

The content of essential and toxic elements in wheat bran and flour

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Abstract

The research was conducted with the aim of examining the presence of toxic elements (Pb, Cd, As and Hg) and essential elements (Zn, Cu, Fe and Mn) in selected samples of wheat flour and bran by Inductively Coupled Plasma Mass Spectrometry. The principal component analysis has been used for assessing the variety of samples. Quality results show that the first two principal components, accounting for 84.22% of the total variability, can be considered sufficient for data representation and the first two principal components for toxic elements and essential microelements. Zn (16.38%), Mn (16.19%), Cu (15.73), Fe (15.44%) and Cd (14.99%) have been found the most influential for the first factor coordinate calculation, while the contribution of Hg (89.09%) has been the most important variable for the second factor coordinate calculation.

Keywords: microelements, wheat bran, flour, principal component analysis.

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Wheat is one of the most important crops in the world and is grown in different climates. The importance of wheat lies in the fact that wheat crop covers the largest area in the world of agricultural production. The most important step in wheat processing is milling which produces different types of flour as well as different by-products such as bran, and shorts and also impurities extracted from the process of preparing [1].

In addition to nutrients (high level of vitamins, minerals and dietary fibers), wheat grain also contains a number of elements (Cu, Zn, Fe, Ni and Mn) vital to our biological functions, but hazardous to our health in high concentrations [2–4]. It also may contain certain toxic elements (As, Pb, Hg and Cd) which CERCLA Priority List [5] rated as the first, second, third and seventh in toxicity.

Both nutritional essentiality and toxicity of an element depend on its concentration and dietary intake [6].

Mineral fertilizers and pesticides used in wheat production may be contaminated with insecticides based on toxic elements: Pb, Cd or As. Due to the slow Pb, Cd, As and Hg excretion from organisms, carcinogenic and mutagenic effects caused by chronic toxicity may occur [7], along with acute intoxication caused by toxic doses taken over an extended period of time (six months to two years).

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The quality of bread, pastries and other wheat products depends primarily on the quality of the flour as the basic ingredient, i.e., the quality of the wheat variety as the basic raw material. Considering that bread is consumed on a daily basis, the emphasis should be put on its healthy production. If there are undesirable substances present in bread, there is a risk to human health [8]; thus, wheat-based products have received considerable attention in view of their potential role in transporting of microelements into the human diet [9].

According to the current Regulation on the quantities of pesticide, metals, metalloids and other toxic substances, drugs, anabolic and other substances that could be present in foodstuffs [10], the maximum permissible concentration levels of Pb, Cd, Hg and As in flour are 0.4, 0.1, 0.05 and 1 mg/kg of dry matter, respectively, while the maximum permissible concentration of Cd in bran is 0.05 mg/kg of dry matter.

In addition to Cd, Pb, Hg, and As, Cu, Zn, Fe and Mn were selected for this study, as the consumption of wheat products is also an important source of dietary intake of these essential trace elements and can also influence trace element reference values in the tissues of the world's population [11].

The objective of this research is to test the presence of toxic elements (Pb, Cd, As and Hg) and essential elements (Zn, Cu, Fe and Mn) in flour and bran obtained by milling wheat grown in Banat, Serbia. The results will provide information about the position of the accumulation of the studied elements in certain parts of the grain, and thus enable the production of safe food and prevent these elements from entering the food chain.

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MATERIAL AND METHODS

Material

Wheat samples were collected from ten locations in the whole territory of Banat (the region in Serbia's northern province of Vojvodina; geographic coordinates of Vojvodina: latitude 45.3167° N, longitude 19.8500° E). 30 individual samples were taken from each site and they were mixed to obtain a representative sample for each site. In this way, ten representative samples of wheat were obtained out of 300 individual samples.

Bran and flour of 60% extraction were obtained by milling the representative samples of wheat in the mill Quadrumat Senior.

Preparation and analysis of the wheat bran and flour

The wheat bran and flour samples were prepared in the following way: A 0.5 g sample was dissolved in 10 ml of concentrated HNO_3 and heated under reflux. After dissolution, 10 ml of concentrated perchloric acid was added and heated until the formation of nitrous fumes stopped. The digestion temperature did not exceed 85 °C to prevent loss of As and Hg (the temperature range was from 70 to 85 °C). The solution was placed in a 50 ml volumetric flask and made up to volume with deionized water (18.2 Ω).

Samples were analyzed depending on the type of elements and their concentration by inductively coupled plasma mass spectrometry (Nexion 300 ICP-MS, Perkin Elmer).

Appropriate quality assurance procedures and precautions were carried out to ensure the reliability of the results. Samples were generally carefully handled to avoid contamination. Glassware was properly cleaned and the reagents were of analytical grade. Reagent blank determinations were used to calibrate the instrument readings. A recovery study of the analytical procedure was carried out by spiking and homogenizing several previously analyzed samples with varied amounts of standard solutions of the metals. Recoveries ranged from 97% for Hg and Fe to 102% for Cu and Cd. It is evident that the determined concentrations of toxic elements agreed well with the reported certified values, confirming the accuracy of the procedure applied.

Toxic element index (*TEI*) and essential microelement index (*EMI*)

Central tendency is the most widely used to compare the toxic element and essential microelement content of complex samples determined using multiple assays [12], where samples are ranked based on the mean value and standard deviation of the assays used. Since the units and the scale of the data from various

assays are different, the data in each data set should be transformed into standard scores, a dimensionless quantity derived by subtracting the minimum from the raw data divided by the difference of maximum and minimum values, according to the following equation (for both *TEI* and *EMI*):

$$\bar{x}_i = \frac{\max x_i - x_i}{\max x_i - \min x_i}, \quad \forall i \quad (1)$$

where x_i represents the raw data. When averaged, the standard scores of a sample for different assays give a single unitless value termed as: toxic element index (*TEI*), for toxic elements (Pb, Cd, As and Hg) and essential microelement index (*EMI*), for essential elements (Zn, Cu, Fe and Mn), which is a specific combination of data from different chemical assays with no unit limitation and no variance among the methods.

Statistical analysis

All the experiments were performed with 24 repetitions. Descriptive statistical analyses for calculating the means and the standard error of the mean were performed using the StatSoft Statistica 10 software. All the results obtained were expressed as the mean \pm standard deviation (*SD*). The evaluation of correlation matrix and the principal component analyses (PCA) of the results obtained were performed using the StatSoft Statistica 10 software.

RESULTS AND DISCUSSION

Toxic and essential microelement content in the wheat bran and flour

The concentrations of analyzed microelements in the collected wheat bran and flour samples are shown in Table 1.

Cd content in the analyzed samples of flour ranged between 0.094 and 0.113 mg/kg (Table 1), which is a concentration four times higher than the concentrations found in the tests reported by Tejera *et al.* [13] where the total content of Cd found in the flour samples was 0.023–0.027 mg/kg.

The concentration of Cd found in the bran (0.109–0.138 mg/kg) was higher than the maximum permissible concentration level (0.05 mg/kg) due to phosphate fertilizers used in agricultural production.

Pb content in the flour ranged from 0.076 to 0.122 mg/kg. The mean values of Pb were 0.107 ± 0.010 mg/kg. The results show that Pb concentration in the analyzed flour was higher than the concentrations found in the tests reported by Tejera *et al.* [8], where the total content of Pb found in the flour samples was 0.037–0.056 mg/kg, and also higher than the concentrations found in the tests performed by Zhang *et al.*

Table 1. Descriptive statistics data for toxic element and essential microelement content (mg/kg) in the bran and the flour

Parameter	Pb	Cd	Hg	As	Zn	Cu	Fe	Mn
Bran								
Average	0.143	0.121	0.008	0.028	68.545	9.017	104.23	164.665
St.Dev.	0.046	0.006	0.002	0.014	13.471	2.231	36.508	29.858
Min.	0.105	0.109	0.004	0.014	46.043	1.439	12.045	121.738
Max.	0.290	0.138	0.011	0.074	99.985	13.265	240.48	235.850
Variance	0.002	0.000	0.000	0.000	181.468	4.976	1332.85	891.495
Flour								
Average	0.107	0.105	0.007	0.015	6.342	1.465	9.392	11.302
St.Dev.	0.010	0.004	0.002	0.008	1.688	0.350	2.461	3.734
Min.	0.076	0.094	0.003	0.009	3.200	0.987	6.764	6.150
Max.	0.122	0.113	0.010	0.045	13.070	2.944	20.098	26.829
Variance	0.000	0.000	0.000	0.000	2.849	0.122	6.054	13.943

[13], where Pb concentration was 0.0351 mg/kg. However, the tests carried out by Doe *et al.* [14] and Locatelli [15] showed Pb concentrations in the flour which ranged from 0.22 to 0.34 mg/kg and from 0.49 to 0.89 mg/kg, respectively, which are the concentrations much higher than the ones found in the results obtained in this study.

Pb, As and Hg contents found in the analyzed samples of bran and flour (Table 1) indicate that the average content range of these toxic elements was within permissible limits prescribed by the Regulations [10]. Having in mind that the toxic and cumulative effects which these elements may have on human organism, it is necessary to permanently monitor and determine their content. The results show that Zn concentration found in the flour (Table 1) is 11 times lower than in the bran, which complies with the results obtained by Cubadda *et al.* [16]. Zn concentration is equalized between the concentrations found in the tested flour samples and the concentrations found in previously performed tests [8], where the average Zn content in wheat flour was 6.154 ± 0.313 mg/kg.

According to the obtained results, the content of Fe found in the bran varied between 12.45 and 240.48 mg/kg (Table 1), while in the wheat flour it was between 6.76 and 20.09 mg/kg.

The results show that Fe concentration found in the bran was higher than the concentration found in the flour, which complies with the results obtained by Milićević *et al.* [17] who investigated the reactions of different wheat genotypes which were results of application of different types of fertilization and concluded that the mineral nutrition significantly affected the concentration of Fe in the grain.

Analyzing deficiencies in human nutrition, the World Health Organization (WHO) and nutritionists stress the important role of dietary fibers in health maintenance. Pastry products are consumed on a daily

basis, which makes them suitable to compensate for fiber deficiency in food. Being rich in dietary fibers, wheat bran is used in bread and pastry production; therefore, monitoring of the content of toxic elements in both flour and bran is required to ensure production of safe and healthy food and to prevent them from entering the food chain.

TEI and EMI calculations

TEI and EMI score results, calculated using Eq. (1), for the bran and flour samples are presented in Fig. 1. Positive scores in Fig. 1 show increased toxic element and essential microelement content, and these values belong to areas close to larger towns, *i.e.*, areas with higher population density.

The positive TEI values indicate higher levels of toxic elements mainly due to the high level of Pb content (0.29 mg/kg) and of Cd content whose average concentration is 2.4 times higher than the maximum permissible concentration prescribed in the regulations [10].

The results (Fig. 1) show that the concentrations of tested elements are the highest in peripheral parts of the grain, *i.e.*, in the bran, which is especially significant for production of either whole meal bread or bread with added bran.

The positive EMI values account for high concentrations of essential microelements due to high levels of Zn, Fe and Mn concentrations.

Correlation analysis

The data in Table 2 show a correlation between toxic element and essential microelement assays in the bran and the flour samples. These data showed that the contents of Pb, Zn, and Cu found in the flour are independent from the contents of these metals found in the bran. As and Fe contents in the flour are even negatively correlated to the contents of these metals in the bran. Cd and Hg contents in the flour are positively correlated to the contents of these metals in the bran,

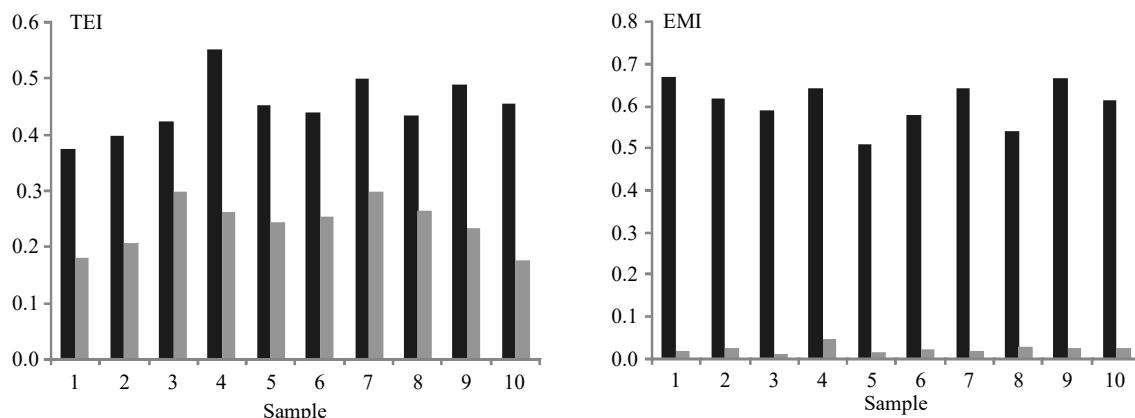


Figure 1. TEI and EMI score results for bran (■) and flour (□).

Table 2. Pearson's correlation coefficients between toxic element and essential microelement assays in the bran and flour samples, with statistical significance expressed as p-level values, written in small parentheses; * – significant at $p < 0.05$ level, ** – significant at $p < 0.10$ level; unmarked correlation coefficients were statistically insignificant

	Flour								
	Pb	Cd	Hg	As	Zn	Cu	Fe	Mn	
Bran	Pb	0.27 (0.145)	-0.03 (0.887)	0.42* (0.022)	-0.05 (0.794)	-0.14 (0.451)	-0.26 (0.159)	-0.17 (0.382)	-0.17 (0.35)
	Cd	-0.16 (0.395)	0.48* (0.007)	-0.24 (0.211)	0.06 (0.749)	0.33** (0.078)	0.25 (0.188)	0.31** (0.096)	0.19 (0.32)
	Hg	-0.04 (0.836)	0.12 (0.512)	0.65* (0.000)	0.33** (0.078)	-0.25 (0.188)	-0.16 (0.413)	-0.23 (0.227)	-0.28 (0.13)
	As	0.14 (0.446)	0.23 (0.222)	0.04 (0.825)	-0.10 (0.595)	-0.01 (0.940)	0.13 (0.496)	-0.06 (0.736)	-0.12 (0.54)
	Zn	0.05 (0.787)	0.01 (0.973)	-0.30 (0.102)	-0.19 (0.303)	0.26 (0.159)	0.17 (0.374)	0.06 (0.739)	-0.10 (0.61)
	Cu	-0.10 (0.613)	-0.14 (0.459)	-0.23 (0.227)	-0.22 (0.237)	-0.01 (0.974)	0.13 (0.502)	0.03 (0.885)	-0.10 (0.59)
	Fe	-0.24 (0.204)	0.07 (0.719)	-0.05 (0.788)	-0.03 (0.864)	-0.05 (0.779)	-0.07 (0.701)	-0.06 (0.736)	-0.38* (0.03)
	Mn	0.09 (0.645)	0.05 (0.791)	-0.03 (0.871)	0.10 (0.584)	0.33** (0.077)	0.36** (0.054)	0.28 (0.131)	0.36** (0.05)

0.48 and 0.65, (statistically significant at $p < 0.05$ level, 95% confidence limit), which indicates that the contents of Cd and Hg in the flour are directly related to their contents in the bran. The content of Cd and Hg in the bran and flour may be associated with their content in the soil where wheat is grown. Correlation between Mn content in the flour and bran is statistically significant at $p < 0.10$ level.

The correlation between Hg in the flour and Pb in the bran, and also between Mn in the flour and Fe in the bran were observed, statistically significant at $p < 0.05$ level. Cd content in the bran correlates Zn and Fe contents in the flour samples, statistically significant at $p < 0.10$ level, while Hg content in the bran correlates As content in the flour samples, at $p < 0.10$ level.

Mn content in the bran correlates both Zn and Cu contents in the flour at $p < 0.10$ level.

Principal component analyses (PCA)

The PCA allows a considerable reduction in a number of variables and the detection of a structure in the relationship between measuring parameters that give complimentary information. The eigenvalues for successive factors are displayed on a so-called "scree-plot", Fig. 2. The number of factors retained in the model for proper classification of experimental data, in original matrix into loading (bran and flour samples) and score (toxic element and essential microelement content) matrices were determined by application of Kaiser and Rice's rule [18]. This criterion retains only principal components with eigenvalues > 1 . All samples

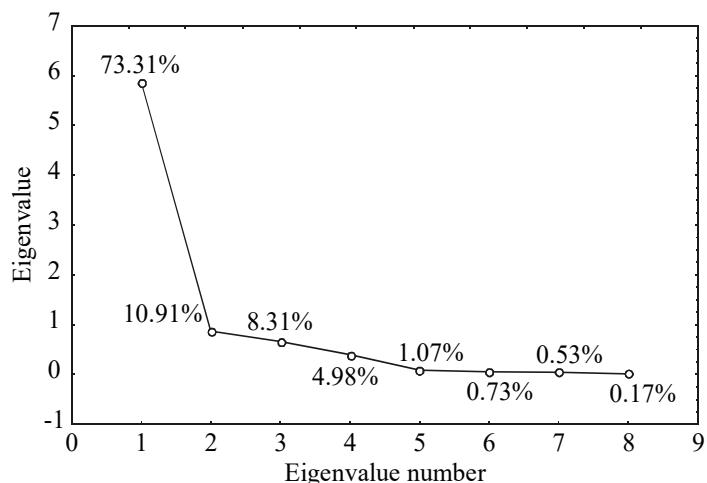


Figure 2. Scree-plot.

having different toxic elements and essential microelement content are shown by descriptive analysis (Table 1) and predicted by the PCA score plot (Fig. 3). The full auto scaled data matrix consisting of different bran and flour samples is submitted to the PCA.

For visualizing the data trends and the discriminating efficiency of the descriptors used, a scatter plot of samples using the first two principal components (PCs) issued from the PCA of the data matrix is obtained (Fig. 3). As can be seen, there is a neat separation of the observed samples, according to the assays used. Quality results show that the first two principal components, accounting for 84.22% of the total variability, can be considered sufficient for data representation and the first two principal components for toxic ele-

ments and essential microelements. Zn (16.38%), Mn (16.19%), Cu (15.73), Fe (15.44%) and Cd (14.99%) have been found the most influential for the first factor coordinate calculation, while the contribution of Hg (89.09%) has been the most important variable for the second factor coordinate calculation.

The influence of toxic elements and essential microelements can be observed in Fig. 3, with higher Cd, Zn, Cu, Mn and Fe contents, and on the left side of the graphic. The PCA graphic showed good discrimination characteristics between the bran and flour samples, which were found different mostly due to the contents of Cd, Zn, Cu, Mn and Fe. The samples having the highest TEI and EMI content are located on the left side of PCA biplot graphic.

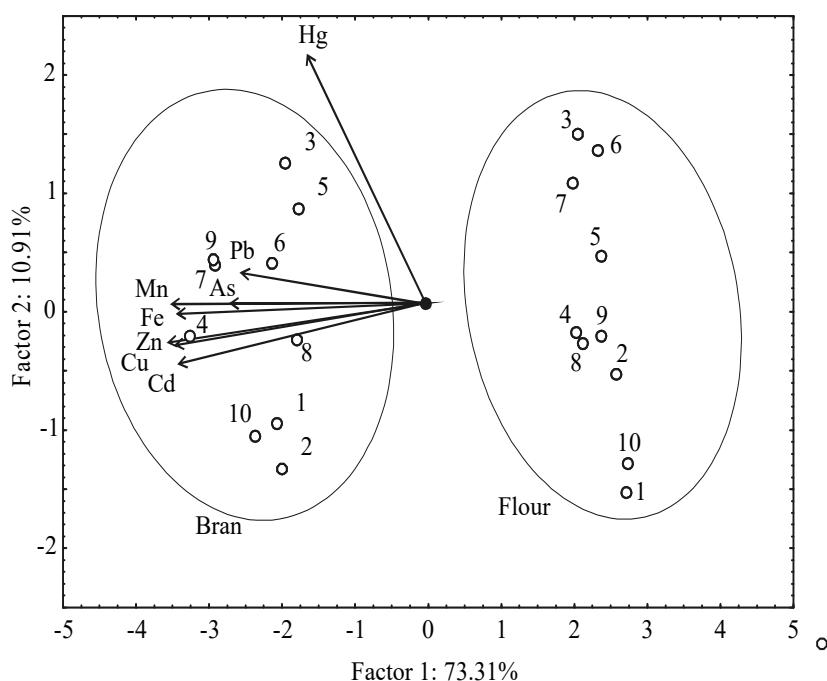


Figure 3. Biplot for toxic element and essential microelement content in the bran and flour.

CONCLUSION

Considering the results of the analysis of toxic elements and essential microelements in the wheat bran and flour, the following could be concluded:

The Pearson table is a useful tool for showing correlation between chemical elements in the bran compared to the content of the elements analyzed in the flour. The content of Cd and Hg in the flour is positively correlated to the content of these metals in the bran, 0.48 and 0.65, which indicates that the content of Cd and Hg in the flour is directly related to their content in the bran.

The Cd concentrations found in the bran, 0.109–0.138 mg/kg, are higher than the maximum permissible concentration levels prescribed by the Serbian legislation (0.05 mg/kg).

The content of Fe in the bran varied between 12.45 and 240.48 mg/kg, while in the wheat flour it ranged between 6.76–20.09 mg/kg. Since the content of this metal, whose role is very important in human nutrition, is less in the flour than in the bran, it would be advisable to fortify the flour used in bread production with iron by adding wheat bran safe from toxic elements.

According to the PCA analysis it has been found that there is a far less quantity of analyzed elements in the wheat bran than in the flour. The factor axes indicate the samples with increased content of certain elements. Quality results show that the first two principal components, accounting for 84.22% of the total variability, can be considered sufficient for data representation and the first two principal components for toxic elements and essential microelements. Zn (16.38%), Mn (16.19%), Cu (15.73), Fe (15.44%) and Cd (14.99%) have been found the most influential for the first factor coordinate calculation, while the contribution of Hg (89.09%) has been the most important variables for the second factor coordinate calculation.

Monitoring of the content of toxic elements in flour and bran is required to ensure production of safe and healthy food, particularly if wheat bran is used for fiber deficiency compensation in wheat based products.

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IZVOD**SADRŽAJ ESENCIJALNIH I TOKSIČNIH ELEMENATA U PŠENIČNIM MEKINJAMA I BRAŠNU**

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(Naučni rad)

Žitarice, brašno i hleb predstavljaju, direktno ili indirektno, jednu od osnovnih komponenti ljudske ishrane, pa je i njihov uticaj na zdravlje ljudi veliki. Zbog značaja koje ove namirnice imaju u ljudskoj ishrani, potrebno je znati njihov mineralni sastav koji je i direktni indikator hranljive vrednosti ovih proizvoda kao i eventualnog zagađenja ovih proizvoda kao posledica zagađenja životne sredine ili posledica primenjenih agrotehničkih mera. Istraživanje je sprovedeno sa ciljem ispitivanja prisustva toksičnih elemenata (Pb, Cd, As i Hg) kao i esencijalnih elemenata (Zn, Cu, Fe i Mn) na odabranim uzorcima pšeničnih mekinja i brašna primenom metode ICP-MS. Praćena je korelacija između sadržaja toksičnih mikroelemenata u pšeničnim mekinjama i brašnu. Rezultati pokazuju statistički značajnu korelaciju (na nivou značajnosti $p < 0,05$) između Cd (0,48), kao i Hg (0,65) u brašnu i mekinjama. Izmerene koncentracije Cd u mekinjama, 0,109–0,138 mg/kg, su veće od maksimalno dozvoljene, koja je propisana Pravilnikom RS (naveden u literaturi) (0,05 mg/kg). Sadržaj Fe u mekinjama se kretao od 12,45–240,48 mg/kg, a u pšeničnom brašnu od 6,76–20,09 mg/kg. Kako je sadržaj ovog, u ishrani ljudi važnog metala, manji u brašnu u poređenju sa mekinjama može se preporučiti da se prilikom prozvodnje hleba vrši njegovo obogaćivanje gvožđem korišćenjem pšeničnih mekinja ili celih zrna pšenice. Analiza glavnih komponenata pokazuje da je samo jedna faktorska koordinata dovoljna za opisivanje 73,31% ukupne varijanse, a da druga faktorska koordinata opisuje 10,91% varijabilnosti sistema. Pošto ove dve koordinate opisuju zajedno 84,22% ukupne varijanse, sistema može se smatrati da one opisuju sistem na zadovoljavajućem nivou.

Ključne reči: Mikroelementi • Mekinje • Brašno • Analiza glavnih komponenti