www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 633.11:664.71:[546.47+546.72 (497.11)
DOI: 10.5937/ffr0-42946

Original research paper

MICRONUTRIENT COMPOSITION OF MILLING STREAMS OF TRADITIONAL WHEAT CULTIVARS FROM SERBIA

Dragan R. Živančev*[1], Maja S. Buljovčić[2], Jordana M. Ninkov[1], Igor B. Antić[2], Sanja Z. Mikić[1], Simona Ž. Jaćimović[1], Bojan Đ. Jocković[1]

[1]Institute of Field and Vegetable Crops, Small Grains Department, 21101 Novi Sad, Maksima Gorkog 30, Serbia
[2]University of Novi Sad, Faculty of Technology, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia

Abstract: The deficiency of micronutrients in the human diet is widely recognised as a global problem. The objective of this study was to assess the content of two essential minerals, iron and zinc, in milling streams of old heritage wheat cultivars that have not been produced for more than a half-century to discover potential parent lines with high mineral content for breeding. Kernel size of seven Serbian old wheat cultivars was evaluated for ash content, thousand-grain weight and share of specific kernel fractions. Three cultivars, Crnozrna, Rumska Crvenka and Stara Banatka, were selected as the most appropriate for milling and further analysis of mineral content in the milling streams. The cultivars were milled in a laboratory mill to obtain six flour and two by-product streams. At the same time, iron and zinc content was determined on a graphite furnace atomic absorption spectrometer. After mixing all six flour streams obtained from each cultivar, three different flour types were obtained. The flour with the highest ash content was the "1100" type originating from cultivar Stara Banatka, characterized by the highest iron and zinc content (54.9 mg/kg d. b. and 5.407 mg/kg d. b., respectively). It was estimated that consumption of a daily average bread portion (166 g according to national statistical data) made from Stara Banatka flour could provide about 90% of the recommended daily intake for iron. Therefore, Stara Banatka may be a target cultivar for wheat breeders searching for high-iron parent wheat lines.

Key words: *heritage varieties, ash, high-mineral lines, iron, zinc, daily-intake*

INTRODUCTION

Wheat (*Triticum aestivum* L. subsp. *aestivum*) is the most important cereal for breadmaking due to the unique viscoelastic properties of its flour and dough. Current production of wheat around the globe is above 730 million tons per year during the 5 years (2016 - 2020), according to the Food and Agriculture Organisation of the United Nations (FAO) (Food Balance Sheet, 2023). Among the young population in Serbia, the consumption of white bread is still dominant at 55 per cent, whereas the consumption of half-white bread is below 15% (Šereš et al., 2017). Wheat is a substantial source of carbohydrates (~55%), providing 20% of the daily required calories. It could contain a substantial amount of different

Corresponding author: Phone: +381214898208 Fax: +38121450725
E-mail address: dragan.zivancev@ifvcns.ns.ac.rs

micronutrients, such as zinc (Zn) and iron (Fe) (Kumar et al., 2011). However, Zn and Fe deficiency represents a big issue around the globe. In a few countries in Africa and south Asia, almost every second child suffers from Zn deficiency (Victora et al., 2021) and in poor and developing countries about 60% of children below 5 years are anaemic (Zlotkin & Dewey, 2021), which is a direct consequence of Fe deficiency. Iron deficiency is also usual among women, being higher in developing than in industrial countries (Zimmermann & Hurrell, 2007). Besides, every third surgical patient is anaemic, according to the research of Füllenbach et al. (2019).

The wheat milling process has the task to break down the wheat kernel and to divide the endosperm part from germ and bran. The gained parts of the endosperm are, thereafter, reduced into flour particles. During the milling process, wheat flour is separated and classified into different types based on their ash content. The classification of flour into different types is not uniformly regulated in European countries (Zanirato, 2013). The milling process affects the mineral content of the end flour since different milling streams contain different amounts of minerals. It was observed that wheat bran possessed a higher amount of Se (Ferretti and Levander, 1974), Zn and Fe (Eagling et al. 2014) than flour streams. On the other hand, Fe and Zn from whole grain flour and wheat bran are less bioavailable than from flour milling streams (Erdman, 1981) due to the presence of phytic acid (PA) in cereal kernels (Pandey, Szakacs, Soccol, Rodriguez-Leon & Soccol, 2001). Having a high chelating activity, PA can bind minerals as a ligand in a coordinate complex, (Bock, 2000) and together with the ash content (flour type) determine the available mineral content in flour.

Therefore, it is necessary to search the available germplasm for wheat cultivars that could be used for breeding new high-mineral lines. One of the existing strategies for preventing micronutrient deficiency through plant breeding or agronomic practices is biofortification (Bouis, Hotz, McClafferty, Meenakshi & Pfeiffer et al., 2011). Currently, several studies reported differences among a few wheat cultivars in mineral concentration in kernels (Garvin, Welch, & Finley, 2006; Morgounov et al., 2006, Zhao et al., 2009). Few studies analysed the distribution of Fe and Zn in milling streams (Eagling et al., 2014, Živančev et al., 2021), but focusing only on modern cultivars.

Therefore, the scope of this study was to examine the mineral content of milling streams from old heritage Serbian wheat cultivars that exist only in germplasm collections to find wheat cultivars that could be used as a parent of new high-mineral lines. The aim of the study was also to assess the nutritional value of old wheat cultivars by the content of Fe and Zn in the milling streams.

MATERIALS AND METHODS

Grain materials

To select suitable cultivars for the analysis, seven Serbian traditional bread-wheat cultivars chosen from the collection of FAO GRAINEFIT BSF-4 FAO project were examined (listed in Table 1). Cultivation of these cultivars was performed at the experimental field Rimski šančevi, Novi Sad, Serbia (45°20’ N, 19°51’ E) during 2021/2022 season.

Methods of cultivar triage for mineral analysis

The selection of three cultivars for milling and determination of mineral content in milling streams was done based on the ash content analysis (according to the method ISO 749 EN, 1977), thousand-grain weight (TGW) (ISO 520 EN, 2010) and kernel size. TGW was expressed on dry basis matter (d.b.). Kernel size distribution was determined using a Pfeuffer device Sortimat (Kitzingen, Germany) sifting 100 g of clean grain samples of every wheat cultivar for 1 min through three sieves with openings 20 × 2.8 mm, 20 × 2.5 mm 20 × 2.2 mm and bottom. Analyses were performed to select cultivars with two extremes and average parameter values (approximately maximum, mean and minimum) to gain a wide range of mineral content of flour streams.

Milling

The 3000 g of each of the three wheat cultivars was first stabilised at room temperature. After that, the moisture was determined according to the method ISO 712 EN, 2012 and increased to 13.5%. Also, the samples were tempered for 24 h at room temperature. Moisture content was increased to 15.0% half an hour before milling in a Bühler pneumatic laboratory mill MLU 202 (Uzwil, Switzerland).

Table 1.
Cultivar name, year of the cultivar collection and cultivar's DOI number

Name	Collection year	DOI
Belgrade 1	1929	10.18730/ZAEHP
Rumska Crvenka	1958	10.18730/ZAF77
Crnozrna	1964	10.18730/ZAFXX
Crvenica	1949	10.18730/ZAEJQ
Šević́eva Šišulja	1962	10.18730/ZAF66
Stara Banatka	1950	10.18730/ZAG0*
Serbia 63	1900	10.18730/ZAEGN

The 8 milling streams of the chosen wheat cultivars were gained from three break passages (B1-B3); three reduction passages (M1-M3) – a total of six flour fractions and two by-products: bran and short fractions.

Iron and zinc determination

Homogenized samples – each milling fraction and whole grains of three chosen Serbian old wheat cultivars were weighted (about 0.5 g) and transferred into the Teflon vessels for microwave digestion. Concentrated nitric acid (8 mL, ccHNO3, 69%, J. T. Baker (Center Valley, USA)) and hydrogen peroxide (1 mL, H_2O_2, 30% J. T. Baker (Center Valley, USA)) were added. Microwave digestion system (Ethos One, Milestone, Italy) equipped with a segmented rotor holding for 10 microwave vessels of high pressure (HPR-1000/10S) and an internal temperature sensor were used for the digestion of the samples. During each sequence, three Teflon vessels that contained the mixture of HNO_3 /H_2O_2 were provided as a blank sample to assess the contamination. The samples were digested for 40 min at 200 °C and a power of 1600 W according to the manufacturer's recommendation (Milestone Ethos Microwave Digestion System method). After cooling at room temperature, the digests were diluted using ultra-pure water (Milli-Q, Simplicity, Millipore, France of 18.2 MΩ cm resistivity) into a 25 ml plastic flask and finally transferred to a previously acid-cleaned and labelled polypropylene vessel for further analysis. From each sample, duplicates were digested and each sample solution was analyzed in triplicates.

Graphite furnace atomic absorption spectrometer (Agilent Technologies, Santa Clara, USA) (GFAAS) with deuterium background correction, was used to quantify Fe and Zn concentrations in sample digests. The assembly was operated from an interfaced computer running SpectraA software. Argon (5.0, Messer, Beograd, Serbia) was used as the inert gas. Agilent Technologies hollow cathode lamps were used as line sources for all analytes. Quantification of Fe and Zn was done using acidified aqueous metal standards (J. T. Baker, Center Valley, USA) by an external calibration procedure. Calibration standards were prepared using stock solutions in the same acid matrix used for the investigated samples. Each recording was repeated three times. The wavelengths used for the determination of Fe and Zn were 372.0 nm and 307.6 nm, respectively.

Quality assurance

The method performance was checked using recovery experiments on the following spiking levels (n = 4): Zn (2.00 and 20.0 mg/kg) and Fe (10.0 and 200 mg/kg). The repeatability of the method was determined as the relative standard deviation (RSD, in %) of the heavy elements content in fortified samples. Other parameters for the method verification were: limits of detection (LOD) and quantification (LOQ), recovery, and instrumental linearity (expressed as correlation coefficients R^2), summarized in Table 2.

Table 2.
Summary of the method verification of the GFAAS method for Fe and Zn determination

Heavy metal	LOQ (mg/kg)	Recovery*(%)		Average recovery, (%)	RSD (%)	R^2
		Level[a]	Level[b]			
Zn	1.00	92.4±4.5	98.1±5.2	95.3±5.3	5.6	0.995
Fe	1.35	90.5±9.1	94.7±7.4	92.7±7.7	8.3	0.995

**Recovery is expressed as a mean value of 4 replicates ± standard deviation (SD)*

Phosphorus (P) analysis

Every milling fraction and whole grain of three selected cultivars were analysed for phosphorus (P) after microwave digesting the samples in concentrated HNO_3 and H_2O_2 (0.5 g samples in digestion solution of 10 mL HNO_3 + 2 mL H_2O_2, Vt = 50 mL) by stepwise heating up to 180 °C using a Milestone Vario EL III for 35 min. The concentration of P was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Vista Pro-Axial, Varian). Quality assurance and quality control were conducted on the results of successfully passed AFPS Animal Feeds PT Scheme provided by LGC. The prepared samples were analysed in two replicates.

Statistical analysis and calculations

Kernel properties (TGW and kernel size distribution : >2.8 mm, 2.8/2.5 mm, 2.5/2.2 mm and <2.2 mm) of wheat cultivars and the total content of Zn, Fe, and P of every milling fraction were tested for significance by using one-way analysis of variance (ANOVA) by InfoStat software Version 2016e (UNC, Argentina). Cumulative curves of ash and mineral content (Zn and Fe) from milling streams of Serbian old wheat cultivars were computed on a dry matter basis (d.m. basis), by the formula according to Fišteš and Tanović (2014). Multivariate analysis of principal components (PCA) was used to find retrieval of underlying interdependences of kernel size distribution and ash content, Zn, Fe and phosphorous content, and Serbian old wheat cultivars by using XLSTAT-Pro, demo version, Version 5.03, 2014 software (Addinsoft, Paris, France). Also, the same statistical analysis was applied for the retrieval of underlying interdependences of kernel size and ash content, the content of phosphorus and each micronutrient independently of milling fractions of Serbian wheat cultivars.

The estimation of iron intake trough consumption of an average bread portion in Serbia, was calculated according to Equation 1:

$$m_m = \frac{c_m \times m_f}{1000} \quad (1)$$

m_m (mg d.m.) – amount of iron provided by an average portion of 60%-flour bread); c_m (mg/kg d.b.) – iron content in 60% flour; m_f (g d.m.) – flour mass necessary for the production of an average bread portion in Serbia (calculated according to Eguation 2):

$$m_f = \frac{166\ g \times 86\%}{137\%} \quad (2)$$

166 g –average daily bread portion consumed in Serbia (Household Budget Survey, 2021), 137% – average yield of white bread (%) (Kovačević, 2011), 86% – percentage of dry ingredients in flour according to the standard Basic Straight-Dough Bread-Baking Method – Long Fermentation Method 10-09 (AACC, 2000).

RESULTS AND DISCUSSION

Grain size

To select three wheat cultivars suitable for milling and subsequent analysis of Zn and Fe content in milling streams, kernel properties (TGW and kernel size distri-bution: >2.8 mm, 2.8/2.5 mm, 2.5/2.2 mm and <2.2 mm) were measured (Table 3). The one-way ANOVA showed that wheat cultivar Rumska Crvenka possessed significantly the highest ash content and percentage of kernels over 2.8 mm, whereas shares of other kernel fractions (2.8/2.5 mm, 2.5/2.2 mm and <2.2 mm) were significantly the lowest. On the other hand, the ash content in the wheat cultivar Stara Banatka was significantly the lowest, whereas the share of kernel fractions above 2.5 mm was significantly the highest. Besides Rumska Crvenka and Stara Banatka, wheat cultivar Crnozrna was also included for further examination based on its statistically lowest TGW and percentage of kernels above 2.8 mm. Range values for kernel size parameters of Serbian old wheat cultivars were similar/comparable to those of modern wheat cultivars (Živančev et al., 2021). However, the kernels of old wheat cultivars were smaller than those of modern ones since modern Serbian wheat cultivars Simonida, NS Todorka and Zvezdana, according to Živančev et al. (2021), had TGW > 35 g d.b. and over 50% kernels with size frac-tion above 2.8/2.5 mm.

Fe and Zn content in the whole wheat grains

The total Fe, Zn, and P content in whole wheat grains of 3 selected Serbian old wheat cultivars is presented in Table 4. The content of Fe was not statistically different among the cultivars while the content of P was statistically higher in Rumska Crvenka compared to the other two wheat cultivars. Also, Zn content was statis-

Table 3.
Mean values of ash content, thousand grain weight (TGW) and grain sizes of seven wheat cultivars analysed by one-way ANOVA

Wheat Cultivar	Ash content (%)	TGW (g d.b.)	>2.8 mm (%)	2.8/2.5 mm (%)	2.5/2.2 mm (%)	<2.2 mm (%)
Belgrade 1	1.73±0.03[a]	34.26±1.46[a]	29.53±1.25[b]	58.40±0.72[b]	7.50±0.35[de]	4.27±0.85[d]
Rumska Crvenka	1.69±0.01[a]	32.15±1.41[ab]	36.13±0.46[a]	49.63±1.60[d]	7.87±0.83[d]	6.03±0.64[d]
Crnozrna	1.65±0.03[ab]	27.31±0.77[b]	15.03±1.07[d]	59.53±0.65[b]	12.97±1.05[c]	12.53±0.06[c]
Crvenica	1.53±0.02[bc]	27.88±1.24[b]	6.80±0.10[e]	60.3±0.75[ab]	17.23±0.64[b]	15.27±0.91[b]
Ševićeva Šišulja	1.53±0.07[bc]	32.01±1.89[ab]	35.17±0.65[a]	54.03±1.29[c]	5.80±0.61[e]	4.67±0.21[d]
Stara Banatka	1.49±0.04[c]	31.03±1.54[ab]	24.60±0.10[c]	63.43±0.57[a]	7.10±0.10[de]	4.57±0.50[d]
Serbia 63	1.46±.0.06[c]	28.18±4.75[ab]	3.10±0.17[f]	49.97±1.77[d]	24.40±0.85[a]	22.23±1.00[a]

Different letters within column indicate significant difference according to Tukey's test at 0.05 probability level

Table 4.
Iron, zinc and phosphorus content in whole grains of selected Serbian old wheat cultivars

Wheat Cultivar	Fe mg/kg d.m.	Zn mg/kg d.m.	P mg/kg d.m.
Crnozrna	38.38±1.24[a]	19.31±0.02[b]	2932±31.11[b]
Rumska Crvenka	40.73±2.55[a]	34.40±1.90[a]	3503±36.77[a]
Stara Banatka	42.29±2.38[a]	13.86±0.87[c]	2954±33.94[b]

Different letters within column indicate significant difference according to Tukey's test at 0.05 probability level

tically highest in Rumska Crvena but lowest in Stara Banatka.

The iron range determined in the present selection of old wheat grains (38.38–42.29 mg/kg) was in line with the findings from the study by Zhao et al. (2009). Furthermore, the Zn level in Rumska Crvenka reached the maximum value (34.5 mg/kg) reported in the above-mentioned study. Stara Banatka and Crnozrna grains were much higher in iron than in zinc.

PCA analysis of mineral content in wheat grains

PCA was performed to estimate the potential use of old wheat cultivars as parents of new high-mineral lines (Fig. 1). Zinc in whole wheat grains was positively related to ash content and close to phosphorus (P). Rumska Crvenka was positioned close to these parameters, indicating a high content of Zn, P and ash in this cultivar. A real assessment of the bioavailability of micronutrients in cereals is not possible without the phosphorus content, because approximately two-thirds of the total P in wheat grain is phytates that bound minerals (Steiner et al., 2007). Therefore, this may indicate the low bioavailability of micronutrients in the set of Serbian old cultivars with high content of P and Zn. Iron content in whole wheat grains showed a positive relationship with TGW and percentage of grain fractions >2.8 and 2.8/2.5 mm. Stara Banatka was positioned close to these parameters, implying high values of Fe, TGW and kernel size in this cultivar. Also, the biplot of PCA depicted that Fe content was unrelated with Zn content. Furthermore, Crnozrna was not close to any of the measured variables.

Extraction yield and ash content

The highest flour yield in all three cultivars was gained from the first reduction passage M1 with a range of 29.33-31.00% (Table 5.). The wheat cultivar with the lowest ash content in 4 flour fractions (B1, B2, M1, M2 and M3) of all three examined cultivars was Rumska Crvenka (Table 5.). The calculated ash content in a mixture of all flour streams was below 0.45% (d. b.). Therefore, it was possible to produce extra white flour Type '400' (Pravilnik, 2018.) from the milling streams of Rumska Crvenka. The ash content in a blend of all six flour streams of this cultivar was below 0.40% (d. b.) comprising more than 53% of flour yield (Y) (Fig. 2 A). The wheat cultivar with the highest ash content in all six flour fractions was Stara Banatka (Table 5). The ash content in the blend of six flour streams was above 1.00% (d.b.) comprising more than 51% of flour yield (Fig. 2 C). Therefore, the milling streams of Stara Banatka yielded dark flour Type '1100' (Pravilnik, 2018). It was possible to produce white flour Type '500' (Pravilnik, 2018) from Crnozrna, since the ash content in the blend of flour streams was 0.518% (d. b.) (Fig. 2 B). Additionally, the ash content of flour milling streams from the traditional cultivars Rumska Crvenka and Stara Banatka quite differentiated from those of modern wheat cultivars, based on the findings of Živančev et al. (2021). Namely, ash content of B1, B2, B3, M1 and M2 of flour streams Rumska Crvenka cultivar were lower than modern cultivars, whereas the B1, B2, M1, M2 and M3 of flour streams Stara Banatka cultivar was higher. Also, millstream yields of flour fractions were lower.

Fe and Zn content of milling streams

The levels of Zn in the milling streams of Stara Banatka were similar to those reported by Guttieri et al. (2015), whereas in the case of two other wheat cultivars were lower. All six flour streams of Crnozrna had zinc below 10 mg/kg d.m. which is the lowest concentration in the flour stream reported by Guttieri, Seaburn, Liu, Baezinger and Waters (2015).

Table 5.
Extraction yield and ash content of 8 milling fractions from a Bühler laboratory mill MLU 202

Wheat Cultivar	B1	B2	B3	M1	M2	M3	Total extraction yield	Shorts	Bran
				Extraction yield (%)					
Crnozrna	2.00	5.33	1.33	31.00	8.33	5.67	53.67	35.70	10.67
Rumska Crvenka	3.57	5.00	1.43	28.21	8.57	6.79	53.57	35.36	11.07
Stara Banatka	2.33	5.67	1.33	29.33	7.00	5.67	51.33	39.67	9.00
				Ash content (%) on dry matter basis[a]					
Crnozrna	0.379	0.714	1.097	0.406	0.668	0.643	0.518	2.142	6.329
Rumska Crvenka	0.200	0.477	1.301	0.312	0.472	0.432	0.386	2.850	6.637
Stara Banatka	0.957	1.198	1.498	0.837	1.206	1.389	1.011	1.954	7.197

[a]*Mean of two replicates*

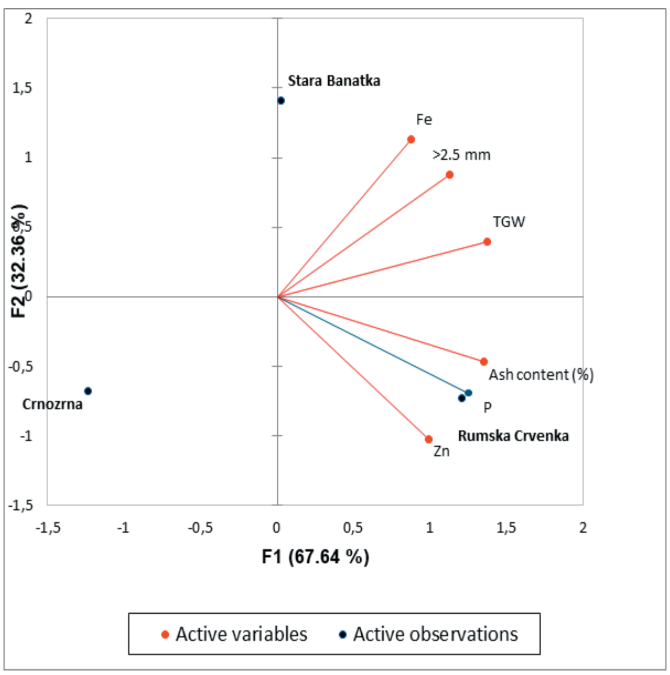
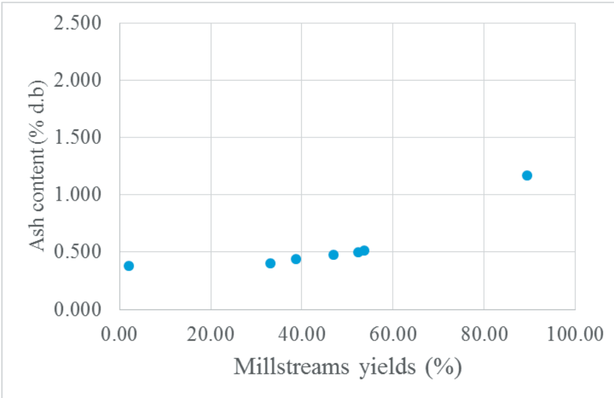
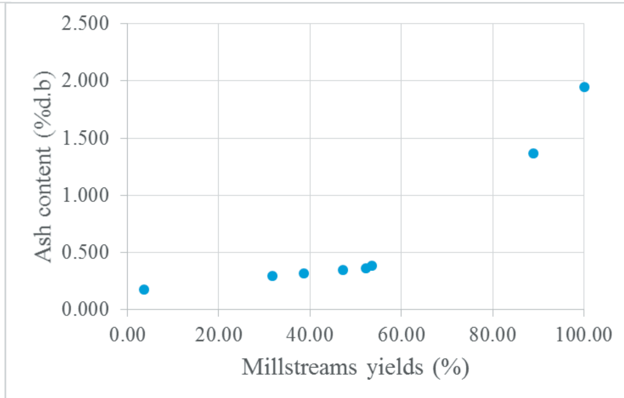


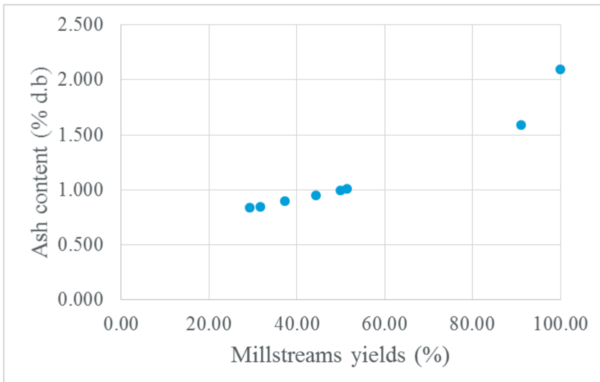
Figure 1. PCA biplot of ash content, TGW >2.8 and >2.5 mm and Fe, Zn and P content in whole grains of the selected heritage wheat cultivars



A)



B)



C)

Figure 2. Cumulative ash content (% d. b.) curves for wheat cultivars Crnozrna (A), Rumska Crvenka (B) and Stara Banatka (C)

Table 6.
Mean values of total zinc and iron in the milling streams of old wheat cultivars

Milling streams	**Crnozrna**		**Rumska Crvenka**		**Stara Banatka**	
	Zn mg/kg d.m.	**Fe** mg/kg d.m.	**Zn** mg/kg d.m.	**Fe** mg/kg d.m.	**Zn** mg/kg d.m.	**Fe** mg/kg d.m.
B1	0.4 ± 0.1^{b}	5.4 ± 0.02^{b}	1.0 ± 0.3^{b}	9.1 ± 0.3^{b}	8.2 ± 0.9^{a}	16.01 ± 1.5^{a}
B2	4.4 ± 0.8^{b}	10.3 ± 0.6^{b}	10.9 ± 1.0^{a}	5.7 ± 0.5^{c}	13.1 ± 1.2^{a}	65.2 ± 1.5^{a}
B3	9.3 ± 0.8^{b}	29.2 ± 1.6^{c}	12.4 ± 0.7^{a}	84.7 ± 0.4^{b}	11.8 ± 0.1^{ab}	162.6 ± 5.6^{a}
M1	4.3 ± 0.02^{a}	13.5 ± 0.6^{b}	1.7 ± 0.3^{b}	11.1 ± 1.6^{b}	4.0 ± 0.5^{a}	46.5 ± 3.8^{a}
M2	1.8 ± 0.6^{b}	18.0 ± 1.6^{b}	2.1 ± 0.1^{b}	15.9 ± 0.6^{b}	5.6 ± 0.1^{a}	44.3 ± 2.1^{a}
M3	1.5 ± 0.1^{c}	99.1 ± 1.2^{a}	4.2 ± 0.4^{b}	21.4 ± 1.2^{c}	5.8 ± 0.3^{a}	92.2 ± 2.2^{b}
Shorts	41.3 ± 1.7^{a}	172.2 ± 7.2^{b}	40.9 ± 0.9^{a}	393.7 ± 6.0 ^{a}	44.8 ±2.1^{a}	388.1 ± 4.6^{a}
Bran	54.7 ±1.5^{b}	341.7 ± 5.6^{c}	81.7 ± 2.0^{a}	429.7 ± 2.9^{b}	47.6 ±3.3^{b}	606.3 ± 3.0^{a}

Means±SD with different letters in the same row are significantly different according to Tukey's test (P < 0.05)

Table 7.
Total phosphorous content in milling streams of old wheat cultivars

Milling streams	**P content** (mg/kg d.m.)		
	Crnozrna	**Rumska Crvenka**	**Stara Banatka**
B1	1134±18.4^{a}	785±7.1^{c}	877±9.9^{b}
B2	1205±21.2^{a}	883±12.7^{c}	1035±21.2^{b}
B3	2118±24.0^{b}	2015±21.2^{b}	2305±49.5^{a}
M1	1007±12.7^{a}	1049±12.7^{a}	1067±29.7^{a}
M2	1055±21.2^{b}	1177±11.3^{c}	1303±32.5^{a}
M3	914±9.9^{c}	1189±15.6^{a}	989±9.9^{b}
Shorts	3857±38.2^{c}	5069±80.6^{a}	4121±56.6^{b}
Bran	9891±99.0^{c}	11460±113.1^{b}	12870±169.7^{a}

Means±SD with different letters in the same row are significantly different according to Tukey's test (P < 0.05)

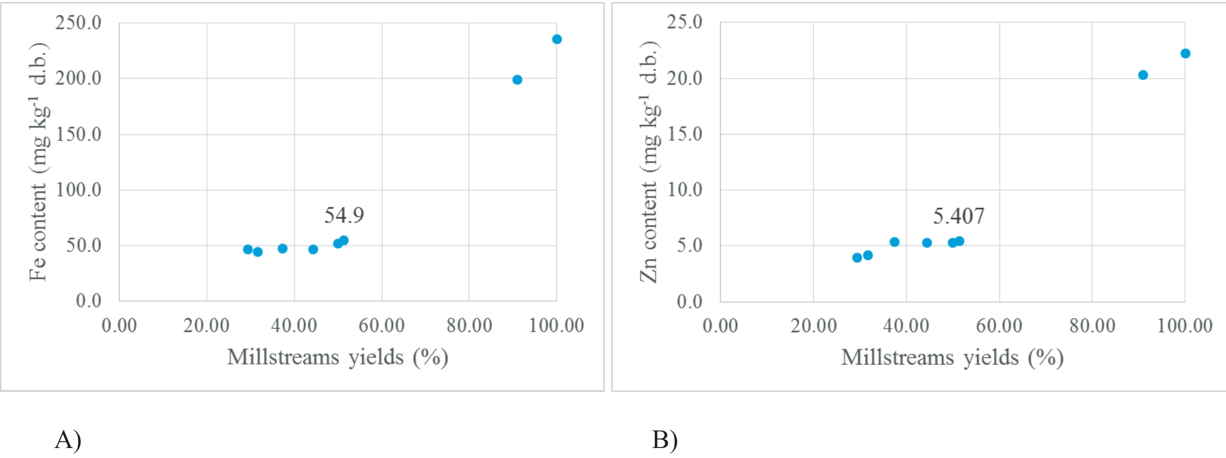


Figure 3. Cumulative iron (A) and zinc (B) content (% d. b.) curves for wheat cultivar Stara Banatka

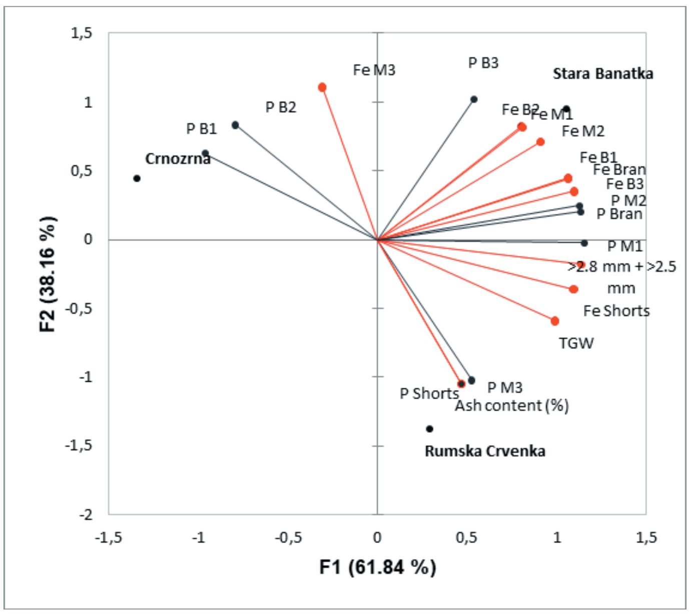


Figure 4. PCA biplot of ash content, TGW, >2.8 and >2.5 mm, Fe and P content in milling streams

Similar observation was made in the case of Rumska Crvenka but for four flour streams (B1, M1, M2 and M3) (Table 6). The iron content in all flour fractions was higher in Stara Banatka compared to the milling fractions from Crnozrna and Rumska Crvenka (Table 6). Moreover, the total iron content in all six flour fractions of Stara Banatka cultivar ranged from 16.01 to 162.6 mg/kg which was above the range reported by Eagling et al. (2014) and Živančev et al. (2021) who examined iron content in milling streams of modern wheat germplasm with high and low iron content in the kernel. The reason for this was probably due to initially high iron content in the grains of Stara Banatka. Furthermore, five millings streams of Stara Banatka cultivar possessed significantly higher Zn (B1, B2, M1, M2 and M3) and Fe (B1, B2, B3, M1 and M2) than Crnozrna and Rumska Crvenka cultivars. The iron contents in the milling by-products from this study were almost twice as high as those reported by Eagling et al. (2014) and Guttieri et al. (2015). The reason for this variation might be due to differences in the method of micronutrient determination; in these studies inductively coupled plasma-optical emission spectroscopy was used to measure the iron levels. The total content of P in the milling streams of traditional Serbian wheat cultivars is displayed in Table 6. Although P content of flour streams B1 and B2 from Stara Banatka was statistically lower in comparison to Crnozrna, the ash content showed an opposite trend. Also, a similar situation was noticed in flour stream M3 from

Stara Banatka where P content was statistically lower in comparison to Rumska Crvenka cultivar.

PCA analysis of milling streams

The calculated Fe and Zn content according to the equation for cumulative flour ash curves (Fišteš and Tanović, 2014) for all six flour fractions of the Stara Banatka cultivar was 54.9 mg kg/d.b. (Fig. 2 (A) and 5.407 mg kg/d.b. (Fig. 2 (B), respectively. Iron content in the old cultivar was almost twice time higher compared to white wheat flour obtained by processing hard and soft wheat from the study of Heshe, Haki, Woldegiorgis and Gemede (2016), whereas the Zn content was on the level of white flour from soft wheat. A biplot diagram of ash content, TGW and percentage of grain kernels >2.8 and >2.5 mm and content of Fe, Zn of examined wheat cultivars is presented in Fig. 4. PCA analysis was performed to find the relationship between grain size traits, Fe, and P content in the milling streams. Factor 1 (F1) and Factor 2 (F2) accounted for 61.84% and 38.16% of the total variance, respectively. It can be seen that the P content of milling streams M3 and shorts correlates with ash content. Rumska Crvenka cultivar was positioned close to these parameters across the F2. Also, the mentioned parameters together with TGW negatively correlated with Fe content in milling streams M3. Therefore, the low ash contents and TGW value could indicate high Fe contents in milling streams M3. Similar to the interpretation of the biplot along the F2, the iron content of B1, Bran, B3, Shots and P content of M2, Bran, and M1 positively correlates with grain fractions >2.8 and >2.5 mm. Stara Banatka cultivar was positioned close to these parameters across the F1. The Crnozrna cultivar showed a negative correlation with the mentioned variables since it was positioned on the negative part of the biplot along F1. Furthermore, the Fe content of flour streams B1 and B2 was unrelated to P content in the same streams, whereas the Fe content of flour stream M3 was negatively correlated to P content. This implies that Fe from flour streams of wheat cultivar Stara Banatka could be more available than that from Rumska Crvenka and Crnozrna

To assess the nutritional values of dark flour obtained from wheat cultivars Stara Banatka, intake of Fe through consumption of average bread portion in Serbia was calculated. The estimated daily intake of total Fe by consuming an average bread portion in Serbia produced from type '1100' flour originating from wheat cultivars Stara Banatka was 9.61 mg/kg which is 87% of the recommended daily intake for adult males according to the Dietary Reference Values for Nutrients Summary Report (EFSA, 2017). The amount is twice higher than the dietary Fe intake estimated in the study by Živančev et al. (2021) by consuming a portion of dark, type "1100", bread made from modern wheat cultivars. This implies that our ancestors may have had higher dietary iron intake when consuming bread obtained from flour of the wheat cultivar Stara Banatka.

CONCLUSIONS

The results of this research indicate that the grains of Serbian old wheat cultivars were small and that the cultivars with a high Fe content in the grain had a low Zn content. Also, three different types of flour were obtained by laboratory milling of three different wheat cultivars. Namely, wheat flour types '400', '500'and '1100' were produced from the cultivars Rumska Crvenka, Crnozrna and Stara Banatka, respectively. Furthermore, the content of examined micronutrients in five flour streams of wheat cultivar Stara Banatka was statistically higher than in flour streams of other two old wheat cultivars. Also, the Fe content in the flour mixture of all flour streams of wheat cultivar Stara Banatka was 54.9 mg/kg d.m. According to our estimation, an average bread portion could provide about 90% of the recommended daily iron intake. Therefore, wheat cultivar Stara Banatka can be used as a parent of new high-iron wheat lines.

ACKNOWLEDGEMENTS

Authors would like to express their gratitude to the Provincial Secretariat for Science and Technological Development of Vojvodina, project "Influence of milling process of traditional wheat cultivars from Vojvodina on the content of essential minerals in wheat flour", grant 142-451-2313/2022-01/01 and the Benefit-Sharing Fund of the International Treaty on Plant Genetic Resources for Food and Agriculture project "Redesigning the exploitation of small grains genetic resources towards increased sustainability of grain-value chain and improved farmers' livelihoods in Serbia and Bulgaria—GRAINEFIT", grant PR-166-Serbia.

REFERENCES

AACC (2000). Straight-Dough Bread-Baking Method – Long Fermentation Method 10-09. In AACC Methods (10th ed.) American Association of Cereal Chemistry, USA.

Bock, M.A., 2000. Minor constituents of cereals. In K. Kulp & J.G. Jr. Ponte (Eds.), *Handbook of Cereal Science and Technology, 2nd ed.* (pp. 479-504). New York: Marcel Dekker Inc.

Bouis, H. E., Hotz, C., McClafferty, B., Meenakshi, J. V., & Pfeiffer, W. H. (2011). Biofortification: A new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin, 32*(1_suppl), S31–S40. https://doi.org/10.1177/15648265110321s105

Eagling, T., Neal, A. L., McGrath, S. P., Fairweather-Tait, S., Shewry, P. R., & Zhao, F.-J. (2014). Distribution and speciation of iron and zinc in grain of two wheat genotypes. *Journal of Agricultural and Food Chemistry, 62*(3), 708–716. https://doi.org/10.1021/jf403331p

EFSA. (2017). Dietary Reference Values for nutrients - Summary report. EFSA Supporting Publications, *14*(12). https://doi.org/10.2903/sp.efsa.2017.e15121 Accessed: 2020 October 21.

Erdman, J. W., Jr. (1981). Bioavailability of trace minerals from cereals and legumes. *Cereal Chemistry*, 58, 21–26. www.cerealsgrains.org. https://www.cerealsgrains.org/publications/cc/backissues/1981/Documents/CC1981a05.html

Ferretti, R. J., & Levander, O. A. (1974). Effect of milling and processing on the selenium content of grains and cereal products. *Journal of Agricultural and Food Chemistry, 22*(6), 1049–1051. https://doi.org/10.1021/jf60196a057

Fišteš, A., & Tanović, G. (2014). *Practice book in milling technology* (p. 75). Novi Sad: University of Novi Sad, Faculty of Technology.

Füllenbach, C., Stein, P., Glaser, P., Triphaus, C., Lindau, S., Choorapoikayil, S., Schmitt, E., Zacharowski, K., Hintereder, G., Hennig, G., Homann, C., Stepp, H., Spahn, G. H., Kaserer, A., Schedler, A., Meybohm, P., & Spahn, D. R. (2019). Screening for iron deficiency in surgical patients based on noninvasive zinc protoporphyrin measurements. *Transfusion, 60*(1), 62–72. https://doi.org/10.1111/trf.15577

Food Balance Sheet. (2023). FAO. Retrieved Jan. 13, 2023 from http://www.fao.org/faostat/en/#data/FBS.

Garvin, D. F., Welch, R. M., & Finley, J. W. (2006). Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *Journal of the Science of Food and Agriculture, 86*(13), 2213–2220. https://doi.org/10.1002/jsfa.2601

Guttieri, M. J., Seabourn, B. W., Liu, C., Baenziger, P. S., & Waters, B. M. (2015). Distribution of cadmium, iron, and zinc in millstreams of hard winter wheat (*Triticum aestivum* L.). *Journal of Agricultural and Food Chemistry, 63*(49), 10681–10688. https://doi.org/10.1021/acs.jafc.5b04337

Household Budget Survey. (2021). Statistical Office of the Republic of Serbia. Retrieved Feb. 01, 2020 from https://publikacije.stat.gov.rs/G2022/Pdf/G20225687.pdf

Heshe, G. G., Haki, G. D., Woldegiorgis, A. Z., & Gemede, H. F. (2016). Effect of conventional milling on the nutritional value and antioxidant capacity of wheat types common in Ethiopia and a recovery attempt with bran supplementation in bread. *Food Science & Nutrition, 4*(4), 534. https://doi.org/10.1002/fsn3.315

ISO 520 (2010) Cereals and pulses - Determination of the mass of 1000 grains. International Organization for standardization. Brussels, Belgium: European Committee for Standardization.

ISO 712 (2012) Cereals and cereal products – Determination of moisture content - Reference method. International Organization for standardization. Brussels, Belgium: European Committee for Standardization.

ISO 749 (1977) Cereals and cereal products - Determination of moisture content - Reference method. International Organization for standardization. Brussels, Belgium: European Committee for Standardization.

Kovačević, M. 2011. Tehnološki postupak proizvodnje hleba [Bread Processing]. In R. Perušković, (Ed.), *Praktično pekarstvo* [Practical Breadmaking], pp. 79–148. Novi Sad, Serbia: Progres.

Kumar, P., Yadava, R., Gollen, B., Kumar, S., Verma, R., & Yadav, S. (2011). Nutritional contents and medicinal properties of wheat: A review. www.semanticscholar.org. https://www.semanticscholar.org/paper/Nutritional-Contents-and-Medicinal-Properties-of-A-Kumar-Yadava/8589470fe0581f3ec005385fadedb9e8e1c810f0

Morgounov, A., Gómez-Becerra, H. F., Abugalieva, A., Dzhunusova, M., Yessimbekova, M., Muminjanov, H., Zelenskiy, Y., Ozturk, L., & Cakmak, I. (2006). Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica, 155*(1-2), 193–203. https://doi.org/10.1007/s10681-006-9321-2

Pandey, A., Szakacs, G., Soccol, C. R., Rodriguez-Leon, J. A., & Soccol, V. T. (2001). Production, purification and properties of microbial phytases. *Bioresource Technology, 77*(3), 203–214. https://doi.org/10.1016/s0960-8524(00)00139-5

Pravilnik. (2018). Pravilnik o kvalitetu žita, mlinskih i pekarskih proizvoda i testenina. (2018). *Službeni glasnik RS, 68/2016 i 56/2018.*

Šereš, Z., Simović, D., Grujičić, M., Maravić, N., Kiš, F., Dokić, L., Nikolić, I., Đorđević, M., & Šaranović, Ž. (2017). Bread as indicator of age-changing dietary habits among young people. *Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku, 6*(2) 48-89. https://hrcak.srce.hr/file/292862

Steiner, T., Mosenthin, R., Zimmermann, B., Greiner, R., & Roth, S. (2007). Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Animal Feed Science and Technology, 133*(3-4), 320–334.

https://doi.org/10.1016/j.anifeedsci.2006.04.007

Victora, C. G., Christian, P., Vidaletti, L. P., Gatica-Domínguez, G., Menon, P., & Black, R. E. (2021). Revisiting maternal and child undernutrition in low-income and middle-income countries: variable progress towards an unfinished agenda. *The Lancet, 397*. https://doi.org/10.1016/s0140-6736(21)00394-9

Zanirato, S. (2013). TFIF International Grain Congress on "Wheat, Flour, Climatic Changes and New Trends". Wheat flour standards in European Union. Antalya. Retrieved Jan. 19, 2023 from http://www.tusaf.org/Eklenti/367,sandro-zaniratowheat-flour-standards-in-eupdf.pdf?0

Zhao, F. J., Su, Y. H., Dunham, S. J., Rakszegi, M., Bedo, Z., McGrath, S. P., & Shewry, P. R. (2009). Variation in mineral micronutrient concentrations grain of wheat lines of diverse origin. *Journal of Cereal Science, 49*(2), 290–295. https://doi.org/https://doi.org/10.1016/j.jcs.2008.11.007

Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional iron deficiency. *The Lancet, 370*(9586), 511–520. https://doi.org/10.1016/s0140-6736(07)61235-5

Živančev, D., Ninkov, J., Jocković, B., Momčilović, V., Torbica, A., Mirosavljević, M., Belović, M., Aćin, V., & Ilin, S. (2021). Distribution of iron, zinc and manganese in milling streams of common Serbian wheat cultivars: Preliminary survey. *International Journal of Food Science & Technology, 56*(6), 3099–3110. https://doi.org/10.1111/ijfs.14953

Zlotkin, S., & Dewey, K. G. (2021). Perspective: Putting the youngest among us into the nutrition "call for action" for food fortification strategies. *The American Journal of Clinical Nutrition, 114*(4), 1257–1260.https://doi.org/10.1093/ajcn/nqab207

SASTAV MIKRONUTRIJENATA PROIZVODA MLEVENJA TRADICIONALNIH SORTI PŠENICE POREKLOM IZ SRBIJE

Dragan R. Živančev*[1], Maja S. Buljovčić[2], Jordana M. Ninkov[1], Igor B. Antić[2], Sanja Z. Mikić[1], Simona Ž. Jaćimović[1], Bojan Đ. Jocković[1]

[1]Institut za ratarstvo i povrtarstvo, Departman za strna žita, Maksima Gorkog 30, 21101 Novi Sad, Serbia
[2]Univerzitet u Novom Sadu, Tehnološki fakultet, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Sažetak: Nedostatak mikronutrijenata u ljudskoj ishrani predstavlja globalni problem. Cilj ovog rada je određivanje sadržaja dva esencijalna metala, gvožđa (Fe) i cinka (Zn) u frakcijama dobijenim pri mlevenju tradicionalnih starih sorti pšenice koje se nisu proizvodile više od pola veka u cilju pronalaženja frakcije sa visokim sadržajem esencijalnih metala koje bi poslužile za oplemenjivanje. Kod sedam starih srpskih sorti pšenica određeni su sledeći parametri veličine zrna: sadržaj pepela, masa hiljadu zrna i udeo specifičnih frakcija zrna. Crnozrna, Rumska Crvenka i Stara Banatka bile su odabrane sorte kao najpogodnije za mlevenje, radi kvantitativnog određivanja sadržaja Fe i Zn u dobijenim frakcijama. Mlevenje je izvršeno na laboratorijskom mlinu da bi se dobilo šest proizvoda mlevanja i dva nusproizvoda, a sadržaj Fe i Zn određen je atomskim apsorpcionim spektrometrom sa grafitnom kivetom. Namešavanjem šest proizvoda mlevenja svake sorte dobijene su tri različite vrste brašna. Najveći sadržaj pepela određen je za brašno tip „1100“, dobijeno iz sorte Stare Banatke, koje je takođe imalo najveći sadržaj Fe i Zn (54,9 mg/kg s.m. i 5,407 mg/kg s.m., redom). Konzumiranjem standardne porcije hleba u Srbiji (166 g/dan) dobijene od brašna Stare Banatke bilo bi moguće obezbediti oko 90% preporučenog dnevnog unosa Fe. Stoga, Stara Banatka predstavlja sortu koja bi mogla da bude izvor visokog sadržaja Fe i da se koristi za oplemenjivanje, radi stvranja novih linija pšenice sa visokim sadržajem ovog minerala.

Ključne reči: *stare sorte, pepeo, visoko mineralne linije, gvožđe, cink, dnevni unos*

Received: *20 February 2023/* ***Received in revised form:*** *22 March 2023/* ***Accepted:*** *27 March 2023*

Available online: *April 2023*