples harvested from trials fertilized exclusive mineral were dominant species from *Poaceae* familly (*Festuca rupicola*), followed by *Calamagrostis epigejos*. The forages from organic - mineral fertilized trials were divided generally in two groups: one formed with GP5 and GP6, with a floristic composition closely to organic fertilisation, and the second by GP7 related with forages from exclusive mineral fertilisation. From other botanical family were present *Rosa canina*, *Filipendula vulgaris*, *Galium verum* and *Inula britanica*.

After harvesting the plants were dried at 60^{o}C with air circulation. The grounded was the next step in conditioning processes.

Fertilizers

It was used mineral (15:15:15 NPK complex, ammonium nitrate, superphosphat, potassium salt) and organic fertilizers (fermented sheep manure) over the period 2003-2008. The mineral fertilizers were applied yearly and fermented sheep manure at each two years.

Chemical determination of crude fiber

Chemical data for crude fiber contents were obtained using indications of JAOAC 962.09/1990 method, when the samples are sequentially refluxed in dilute base followed by dilute acid.

FTIR calibration model

The "FTIR-CF" model was performed using PLS (Partial Last Square) regression, implemented in Panorama software (Variant 3, LabCognition, 2009). For input data were used the chemical results for crude fiber and the values of reflectance from 190 FTIR spectra scanned with V670 Spectrophotometer instrument by Abble-Jasco. The FTIR scan (Figure 2) was made in the range 500 – 4000cm^{-1} in triplicate for all the grounded dried samples. The validation of "FTIR-CF" calibration model was made using control samples harvested from the same experimental trials in August 2009.

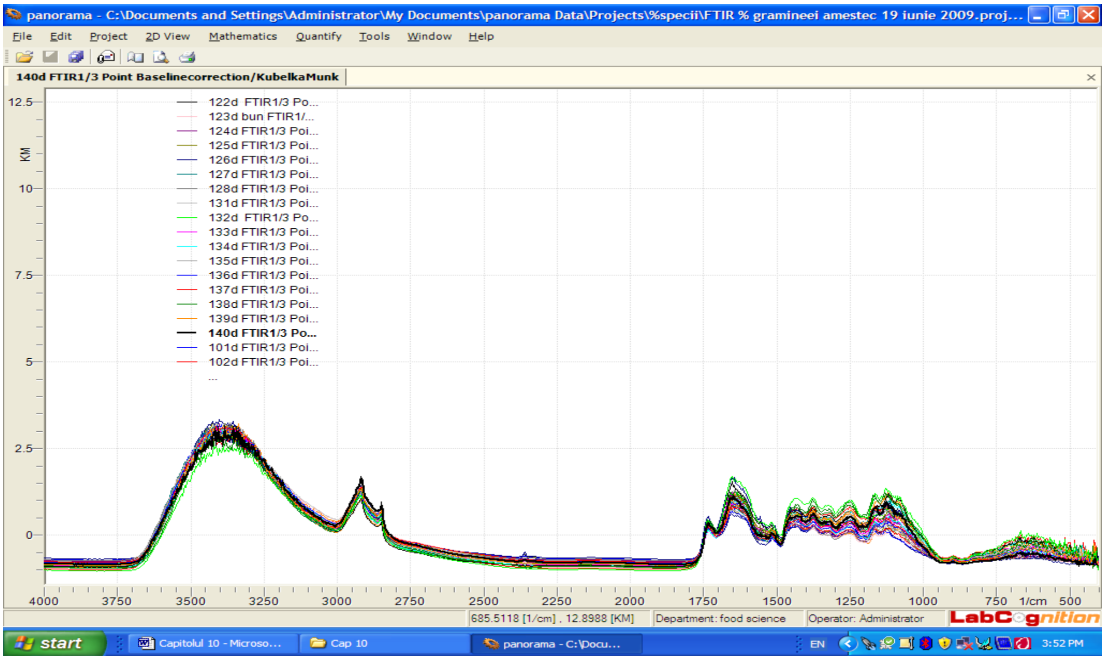

Figure 2. **Overlay of FTIR spectra of some forages from studied permanent grassland**

RESULTS AND DISCUSIONS

The optimum spectral ranges selected to perform the „FTIR-CF” model are presented in Table 1. With these spectral data, using modelling software Panorama (version 3, LabCognition 2009), was performed the prediction model for crude fiber by PLS. Statistical parameters of „FTIR-CF” model are given in Table 2, and the correlations between the values predicted with model and those determinated chemically for this parameter are in Figure 3.

Table 1

Calibration data for the „FTIR-CF” model with 4 spectral ranges

Selected spectral ranges	Wavelengths
[1008.8042 .. 1462.0910]	236
[1541.1751 .. 1726.3476]	97
[2715.8630 .. 2982.0485]	139
[3221.2296 .. 3576.1435]	185

Figure 3 **Prediction of crude fiber by „FTIR-CF” model with 4 spectral ranges**

Table 2

Statistical parameters for „FTIR-CF" model with 4 spectral ranges

R^2	0.8167
RMSEC	2.5315
SD	4.7184
„FTIR-CF" model -FTIR-crude fiber model	

Referring to the model prediction of crude fiber "FTIR-CF", it is possible to affirm that the statistical parameters are R^2 = 0.8167, RMSEC = 2.5315 and standard deviation = 4.7184, witch reflect the medium quality of it.

The predicted results for the 12 control samples of forages harvested from the permanent grassland in August 2009 are shown in *table 3*.The differences between chemical results and those predicted by FTIR calibration model were situated in the range 0.240 - 2.391%. These results encourage the continuation of beginning studies using a high number of forages samples, to characterize better the entire concentration values of this qualitative parameter of forages from permanent grassland.

Table 3

The results of crude fiber (%) prediction for the control samples forages (August 2009) by „FTIR-CF" calibration model with 4 spectral ranges

Sample's name	Crude fiber (%)		
	Real (chemical method)	Predicted (FTIR model)	Differences between Real - Predicted
201d	28.240	25.849	2.391
202d	25.990	27.306	-1.316
205d	27.590	28.506	-0.916
206d	28.730	27.729	1.001
207d1	31.100	29.863	1.237
207d2	31.100	30.512	0.588
208d1	31.100	31.340	-0.240
208d2	31.100	29.802	1.298
209d1	27.200	25.929	1.271
209d2	27.200	26.043	1.157
210d1	28.380	28.650	-0.270
210d2	28.380	30.334	-1.954

This high number of samples to perform a good calibration model to predict the crude fiber by FTIR spectrometry can be explain by the complexity of compounds which form crude fiber of forages based on the diversity of plants species witch formed the complex matrix of floristically composition of studied permanent grassland.

CONCLUSIONS

This PLS regression model "FTIR-CF" with 4 selected spectral ranges is promising to be used with success to determine routinely qualitative and quantitetivelly this parameter for the forages samples harvested from the studied permanent grassland. But this will be possible after the introduction of a high number of samples, to characterize better the complexity of chemical compounds which form crude fiber of forages, the mirror of the diversity of plants species which contribute to the floristically composition.

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