

The Physico-chemical and Spectroscopic Composition Characterization of Oat Grains and Oat Oil Samples

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In the present paper the chemical composition (protein, ash, crude fiber, total fat, total carbohydrates and moisture) of oat samples was determined. There were observed differences regarding the content of protein, crude fiber and total fat in the studied oat samples while ash, total carbohydrates and moisture content were approximately similar. The oat oils extracted by Soxtec system was analyzed using spectroscopic methods (¹H-NMR and FT-IR) coupled with the chemometrical method Principal Component Analysis. Using ¹H-NMR spectroscopy data and systems of chemometric equations, the composition of oat oils on four classes of fatty acids was established. The statistical processing of the data obtained from ¹H-NMR and FT-IR spectroscopy illustrated a tendency to group of samples P1, P2, P3 and P4 with similar fatty acids composition and samples P5 and P6 with different one. This study can be used in authentication purposes.

Keywords: oat, oat oil, ¹H-NMR spectroscopy, FT-IR, PCA

Oat (*Avena sativa* L.) can be regarded uniquely among the grain because it contains high quantities of valuable nutrients (soluble fibers, proteins, unsaturated fatty acids, vitamins, minerals and phytochemicals) and has multifunctional characteristics [1-3]. Oat grain is characterized by a good taste, dietary properties because of its activity concerning stimulating metabolic changes in the body. All these make its high nutritive value for both people and animals [4, 5].

A substantial amount of fiber of oat is β -glucan ((1–3)(1–4)- β -D-glucan). β -glucan is a polysaccharide that can reduce the concentration of serum cholesterol, attenuate blood glucose level, slow insulin response in the blood, and maintain the balance of intestinal flora [6-10].

Oats protein contain large amounts of essential amino acids in comparison to wheat, thus its use in the production of wheat bread should improve biological value of the final product. The addition of oat products in the production of wheat bread is accompanied by decreasing sensorial quality (i.e. volume and structure), caused by gluten weakening. Using oats as raw materials into bread dough is possible only to a certain quantity, limited by organoleptic quality of the final product. There were many studies regarding the formulation for wheat bread in which part of wheat flour was replaced by residual oat flour and concerning on the influence of this ingredient on sensory and nutritional properties of the products with special consideration to content and biological value of the proteins [11,12].

The nutritional interest in oat products has focused on the fact that oat is a source of dietary fiber, but, also, oat oil has a nutritional and technological potential. Because of low content of oil in comparison with oil seed crop, oats have not been used as a source of edible oil. Nevertheless, oats is an excellent source of unsaturated fatty acids because contain much higher levels of lipid than any other cereal grain [13].

In this study the chemical composition was determined – protein, ash, crude fiber, total fat, total carbohydrates and moisture – for six samples of oat grain. In addition, there

were analyzed the oils (extracted by Soxtec system) from six samples of oat, using ¹H-NMR and FT-IR spectroscopy. The ¹H-NMR and FT-IR analyses were conducted directly on the oat oil samples, without any sample derivatization (as triglycerides). The data obtained through ¹H-NMR spectroscopy (the composition of oat oil samples on four classes of fatty acids: tri-unsaturated, di-unsaturated, mono-unsaturated and saturated) and FT-IR spectroscopy (transformation of the FT-IR spectra in vectors containing 31 values) were processed using the chemometrical method Principal Component Analysis. The results obtained showed similarities and differences between oat oil samples investigated that can be used in further authenticity studies.

Experimental part

The authentic Romanian oat variety samples (P1 – P4 the species *Avena sativa* L.) were provided from research Station. Two types of oats – P5 – nude oat and respectively, P6 – oat produced in Ukraine (table 1) were analyzed. All oat samples are spring varieties from 2012 crop year. All oat samples (except *Avena nuda* L.) were dehulled manually and milled using Sample Mill MRC SM-1, Laboratory Equipment.

Physico-chemical analysis of oat samples

The chemical composition of oat samples was determined by the following standard methods:

- moisture content was determined using SR EN ISO 712:2009 method [14];

- nitrogen content was measured by the Kjeldahl procedure (using the Kjeltec 2300, Tecator Digestor Auto, Tecator Scrubber, FOSS), and the protein content was calculated by multiplying the nitrogen content by 6, 25 [15];

-ash content was determined by weighing the sample before and after burning at 750°C for 4 h.

- Lipid Extraction was performed using a Soxtec system (Avanti 2055, Foss). The Avanti 2055 has the capacity to perform extractions on six separate samples simultaneously. The solvent used for the extractions was petroleum ether [16].

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Sample	Oats variety	Geographical origin
P1	Mures	Agricultural Research and Development Station Turda, Romania
P2	Jeremy	Agricultural Research and Development Station Lovrin, Romania
P3	Lovrin 1	Agricultural Research and Development Station Lovrin, Romania
P4	Mures	Experimental Farm "Moara Domneasca", Ilfov, Romania
P5	Avena nuda L.	S.C Transapicola S.R.L, Târgu Mures , Romania
P6	Unknown	Ukraine

Table 1
OATS SAMPLES USED IN THIS STUDY

- crude fiber was determined using the FibreTherm FT 12 system. The method consists in boiling oatmeal sample with sulfuric acid. The residue is separated by filtration, then washed, dried and weighed. The residue obtained was calcined and the mass loss resulting from calcinations corresponds to the mass of crude fiber from the analyzed sample.

The content of total carbohydrates was calculated using the following formula:

$$\text{Total carbohydrates} = 100 - (\text{protein} + \text{ash} + \text{lipid} + \text{moisture})$$

Spectroscopic analyses of oat oil samples

The oat oil samples were obtained by Soxtec protocol [16].

The ¹H-NMR and FT-IR analyses were conducted directly on the oat oil samples, without any sample derivatization (as triglycerides).

¹H-NMR analyses of oat oil samples

The oil sample was dissolved in CDCl₃ (1:9 v/v). The sample was sonicated for 5 min, for degassing and mixing.

The ¹H-NMR spectra of the oat oils extracted were recorded on a Varian INOVA 400 spectrometer, operating at 9.4 Tesla, corresponding to the resonance frequency of 399.95 MHz for the ¹H nucleus, equipped with a direct detection four nuclei probe head and field gradients on z axis. Samples were analyzed in 5 mm NMR tubes (Norell 507). The chemical shifts are reported in ppm, using the TMS as internal standard. Typical parameters for ¹H-NMR spectra were: 45° pulse, 2.05 s acquisition times, 6.4 KHz spectral window, 32 scans, 26 K data points. The FID was not processed prior to Fourier transform. The average acquisition time of the ¹H-NMR spectra was approximately 2 min. The sample preparation concerned in dilution of 20 μL of oat oil in 80 μL of CDCl₃.

The statistic analyses (Principal Component Analysis) used to investigate the compositional differences between oat oils was carried out using the XLSTAT software.

FT-IR analyses of oat oil samples

FT-IR spectra were recorded on a Bruker Vertex 70 Spectrometer, with horizontal device for attenuated reflectance and diamond crystal, on a spectral window ranging from 4000 to 400 cm⁻¹, at a spectral resolution of 2 cm cm⁻¹ [17]. Spectra were recorded without any sample preparation and were processed with OPUS 5.5 program (Bruker). The compositional differences between oat oils,

using FT-IR spectroscopy were established by Principal Component Analysis. Differentiation of the samples was performed using the relative intensity of absorption bands. Using OPUS 5.5 program, the peak intensity of the absorption band corresponding to the main classes of chemical compounds identified in the IR spectrum was measured. The area between 3050 and 4000 cm⁻¹ was eliminated from the study because it contains information which is not relevant for oils discrimination (water absorbance) and it is also a source of noise in the spectrum. From spectral range 2800-3050 cm⁻¹, were obtained six values of the absorption band intensity (every 50 cm⁻¹). In the same way another twenty-five values between 600 and 1800 cm⁻¹ (every 50 cm cm⁻¹) were obtained. In total, each IR spectrum was represented as a vector with 31 values.

Results and discussions

Physico-chemical analysis of oat samples

The physico-chemical analyses of oat samples are presented in table 2.

As it can be shown from table 2, the highest content of protein is found in P2 and P3 samples; both samples are from the same geographical area (Lovrin); P1 sample from Turda have had the highest content of crude fiber. Regarding the composition of ash, total carbohydrates and moisture is almost identical for all six samples. The highest content of total fat was found in sample P4.

Spectroscopic analyses of oat oil samples

¹H-NMR analyses of oat oil samples

¹H-NMR spectra of oat oils have the same shape, but the differences between them are reflected on the integral values of the characteristic peaks. Figure 1 shows the ¹H-NMR spectrum of oat oil (sample P1) and the chemical shifts and peak assignment of ¹H-NMR spectra, according to the literature specifications [18-20].

Based on ¹H-NMR spectra and using systems of chemometric equations the composition of oat oils was determined on four classes of fatty acids: tri-unsaturated, di-unsaturated, mono-unsaturated and saturated [19] (table 3).

It can be shown in table 3 that the composition of tri-unsaturated and di-unsaturated fatty acids is similar in all six samples of oat oils. We can also notice that the highest amount of mono-unsaturated fatty acids and the lowest amount of saturated fatty acids are found in oat oil samples P5 and P6.

Sample	Protein (%)	Ash (%)	Crude fiber (%)	Total fat (%)	Total carbohydrates (%)	Moisture (%)
P1	14.31	2.05	4.36	4.46	68.78	10.40
P2	21.31	1.96	3.26	5.31	60.92	10.50
P3	21.03	2.02	3.65	5.14	61.34	10.47
P4	17.26	2.34	2.62	6.70	63.64	10.39
P5	14.95	2.00	1.90	6.54	65.70	10.81
P6	16.62	1.50	1.96	6.10	64.68	11.10

Table 2
CHEMICAL COMPOSITION OF
OAT GRAIN SAMPLES

Signal	δ (ppm)	Proton	Compound
A	0,95	-CH=CH-CH ₂ -CH ₃	linolenic acid
B	0,85	-CH ₂ -CH ₂ -CH ₂ -CH ₃	all acids except linolenic acid
C	1,25	-(CH ₂) _n -	all fatty acids
D	1,6	-CH ₂ -CH ₂ -OCO-	all fatty acids, β -methylene protons
E	2,02	-CH ₂ -CH=CH-	all unsaturated fatty acids
F	2,3	-C H ₂ -OCO-	all fatty acids, α -methylene protons
G	2,75	-CH=CH-CH ₂ -CH=CH-	linoleic acid and linolenic acid
H	4,19	-CH ₂ -OCOR	all fatty acids
I	5,24	-C H-OCOR	all unsaturated fatty acids
J	5,35	-CH=CH-	all unsaturated fatty acids

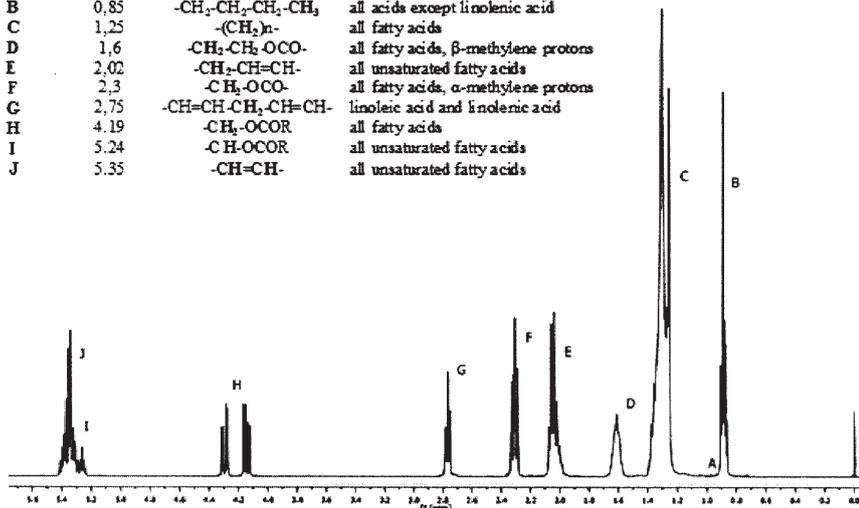


Fig.1. ¹H-NMR spectrum of oat oil (sample P1)

Oat oil samples	Fatty acids			
	Tri-unsaturated	Di-unsaturated	Mono-unsaturated	Saturated
P1	4.59±0.21	35.06±1.74	39.24±1.97	21.11±1.01
P2	4.53±0.22	32.67±1.61	40.43±1.56	22.37±1.25
P3	4.62±0.19	33.81±1.59	38.02±1.85	23.56±0.98
P4	4.74±0.24	31.62±1.45	38.98±1.79	24.66±1.12
P5	4.07±0.18	38.85±1.85	50.75±2.43	6.34±0.36
P6	5.66±0.27	38.44±1.98	52.70±2.51	3.20±0.14

Table 3
COMPOSITION OF OAT OIL
SAMPLES ON FOUR CLASSES OF
FATTY ACIDS

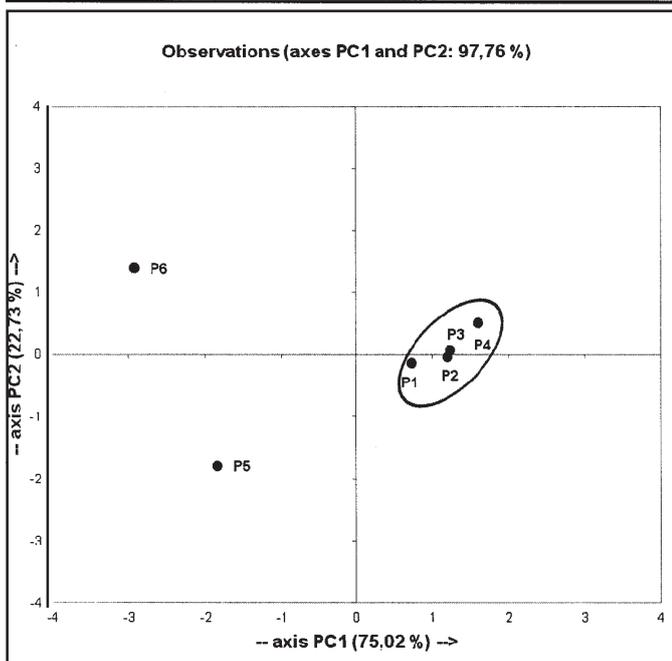


Fig.2. Principal Component scores PC1/PC2 plot for the oat oils obtained using ¹H-NMR spectroscopy (composition of fatty acids)

By statistical processing of the data obtained through ¹H-NMR spectroscopy (fatty acids composition) for oat oil samples the PC1/PC2 representation was obtained (fig. 2).

It can be noticed from figure 2 that the oat oil samples P1, P2, P3 and P4 (*Avena sativa* L.) have the tendency to group and the samples are very well differentiated from the other two oil samples P5 and P6. This can be explained by the fact that oat oil sample P5 and P6 are from oat with different characteristics (P5 - *Avena nuda* L., and do not require hulling, and commercial dehulling oat P6). Additional, P6 is differentiated (quadrant 1) by different cultivation techniques and pedo-climatic, being an imported variety (Ukraine).

FT-IR analyses of oat oil samples

The overlapped FT-IR spectra of the oat oil samples are shown in figure 3.

To establish the differences between oat oils, the FT-IR spectrum was transformed in a vector of 31 numerical values as previously described and the data were processed using the Principal Component Analysis method (fig.4).

After statistical processing of the data obtained from FT-IR spectra it can be noticed in figure 4 that there is the same situation as in the case of statistical processing of the NMR data – sample P1 – P4 were grouped while oat oil samples P5 and P6 were found in two different quadrants.

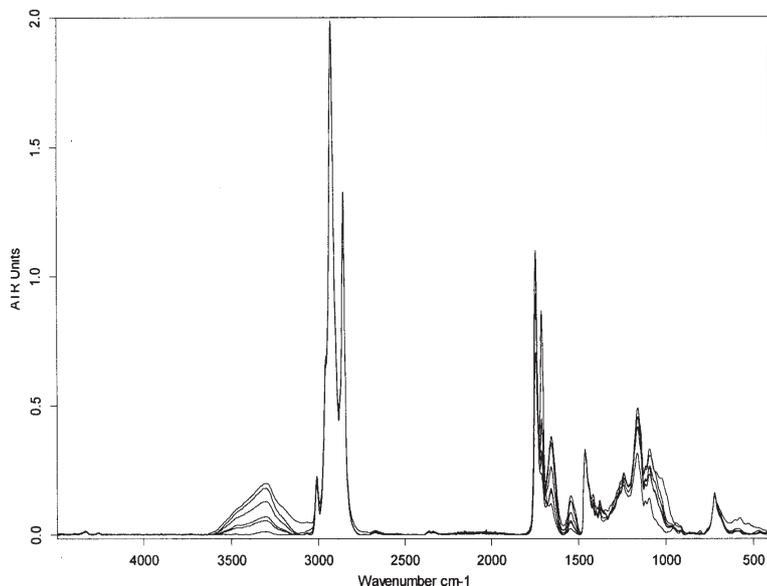


Fig.3. The overlapped FT-IR spectra of oat oil samples

Observations (axes PC1 and PC2: 84,08 %)

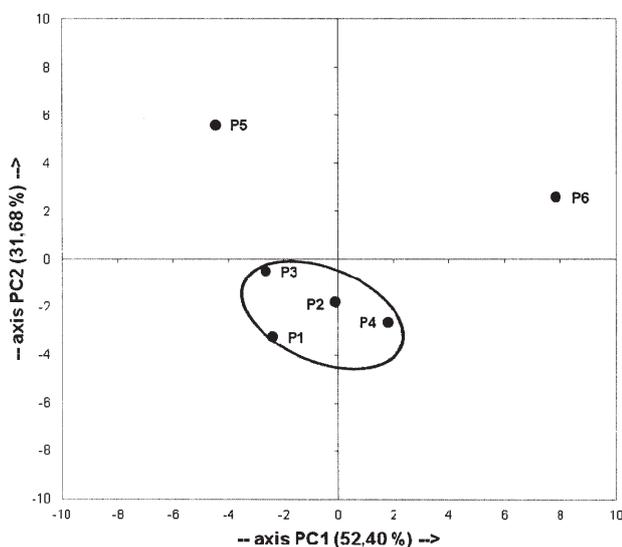


Fig.4. Principal Component scores PC1/PC2 plot for the oat oils using FT-IR spectra (vectors of 31 numerical values) processed by PCA

Conclusions

The chemical composition (protein, ash, crude fiber, total fat, total carbohydrates and moisture) of oat grain samples was determined. Also, the oat oil samples using spectroscopic methods $^1\text{H-NMR}$ and FT-IR were analyzed. Compositional differences in the fatty acids profiles of oat oils were observed. Statistical processing PCA (Principal Component Analysis) of the data obtained by NMR and FT-IR spectroscopy showed a tendency to group of samples P1, P2, P3 and P4 (*Avena sativa* L.), while samples P5 and P6 were found in different quadrants. The results are important for further authentication studies for different types of cereals and pseudocereals.

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