CHEMICAL COMPOSITIONS AS QUALITY PARAMETERS OF ZP SOYBEAN AND WHEAT GENOTYPES

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This research is focused on the analysis of chemical characteristics of ZP soybean and wheat genotypes, as well as, on nutritional differences between this two complementary plant species. The experimental material consisted of two bread (ZP 96/I and ZP 87/Ip), two durum (ZP 34/I ZP and ZP DSP/01-66M) wheat genotypes and four soybean varieties (Nena,

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Lidija, Lana and Bosa) of different genetic background. All ZP soybean genotypes, except the Lana, had over 40% of total proteins by dry matter. Lana and Lidija, variety of recent creation, developed as a result of selection for specific traits, had high oil content. Wheat genotypes had much a lower content of ash, oil, total and water soluble proteins than soybean cultivars. The highest oil, total and water soluble proteins content was detected in grain of durum genotype ZP DSP/01-66M. Lignin content varies much more among soybean than among the wheat genotypes. Generally, contents of total phenolics, carotenes and tocopherol were more abundant in ZP soybean than bread and durum wheat genotypes.

Key words: antinutritional components*,* protein, oil, ZP genotypes

INTRODUCTION

The genetic improvement of soybean and wheat germplasm contributes to advances in production and food processing industry by developing high-yielding and high-quality cultivars, hereby enhancing value-added and healthy properties of final products. The quality of some products has been associated with the content of some component of the protein fraction of the seed. Out of all cereals and legumes soybean has the highest percent of proteins (over 40% dry matter, on the average). However, the significance of soybean proteins in nutrition depends not only on its quantity but primarily on its quality. Betaconglycinin and glycinin are the two primary components of soybean storage proteins (WILSON, 1987). For those components significant differences among genotypes and environments have been reported (MURPHY and RESURRECIN1984, FEHR, 2003, TAŠKI-HAJDUKOVIĆ *et al*., 2008). One of the most important chemical characteristics of soybean proteins is the amino acid content that determines nutritive values of proteins. However, a high nutritive value and the use of soybean proteins are limitated by a great number of antinutritional factors (trypsin inhibitors, hemaglutinins, isoflavones, saponins, lipoxygenase, phytic acid, flotulen acid, urease etc).

Soybean contains approximately 20% oil and it ranks second among all edible legumes by oil quality (a higher content of about 48% oil in dry matter was recorded in peanut). Oil content is a quantitative trait, and its level is determined by genetic background, environmental variables and interaction between genotype and environment (ŽILIĆ *et al*., 2006, PERIĆ *et al*. 2009). Nutritional and functional properties of the oils are determined by their fatty acid composition and distribution pattern within triaglycerols, and content and composition of natural antioxidants (SUDARIC *et al*, 2008).

Wheat has traditionally been selected for functionality, for example, baking or biscuit values. However, little attention has generally been given to the nutritional value of the grain and its improvement through breeding programmes. Nutritional values of wheat vary according to their nutrient content and digestibility. Variation of nutrient content is under genetic and environmental control. Wheat grains are

mainly composed of carbohydrates (65–75% starch and fibre) and proteins (7–12%), but also contain lipids (2–6%), water (12–14%) and micronutrients (POMERANZ, 1988). Starch is the most abundant component of wheat and is present in the endosperm. It consists of glucose polymers, amylose and amylopectin. Typical levels of amylose and amylopectin are 25–28% and 72–75%, respectively (COLONNA and BULEON, 1992). Wheat grain proteins can be divided in two main groups: the gluten and non-gluten proteins. Non-gluten proteins (ca. 15–20% of total wheat proteins) consist of albumins and globulins. Gluten proteins (ca. 80–85% of total wheat proteins) are the main storage proteins of wheat. Currently, in Serbia there is no official soybean and wheat classification system based on specific end use. Soybean and wheat class has been a major basis for determining end-product functionality. Besides, considering that a significant number of metabolic disorders and diseases are caused by malnutrition, and the fact that the majority of the world population consumes soybean and wheat for food as complementary plant species, one of the important breeding objectives in the Maize Research Institute is the development of genotypes with the improved nutritive value. Therefore, this research is focused on the analysis of chemical characteristics of ZP soybean and wheat genotypes (standard chemical composition, dietary fibres, antioxidants and enzymes), as well as, on nutritional differences between this two complementary plant species. A more detailed knowledge of chemical properties of soybean and wheat genotypes will be have benefit in the future selection of genotypes with improved nutritional quality.

MATERIALS AND METHODS

Soybean and wheat samples.-The plant material consisted of two bread (*Triticum aestivum* L.) (ZP 96/I and ZP 87/Ip), two durum (*Triticum turgidum* Desf.) (ZP 34/I ZP and ZP DSP/01-66M) wheat genotypes and four soybean varieties (*Glycine max* L. Merit) (Nena, Lidija, Lana and Bosa) of different genetic background recently developed at the Maize Research Institute, Zemun Polje, (MRIZP), Serbia. The genotypes were chosen on the basis of their differences in agronomic traits. Kernels were collected at full maturity stage from plants grown in a field-trial at the MRIZP in the 2007-2008 growing season. Standard agronomic practices were used to provide adequate nutrition and maintain the plots disease-free. The wholemeal flour (particle size<500 μm), obtained by grinding kernels on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden) was used in analyses.

Analytical procedures.-The standard chemical methods (OFFICIAL GAZETTE OF SFRY, 1987) were applied to determine the content of starch and total proteins. The content of water soluble proteins was analysed by the modified Osborne method (LANDRY and MOUREAUX, 1970). The results are given as % of d.w., as well % of total proteins (protein solubility index-NSI).

The content of dietary fibres, hemicellulose, cellulose, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) was determined by Van Soest detergent method (VAN SOEST, 1963) modified by MERTENS (1992). The method is based on the fibres solubility in neutral, acid and alkali reagents.

For the DPPH test the soybean and wheat kernel extract was prepared by dissolving 0.3 g of flour in 10 ml of 70% (v/v) acetone. After continuous shaking for 30 min at room temperature, the solution was centrifuged for 20 min at 20 000 g. An aliquot of extract (0.1 ml) was mixed with ethanol DPPH$^{\bullet}$ solution (0.5 mM, 0.25 ml) and acetate buffer (100 mM, pH 5.5, 0.5 ml). After 30 min standing in the dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. The results are expressed as an IC_{50} value that represents the amount of flour (in mg) providing 50% inhibition of DPPH$^{\bullet}$ radicals (KOLEČKÁŘ *et al.,* 2007). Total phenolics were determined by the method of SINGLETON and ROSSI (1965), using the same extract as for the DPPH$^{\bullet}$ test. The total phenolics content was calculated as a catechin equivalent (eq.) from the calibration curve of catechin standard solutions and expressed as mg of catechin per g of dry matter (d.m.). All the assays were done in duplicate, from two separate extractions.

The carotene content was extracted from 11 g of flour with three volumes of the petrolether: acetone (70:30 v/v) mixture at 60-70°C for one hour. After separation on the column made of the Al_2O_3, Na_2SO_4 and MgO (3:2:1 w/w) mixture, the absorbance of fluent (petrolether: acetone) was measured spectrophotometrically at 447 nm (TURČIĆ, 1976). The tocopherol content was determined by the HPLC method (OUFNAC, 2007). The fluorescence detector was set at 290 nm excitation and 330 emissions. Analyses were done in three replications.

For determination of the POD activity, crude homogenate of flour (0.5 g) was prepared by constant stirring of (0.5 g) flour in 10 ml of 0.1 M K-phosphate buffer, pH 7.6 at 4°C, for 1 hour. After centrifugation at 20 000 g for 15 min, the obtained supernatant was used to determine the POD activity. The reaction mixture (1 ml) consisted of 0.1 mM ferulic acid, 1 mM H_2O_2 and an aliquot of extract containing about 4 mg of the sample, in 100 mM K-phosphate buffer, pH 5.5. The initial rate of the absorbance changes at 286 nm ($\varepsilon = 1.68 \times 10^4 \ M^{-1} \ cm^{-1}$) was measured. The enzyme activity calculation was done on the sample dry mass basis (HADŽI-TAŠKOVIĆ ŠUKALOVIĆ *et al.,* 2003).

The lipoxygenase (LOX) (EC 1.13.11.12) activity was determined in the crude flour homogenate prepared by shaking the sample with five volumes of 0.2 M sodium phosphate buffer (pH 6.5) at 4°C for 120 min. The supernatant obtained by centrifugation at 20 000 g for 15 min, was used to measure the LOX activity. The assay mixture consisted of 50 mM linoleic acid in 0.2 M borate buffer, pH 9.0, and an aliquot of the sample. The initial rate of the absorbance changes at 234 nm ($\varepsilon = 2.5 \times 10^4 \ M^{-1} \ cm^{-1}$) was recorded. The activity of LOX was expressed in μmol of conjugated diene formed per minute and g d.w. (AXELROD *et al.,* 1981).

Statistical analyses.-All chemical analyses were performed with replicates and the results were statistically analysed. Significant statistical differences of observed chemical maize parameters means were determined by the Fisher´s least significant differences (LSD) test, after the analysis of variance (ANOVA) for trials set up according to the RCB design.

RESULTS AND DISCUSSION

Soybean is a plant with particularly high contents of both, proteins and oil, which was also confirmed by our results (Table 1). According to gained results all ZP soybean genotypes, except the Lana, had over 40% of total proteins by dry matter. Nena ranks first among all tested genotypes regarding the content of total proteins contained 43.30% proteins on the average. However, the oil content was the lowest in this genotype (only 19.33%). Although, the content of total proteins of the Lana was lower by 10% than of the Nena, the former had the oil content higher by 15% than the latter (Table 1). Lana and Lidija, genotypes of recent creation, developed as a result of selection for specific traits, had high oil content. The oil yield in these genotypes amounted to approximately 1,120 kg ha^{-1}. Lidija and Bosa are very similar in respect to contents of total and water soluble proteins. The solubility index in both amounted to 80.0% of the total protein content (Table 2). On the other hand, seed of the Nena had the highest content of water soluble proteins, even 35.0% of d.m. (Table 1).

Table 1. The basic chemical content and soluble proteins in kernels of ZP soybean and wheat genotypes

Genotypes	Ash (%)	Oil (%)	Total proteins (%)	Water soluble proteins (%)	NSI (%)
Nena	4.71[b]	19.33[d]	43.30[a]	35.05[a]	80.83[a]
Lidija	4.55[c]	22.15[b]	40.45[b]	32.41[b]	80.04[b]
Lana	4.82[ab]	22.64[a]	38.70[c]	28.56[c]	73.63[c]
Bosa	4.94[a]	21.12[c]	40.70[b]	32.63[b]	80.12[b]
ZP 96/I	1.82[ef]	2.74[f]	12.03[f]	3.91[e]	32.50[e]
ZP 87/Ip	1.80[f]	1.69[h]	12.13[f]	3.67[f]	30.25[f]
ZP 34/I	1.95[e]	2.69[g]	13.03[e]	3.53[f]	27.09[g]
ZP DSP/01-66M	2.32[d]	3.33[e]	17.52[d]	5.83[d]	33.27[d]
LSD$_{0.05}$	0.129	0.100	0.313	0.201	0.651
CV (%)	3.30	0.68	0.97	0.94	1.01

[a-h] Means followed by the same letter within the same row are not significantly different P<0.05); CV-coefficient of variation

Protein and oil content are negatively correlated (r=-0, 963). Negative correlations between protein and oil, as well as with yield, are well documented in the soybean genetics and breeding literature (BURTON, 1985; WILCOX and CAVINS, 1995; WILCOX, 1998; COBER and VOLDENG, 2000, CHUNG *et al*, 2003, PERIC *et al*., 2009).

Wheat genotypes had much a lower content of ash, oil, total and water soluble proteins than soybean cultivars. According to our results, the highest oil, total and water soluble proteins content was detected in grain of durum genotype ZP DSP/01-66M (3.33, 17.52 and 5.83% d.m., respectively) (Table 1). In contrast with the soybean protein which is rich in lysine, the wheat protein has a high content of

essentially sulphur containing amino acid, methionine and cystine. Therefore, soybean and wheat are complementary plant species for food.

Fibre components are one of the most important nutritional and technological factors of the soybean and wheat kernel. The dietary fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. A large part of fibre content variations depends of their genetic background. The content of cellulose, hemicellulose, NDF, ADF and lignin in kernels of the investigated genotypes is presented in Table 2. The lignin content vary from 0, 64% (Lidija) to 1, 67% (Bosa) in soybean seed. This is in accordinance with results of ALVARES *et al,* 1997; PANOBIANCO *et al*, 1999, and MARWANTO, 2003, who find that lignin content in soybean seed varies among genotypes. On the other hand, lignin content did not vary greatly among the wheat genotypes. Significant differences in the cellulose, hemicellulose, NDF and ADF content between soybean and wheat genotypes were found (P<0.05). The ADF content ranged from 2.91% in the bread wheat genotype ZP 87/Ip to 9.84% in the soybean genotype Lana. The wheat genotypes had the highest content of hemicellulose ranging from 59.89 to 70.12%, as well as, NDF ranging from 62.80 to 73.24% (Table 2). The content of all of these components, except lignin, were in correlation with a high digestibility. Our results showed that wheat is an important source of dietary fibres. This property of wheat is of a considerable industrial importance, particularly for the food industry where the digestibility is one of the most important nutrition factors of end products.

Table 2. The content of dietary fiber in the kernels of ZP soya bean and wheat genotypes.

Genotypes	Cellulose (%)	Hemicellulose (%)	NDF (%)	ADF (%)	Lignin (%)
Nena	8.91[a]	6.31[e]	16.07[e]	9.76[a]	0.85[c]
Lana	8.42[b]	3.02[h]	12.86[h]	9.84[a]	1.38[b]
Lidija	8.51[b]	4.13[g]	13.28[g]	9.15[b]	0.64[d]
Bosa	7.02[c]	5.31[f]	14.01[f]	8.69[b]	1.67[a]
ZP 96/I	2.35[d]	69.12[b]	72.32[b]	3.20[cd]	0.85[c]
ZP 87/Ip	2.10[e]	59.89[d]	62.80[d]	2.91[d]	0.81[cd]
ZP 34/I	2.29[de]	66.04[c]	69.27[c]	3.23[c]	0.94[c]
ZP DSP/01-66M	2.18[de]	70.12[a]	73.24[a]	3.12[cd]	0.94[c]
LSD$_{0.05}$	0.205	0.175	0.280	0.311	0.175
CV (%)	3.34	0.42	0.57	4.17	14.56

[a-h] Means followed by the same letter within the same row are not significantly different P<0.05); CV-coefficient of variation

Carotene concentration of bread and durum wheat genotypes adapted to south-east European conditions, ranged from 2.29 to 3.02 mg kg^{-1} and 4.02 to 6.64 mg kg^{-1}, respectively (Table 3). The carotene content was higher than that reported by PANFILI *et al.,* (2004) for bread (0.1-2.4 mg kg^{-1}) and durum wheat (1.5-4.0 mg

kg^{-1}). A higher carotene content in durum than bread wheat was in agreement with the results of ADOM and LIU, (2003).

LEENHARDT *et al* (2006) show significant varietal variation of the total carotenoid concentration. According to our results, the carotene content was statistically significantly higher in seed of the Nena (6.12 µg g^{-1}) than in Lana (5.74 µg g^{-1}), Lidija (5.17 µg g^{-1}) and Bosa (4.86 µg g^{-1}).

Table 3. The content of antioxidants in kernels ZP soybean and wheat genotypes

Genotypes	Carotenes (µg g^{-1})	Total phenolics (catechin eq, mg g^{-1})	DPPH - scavenging activity (IC_{50}, mg)	α tocopherol (µg g^{-1})	β+γ tocopherol (µg g^{-1})
Nena	6.12[b]	3.87[b]	6.5[g]	27.06[a]	-
Lana	5.74[c]	4.41[c]	7.41[f]	18.11[b]	-
Lidija	5.17[d]	3.62[a]	8.28[d]	15.00[c]	-
Bosa	4.86[e]	2.86[d]	7.66[e]	15.25[d]	-
ZP 96/I	3.02[g]	1.28[e]	11.78[a]	9.56[h]	4.37[b]
ZP 87/Ip	2.29[h]	1.28[e]	11.72[a]	10.23[f]	4.69[a]
ZP 34/I	4.02[f]	1.60[e]	10.77[b]	11.06[g]	3.78[c]
ZP DSP/01-66M	6.64[a]	1.57[e]	10.18[c]	13.16[e]	4.01[c]
$LSD_{0.05}$	0.124	0.106	0.167	0.053	0.236
CV (%)	2.26	4.68	1.51	0.31	3.34

a-c Within rows, means followed by the same letter are not significantly different according to LSD (P < 0.05), CV-coefficient of variation; n.d.-not deretminated

Phenols, secondary metabolites in plants, have an antioxidant, antimutagenic, antimicrobial and anti-inflammatory role. Due to these properties, phenolic compounds are attractive for food. The highest (4.41 mg catechins g^{-1}), i.e. lowest (2.86 mg catechins g^{-1}) content of total phenols was recorded in seed of the Lana, i.e. Bosa, respectively, (Table 3). The total phenolics content in analysed ZP soybean genotypes was higher than that reported by FERNANDEZ-OROZCO *et al*., (2007). Quantitative variability in the total phenols content of the analysed soybean varieties by MALENČIĆ *et al.,* (2007) ranged from 2.70 to 4, 88 mg catechins g^{-1}. Same authors also reported that the antioxidant activity increased proportionally to the phenolic content and that a linear relationship between the DPPH radical scavenging activity and total phenolics was established ($r^2 = 0.56$). The total antioxidant activity (IC_{50}) is the effective concentration at which DPPH radicals are scavenged by 50%. In our study, the highest total antioxidative activity was recorded in genotype Nena. According to our results 6.5mg of flour scavenged 50% of DPPH radical, while the Lana had the lowest antioxidative activity, 8.28 mg of flour scavenged 50% of DPPH radical (Table 3). The wholemeal flour from durum wheat having a lower IC_{50} showed a stronger DPPH scavenging activity than bread wheat. The IC_{50} values ranged from 10.18 to 10.77 mg d.m. and on the average 11.75 mg d.m in durum and bread genotypes, respectively. The average value of the total phenolic

content was 1.28 mg g^{-1} d.m. and 1.59 mg g^{-1} d.m. in bread and durum wheat, respectively (Table 3). Phenolics are highly concentrated in bran layers (LI *et al.,* 2005), carotenoids in outer kernel layers, opposite to vitamin E, found mostly in the germ. Therefore, the whole grain products could be considered as food products with maximum health benefits.

Soybean [*Glycine max* (L.) Merr.] is an important source of tocopherols which have health-beneficial properties. The content of the antioxidant α-tocopherol was considerably higher in seed of genotype Nena (27, 06 µg g^{-1}) than in Lana (18.11 µg g^{-1}), Bosa (15.25 µg g^{-1}) and Lidija (15.00 µg g^{-1}) (Table 3). In our study, β and γ-tocopherol were not determined. SEGUIN *et al* (2009) observed the large variation among soybean genotypes for α-tocopherol, the relatively high stability of genotypes performance across environments, and the lack of negative correlation with other important seed characteristics. Concentrations of α-tochopherol in bread wheat genotypes were 9.56 to 10.23 µg g^{-1} d.m., respectively. This values were higher than that of 3.36-10.09 µg g^{-1} reported for bread wheat by MOORE *et al.,* (2005) and slightly lower than that of 15.9 µg g^{-1} previously found in bread wheat by PANFILI *et al.,* (2003). The highest α-tocopherol content was estimated in kernels of durum wheat ZP DSP/01-66M (13.16 µg g^{-1} d.m.). The content of β+γ tocopherol was much lower than α-tocopherol in both wheat species (Table 3).

Generally, contents of total phenolics, carotenes and tocopherol were more abundant in ZP soybean than bread and durum wheat genotypes. All these nutrients with antioxidant properties influenced the capacity of DPPH$^•$ scavenging. Because of the ability to reduce free radicals all these antioxidants protect polyunsaturated fatty acids from oxidative damage. Therefore, it is natural that high oil plants have a high content of antioxidants, confirmed by our results.

The activity of peroxidase and lipoxygenase in soybean and wheat seed can significantly decrease both, the nutrition value and the storage stability. These enzymes can participate in a great number of oxidative reactions, such as colour change, degradation of chlorophyll and auxins, oxidation of phenols, carotenes and vitamins. Many of these factors are also associated with the flavour, colour, texture and nutritional qualities of foods. According to the POD activities, the observed soybean genotypes could be classified into two groups: 1) genotypes with a low activity (epep genotypes, Nena, Lidija, Lana, 0.19, 0,17 and 0,16 µmol *guaiacol* mg^{-1} prot. min^{-1}, respectively) and 2) the genotype Bosa that is heterozygous for this trait (Epep) and with the POD activity of 1.36 µmol *guaiacol* mg^{-1} prot. min^{-1} (Figure 1). The POD activity was significantly differed between the two wheat species (P<0.05), and ranged from 0.54 to 0.72 µmol *guaiacol* mg^{-1} prot. min^{-1} in bread and from 0.33 to 0.37 µmol *guaiacol* mg^{-1} prot. min^{-1} in durum wheat (Figure 1). Lipoxygenase isoenzymes catalyse peroxidation of fatty acids, while, WANG and HILDEBRAND (1988) confirmed that linolenic (C18:2) and linolenic (C18:3) acids were main substrates of the lipoxygenase activity. The low, i.e. high, LOX-1 activities were recorded in the genotypes Nena (3.01 µmol mg^{-1} prot. min^{-1}), i.e. Lana (6.37 µmol mg^{-1} prot. min-1), respectively (Figure 2). In comparison with soybean, LOX-1 was lower in all analysed wheat genotypes. The activity of LOX-1 calculated as nmoles

of linoleic acid oxidised per g protein and min was 27 and 15 of bread (ZP 87/Ip and ZP 96/I), 5 and 2 of durum wheat genotypes (ZP 34/I and ZP DSP/01-66M), respectively (Figure 2). Our results are in accordance with the data obtained by LEENHARDT *et al.,* (2006) that durum wheat possess lower activities of LOX and POD enzymes than bread wheat. Same authors suggested that in a perspective of bread-making the ratio between the total carotenoid concentration and the LOX activity would be a suitable criterion for wheat breeding programmes.
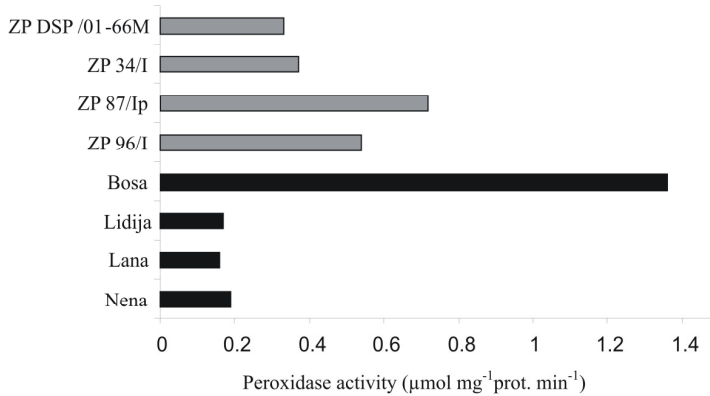

Figure 1. Peroxidase activity in kernels of ZP soybean and wheat genotypes.
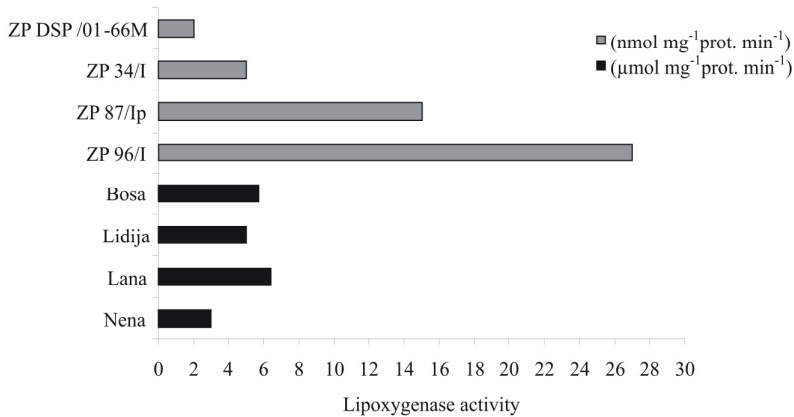

Figure 2. Lipoxygenase 1 activity in kernels of ZP soybean and wheat genotypes

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HEMIJSKA KOMPOZICIJA KAO PARAMETAR KVALITETA ZP SORTI SOJE I PŠENICE

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I z v o d

Cilj ovog rada je analiza hemijskih karakteristika ZP genotipova soje i pšenice, kao i nutritivne razlike između ove dve komplementarne biljne vrste. Eksperimentalni mateirjal obuhvata dva hlebna, dva durum genotipa pšenice i četiri ZP genotipa soje različitog genetočkog porekla. Svi ZP genotipovi soje osim Lane, imaju preko 40% ukupnih proteina. Lana i Lidija, nedavno stvorene sorte, nastale kao rezultat selekcije za specifična svojstva, imaku visok sadržaj ulja. Genotipovi pšenice imaju znatno niži sadržaj pepela, ulja, ukupnih i u vodi rastvorljivih proteina negp genotipovi soje. Najviši sadržaj ulja, ukupnih i u vodi rastvorljivih proteina je nađen u durum genotipu ZP DSP/01-66M. Sadržaj lignina varira značajno više između genotipova soje nego pšenice. Generalno, sadržaj ukupnih fenola, karotina, i tokoferola je obilniji u ZP genotipovima soje nego u pšenici.