RESEARCH ON PROTEIN CONTENT OF A COB AND STALK OF MAIZE BY FT-NIR SPECTROMETRY

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Abstract
Using Near Infrared Spectrometry has become lately quite a developed technique in the different physic-chemical parameters of the feed as a procedure is extremely elegant and precise use. This paper aims to highlight a way of direct analysis method undestructive protein using near infrared spectrometry in conjunction with reflected attenuated total. Tests were conducted on samples of maize cob and maize stalks from The Research - Agricultural Development Turda. Samples were done at each variant separately. Each sample was subjected to destructive method calculations: for determination of protein we use Kjeldahl method, and then the spectra were collected with NIR spectrum. After we built mathematical models for both the cob and stalks, based on these techniques and multivariate analysis allows the determination of an error prediction for the best protein 0.5%, we validate the method with samples from different years (2006 and 2007).

Key words: NIR, protein, non-destructive methods, feed, maize.

INTRODUCTION

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.

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.

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.

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 its widespread use and acceptance among scientists as a valid analytical technique.

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 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 maize cob and maize stalks were obtained from The Research – Agricultural Development Turda from during the period from 2005. The samples from 2005 were used solely for calibration. The samples from 2005 were all from The Research – Agricultural Development Turda 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 determination of protein. Using these values for spectra we build a mathematical model for direct determination of these chemical properties of the samples. For this calculation Spectrum Quant+ v4.60 is used. The multivariate (multiple wavelength) calibration techniques (e.g., principal components analysis or partial least squares) are often employed to extract the desired chemical information. Careful development of a set of calibration samples and application of multivariate calibration techniques is essential for near infrared analytical methods.

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.

RESULTS AND DISCUSSION

Forty three different samples of maize cob and maize stalks were supplied and measured with no additional milling or grinding (no processing of spectra). Spectra were recorded by filling a standard sample cup with the sample and scanning in interleaved mode. This mode of operation alternately takes a background spectrum as well as the rationed spectrum which minimizes changes in atmospheric effects.

Eight 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 8 cm^{-1} resolution with 2 cm^{-1} step. 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 maize cob and maize stalks from these spectra.

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

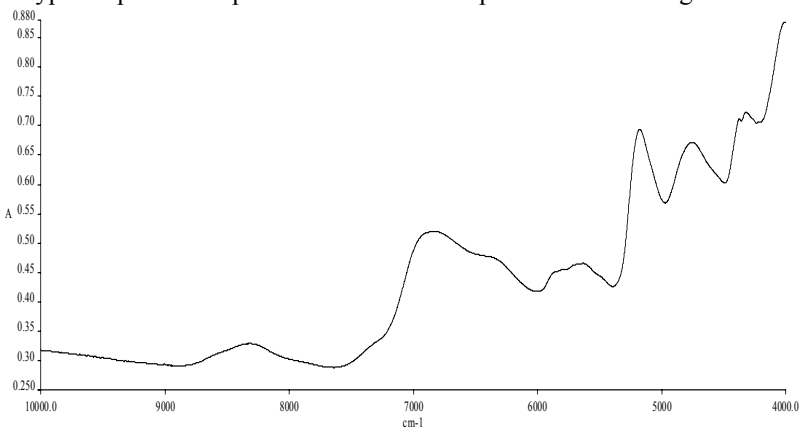

Fig 1 – Typical spectrum of sample

A partial least squares analysis (PLS) was performed on the data (43 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.

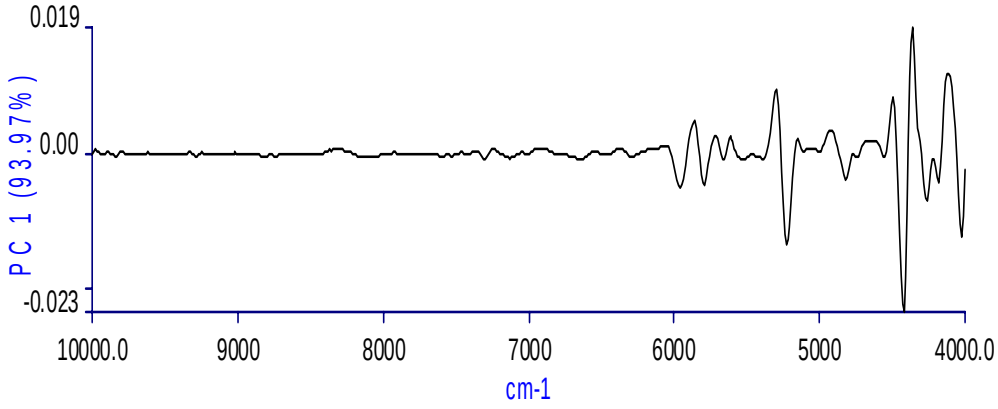

Fig. 2 – Evolution of PC 1 with wavelength

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.

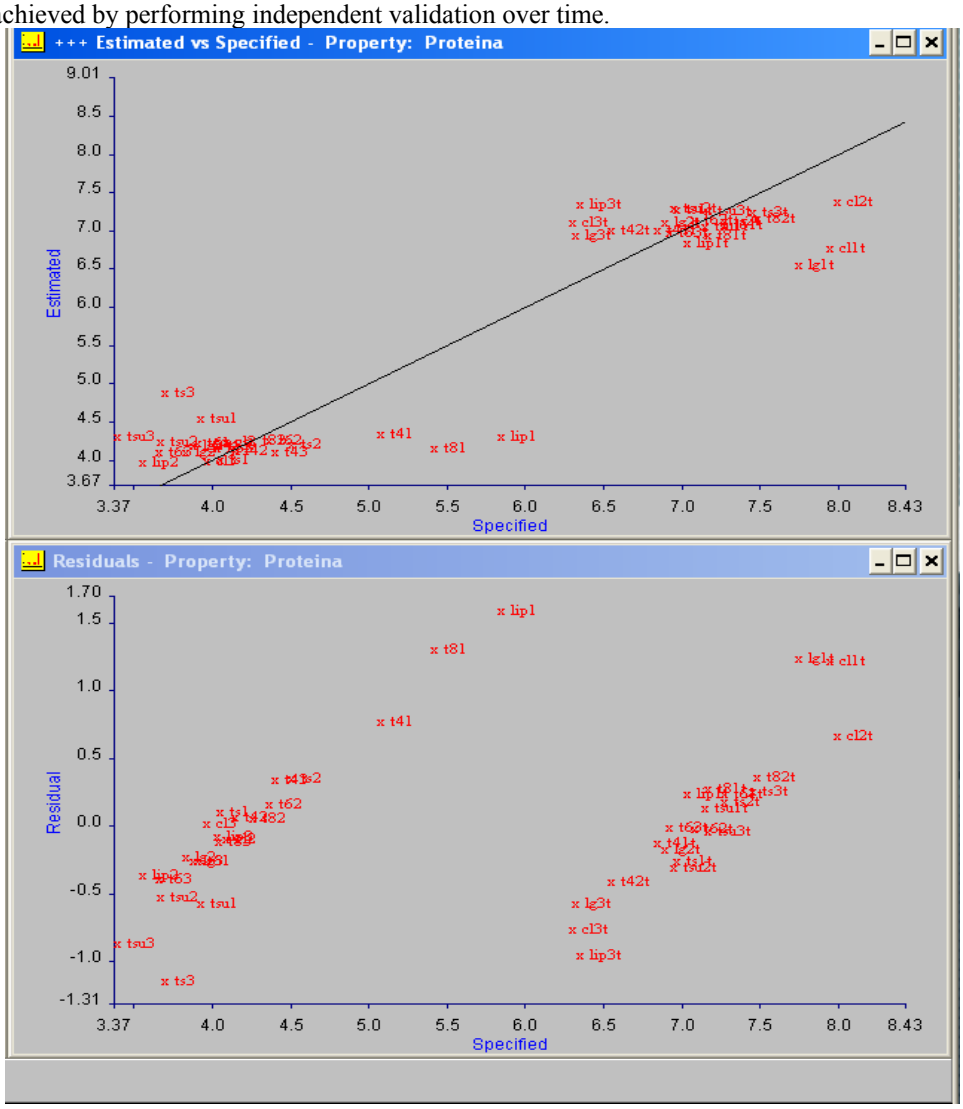

Fig 3 – Estimated vs. Specified and Residual vs. Specified plot for protein

Figure 3 is the illustrated plots of Estimated versus Specified values, first for protein. This provides an adequate starting point for the calibration model.

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

Table 1

Regression model summaries

Method Name:	pls2-1
Ident:	Spectrum QUANT+ v4.51
No. of properties:	2
No. of standards:	43
Calibrated:	Yes
Calculation Parameters:	
Algorithm:	PLS2
Range:	10000 to 4000 cm-1
Interval:	2 cm-1
Analysis Type:	Original Units
Scaling (Spectra):	Mean
Scaling (Property):	Mean
Smooth:	None
Baseline correction:	Derivative
Order:	2
Normalization:	SNV (with de-trending)
Ordinate threshold:	
Upper threshold:	1.5 log(1/R)
Lower threshold:	None
Number of factors:	
Minimum:	1
Maximum:	100
Blank regions:	None

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 PLS2

Sample	Name	Protein	Normalizat	RMS Error	P-P Error	Total M-Dist	Residual Ratio
1	**811TD1R1V1**	7.410	0.06733	1.02E-05	7.33E-05	4.3010	14.020
2	**811TD1R1V2**	7.693	0.06533	8.90E-06	5.28E-05	4.7350	11.420
3	**811TD1R1V3**	7.165	0.08009	9.88E-06	7.01E-05	3.2030	9.362
4	**811TD1R1V4**	7.551	0.06421	8.77E-06	7.92E-05	1.8390	11.480
5	**811TD1R1V5**	7.801	0.06693	9.94E-06	8.06E-05	3.5980	13.570
6	**811TD1R1V6**	6.989	0.07722	1.17E-05	9.34E-05	1.7780	14.080
7	**811TD1R1V7**	7.289	0.07105	8.05E-06	5.49E-05	1.8250	7.900
8	**811TD1R1V8**	7.493	0.069	9.47E-06	6.21E-05	3.7240	11.600
49	**812TD2R1V1**	6.577	0.08152	8.25E-06	6.18E-05	1.006	6.305
50	**812TD2R1V2**	5.881	0.08948	8.72E-06	8.47E-05	1.035	5.843
51	**812TD2R1V3**	6.501	0.0799	8.84E-06	7.70E-05	1.699	7.537
52	**812TD2R1V4**	6.185	0.08267	8.10E-06	5.97E-05	1.429	5.909
53	**812TD2R1V5**	6.596	0.07359	7.93E-06	6.62E-05	2.65	7.138
54	**812TD2R1V6**	6.722	0.08054	7.13E-06	5.10E-05	0.9853	4.818
55	**812TD2R1V7**	6.757	0.07528	7.93E-06	5.44E-05	2.053	6.827
56	**812TD2R1V8**	6.844	0.07459	8.00E-06	6.32E-05	1.552	7.079
97	**811TD1R1V1**	6.043	0.07464	8.45E-06	7.18E-05	2.83	7.883
98	**811TD1R1V2**	6.757	0.07505	8.73E-06	6.17E-05	3.039	8.317
99	**811TD1R1V3**	6.317	0.06996	8.72E-06	6.34E-05	2.792	9.553
100	**811TD1R1V4**	6.229	0.07061	9.14E-06	8.30E-05	3.188	10.320
101	**811TD1R1V5**	6.200	0.07168	9.43E-06	7.07E-05	2.668	10.640
102	**811TD1R1V6**	6.610	0.07712	9.20E-06	6.26E-05	2.484	8.764
103	**811TD1R1V7**	6.757	0.07505	8.73E-06	6.17E-05	3.039	8.317
104	**811TD1R1V8**	6.876	0.07474	9.33E-06	6.60E-05	3.755	9.580
145	**812TD2R1V1**	6.061	0.0776	9.81E-06	7.62E-05	3.08	9.838
146	**812TD2R1V2**	6.450	0.06836	7.85E-06	5.45E-05	2.478	8.118
147	**812TD2R1V3**	6.444	0.07583	9.03E-06	6.09E-05	3.114	8.731
148	**812TD2R1V4**	6.215	0.06995	9.85E-06	9.71E-05	1.927	12.200
149	**812TD2R1V5**	6.673	0.07773	8.59E-06	6.02E-05	3.328	7.516
150	**812TD2R1V6**	6.518	0.0669	9.76E-06	6.08E-05	3.894	13.090
151	**812TD2R1V7**	6.699	0.06968	9.97E-06	7.39E-05	3.799	12.590
152	**812TD2R1V8**	6.797	0.07683	9.93E-06	8.55E-05	2.946	10.270

The samples we named after the hybrid type, sow 811 means type of early hybrid and 812 means type of mid-early hybrid, ”S” means cob wax stage and ”T” means stalk in cob’s wax stage, D means density and from early hybrids: V1 means the variety Turda Super, V2 means the variety Turda 165, V3 means the variety Turda 145, V4 means the variety Turda SU – 182, V5 means the variety Turda Mold 188, V6 means the variety LG 2244, V7 means the variety Clarica and V8 means the variety Lipessa. From mid-early hybrids we have: V1 how means the variety Saturn, V2 means the variety Turda Star, V3 means the variety Turda Favorit, V4 means the variety Turda 201, V5 means the variety Minerva, V6 means the variety LG 2305, V7 means the variety Ribera and V8 means the variety Sandrina.

From 1 to 96 we present samples from 2006 and from 97-192 we present samples from 2007.

CONCLUSIONS

The example detailed here illustrates that it is possible to determine a number of properties present in ground wheat 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 in ground maize with very good standard error of prediction. This proves that FT-NIR spectroscopy is an extremely reliable, non-destructive and rapid technique for the quantity of many chemical and physical properties.

The results obtained in this work demonstrate that several compositional fractions of forage from different types of maize can be accurately predicted by NIRS on fresh plant material. Fiber optics technology, on the other hand, shows some potential, but results are not acceptable so far.

The dates are predicted with Spectrum Quant+ v4.60 software, which aloud as to build a mathematical model for direct determination of these chemical properties. The samples are cob and maize strains which together forms maize silo. The protein content is between 4,058-7,912 %. After Giardini and Baldoni (2002) the protein content for maize silo must be between 4,5-5,5%.

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