

## INFLUENCE OF GROWING CONDITIONS AND TECHNOLOGICAL PROCESSING ON PROPERTIES OF FLOURS ASSESSED BY SPECTROSCOPIC METHODS.

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## SUMMARY

Complex analysis of the influence of growing and technological processing conditions on selected properties of group of commercial samples of Slovak and Hungarian wheat, spelt and rye flours prepared following organically and conventional production practices was performed. Solid flour samples were analyzed to ash and dry matter content as well as to minerals and trace elements content. Extracts of flours in 50% ethanol were treated by EPR and UV-VIS spectroscopy to determine the total polyphenols and flavonoids content as well as some other characteristics of antioxidant properties, including ABTS<sup>•+</sup> and DPPH tests. Multivariate statistical analysis was subsequently applied on the whole dataset of experimental characteristics in order to assess the influence of way of production (organic vs. conventional) and technological processing (grinding) on sample properties. Besides that, the possibility of flours differentiation according to the previously mentioned characteristics as well as their origin was tested. By means of canonical discrimination analysis, flour samples were with > 95% correctness discriminated according to their country of origin. Taking into account the way of production, organically grown samples were with ~ 92% discrimination score differentiated from that produced by conventional production practices. Classification of samples according to the way of technological processing (grinding) reached ~ 96% and according to varietal composition, 100% correct classification. As the most significant experimental characteristics for the purposes of flour samples differentiation, minerals and trace elements content, as well as flavonoids content and radical-scavenging activity of extracts were recognized. The prediction ability of the statistical models was in all cases > 92%, in case of varietal composition, even 100%.

## INTRODUCTION

Besides the traditional role of cereals as a source of nutrients, in the recent years, their growing utilization in so called "functional foods" has been noticed, utilizing either whole cereals or some their components. The conventional definition of functional foods is that it is a food where a new ingredient (or more of an existing ingredient) has been added and the new so-prepared product has a new function, frequently related e.g., to health-promotion or disease prevention. Functional foods must have the character of foods and their positive impact will be reflected already in the consumption quantity corresponding to normal food. They are not pills or other form of medication or supplements<sup>1</sup>. One type of cereals with high potential in functional foods preparation represents spelt (*Triticum spelta*, L.) – a hexaploid species of wheat, being in the past an important staple in parts of Europe. Nowadays, it has again found a growing importance in market as a health food or a component of health beneficial foods, due to its agronomic, compositional and medical characteristics. This cereal has similar chemical composition as common wheat but its content of nutritionally important compounds is higher. According to some previously published analytical data, it contains about 57.9% carbohydrates (excluding 9.2% fibre), 17.0% protein and 3.0 % fat, as well as dietary minerals and vitamins<sup>2</sup>. Due to its moderate amount of gluten, it is suitable for baking purposes. Spelt is most frequently available in several forms (products) e.g., as coarse pale bread, biscuits, crackers or as spelt pasta. Besides that, grain of spelt is used in brewing industry and/or as feeding<sup>3-5</sup>. Comparison of some characteristics of wheat, spelt and rye whole meal flours is presented in Table 1<sup>6,7</sup>.

**Table I.** Comparison of selected averaged characteristics of wheat, spelt and rye whole meal flours.

<i>Parameter</i>	<i>Wheat</i> <sup>6</sup>	<i>Spelt</i> <sup>7</sup>	<i>Rye</i> <sup>6</sup>
<i>Proteins (g/100g)</i>	11.55	12.8	11.27
<i>Saccharides (g/100g)</i>	71.03	62	71.76
<i>Dietary fiber (g/100g)</i>	9.1	11.4	13.3
<i>Minerals (as ash, g/100g)</i>	1.62	1.8	1.72
<i>Riboflavin (mg/100g)</i>	0.11	0.15	0.14

For the preparation of functional foods containing cereals or its components, it is necessary to consider several factors. Although the content of the main components in the grain changes within the respective variety only statistically, significant effect on the chemical composition of grain could have soil composition, climatic and agro-technical conditions, and last but not least, the technological procedures/processes used in subsequent post-harvesting treatment<sup>8</sup>. All these factors can significantly affect the properties of grains/flours and the content of potentially interesting constituents, predetermining thus their use in functional foods production. In this context, procedures for the isolation of individual components of interest from cereal are of great importance, as well. Therefore, it is necessary to deal in details with the problems of the isolation of functional components from flour and aspects

of their stability. Besides that, it is also important to deal with the effects of isolation/extraction conditions on the content of functional components (e.g., polyphenols, flavonoids, organic acids, ...).

This contribution, as a part of a complex study, focused on potential of some cereals to serve as functional foods components themselves, or after their additivation / doping by mixing with some other components of natural origin with known antioxidant potential and health beneficial effects (e.g., anthocyanins, flour or medical herbs extracts). As an input information for further processing, the influence of the origin & growing condition (organic vs. conventional farming practices), technological processing (degree of grinding) as well as varietal composition on selected properties of group of 24 commercial wheat, spelt and rye commercial flour samples of Slovak and Hungarian origin was performed. The basic characteristics of solid samples, i.e., dry matter and ash content, but also minerals and trace elements content was evaluated. Besides that, antioxidant properties of flours extracts were assessed by means of UV-VIS and EPR spectroscopy, involving several assays. Amino acids profile was determined by HPLC-MS system. Results obtained were correlated via simple correlations with total polyphenols and flavonoids content. Besides that, the multivariate statistical analysis was used to evaluate the influence of origin, varietal composition, way of production and technological processing on sample properties. In addition, the possibilities of some flour properties (ash, dry matter content) prediction from spectral characteristics, was tested.

## EXPERIMENTAL

### Samples

For the purposes of this study, 24 commercial samples of Slovak and Hungarian organic and conventional smooth, semi-smooth, rough and whole meal wheat, spelt and rye flours were selected. Detailed description of samples is given in Table II.

**Table II.** Basic characteristics of the samples of Slovak and Hungarian organic (O) and conventional (C) flours under study.

ID	Sample characterisation	Country of origin*	Way of production**	ID	Sample characterisation	Country of origin*	Way of production**
F1	Wheat, whole meal, smooth	SK	O	F13	Rye, whole meal, smooth	SK	C
F2	Wheat, whole meal	HU	O	F14	Spelt, whole meal, smooth	SK	O
F3	Wheat, whole meal, stone mill	HU	O	F15	Spelt, whole meal	HU	O
F4	Wheat, smooth	HU	O	F16	Spelt, whole meal, stone mill	HU	O
F5	Wheat, whole meal, smooth	SK	C	F17	Spelt, smooth	HU	O
F6	Wheat, semi-smooth	SK	C	F18	Spelt, whole meal, smooth	SK	C
F7	Wheat, rough	SK	C	F19	Spelt, smooth	SK	O
F8	Wheat, smooth	SK	C	F20	Spelt, whole meal, rough	SK	O
F9	Rye, whole meal, smooth	SK	O	F21	Spelt, smooth	HU	O
F10	Rye, whole meal	HU	O	F22	Spelt, whole meal	HU	O
F11	Rye, whole meal, stone mill	HU	O	F23	Spelt, whole meal	HU	O
F12	Rye, smooth	HU	O	F24	Spelt, semi-smooth	HU	O

\* HU – Hungary, SK- Slovak Republic; \*\*O-organic, C-conventional

### Basic characteristics of solid flours

Solid flour samples were evaluated to dry matter and ash content using the standard AOAC methods<sup>9,10</sup>. Minerals and trace elements content (Ca, Cu, Fe, K, Mg, Na and Zn) was determined using the atomic absorption spectrometry – AAS. For these purposes, Perkin Elmer 4100 (Perkin Elmer, USA) spectrometer, equipped with a deuterium lamp background-correction system was employed, using an air/acetylene flame and the flame-ionization detector<sup>11</sup>. Besides that, total nitrogen content was determined using the routine Kjeldahl method<sup>12</sup>.

### Extracts preparation

For EPR and UV-VIS experiments, exactly 50 ml of 50% ethanol/water solution (v/v) was poured over 2.5 g of respective flour sample and the extraction was performed at ambient temperature. The mixture was shaken on laboratory shaker (Innova 2000, USA) for 1h at 150 rpm. Subsequently, the supernatant was separated from the solid matter using the laboratory ultracentrifuge (SciQuip, UK) at 9200 rpm at ambient temperature during 10 min. The so prepared extracts were stored at ambient temperature in darkness between the experiments. For HPLC analysis, 1g of flour sample was mixed with 20 ml of acetic acid solution in water (0.1 M) and the aliquot of internal standard d3-Glu (50 µl) was added. The mixture was shaken at 150 rpm for 30 min in laboratory shaker at ambient temperature and centrifuged at 10 000 rpm for 10 min at the temperature of 0 °C. After the filtration, the supernatant (200 µl) was mixed with 100 µl of isotopically labelled internal standard and 800 µl 0.1% solution of acetic acid (in water, v/v) and used in analysis.

### HPLC-MS, UV-VIS and EPR spectral characteristics

Amino acids (AA) profile and their quantification was performed by HPLC-MS-MS with positive electrospray ionization using an Agilent 6410 Triple Quad detector (Agilent Technologies, Palo Alto, USA) coupled to a HPLC system Agilent 1200 series (Agilent Technologies, Palo Alto, USA) consisting of a binary pump, a vacuum degasser, a autosampler, and a thermostated column

compartment. The analytical separation was performed on an a Purospher® STAR RP-8ec (150 mm x 4.6 mm, 3 µm) (Merck, Darmstadt, Germany) using an isocratic mixture of 100 ml acetonitrile and 500 ml of water solution of perfluorooctanoic acid (PFOA) (0.05 mM) at the flow-rate of 0.5 ml/min at temperature of 25 °C. Free individual amino acids was quantified using linear calibration curve established with standard solution using the L-amino acids kit containing the 98% purity standards of 20 amino acids (Sigma – Aldrich). As an internal standards, a lyophilised mixture of isotopically labelled L-amino acids (ChromSystems, München, Germany) and standard of d3 -glutamic acid (d3-Glu, 97%, Cambridge Isotope Laboratories, Andover, USA) were used. Quantification was performed by comparison of the peak area ratio of selected amino acids with relevant internal standard monitored using the MRM transitions. The following instrumental (ion source) parameters were used for amino acids analysis: drying gas (N<sub>2</sub>) flow, 8 L.min<sup>-1</sup>; gas temperature, 320 °C; nebulizer pressure 50 psi; capillary voltage, 3.0 kV; fragmentor voltage, (50 – 100) V (depending on analyte); collision energy, (2 – 30) eV (depending on analyte); dwell time, 50 ms. All the samples were analysed in two parallel repetitions. The determined concentrations of 21 free amino acids were counted together and the result was presented as total amino acids content.

UV-VIS experiments were carried out with both, extracts and solid samples, using a UV-VIS-NIR spectrophotometer Shimadzu 3600 with accessory. The experiments with solid samples were performed in attenuated reflectance mode using the Large Integrating Sphere Assembly LISR 3100 (Shimadzu, Kyoto, Japan) employing quartz cell enabling reflection measurements from the surface of defined upper layer of flours samples. The reflectance spectrum of the respective flour sample was recorded in triplicates in the spectral range from 200 to 2500 nm and averaged for use in the data analysis. Flours reflectance spectra visualisation and comparison was performed using the spectroscopic data evaluation environment of Panorama 3.1 (Labcognition GmbH & Co. KG) enabling the multivariate transformation of spectra by the method of principal component factoring (PCF). Before the PCF all the spectra were smoothed by the Savitzky-Golay method to remove baseline shifts and superimposed peaks and normalised by data scaling and centering techniques. Radical-scavenging activity of solid samples was characterized by UV-VIS, employing the modified Quencher method<sup>13</sup>. The content of total polyphenols (TPC) expressed as Gallic acid equivalents<sup>14</sup> and of flavonoids (TFC) expressed as Rutine equivalents was determined in the extracts. Besides that, radical-scavenging activities of extracts were evaluated using the solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) cation-radical (ABTS<sup>+</sup>), respectively<sup>15</sup>. All the experiments were performed in duplicates.

The entire EPR experiments were performed in duplicates, using a portable X-band EPR spectrometer e-scan (Bruker Biospin, GmbH, Karlsruhe, Germany) with accessory. The ability of flour extracts to terminate DPPH and ABTS<sup>+</sup> radicals was evaluated. The ABTS<sup>+</sup>/DPPH radical-scavenging activities were expressed as Trolox equivalents (TEAC<sub>ABTS<sup>+</sup>/DPPH</sub>)<sup>14</sup>.

Multivariate statistical methods were used to distinguish the samples according to selected characteristic, i.e., origin, way of production, varietal composition, way of technological processing, employing methods of principal component analysis, principal component factoring, canonical, and k<sup>th</sup> neighbour discriminant analysis and classification. These calculation were performed by means of Unistat<sup>®</sup>6.1 (Unistat, London, United Kingdom) statistical software, taking into consideration all the experimental data obtained from HPLC-MS, AAS, UV-VIS and EPR experiments. The recognizability of discriminant model was determined as the percentage of the correctly classified samples in the training data set. The prediction ability was tested, as the percentage of the samples correctly classified in the leave-multiple-out cross-validation approach<sup>16</sup>.

## RESULTS AND DISCUSSION

As the basic parameters of the studied flour samples, ash and dry matter content were determined. Results obtained indicated, that there is not a clear difference between the samples following neither from their origin, way of production, nor varietal composition, as the results within the common groups are statistically distributed.

**Table III.** Averaged results (mean± SD, n=2) of some characteristics of solid flour samples and their extracts in 50 % EtOH (v/v).

ID	Ash [%]	Dry matter [%]	Total nitrogen content [%]	Total AA content [mg.kg <sup>-1</sup> ]	Quencher [mg.kg <sup>-1</sup> ]	TPC [mg.kg <sup>-1</sup> ]	TFC [mg.kg <sup>-1</sup> ]
F1	1.9±0.0	89.2±0.0	1.8±0.1	919.3±0.9	11.3±1.0	1433.2±16.6	310.3±43.2
F2	1.6±0.0	89.2±0.0	2.3±0.1	1473.6±5.3	12.7±2.3	1734.7±70.4	163.8±2.5
F3	1.9±0.4	89.4±0.1	2.3±0.1	1448.1±3.5	11.8±1.4	1719.2±55.6	185.8±3.8
F4	0.8±0.0	89.0±0.0	2.3±0.1	989.0±6.5	10.8±2.0	1548.2±51.2	560.9±19.3
F5	1.9±0.0	88.7±0.1	1.9±0.1	1273.1±8.5	14.2±1.7	1580.6±29.5	127.5±1.8
F6	0.6±0.4	88.5±0.1	1.6±0.1	503.0±6.7	8.3±3.1	1492.7±3.7	441.5±9.6
F7	0.4±0.0	89.4±0.0	1.7±0.1	482.4±8.0	8.6±1.7	1273.5±55.2	294.7±15.7
F8	0.5±0.0	88.5±0.0	1.7±0.1	526.9±2.9	10.9±2.4	1330.0±39.4	182.5±6.1
F9	1.6±0.0	89.4±0.1	1.3±0.1	2173.9±1.3	11.0±1.3	1376.3±3.4	432.9±6.6
F10	1.7±0.0	89.5±0.3	1.6±0.1	2488.8±12.8	14.6±0.2	1395.6±15.6	558.5±4.4
F11	1.9±0.0	89.0±0.1	1.2±0.1	1736.2±28.5	12.9±0.9	1677.5±71.2	480.9±15.9
F12	0.7±0.0	88.2±0.8	0.8±0.0	1168.9±7.5	10.2±1.2	833.1±12.9	417.0±4.3
F13	1.6±0.0	89.2±0.0	1.3±0.1	1733.9±3.2	13.2±1.6	1328.3±81.3	770.5±12.1
F14	2.1±0.0	89.7±0.1	2.3±0.1	1495.8±30.7	12.4±2.0	1825.8±221.4	271.8±37.8
F15	2.2±0.0	89.8±0.0	2.6±0.1	1488.1±5.9	13.9±3.1	1964.9±427.5	214.8±25.7

**Table III (cont).** Averaged results (mean± SD, n=2) of some characteristics of solid flour samples and their extracts in 50 % EtOH (v/v).

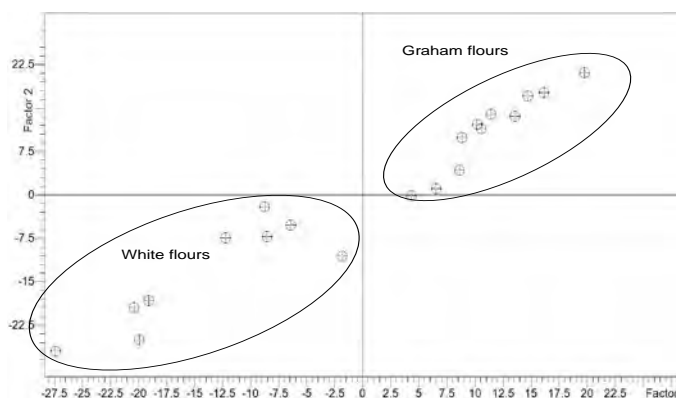
ID	Ash [%]	Dry matter [%]	Total nitrogen content [%]	Total AA content [mg.kg <sup>-1</sup> ]	Quencher [mg.kg <sup>-1</sup> ]	TPC [mg.kg <sup>-1</sup> ]	TFC [mg.kg <sup>-1</sup> ]
F16	2.1±0.0	89.8±0.0	2.6±0.1	1428.9±18.2	12.0±3.2	1569.8±3.5	182.7±0.6
F17	1.0±0.0	89.3±0.0	2.4±0.1	818.6±2.1	9.5±3.4	1392.3±0.8	114.9±13.4
F18	2.5±0.0	90.3±0.0	2.2±0.1	1521.9±19.4	12.1±3.3	1411.5±74.6	274.9±6.9
F19	1.1±0.0	89.4±0.0	2.3±0.1	1002.2±6.6	13.0±3.2	1390.9±44.4	143.5±14.8
F20	1.4±0.0	89.8±0.0	2.1±0.1	1263.5±38.9	9.6±2.0	1433.0±54.6	129.8±4.5
F21	0.8±0.0	89.7±0.0	2.1±0.1	771.0±6.6	10.8±0.1	1339.3±64.5	187.8±6.7
F22	2.1±0.0	90.1±0.1	2.2±0.1	1379.1±17.3	12.8±1.2	1728.2±28.6	181.2±13.1
F23	1.1±0.0	88.4±0.1	1.5±0.1	810.1±7.9	10.2±1.1	1276.8±26.5	143.5±3.4
F24	1.1±0.0	89.0±0.3	1.3±0.1	722.0±0.2	9.8±0.6	1110.1±57.8	95.0±6.1

Regarding the total nitrogen content, it is obvious that samples from Hungary reveal in average its slightly higher contents than the Slovak ones, exception for some case, where practically either none or only statistically negligible differences were found. There is also not clear relationship between the nitrogen content and the varietal composition of flours, the same for the way of production. For the later mentioned, with respect to presupposed much more intensive fertilization in conventional farming, one could expect at least slightly higher content of nitrogen, but this expectation was not supported by the results. Results presented in Table III also indicate that there exist some relationship between the amino acids content and the way of sample processing (grinding), which is most probably the result of modified extraction ability of amino acids from respective flours in dependence on the degree of grain micronization. Practically the same, unambiguous trends and relationships were obtained also for other evaluated parameters of flours and their extracts, as was also observed for the results of Quencher assay, content of polyphenols or flavonoids.

Correlation matrix of all determined characteristics (data not presented) indicates moderate correlations of majority of determined characteristics, revealing R<sup>2</sup> values within the range of <0.4-0.6>; however, in some cases, strong correlations were confirmed e.g. between the total amino acids content and TEAC values evaluated both from UV-VIS and EPR measurements with ABTS<sup>•+</sup> and DPPH radicals.

However, considering the results of all methods and assays used, it can be concluded, that any single analytical method or their combination and/or simple mutual correlation do not offer an unambiguous answer on the topics of the similarities/differences between the samples from their origin, way of production, processing or varietal composition points of view. Thus, multivariate statistical analysis was employed to find the similarities and differences within the group of samples of common characteristics and between the individual groups and to perform the differentiation and classification of individual flour samples.

By the method of principal component factoring (PCF), the whole UV-VIS-NIR reflection spectra were processed, separately for respective spectral regions (UV, Visible and NIR). While the spectra from UV and NIR regions do not offer clear differentiation of samples into subgroups by any of chosen criteria (origin, way of production, varietal composition, way of grinding & processing), this is not the case of VIS-spectra (part of complex reflection spectra recorded in the spectral range 330-780 nm). As is clearly indicated on Fig. 1, from the UV-VIS-NIR reflection measurements of flours the data obtained in the visible spectral range, it is possible to differentiate the wheat flours according to way of grinding. From practical reasons, samples were classified only into two sub-groups – one for whole meal flours (labelled as graham) and the 2<sup>nd</sup> for the remaining samples (refer to Table II for further details).



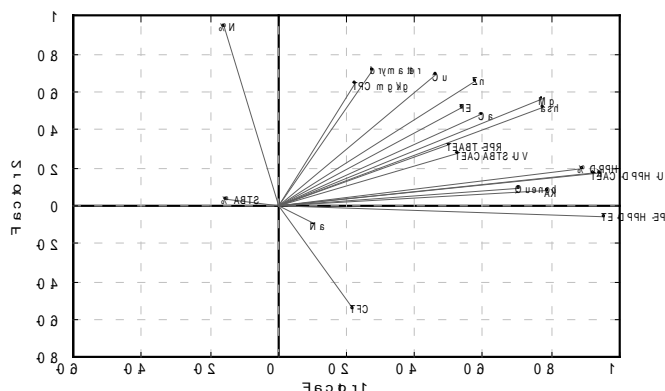
**Figure 1.** Wheat, rye and spelt flours spectral reflection data projection by principal component factoring (each point represents a reflection spectrum of sample in VIS spectral region). For the differentiation purposes, flour samples were classified into two sub-groups as graham (whole meal) and white (the rest).

Very promising results offered the processing of the entire group of experimental characteristics by the methods of principal component analysis, canonical discrimination analysis and  $k^{\text{th}}$  nearest neighbour classification. Principle component analysis was able to explain in all studied cases the variability of the dataset by 78% and 83% of the variability of the dataset (taking into consideration the cumulative % of variance of first 3 and 4 principal components, respectively). Eigenvalues indicates that in the differentiation by PCA, very important role have the total nitrogen content, content of amino acids and total polyphenols. The other characteristics contributed to principal components construction by various, but usually lower significance.

**Table IV.** Results of canonical discrimination analysis,  $k^{\text{th}}$  nearest neighbour classification and prediction tests by means of CDA, of flour samples under study and their extracts according to various discrimination parameters. The whole dataset of experimental characteristics was taken for the discrimination and classification. Results are expressed as % of correctly classified samples.

Method		Origin [%]	Way of farming [%]	Varietal composition [%]	Technological processing (grinding) [%]
Canonical discrimination		95.8	91.7	100	95.8
$K^{\text{th}}$ nearest neighbour	$k=1$	100	100	100	100
	$k=2$	95.8	100	100	95.8
Prediction ability by CDA		92.9	97.7	100	97.7

CDA, in dependence on the selected discrimination criterion, possessed very high recognition scores, reflecting the successfulness of the discrimination of the samples into respective groups on the basis of their experimental characteristics, as clearly indicated in Table IV. The lowest recognition was obtained in classification of samples according the way of production (farming), in which 3 organically and 1 conventionally produced sample were misclassified. As regards the importance of individual characteristics for the discrimination, it should be noted here that it vary with the selected criterion, however, important role of individual minerals and trace elements, followed by TPC/TFC and last but not least, individual characteristics of antioxidant properties. Similar results give also processing of the data by the principal component factoring in varimax rotation. As follows from the plot of factors depicted on Fig. 2, 4 vectors are lying in different sectors of the plot than those of rest of the group, corresponding to the content of nitrogen, total flavonoid content, as well as sodium content and ABTS<sup>•+</sup> radical-scavenging ability assessed by UV-VIS.



**Figure 2.** Plot of factors (Varimax rotation) indicating the importance of individual experimental characteristics of flour samples and their extracts for the purposes of samples differentiation according to chosen criteria.

Prediction ability is one of the most important characteristics of the classification model/procedure, reflecting the ability of the statistical model to correctly classify the unknown samples (samples of unknown affiliation), just on the basis of comparison of its characteristics with those of already classified samples. For the purposes of this study, the prediction ability was tested by CDA in leave-multiple-out approach, assigning in turn up to 20% of the samples as unknown. Percentage of correctly predicted samples reached in each cases high value, as indicated in Table II, the lowest in prediction of sample origin and the highest in the prediction of sample varietal composition.

## CONCLUSION

The spectral characteristics of 24 commercial flour samples and their extracts in 50% EtOH were studied by UV-VIS-NIR, EPR, AAS and HPLC-MS methods, in order to assess the influence of several factors, i.e., origin of sample, growing conditions, varietal composition and technological processing on their properties. As follows from the results, besides the antioxidant properties, the content of minerals and trace elements, but also the total nitrogen and amino acids content is significantly influenced by the above-mentioned conditions. Additional experiments with samples of identical origin but various growing conditions are in progress, in

order to specify the most proper conditions for grain production and processing, from functional components content and health-beneficial properties maintenance points of view.

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