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ORIGINAL RESEARCH PAPER

WHEAT FLOUR HUMIDITY VARIATION WITH UV-VIS RADIATION DOSE REVEALED BY SPECTRAL AND CHEMOMETRIC STUDIES

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Abstract: The cells' exposure to UV radiation induces mutations of the cellular components by its action on DNA, protein synthesis and enzymatic activities. Different varieties of wheat flour were treated with UV-B, UV-A, Vis radiation and compared with untreated samples. The IR spectra for these components were recorded with a Bruker FTIR spectrophotometer using an ATR method, at 4 cm^{-1} resolution. The paper proposes a comparative study of unmaturing flour behavior under UV-Vis and natural radiations in order to observe the physico-chemical changing by FTIR spectroscopy. At small doses of irradiation (up to 2 h) the humidity of the samples decreases and then it significantly increases, most pronounced in Gruia's case where the humidity is reaching 74.4% of the initial value. Middle infrared spectral studies reveal an inverse weak linear correlation between Amide I region (1650 cm^{-1}) (R-squared value: -0.3168) and an inverse medium linear correlation assigned to area alcohol O-H band at 3290 cm^{-1} (R-squared value: -0.6064) with the irradiation dose variables. Strong direct linear correlations confirmed by R-squared value: 0.7835 are found between alcohol O-H band at 3290 cm^{-1} and humidity percentage parameter.

Keywords: *FTIR spectroscopy and chemometrics, irradiation, maturation, wheat flour*

INTRODUCTION

The maturing flour is a complex biophysical process taking place slow in flour by grinding wheat grains and its results in improving the characteristics of bread. Freshly milled flour forms sticky, non-elastic dough with a low water absorption. The maturation process is a complex and interrelated set of phenomena that occurs in wheat flours after grinding, influenced by numerous physical, chemical and biochemical properties, which can produce the substantial transformation characteristics of bread, especially for flours with low and medium power [1].

There are several chemical processes that occur during maturation flour. Two processes that occur during storage of flour are essential: compounds oxidation by lipases and proteases and the development of rancid taste. Complex processes during aging flour are influenced by the type of flour, storage conditions and packaging materials [2].

Wheat has distinguished itself from all other cereals because of its unique dough-forming properties [3]. These properties of materials are due to the gluten - protein complex. The main processes that lead to improved properties are due to mature bakery flour protides complex change of flours. Improving the quality of gluten in flour during maturation is especially evident since its original quality is lower. In the weak flour parties, after such changes are improved rheological properties of gluten and dough, it improves the volume, crust appearance, crumb porosity [4].

The main changes in wheat flour after grinding, which occur during maturation, are uniformity of moisture flour based on environmental parameters in steady state. Biochemical modification of the main ingredients of flour, carbohydrates, lipids, protides with increasing acidity are due to the release of fatty acids under the action of lipase and improving technology traits gluten-forming proteins and chemical oxidation / enzyme essential fatty acids, carotenoid pigments and-SH groups, with the opening of the flour color and the formation of -S-S- links with increasing flour strength / improvement of rheological properties of gluten [5].

In the natural maturation the primary role they have free polyunsaturated fatty acids, formed by hydrolysis of flour lipids, which in the presence of oxygen in the air are oxidized to hydroperoxides lipoxygenase enzyme. Immediately after grinding, depending on initial moisture content of the flour and storage conditions (relative humidity, temperature, degree of aeration), there is a variation of water activity (aw) in order to establish the proper equilibrium state air parameters from storage in case of natural maturation [6].

During normal maturation of flours, in the absence of microbiological processes, the amount of monosaccharides remains constant. Among carbohydrates, sucrose does not change quantitative and maltose accumulated in significant quantities. Changes in the amount of maltose are correlated with amylase activity of flour, which drops more rapidly in the first 7 days and remains constant after 30 days from grist. The explanation of this phenomenon is that at maturity there is a growing resistance of starch granules and by compacting their structure amylolytic enzyme action, each type of starch granule enzyme reacts to attack in a manner characteristic [7].

It is known that infrared investigative techniques reveal the molecular changes during different processes.

FTIR imaging coupled with statistical analysis was used to map the compositional and structural and several distinct populations of endosperm cells and wheat could be identified by spectral features. Also structural differences within the polysaccharides, the proteins and DNA could be distinguished [8-10].
FTIR spectroscopy and chemometrics have been combined to detect adulteration in food samples [11]. After chemometric analyzing, the samples were correctly classified by the applied model.
In this article there are studies of the ultraviolet radiation behavior acting on the biochemical and biophysical characteristics of Crina, Gruia and Flamura 85 flour types.

MATERIALS AND METHODS

Water content measurement

Uniform moisture flour based on environmental parameters is a main parameter change after grinding flour. To establish conditions that can hasten the milled flour maturation process were studied the humidity variation depending on the time of radiation exposure to non-ionizing radiation. The moisture and sample weight were measured with Kern MLB 50-3 Moisture Balance (Figure 1). Three flour samples (Flamura 85, Gruia, Crina) were exposed at 60 min, 120 min and 180 min time irradiation. Humidity flour is very important not only for the validity of the product, but also for determining the solids [10].

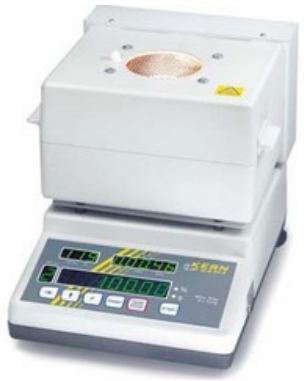

Figure 1. *Kern MLB 50-3 Moisture Balance*

It is necessary to know the dry mass of material in order to determine the amount of substances they contain. Flour sample weight must be adjusted to moisture content.
The moisture content of flour depends largely on climatic conditions during harvesting and storage space humidity. Values above 15% humidity threaten the validity of the product [10].

Spectral measurement. Statistical data processing

The flour samples were investigated with FTIR-ATR spectrometric methods.
The MIR spectrum was recorded with ATR technique from Tensor 27, Bruker at 4 cm^{-1} resolution. The ORIGINI 8 and The USCRAMBLE X, Camo, Norway software statistical software has been used for data processing.
Note the middle infrared spectral difference between different types of flour. Using statistical data processing software were compared the characteristics (humidity percentage, irradiation time and absorbance intensity) with each different types at 1, 2, 3 hours time exposure UV irradiation. FTIR spectra highlighted differences between the flour types investigated.

RESULTS AND DISCUSSION

Water practically is present in every food product. Water content has a significant importance for several reasons. Determination of water content is therefore most frequently used to analyze food. They are classified into direct and indirect methods. Direct methods aimed at determining the water itself. Physical techniques indirect methods are based on a separation of water [9, 10, 12].
Heating techniques that measure a mass loss in certain circumstances, are mainly of concern because they can not distinguish between water and other volatile substances. Direct chemical methods are based on a chemical reaction of water molecules. Indirect methods can measure or test a property that depends on water content, or water molecules apparent response to a physical influence [10, 12].
The flour maturation takes place due the sulfhydryl groups' oxidation from protein structures, proteolysis' enzymes and proteolysis activators. The high content of oxygen sensitive lipid nutrients makes the flour susceptible to oxidation.

The humidity percentage variation

To emphasize the humidity variation with irradiation time, the Unscramble statistical software has been used for data processing. All investigated samples were characterized by the following variables: humidity percent and infrared absorbance at different wave number (Table 1).
It can be observed (Table 2) that the humidity decreases after an hour emphasized at Flamura 85, reaches 79% of its initial value, after only two hours decreases at 76.4% and after three hours returns at 77.3%. In Crina's case the humidity decreases less than Flamura 85, at 83% after an hour and after three hours it reaches 78.3% of the initial value. In Gruia's case the value reached after two hours irradiation is lower, the humidity reaching 74.4% of the initial value and after three hours it returns at the highest value of the three varieties of wheat, 78.6%.
Note in Gruia's case the highest variation of the humidity percentage of the initial value after the three hour irradiation.
In conclusion, for all types of flour, the humidity decreases with increasing radiation dose up to a certain level and then increases.

Spectral analysis

The middle-infrared (MIR) spectrum of Flamura 85, Crina and Gruia wheat flour are presented in Figures 2 – 4. The main infrared spectral corresponding assignment of Crina, Flamura 85 and Gruia wheat flour middle-infrared spectrum are related in Table 3.
In analyzing samples from cereal grain and grain products by near-infrared (NIR) spectroscopy has a clear advantage in sampling over mid-infrared (MIR) spectroscopy because of the more effective sample penetration by light at shorter wavelengths. The advantage of MIR spectra consists in containing bands of all the fundamental functions of vibration, symmetric stretching, asymmetric stretching, bending, rocking, wagging, and twisting [13].

Table 1. *Humidity value and the main absorption intensity in middle infrared spectrum of Flamura 85, Crina and Gruia wheat flour*

Wheat flour type		Humidity [%]	Absorbance at:					
			1004 cm^{-1}	**1078 cm^{-1}**	**1148 cm^{-1}**	**1650 cm^{-1}**	**2926 cm^{-1}**	**3290 cm^{-1}**
Flamura 85	Control	10.5	0.1711	0.0918	0.0475	0.0315	0.0095	0.0288
	Irradiated 1 h	8.3	0.1824	0.0918	0.0637	0.0322	0.0273	0.0245
	Irradiated 2 h	8.02	0.143	0.075	0.0358	0.0191	0.0056	0.0177
	Irradiated 3 h	8.12	0.1574	0.082	0.0455	0.0289	0.0086	0.0211
Crina	Control	10.27	0.1188	0.0751	0.0494	0.0391	0.0185	0.032
	Irradiated 1 h	8.52	0.1181	0.0735	0.0487	0.0364	0.0177	0.0243
	Irradiated 2 h	7.84	0.1137	0.071	0.0475	0.0343	0.0153	0.0225
	Irradiated 3 h	8.04	0.1152	0.072	0.0481	0.0364	0.0153	0.0232
Gruia	Control	10.29	0.2015	0.1028	0.0581	0.0296	0.0162	0.0352
	Irradiated 1	8.32	0.1814	0.0965	0.0517	0.0247	0.0112	0.0274
	Irradiated 2 h	7.66	0.1723	0.0873	0.0462	0.0178	0.0083	0.012
	Irradiated 3 h	8.09	0.1905	0.0983	0.0535	0.0235	0.0092	0.0274

Table 2. *Variation of humidity percentage from initial value during irradiation*

Wheat flour type		Humidity [%]	Percentage of humidity from initial value %
Flamura 85	Control sample	10.5	100
	Irradiated 1 hour	8.3	79↓
	Irradiated 2 hours	8.02	76.4↓
	Irradiated 3 hours	**8.12**	**77.3↑**
Crina	Control sample	10.27	100
	Irradiated 1 hour	8.52	83↓
	Irradiated 2 hours	7.84	76.3↓
	Irradiated 3 hours	**8.04**	**78.3↑**
Gruia	Control sample	10.29	100
	Irradiated 1 hour	8.32	80.8↓
	Irradiated 2 hours	7.66	74.4↓
	Irradiated 3 hours	**8.09**	**78.6↑**

Structural elucidation achieved by performing FTIR revealed that all wheat flour samples analyzed contained some similar group frequencies.

These frequencies are represented by a strong absorption centered at 1000 cm^{-1} (I), a amide II band at 1530 cm^{-1} (II), a amide I band at 1650 cm^{-1} (III), a weak absorption centered at 2926 cm^{-1} (IV) and a broad absorption band from 3500–3000 cm^{-1} (V).

We attribute the bands at I, II and V to be due to hydroxyl group O–H stretching, alcohol related C–O stretching and aliphatic organic compound related C–H stretching, respectively, as illustrated in Figures 2, 3 and 4 [14]. The absorption bands centered at 1650 and 1530 cm^{-1} are attributed to protein group frequencies due to the related amide I and amide II functional groups. The fingerprint region in MIR from 1900 to 700 cm^{-1} contains the characteristic C–O and C–N stretching bands [13].

Table 3. *The main corresponding assignment of Crina, Flamura 85 and Gruia wheat flour middle-infrared spectrum*

Wavenumber [cm^{-1}]	Assignment	Ref. no
1004	C–O and C–N stretching bands	14, 15
1530	Amide II (N–H band and C–N stretch)	13, 15
1650	Amide I arises from the stretch of C=O of the peptide group in the protein	13, 15
2926	CH_2 stretching vibration	13, 14, 15
3290	Broad an alcohol O-H band	13, 14, 15

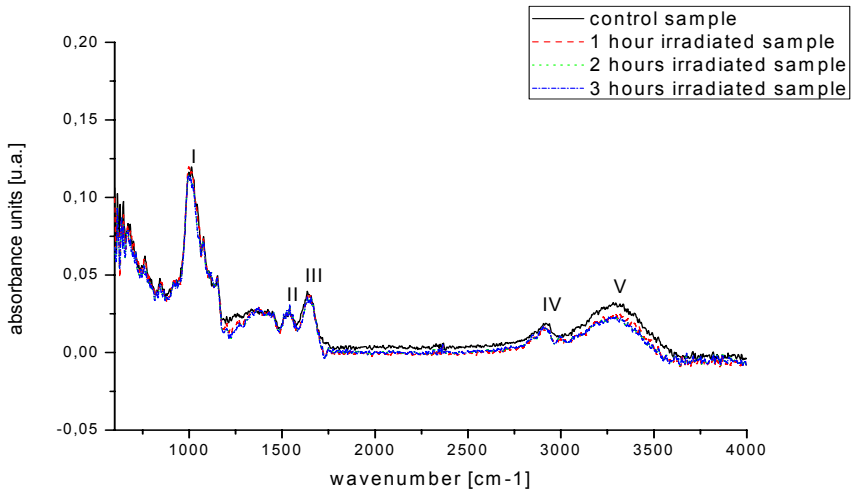

Figure 2. *Middle-infrared (MIR) spectrum of Crina wheat flour*

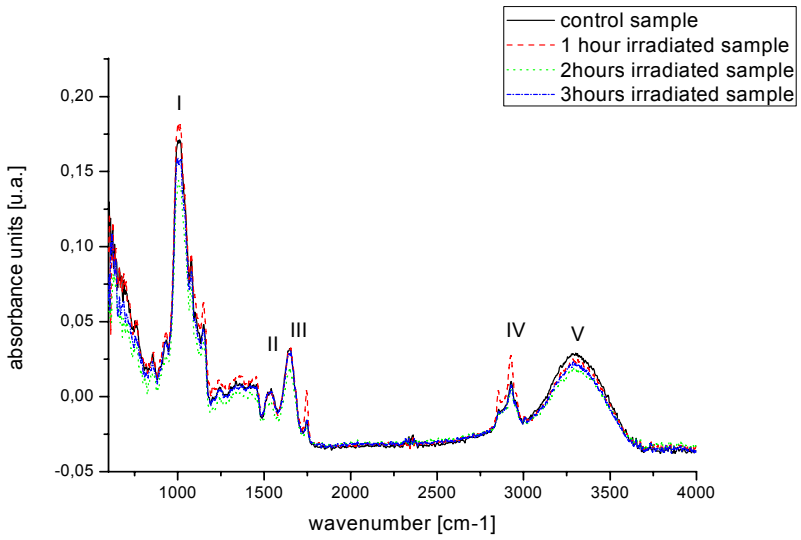

Figure 3. *Middle-infrared (MIR) spectrum of Flamura 85 wheat flour*

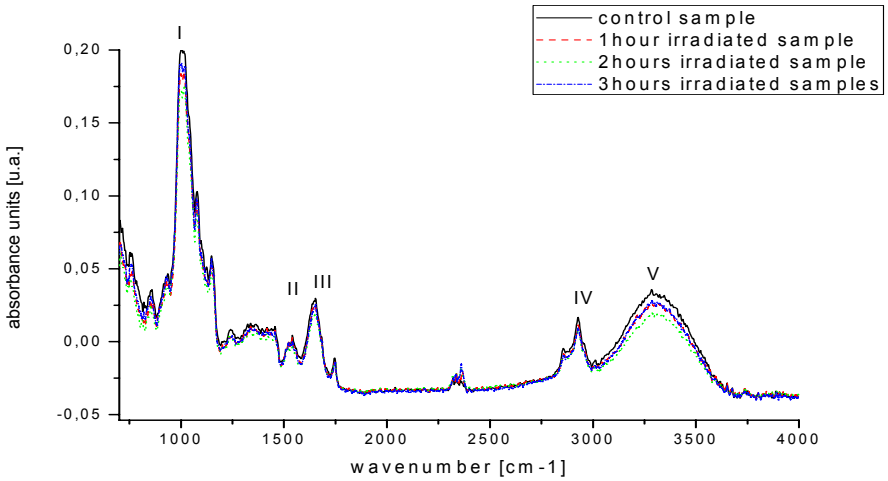

Figure 4. *Middle-infrared (MIR) spectrum of Gruia wheat flour*

Structural elucidation achieved by performing FTIR revealed that all wheat flour samples analyzed contained some similar group frequencies which are common to all spectra.

The bands occur in the fingerprint region (usually between 1250 and 1000 cm^{-1}) are subject to disorderly shifts from quite small structural changes. Fortunately they are very intense in the IR, which usually enables them to be identified.

The differences between the main peak positions revealed structural changes under irradiation at the molecular level. In our study structural changes revealed by spectral analysis are correlated with statistical and chemometric studies.

Statistical and chemometric analysis

As a validation model of the experimental observations we chose to use specialized Camo Unscrambler X version 10.1 software for statistical and chemometric data processing.

Statistical analysis of absorption intensity dependences can be done by multiple regression and correlation task.

In Figures 5 – 8 are illustrated intensities of the not irradiated and irradiated corresponding infrared absorption peaks for one hour, two hours and three hours of samples: Flamura 85, Crina and Gruia.

By comparing the four figures clear differences can be observed corresponding to absorption peaks of 2926 and of 3290 cm^{-1}.

This remark is confirmed by the linear correlation between the irradiation time and the intensity of the absorption peaks at 2926 cm^{-1} and 3290 cm^{-1} (Table 4). The intensity of the bonds has an average value for 2926 cm^{-1} peak and a strong one for 3290 cm^{-1}. We therefore conclude that there is a strong dependence between vibration molecule behavior in infrared region (3290 cm^{-1}) and the irradiation dose of wheat samples.

The study of linear correlation coefficients between variables in our case the absorption intensity, humidity percentage and the irradiation time are extremely important. The strong correlation coefficients, higher than 0.7 values, show that variables are responsible for data variation.

A univariate regression model can be found looking at the correlation between each absorption intensity value at 3290 cm^{-1} and humidity percentage variable parameter (Table 5). Studying the regression line between the humidity parameter and intensity of absorption corresponding the 3290 cm^{-1} peak we can observe a good correlation: R-squared value is 0.7835 (Figure 9).

Table 4. *Linear correlation coefficients between different peaks absorption intensity in middle infrared spectra and irradiation time parameter*

Irradiation time, h	Absorbance at:					
	1004 cm^{-1}	1078 cm^{-1}	1148 cm^{-1}	1650 cm^{-1}	2926 cm^{-1}	3290 cm^{-1}
1	-0.1656	-0.2757	-0.3296	-0.3168	-0.3892	-0.6064

Table 5. *Linear correlation coefficients between different absorption intensity in middle infrared spectra and humidity percentage parameter*

Humidity [%]	Absorbance at:					
	1004cm^{-1}	1078cm^{-1}	1148 cm^{-1}	1650 cm^{-1}	2926 cm^{-1}	3290 cm^{-1}
1	0.1636	0.2946	0.2388	0.4141	0.1888	0.7835

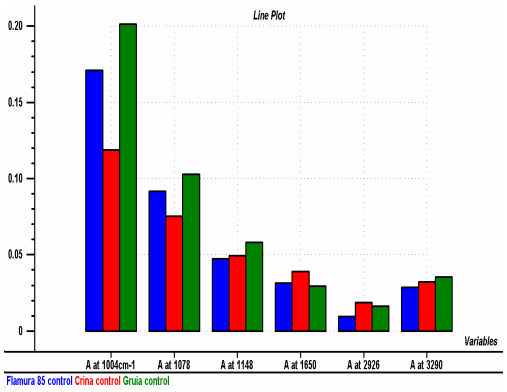

Figure 5. *Peaks absorption intensity corresponding to non irradiated samples: Flamura 85, Crina and Gruia*

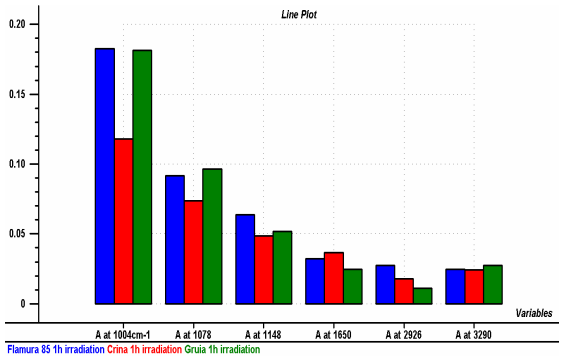

Figure 6. *Peaks absorption intensity corresponding to samples irradiated for one hour: Flamura 85, Crina and Gruia*

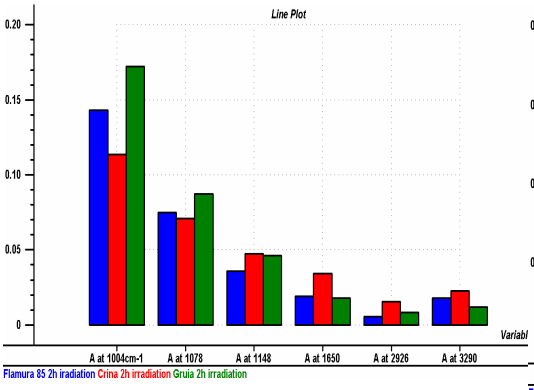

Figure 7. *Peaks absorption intensity corresponding to samples irradiated for two hours: Flamura 85, Crina and Gruia*

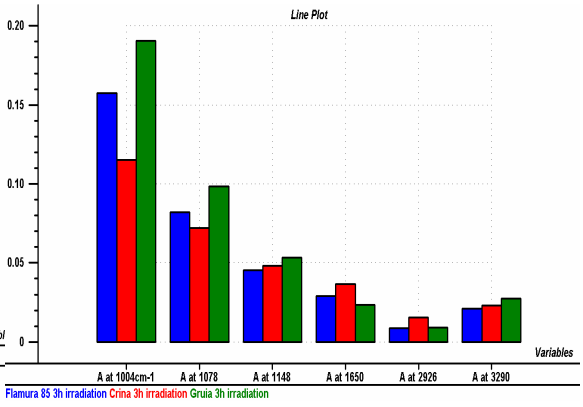

Figure 8. *Peaks absorption intensity corresponding to samples irradiated for three hours: Flamura 85, Crina and Gruia*

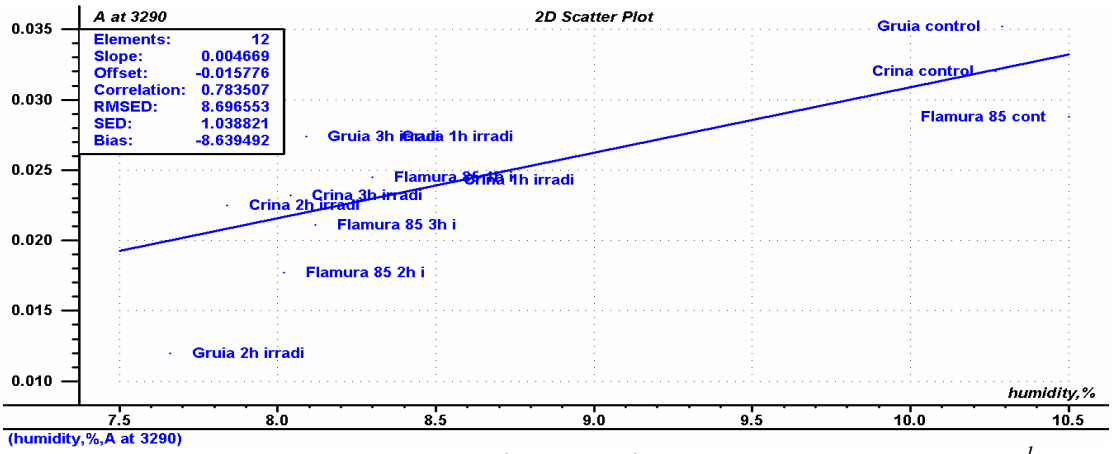

Figure 9. *Univariate regression between absorption intensity at 3290 cm^{-1} and humidity percentage parameter*

CONCLUSIONS

The studies on non-ionizing radiation impact are particularly focused on the sterilization and preservation of the products, but the molecular bases of these changes occurred after irradiation is not yet completely known.

After different wheat flour samples irradiation with different doses, a less common behavior is emphasized: at small doses of irradiation (up to two hours) the humidity of the sample decreases and then it significantly increases, probably due to biophysical changes of the cell membrane induced by radiation intensity. We obtained the most significant variation in Gruia's case.

Between molecular vibration behavior in Amide I region (1650 cm^{-1}) and the irradiation dose of the wheat samples there is an inverse week correlation (R-squared value -0.3168), its intensity increasing to an inverse medium correlation assigned to area alcohol O-H band at 3290 cm^{-1} (R-squared value -0.6064).

Between molecular vibration behavior in infrared spectrum area (3290 cm^{-1}) and humidity percentage parameter of the wheat samples there is a strong direct correlation confirmed by R-squared value (0.7835).

The increasing absorption intensity on alcohol O-H band is correlated with the increasing humidity percentage and with decreasing dose irradiation.

So, the FTIR studies associated with statistical and chemometric analysis confirm the biochemical modification of the wheat flours occurred during irradiation.

This preliminary study will allow us in the future to deepen further, explain and associate the changes induced by not ionized irradiation of the biochemical and biophysical parameters that characterize the plant cell.

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