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Grain characteristics and composition of maize specialty hybrids

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Abstract

Improved nutritive and technological maize grain value is very important for its use in diets. In this work, the chemical composition and potential beneficial components, including total and soluble proteins, tryptophan, starch, sugars (sucrose and reducing sugars), and fibres were investigated in flour of eight specialty maize hybrids from Maize Research Institute Zemun Polje (ZP): two sweet, popping, red, white, waxy, yellow semiflint and yellow dent maize hybrids. In addition, digestibility of grain dry matter and viscosity of maize flour were determined. The highest nutritive value was recorded in sweet maize hybrids ZP 504su and ZP 531su which had the highest content of total protein, albumin, tryptophan, sugars and dietary fibres. Besides, low content of starch (55.32% and 54.59%, respectively) and lignin (0.39% and 0.45%) affected the highest dry matter digestibility (92.69% and 91.07%) of sweet maize flour. However, functional properties of ZP sweet hybrids were not satisfactory for food and industrial applications. In contrast, flour of ZP waxy maize hybrid was characterised by a clear and a high peak viscosity. All hybrids could be classified according to the sucrose content in three groups: a) > 4% (sweet and red hybrids-ZP 504su, ZP Rumenka), b) from 3 to 4% (waxy, standard dent and semi flint hybrids-ZP 704wx, ZP 434, ZP 633) and c) from 2 to 3% (sweet, white and popping maize hybrids-ZP 531su, ZP 74b, ZP 611k).α-Zein was the dominant protein fraction in all genotypes except the sweet maize hybrids, making 22.45% to 29.25% of the total protein content.

Additional key words: carbohydrates; digestibility; protein fractions; speciality maize hybrids; viscosity; *Zea mays.*

Resumen

Características y composición del grano de híbridos de maíz de especialidad

La mejora en el valor nutritivo del maíz de grano es muy importante en las dietas. En este trabajo, se investigaron la composición química y componentes potencialmente beneficiosos (proteínas totales y solubles, triptófano, almidón, azúcares y fibras) en la harina de ocho híbridos de maíz de especialidad del Instituto de Investigación del Maíz Zemun Polje (ZP): dos dulces, reventón, rojo, blanco, ceroso, amarillo semivítreo y amarillo dentado. Se determinaron, además, la digestibilidad de la materia seca del grano y la viscosidad de la harina. El valor nutritivo más alto se registró en los híbridos dulces ZP 504su y ZP 531su, que presentaron el mayor contenido de proteínas totales, albúmina, triptófano, azúcares y fibras. Además, su bajo contenido de almidón (55,32% y 54,59%, respectivamente) y lignina (0,39% y 0,45%), afectó a la mayor digestibilidad de la materia seca (92,69% y 91,07%) de la harina de estos híbridos. Sin embargo, sus propiedades funcionales no fueron satisfactorias para la alimentación y usos industriales. Por el contrario, la harina de maíz híbrido ZP ceroso se caracterizó por una alta viscosidad. Según el contenido de sacarosa, los híbridos podrían ser clasificados en tres grupos: a) > 4% (ZP 504su, ZP Rumenka), b) 3-4% (ZP 704wx, ZP 434, ZP 633) y c) 2-3% (ZP 531su, ZP 74b, ZP 611k). La fracción proteica dominante en todos los genotipos fue la α-zeína, excepto en los híbridos dulces, donde constituyen el 22,45-29,25% del contenido total de proteínas.

Palabras clave adicionales: digestibilidad; fracciones proteicas; hidratos de carbono; híbridos de maíz de especialidad; viscosidad; *Zea mays*.

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Abbreviations used: ADF (acid detergent fibre), ADL (lignin), CDOMD (cellulase digestible organic matter of the dry matter), DM (dry matter), MRIZP (Maize Research Institute «Zemun Polje»), NDF (neutral detergent fibre), QPM (quality protein maize), RCB (random block design).

Introduction

Maize (*Zea mays* L.) ranks as the third most important cereal grain in the world. Traditional criteria for selecting maize hybrids have been based primarily on agronomic factors, including grain production, disease resistance, drought tolerance and storage characteristics. Little emphasis has been placed on the nutritional value of maize for food and feed. While the majority of the product in developing countries is for human consumption, in the developed world it is mainly used for industrial purposes and animal feed (FAO, 1992). Because of its importance, the genetic improvement of maize has played a key role in the development of genotypes with high technological and nutritional values. Specialty maize hybrids are the result of selection for improved chemical composition of the grain compared to standard hybrids. Many of these hybrids including high lysine, high oil, waxy, white and sugary, among others, have been the subject of a renewed interest because of their improvements in agronomic performances, commitments by marketers to preserve the identity of specialty grain, and the advance in our understanding of digestion and nutrient requirements.

A selection pressure by both humans and nature has resulted in various maize types, generally classified by properties of their grain endosperm. The most common types of maize include flint, flour, dent, popping, sweet and waxy (Knott *et al.,* 1995). The physical appearance of each grain type was determined by its pattern of the endosperm composition, as well as, quantity and quality of the endosperm. Grains of flint maize have mostly hard, glassy endosperm with smooth, hard seed coats (pericarps). Usually, yellow flint maize has a high content of proteins and β-carotene. In other maize types β-carotene with the highest pro-vitamin A activity is present in a relatively low concentration. Flour maize endosperm is made of soft starch with thin pericarps. Dent maize with flinty sides and soft cores of starch that cause the end of the grains to collapse or dent during drying fall between the flint and flour types. Although the majority of the dent maize types has a yellow endosperm, white, red and blue dents are very popular in human food products. Red and blue coloration comes from phenolic compounds which have antioxidant properties (Cortés *et al.,* 2006). Red and blue maize have a coarser, sweeter and nuttier taste than other maize grown for flour or meal. Although most of the products (cooking oil, various maize grits, meals, flour starches, sweeteners, alcohol, paper, adhesives, cosmetics, citric acid, glutamic acids, etc.) are made from dent maize, other types of maize are becoming more and more important. Unlike dent maize, sweet maize is grown primarily for fresh consumption, not feed or flour, although USDA researchers have developed a technique to produce a high-fibre, no-calorie flour from sweet maize pericarps (Burge and Duensing, 1989). The wrinkled, glass appearance of sweet maize grains is a result of a sugary gene that retards the normal conversion of sugar to starch during the endosperm development. The sucrose content changes during the endosperm development and reaches its peak 23-25 days after pollination when grain is consumed (Pajic and Radosavljevic, 1987). Field maize contains approximately 4% of sucrose to immature milky stage. Standard sweet maize with the *sugary1* (*su1*) mutant at the same stage contains approximately 10% sucrose. Following harvest or if left on the stalk too long, sucrose in *su1* standard sweet maize is rapidly conversed to starch. Grains can loose as much as 50% of their sucrose at room temperature 24 hours after harvest (Amir *et al.,* 1971). Waxy maize is a starch variant of normal maize which contains 100% amylopectin whereas normal maize contains 75% amylopectin and 25% of amylose. Waxy maize is used by wet-maize millers to produce waxy starch which is utilised by the food industry as a stabiliser and in the paper industry as an adhesive (Ptaszek *et al.,* 2009). Popping maize has a hard, flinty endosperm that surrounds a small amount of soft moist starch in the centre. Heating the grain turns this moisture into steam which expands, splits the pericarp and causes the endosperm to explode, turning the grain inside out. Most commercial varieties expand 30-40 times their volume. Among the most important types of maize are high lysine maize, namely *opaque-2* and quality protein maize (QPM) and high-oil content genotypes with more than 6% of oil high in polyunsaturated essential fatty acids (Graham *et al.,* 1990).

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 important breeding objectives in the Maize Research Institute is the development of genotypes with the improved nutritive value. Therefore, this research was focused on the analysis of chemical characteristics of specialty maize genotypes (non fibre-carbohydrates, dietary fibres, protein fractions), as well as, their effect on grain dry matter digestibility and flour viscosity. A more detailed knowledge of chemical properties of specialty maize genotypes will be beneficial in the production of maize food with improved nutritional quality.

Material and methods

Plant materials

The kernels of eight specialty maize (*Zea mays* L.) hybrids developed at the Maize Research Institute Zemun Polje (MRIZP), Belgrade, Serbia, were used: two sweet maize hybrids, ZP 504su and ZP 531su; popping maize hybrid ZP 611k; ZP Rumenka with dark red pericarp and yellow endosperm; hybrid ZP 74b which is characterised by the white colour of grains; waxy hybrid ZP 704wx; semiflint hybrid ZP 633 with pronounced yellow grains and dent hybrid ZP 434 with the standard chemical composition. All selected maize genotypes except ZP 704wx are commercialized, and their detailed characterization was important for the expansion of their use. Grains were collected in full maturity stage from plants grown in a field-trial at the MRIZP location under the same conditions in 2008 growing season. The experiment was set up by a random block design (RCB) with two replications. Area of individual plots for each replication was 21 m^2, and the crop density was 50,000 plants ha^{-1}. Standard agronomic practices were used to provide adequate nutrition and keep the plots disease-free. Grain of plants from the two inner rows was used for the analysis. The wholemeal flour (particle size < 500 µm), obtained by grinding maize grains on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden) was used in the analyses.

Analytical procedures

Different protein fractions were obtained by successive extractions of defatted flour with a series of solvents (in a ratio 1:10 w/v) according to the Landry and Moureaux (1970) method, with some modifications. To obtain the first fraction, distilled water was added to the sample powder and the mixture was stirred three times for 30 min at 4°C. The residue was extracted three times with the same volume of 0.5 M NaCl. To obtain the third fraction, the residual material was stirred with 70% ethanol, and for the last fraction, 0.0125 M borate buffer, pH 10 with 5% sodium dodecyl sulphate (SDS) was used with stirring for 30 min at 4°C. The solid material was isolated from extracts by centrifugation at 20,000 × *g* for 15 min. The supernatant was used for the analysis. For each solvent, supernatants were combined to give the total extract. The final volume of each protein extract was 50 mL. Fractions I and II contained albumin and globulin, respectively, fraction III α-zein and fraction VI contained the true glutelin (G3-glutelins). Ten milliliters of protein extract was evaporated 12 h at 100°C. The protein content in each fraction was calculated from the nitrogen content determined by the micro Kjeldahl method, using 6.25 as the conversion factor. The results are given as the percentage of the dry matter (DM), as well as the percentage of the 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^{-1}.

To obtain the pasting curve of flour of various maize hybrids, changes in the apparent viscosity of an aqueous suspension were determined as follows. The flour slurry (8% starch suspension, total mass of 500 g) was heated in a Brabender Viscograph at a rate of 1.5°C min^{-1} from 25 to 95°C, held at the maximum temperature for 30 min, and then cooled at a rate of 1.5°C min^{-1} to 50ºC. The Brabender Viscograph (model PT 100) (Brabender Instrument Inc, Duisburg, Germany) was operated according to the method for using the Brabender Amylograph (ICC, 1992).

Mono and disaccharide composition was determined by HPLC using a method suggested in FCC (2004). The separations were performed on Carbohydrate column (Waters, USA). Refractive index detector Waters model 410 was used. Mobile phase acetonitrile: water (83:17 v/v) at a flow rate of 2 mL min^{-1} was used. Defatted samples were extracted in 70% ethanol (flour: ethanol ratio 1:10 w/v), *i.e.* 0.5 g of each sample was dispersed in 70% ethanol and stirred on magnetic stirrer for 1 hour at 50°C. Extracts were centrifuged at 13,500 × *g* for 15 min. Supernatants were evaporated to dryness at 55°C under vacuum. Residues were resolved with 20 mL of Milli Q water using ultrasound bath, filtrate through Watman No 4 and let pass through activated kationic (United Waters, England) and anionic (Zerolit 225, Rohh-Haas, USA) column. Prior to injecting samples were diluted with mobile phase (1:1 v/v), and filtered through 0.25 μm sample filter (Nucleopore, USA). Injection volume of the samples was 25 μL. As a standard we used 0.1% solution of xylose, arabinose, glucose, fructose, maltose and sucrose (Sigma, USA).

The content of dietary fibres, hemicellulose, cellulose, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) was determined by the Van Soest detergent method (Van Soest, 1963) modified by Mertens (1992) using Fibertec system. The method is based on the fibres solubility in neutral, acid and alkali reagents. NDF was measured by boiling maize flour sample (1 g) in 100 mL of a special detergent under a neutral (pH 7) condition during 60 min and filtering the boiled sample. Solution for hydrolysis contained sodium tetraborate-10-hydrate (6.81 g), sodium salt dihydrate (18.61 g), dodecyl sulfate (30 g), disodium hydrogenphosphate-12-hydrate (7.77 g) and methil celosol (10 mL) dissolved in 1000 mL of distilled water. The liquid that passed through the sintered disc filter contained starch, sugar, protein and other compounds that were dissolved. The residue of the sample that was not dissolved remained on the sintered disc filter is called NDF. After drying, NDF was calculated as a percentage of the original sample. ADF was determined in much the same way, except that a different detergent was used under acid (pH 2) conditions. Cetyl trimethim ammonium bromide and 0.5 M H_2SO_4 were used for hydrolysis. The sample was boiled and filtered as in the NDF procedure. Because of the different detergent and acid conditions, hemicellulose and cell solubles were dissolved and filtered away. The residue left was ADF and consisted mainly of cellulose and lignin. ADF was related to dry matter digestibility and was used to predict net energy content. ADL was measured by further treating ADF with strong acid (72% H_2SO_4) which dissolved cellulose. After filtering and drying, the ADL was calculated as a percentage of the original sample. All the results were given as the percentage of DM. Content of hemicellulose was obtained as the difference between NDF and ADF content, while the cellulose content was calculated as the difference between ADF and lignin content.

The dry matter digestibility was determined using cellulase digestible organic matter of the dry matter (CDOMD) method (De Boever *et al.,* 1986). The digestibility was obtained by a successive enzymatic hydrolysis of maize flour with solutions of pepsin and cellulase in a ratio 1:100 w/v. The enzymatic procedure comprised three steps: (1) hydrolysis with 0.2% pepsin solution (Merck 2000FIP U g^{-1} Art 7190) in 0.1 M HCl at 40°C for 24 h; (2) starch hydrolysis in same solution at 80°C for 45 min; (3) hydrolysis with 0.1% cellulase solution (Onozuka R10) in 0.05 M of sodium acetate, pH 4.6 at 40°C for 24 h. After filtration the weight of sample undigested DM was measured. Digestibility was expressed as a % of digested organic matter of total DM.

The standard chemical methods (Official Gazette of SFRY, 1987) were applied to determine the content of starch and total proteins.

Physical procedures

After measuring the weight of grains, pericarp, germ, and endosperm were isolated by hand-dissection of duplicate samples previously soaked in water for 12 h. Mass of each part of grain after drying was measured and their share of the whole grain weight was calculated. Percentage share of hard and soft endosperm was determined by Stenvert-Pomeranz method (Radosavljevic *et al.,* 2000).

Statistical analyses

All chemical analyses were performed in three replicates and the results were statistically analysed. Significant statistical differences of observed chemical maize variables 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. This experiment was one factorial design with a genotype and a replication as the sources of variation. For the analysis of variance MSTAT software was used. Correlations between variables were examined using the Pearson correlation.

Results and discussion

Protein fractions in grains of maize specialty hybrids

The protein solubility is an important functional property that affects the utilisation and nutritional

Table 1. The content of albumin, globulin, α-zein, G3-glutelin and tryptophan in the grains of ZP specialty maize hybrids. The results are presented as % of dry matter (1) and % of total protein (2)

Hybrids[1]	Protein	Albumin		Globulin		α-Zein		G3-Glutelin		Tryptophan
	(1)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)
ZP 504su	13.27^{a}	2.99^{a}	21.54^{a}	1.10^{bc}	7.93^{c}	2.46^{e}	17.72^{d}	2.90^{a}	20.89^{a}	0.097^{ab}
ZP 531su	12.01^{c}	2.70^{b}	21.60^{a}	1.02^{c}	8.16^{c}	2.24^{g}	17.92^{d}	2.38^{b}	19.04^{c}	0.100^{a}
ZP 74b	10.49^{e}	1.12^{f}	10.61^{d}	1.20^{a}	11.36^{a}	2.82^{b}	26.70^{b}	1.78^{f}	16.86^{e}	0.059^{cd}
ZP 611k	12.41^{b}	1.17^{f}	9.43^{e}	0.83^{d}	6.69^{d}	3.36^{a}	29.25^{a}	2.35^{b}	18.82^{b}	0.055^{cd}
ZP 704wx	11.14^{d}	1.56^{c}	13.76^{b}	1.17^{ab}	10.32^{b}	2.58^{d}	22.75^{c}	2.32^{b}	20.37^{a}	0.086^{b}
ZP Rumenka	11.42^{d}	1.49^{d}	12.76^{c}	1.17^{ab}	10.02^{b}	2.70^{c}	23.12^{c}	2.19^{c}	18.75^{c}	0.050^{d}
ZP 434	10.42^{e}	1.32^{e}	10.90^{d}	1.10^{bc}	10.34^{b}	2.87^{b}	26.97^{b}	1.90^{e}	17.86^{d}	0.065^{c}
ZP 633	10.13^{e}	1.29^{e}	12.43^{c}	1.17^{ab}	11.27^{a}	2.33^{f}	22.45^{c}	2.04^{d}	19.65^{b}	0.052^{d}
$LSD_{0.05}$	0.292	0.075	0.632	0.092	0.803	0.084	0.659	0.065	0.596	0.011

[1] su-sweet, b-white, k-popping, wx-waxy, Rumenka-red, 434-yellow dent, 633-yellow semiflint corn. [a-f] Means followed by the same letter within the same columns are not significantly different ($p < 0.05$).

value of maize grains. Protein fractions were isolated according to their solubility in different solutions. The results are presented in Table 1. α-Zein was the dominant protein fraction in all genotypes except in sweet maize hybrids, making 22.45% to 29.25% of the total protein content. The highest α-zein content was detected in grains of popping maize hybrid ZP 611k, 3.36% of dry matter. The sweet maize hybrids had a significantly higher albumin content than other specialty ZP hybrids ($p < 0.05$). The content of albumin was 21.54% and 21.60% of total protein in ZP 504su and ZP 531su sweet hybrids, respectively. In the grains of the other specialty hybrids, the albumin content was lower by 38% to 57%, ranging from 9.43% of total protein in grains of popping maize hybrid ZP 611k to 13.76% of total protein in waxy genotypes ZP 704wx. Globulin was the lowest fraction in all the analyzed samples (6.69 to 11.36% of total protein). The G3-glutelin fraction is true glutelin with a high content of two essential amino acids, cysteine and methionine. The content of G3-glutelins was lowest in grains of white maize ZP 74b and standard dent hybrids ZP 434, *i.e.* 16.86% and 17.86% of total protein, respectively. For the most part zein is present in the maize endosperm, glutelin is distributed between the endosperm and the germ, while albumins and globulins are present mainly in the germ (Shukla and Cheryan, 2001). Grains of sweet hybrids ZP 504su and ZP 531su with the highest content of the albumin fraction had the highest portion of germ, *i.e.* 23.2 and 19.6% of the grain weight, respectively. In addition, grains of white maize ZP 74b and popping maize hybrid ZP 611k with the lowest content of the albumin fraction had the lowest portion of germ (11.8 and 11.1%, respectively). However, popping maize hybrid ZP 611k had the highest portion of hard endosperm per grain weight (75.4%) as well as the highest content of α-zein (Tables 1 and 2). The content of individual protein fractions found in the grains of ZP maize hybrids was consistent with the results obtained by Fageer and El Tinay (2004). These authors reported that the content of true glutelin (G3-glutelins) had 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%.

Content of tryptophan in grains of maize specialty hybrids

Content of total protein ranged from 10.13 to13.27% in grain of the analyzed specialty maize genotypes (Table 1). However, 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 variable for evaluating the nutritional quality of the grain protein (Hernandez and Bates, 1969). According to our results, the highest tryptophan content was detected in kernels of sweet hybrids (0.097% and 0.100% DM, respectively). Waxy maize had a high tryptophan content (0.086% DM), however white (ZP 74b), red (ZP Rumenka), standard dent (ZP 434), semiflint (ZP 633) and popping maize had a low tryptophan content, ranging from 0.050% to 0.065% of dry matter (Table 1). Tryptophan contents determined in the analyzed maize hybrids were consis-

Table 2. The portion of pericarp, germ and endosperm per grain weight of ZP specialty maize hybrids (%)

Hybrid[1]	Pericarp	Germ	Whole endosperm	Hard endosperm	Soft endosperm
ZP 504su	7.1^{e}	23.2^{a}	69.3^{e}	61.1^{d}	38.9^{c}
ZP 531su	9.0^{b}	19.6^{b}	71.4^{d}	58.5^{f}	41.5^{a}
ZP 74b	8.6^{c}	11.8^{e}	79.6^{c}	65.6^{b}	34.4^{e}
ZP 611k	9.8^{a}	11.1^{f}	79.1^{c}	75.4^{a}	24.6^{f}
ZP 704wx	6.5^{f}	13.0^{d}	80.5^{b}	60.3^{e}	39.7^{b}
ZP Rumenka	8.2^{d}	14.0^{c}	71.8^{d}	60.2^{e}	39.8^{b}
ZP 434	7.2^{e}	12.9^{d}	79.9^{b}	58.8^{f}	41.2^{a}
ZP 633	5.2^{g}	12.7^{d}	82.1^{a}	64.1^{c}	36.9^{d}
$LSD_{0.05}$	0.367	0.654	0.689	0.735	0.692

[1] See Table 1. $^{a-f}$ Means followed by the same letter within the same columns are not significantly different ($p < 0.05$).

tent with the average tryptophan content (0.072% DM) of maize genotypes presented by Vyn and Tollenaar (1998). 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. The tryptophan content was negatively correlated to the content of globulin and α-zein concentrations ($r = -0.38$ and $r = -0.75$, respectively, $p < 0.05$) and positively correlated to the content of albumin and G3-glutelin content ($r = 0.86$ and $r = 0.52$, respectively, $p < 0.05$). Zein is particularly rich in glutamic acid (21–26%), leucine (20%), proline (10%) and alanine (10%), but deficient in basic and acidic amino acids (Shukla and Cheryan, 2001). The notable absence of tryptophan and lysine in zein accounts for its negative dietary nitrogen balance. The need to improve the nutritional value of maize varieties, both for animal feed and human consumption, resulted in development of QPM-quality protein maize, with increased levels of tryptophan and lysine (Vasal, 2000). Sweet and waxy hybrids analyzed in our study were at the QPM level for tryptophan content and thus could be used in breeding programs for improving protein quality of standard maize hybrids. The QPM threshold values for tryptophan content are 0.070% (endosperm) and 0.075% (whole grain) (Vasal *et al.,* 1996). The nutritional benefits of QPM for people, who depend on maize for their energy and pro-ein 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).

Physical structures of specialty maize hybrids grains

The weight distribution of different parts of ZP maize grains is shown in Table 2. According to our results endosperm, the largest structure, ranged from 69.3 to 82.1% of the maize grain weight, while germ ranged from 11.1 to 23.2% and pericarp from 5.2 to 9.8%. Our results of pericarp, germ and endosperm portion per grain weight is in accordance with results previously published by Radosavljevic *et al.* (2006). Considering differences in distribution of chemical composition among anatomical parts of maize grain, the study of pericarp, germ and endosperm portion per whole grain weight and the microscopic structure of these anatomical components is very important (Wolf *et al.,* 1969). Maize pericarp is characterized by a high crude fibre content of about 87%, which is constituted mainly of hemicellulose (about 67%), cellulose (about 23%) and lignin (about 0.1%) (Burge and Duensing, 1989). However, according to our results, portion of pericarp per whole grain weight was negatively correlated to the content of hemicellulose ($r = -0.32$, $p < 0.05$) and positively correlated to the content of cellulose and lignin ($r = 0.58$ and $r = 0.49$, respectively, $p < 0.05$). The maize pericarp is used mainly as a feed, although in recent years interest has developed in it as a source of dietary fibre (Burge and Duensing, 1989). The germ is characterized by a high crude fat content, averaging about 33%. The germ also contains a relatively high level of protein (about 18.4%) and minerals. The quality of germ proteins is much higher than that of endosperm proteins and is obviously superior to the quality of whole kernel protein (Landry *et al.,* 2004). Germ of the high-protein varieties is larger than that of common maize but about half the size of high-oil varieties (Landry and Moureaux, 1980). According to our results, grains of sweet hybrids ZP 504su and ZP 531su with the highest portion of germ had the highest content of total proteins-13.27 and 12.10%, respectively (Tables 1 and 2). The maize germ processed to produce oil gives as a by-product maize germ meal, is used as an animal feedstuff. Some attempts have been made to use these by-products for humans in food mixes and formulations. Finally, endosperm contains high level of starch (about 88%) and protein level of about 8%. Crude fat content in endosperm is relatively low. The weight distribution of hard and soft endosperm of ZP maize speciality hybrids ranged from 58.5 to 75.4% and for 24.6 to 41.5%, respectively (Table 2). Tested maize speciality hybrids ZP 531su, ZP 434 and ZP 704wx with the highest proportion of soft endosperm in grain weight (41.5, 41.2 and 39.7%, respectively) had the highest content of tryptophan (0.100, 0.065, and 0.086%, respectively). However, in sweet maize hybrid ZP 504su with somewhat lower portion of soft endosperm in grain weight (38.9%) a high content of tryptophan (0.097%) was also identified. This hybrid had the highest portion of germ with a high content of protein quality (Tables 1 and 2). Jaeger *et al.* (2006) concluded that cattle fed dry-rolled maize hybrids with greater proportions of soft endosperm had a faster growth than cattle fed hybrids with a harder endosperm. Selecting for these softer kernel traits may improve the efficiency of gain in feedlot cattle. Also, soft endosperm resulted in higher final ethanol concentrations compared to ground maize or hard endosperm (Wang *et al.,* 2010).

Content of non-fibre carbohydrates in grains of maize specialty hybrids

The content of non-fibre carbohydrates (starch, sucrose and reducing sugars), of specialty maize hybrids is presented in Table 2. A low content of starch was determined in kernels of both sweet maize hybrids ZP 504su and ZP 531su (55.32% and 54.59%, respectively). When mature, the maize kernel contains carbohydrates other than starch in small amounts. Total sugars in the kernel range between 1 and 3%, with sucrose, the major component, found mostly in the germ. Grain of all investigated specialty hybrids contained sucrose, however arabinose and maltose were not detected. All hybrids could be classified according to the sucrose content in three groups: a) > 4% (sweet and red hybrids-ZP 504su, ZP Rumenka), b) from 3 to 4% (waxy, standard dent and semi flint hybrids-ZP 704wx, ZP 434, ZP 633) and c) from 2 to 3% (sweet, white and popping maize hybrids-ZP 531su, ZP 74b, ZP 611k). Significant differences in the sucrose content between genotypes within the same group were not found ($p < 0.05$). It should be noted that the maize genotype ZP Rumenka with red grain had sucrose content above the average for the dent type of maize hybrids (4.15%) (Table 3). This hybrid also had a high portion of germ in the grain weight (14.0%) (Table 2). However, a high sucrose content could be associated with a high content of phenolic compounds, which often build glycosides. According to our previous research, content of total phenol in grain of this maize genotype was 2.76 CE mg g^{-1} DM (Zilic *et al.,* 2010). Quantitative variability in the content of sucrose of the North American maize genotypes analysed by Kereliuk *et al.* (1995) ranged

Table 3. The content of non-fibre carbohydrates in the grains of ZP specialty maize hybrids (% of dry matter)

Hybrid[1]	Starch	Xylose	Arabinose	Fructose	Glucose	Sucrose	Maltose
ZP 504su	55.32^{f}	—	—	—	1.59^{b}	4.25^{a}	—
ZP 531su	54.59^{g}	1.84^{a}	—	1.93^{a}	1.96^{a}	2.75^{d}	—
ZP 74b	67.19^{d}	—	—	—	—	2.39^{e}	—
ZP 611k	67.14^{d}	—	—	—	—	2.68^{d}	—
ZP 704wx	69.01^{b}	—	—	—	—	3.20^{bc}	—
ZP Rumenka	65.38^{e}	—	—	—	—	4.15^{a}	—
ZP 434	67.92^{c}	—	—	—	—	3.36^{b}	—
ZP 633	69.92^{a}	—	—	—	—	3.37^{b}	—
$LSD_{0.05}$	0.562	—	—	—	0.127	0.164	—

[1] See Table 1. $^{a\text{-}f}$ Means followed by the same letter within the same columns are not significantly different ($p < 0.05$). —: not detected.

from 2.79% in the USA maize kernels to 3.40% in the Alberta maize and 3.65% in the Ontario hybrids. Same authors also reported that North American genotypes did not contain significant levels of glucose, fructose and raffinose, each averaging 0.28%. In the present study, we showed the sweet maize hybrid ZP 531su had 1.84% of xylose, 1.93% of fructose and 1.96% of glucose. Also, sweet maize ZP 504su consisted of a high percentage of glucose (1.59%), but fructose and xylose were absent. Carbonyl groups of reducing sugars react with free amino groups of proteins in the process of Maillard reaction (Maillard, 1912). Therefore, reducing sugars potentially may contribute to changes in food palatability, as well as, other nutritional effects, such as excessive browning, volume and tenderness reduction during cake preparation, and formation of mutagenic compounds (Didzbalis and Ho, 2001). However, some of Maillard reaction products are also known to exhibit antioxidant activities (Antony *et al.,* 2002). Also, many products of Maillard reaction (melanoidins) are mainly insoluble and are being largely indigestible by humans, so they have been proposed to behave as dietary fibre.

Content of dietary fibre in grains of maize specialty hybrids

Fibre components are one of the most important nutritional and technological factors of the maize grain. The dietary fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. The content of cellulose, hemicellulose, NDF, ADF and lignin in grains of the investigated genotypes is presented in Table 3. The cellulose and ADF contents did not vary greatly among the investigated ZP genotypes. Nevertheless, significant differences in the hemicellulose, NDF and the lignin content between specialty maize hybrids were found ($p < 0.05$). In the kernels of the tested specialty hybrids, the ADF content ranged from 3.63% in semiflint hybrid ZP 633 to 4.76% in popping maize genotype ZP 611k. The sweet maize hybrids ZP 504su and ZP 531su had the highest content of hemicellulose (10.29% and 8.56%, respectively) and NDF (14.72% and 14.07%, respectively). Values obtained overlapped with the range from 2.2% to 5.5% for the ADF content and from 8.0% to 17.4% for the NDF content reported by Reynolds *et al.* (2005) for maize hybrids originating from Germany, southern France, northern France and Italy. The NDF content for waxy maize (14.3%) was lower than that reported by Dado (1999). The lowest lignin content was detected in grains of the waxy hybrid ZP 704wx (0.29% of dry matter). The fibre in the maize grains, as with most other cereal grains, is predominantly located in the hull and germ fractions of the grain. The hull fraction makes approximately 7% of the total grain weight and contributes 51% of the total grain fibre, while the germ fraction makes 12% of the grain weight and contributes 16% of the total grain fibre (Watson, 1987). Therefore, removal of these fibre-rich maize fractions should have a significant impact on the nutrient composition of maize and potentially enhance the nutritional value of maize. Our results are in accordance with results obtained by Watson (1987) (Tables 2 and 4).

Table 4. The content of dietary fibres in the grains of ZP specialty maize hybrids (% of dry matter)

Hybrid[1]	Cellulose	Hemicellulose	NDF	ADF	Lignin
ZP 504su	4.04^{a}	10.29^{a}	14.72^{a}	4.43^{ab}	0.39^{c}
ZP 531su	4.08^{a}	9.56^{b}	14.07^{b}	4.48^{ab}	0.45^{c}
ZP 74b	3.57^{b}	7.66^{d}	11.87^{e}	4.22^{bc}	0.66^{ab}
ZP 611k	4.15^{a}	7.29^{de}	12.06^{de}	4.76^{a}	0.62^{b}
ZP 704wx	3.48^{bc}	8.66^{c}	12.43^{c}	3.79^{d}	0.29^{c}
ZP Rumenka	3.27^{cd}	7.07^{e}	11.02^{f}	3.96^{cd}	0.69^{ab}
ZP 434	3.11^{d}	7.22^{de}	11.13^{f}	3.92^{cd}	0.80^{a}
ZP 633	3.30^{cd}	8.79^{c}	12.41^{cd}	3.63^{d}	0.36^{c}
$LSD_{0.05}$	0.256	0.530	0.363	0.379	0.130

[1] See Table 1. $^{a\text{-}f}$ Means followed by the same letter within the same columns are not significantly different ($p < 0.05$).

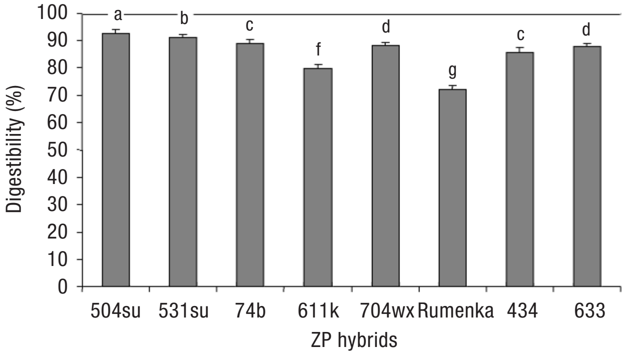

Figure 1. Grains dry matter digestibility of ZP specialty maize hybrids. Bars with different letters are significantly different ($p < 0.05$). Hybrids nomenclature: see Table 1.

Digestibility of maize grain dry matter

As shown in Figure 1 grains of both sweet maize hybrids were most digestible by enzymes. The dry matter digestion of ZP 504su and ZP 531su grains was 92.69% and 91.07%, respectively. In comparison with other investigated specialty genotypes these genotypes had the lowest content of starch, a low content of lignin (0.39% and 0.45%, respectively), and the highest content of soluble carbohydrates, sugar, albumin, hemicellulose and NDF. The content of all these components in the sweet grains had a positive correlation with digestibility of grain dry matter. The standard dent (ZP 434), popping (ZP 611k) and red (ZP Rumenka) maize hybrids with the highest content of lignin (0.80%, 0.62% and 0.69%, respectively) and the lowest content of hemicellulose (7.22%, 7.29% and 7.07%, respectively) and NDF (11.13%, 12.06% and 11.02%, respectively) had grains with the lowest dry matter digestibility (85.64%, 79.84% and 72.13%, respectively). Our results are in accordance with literature showing that sugars are completely digestible carbohydrates, hemicellulose is poorly digestible fibre and lignin is an indigestible dietary fibre. According to Sosulski and Cadden (1982), lignin is the most chemically active component of the cell walls, being responsible for interactions with other dietary components and for decreasing bioavailability of nutrients. Generally, in our study, the grain dry matter digestion was negatively correlated to the lignin content ($r = -0.53$, $p < 0.05$), and positively correlated to the hemicellulose and NDF ($r = 0.80$ and 0.71, respectively, $p < 0.05$). Besides, the maize grain digestion is influenced by the structure of starch (Hasjim *et al.,* 2009). In our experiments, dry matter of ZP waxy maize grains was more digestible by 2.5% than that of normal (dent) ZP hybrid. A high degree of branching, as noted in waxy amylopectin, was shown to disrupt the granular structure of starch and to increase its susceptibility to the attack by enzymes and its digestibility *in vitro* (Mohd and Wootton, 1984). Accordingly, a higher content of rapidly digestible starch was found in waxy (87%) than in normal maize starch (84%) (Wongsagonsup *et al.,* 2008). Also, our results confirmed the investigation carried out by Weaver *et al.* (1998) that differences in the protein digestibility were related to enzyme susceptibility of the major storage protein, prolamin (zein). Results presented in Table 1 and Figure 1 show that samples with a high content of α-zein had low kernel digestibility. The digestion of the grain dry matter was negatively correlated to the zein content ($r = -0.47$, $p < 0.05$), and positively correlated to other protein fractions, albumin, globulin and glutelin ($r = 0.54$, $r = 0.035$ and $r = 0.25$, respectively, $p < 0.05$). According to Fageer and El Tinay (2004) digestibility of maize grain protein ranged from 12.6 to 16.4%. A genotype with the protein digestibility of 16.4% had the lowest content of zein (31.9%) and very high content of albumin and globulin (21.9%) in relation to other genotypes. The standard dent (ZP 434), popping (ZP 611k) and red (ZP Rumenka) maize hybrids with the highest content of zein and the lowest digestion of the grain dry matter could be used for zein isolation and its utilization as an industrial polymer.

Viscosity of flour made from grains of maize specialty hybrids

Pasting properties of maize flour and digestion depend on different chemical components of grains, as well as the interactions of these components. Starch constitutes are the most abundant components in maize flours. It seems that the pasting properties were more affected by starch type and quantity rather than by protein presence. Further, lipids and their interaction with starch played an effective role in gelatinisation, pasting and cooling stage of paste viscosity. The components of starch, amylopectin and amylose have different roles in retrogradation. Published evidence suggests that changes in the amylopectin are the main cause for what we call retrogradation because these changes are responsible for all long-term rheological and structural changes. Changes in amylose, however, are responsible for the short-term rheological and structural changes (Gudmundsson, 1994). Many studies support the idea that starch retrogradation can have major effects on the texture and the digestibility of starchy food products (Ring *et al.,* 1987). Our results of the apparent viscosity referring to flour of semiflint yellow, waxy and sweet maize grains are presented in Figure 2. The flours of yellow dent, white, red and popping maize had the same pasting behaviour as flour of semiflint yellow maize grains, therefore the results of apparent viscosity of these samples, due to the visibility of pasting curves, are not presented. The yellow dent and semiflint (ZP 434, ZP 633), white (ZP 74b) and red (ZP Rumenka) maize flours produced amylograms with viscosities that were typical for normal (dent) maize flour. Such flour types are characterised by moderate pasting viscosities with clear peak viscosity. Prolonged cooking at 95°C for 30 min resulted in a constant increase of viscosity in the samples. The sweet maize hybrid ZP 531su gave a hardly detectable viscosity peak. The lower starch and zein contents and a higher content of sugars were associated with very low viscosity and completely different gelatinisation properties in comparison with other analysed maize samples. In starch based gel or paste systems, a sugar addition has been reported to increase gelatinization (starch melting) temperature and enthalpy. However, various studies reported that certain sugars could retard or accelerate starch recrystallisation (Slade and Levine, 1987). This could imply that sugars can behave differently, possibly depending on the host system and storage conditions. Also, Schober *et al.* (2008) reported that zein had some of the properties of wheat gluten but was not able to form viscoelastic fibrils at room temperature, though it could be made functional in this way at higher temperatures. Flour of ZP waxy maize hybrid is characterised by clear and high peak viscosity. The viscosity of the paste increases to the point where the number of swollen-intact starch granules is maximal. The peak viscosity is indicative of a water-binding capacity. However, prolonged cooking at 95°C for 30 min did not result in a constant increase of viscosity in the sample. When gelatinised starch cools, amylose retrogrades, resulting in an increase in viscosity named setback. But, the retrogradation properties of waxy starch are different and highly affected by the molecular size, average chain length and the distribution (polydispersity) of amylopectin branch chains (Karlsson *et al.,* 2007). The clarity and visco stability of amylopectin waxy starch improves smoothness and creaminess of tinned food and dairy products, as well as, freeze-thaw stability of frozen foods. It gives a more desirable texture and appearance to dry foods and mixes (Fergason, 2001). Waxy maize starch is also starting material for the production of maltodextrins because of improved water solubility after drying and greater solution stability and clarity.

Through conventional breeding and biotechnology, the chemical composition of maize has been modified to better meet the needs of livestock feeders, the food industry, and industrial users of maize. Specialty maize have been genetically altered to improve their starch, protein, or oil content, depending on their intended use. From the results obtained, it can be concluded that the Serbian's maize genetic resources contain a wealth of benefits, including new opportunities for improving nutrition, and multiple uses of maize and maize products.

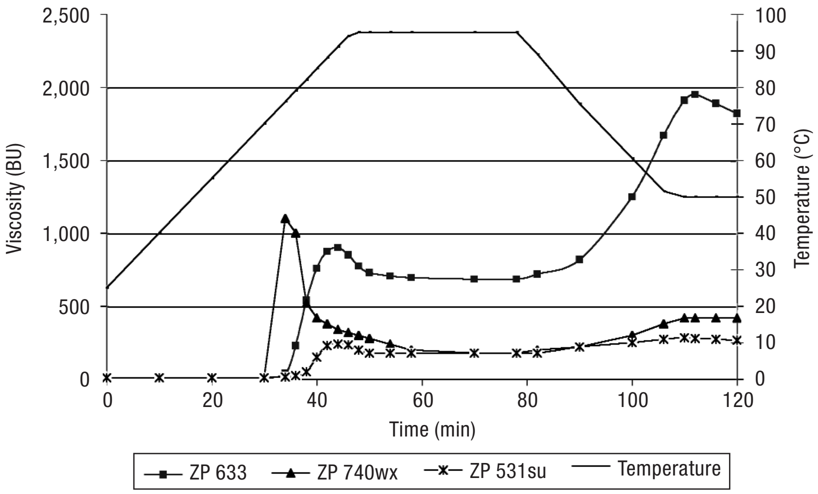

Figure 2. Brabender amylograph viscosity curves of flour paste of specialty maize hybrids. Hybrids nomenclature: see Table 1.

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