

ORIGINAL RESEARCH PAPER

**PREDICTION OF THE ASH CONTENT OF WHEAT FLOURS USING
SPECTRAL AND CHEMOMETRIC METHODS**

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Fourier transform infrared (FTIR) spectroscopy has been considered an important method in evaluating structural properties of biomolecules. The assignment of the spectra indicated combination bands of the chemical bonds (O–H, C–H, C–C, etc.) that compose the chemical constituents of biomass. This paper presents, for several regional common wheat varieties (*Triticum aestivum* L.): Crina, Dropia, Flamura, Gruia, Haiduc and wheat from Vaslui area, the comparative analyses of the infrared absorption spectra for two spectral ranges: near-infrared (NIR) region, in the corresponding wavelength of 700-1000 nm, and middle-infrared (MIR) region, in the corresponding wavenumber 600-1600 cm^{-1} . The positions of the characteristic absorption peaks were observed at 877.49 and 941.03 nm in NIR region and 756.50, 857.86, 929.75, 999.83, 1076.34, 1150.08, 1244.92, 1336.95, 1415.46 and 1538.35 cm^{-1} in MIR region. The information obtained from the analysis of NIR-MIR spectra is used to rapidly determine some of physico-chemical parameters of the wheat species-specific part. The aim of this study is to apply FT-NIR and FT-MIR spectroscopy to correlate those signals by the StatCorr analyze. A fast technique such as chemometric analysis on the basis of NIR and MIR spectra was used to predict the amount of ash from the flour sample. R square of prediction model is 0.7 and Root Mean Square Error of Prediction (RMSEP) using two Principal Component PC_s is 1.5 % which certifies the calibration model.

Keywords: wheat, stretching, bending, ash, chemometric

Introduction

The necessary raw material milling and baking industry technological characteristics of wheat, like humidity, moisture content, hectolitre weight, protein content, wet gluten content, gluten-index index strain and index fall, are determined by physical and chemical analysis. These characterize the wheat quality as raw material for bread wheat and assess the overall quality of the annual harvest.

The analyses involved in the determination of these parameters which require standard procedure are expensive, time consuming and not especially accurate, thus there is the concern to develop alternative methods to be applied (Cocchi *et al.*, 2005). For this reason, a fast technique such as NIR and MIR infrared spectra acquired on the wheat flour samples may be a useful choice.

Many factors affect the variability of bread wheat flour, including genetic variability, cultivation practices and environmental effects. All this variability renewable and their interactions significantly influence the quality of wheat flour. Also, the modifications of food protein by microbial and enzymatic inactivation have received considerable attention (Ahmed *et al.*, 2007). So it is necessary to know at any time the physico-chemical properties of each type of flour.

Usually the ash contents were evaluated by using standard methods (Collin *et al.*, 2007; Kamil *et al.*, 2011).

NIR-IR and MIR-IR spectroscopic techniques detect both physical and chemical differences between samples. The region from about 1500 to 500 cm^{-1} contains a series of absorptions due to the bending vibrations within the molecule. In this domain, called the fingerprint region (Sun, 2009), each different compound produces a different pattern of vibrations. Unlike mid-infrared spectra, the peaks in NIR do not assign as “fingerprints” for certain bonds or certain compounds. The NIR signal is a cumulative effect of the chemical bonds at the specific wavelength. In consequence, the entire NIR scanned region was used to distinguish bread wheat samples of different quality. Therefore, the NIR and MIR infrared spectroscopy, alongside the rheology study, has been considered important in evaluating the structural changes of biomolecules (Liu *et al.*, 2010; Aït Kaddour *et al.*, 2008).

The chemometric analysing techniques are able to distinguish more easily between closely related compounds. A review on multivariate calibration has been written by Brereton (2000) (Brereton, 2000). The samples matrices for calibration model are extremely important. Chemometric methods such as correlation and regression are essential, and therefore it is vital that the calibration samples cover all possible sources of variation that can be found in the predicted samples (<http://www.camo.com>, Brereton, 2007).

One hundred samples of wheat flour were used in Ferrãoa and Davanzo study for the determination of both the protein and the ash contents of wheat flours by using spectroscopic data in the mid-infrared region obtained with a horizontal attenuated total reflectance (HATR) accessory. The partial least square (PLS) regression analyses to construct the calibration model, were made by using the PLS method of the Turbo Quant Analyst v1.1, a software from Nicolet. Different types of calibration were tested using raw spectra, first derivative spectra, second derivative spectra and those corrected by multiplicative signal correction (MSC).

The different calibration models for protein and ash content was obtained with the following pretreatments data: mean centered (MC) values (R^2 :0.847; RMSEC 0.388), with auto-scaling (mean centered and variance scaled (VS) methods were applied) (R^2 :0.863; RMSEC (%): 0.367) and with pretreated only by variance scaling (VS) (R^2 :0.153; RMSEC (%): 1.577) (Ferrãoa and Davanzo, 2005).

Near infrared spectroscopy region (4000 - 12000 cm⁻¹) was used in predicting simultaneously the total ash and acid-insoluble ash contents of 72 *Prunellae Spica* samples which were obtained from ten production regions in China. Calibration models based on PLS were accurate for the prediction of total ash ($R^2 = 0.914$; RMSEP = 0.373), and acid-insoluble ash ($R^2 = 0.905$; RMSEP = 0.452) was employed for the data process, including spectra pretreatment using OPUS (Optics User Software) version 5.0 from Bruker (Yulan and Bingren, 2009).

Trafela *et al.* (2007) report a multianalytical non-destructive approach to historical paper analysis using FT-IR and NIR with chemometric data evaluation. For calibration, partial least-squares analysis (PLS) from Spectrum Quant software (Perkin-Elmer, Waltham, MA) was used. A model was built by using different data pretreatments and different wavenumber intervals for optimal correlations of ash content. The results obtained are: optimal wavenumber intervals (4000-6500 cm⁻¹), number of samples 144, SEP (standard error of prediction, the average error between the measured and the estimated property values of samples) is 0.81 and R (the Correlation Coefficient of validation model) is 0.9741 (Trafela *et al.*, 2007).

In our study, the analysis of the molecular components using the vibrational spectroscopy is presented. The correlation between the absorption peaks values corresponding to the most NIR and MIR representative region was made in order to identify where the strongest connections are. This way it provides complementary information from a simple NIR infrared scanning.

A multivariate calibration procedure, such as multi linear wavelength is used to find the coefficients by least squares regression. In this context, our research work is aimed at evaluating the possibility to build a calibration model able to perform the prediction of the different ash wheat flours, only on the basis of the fingerprint MIR spectra. This can lead to a rapid and accurate method for ascertaining the ash amount.

Materials and methods

Wheat samples

In our study, the species of investigated wheat flour are: Crina, Drop, Flamura, Gruia, Haider and wheat from Vaslui area. DROPIA is a type created at the Institute of Agricultural Research and Development (I.C.D.A) Fundulea and approved in 1993. It is resistant through winter, drop resistant, middle resistant to red robin. Due to these characteristics, the flour has a good baking quality, being one of the most favoured cereals in this region.

CRINA is a type created at I.C.D.A Fundulea and approved in 2001. Its characteristics are: drop resistant, middle resistant to red robin and the baking qualities are good and very good. Flamura 85 type was created at I.C.D.A Fundulea and approved in 1989. Its physiological features are: drop resistant kind of cereal, resistant through winter and drought conditions and the baking quality is very good. GRUIA presents a good resistance to winter and the quality characteristics are close to a the Drovia variety, which tends to have more tenacious gluten.

Triticale varieties (Haiduc) have a good production performance, because they are less demanding on the ground and climate compared to the genotypes of wheat (Leonte *et al.*, 2007, <http://www.agricultor.ro>).

Ash content measuring

The minerals in wheat grain are located mainly in its peripheral layers. The flours of high extraction containing the grain envelope part have higher ash content than flour from low extraction by the endosperm which is poorer in mineral substances. The value of ash content, in both wheat and especially in the flour is a basic quality index, differentiating the extraction flour types. In our study the ash content was obtained according to STAS 90-1988 (Bordei *et al.*, 2007).

NIR spectroscopy

NIR Omega G Analyser is a tool designed for precise and accurate measurements of parameters of whole grain cereal grains (corn, soybeans, rice, wheat and oats). FT-NIR spectroscopy is a fast and efficient solution for non-destructive analysis of heterogeneous and granular samples which can be analyzed without the reagents or sample preparation in advance. Applications for raw material identification and quantitative analysis of the product can be performed quickly.

In our study, the different wheat samples were analyzed with the workhorse of Bruins Instruments NIR grain analyzers Omega Analyzer G™. The spectral transmission range is between 730 to 1100 nm and the scan increment is 5nm. All wheat samples were stored at 24°C room temperature. The environmental parameters were monitored with a weather station.

MIR spectroscopy

The FTIR transmission spectra were recorded on a Tensor 27, Bruker FTIR spectrometer. The measurement was carried out at 22°C in triplicates. A total of 32 scans were recorded at 8 cm⁻¹ resolution. All spectra were acquired in absorbance mode using Opus 6.0 Bruker software. MIR spectra of the different wheat flour type were recorded using an ATR unit (Attenuated Total Reflectance), a powerful and versatile tool in qualitatively and quantitatively investigating chemical processes. The advantage of infrared analysis in the middle region is that it takes only a few seconds.

Statistical correlation and regression

For quantitative analysis of ash content of unknown flour was used the Unscrambler X software (Camo, Norway). Only raw spectra without different data pretreatments were used as data for building the calibration models. We focused on absorption influence at particular wavenumbers for a good statistical correlations quality. Because of that, at this preliminary study, only a small number of samples was used. These models were developed using corresponding absorption peak values assigned at 877.49 and 941.03 nm wavelengths in NIR region for the six standard samples (Crina, Dropia, Flamura, Gruia, Haiduc and wheat from Vaslui area) and at 756.50, 857.86, 929.75, 999.83, 1076.34, 1150.08, 1244.92, 1336.95,

1415.46 and 1538.35 cm^{-1} wavenumber of MIR region for the same samples. The fingerprint spectral range identified between 600 to 1500 cm^{-1} is used for calibration and prediction processes.

All the regression analyses presented in this paper to predict the ash amount in the unknown flour samples were made by using the PLS method of the Unscrambler X software and this PLS models were optimized by cross-validation. The variables are separated into two matrices denominated X (formed by the ATR signals at different wavenumbers and the NIR signals at different wavelengths), and Y (formed by the percentage of ash for each sample). The accuracy of the prediction equations was given by squared coefficient of determination in prediction (R^2) and Root Mean Square Error of Prediction (RMSEP). The Statistical Correlation component (StatCorr) from the Unscrambler X software is used to highlight the relation between NIR and MIR spectral properties. The used Correlation method allows us to determine which chemical bonds, obtained from NIR spectra, most influence the characteristics of wheat.

Results and discussion

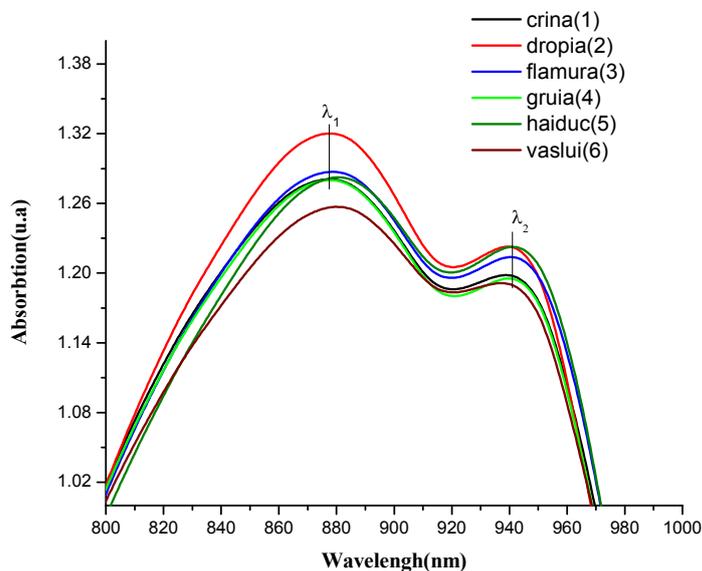
Description of NIR and MIR infrared spectra

Major food components are generally complex molecules resulting from the polymerization of monomers which exhibit specific chemical groups such as carboxylic and amine functions in amino acids. First it is necessary to identify the characteristic absorption bands of these groups in the near- and mid-infrared regions (Sun, 2009).

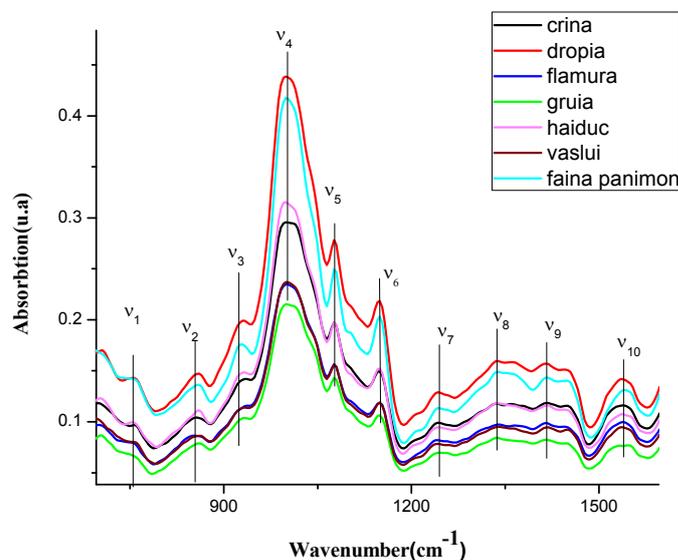
The NIR spectra and the MIR spectra after smoothing correction obtained by Savitzky Golay method with two polynomial orders are presented in Figure 1A and B respectively. The main constituents can be identified on the NIR and MIR spectra by strong and distinctive absorption bands.

The identification and chemical assignment of the different NIR bands for biological samples have already been proposed (Aït Kaddour *et al.*, 2007; Alava *et al.*, 2001; Olewnik *et al.*, 2004; Paradkar *et al.*, 2002). From the NIR spectra, it is difficult to extract directly useful information because of the low signal-to-noise ratio, large baseline changes from one spectrum to another one and broad absorption peaks (Aït Kaddour *et al.* 2008), so the MIR spectra will be analyzed to correlate with the NIR spectra for complementary information.

The principal MIR bands and the assigning were summarized in Table 1. Furthermore, the most relevant allocations will be briefly described.



(A) $\lambda_1 = 877.49 \text{ nm}$ $\lambda_2 = 941.03 \text{ nm}$



(B) $v_1=756.50 \text{ cm}^{-1}$; $v_2=857.86 \text{ cm}^{-1}$; $v_3=929.75 \text{ cm}^{-1}$; $v_4=999.83 \text{ cm}^{-1}$; $v_5= 1076.34 \text{ cm}^{-1}$; $v_6=1150.08 \text{ cm}^{-1}$; $v_7=1244.92 \text{ cm}^{-1}$; $v_8=1336.95 \text{ cm}^{-1}$; $v_9=1415.46 \text{ cm}^{-1}$; $v_{10}=1538.35 \text{ cm}^{-1}$

Figure 1. NIR spectra for Crina, Dropia, Flamura, Gruia, Haiduc and wheat from Vaslui area. **(A)** The main absorption bands are assigned to 877.49 and 941.03 nm wavelength. **(B)** The main absorption bands are assigned to 756.50, 857.86, 929.75, 999.83, 1076.34, 1150.08, 1244.92, 1336.95, 1415.46 and 1538.35 cm^{-1} wavenumber.

Table 1. Suggested MIR spectral absorption band assignments of wheat flour samples

No.	Wavenumber (cm-1)	Vibration	*Assignment	References
v ₁	756.50	δ (COH), δ (CCH) δ (OCH)	Isolated Carbohydrates	Sun, 2009
v ₂	857.86	δ (COH), δ (CCH) δ (OCH)	Isolated Carbohydrates	Sun, 2009
v ₃	929.75	δ (COH), δ (CCH) δ (OCH)	Isolated polysaccharides (Cellulose)	Kačuráková et al., 2000 ; Sun, 2009
v ₄	999.83	δ (COH) ν (C-O)	Polysaccharides Starch Oligosaccharide (mannose, galactose)	Movasaghia et al., 2008, Carbonaro et al., 2008
v ₅	1076.34	ν (C-O) ν (C-C)	Polysaccharides Starch	Henczov et al., 2008 Carbonaro et al., 2008
v ₆	1150.08	ν (C-O) ν(C-C)	Polysaccharides Starch	Kaddour et al. 2008; Movasaghia et al., 2008 Ait Kaddour et al., 2008
v ₇	1244.92	δ(N-H) ν(C-H) Amide III	Protein	Ait Kaddour et al., 2008
v ₈	1336.95	δ(CH)	Phospholipids	Movasaghia et al., 2008
v ₉	1415.46	δ (C-H) δ (N-H) ν (C-H)	Phospholipids	Movasaghia et al., 2008
v ₁₀	1538.35	β-sheet of Amide II	Protein	J. Ahmed et al. 2009 Zanyar Movasaghia et al.,2008

*ν—stretching vibration; δ—deformation (bending);

The observed vibrational bands can be attributed to the following modes: the most intense band at 999.83 cm⁻¹ is aimed to identifying its components through the corresponding peak frequencies and also to determining the corresponding relative intensities, which are characteristic of the carbohydrates (Movasaghia *et al.*, 2008; Carbonaro *et al.*, 2008); the relative intense absorption band around 1076 cm-1 can be attributed to C–O and C–C stretching and are assignment to polysaccharides region (Carbonaro *et al.*, 2008). The MIR bands at 1150 cm⁻¹ are associated with the coupled C–O and C–C stretching vibrations that can be principally assigned to carbohydrates molecules like starch.

The structural repeat unit of proteins gives three major characteristic midinfrared bands, named amide I, II, and III.

In our spectral domain study, only amide II and III are present. The amide III region (1350– 1200 cm⁻¹) of the mid-infrared corresponds to N-H in-plane bending

(40–60%) coupled with C-N stretching (18–40%) molecular vibrations (Seabourn *et al.*, 2008); the amide III spectral region, usually neglected in MIR studies due to its relative weakness, is very useful because the characteristic water bend is not present (Anderle and Mendelsohn, 1987; Cai and Singh, 2004) (Aït Kaddour *et al.*, 2008); the band around 1420 cm^{-1} in the region of C–H bending modes and perhaps coming from the deformational vibrations of the CH_2 functional groups (Bhattacharjee *et al.*, 2008).

It should be noticed that the main MIR investigated area (750 to 1450 cm^{-1}) is the characteristic of main energy source of wheat flour: various forms of carbohydrates (sugars, fibres, and starches). The region from 1450 to 1550 cm^{-1} is attributed to lipids compounds.

Correlation analyses

The proportion of each NIR absorbance possibly depends on all other MIR absorbances, so there may be some correlation between them (Brereton, 2003). The statistical STATCORR analysis by the Unscrambler X software method has been used to identify correlations between the MIR and NIR spectral regions. In Table 2 are presented the parameter CorrVal correlations coefficients between NIR and MIR absorbance (A) obtained.

Table 2. Correlations coefficients between NIR absorbance [A (880.75 nm); A (941.03 nm)] and MIR absorbance [A (756.50); A (857.86); A (929.75), A (999.83), A (1076.34), A (1150.08), A (1244.92), A (1336.95), A (1415.46) and A (1538.35) cm^{-1}]

A NIR (nm)	A(880.75)	A(941.03)
A(756.50)	0.8218896	0.7187595
A(857.86)	0.809162	0.7184651
A(929.75)	0.8110234	0.6942608
A(999.83)	0.8165486	0.6874613
A(1076.34)	0.8291296	0.6714125
A(1150.08)	0.8229736	0.6631619
A(1244.92)	0.8090265	0.6620842
A(1336.95)	0.8036662	0.6805224
A(1415.46)	0.814926	0.6746333
A(1538.35)	0.7517601	0.6234276

Note that between specific absorption bands from MIR region (750 - 1450 cm^{-1}) corresponding to carbohydrate compounds there is a close correlation with the absorption from NIR region at 880.75 nm . For 941.03 nm (NIR region), the degree of correlation decreases.

Multivariate model

A partial least square regression (PLS) including the absorbance corresponding to mainly FTIR pick results in a multivariate prediction correlation of 0.83 (Figure 2)

and a root mean square error of cross correlation is 0.15% (Figure 6). Regression models using ten wavelengths in the 750–1550 nm range provide us with lower prediction errors.

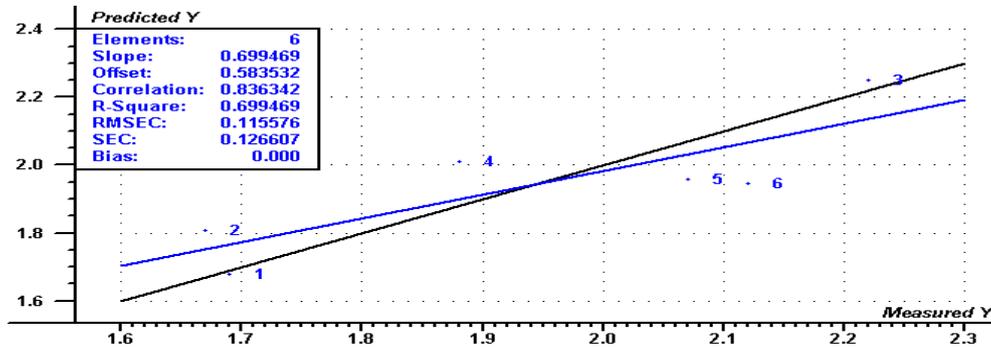


Figure 2. PLS1 (Leverage Correction) Regression overview; The statistical elements (correlation, R2, RMSEC) are illustrate on figure

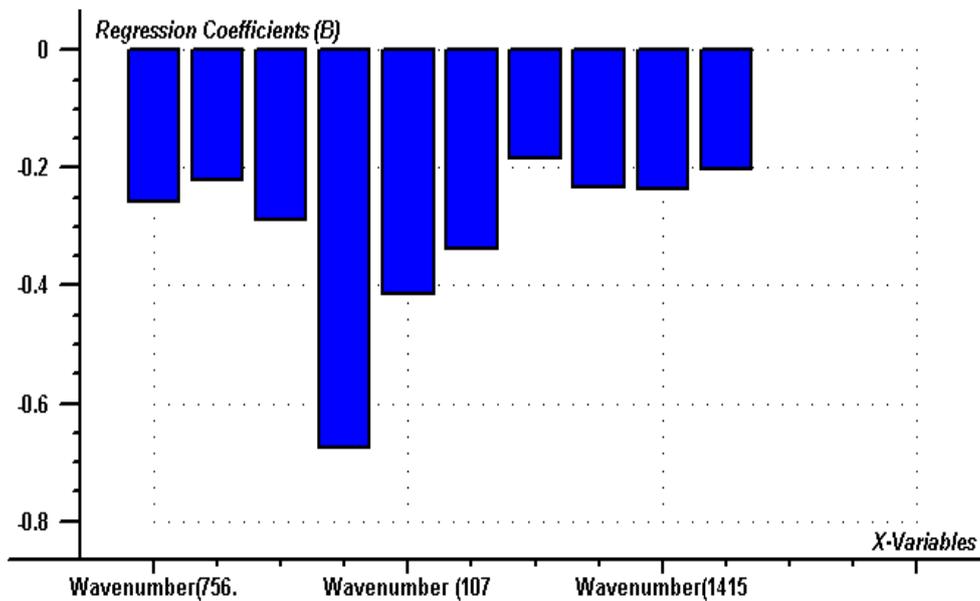


Figure 3. Plot of the Regression Coefficients (B)

For every absorption band, the regression coefficient B is obtained (Figure 3). The B coefficients enable us to write the model equation relating the amount of ash to the each absorbance band:

$$\text{Amount of ash} = B_0 + B_1 * A(756.50) + B_2 * A(857.86) + \dots$$

The purpose of making a regression model is most of the time to be able to predict the response value of new samples that are measured in the future, in our case wheat flour sample named Panimon.

In Figure 4, the predicted values of ash content for Crina, Dropia, Flamura, Gruia, Haiduc and wheat from Vaslui area are represented in Figure 5 the predicted value of ash content for Panimon wheat flour is shown too.

In Table 3, the numeric values of ash content predicted for Crina, Dropia, Flamura, Gruia, Haiduc, wheat from Vaslui area and Panimon wheat with standard deviations are presented.

The purpose of making a regression model is most of the time to be able to predict the response value of new samples that are measured in the future.

The evaluation of calibration and the validation of the prediction model is made by root mean square error value. Usually more samples and all wavenumbers from spectral infrared region were used to obtain the highest performance of regression models. In our case the RMSEP value is 1.5% (Figure 6). This result is probably due to the analysis of a lower number of samples. However the results are encouraging if we consider the spectral analyses for establishing the optimum wavenumber region involved in ash content prediction, which is smaller than all wavenumber infrared spectra, but more accurate.

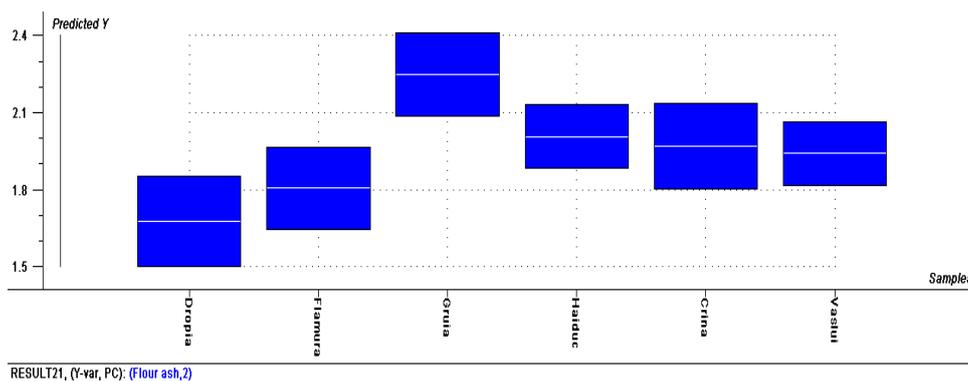


Figure 4. Predicted values of ash content for Crina, Dropia, Flamura, Gruia, Haiduc and wheat from Vaslui area

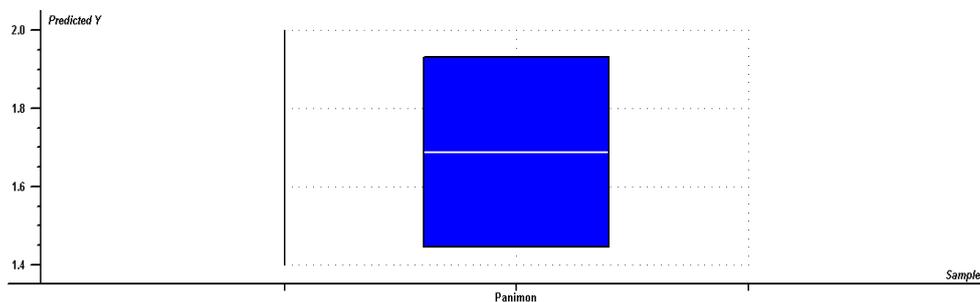
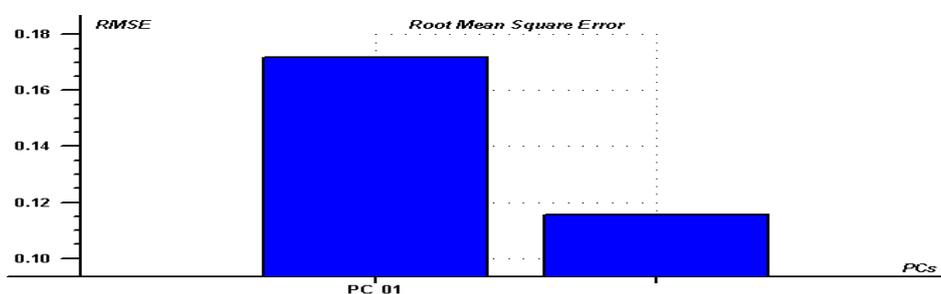


Figure 5. Predicted value of ash content for Panimon wheat

Table 3. Numeric values of ash content predicted for Crina, Drop, Flamura, Gruia, Haider, wheat from Vaslui area and Panimon wheat

Sample	Predicted ash	Deviation
Dropia	1.679	0.177
Flamura	1.809	0.162
Gruia	2.250	0.165
Haiduc	2.009	0.125
Crina	1.958	0.169
Vaslui	1.945	0.125
Panimon	1.688	0.244

**Figure 6.** Root mean square error for calibration model after two Principal Component PCs

Conclusions

Chemometrics methods were applied in the current study to extract information from the spectra for the total ash by analyzing the influence of absorption at particular wavenumbers of the wheat flour samples.

The analysis of the MIR spectra demonstrated that absorption bands can be mostly assigned to starch (1150.08, 1076.34, and 999.83 cm^{-1}), proteins (1538.35, 1244.92 cm^{-1}) and lipids (1415.46, and 1336.95 cm^{-1}).

The NIR band located at 880.75 nm seems to be highly correlated with the MIR band at 1076.34 and 1150.08 cm^{-1} . This implies that the NIR band at 880.75 nm can be mostly assigned to C–O and C–C stretching vibrations assigned to starch compound.

Based on the ash content in flours can be achieved the classification per quality class. Chemometric results showed that MIR absorbance was suited to determine ash content non destructively and quickly in wheat flour samples.

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