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Contents of Amino Acids in Grains of Different Bread Wheat Genotypes

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Abstract

This paper analyzes 10 different genotypes of the bread wheat by method of chromatography to identify the presence of free amino acids. The contents of the identified amino acids have been determined by spectrophotometric method. The results of the qualitative analysis showed the great deal of variability in the amino acid composition for each of the examined genotypes. Quantitative analysis of the free amino acids in the grains indicated their high content (over the 100 mg ml^{-1}) in wheat genotypes San Pastore, Becker, Lihnida and Ana Morava, while their lowermost content was in the genotypes Uras (73 mg ml^{-1}) and Jawa (75 mg ml^{-1}). By using chromatography in the examined wheat grains have been determined that the most present amino acids were glutamic acid, glycine, sarcosine, valine, norvaline and tryptophan. The most present of all examined amino acids was glutamic acid, which was identified in nine examined wheat genotypes. The highest content of glumatic acid was found in wheat genotype Uras (6,52 mg ml^{-1}). Among the essential amino acids has been found the highest content of norvalin (2,56 mg ml^{-1}) and valin (2,32 mg ml^{-1}). The wheat grains of the genotypes Becker, San Pastore and Ana Morava had the largest number of the determined essential amino acids (five), indicating their high nutritional value.

Key words: wheat, amino acids, genotypes, contents

Introduction

Wheat grain is a major cereal crop used in milling and baking industries, as well as in the human nutrition. The major components of the wheat flour are carbohydrates (between 60-80%), proteins (6-28%), fiber (~10%), oil (~2%), minerals and vitamins (Lasztity, 1995). Wheat proteins deposited in the grain's endosperm are divided into two main groups: the salt soluble fraction (albumins and globulins) and the

gluten fraction (gliadins and glutenins). Gluten is in the relationship with the viscoelastic properties of dough and because of that has important role in the process of making the bread, biscuits, cakes and other bakery products (Torbica et al., 2007; Živančev et al., 2010). In the combination with other food proteins such as those from legumes, oil seeds or animal products, wheat proteins play important role in human nutrition, as source of the essential amino acids.

The proteins and amino acids also have several significant roles for the plants, for example they regulate ion transport and affect the synthesis and activity of enzymes and gene expression (Rai, 2002). The proline- and glutamine-rich storage proteins of the wheat are the sources of nitrogen and amino acids for the embryo in the first phase of the seed development. Therefore, it is likely that endogenous cereal proteases synthesized during germination are capable for extensively hydrolysis of these proteins (Hartmann et al., 2006). These endopeptidases and exopeptidases have specific capability for the fragmentation of gluten protein and peptides (Gessendorfer et al., 2011).

On the other side, important source of nitrogen required for the synthesis of amino acids and proteins in the plants is nitrogen nutrition (Neuberg et al., 2010; Pavlík et al., 2010). Nitrogen fertilisation can be used for nutritional improvement of the human diet by increasing and maintaining protein and essential amino acid contents (Thanapornpoonpong et al., 2008). According to Tilsner et al. (2005) under low N supply, the amino acid contents are comparable at all leaf ages and decrease slightly from young to mature leaves, while higher N supply also causes an overall increase in amino acid content (Atanasova, 2008).

For sustainable wheat production with reduced inputs of the agrochemicals it is necessary to create cultivars with enhanced quality for specific end-uses, example for biofuels and in the food industry. Some cultivars are generally considered to be more consistent in quality than others (Shewry, 2007).

The purpose of this investigation was the analysis of composition of amino acid and determination of concentration in 10 genetically divergent bread wheat cultivars.

Materials and methods

The presence of amino acids and their content were determinated in the grain samples of the 10 wheat genotypes: Uras, Becker, San Patore, Jawa, Ceska 488, Moulin, Lodan, Lepenica, Lihnida and Ana Morava. Amino acids have been extracted from the wheat grain with ethanol (diluted to 80%), while the sedimentation of the dissolved proteins has been done with chlorophorm (Grujić-Injac, 1962). Identification of amino-acid in the prepared extracts has been done by using the method of chromatography. The method of spectrophotometry was used for determination of the total content of the identified amino acids. Total concentration of the free amino acids was determined by standard curved line for tyrozine, while concentration for each amino acid was determined by standard curved line for glycine (Trajković et al., 1983; Džamić, 1989).

Tab 1. The results of the qualitative analysis of the amino acides in the examined wheat genotypes
Rezultati kvalitativne analize aminokiselina u ispitivanim sortama pšenice

Amino acids *Aminokiseline*	San Pastore	Uras	Lodan	Becker	Jawa	Moulin	Ceska 488	Lepenica	Lihnida	Ana Morava
Sarcosine		+	+		+		+	+	+	
Proline				+	+	+				+
Oxyproline							+			
Glycine	+	+	+		+		+	+	+	
Threonine			+		+					+
Glutamic acid	+	+	+	+		+	+	+	+	+
Valine	+	+				+	+			+
Norvaline			+	+	+			+	+	
Methionine	+							+		+
Tryptophan				+		+	+	+		+
Nor-leucine				+					+	
Phenylalanine	+	+				+				+
Cistin-clorhydrate	+			+				+		
Arginine-clorhidrate			+	+						
Arginine	+									

Results and discussion

The analysis of amino acids in the wheat grain showed differences among examined wheat genotypes.

The results of the qualitative analysis showed the variability in the amino acid composition in the examined wheat genotypes. For all analyzed cultivars has been identified 15 different amino acids. In the each of the examined cultivars has been identified different number of amino acids (between 5-7), as well as the different composition of identified amino acids (Tab. 1). According to the results of the analysis the most present amino acids in the examined grain samples were glutamic acid, glycine, sarcosine, valine, norvaline and tryptophan. It is well known, that glutamic acid and glycine are principal amino acids in all cereal protein fractions.

Quantitative analysis of the free amino acids in their grains indicated their high content (over the 100 mg ml^{-1}) in the wheat genotypes San Pastore, Becker, Lihnida and Ana Morava (Tab. 2).

Tab. 2. Total content of free amino-acids in grains of the examined wheat genotypes
Ukupni sadržaj aminokiselina u zrnu ispitivanih genotipova pšenice

Cultivar of wheat (*Triticum aestivum*) *Sorta pšenice* (*Triticum aestivum*)	Concentration (mg ml^{-1}) *Koncentracija* (mg ml^{-1})	%
San Pastore	113	11.3
Uras	73	7.3
Lodan	89	8.9
Becker	130	13.0
Jawa	75	7.5
Moulin	98	9.8
Ceska 488	90	9.0
Lepenica	82	8.2
Lihnida	106	10.6
Ana Morava	114	11.4

The total content of free amino acids in the analyzed wheat genotypes varies between 73 mg ml^{-1} and 130 mg ml^{-1}. The highest content of the free amino acid was found in the wheat genotype Becker (130 mg ml^{-1}), while the lowest was in the genotype Uras (73 mg ml^{-1}).

The total content of the essential amino acids varies in the grain of the diferent wheat genotypes, too. Amino acid valine and tryptophan were present in the grains of the five examined wheat genotypes. Valine was identified in wheat genotypes San Pastore, Uras, Moulin, Ceska 488 and Ana Morava, while tryptophan was identified in genotypes Becker, Moulin, Ceska 488, Lepenica and Ana Morava. Total content of valine was in ratio from 1.0 mg ml^{-1} (Uras) to 2.32 mg ml^{-1} (San Pastore), while ratio of concentration of tryptophan was from 1.25 mg ml^{-1} (Lepenica) to 2.24 (Ceska 488).

Tab. 3. Total content of essential amino acids (EAA) in the grains of the examined wheat genotypes
Ukupan sadržaj esencijalnih aminokiselina (EAA) u zrnu ispitivanih genotipova pšenice

Cultivar *Sorta*	Met	Thr	Val	Nor-valin	Nor-leucin	Phe	Arg	Arginine - clorhy drate	Trp	Cyistin-clorhy drate
San Pastore	1.0	-	2.32	-		0.84	0.94	-	-	0.86
Uras			1.0			0.94				
Lodan		1.0		2.56				0.83		
Becker				2.08	1.06			0.83	2.07	0.92
Jawa		0.92		1.0						
Moulin			2.14			1.0			1.82	
Ceska 488			2.22						2.24	
Lepenica	1.43				1.18				1.25	1.14
Lihnida				1.23	1.0					
Ana Morava	1.24	0.96	2.24			1.0			1.62	

Tab. 4. Total content of non-essential amino acids (NEAA) in grain of the examined wheat genotypes
Ukupan sadržaj neesencijalnih aminokiselina (NEAA) u zrnu ispitivanih genotipova pšenice

Genotype	Glu	Pro	OxyPro	Gly	Sarc
San Pastore	4.25			0.95	
Uras	6.52			0.8	1.0
Lodan	4.82			1.24	1.0
Becker	2.74	1.0			
Jawa		2.2		1.0	1.0
Moulin	2.26	1.0			
Ceska 488	2.12		0.98	0.96	0.8
Lepenica	3.68		1.36	1.0	1.0
Lihnida	2.85			1.0	0.8
Ana Morava	2.86	1.34			

Methionine was identified in only three examined genotypes: San Pastore, Lepenica and Ana Morava. The wheat genotype Lepenica had the highest content of methionine (1.43 mg ml^{-1}) and genotype San Pastore consists the lowest content of methionine (1.0 mg ml^{-1}), Tab. 3.

Threonin, that is very important for nutrition as one of limiting essential amino acids, was identified in grain of wheat genotypes Lodan, Jawa and Ana Morava with total content ~1.0 mg ml^{-1}. Similar total content for nor-leucine was identified in wheat genotypes Becker, Lepenica and Lihnida ~ 1.0 mg ml^{-1}. The content of phenyl alanine was ~1.0 mg ml^{-1} in four wheat genotypes San Pastore, Uras, Moulin and Ana Morava. Arginine was identified only in San Pastore with determined content of 0.94 mg ml^{-1}, while arginine-chlorhydrate was identified in wheat genotypes Lodan and Becker (0.83 mg ml^{-1}), Tab. 3.

The grains of the examined genotypes Becker, San Pastore and Ana Morava had the largest number of the determined essential amino acids (five), indicating their high nutritional value.

Glutamic acid is very important for nitrogen metabolism in cell. In gliadins storage proteins glutamic acid is present as glutamine. In this investigation glutamic acid was identified in nine wheat cultivars, while it was not identified only in wheat genitype Jawa. The Ceska 488 had the lowest total content (2.12 mg ml^{-1}), while the wheat genotype Uras contained 6.52 mg ml^{-1} of glutamic acid (Tab. 4). Similar results in aestivum and durum wheat cultivars were found in the ratio from 4.25 to 8.75 mg ml^{-1} by earleir research (Knežević et al., 2009).

Amino acid glycine was identified in content ~1.0 mg ml^{-1} in grain of seven wheat genotypes: San Pastora, Uras, Lodan, Jawa, Ceska 488, Lepenica and Lihnida (Tab. 4).

Sarcosine was identified with content ~1.0 mg ml^{-1} in six wheat genotypes Uras, Lodan, Jawa, Ceska 488, Lepenica and Lihnida. Concentration of proline was the lowest 1.0 mg ml^{-1} in genotypes Becker, Moulin, than 1.34 mg ml^{-1} in genotype Ana Morava, and the highest 2.2 mg ml^{-1} in genotype Jawa (Tab. 4). The high proline content has an influence to the secondary structure of gliadin polypeptides. This results were similar in the earlier investigation (Knežević et al., 2007).

Conclusion

The amino acid composition of analyzed bread wheat genotypes was different. For each genotype was identified between five to nine free amino acids, but each genotype had specific composition of amino acids. Analyzed wheat genotypes differed according to amino acid concentration. Glutamic acid had the highest concentration in the examined wheat genotypes. The presence of essential amino acids varies between two (Uras and Ceska 488) to five (San Pastore, Becker and Ana Morava). The highest concentration of essential amino acids was established for norvaline (2.56 mg ml^{-1}) and valine (2.32 mg ml^{-1}), while the lowest concentrations were registered for methionine, threonine, arginine, phenylalanine (~1.0 mg ml^{-1}). For increasing of amino acid content as well composition of free essential amino acids in grain of wheat we need to increase

our knowledge about mechanisms of the control grain protein accumulation at the molecular, biochemical and physiological levels. Considering that amino acid composition of wheat grain proteins is genetically determined, it mean that changes of amino-acid composition is possible realize through changes of composition of protein fraction and its proportion. Also, for improving nutritional value are necessary to select wheat genotypes in terms of essential amino acids content and higher protein content. Changes of proportion of storage proteins could have advantage for nutritional value.

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Sadržaj aminokiselina u zrnu različitih tipova hlebne pšenice

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Sažetak

U radu je ispitivano 10 različitih genotipova hlebne pšenice metodom hromatografije za identifikaciju prisustva slobodnih aminokiselina. Sadržaj identifikovanih aminokiselina je utvrdjen spektrofotometrijskom metodom. Rezultati kvalitativne metode su ukazali na veliki varijabilitet po pitanju sastava aminokiselina za svaki ispitivani genotip. Kvantitativna analiza slobodnih aminokiselina u zrnu pšenice ukazala je na njihov visok sadržaj (više od 100 mg ml^{-1}) u genotipovima pšenice San Pastore, Becker, Lihnida i Ana Morava, dok je najniži sadržaj kod genotipova Uras (73 mg ml^{-1}) i Jawa (75 mg ml^{-1}). Hromatografijom je u ispitivanim zrnima pšenice utvrdjeno da su najprisutnije aminokiseline: glutaminska kiselina, glicin, sarkozin, valin, norvalin i triptofan. Od svih ispitivanih aminokiselina, najprisutnija je glutaminska kiselina koja je identifikovana kod devet ispitivanih pšeničnih genotipova. Najviši sadržaj glutaminske kiseline ustanovljen je kod genotipa pšenice Uras (6,52 mg ml^{-1}). Po pitanju esencijalnih aminokiselina utvrdjen je najviši sadržaj norvalina (2,56 mg ml^{-1}) i valina (2,32 mg ml^{-1}). Zrna pšenice genotipova Becker, San Pastore i Ana Morava imala su najveći broj utvdjenih esencijalnih aminokiselina (pet), što ukazuje na visoku nutritivnu vrednost.

Ključne reči: pšenica, aminokiseline, genotipovi, sadržaj

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