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PROTEIN AND TRYPTOPHAN CONTENT IN KERNELS OF MAIZE HYBRIDS*

SLAĐANA ŽILIĆ, DRAGANA IGNJATOVIĆ-MICIĆ, GORAN SARATLIĆ, GORAN STANKOVIĆ, NENAD DELIĆ, MILOMIR FILIPOVIĆ, BRANKA KRESOVIĆ[1]

SUMMARY: In this work, proteins and tryptophan content were analyzed in kernels of semi-flint, semi-dent, dent and specialty ZP maize hybrids. Protein content, as well as protein fraction, differed significantly among the analyzed maize genotypes. Globulin was the lowest fraction in kernels of all maize hybrids (6.69-11.03% of total protein). The content of albumin was the highest in sweet hybrids ZP 441su and ZP 461su i.e. 20.27% and 19.76% of total protein, respectively. α-Zein and G-3 glutelin were dominant protein fractions in all maize genotypes. The highest content of α-zein and G-3 glutelin was in the kernels of popping maize hybrid ZP 611k, i.e. 29.25 and 25.71% of total protein, respectively. All hybrids could be classified according to the tryptophan content in three groups – over 0.08% (sweet and waxy hybrids), from 0.06 to 0.07% (three standard dent hybrids, one semi-flint and two semi-dent hybrid) and from 0.05 to 0.06% (two semi-flint, one semi-dent hybrids and popping).

Key words: *maize hybrids, protein fractions, tryptophan*

INTRODUCTION

The diets consumed in developed countries usually contain various sources of dietary protein (cereals, legume, meat, etc.). However, in some less developed countries in which a single cereal may account for a major part of the total protein intake, the nutritional quality of the protein as well as the amount may be important.

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[1] Slađana Žilić, PhD, scientific associate; Dragana Ignjatović-Micić, PhD, senior scientific associate; Goran Saratlić, PhD, scientific associate; Goran Stanković, MSci, researcher associate, Nenad Delić, PhD, scientific associate; Milomir Filipović, PhD, scientific associate; Branka Kresović, PhD, scientific associate; Maize Research Institute „Zemun Polje", Slobodana Bajića 1, Zemun-Belgrade, Serbia
Corresponding author: Slađana Žilić, Maize Research Institute „Zemun Polje", Tel.: +381 11 37 56 704, fax: +381 11 37 54 994, e-mail: szilic@mrizp.rs
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Cereals are a major source of dietary protein for humans. Cereal grains have a low protein content and the protein quality is limited by deficiencies in some essential amino acids, mainly lysine and tryptophan. The contents of amino acids in whole cereal grains are largely determined by the starchy endosperms which typically comprise about 80% of the grain dry weight and consist of starch and prolamin rich proteins. The aleurone and embryo tissues of grains contain higher contents of essential amino acids but these are often not available for human nutrition as they are removed by milling (wheat), polishing (rice), pearling (barley) and decorticating (sorghum) (Jensen and Martens, 1983).

The protein quality of maize is similar to other cereals except rice and oats, in which the major storage proteins are related to the 11S globulins („legumins") of legumes with the prolamins (zein) being only minor components. Protein solubility is an important functional property that affects the utilization and nutritional value of cereal grain. According to the solubility in different solvents, proteins from maize grain can be separated on albumins - water soluble, globulins - salt soluble, zeins - alcohol soluble and glutelins - alkali soluble. The albumins and globulins represent about 6% of total protein, while glutelins account for approximately 30-45% (Konzak, 1977). Zeins and glutelins constitute storage proteins of maize kernels. Zein fraction accounts for about 50 % of the total endosperm protein. Zeins can be separated into four distinct subfractions: α, β, γ, and δ. α-Zein are by far the most abundant, making up to approximately 70 % of the total. It is located in large central portion of the protein body with β- and γ-zeins surrounding it on periphery, which can be the reason for higher thermo stability of zein fraction (Lending and Larkins, 1989). Also, zein has some of the properties of wheat gluten but is not able to form viscoelastic fibrils at room temperature, though it can be made functional in this way at higher temperatures (Bugusu et al., 2001; Schober et al., 2008). Three glutelin subgroups, denoted G1, G2 and G3, constitute alkali soluble maize storage protein. Interpolypeptide disulfide bonds make glutelin poorly soluble (Landry and Moureaux, 1981), except for G3-glutelins, which have an amino acid composition similar to that of salt-soluble proteins.

Zein is characterized by high contents of glutamine, leucine, proline, and partially is devoid of two essential amino acids - lysine and tryptophan, which determine corn protein as nutritionally inadequate. In contrast, albumins and globulins are high in lysine and arginine while glutelins are intermediate.

To improve the nutritional value of maize varieties, both for animal feed and for human consumption, the content and quality of amino acids of grain storage proteins have to be changed. The first biofortified crop was *opaque*-2 (*o*2) maize, bred for its improved protein quality. At the time, this discovery was considered a potentially major breakthrough to help reduce protein deficiency, which was then considered the world's most pressing nutrition problem. Subsequent conventional breeding led to *o*2 maize varieties with improved agronomic characteristics, now known as QPM - quality protein maize (Vasal, 2000).

This research is focused on analyses of protein and tryptophan content in kernels of standard, sweet, popping and waxy ZP maize hybrids. A more detailed knowledge of chemical properties of ZP maize genotypes will be beneficial in the future selection of maize hybrids, as well as production of maize food with improved nutritional quality.

MATERIALS AND METHODS

The kernels of 13 maize (*Zea mays* L.) hybrids developed at the Maize Research Institute, Zemun Polje, (MRIZP) Belgrade, Serbia, were used for this study. Kernels were collected at full maturity stage from plants grown in a field-trial at the MRIZP in 2008 growing season. The maize genotypes were chosen on the basis of their differences in agronomic traits and physical characteristics of the kernels: three semi-flint hybrids (ZP 209, ZP 745, ZP Prado), three semi-dent hybrids (ZP 505, ZP 643, ZP 717), three dent hybrids (ZP 434, ZP 544, ZP 688) and four specialty maize hybrids (popping ZP 611k; waxy ZP 704xw and two sweet ZP 441su, ZP 461su). The wholemeal flour (particle size<500 µm), obtained by grounding maize on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden) was used in the analyses.

Different protein fractions were obtained by succesive extractions of defatted maize flour with a series of solvents (in a ratio 1:10 w/v) according to the Landry and Moureaux (1970) method, with some modifications. Distilled water, 0.5 M NaCl, 70% ethanol and 0.0125 M borate buffer, pH 10, containing 5% sodium dodecyl sulfate (SDS), were used to extract albumin, globulin, α-zein and glutelin (G3-glutelin) fractions, respectively. Extraction of each protein fraction was done by repeated stirring three times for 30 min at 4°C, followed by centrifugation at 20 000 g for 15 min. Protein content was calculated, in each fraction, from the nitrogen content determined by micro Kjeldahl method, using 6.25 as the conversion factor. The results are given as percentage of total protein (protein solubility index).

Tryptophan content was determined according to Nurit et al. (2009). Shortly, flour hydrolysate (obtained by overnight digestion with papain solution at 65°C) was added to 3 ml reagent containing Fe^{+3} (1 g $FeCl_3$ dissolved in 50 ml 7 N H_2SO_4), 30 N H_2SO_4 and 0.1 M glyoxilic acid. After incubation at 65°C for 30 min, absorption was read at 560 nm. Tryptophan content was calculated using a standard (calibration) curve, developed with known amounts of tryptophan, ranging from 0 to 30 µg/ml. Besides, tryptophan content quality index (QI), defined as tryptophan to protein ratio in the sample, was also calculated.

All chemical analyses were performed in three replicates and the results were statistically analysed. Significant statistical differences of observed chemical maize parameter 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. Correlations between parameters were examined using the Peterson correlation.

RESULTS AND DISCUSSION

Data in Table 1 indicate that protein content, as well as soluble protein fractions among analyzed maize genotypes, differs significantly ($P < 0.05$). The sweet and popping hybrids ZP 441su and ZP 611k had higher content of total protein (12.73 and 12.41% of d.m., respectively) than any of the other eleven maize genotypes, which protein content varied from 9.11% (ZP 688) to 11.69% (ZP 461su).

Protein fractions were isolated according to their solubility in different solutions. In our study globulin was the lowest fraction in kernels of all maize hybrids (6.69-11.03% of total protein). The content of albumin was 20.27% and 19.76% of total protein in ZP 441su and ZP 461su hybrids, respectively. In the kernels of the other hybrids, the albumin content was lower by 33% to 54%, ranging from 9.43% of total protein in kernels of popping maize hybrid ZP 611k to 15.48% of total protein in typical dent genotypes ZP 688. Also, typical dent genotypes ZP 434 and ZP 544 had higher content of albumin (14.08 and 14.32% of total protein, respectively) than other standard semi-flint and semi-dent hybrids. Content of soluble proteins is affected by the kernel structure. Bulk of the proteins in a mature maize kernel is present in the endosperm and germ, but the germ protein is superior in both quantity and quality. The albumins and globulins are present mainly in the germ (Shukla and Cheryan, 2001). The kernel of sweet hybrid ZP 441su with the highest content of albumin fraction had the highest portion of germ, i.e. 20.0% of the grain weight. Also, kernels of popping maize hybrid ZP 611k with the lowest portion of germ (9.7%) had the lowest content of albumin fraction (Table 2).

α-Zein and G-3 glutelin were the dominant protein fractions in all maize genotypes. The highest content of α-zein and G-3 glutelin was in the kernel of popping maize hybrid ZP 611k i.e. 29.25 and 25.71% of total protein, respectively. Almost all the zein is present in the maize endosperm, whereas glutelin is distributed between the endosperm and the germ (Shukla and Cheryan, 2001). In normal maize, proportions of various endosperm storage protein fractions, on an average, are: albumins (3%), globulins (3%), zeins (60%) and glutelins (34%) (Prasanna et al., 2001). Zeins have never been detected in any part of the plant other than the kernel. In our study the popping maize hybrid ZP 611k had the highest portion of hard endosperm per the grain weight (80.0%) (Table 2). Semi-dent and dent maize hybrids (ZP 505 and ZP 434) had higher content of α-zein (25.5 and 24.26% of total protein, respectively) than other semi-flint, semi-dent and dent hybrids. The lowest content of α-zein was in kernels of sweet maize ZP 441su (17.05% of total protein) and in the kernels of dent maize hybrids ZP 688 (18.44% of total protein). Malumba et al. (2008) reported that zein was the most abundant protein group extracted from the whole maize kernel, followed by glutelin-G1 and glutelin-G3. Content of zein was 33.5%, while G-3 glutelin was 18.1%. Differences observed between the present study and previous study is possibly due to the maize genotypes used. A by-product (so-called "gluten-meal") of corn-starch manufacturing can be relied on for the supply of zein sources, but it is not effectively used for food materials. The main reason is that the maize protein is, not only poor in its nutritive value (low in tryptophan and lysine), but also cumbersome for food processing because of its gummy cohesion in a hydrated state. Deamidation, or partial cleavage of the peptide bond, leads to a pronounced change in the functional properties of zein. As the fragmentation reaction proceeds, however, a favorable feature, represented by antioxidation, vanishes. Additionally, the possibility cannot be excluded that a crude zein preparation contains physiologically active substances which benefit rather than damage health (Chiue et al., 1997). The highest content of G-3 glutelin was in kernels of semi-dent maize hybrids ZP 643 and ZP Prado i.e. 23.44 and 24.06% of total protein, respectively. However, the kernel of sweet hybrid ZP 461su had the lowest content of G3-glutelin i.e. 16.25% of total protein. Our results are in accordance with results obtained by Fageer and El Tinay (2004). These authors reported that the content of true glutelin (G3-glutelins) varied from 10.8% to 21.9% in grains of twelve maize genotypes, as well as albumins and globulins from 16.8% to 22.7%.

Table 1. Content of soluble protein fractions in the kernel of ZP maize hybrids. Results are presented as % of total protein

Tabela 1. Sadržaj rastvorljivih proteina u zrnu ZP hibrida kukuruza. Rezultati su iskazani kao % od ukupnih proteina

Hybrids	Protein	Albumin	Globulin	α-Zein	G3-Glutelin
ZP 209	10.15g	12.71h	9.36d	22.48d	21.38ef
ZP 434	9.52j	14.08e	9.98bc	24.26c	19.96h
ZP 441su	12.73a	20.27a	7.62f	17.05i	20.42gh
ZP 461su	11.69c	19.76b	7.10g	20.79g	16.25i
ZP 505	9.70i	12.26i	11.03a	25.05b	20.31gh
ZP 544	9.50jk	14.32d	9.68c	22.84e	21.26f
ZP 611k	12.41b	9.43l	6.69h	29.25a	25.71a
ZP 643	9.43k	12.94f	8.59e	22.80e	23.44c
ZP 688	9.11l	15.48c	10.76a	18.44h	20.64g
ZP 704wx	11.34d	13.76e	10.32b	22.75e	20.37gh
ZP 717	9.98h	11.72j	8.52e	22.24f	22.34d
ZP 745	10.41f	13.06g	8.84e	22.00f	21.71e
ZP Prado	10.85e	10.78k	9.86c	20.92g	24.06b
LSD0.05	0.099	0.281	0.351	0.291	0.477

[a-l] Means followed by the same letter within the same columns are not significantly different (P<0.05)
[a-l] Srednje vrednosti označene istim slovima unutar iste kolone nisu značajno različite (P<0.05)

Table 2. The portion of pericarp, germ and endosperm per kernel weight of ZP maize hybrids (%)
Tabela 2. Udeo perikarpa, klice i endosperma u zrnu ZP hibrida kukuruza (%)

Hybrids	Pericarp	Germ	Whole endosperm	Hard endosperm	Soft endosperm
ZP 209	6.0e	11.9de	82.1a	71.5d	28.5f
ZP 434	7.3c	13.3c	79.4d	62.5g	37.5c
ZP 441su	6.2e	20.0a	73.7e	61.0h	38.9b
ZP 461su	9.0a	19.1b	71.9f	58.5i	41.5a
ZP 505	6.2e	12.2d	81.7b	71.1de	28.9f
ZP 544	6.4d	13.0c	80.6	61.5h	38.5b
ZP 611k	8.6b	9.7f	81.7b	80.0a	20.0i
ZP 643	7.8c	12.6cd	79.6d	69.1f	30.9e
ZP 688	6.7d	11.8de	81.5b	61.3h	38.7b
ZP 704wx	5.8ef	13.4c	80.8c	65.7g	34.3d
ZP 717	6.1e	12.6cd	81.4b	72.2c	27.8g
ZP 745	6.3de	11.4e	82.3a	73.1b	26.9h
ZP Prado	6.1e	12.9cd	81.1bc	71.8d	28.2fg
LSD0.05	0.325	0.480	0.534	0.582	0.560

[a-i] Means followed by the same letter within the same columns are not significantly different (P<0.05)
[a-i] Srednje vrednosti označene istim slovima unutar iste kolone nisu značajno različite (P<0.05)

In our study, the albumin content was negatively correlated to the content of globulin, α-zein and G3-glutelin concentrations ($r = 0.20$, $r = 0.70$ and $r = 0.80$ respectively, $P < 0.05$). However, α-zein content was positively correlated to the content of G3-glutelin ($r = 0.44$, $P < 0.05$).

The quality of maize proteins is poor because they are deficient in the essential amino acids, lysine and tryptophan (Shewry, 2007). Since these two amino acids are highly correlated, tryptophan is usually used as a single parameter for evaluating the nutritional quality of the grain protein (Hernandez and Bates, 1969a). Results of tryptophan analysis in kernels of maize genotypes determined by Nurit et al. (2009) colorimetric method and levels of statistical significance obtained from analysis of variance, are summarized in Table 3

The data indicate that the levels of tryptophan in kernels of semi-flint hybrids ZP 209 and ZP 745, semi-dent hybrid ZP 505 and popping maize ZP 611k were similar and lower than the other maize genotypes (in average 0.055% of d.m.). According to our results, the highest tryptophan content was detected in kernels of sweet hybrid ZP 441su i.e. 0.093% of d.m. Also, this hybrid had the highest portion of germ, i.e. 20.0% of the grain weight (Table 2), as well as the highest content of albumin. However, this hybrid had the highest content of total protein and this could be the reason for QI somewhat below the QPM treshold level, i.e. 0.72. (Table 3).

Table 3. Tryptophan content and quality index in in the kernel of ZP maize hybrids
Tabela 3. Indeks kvaliteta proteina i sadržaj triptofana u zrnu ZP hibrida kukuruza

Hybrids	Tryptophan (%)	QI
ZP 209	0.056ef	0.56g
ZP 434	0.066bcd	0.70cd
ZP 441su	0.093a	0.72bc
ZP 461su	0.083a	0.72bc
ZP 505	0.058def	0.60f
ZP 544	0.070bc	0.73ab
ZP 611k	0.055ef	0.443i
ZP 643	0.071b	0.75a
ZP 688	0.061cde	0.67d
ZP 704wx	0.086a	0.75a
ZP 717	0.063bcde	0.64e
ZP 745	0.051f	0.49h
ZP Prado	0.062bcde	0.57f
LSD0.05	0.015	0.022

[a-g] Means followed by the same letter within the same columns are not significantly different ($P<0.05$)

[ag] Srednje vrednosti označene istim slovima unutar iste kolone nisu značajno različite ($P<0.05$)

Waxy and sweet maize (ZP 704wx and ZP 461su) also had high tryptophan content (0.086 and 0.083% of d.m., respectively) and QI (0.75 and 0.72, respectively) (Table 3). Content of tryptophan in kernels of typical dent hybrids (ZP 434, ZP 544 and ZP 688) ranged from 0.061 to 0.070% of d.m. It was interesting that semi-dent hybrid ZP 643 had higher content of tryptophan (0.071% of d.m.) and QI (0.75) than typical dent hybrids (Table 3). The maize genotypes analyzed by Vyu and Yollenaar (1998) contained on average 0.072% of tryptophan. The values obtained in our study were close to this average. In the study of Ignjatović-Micić, et al., (2009) content of triptophan in kernels of normal and *opaque*-2 maize inbred lines ranged from 0.071 to 0.136% of d.m.

Three out of 13 ZP hybrids had tryptophan over the QPM threshold limit, but five hybrids had QI close to the threshold value (ZP 441su, ZP 461su, ZP 544, ZP 643 and ZP 704wx) (Table 3). The QPM threshold values for tryptophan content are 0.07% (endosperm) and 0.075% (whole grain), while for QI they are 0.7 and 0.8%, respectively (Vasal, et al., 1996). The nutritional benefits of QPM for people, who depend on maize for their energy and protein intake, and for other nutrients, are indeed quite significant. QPM protein contains, in general, 55% more tryptophan, 30% more lysine and 38% less leucine than that of normal maize (Prasanna et al., 2001).

Our results are in accordance with the data presented by Segal et al. (2003) and Huang et al. (2006) that the decrease in zein resulted in the increased grain lysine and tryptophan content. Tryptophan content was negatively correlated to contents of globulin, α-zein and G3-glutelin ($r = 0.27$, $r = 0.53$ and $r = 0.51$ respectively, $P < 0.05$) and positively correlated to the albumin content ($r = 0.78$, $P < 0.05$).

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 maize as the main bread grain, one of the future important breeding objectives in the Maize Research Institute will be development of maize genotypes with the improved nutritive value.

CONCLUSION

Currently, limited information is available on the biochemical and genetic mechanisms that regulate high-proteins. Essentially two avenues of improving maize protein have to be utilized. The first is through traditional breeding using high-protein germplasm and the second is the use of biotechnology.

Our results showed differences in the concentration of soluble proteins and tryptophan among studied ZP maize genotypes. The highest content of albumin and tryptophan was in kernels of sweet maize hybrids. Popping maize hybrid had the highest content of α-zein and the lowest content of tryptophan. Standard dent hybrids had higher content of albumin than other standard maize hybrids. Among the standard ZP hybrids, semi-dent and dent hybrids (ZP 643, ZP 544) had the highest content of tryptophan.

In conclusion, it appears that among ZP maize genotypes there are genotypes with different amount of protein fractions and tryptophan that should be bred in the future for a desired level and quality of the protein components.

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SADRŽAJ PROTEINA I TRIPTOFANA U ZRNU HIBRIDA KUKURUZA

SLAĐANA ŽILIĆ, DRAGANA IGNJATOVIĆ-MICIĆ, GORAN SARATLIĆ, GORAN STANKOVIĆ, NENAD DELIĆ, MILOMIR FILIPOVIĆ, BRANKA KRESOVIĆ

Izvod

U ovom radu ispitivan je sadržaj rastvorljivih proteina i triptofana u zrnu polutvrdunaca, poluzubana, zubana i specifičnih ZP hibrida kukuruza. Sadržaj ukupnih proteina, kao i sadržaj rastvorljivih proteinskih frakcija se statistički razlikovao između analiziranih genotipova kukuruza. Globulin je bio najmanjie zastupljena frakcija u kukuruznom zrnu (6.69-11.03% od ukupnih proteina). Sadržaj albuminske frakcije u zrnu hibrida šećeraca ZP 441su i ZP 461su iznosio je 20.27% i 19.76% od ukupnih proteina, dok je u zrnu ostalih hibrida bio značajno niži. α-zein i G3-glutelin bile su dominantne frakcije u zrnu svih ispitivanih genotipova kukuruza. Najviši sadržaj α-zeina i G3-glutelina bio je u zrnu kukuruza kokičara ZP 611k i iznosio je 29.25% i 25.71% od ukupnih proteina. Prema sadržaju triptofana svi analizirani hibridi mogu se podeliti u tri grupe: preko 0.08% (šećerci i voksi hibridi – ZP 441su, ZP 461su i ZP 704wx), od 0.06 do 0.07% (tri standardna zubana - ZP 434, ZP 544, ZP 688, poluzubani - ZP 643, ZP 717 i polutvrdunac ZP Prado), od 0.05 do 0.06% (dva polutvrdunca – ZP 209, ZP 745, jedan poluzuban i kokičar – ZP 505, ZP 611k).

Ključne reči: hibridi kukuruza, rastvorljive proteinske frakcije, triptofan.

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