

**PROTEIN CONTENT AND AMINO ACID COMPOSITION IN SEED OF BREAD
WHEAT (*Triticum aestivum* L.)**

Dušan UROŠEVIĆ¹, Desimir KNEŽEVIĆ², Gordana BRANKOVIĆ³,
Aleksandra Yu. NOVOSELSKAYA-DRAGOVICH⁴, Alexander M. KUDRYAVTSEV⁴,
Mirela MATKOVIĆ STOJŠIN⁵, Danica MIĆANOVIĆ⁶, Veselinka ZEČEVIĆ⁷

¹Maize Research Institute Zemun Polje, Zemun Serbia

²University of Pristina, temporary settled in Kosovska Mitrovica, Faculty of Agriculture, Lešak,
Lešak, Kosovo and Metohija, Serbia

³University of Belgrade, Faculty of Agriculture Zemun, Belgrade, Serbia

⁴Russian Academy of Sciences Laboratory of Plant Genetics, “N.I.Vavilov” Institute of General
Genetics RAS, 119333 Moscow, Russia

⁵Tamiš Research and Development Institute, Pančevo Serbia

⁶Serbian Chamber of Commerce and Industry, Belgrade, Serbia

⁷Institute for Vegetable Crops, Smederevska Palanka, Serbia

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The aim of this study was determination of protein content and amino acid composition in seeds of bread wheat, with particular focus of evaluation essential amino acids (EAAs). For analysis used flour samples of grained seed of 10 wheat variety, which selected in different breeding center (in Novi Sad and Kragujevac, Serbia). Kjeldahl method was used for determination of nitrogen (N) contents which value multiplied with coefficient 5.7 for computing protein content (protein contents = 5.7 x % N contents). Amino acids analyses of wheat samples were performed by ion exchange chromatography, followed by

Corresponding author: Dušan Urošević, Maize Research Institute Zemun Polje, Slobodana Bajića 1. 11080 Zemun Serbia; e-mail: dusan.urosevic@gmail.com

the ninhydrin colour reaction and photometric detection at 570 nm and 440 nm (for proline). The results showed that the mean protein content for wheat varied from the lowest value 10.24% in Ljubičevka to the highest 14.21% in Fortuna variety. The mean contents (g 100 g⁻¹ protein) of nonessential amino acids (NEAAs) were aspartic acid 5.42%, serine 4.23%, glutamic acid 18.51%, proline 12.18%, glycine 4.17%, alanine 3.64%, tyrosine 2.52%, arginine 5.02%, while mean contents of essential amino acids (EAAs) were threonine 2.86%, valine 4.52%, methionine 1.28%, isoleucine 3.87%, leucine 5.87%, phenylalanine 4.62%, histidine 2.46%, lysine 2.91%. The wheat variety contained relatively high concentration of the most deficient EAAs. The variety Ljubičevka contained relatively high concentration of the most deficient EAAs (threonine, valine, leucine, lysine,). The highest value (29.31 g 100 g⁻¹ protein) of total essential amino acids (TEAA), 59.79 g 100 g⁻¹ protein of total nonessential amino acids (TNEAA) as well as the highest value (90.35 g 100 g⁻¹ protein) of total amino acids (TAA). Obtained results provide variability of wheat variety on the base of amino acid contents and indicate reliable variety for selecting desirable parents in breeding program for improving nutrient quality.

Keywords: amino acid composition, amino acid score protein content

INTRODUCTION

Wheat species and wheat products are the most important source of cereal protein in human food as well sources for amino acids, fats, minerals and vitamins (BRAUN *et al.*, 2010; POPOVIĆ *et al.*, 2020). The wheat grain contains carbohydrates 60-75%, water-12%, proteins-12-18%, crude fiber-2.0%, fat-1.5-2.0% and minerals ~1.80% (ŠRAMKOVÁ *et al.*, 2009). Grain protein content generally varies from 10-15% in wheat varieties grown under field condition (SHEWRY *et al.*, 2015), but there are different data for variation values of grain protein depend of variety, region as well as and applied scientific farming measure (BRANKOVIĆ *et al.*, 2018). WANG *et al.*, (2019) in their study found that grain protein content of wheat vary from 11.8% to 18.0%, and in average 14.9%. Similar variation of the protein content in wheat established in range from 12.47 % to 17. 0%, with an average value of 14.18 % (ARAPI *et al.*, 2013). The protein content of flour, content of wet and dry gluten and proportion of high molecular weight protein (glutenin) and low molecular weight protein content (low molecular weight glutenin, gliadins, globulins and albumins) have influence on physicochemical properties of dough, rheological and baking quality and quality of end products (DHAKA and KHATKAR, 2015; XUE *et al.*, 2019).

The presence of amino acids in wheat variety are due to the genetic control and environmental factors (nutrition) as well as their interaction (UMRANI *et al.*, 2013; LAZE *et al.*, 2019). The wheat processing for food products influence on decreasing concentration of essential amino acids (ANJUM *et al.* 2005). For improving nutritional values of wheat food product it is important increasing the protein content in seed, and particularly improving balance of amino acid composition of seed protein. Numerous analysis of amino acids in the grain showed differences between examined genotypes, influenced by genetic and environmental factor (KNEZEVIĆ *et al.*, 2009; ALIJOŠIUS *et al.*, 2016). Also, amino acids have role in determining quality of wheat grain, flour, dough and bread (CURTIS *et al.*, 2016). Glutamic acid and proline

as well arginine and threonine content in seed storage proteins gliadin and glutenins is related to technological quality properties of flour and dough, i.e. contribute to enhance dough extensibility, viscosity and loaf volume (FERMIN *et al.*, 2005). Glutamic acid is main amino acids in all cereal protein, which played an important role in metabolism of nitrogen in cell in addition, and in the form of glutamine is an important component of the gluten proteins (KNEŽEVIĆ *et al.*, 2013; ABDELALEEM and AL-AZAB, 2021; LOOKHART *et al.*, 2001). Gluten is complex protein that consist gliadin and glutenins which have share about 80-85% of total weight content of protein in seed (WIESER, 2007). The mixture of gliadin and glutenin, with water formed rubbery viscoelastic matrix (dough) which viscous properties determined by gliadins and the strength and elasticity determined by glutenins (KNEZEVIC *et al.*, 2017).

The objectives of this study were (i) identification of amino acid composition (ii) determination of concentration of amino acids in grain wheat (iii) evaluation of variability of wheat varieties according to total content of amino acids as well total content of essential and non essential amino acids variation of concentration (iv) variability of grain protein content in bread wheat and (v) estimation of relationship between amino acid composition and protein content.

MATERIAL AND METHODS

The seed sample (0.5 g) of ten bread wheat was used for milling and obtaining flour which used for extraction of amino acids. The wheat varieties are originating from two selection centres in Republik Serbia, five variety selected in Institute of field and vegetable crops in Novi Sad (Fortuna Sasanka, Danica, Somborka, Kremna) and five in Center for small Grains in Kragujevac (Kosmajka, Šumadija, Morava, KG 56 S, Ljubičevka). Amino acids analyses of samples were performed by ion exchange chromatography using an automatic amino acid analyzer Biochrom 30+ (Biochrom, Cambridge, UK), according to SPACKMAN *et al.* (1958). The technique was based on amino acid separation using strong cation exchange chromatography, followed by the ninhydrin colour reaction and photometric detection at 570 nm and 440 nm (for proline). Samples of wheat were previously hydrolysed in 6M HCl (Merck, Germany) at 110 °C for 24 h. After hydrolysis, samples were cooled to room temperature and dissolved in 25 ml of Loading buffer (pH 2.2) (Biochrom, Cambridge, UK). Subsequently, prepared samples were filtered through 0.22 µm pore size PTFE filter (Plano, Texas, USA) and the filtrate was transferred into a vial (Agilent Technologies, USA) and stored in a refrigerator prior to analysis. The amino acid peaks were identified by comparison of retention times with retention times of amino acid standard purchased from Sigma Aldrich (Amino Acid Standard Solution (Sigma-Aldrich, St. Louis, USA). Results were expressed as mass of amino acid (g) in 100 g of protein/sample (TOMIČIĆ *et al.*, 2020).

The EAA in the test materials was evaluated by amino acid score (AAS), which was observed by determining the ratio of each EAA content to FAO/WHO amino acid recommendations (FAO/WHO 1973). The relevant formula was shown as follows:

$$\text{AAS(\%)} = \frac{\text{Amino acid per 100 g test protein (g)}}{\text{Amino acid per 100 g reference protein (g)}} \times 100$$

Improving potential of A = $\frac{A_{max}-\bar{A}}{\bar{A}}$ (LI *et al.* 1995).

A-Amino acid, refers to individual quality index

Total protein content was determined according to the Kjeldahl's method (AOAC, Method 979, 09), (AOAC, 1995) which used for determination of nitrogen (N) contents and obtained value was multiplied by coefficient 5.7 for computing protein content ($p\% = N \times 5.7$).

Correlation matrix analysis by the principal components method (PCA) were performed in order to express the inter-relationships between analysed amino-acids using IBM SPSS Statistics, Trial Version 22.0.

RESULTS

The outcomes of this research included identified content of amino acids and differences among wheat varieties according to variation of amino acid content and grain protein content. Also, the different values of concentration of total amino acids as well total essential amino acids and total non essential amino acids were established. The analysis of amino acid concentrations in grain of wheat (in grams per 100 g of protein) showed different values in different wheat genotypes. The concentration of amino acids varied depending on wheat variety.

Non essential amino acids

Humans and animals are able to synthesize the nine non-essential amino acids out of the 20 amino acids. In this study the highest amount of amino acid alanine was found in wheat variety Ljubičevka (4.32%) followed by variety Morava (4.24%), while the lowest content of alanine was determined for genotype Fortuna (3.83%). The mean value of alanine content was 4.04% for all analysed genotypes. The aspartic acid concentration varied from 5.28% (Sasanka and Somborka) to 5.72% (Ljubičevka) with mean value 5.42% in all analyzed genotypes. Concentration of cysteine varied from 1.62% in Fortuna to 2.18% in Somborka with mean value 2.00%. The glutamic acid concentration varied in the range from 16.74% in Fortuna variety to 20.22% in Ljubičevka variety with mean value 18.51% for all ten varieties. The highest value of the content of amino acid proline was found in variety Fortuna (12.95%) followed by variety Ljubičevka (12.82%) and Sasanka (12.80%), while the lowest content was found in wheat variety KG 56S (11.00%) with the mean value 12.15%. The highest content of amino acid glycine had genotype Ljubičevka (4.45%) followed by varieties Morava and Šumadija (4.36%), while the lowest content was established in variety Kremna (3.94%), with mean value 4.17% for all analysed genotypes. The concentration of serine was in the range from 3.94% in Kremna variety to 5.17% in KG 56 S variety, with mean value 4.23% for all ten analysed varieties. The Fortuna variety contains the highest amount of amino acid tyrosine (2.99%) followed by variety Kremna (2.93%), while the lowest content of tyrosine was found in genotype Kosmajka (2.02%). The mean value of tyrosine content was 2.52% for all analysed genotypes. Amino acids arginine had the the highest amount in KG 56 S variety (5.42%) followed by variety Morava (5.34%) and Fortuna (5.32%) while the lowest content of arginine was found in genotype Kremna (4.58%). The mean value of arginine content was 5.02% for all analysed genotypes (table 1).

Table 1. Amino acid profiles of wheat samples (g 100 g⁻¹ protein)

Amino acid (g 100 g ⁻¹ protein)	Fortuna	Sasanka	Danica	Sombo rka	Kremn a	Kos maj ka	Šumad ija	Morav a	KG 56 S	Ljubič evka	Mean AA	Min	Ma x	CV (%)
Alanine	3.83	3.89	4.02	3.94	3.88	4.00	4.13	4.24	4.14	4.32	4.038	3.83	4.32	4.05
Aspartic acid	5.41	5.28	5.54	5.28	5.30	5.30	5.43	5.64	5.35	5.72	5.425	5.28	5.72	2.92
Cysteine	1.62	1.98	2.11	2.18	1.95	1.98	1.85	2.12	2.08	2.17	2.004	1.62	2.18	8.55
Glutamic acid	16.74	17.82	17.79	17.93	17.33	18.59	19.51	19.72	19.41	20.22	18.506	16.74	20.22	6.26
Glycine	4.07	3.95	4.18	3.98	3.94	4.17	4.36	4.36	4.25	4.45	4.171	3.94	4.45	4.41
Proline	12.95	12.8	12.04	11.49	12.35	11.70	11.88	12.44	11.00	12.82	12.147	11.00	12.95	5.26
Serine	4.04	4.14	4.11	4.05	3.94	4.03	4.00	4.4	5.17	4.43	4.231	3.94	5.17	8.70
Tyrosine	2.99	2.13	2.16	2.60	2.93	2.02	2.46	2.59	2.75	2.61	2.524	2.02	2.99	13.18
Arginine	5.32	4.86	4.92	5.00	4.58	4.61	4.90	5.34	5.42	5.22	5.017	4.58	5.42	5.96
Histidine*	2.70	2.76	2.82	2.77	2.86	2.65	2.53	2.73	2.73	2.81	2.736	2.53	2.86	3.46
Isoleucine*	3.95	3.93	3.83	3.90	4.31	3.68	3.65	3.87	3.51	4.06	3.869	3.51	4.31	5.82
Leucine*	5.50	5.63	5.62	5.52	5.90	5.57	5.46	5.92	7.55	5.98	5.865	5.46	7.55	10.60
Lysine*	2.89	2.76	3.03	2.80	2.76	2.90	2.92	3.02	2.97	3.06	2.911	2.76	3.06	3.80
Methionine*	1.28	1.20	1.23	1.34	1.40	1.13	1.14	1.33	1.36	1.36	1.277	1.13	1.4	7.57
Phenylalanine*	3.70	4.10	4.64	4.34	4.03	4.93	5.84	5.22	3.95	5.45	4.620	3.70	5.84	15.52
Threonine*	2.87	2.82	2.89	2.82	2.74	2.79	2.76	3.00	2.89	3.06	2.864	2.74	3.06	3.57
Valine*	4.37	4.51	4.51	4.47	4.65	4.47	4.41	4.69	4.34	4.78	4.520	4.34	4.78	3.18
Tryptophan*	1.12	1.32	1.29	1.05	0.99	1.27	1.17	1.27	1.25	1.32	1.205	0.99	1.32	9.70
TEAA	28.38	29.03	29.86	29.01	29.64	29.39	29.88	31.05	30.55	31.88	29.867	28.4	31.88	3.50
TNEAA	56.97	56.85	56.87	56.45	56.2	56.40	58.52	60.85	59.57	61.96	57.664	56.2	61.96	3.58
TAA	85.35	85.88	86.73	85.46	85.84	85.79	88.04	91.90	90.12	93.84	87.531	85.4	93.84	3.47
Share TEAA/TAA	33.25	33.80	34.43	33.95	34.53	34.26	33.80	33.79	33.90	33.97	34.12	33.3	34.53	1.08
Share TNEAA/TAA	66.75	66.20	65.57	66.05	65.47	65.74	66.20	66.21	66.10	66.03	65.88	65.5	66.75	0.56

Essential amino acids are indicated by an asterisk (*). Abbreviations: TEAA, total essential amino acids; TNEAA, total nonessential amino acids; TAA, total amino acids.

Essential amino acids

Essential amino acids can not be synthesized by metabolisms of human and animals and must be provided by foods. Amino acids histidine had the highest concentration in Kremna (2.86%) followed by variety Danica (2.82%) and Ljubičevka (2.81%) while the lowest content of histidine was found in variety Šumadija (2.53%). The mean value of histidine concentration was 2.74% for all analysed varieties. The highest content of amino acid isoleucine had genotype Kremna (4.31%) followed by variety Ljubičevka (4.06%), while the lowest content was established in variety KG 56 S (3.51%), with mean value 3.87% for all analysed genotypes. The KG 56 S variety contained the highest content of amino acid leucine (7.55%) followed by variety Ljubičevka (5.98%), while the lowest content was found in variety Šumadija (5.46%). The mean value of leucine content was 5.86% for all analysed genotypes. The concentration of lysine

varied from the highest in Ljubičevka variety (3.06%) followed by Danica (3.03%) and Morava (3.02%) to the lowest content of lysine found in varieties Kremna and Sasanka (2.76%), with the mean value 2.91% for all ten analysed varieties. The highest concentration of amino acid methionine was found in Kremna (1.40%) followed by variety KG 56 S (1.36%) and Ljubičevka (1.36%) while the lowest content of methionine was found in wheat variety Kosmajka (1.13%). The mean value of methionine concentration was 1.28% for all analysed genotypes. The Šumadija variety contained the highest concentration of amino acid phenylalanine (5.84%) followed by variety Ljubičevka (5.45%), while the lowest content of phenylalanine was found in genotype Fortuna (3.70%). The mean value of phenylalanine content was 4.62% for all analysed genotypes. The concentration of threonine varied from the highest in Ljubičevka variety (3.06%) followed by variety Morava (3.00%) to the lowest content found in genotype Kremna (2.74%) with mean value 2.86% for all ten analysed varieties. The Ljubičevka variety contained the highest amount of amino acid valine (4.78%) followed by variety Morava (4.69%) and Kremna (4.65%), while the lowest content of valine was found in genotype KG 56 S (4.34%). The mean value of valine content was 4.52% for all analysed genotypes. Amino acid tryptophan had the highest concentration in Ljubičevka and Sasanka (1.32%) and the lowest concentration in Kremna (0.99%) with mean value 1.20% (table 1).

Total content of amino acids (TAA) in g 100 g⁻¹ of protein, was the highest in variety Ljubičevka (93.84%) followed by variety Morava (91.90%) whereas the lowest value was found in variety Fortuna (85.35%) with average value 87.53% for all ten wheat varieties (table 1).

Total amount of non essential amino acids (TNEAA) varied depending of wheat variety. The highest concentration (g 100 g⁻¹ of protein) of non essential amino acids was shown for variety Ljubičevka (61.96%) followed by variety Morava (60.85%) whereas the lowest value of TNEAA was found in Kremna (56.20%). The mean value of TEAA was 57.67% for all ten varieties (table 1).

The concentration of essential amino acids (TEAA) in each analyzed variety was lower than concentration of nonessential amino acids. The highest concentration of TEAA was found in variety Ljubičevka (31.88%) followed by variety Morava (31.05%) whereas the lowest concentration was shown for variety Fortuna (28.38%) with average value 29.87% for all ten wheat varieties (table 1).

The lowest share of TEAA in TAA (33.25%) was established for variety Fortuna which had the lowest amount of TEAA (28.38%). Although the highest concentration of TEAA was shown for the cultivar Ljubičevka (31.88%), the share of TEAA / TAA (31.88/93.84) was 33.97%, but it was not the highest among varieties. The highest share of TEAA in the total content of amino acids was in the cultivar Kremna (34.53%), which had a lower concentration of TEAA (29.64%) than the variety Ljubičevka (31.88%) and than the varieties Morava (31.05%) and KG 56 S (30.55%). Also, in varieties Danica and Kosmajka the share of TEAA in TAA was 34.43% and 34.26%, respectively and higher than in varieties which had the higher amount of TEAA (Šumadija, KG 56 S, Morava and Ljubičevka) table 1.

The highest value of coefficient of variation (CV) was observed for phenylalanine (15.52%), and the lowest for aspartic acid (2.92%) (table 2). TEAA, TNEAA, TAA, share TEAA/TAA, share TNEAA/TAA varied with CV of 3.50%, 3.58%, 3.47%, 1.08%, and 0.56%, respectively (table 1).

In comparison, varieties derived from breeding center in Novi Sad and from breeding center in Kragujevac had differences in average values of nonessential and essential amino acids (table 2, 3).

Table 2. Nonessential amino acid content of wheat samples (in g 100 g⁻¹ protein)

Non essential amino acid (g 100 g ⁻¹ protein)	Varieties from breeding Center Novi Sad						Varieties from breeding Center Kragujevac						Average NEEA
	Fortuna	Šaška	Danica	Somborka	Kremna	Average NEEA	Kosmajka	Šumadija	Morava	KG 56 S	Ljubičevka	Average NEEA	
Alanine	3.83	3.89	4.02	3.94	3.88	3.91	4.0	4.13	4.24	4.14	4.32	4.17	4.038
Aspartic acid	5.41	5.28	5.54	5.28	5.3	5.36	5.3	5.43	5.64	5.35	5.72	5.49	5.425
Cystine	1.62	1.98	2.11	2.18	1.95	1.97	1.98	1.85	2.12	2.08	2.17	2.04	2.004
Glutamic acid	16.74	17.82	17.79	17.93	17.33	17.52	18.59	19.51	19.72	19.41	20.22	19.49	18.506
Glycine	4.07	3.95	4.18	3.98	3.94	4.02	4.17	4.36	4.36	4.25	4.45	4.32	4.171
Proline	12.95	12.8	12.04	11.49	12.35	12.33	11.7	11.88	12.44	11	12.82	11.97	12.147
Serine	4.04	4.14	4.11	4.05	3.94	4.056	4.03	4	4.4	5.17	4.43	4.41	4.231
Tyrosine	2.99	2.13	2.16	2.6	2.93	2.56	2.02	2.46	2.59	2.75	2.61	2.49	2.524
Arginine	5.32	4.86	4.92	5	4.58	4.94	4.61	4.9	5.34	5.42	5.22	5.10	5.017
TNEAA	56.97	56.85	56.87	56.45	56.2	56.67	56.4	58.52	60.85	59.57	61.96	59.46	58.07
TAA (NEAA+EAA)	85.35	85.88	86.73	85.46	85.84	85.85	85.79	88.4	91.9	90.12	93.84	90.01	87.93
Share TNEAA/TAA	66.75	66.20	65.57	66.05	65.47	66.01	65.74	66.20	66.21	66.10	66.03	66.06	66.04

The average value of total nonessential amino acids. In varieties from Kragujevac, was 59.46% which is higher than in varieties from Novi Sad with the the average value of total nonessential amino acids of 56.67%. The mean share TEAA/TAA was 66.06% for varieties from Kragujevac which is similar to mean of share TEAA/TAA for varieties from Novi Sad 66.01% (table 2).

The value of total essential amino acids was on average higher in varieties from Kragujevac (30.55%) than in varieties from Novi Sad (29.18%) table 2. The mean value of share of total essential amino acids in content of total amino acids was 33.94% for varieties from Kragujevac, which is similar to the mean value of share of total essential amino acids in content of total amino acids for varieties from Novi Sad 33.99% (table 3).

Table 3. Essential amino acid content of wheat samples (in g 100 g⁻¹ protein)

Essential amino acid (g 100 g ⁻¹ protein)	Varieties from breeding Center Novi Sad							Varieties from breeding Center Kragujevac						Average EEA
	Fortuna	Šašanka	Danica	Somborka	Kremena	Average EEA	Kosmajka	Šumadija	Morava	KG 56 S	Ljubičevka	Average EEA		
Histidine	2.7	2.76	2.82	2.77	2.86	2.78	2.65	2.53	2.73	2.73	2.81	2.69	2.736	
Isoleucine	3.95	3.93	3.83	3.9	4.31	3.98	3.68	3.65	3.87	3.51	4.06	3.75	3.869	
Leucine	5.5	5.63	5.62	5.52	5.9	5.63	5.57	5.46	5.92	7.55	5.98	6.10	5.865	
Lysine	2.89	2.76	3.03	2.8	2.76	2.85	2.9	2.92	3.02	2.97	3.06	2.97	2.911	
Methionine	1.28	1.2	1.23	1.34	1.4	1.29	1.13	1.14	1.33	1.36	1.36	1.26	1.277	
Phenylalanine	3.7	4.1	4.64	4.34	4.03	4.16	4.93	5.84	5.22	3.95	5.45	5.08	4.62	
Threonine	2.87	2.82	2.89	2.82	2.74	2.83	2.79	2.76	3.00	2.89	3.06	2.90	2.864	
Valine	4.37	4.51	4.51	4.47	4.65	4.50	4.47	4.41	4.69	4.34	4.78	4.54	4.52	
Tryptophan	1.12	1.32	1.29	1.05	0.99	1.15	1.27	1.17	1.27	1.25	1.32	1.26	1.205	
TEAA	28.38	29.03	29.86	29.01	29.64	29.18	29.39	29.88	31.05	30.55	31.88	30.55	29.867	
TAA (NEAA+EAA)	85.35	85.88	86.73	85.46	85.84	85.85	85.79	88.4	91.9	90.12	93.84	90.01	87.531	
Share TEAA/TAA	33.25	33.80	34.43	33.95	34.53	33.99	34.26	33.80	33.79	33.90	33.97	33.94	33.97	

Association among amino acid contents in seed of wheat

From the biplot shown in Figure 1., it can be seen that aspartic acid and serine are in the strongest positive correlation (an sharp angle). The aspartic acid and serine are in strong correlation with glycine and with alanine, glutamic acid and arginine, while aspartic acid and serine had negative correlation with tyrosine. Also alanine with glutamic acid showed strong correlation and both of these amino acids had positive correlation with cysteine. aspartic acid, serine and glycine, while negative correlation exists with arginine, proline and tyrosine. Amino acid proline was in positive correlation with arginine, aspartic acid, serine and tyrosine while proline had negative correlation with glycine, alanine, glutamic acid and cysteine. Amino acid arginine was in strong positive correlation with aspartic acid, serine, glycine, proline and tyrosine while arginine showed negative correlation with alanine, glutamic acid and cysteine. Amino acid tyrosine was not in positive correlation with cysteine, glutamic acid, alanine, aspartic acid, serine and glycine. Also, cysteine is not positively correlated with arginine, proline, aspartic acid and tyrosine (obtuse angle) Fig. 1.

Among essential amino acids, the highest positive correlation was estimated between histidine and methionine and these two amino acids are in positive correlation with isoleucine and valine. The strong positive correlation was also shown for tryptophan with phenylalanine. Amino acids methionine and histidine showed positive correlation with isoleucine, lysine, valine, leucine and threonine, while methionine and histidine had negative correlation with tryptophan and phenylalanine. Amino acid valine had positive correlation with all eight determined essential amino acids histidine, methionine, isoleucine, threonine, lysine, tryptophan and phenylalanine.

Positive correlation was observed between threonine with tryptophan, leucine, phenylalanine, valine, histidine, methionine and isoleucine, while negative correlation was assessed between threonine and lysine. Amino acid lysine had positive correlation with isoleucine, methionine, histidine and valine, while negative correlation of lysine was shown with phenylalanine, tryptophan and threonine. Amino acids tryptophan and phenylalanine showed positive correlation with threonine, leucine and valine, while negative correlation of tryptophan and phenylalanine was observed with histidine, methionine, isoleucine and lysine. Leucine was positively associated with seven essential amino acids (phenylalanine, tryptophan, threonine, valine, histidine, methionine and isoleucine) and negatively with lysine (Fig. 2).

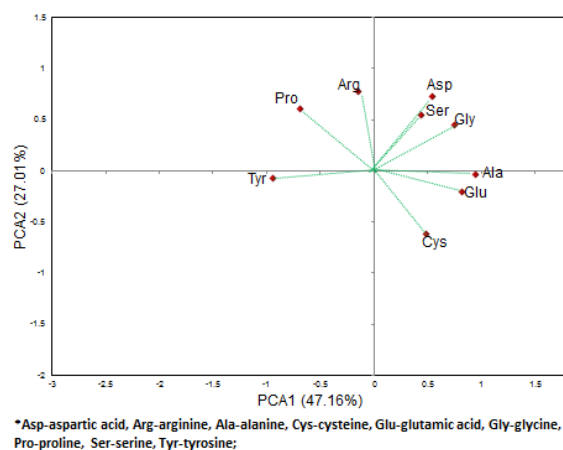


Figure 1. PCA ordinates of non-essential amino acids in the tested samples

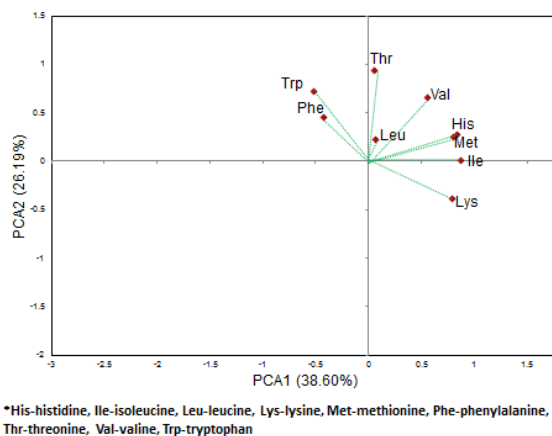
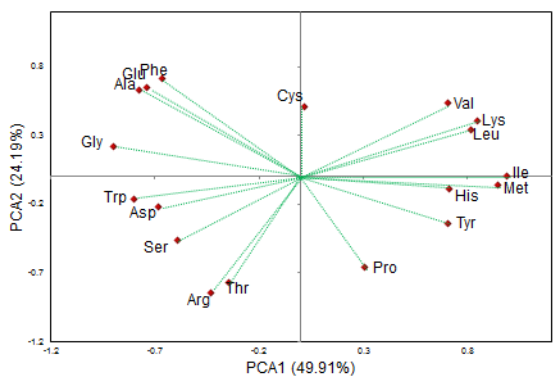


Figure 2. PCA ordinates of essential amino acids in the tested samples

The high positive correlation was estimated between lysine and leucine and this two AA with valine, than isoleucine and methionine and this two AA with histidine and tyrosine. Amino acid cysteine had positive correlation with valine, lysine, leucine, phenylalanine, glutamic acid, alanine, isoleucine, and glycine, while negative correlation was shown with tryptophan, aspartic acid, serine, arginine, threonine, proline, tyrosine, methionine and histidine. The higher positive correlation was observed for arginine with threonine, than for aspartic acid with tryptophan.

These four amino acids are positively correlated with serine. The strong positive correlation was shown for amino acids alanine and glutamic acid and both of them with phenylalanine. These three amino acids (alanine, glutamic acid, phenylalanine) were in positive correlation with cysteine, glycine, tryptophan, aspartic acid and serine. Proline had positive correlation with tyrosine, histidine, methionine, isoleucine, threonine, arginine, serine, leucine, lysine and valine, while negative correlation showed with aspartic acid, tryptophan, glycine, alanine, glutamic acid, phenylalanine and cysteine. The amino acids valine, lysine and leucine showed negative correlation with alanine, glutamic acid, phenylalanine and glycine, as well with threonine, arginine, tryptophan, aspartic acid and serine. Also, negative correlation of amino acids isoleucine, methionine, histidine, tyrosine was found with alanine, glutamic acid, phenylalanine and glycine, as well with threonine, arginine, tryptophan, aspartic acid and serine (Fig. 3). The five groups of highly associated amino acids among tested bread wheat varieties were observed: I-alanine, glutamic acid, phenylalanine; II-valine, lysine, leucine; III-isoleucine, methionine, histidine; IV-arginine, threonine; V- tryptophan, aspartic acid (Fig. 3).



*Asp-aspartic acid, Arg-arginine, Ala-alanine, Cys-cysteine, Glu-glutamic acid, Gly-glycine, Pro-proline, Ser-serine, Tyr-tyrosine; *His-histidine, Ile-isoleucine, Leu-leucine, Lys-lysine, Met-methionine, Phe-phenylalanine, Thr-threonine, Val-valine, Trp-tryptophan

Figure 3. PCA ordination of total amino acids in the tested samples

Variation of protein content in seeds of wheat varieties

Wheat grain is important source of protein for the human nutrition. The quality of wheat is influenced with amount of protein and its nutritive value. This is the main reason for improving protein content in wheat varieties. In this study the protein content in wheat flour varied from

10.24% (Ljubičevka) to 14.21% (Fortuna), with an average of 12.16%. The protein content varied depending on wheat genotype. The high protein content was observed in Šumadija (13.76%) and Kremna (13.18%) which followed by Somborka (12.71%), Danica (12.44%) and Sasanka (12.32%). The protein content below the average value of all analysed varieties was identified in Kosmajka (11.47%), followed by Morava (10.74%) and KG 56 S (10.30%) Figure 4.

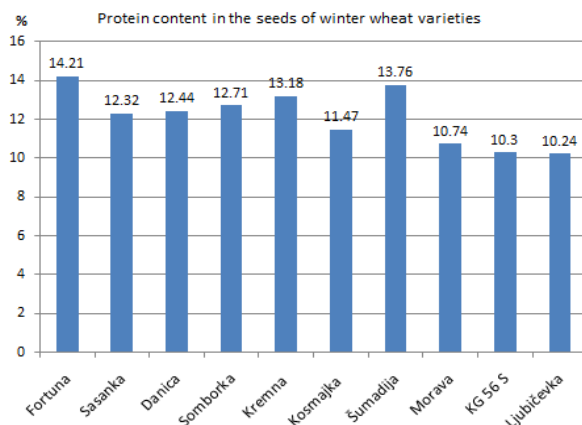


Figure 4. Protein content in the seeds of winter wheat varieties

DISCUSSION

Protein content of wheat grain is indicator of quality value of flour, dough and food products made from them such as spaghetti, bread, cake, biscuits and pastries (MENKOVSKA *et al.*, 2002; 2015; DJUKIC and KNEZEVIC, 2013; KNEŽEVIĆ *et al.*, 2016a;). The content of wheat proteins and composition of amino acids are influenced by genotype, ecological factors (soil fertility, soil salinity, humidity, temperature, precipitation), cultivation technology (rate and time of sowing, and fertilizer application) (ANJUM *et al.*, 2005; ZECEVIC *et al.*, 2014; LAZE *et al.*, 2019). The wheat protein content was positively correlated with temperature during grain filling, and negatively correlated with precipitation (HADŽI-TAŠKOVIĆ ŠUKALOVIĆ *et al.*, 2013; BRANKOVIĆ *et al.*, 2015). The protein content decreases if rainy, cold and wet weather prevails after pollination, but increases if precipitation prevails during vegetative development of wheat, with warm and dry weather during the generative phases (LOOKHART *et al.*, 2001; TORBICA *et al.*, 2011). The plant nutrition for optimal growing requires appropriate amount of mineral fertilizer, adequate time of its application, and wet soil conditions to make mineral elements in accessible forms for the efficiency of their root uptake and translocation into stem and leaf for use in the process of photosynthesis (XIE *et al.*, 2017; LJUBIČIĆ *et al.*, 2021). Nitrogen fertilizer have important role for protein synthesis (ŠIĆ *et al.*, 2013; KNEŽEVIĆ *et al.*, 2016b; STUPAR *et al.*, 2018). The prolonged period with extremely high temperatures affects the reduction of accumulation of organic matter in grain as well as yield. Also, hot weather influences changes of

the content and the composition of proteins (DJUKIĆ *et al.*, 2019). Changes in protein content and composition affect rheological and baking quality of wheat flour (NUTTALL *et al.*, 2017; KNEŽEVIĆ *et al.*, 2020). It is especially important to have mineral elements in an accessible form in the grain filling phase (GALLEJONES *et al.*, 2012). Mineral elements, nitrogen, phosphorus, potassium are essential elements for protein synthesis and growth of wheat plants. Efficiency of nitrogen absorption and utilization depends on genotypes and environmental conditions (ZEČEVIĆ *et al.*, 2005). In nitrogen metabolism, other elements play a significant role, such as sulfur having role in metabolism of the enzyme nitrate reductase. The sulfur in mineral nutrition have positive influence on nitrogen absorption and protein content (ZÖRB *et al.*, 2012). Increasing sulfur application by fertilization in S-limited soils, especially under dry conditions, alleviate negative effect of high temperature on wheat yield and increase the content of protein and its quality in flour (TAO *et al.*, 2018). The sulfur fertilization can increase the plant content of sulfur amino acids, which favors synthesis of proteins rich in S, cysteine (Cys) and methionine (Met) influencing the changes of the proportions of glutenin and gliadin in total protein content, and further affecting the quality of flour.

The protein content and amount and composition of amino acid in wheat grain, especially essential amino acids have important share in determining nutritional quality of food product for human and animals (FAO, 2002). The wheat varieties with high content of essential amino acids have advantages for production in less developed countries in which cereals represents major sources of total protein (SHEWRY, 2007). However, content of essential amino acids (lysine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, tryptophan) is low and limited in wheat grain. The content of essential amino acid accounted for share 34.12% in the total content of amino acids on average of all ten analyzed varieties. Among them in this study the lowest value of AA content was found for methionine (1.28%), tryptophan (1.20%), histidine (2.78%), threonine (2.86%) and lysine (2.91%). Quantitative analysis of protein in wheat showed that the highest percentage of all identified non-essential amino acids had glutamic acid (18.51%) and proline (12.15%), and this is in agreement with other research (KNEZEVIC *et al.*, 2009; KNEŽEVIĆ *et al.*, 2013; ALIJOŠIUS *et al.*, 2016; LAZE *et al.*, 2019; SIDDIQI *et al.*, 2020). Amino acid proline have important role in wheat plant response to environmental stress condition. The role of proline is contribution to stabilizing redox potential activity of cells and protecting membranes and proteins from harmful effects of high ion concentrations and extreme temperatures (SZABADOS and SAVOURÉ, 2010; BESALIEV *et al.*, 2021). Amino acids play different roles in plants. They can act as stress-reducing agents, source of nitrogen and hormone precursors, thereby inducing growth parameters (HAMMAD and ALI, 2014). Amino acids play different roles in plants. They can act as stress-reducing agents, source of nitrogen and hormone precursors, thereby inducing growth parameters (HAMMAD and ALI, 2014). So, foliar application of aspartic acid leads to the efficient increase in content of carbohydrates, proteins, and to the efficient absorption of nitrogen, phosphorus, potassium increasing plant height, number of tillers m⁻², number of grains spike⁻¹, 1000-grains weight and grain yield (EL-AWADI *et al.*, 2019).

Amino acids are very important role in advancing yield, quality and adaptation to biotic and abiotic stress condition in wheat plant growth period. For example, alanine and glycine have influence on velocity of plant growth and initiating chlorophyll formation and chlorophyll

concentration what magnifies intensity of photosynthesis. Phenylalanine contribute in enhancing plant cell growth and formation of embryo. Methionine contributes to the faster ripening and has role in root activation, as well as valine has influence on faster growth of plant and root as well on seed production. Histidine and isoleucine contributes to enlargement of plant growth and early yielding, while lysine influence to increasing shoot system, growth, and early yield. Tryptophan has role in formation of plant hormones which stimulate plant growth (auxins - IAA) and have a role in the early yielding. Serine has important role for hormone balance of plant, activating chlorophyll (MA and WANG, 2016; BAQIR *et al.*, 2019; FEDURAEV *et al.*, 2020). Similar as lysine, the glutamic acid influences increasing of the shoot system, growth, and early yielding (HAROUN *et al.*, 2010; MAZHER *et al.*, 2011; MATYSIAK *et al.*, 2020). Amino acid arginine enhances root formation, cell division and chlorophyll formation (HOZAYN *et al.*, 2010).

In old Kragujevac's wheat varieties (Kosmajka, Šumadija, Morava, KG 56 S and Ljubičevka) had higher content of glycine than in remain five varieties which is younger according to selected time in breeding Selection Center in Novi Sad. Therefore, moderate concentration of glycine and glutamine leads to the increase of yield, leaf chlorophyll content, content of vitamins, while the application of higher concentration of 1000 mg L⁻¹ glycine increases concentration of leaf iron (NOROOZLO *et al.*, 2019).

CONCLUSION

Examination of amino acid composition and associations of seeds proteins in Serbian bread wheat varieties provided estimation of nutritional value of proteins in analyzed wheat varieties. The established content of essential and nonessential amino acids indicated quality of flour, dough and bread. The grain protein content varied from the lowest 10.24% in Ljubičevka variety to the highest in Fortuna variety 14.21%, with the average value 12.16% for the analyzed wheat varieties. The variability of protein content in wheat grain, as well of total essential amino acids, total nonessential amino acids, and total amino acids were assessed. For each variety the nine essential and the nine non essential amino acids of total 18 amino acids were identified. The content of essential amino acids varied in ratio between 28.38% in Fortuna variety and 31.88% in Ljubičevka variety. The content of total amino acids was the lowest in Fortuna (85.35%) and the highest in Ljubičevka (93.94%), with average value 87.53% for all ten wheat varieties. However, in wheat variety Kremna, the highest share of essential amino acids was observed (34.53%) in total content of amino acids and the lowest share of essential amino acids had variety Fortuna (33.25%). The varieties from breeding center in Kragujevac (Kosmajka Šumadija, Morava, KG 56 S, Ljubičevka) on average had higher content of total amino acids (90.01%) in comparison to wheat from breeding center in Novi Sad (Fortuna, Sasanka, Danica, Somborka, Kremna) with average content value 85.85% of total amino acids, underlying genetic differences of initial breeding germplasm and different breeding strategies. Also, in Kragujevac's wheat varieties the higher content of essential amino acids (30.55%) was identified in comparison to varieties from Novi Sad breeding center (29.18%), while the share of essential amino acids in content of total amino acids was approximately equal (33.94% and 33.99%). Among individual amino acids the highest content was identified for glutamic acid on average (18.51%) and afterwards for proline (12.15%), whereas the lowest content was observed for tryptophan. The highest value of coefficient of variation (CV) was observed for phenylalanine

(15.52%), and the lowest for aspartic acid (2.92%). TEAA, TNEAA, TAA, share TEAA/TAA, share TNEAA/TAA varied with CV of 3.50%, 3.58%, 3.47%, 1.08%, and 0.56%, respectively. The five groups of highly associated amino acids among tested bread wheat varieties were observed: I-alanine, glutamic acid, phenylalanine; II-valine, lysine, leucine; III-isoleucine, methionine, histidine; IV-arginine, threonine; V- tryptophan, aspartic acid.

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SADRŽAJ PROTEINA I SASTAV AMINOKISELINA U SEMENU HLEBNE PŠENICE (*Triticum aestivum* L.)

Dušan UROŠEVIĆ¹, Desimir KNEŽEVIĆ², Gordana BRANKOVIĆ³,
Aleksandra Yu. NOVOSELSKAYA-DRAGOVICH⁴, Alexander M. KUDRYAVTSEV⁴,
Mirela MATKOVIĆ STOJŠIN⁵, Danica MIĆANOVIĆ⁶, Veselinka ZEČEVIĆ⁷

¹Institut za kukuruz Zemun Polje, Zemun Srbija

²Univerzitet u Prištini, sa privremenim sedištem u Kosovskoj Mitrovici, Poljoprivredni fakultet,
Lešak, Lešak, Kosovo i Metohija, Srbija,

³Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd Beograd, Srbija

⁴Ruska akademije nauka, Laboratorija za biljnu genetiku, Institut za opštu genetiku
“N.I.Vavilov” RANMoskva, Rusija

⁵Instraživačko Razvojni Institut “Tamiš” Pančevo, Pančevo Serbia

⁶Privredna komora Srbije, Beograd

Institute, za povrtarstvo, Smederevska Palanka, Srbija

Izvod

Cilj ovog istraživanja je bio određivanje sadržaja proteina i sastava aminokiselina u semenu hlebne pšenice, sa posebnim fokusom na procenu esencijalnih aminokiselina (EAA). Za analizu su korišćeni uzorci brašna mlevenog semena 10 sorti pšenice, koje su selekcionisane u različitim oplemenjivačkim centrima (u Novom Sadu i Kragujevcu, Srbija). Za određivanje sadržaja proteinakorišćen je Kjeldahlov metod za određivanje sadržaja azota (N) čija je vrednost pomnožena sa koeficijentom 5,7 (sadržaj proteina = 5,7 k % N sadržaj). Za analizu aminokiselina uzoraka pšenice korišćena je jonoizmenjivačka hromatografija, reakcija bojenja ninhidrinom i fotometrijska detekcija na 570 nm i 440 nm (za prolin). Rezultati su pokazali da je srednji sadržaj proteina pšenice varirao od najmanje vrednosti 10,24% kod Ljubičevke do najveće 14,21% kod sorte Fortuna. Srednja vrednost sadržaja (g 100 g⁻¹ proteina) neesencijalnih aminokiselina (NEAA) je nađena za asparaginsku kiselinu 5,42%, serin 4,23%, glutaminsku kiselinu 18,51%, prolin 12,18%, glicin 4,17%, alanin 3,64%, tirozin 2,52%, dok je srednja vrednost sadržaja esencijalnih aminokiselina (EAA) bila za treonin 2,86%, valin 4,52%, metionin 1,28%, izoleucin 3,87%, leucin 5,87%, fenilalanin 4,62%, histidin 2,46% i lizin 1,2%. Sorta pšenice je sadržala relativno visoku koncentraciju najdeficitarnijih EAA. Kod sorte Ljubičevka je nađena relativno visoka koncentracija najdeficitarnijih EAA (treonin, valin, leucin, lizin). Najveći sadržaj (29,31 g 100 g⁻¹ proteina), ukupnih esencijalnih amino kiselina (TEAA), i najveći sadržaj (59,79 g 100 g⁻¹ proteina) ukupnih neesencijalnih amino kiselina (TNEAA) kao i najveća vrednost (90,35 g 100 g⁻¹ proteina) ukupnih aminokiselina (TAA). Dobijeni rezultati predstavljaju varijabilnost sorti pšenice na osnovu sadržaja aminokiselina i ukazuju na pouzdanu sortu za odabir poželjnih roditelja u oplemenjivačkom programu za poboljšanje kvaliteta hranljivih materija.

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