Determination of essential and toxic elements in products of milling wheat

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Abstract

The bread wheat bran is used as a raw material rich in dietary fiber in the production. Therefore, it is necessary to monitor the content of essential and toxic elements in the flour and bran. This paper investigates essential (Zn, Cu, Fe and Mn) and toxic (Pb, Cd, Hg and As) elements in products of milling wheat grown in the whole territory of Banat, the region in Serbia. Inductively coupled plasma mass spectrometry was used for analysis. The mean contents of the following elements Pb, Cd, Hg, As, Fe, Mn and Zn in wheat kernels were 0.143, 0.007, 0.017, 35.7643, 50.865 and 21.174 mg/kg, respectively. Cluster analysis (CA) and principal component analysis (PCA) was applied to discriminate and to group the different samples, according to element content. Quality results show that the first two principal components, accounting for 80.17% of the total variance, can be considered sufficient for data representation and the first two principal components of toxic elements and essential microelements. Cd (15.28%), Zn (17.91%), Cu (17.08), Fe (16.91%) and Mn (17.54%), have been found the most influential for the first factor coordinate calculation, while the contribution of Pb (27.93%) and Hg (61.86%) has been the most important variable for the second factor coordinate calculation.

Keywords: essential elements, toxic elements, wheat, principal component analysis.

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Wheat is one of the oldest cultivated plants in the world. The fact that wheat occupies the largest surfaces in the world's agricultural production explains its significance. The grain, thanks to its nutritional value, represents the most relevant farming product used in the human nutrition and international trade.

Generally speaking, cereals are necessary for a healthy diet and nowadays a daily consumption of between 4 and 6 portions of cereal derived products is recommended due to their content in fiber, trace minerals and vitamins, which are supposed to prevent various diseases [1,2]. All cereal derived products are rich in carbohydrates and therefore, the base of a well-balanced and healthy diet. This is the reason for the importance of this group of food.

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 [3––6]. It also may contain certain toxic elements (As, Pb, Hg and Cd) which CERCLA Priority List [7] rated according to toxicity as the first, second, third and seventh, respectively.

The wheat production uses fertilizers and raw materials which can be contaminated with following toxic elements: lead, cadmium, arsenic, so an adequate and controlled use of phosphate fertilizers in agricultural production is recommended [8].

Cadmium is particularly stressed here because it is present in phosphate fertilizers and can be found in raw phosphate within the range of 1 to 110 mg/kg, depending on its origin [9]. Both nutritional essentiality and toxicity of an element depend on its concentration and daily intake [10].

The food, due to environmental, may also contain microelements or which body has a tolerance level and above this level these may adversely affect biochemical system [11]. Therefore, it is necessary to assess the adequacy and safety of common daily diet such as wheat flour by monitoring the concentration of toxic and essential elements and to establish their baseline level.

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 wheat, flour and bran obtained by milling wheat grown in Banat region, Serbia. This study was conducted in June 2010.

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Since these elements are present in minute quantities, highly sensitive and accurate analytical techniques were practiced for their measurement. Pattern recognition techniques (principal component analysis – PCA and cluster analysis – CA) were applied to the experimental data (used as descriptors) to characterize and differentiate among the observed samples. These two analyses were used to discriminate different samples, collected at various locations in Banat region.

MATERIAL AND METHODS

Material

Wheat samples were collected from ten locations in the whole territory of Banat (the region Vojvodina, province of Serbia; geographic coordinates of Vojvodina: latitude 45.3167° N, longitude 19.8500° E). The origins of the samples are presented in Table 1.

Table 1. Origins of the samples (geographic coordinates)

Town	Lantitude (N)	Longitude (E)
B. Monostor	45°57'24"	20°16'33"
Coka	45°56’33”	20°08’35”
Elemir	45°26’58”	20°18’11”
Kikinda	45°49’48”	20°27’41”
Kumane	45°32’12”	20°13’25”
Melenci	45°30’30”	20°19’01”
N. Becej	45°36’06”	20°08’22”
Perlez	45°12’17”	20°22’32”
Senta	45°55’41”	20°04’53”
Tornjos	45°52’25”	19°49’32”

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, as reported by Ludajić *et al*. [12].

Bran and flour of 60% extraction were obtained by milling the representative samples of wheat in the mill Brabender Quadrumat Senior (Brabender® GmbH & Co., Duisburg, Germany).

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, Richmond, CA, USA).

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.

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 ± standard deviation (*SD*). Principal component analysis (PCA) was used to discover the possible correlations among measured parameters, while cluster analysis (CA) is used to classify objects into groups. The evaluation of the results obtained was performed using the StatSoft Statistica 10 software.

RESULTS AND DISCUSSION

The element concentrations in wheat kernel are summarized in Table 2.

The most frequently occurring patterns for wheat were Mn > Fe > Zn > Cu > Pb > Cd > As > Hg. Also, it should be stressed that in some instances the contents of Cd were higher than the ones set by Serbian regulation [13].

The mean values of Pb were round 0.143 mg/kg. The results show that Pb concentration in the analyzed wheat was lower than the concentrations found in the tests reported by Nan *et al*. [3], where the total content of Pb found in the flour samples was 0.520–1.030 mg/kg, and also lower than the concentrations found in the tests performed by Hussan *et al*. [14] where the Pb concentration was 0.30 mg/kg. The measured content of lead in wheat grain may be attributed to: its higher content in soil, which is in correlation with an intensity of traffic and industrial production, and the fact that the most polluted wheat grain is found in the area of longitudinal furrow, so the most significant quantity of dirt can be found on its surface. According to Ludajic and Filipovic [15], the Cd ion content in the samples of wheat grown in Central Banat over the period 2003–

Table 2. Descriptive statistics data for toxic elements and essential microelements content (mg/kg) in the wheat kernel, the bran and the flour; SD – standard deviation

Bran	Pb	Cd	Hg	As	Zn	Cu	Fe	Mn
Average	0.143	0.121	0.008	0.028	68.545	9.017	104.232	164.665
SD	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	240.488	235.850
Variance	0.002	0.000	0.000	0.000	181.468	4.976	1332.85	891.495
Flour	Pb	Cd	Hg	As	Zn	Cu	Fe	Mn
Average	0.107	0.105	0.007	0.015	6.342	1.465	9.392	11.302
SD	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
Wheat	Pb	Cd	Hg	As	Zn	Cu	Fe	Mn
Average	0.143	0.114	0.007	0.017	21.174	3.068	35.764	50.865
SD	0.039	0.005	0.002	0.008	6.658	0.653	6.300	9.118
Min.	0.112	0.103	0.003	0.009	12.083	1.794	25.416	30.639
Max.	0.225	0.128	0.012	0.048	48.528	4.741	51.524	69.823
Variance	0.002	0.000	0.000	0.000	44.334	0.427	39.690	83.135

–2007 was lower (0.065 mg/kg) than the obtained concentration levels (Table 2).

Ludajic *et al.* [16] also reported that there is a correlation of Cd content in wheat grain in relation to its content in soil which indicates that wheat adopts Cd from the soil. Cd enters agricultural land via applied phosphorus fertilizers [17].

The concentrations of analyzed microelements in the collected wheat kernel, wheat bran and flour samples are shown in Table 2.

Cd content in the analyzed samples of flour ranged between 0.094 and 0.113 mg/kg (Table 2), which is a concentration four times higher than the concentrations found in the tests reported by Tejera *et al*. [1], 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 showed that Pb concentration in the analyzed flour was higher than the concentrations found in the tests reported by Tejera *et al*. [1], 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 Zhange *et al*. [18] where the Pb concentration was 0.0351 mg/kg. However, the tests carried out by Doe *et al*. [19] and Locatelli [20] 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 2) indicated that the average content range of these toxic elements was within permissible limits prescribed by the Serbian Regulations [13]. Having in mind that the toxic and cumulative effect which these elements may have on the human organism, it is necessary to permanently monitor and determine their content. The results show that Zn concentration found in the flour is 11 times lower than in the bran, which complies with the results obtained by Cubadda *et al*. [21] Zn concentration is equalized between the concentrations found in the tested flour samples and the concentrations found in previously performed tests, where the average Zn content in wheat flour was 6.154±0.313 mg/kg.

According to the results obtained, the content of Fe found in the bran varied between 12.45 and 240.48 mg/kg (Table 2), while in the wheat flour it was between 6.76 and 20.09 mg/kg.

Cluster analyses of metal content profile

A dendrogram of metal content data of different samples using complete linkage as an amalgamation rule and the City block (Manhattan) distance as a measure of the proximity between samples (in eight variables factor space) is shown in Fig. 1. The measuring of this type of distance measurement yields results similar to the Euclidean distance, but in this measuring technique, the effect of single large differences (outliers) is

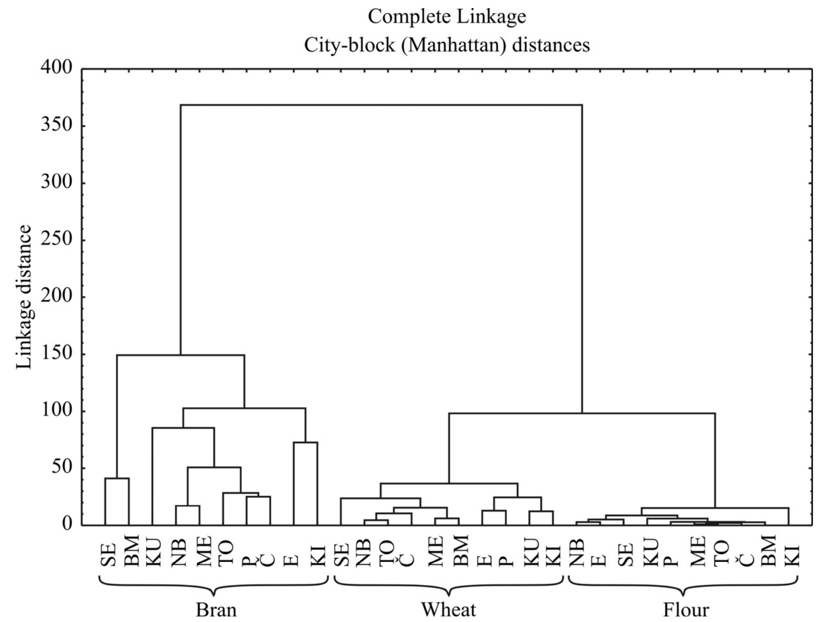

Figure 1. Dendogram of different samples, based on the metal content (determined by ICP-MS analysis). BM – Banatski Monoštor, Č – Čoka, E – Elemir, KI – Kikinda, KU – Kumane, ME – Melenci, NB – Novi Bečej, P – Perlez, SE – Senta, TO – Tornjoš.

dampened (since they are not squared), [22]. The dendrogram based on inductively coupled plasma mass spectrometry (ICP-MS) data showed the proper distinction between different samples and groups of samples. As shown in Fig. 1, there is similarity in the metal content between samples of flour (with lower metal content compared to other samples); a second group, containing the samples of wheat kernel; and the third cluster which comprised samples of bran, with the increased metal content. The linkage distance (shown on the ordinate axis) between the first two clusters was evident (nearly 100), while the distance between the second and the third cluster was more than 350.

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 [22–24]. All samples having different toxic element and essential microelement content are shown by descriptive analysis (Table 2) and predicted by the PCA score plot (Figure 2). Centered

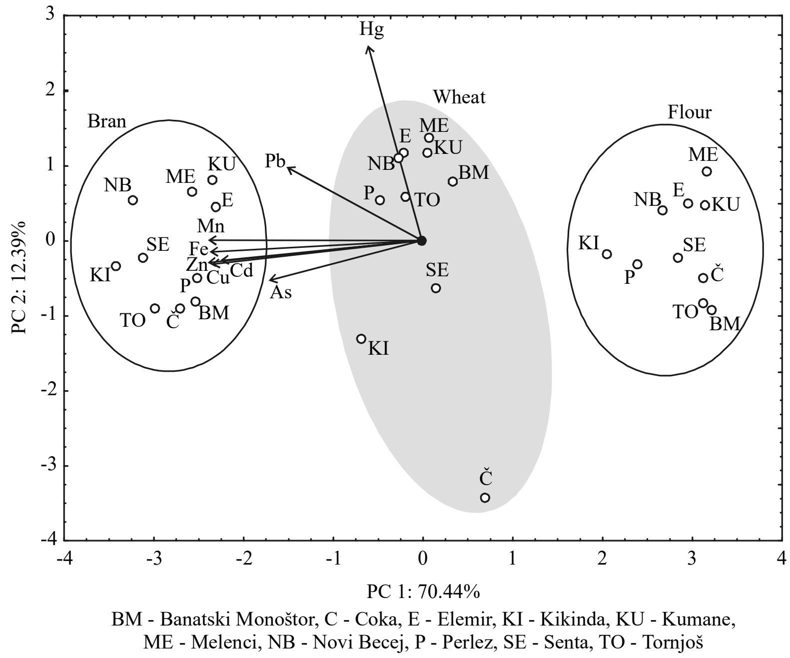

Figure 2. Biplot for toxic element and essential microelement content in the bran, wheat and flour.

logratio (clr) transformation is performed on dataset, before PCA is done. This transformation is based on dividing each sample by the geometric mean of its values, and taking the logarithm of the obtained results. This transformation was introduced in papers by Aitchison [25,26], and it was intended to be used with compositional data in the context of PCA. In this article, data is transformed with clr, and the PCA biplot is obtained as a projection of eight-dimension factor space in the first factor plane (defined by the first and the second principal component).

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 (Figure 2). 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 80.17% of the total variance, can be considered sufficient for data representation of toxic elements and essential microelements. The first two eigenvalues were: 5.64 and 1.00. Cd (15.28%), Zn (17.91%), Cu (17.08), Fe (16.91%) and Mn (17.54%), have been found the most influential for the first factor coordinate calculation, while the contribution of Pb (27.93%) and Hg (61.86%) 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. 2, with higher Cd, Zn, Cu, Mn and Fe contents, and on the left side of the graphic (bran samples). 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. An especially high content of Zn was measured in the bran samples collected in the locality of Kikinda. The increased content of Zn in comparison to other analyzed samples is the result of its presence in the soil surrounding the metal foundry, which is a major source of soil contamination with zinc. The wheat samples are shown in the central part of the PCA graph. It can be seen that the content of Cd, Zn, Mn and Fe in wheat is lower compared to the bran samples. Higher content of these elements was observed in Kikinda, Senta and Novi Becej, which is explained by the fact that the wheat samples were taken from the parcels located in the industrial zone. The results also show that the wheat grains transport significant impurities due to the specific structure of the atoms which enables concentration of pollutants originating from various sources on their surface. The flour samples are positioned on the right side of PCA graph, indicating that these samples contain the least Cd, Zn, Cu, Mn and Fe contents, compared to bran and wheat samples.

The amount of essential and toxic elements in relation to the daily bread consumption

The importance of a certain nutritional component to human health is assessed using the data about its nutritional value and not the frequency of its consumption. Hence, health organizations worldwide have started to recommend a daily intake of nutritional and other food components. World institutions which recommend the most relevant data regarding the nutritional value of certain food components are the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

The average content of toxic elements in flour, bran and wheat kernel is shown in Fig. 3, while the average daily consumption of bread and bakery products according to the WHO is shown in Fig. 4.

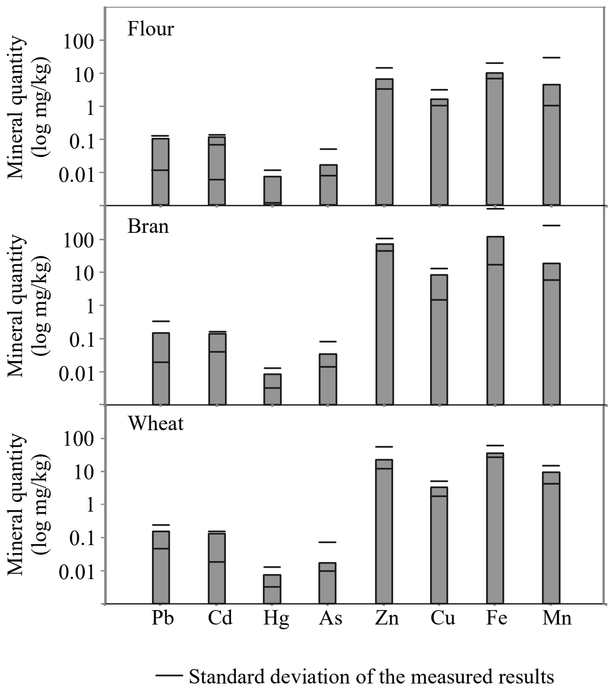

Figure 3. The average content of toxic and essential elements in flour, bran and wheat kernel.

The recommended daily intakes of essential elements are defined in the Republic of Serbia according to the results of investigations on national diet and nutrition for the purpose of prevention and health protection. The recommended daily intakes according to the Regulation on Labelling and Marking of Packaged Foodstuffs [27] for Fe, Zn, Cu and Mn are 14, 15, 2 and 2 mg, respectively. Having analyzed the daily consumption of bread and wheat products, the daily intakes of essential elements were assessed and compared with the relevant dietary reference values. The obtained

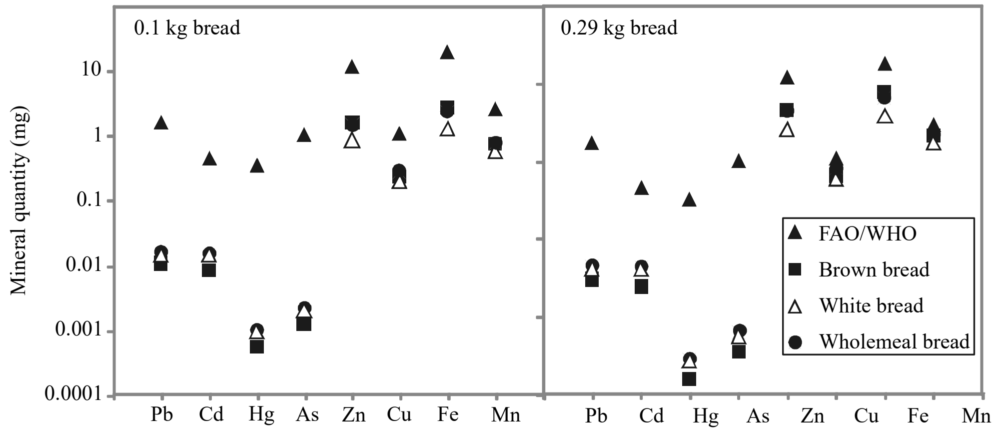

Figure 4. The amounts essential and toxic elements in relation to the daily bread consumption.

data lead to the conclusion that the daily consumption of wholegrain products would meet about 50% of daily needs for copper of an adult, about 23-50% for zinc and about 70% of daily needs for iron. Since the consumption of white flour products is higher in our country, these values are much lower.

The dietary zinc intake normally ranges between 12 and 15 mg of zinc per day. There is a wide tolerance range of normal amounts of zinc intake consumed by food and those that may have harmful effects. The toxic dose of zinc ions is difficult to determine precisely because its effects do not only depend on the amount of zinc salt, but also on its concentration and interaction with other microelements found in food, mainly on Fe, Cu, Ca ions and other.

Joint Expert Committee on Food Additive (JECFA) recommended that the provisional tolerable weekly intake (PTWI) of Cd should be 7 µg/kg or 420 µg per week for an adult bodyweight weighing 60 kg on average [28]. According to the calculations, if the wholegrain products were consumed, the Cd intake would be about 46% of acceptable level. According to JECFA/ /WHO the provisional tolerable weekly intake (PTWI) of Pb is 25 µg/kg bw or 1.5 mg for adults and 4 µg/kg bw for Hg, while for As the acceptable daily intake is 2 µg/kg bw. The obtained results show that the consumption of wholegrain products ensures a daily intake of 12.4–17.7% of acceptable level of Pb, 5% of Hg and 3.5–9% of As.

In the developed countries human diet is characterized by low dietary fiber intake. As bakery products are consumed every day, they can be a very convenient food for fiber deficiency correction.

The World Health Organization recommended a daily fiber intake of 25–38 g where at least one half should be from wheat. According to the Regulation on the quality of grain, milling and bakery products [29] a minimum of 7% bran is added in relation to the total amount of flour used for making wheat bran bread.

The average daily consumption of bread and bakery products in Serbia is 290 g according to the data provided by the Central Institute of Statistics. Figure 4 shows the amounts of essential and toxic elements in relation to the daily wheat bran bread, wholemeal bread and wheat flour bread consumption. Taking into consideration the recommended daily intake and the obtained results, it can be concluded recognized that by consuming the wheat bran bread an adult could meet about 10–22% of the daily needs for zinc, about 11–30% for copper and around 17–37% for iron.

Although copper is one of the elements essential to human health, if its dietary intake is higher than recommended, it may lead to harmful effects. Hence the monitoring of the content of this metal in wheat, flour and bread samples are of great importance.

The obtained results show that Fe content in bran ranged from 12.045 to 240.48 mg/kg (Table 2), while in wheat flour it was between 6.76 and 20.09 mg/kg. As the content of this important metal in the human diet was lower in flour, then in bran, bread production should include bran or wholemeal wheat to fortify bread with iron. Thus the Fe content could be increased to meet the daily needs of the human body for this element and to prevent certain diseases that may occur due to Fe deficiency, such as anemia.

The obtained results show that consumption of wheat bran bread and bakery products ensure that about 8.69–12.71% of acceptable level of Pb intake is met. Different types of flour and semolina are produced by milling, with bran as a by-product. Unlike the central parts of endosperm, lead is found mainly on the surface layer of wheat grain. In order to decrease the Pb intake, surface treatment is necessary before milling.

According to JECFA/WHO [28] the provisional tolerable weekly intake (PTWI) of Hg is 4 µg/kg bw, while for As the acceptable daily intake is 2 µg/kg bw. The daily intake of bread and bakery products enriched with bran and made from analyzed wheat would ensure the intake of 2.5–6% Hg and 1.85–6.3% As. The obtained results support the conclusion based on the research of Cubadda *et al*. [21] that the content of As is higher in bran than in flour.

According to the calculations, the products made from analyzed flour would provide a daily intake of 35% of acceptable level of Cd intake. Chaudri *et al.* [30] reported the decrease by 31% of the Cd content in white flour when compared with wholegrain flour. White flour comes from the central parts of the grain (endosperm), while wholegrain, brown flour of high extraction and bran are found near or on the surface (bran) from grain parts where the Cd content is doubled. Brüggemann and Kumpulainen [31] also reported the decrease by 2–25% of the Cd content, while the maximum decrease was reported in the flour containing the lowest content of ash.

The results show that if the products made of analyzed flour were consumed, the daily intake of Pb would be 11%. Compared with the investigation conducted by Conti *et al*. [32], where the consumption of flour products led to the Pb intake of 1%, the calculated high Pb intake is likely to originate from the lead petrol or from atmospheric deposition of Pb as possible routes of surface contamination.

CONCLUSION

Based on the results of the analysis of essential and toxic elements in products of milling wheat in Banat, it could be concluded that the most frequently occurring pattern of wheat has been Mn (50.865) > Fe (35.764) > Zn (21.174) > Cu (3.068) > Pb (0.143) > Cd (0.114) > As (0.017) >Hg (0.007). The measured content of lead in wheat grain may be attributed to: its higher content in soil, which is in correlation with intensity of traffic and industrial production, and the fact that the most polluted wheat grain is found in the area of longitudinal furrow. Contents of toxic elements (Pb, As and Hg) in all samples of bran and flour are within permissible limits prescribed by the Regulations. Toxic and cumulative effect of Pb, As and Hg may have on the human organism, it is necessary to permanently monitor and determine their content. Using cluster analysis and principal component analysis, the observed samples are successfully grouped, according to the assays used. Daily average consumption of 290 g of either wheat bran bread or wholemeal bakery products contributes to the following intakes 10–22%, 11–30%, 17–37% of zinc, copper and iron, respectively per day. The obtained results show that the consumption of wholegrain products ensures a daily intake of 12.4–17.7% of acceptable level of Pb, 5% of Hg and 3.5–9% of As. A regular quality control of wheat in different agroecological conditions is necessary. It will provide production of healthy and safe foodstuffs and prevent the presence of toxic elements in the food chain. The results are of great importance for bread and pastry manufacturers when deciding whether to use the wheat in the white bread of whole meal wheat product.

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IZVOD

ODREĐIVANJE ESENCIJALNIH I TOKSIČNIH ELEMENATA U PROIZVODIMA MLEVENJA PŠENICE

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Kao sirovina bogata prehrambenim vlaknima u proizvodnji hleba i peciva koriste se pšenične mekinje, te je neophodno praćenje sadržaja toksičnih i esencijalnih elemenata kako u brašnu tako i u mekinjama. U ovom radu ispitan je sadržaj esencijalnih (Zn, Cu, Fe i Mn) i toksičnih elemenata (Pb, Cd, As i Hg) u mlinskim proizvodima žita, gajenog na celoj teritoriji Banata, u region u Srbiji. Analize su rađene masenom spektrometrijom sa indukovanom kuplovanom plazmom (ICP-MS). Pšenično zrno sadrži Pb, Cd, Hg, As, Fe, Mn i Zn u količinama od 0,143, 0,007, 0,017, 35,7643, 50,865 i 21,174 mg/kg, redom. Klaster analiza (CA) i analiza glavnih komponenata (PCA) su primenjene za razdvajanje i grupisanju različitih uzoraka na osnovu sadržaju elemenata. Rezultati pokazuju da su prve dve glavne komponente opisale 80.17% od ukupne varijanse, što se može smatrati dovoljnim za prikazivanje eksperimentalnih podataka za toksične i esencijalne mikroelemenata. Najveći uticaji na izračunavanje prve faktorske kordinate imajali susadržaji: Cd (15.28%), Zn (17.91%), Cu (17.08%), Fe (16.91%) i Mn (17.54%), dok je doprinos sadržaja Pb (27.93%) i Hg (61,86%) bio najveći za izračunavanje druge faktorske kordinate.

Ključne reči: Esencijalni elementi • Toksični elementi • Pšenica • Analiza glavnih komponenti