



UDK 664.641.1+633.15]:641.1

Original research paper

DOI: 10.5937/ffr0-41894

NUTRITIONAL COMPOSITION AND BIOACTIVE PROPERTIES OF THE WHOLEGRAIN FLOUR OBTAINED FROM MAIZE INBRED LINES

Valentina V. Nikolić*, Marijana Z. Simić, Slađana M. Žilić, Natalija B. Kravić, Jelena P. Vančetović, Mile D. Sečanski, Marko G. Vasić

Maize Research Institute Zemun Polje, 11185 Belgrade-Zemun,
Slobodana Bajića 1, Serbia

Abstract: The aim of this study was to assess the chemical composition and bioactive properties of the wholegrain flour obtained from eleven maize inbred lines to identify genotypes with increased potential for the development of hybrids with high nutritional and functional value, suitable for food production. The maize inbreds, including seven standard yellow, two QPM (quality protein maize) and two lines for red kernel hybrids, were grown in the experimental field of the Maize Research Institute at the location of Zemun Polje, Serbia. Wholegrain maize flour was got by grinding the maize grain in a laboratory mill. The assessment of the chemical composition and content of certain bioactive compounds, as well as the total antioxidant capacity, was conducted using standard laboratory procedures. The highest starch content (73.73%) was determined in line L8, while line L10 had the highest protein content (12.82%). Among soluble proteins, the α -zein fraction was dominant in most of the lines, ranging from 0.92% to 3.57%. The highest content of total fibres (NDF) was determined in red kernel line L9 (15.77%). Line L8 was the richest in total carotenoids (21.08 $\mu\text{g } \beta\text{CE/g d.m.}$), while line L7 had the highest total antioxidant capacity (34.30 mmol Trolox/kg d.m.), which can be explained by the presence of anthocyanins in the red grain. Line L1 had the highest content of total sugars (3.36%), and line L4 had the lowest (1.44%). Most of the samples of inbred lines investigated in this study showed good quality parameters regarding chemical composition and bioactive properties. The obtained results may provide some valuable guidelines needed in the following stages of maize breeding and open up various possibilities for the utilization of wholegrain maize flour in the food industry.

Keywords: *maize, wholegrain flour, inbred lines, nutritional composition, bioactive properties*

INTRODUCTION

Maize (*Zea mays* L.) is the most frequently cultivated agricultural plant grown as an essential raw material for food, fodder, energy, and industrial materials. According to the data reported by Statista (2022), 1,089.04 million metric tons of maize were produced worldwide in the 2021/2022 season. In the age of climate change, population growth, and food scarcity,

maize has drawn particular interest from researchers in different scientific fields. Breeders use the information on genetic diversity crucial for the analysis of genetic relationships between lines and for choosing different parental combinations to produce novel, more resilient, and high-yielding maize hybrids with the highest genetic variability (Mladenović Drnić

et al., 2022). The selection and development of inbred lines, the parental components of single-cross hybrids, are expensive and time-consuming procedures in maize breeding programs (Benchimol et al., 2000). Maize hybrids are extensively cultivated, however, only a few inbred lines of maize are being sown, because of the practical and financial benefits of hybrid vigour and grain output (Huang et al., 2015). While consecutive generations of self-pollinated (i.e., inbred) maize plants rapidly decline regarding the vigor and grain yield, these properties of the F1 hybrid frequently exceed those seen for the original parent populations when two inbred lines from unrelated populations are crossed. This phenomenon is known as heterosis (Tollenaar, Ahmadzadeh, & Lee, 2004; Lin et al., 2016).

According to Eckhoff and Watson (2009), the maize grain mostly consists of starch (71.3%), followed by protein (9.91%), oil (4.45%), ash (1.42%), and crude fibre (2.66%). This cereal serves as a significant source of these macronutrients because of the large and widespread use of maize grain in many food products. Considerable grain starch content renders maize both a valuable crop for food and livestock feed and a crucial source of raw materials for industrial use (Radosavljević et al., 2020).

Many epidemiological studies conducted recently have shown that consumption of refined flour has been linked to increased health issues and diseases such as type 2 diabetes, coronary atherosclerosis, chronic cardiovascular disease, colon cancer, and obesity (Cory, Passarelli, Szeto, Tamez & Mattei, 2018; Singh, Metrani, Shivanagoudra, Jayaprakasha & Patil, 2019; Cheng et al., 2021). Unlike refined products of grain milling, wholegrain flour is a source of bioactive components and dietary fiber that possess numerous properties beneficial for health.

Most of these compounds are found in the aleurone layer, which offers this fraction – followed by bran and germ – the highest antioxidant activity. Most of the bran's bioactive elements are bound to fibre, which enables them to transit through the digestive process and arrives intact in the colon, where they foster an antioxidant environment (Gong, Chi, Wang, Zhang & Sun, 2019). Maize grain itself, as well as wholegrain maize flour, which is made by grinding kernels without first removing the germ, is naturally devoid of gluten and is safe for persons with

celiac disease (Parris, Moreau, Johnston, Singh & Dickey, 2006).

The color of maize grains can range from white and yellow to orange, red, burgundy, blue and purple to brown, depending on the genetic background that influences the colour of the pericarp, aleurone, germ, and endosperm. Both provitamin and non-provitamin A carotenoids found in yellow maize have the potential to improve human health. In parts of the world where yellow maize is mostly consumed, an increase in the content of these bioactive nutrients may bring benefits to the diet quality. An evaluation of the carotenoid diversity of new maize inbred lines is a crucial initial step in breeding yellow maize for increased carotenoid concentrations (Menkir, Liu, White, Maziya-Dixon & Rocheford, 2008). The entire kernel of colored maize contains a variety of secondary metabolites, including carotenoids and phenolic compounds, most which are found as phenolic acids and flavonoids, with some types existing in soluble, free, conjugated, or insoluble, bound configurations (Žilić, Serpen, Akilloğlu, Gökmen & Vančetočić, 2012).

The lack of essential amino acids, however, can have a severe effect on both human and animal growth. The average maize's endosperm lacks two crucial amino acids, lysine and tryptophan (Annor & Badu-Apraku, 2016). Quality protein maize (QPM) can provide up to 80% of the human protein demands, while non-QPM genotypes can only provide up to 46% of those needs depending on the genotype (Babu & Prasanna, 2014). Therefore, the development and production of QPM with increased tryptophan and lysine contents could significantly reduce malnutrition and diseases associated with it in low-income countries of the developing world (Amegbor, van Biljon, Shargie, Tarkegne & Labuschagne, 2022).

In cereals breeding and commercial grain/seed production, maize with specific properties and uses needs special consideration. Because of genetic variability, breeding can drastically alter the genotypes' quality traits and influence the distribution of the grain's specific constituents, leading to the development of novel hybrids with multiple applications.

Certain evaluation processes for the desirable traits are required to guarantee successful selection for those qualities. This can be accomplished by carefully selecting parental components with high genetic variability suitable for

subsequent breeding. Hence, the main aim of this research was to investigate the quality parameters of wholegrain flours obtained from eleven maize inbred lines in order to identify genotypes with increased potential for creating a commercial category of maize hybrids with enhanced nutritional, functional, and technological value.

MATERIALS AND METHODS

Plant material

The plant material used in this study comprised seven standard yellow, two QPM, and two inbred maize lines components of red kernel hybrids (Table 1). The maize genotypes were sown in the experimental field at the location of the Maize Research Institute Zemun Polje, Serbia (44°52'N, 20°19'E, 81m asl) according to the randomized complete block design in the 2020 growing season. Standard cropping practices were conducted under rain-fed conditions. The plots were made up of four rows that were each 5 m long, spaced 0.20 m apart from one another within each row, and 0.75 m apart between rows. From two inner rows of each replicate, maize ears were taken at their full physiological maturity stage (R6). To prepare the samples, wholegrain maize flour was produced using a laboratory mill with a mesh size of 0.5 mm (Perten MILL 120 CE, Perten Instruments in Hägersten, Sweden).

Chemicals

All chemicals used were of analytical and high-

performance liquid chromatography (HPLC) grade, and were purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

Chemical analyses

The dry matter content of the wholegrain maize flour was assessed using the conventional drying method at 105 °C to a constant mass. The starch content and soluble carbohydrates were determined according to the Ewers polarimetric method (ISO 10520,1997). The protein content was determined on the BÜCHI Kjeldahl System (AutoKjeldahl Distillation Unit K-350 and Speed Digester K-439, BÜCHI Labortechnik, Switzerland) by the Kjeldahl method number 55.04 as the total nitrogen multiplied by 6.25 (AOAC, 2000). The results are expressed in the percentages of protein per dry matter (d.m.). The oil content was obtained according to the Soxhlet method number 920.39 (AOAC, 2000), on a standard laboratory glass distillation apparatus and a hot water bath (INKOLAB, Croatia). The ash content was determined according to method number 923.03 by the slow combustion of the sample at 550 °C in a muffle furnace (L47, 1200°C, Naber Industrieofenbau, Germany) (AOAC, 2000).

Analysis of soluble protein fractions

Successive extractions of the wholegrain flour with a series of solvents (in a ratio of 1:10, w/v) were performed to get different protein fractions by the method proposed by Landry and Moureaux (1982),

Table 1.
Maize line markers and their parental contribution.

Line marker	Parental contribution
L1	Father component of a QPM hybrid
L2	Mother component of a QPM hybrid
L3	Parent of several commercial hybrids
L4	Mother component of several commercial hybrids
L5	Parent of several commercial hybrids
L6	Parent of several commercial hybrids
L7	Mother component of a red hybrid
L8	Parent of several commercial hybrids
L9	Father component of a red hybrid
L10	Mother component of several commercial hybrids
L11	Mother component of several commercial hybrids

with some modifications. Albumin, globulin, α -zein and glutelin fractions were extracted by distilled water, 0.5 mol/l NaCl, 70% ethanol, and 0.0125 mol/l borate buffer, pH 10, containing 5% sodium dodecyl sulphate, respectively. Each protein fraction was extracted using three rounds of repeated stirring at 4 °C for 30 minutes, followed by 15-minute centrifugation at 20,000 g (Dynamica Velocity 18R Refrigerated Benchtop Centrifuge, DKSH New Zealand Limited, New Zealand). Using the BÜCHI Kjeldahl System (AutoKjeldahl Distillation Unit K-350 and Speed Digester K-439, BÜCHI Labortechnik, Switzerland) nitrogen content was determined as a starting point, and the protein content of each fraction was computed using a conversion factor of 6.25. (Žilić et al., 2010). The results are given as % of dry matter (d.m.).

Analysis of dietary fibre

The content of hemicellulose, cellulose, neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin (ADL) were determined by the Van Soest detergent method modified by Mertens (1992) on the Fibertec system FOSS 2010 Hot Extractor (FOSS Tecator, Hoeganaes, Sweden), using neutral, acid, and alkali reagents. NDF practically represents total insoluble fibres (not soluble in water). ADF mainly consists of cellulose and lignin, while ADL is pure lignin. The content of hemicellulose was obtained as a difference between NDF and ADF contents, while the cellulose content was calculated as the difference between ADF and lignin contents. All the results are given as the percentage per dry matter (d.m.).

Determination of total yellow pigment content

Measurements were performed according to the AACC (1995) method number 14-50 as a reference. In short, 40 ml of 1-butanol saturated with water was used to extract 8 g of sample material. The samples were homogenized for 60 seconds, left at room temperature for 20 minutes, and then homogenized once more for 30 seconds. The absorbance was measured on a spectrophotometer (Agilent 8453 UV-visible Spectroscopy System, Agilent, United States) at 435 nm following centrifugation. Using a conversion factor of 1.6632, the amount of total yellow pigment was determined and expressed as mg of β -carotene equivalent (β CE) per g of d.m. (Žilić et al., 2011).

Analysis of total antioxidant capacity

According to a method proposed by Serpen et al. (2008), the antioxidant capacity of wholegrain maize flour was assessed using the direct or QUENCHER method and the ABTS (2,2-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) reagent. The total antioxidant capacity was represented as the Trolox equivalent antioxidant capacity (TEAC) and reported as mmol of Trolox per kg of dry mass (mmol Trolox Eq/kg). The absorbance was measured at wavelength 734 nm on a spectrophotometer (Agilent 8453 UV-visible Spectroscopy System, Agilent, United States).

Determination of total sugars, reducing sugars and sucrose

Using the Luff-Schoorl method, the content of total sugars, reducing sugars, and sucrose was measured (Egan, Kirk & Sawyer, 1981). The Luff-Schoorl method is based on the reduction of cupric copper to cuprous oxide after the reaction between reducing sugars and an alkaline copper sulphate solution. The method involves measuring unreduced Cu^{2+} ions iodometrically. The conversion of nonreducing sugars into reducing sugars via acid hydrolysis was then used to calculate the total amount of sugars. The percentage of sucrose is determined by dividing total and reducing sugars, or total inverter and natural inverter, respectively. The percentage per d.m. was used to represent all results.

Statistical analyses

Data from at least three separate repetitions were reported as a mean \pm standard deviation. Minitab (v. 19.2.0) Statistical Software was used to conduct the statistical analysis using the one-way ANOVA analysis of variance along with Tukey's test. The difference between the means was considered statistically significant if the probability was $p < 0.05$.

RESULTS AND DISCUSSION

Wholegrain maize flour represents a valuable source of energy, dietary fibre, proteins, and bioactive phytochemicals that can provide many health-beneficial properties. The contents of the main chemical components determined in the wholegrain flour of the investigated maize inbred lines are shown in Table 2. The highest content of starch, the predominant carbohydrate constituent of maize grain, was determined in the wholegrain flour of genotype L8 (73.73%).

Table 2.
Chemical composition of the wholegrain flour of the investigated maize lines (% d.m.)

Inbred line	Starch	SCH*	Protein	Oil	Ash
L1	68.86±0.50 ^e	0.23±0.00 ^g	10.40±0.02 ^{de}	7.15±0.15 ^a	1.58±0.06 ^c
L2	70.07±0.14 ^d	1.66±0.05 ^a	10.70±0.06 ^{cd}	5.41±0.04 ^d	1.71±0.00 ^{ab}
L3	72.25±0.07 ^{bc}	0.61±0.01 ^c	10.03±0.18 ^e	5.92±0.17 ^{bc}	1.44±0.03 ^d
L4	72.48±0.35 ^b	0.73±0.00 ^b	11.38±0.19 ^b	5.98±0.06 ^b	1.31±0.01 ^{ef}
L5	71.41±0.01 ^c	0.37±0.01 ^d	10.70±0.32 ^{cd}	6.04±0.04 ^b	1.36±0.01 ^{de}
L6	70.30±0.10 ^d	0.21±0.00 ^g	10.42±0.11 ^{de}	6.00±0.10 ^b	1.57±0.01 ^c
L7	70.18±0.16 ^d	0.05±0.00 ^h	11.22±0.04 ^{bc}	5.51±0.01 ^{cd}	1.43±0.01 ^d
L8	73.73±0.13 ^a	0.34±0.01 ^{de}	10.40±0.13 ^{de}	4.87±0.21 ^e	1.24±0.01 ^f
L9	68.53±0.16 ^e	0.26±0.00 ^{fg}	10.85±0.13 ^{bcd}	7.23±0.06 ^a	1.34±0.03 ^{de}
L10	67.25±0.10 ^f	0.55±0.00 ^c	12.83±0.06 ^a	6.10±0.10 ^b	1.75±0.03 ^a
L11	69.09±0.12 ^e	0.30±0.01 ^{ef}	10.69±0.01 ^{cd}	5.72±0.06 ^{bcd}	1.63±0.04 ^{bc}

^{a,b,...}Means followed by the same letter within the same row are not significantly different ($\alpha=0.05\%$)

*SCH – soluble carbohydrates

Table 3.
Content of soluble protein fractions in wholegrain flour of the investigated inbred maize lines (% d.m.)

Inbred line	Albumins	Globulins	α -Zein	Glutelin
L1	2.08±0.03 ^b	0.79±0.00 ^{de}	2.04±0.04 ^f	2.28±0.00 ^{bc}
L2	2.21±0.03 ^a	1.60±0.03 ^a	0.92±0.00 ^g	2.28±0.00 ^{bc}
L3	2.17±0.04 ^a	0.77±0.04 ^{ef}	2.63±0.00 ^d	2.04±0.03 ^e
L4	1.49±0.00 ^d	0.86±0.04 ^{cd}	3.13±0.03 ^b	2.26±0.04 ^{bc}
L5	1.42±0.03 ^{de}	0.83±0.00 ^{cde}	3.04±0.03 ^{bc}	2.32±0.00 ^b
L6	1.75±0.00 ^c	0.79±0.00 ^{de}	3.02±0.00 ^c	2.19±0.00 ^{cd}
L7	1.75±0.00 ^c	0.81±0.03 ^{cde}	3.04±0.03 ^{bc}	1.66±0.00 ^g
L8	1.36±0.00 ^e	0.88±0.00 ^c	2.36±0.00 ^e	2.43±0.03 ^a
L9	2.02±0.00 ^b	0.96±0.00 ^b	2.45±0.00 ^e	2.12±0.03 ^{de}
L10	1.73±0.03 ^c	0.96±0.00 ^b	3.57±0.04 ^a	2.08±0.03 ^e
L11	2.02±0.00 ^b	0.70±0.00 ^f	3.09±0.04 ^{bc}	1.82±0.04 ^f

^{a,b,...}Means followed by the same letter within the same row are not significantly different ($\alpha=0.05\%$)

Table 4.
Fibre content in wholegrain flour of the investigated maize inbred lines (% d.m.).

Inbred line	NDF	ADF	ADL	Hemicellulose	Cellulose
L1	9.24±0.54 ^e	2.18±0.30 ^b	0.29±0.01 ^a	7.07±0.23 ^d	1.89±0.30 ^{bc}
L2	11.50±0.21 ^c	3.30±0.06 ^b	0.45±0.05 ^a	8.20±0.16 ^d	2.86±0.11 ^{ab}
L3	9.41±0.34 ^{de}	2.12±0.13 ^b	0.40±0.03 ^a	7.29±0.47 ^d	1.72±0.10 ^c
L4	13.85±0.59 ^b	2.49±0.19 ^b	0.23±0.02 ^a	11.36±0.40 ^{ab}	2.26±0.21 ^{bc}
L5	10.83±0.49 ^{cde}	2.75±0.01 ^b	0.33±0.03 ^a	8.08±0.48 ^d	2.42±0.01 ^{bc}
L6	10.69±0.29 ^{cde}	2.53±0.42 ^b	0.19±0.06 ^a	8.16±0.13 ^d	2.34±0.48 ^{bc}
L7	11.03±0.68 ^{cd}	2.56±0.23 ^b	0.27±0.10 ^a	8.48±0.45 ^{cd}	2.29±0.13 ^{bc}
L8	9.98±0.02 ^{cde}	2.65±0.09 ^b	0.26±0.18 ^a	7.33±0.11 ^d	2.39±0.10 ^{bc}
L9	15.77±0.34 ^a	2.94±0.16 ^b	0.28±0.17 ^a	12.84±0.18 ^a	2.66±0.01 ^{bc}
L10	13.70±0.15 ^b	3.48±0.21 ^a	0.28±0.07 ^a	10.22±0.06 ^{bc}	3.20±0.14 ^a
L11	13.74±0.42 ^b	3.56±0.78 ^a	0.38±0.17 ^a	10.18±1.20 ^{bc}	3.18±0.61 ^a

^{a,b,...}Means followed by the same letter within the same row are not significantly different ($\alpha=0.05\%$)

NDF-neutral detergent fiber, ADF-acid detergent fiber, ADL-lignin

Maize grains on average contain 6% to 12% proteins, which are primarily found in the endosperm (70-79% of the total protein content of the grain) and the germ (18-28% of the total protein content of the grain) (Watson, 2003). Wholegrain flour of the maize inbred L10 had the highest protein content (12.82%), and the lowest content (10.03%) was determined in a yellow dent genotype L3. The lowest oil content was determined in the wholegrain flour of red genotype L7, only 4.87%, while wholegrain flour got from a QPM genotype L1 had the highest oil content, 7.15%. According to Kaur, Singh and Sharma (2019), compared to regular maize, quality protein maize (QPM) refined flour has higher levels of fat (3.90%), protein (10.04%), and carbohydrates (74.83%). Milašinović-Šeremešić, Radosavljević, Srdić, Tomićić and Đuragić (2019) previously reported average oil content of different maize hybrids amounted to 4.14%. Albeit, the oil content determined in the investigated wholegrain maize flours from inbred lines exceeded the maximal allowed values prescribed by the Serbian regulation on the quality requirements for milled maize products (Pravilnik, 2018), namely for wholemeal (4%) and refined maize flour (3%) which is obtained by dry a grinding process (after removal of the grain pericarp, and germ in which most lipids are located). The material investigated in this study comprised of starting genetic material, i.e. inbred lines, some of which may be used in the breeding processes to get high-oil hybrids, in contrast to standard maize hybrids that are commercially used in flour production. The basic chemical composition of the analyzed wholegrain maize flours is mainly in accordance with previously published findings (Milašinović-Šeremešić et al., 2019; Nikolić, Božinović, Vančetović, Radosavljević & Žilić, 2020).

Maize protein solubility is an important functional characteristic that affects both the nutritional value and the utilizable value of maize grains. The largest part of the protein in maize, comprising between 50 and 80% of its total protein, is zein, a storage prolamin. Most zein is made up of α -zeins, which make up around 70% of all zein (Lawton, 2002). The α -zein fraction was dominant in most of the examined samples (Table 3) and ranged from 0.92% in a QPM inbred line (L2) to 3.57% (L10). Unfortunately, the important amino acids lysine, tryptophan, and methionine are scarce or absent in zein

proteins, particularly α -zein (Wang, Geil, Kolling & Padua, 2003). However, even though the QPM genotypes provide a high nutritional value, with the increased endosperm's lysine content, the chalky and soft texture of the grain endosperm poses several issues, including a higher vulnerability to pests and diseases, challenges during harvest and storage, and a lack of direct application (Zhao et al., 2022). Next, in terms of representation were the glutelin and albumin fractions, while the globulin fraction was the least represented in most of the wholegrain flours of the examined maize lines. Ortiz-Martinez, Otero-Pappatheodorou, Serna-Saldívar and García-Lara (2017) reported that, compared to the standard maize genotype, which had 9.10 and 6.83 mg/g of albumin and globulin respectively, the QPM genotype had higher levels of both fractions (21.74 mg/g and 13.55 mg/g). The rise in the albumins and globulins of the QPM is linked to both a lower level of zein production and a higher proportion of germ in the kernel. In some of the inbred lines investigated in our study, namely L3 and L11, the albumin fraction was more represented than the glutelin fraction. With a protein composition of 35 to 45%, glutelins are generally considered the second-largest protein class in maize endosperm (Pérez-Grau, Cortadas, Puigdomènech & Palau, 1986). Because of the significantly higher contents of the limiting essential amino acid lysine and higher rate of digestibility, the water soluble albumins and salt soluble globulins are thought to be the most nutritionally important. The maize protein quality is, therefore, directly correlated with the presence of these two fractions (Ortiz-Martinez et al., 2017). A previous study by Landry and Moureaux (1987) demonstrated that unlike the other protein groups (zeins, G1-, G2-, and G3-glutelins), which showed a steady accumulation until maturity, salt-soluble proteins, made up of albumins and globulins, reached a maximum during development and decreased at maturity in grains or endosperm. A decrease in the absolute level of endosperm albumins appeared to be closely correlated with the decline of salt-soluble proteins, which varied in magnitude and duration. The gradual changes in the amino acid composition of total salt-soluble proteins and the stability of those of the zein and glutelin subgroups as development progressed also pointed to differences in the accumulation rates of albumins and globulins,

as indicated by accumulation kinetics (Landry & Moureaux, 1987).

Given that dietary fibres stand for components crucial for the proper functioning of the human gastrointestinal tract, fibre content is a relevant nutritional and technological indicator of maize grain quality (Table 4). The two main non-starch polysaccharides found in maize grain, particularly in maize bran, are cellulose and hemicellulose (Watson, 2003). The highest content of total fibres (NDF), the most abundant fibre fraction, was determined in the wholegrain flour of genotype L9 (15.77%), and the lowest (9.24%) in L1 samples. Low ADL levels, indicating highly complex and indigestible lignin fraction, were low (0.19-0.45%), suggesting that all the investigated wholegrain maize samples are suitable for food production and human consumption. On the other hand, the findings regarding the content of the lignocellulosic fractions in maize grain reported by Radosavljević et al. (2012) varied significantly from the results of our study, given that the maize was harvested in different physiological stages of maize grain filling, i.e., not intended for human consumption (physiological maturity (R6)), but for silage production (dent phase of grain filling (R5)).

Yellow maize grain is a source of lutein, zeaxanthin, β -cryptoxanthin, α and β -carotene. Their contents in total carotenoids, i.e. yellow pigment, vary depending on the genotype, but lutein and zeaxanthin are the most common. The grain of L8 (21.08 μg $\beta\text{CE/g}$) was the richest in carotenoids, while the grain of line 7 (34.30 mmol Trolox/kg) had the highest antioxidant capacity, which can be explained by the presence of anthocyanins in the red grain (Table 5).

Both genotype and applied agricultural practice have a significant impact on the content of such phytochemicals. Mesarović et al. (2018) reported that among the tested antioxidant properties of three investigated sweet maize hybrids, the lowest variations were determined in total yellow pigment content (2.59%). Five different types of naturally occurring carotenoid compounds, including α -carotene, β -carotene, β -cryptoxanthin, lutein, and zeaxanthin, as well as four different types of tocopherols, are present in maize kernels (Mesarović et al., 2019). Mladenović Drinić, Vukadinović, Srđić, Milašinović-Šeremešić and Andjelković (2021) reported high levels of carotenoids in sweet maize, predominantly lutein, zeaxanthin, and

β + γ -tocopherol, all of which may be beneficial to human health.

As reported by Mladenović Drinić et al. (2019), among 101 inbreds with different kernel types and colour, lutein+zeaxanthin and β -carotene had respective mean contents of 31.34 and 8.72 $\mu\text{g/g}$. The range of β -carotene content was 1.20 to 39.37 $\mu\text{g/g}$. Popcorn inbred lines with orange to dark yellow kernels had the greatest mean values of lutein+zeaxanthin and β -carotene (35.62 and 14.81 $\mu\text{g/g}$, respectively), followed by orange kernel inbred lines (29.67 and 6.26 $\mu\text{g/g}$). Furthermore, Mesarović et al. (2019) reported that the content of the examined carotenoids in the sweet maize hybrids was altered by the use of p-hydroxyphenylpyruvate dioxygenase (HPPD) and acetolactate synthase (ALS) inhibitory herbicides, both with and without foliar fertilization. When compared to the control, the concentration of lutein, zeaxanthin, and β -carotene increased at varying rates following the applied treatments.

The antioxidant capacity ranged from 17.41 mmol Trolox Eq/kg d.m. in wholegrain flour got from yellow genotype L8 to 34.30 mmol Trolox Eq/kg d.m in red genotype L7 sample. The second highest antioxidant capacity was detected in red genotype L9 wholegrain flour (23.39 mmol Trolox Eq/kg d.m.). This can be attributed to the higher content of phenolic compounds, predominantly anthocyanins located in the outer layers of the red kernel maize genotypes. Žilić et al. (2012) closely correlated higher levels of antioxidant capacities to the kernel colour, increasing from white, followed by yellow and orange, to dark red kernel genotypes.

The content of total, total reducing sugars, and sucrose determined in the analyzed samples are shown in Table 6. Wholegrain flours obtained from genotypes L1 and L11 had the highest content of total sugars (3.36% and 3.24%), while line 4 was the lowest (1.44%) (Table 6). The sucrose content in the grains of the examined lines ranged from 1.21% (L10) to 2.23% (L1). The sugar content, especially reducing sugars, plays a role in food production that may affect the functional and sensory properties of the end product, as well as food safety (Cvijanović, Feinle-Bisset, Young & Little, 2015; Mora & Dando, 2021). During the thermal processing of some foods, a Maillard reaction between amino acids and reducing sugars takes place, and acrylamide is one of the undesirable

Table 5.
Total carotenoids and antioxidant capacities of the wholegrain flours

Inbred line	Total yellow pigment ($\mu\text{g } \beta\text{CE/g d.m.}$)	Antioxidant capacity (mmol Trolox Eq/kg d.m.)
L1	11.57±0.04 ^e	23.36±0.51 ^b
L2	16.73±0.06 ^c	20.41±0.07 ^{cd}
L3	14.84±0.06 ^d	21.67±0.43 ^{bc}
L4	14.34±0.09 ^d	19.62±1.07 ^{cde}
L5	16.28±0.21 ^c	21.33±0.28 ^{bc}
L6	17.91±0.02 ^b	19.26±0.50 ^{cde}
L7	10.09±0.11 ^f	34.30±0.13 ^a
L8	21.19±0.43 ^a	17.41±0.71 ^e
L9	17.37±0.00 ^b	23.39±0.01 ^b
L10	11.53±0.02 ^e	17.63±1.15 ^e
L11	9.41±0.04 ^g	18.71±0.76 ^e

a,b,... Means followed by the same letter within the same row are not significantly different ($\alpha=0.05\%$)
 $\beta\text{CE} - \beta$ - carotene equivalents; d.m.- dry matter

Table 6.
Sugar content in the investigated wholegrain flours (% d.m.)

Inbred line	Total sugars	Total reducing sugars	Sucrose
L1	3.36±0.00 ^a	1.01±0.07 ^a	2.24±0.06 ^a
L2	1.56±0.17 ^d	0.24±0.07 ^d	1.26±0.09 ^{bc}
L3	2.16±0.34 ^{cd}	0.24±0.07 ^d	1.83±0.26 ^{abc}
L4	1.44±0.34 ^d	0.15±0.06 ^d	1.23±0.38 ^{bc}
L5	1.92±0.00 ^{cd}	0.19±0.00 ^d	1.64±0.00 ^{bc}
L6	2.52±0.17 ^{bc}	0.63±0.06 ^b	1.80±0.23 ^{abc}
L7	2.52±0.17 ^{bc}	0.48±0.00 ^{bc}	1.94±0.16 ^{ab}
L8	2.04±0.17 ^{cd}	0.29±0.00 ^{cd}	1.67±0.16 ^{bc}
L9	1.92±0.00 ^{cd}	0.29±0.00 ^{cd}	1.55±0.00 ^{abc}
L10	1.56±0.17 ^d	0.29±0.00 ^{cd}	1.21±0.16 ^c
L11	3.24±0.17 ^{ab}	1.01±0.07 ^a	2.12±0.10 ^a

a,b,... Means followed by the same letter within the same row are not significantly different ($\alpha=0.05\%$)

probable carcinogenic products of this process (Žilić et al., 2022).

Acrylamide is created when high temperatures are applied to grains, tubers, storage roots, beans, and other agricultural goods during frying, baking, roasting, toasting, or other processing (Raffan & Halford, 2019). Amino acid asparagine is the primary precursor for the synthesis of acrylamide while reducing sugars play a secondary, yet still important, role in this reaction (Raffan & Halford, 2019). Among the most popular foods eaten globally are corn (maize) tortilla chips and breakfast cereals. In terms of sales volume among savoury and sweet snacks, the tortilla chip market in the US came in first. As a result, acrylamide exposure is in-

creased due to the widespread use of cereal-based goods, generating health concerns and the requirement for acrylamide reduction in these products (Žilić et al., 2022).

CONCLUSIONS

The results regarding the proximate composition and bioactive properties of the wholegrain flour obtained from eleven selected maize genotypes varied as shown by their proximate analyses. The chemical composition of the basic wholegrain flour of the investigated standard yellow-kernel, red-kernel, and QPM maize inbred lines show that the contents of basic chemical components, soluble proteins, fibre, yellow pigments, sugars, as well as antioxidant

capacities varied among the tested genotypes. The findings of this study showed a variety of possibilities for the application of the examined maize inbred lines in the following stages of maize breeding. Most of the whole-grain flours obtained from the inbred line samples displayed chemical composition and bioactive characteristics favourable for food production. Furthermore, residues from the field experiments that include maize grains and plant biomass not suitable for human consumption may find application primarily as components of animal feed or the industrial raw material for different bio-based products.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200040).

REFERENCES

- AACC. (1995). Pigment. Methods 14-50. In: AACC Methods (9th ed.) American Association of Cereal Chemistry, USA.
- Amegbor, I. K., van Biljon, A., Shargie, N., Tarekegne, A., & Labuschagne, M. T. (2022). Grain quality and yield potential of hybrids from quality and non-quality protein maize inbred lines. *Journal of Cereal Science*, 107, 103544. <https://doi.org/10.1016/j.jcs.2022.103544>
- Annor, B., & Badu-Apraku, B. (2016). Gene action controlling grain yield and other agronomic traits of extra-early quality protein maize under stress and non-stress conditions. *Euphytica*, 212, 213-228. <https://doi.org/10.1007/s10681-016-1757-4>
- AOAC. (2000). *Official Methods of Analysis* (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists.
- Babu, R., & Prasanna, B. (2014). Molecular breeding for quality protein maize (QPM). In R. Tuberosa, A. Graner, & E. Frison (Eds.), *Genomics of plant genetic resources* (pp. 490-505). Springer Science+Business Media Dordrecht. <https://doi.org/10.1007/978-94-007-7575-6>
- Benchimol, L. L., de Souza Jr, C. L., Garcia, A. A. F., Kono, P. M. S., Mangolin, C. A., Barbosa, A. M. M., Coelho, A. S. G., & De Souza, A. P. (2000). Genetic diversity in tropical maize inbred lines: heterotic group assignment and hybrid performance determined by RFLP markers. *Plant breeding*, 119(6), 491-496. <https://doi.org/10.1046/j.1439-0523.2000.00539.x>
- Cheng, W., Sun, Y., Fan, M., Li, Y., Wang, L., & Qian, H. (2021). Wheat bran, as the resource of dietary fiber: a review. *Critical Reviews in Food Science and Nutrition*, 62, 1-28. <https://doi.org/10.1080/10408398.2021.1913399>
- Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The role of polyphenols in human health and food systems: A mini-review. *Frontiers in Nutrition*, 5, 87. <https://doi.org/10.3389/fnut.2018.00087>
- Cvijanovic, N., Feinle-Bisset, C., Young, R. L., & Little, T. J. (2015). Oral and intestinal sweet and fat tasting: impact of receptor polymorphisms and dietary modulation for metabolic disease. *Nutrition Reviews*, 73(5), 318-334. <https://doi.org/10.1093/nutrit/nuu026>
- Eckhoff, S. R., & Watson, S. A. (2009). Corn and sorghum starches: Production. In J. N. Bemiller & R. L. Whistler (Eds.), *Starch: Chemistry and technology* (3rd ed., pp. 373-439). Cambridge, MA: Academic Press.
- Egan, H., Kirk, R., & Sawyer, R. (1981). The Luff School method. Sugars and preserves. *Pearson's chemical analysis of foods*, 8, 152-153.
- Gong, L., Chi, H., Wang, J., Zhang, H., & Sun, B. (2019). In vitro fermentabilities of whole wheat as compared with refined wheat in different cultivars. *Journal of Functional Foods*, 52, 505-515. <https://doi.org/10.1016/j.jff.2018.11.027>
- Huang, J., Shang, Z. Q., Man, J. M., Liu, Q. Q., Zhu, C. J., & Wei, C. X. (2015). Comparison of molecular structures and functional properties of high-amylose starches from rice transgenic line and commercial maize. *Food Hydrocolloids*, 46, 172-179. <https://doi.org/10.1016/j.foodhyd.2014.12.019>
- ISO 10520. (1997). *Determination of starch content - Ewers polarimetric method*. International standard, 1, pp. 1-8.
- Kaur, N., Singh, B., & Sharma, S. (2019). Comparison of quality protein maize (QPM) and normal maize with respect to properties of instant porridge. *LWT*, 99, 291-298. <https://doi.org/10.1016/j.lwt.2018.09.070>
- Landry, J., & Moureaux, T. (1982). Distribution and amino acid composition of protein fractions in opaque-2 maize grains. *Phytochemistry*, 21(8), 1865-1869. [https://doi.org/10.1016/0031-9422\(82\)83004-5](https://doi.org/10.1016/0031-9422(82)83004-5)
- Landry, J., & Moureaux, T. (1987). Albumins and globulins in developing maize grains. *Biochimie*, 69(6-7), 691-697. [https://doi.org/10.1016/0300-9084\(87\)90190-8](https://doi.org/10.1016/0300-9084(87)90190-8)
- Lawton, J. W. (2002). Zein: A history of processing and use. *Cereal Chemistry*, 79(1), 1-18. <https://doi.org/10.1094/CCHEM.2002.79.1.1>
- Lin, L., Guo, D., Zhao, L., Zhang, X., Wang, J., Zhang, F., & Wei, C. (2016). Comparative structure of starches from high-amylose maize inbred lines and their hybrids. *Food Hydrocolloids*, 52, 19-28. <https://doi.org/10.1016/j.foodhyd.2015.06.008>
- Menkir, A., Liu, W., White, W. S., Maziya-Dixon, B., & Rocheford, T. (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines. *Food Chemistry*, 109(3), 521-529. <https://doi.org/10.1016/j.foodchem.2008.01.002>
- Mertens, D.R. (1992). Critical conditions in determining detergent fiber. In *Proceedings of the Forage Analysis Workshop* (pp. C1-C8). Denver, Colorado. Omaha, NE: National Forage Testing Association.
- Mesarović J., Srdić, J., Mladenović Drinić, S., Dragičević, V., Simić, M., Brankov M. & Milojković-Opsenica, D. (2018). Antioxidant status of the different sweet maize hybrids under herbicide and foliar fertilizer application. *Genetika*, 50, 1023-1033. <https://doi.org/10.2298/GENSR1803023M>
- Mesarović, J., Srdić, J., Mladenović Drinić, S., Dragičević, V., Simić, M., Brankov, M. & Milojković-Opsenica, D. (2019). Evaluation of the nutritional profile of sweet maize after herbicide and foliar fertilizer

- application. *Journal of Cereal Science*, 87, 132-137. <https://doi.org/10.1016/j.jcs.2019.03.017>
- Milašinović-Šeremešić, M. S., Radosavljević, M., Srdić, J., Ž., Tomičić, Z. M., & Đuragić, O. M. (2019). Physical traits and nutritional quality of selected Serbian maize genotypes differing in kernel hardness and colour. *Food and Feed Research*, 46(1), 51-59. <https://doi.org/10.5937/FFR1901051M>
- Mladenović Drnić, S., Anđelković, V., Srdić, J., Grčić, N., Filipović, M., Kravić, N., & Nikolić, A. (2022). Genetic diversity of maize inbreds with different kernel type using snp markers. In *Book of Proceedings of XIII International Scientific Agriculture Symposium „Agrosym 2022“*, 6-9 October 2022., (pp. 154-159) Jahorina, Bosnia and Herzegovina. http://agrosym.ues.rs.ba/article/showpdf/BOOK_OF_PROCEEDINGS_2022.pdf
- Mladenović Drnić, S., Mesarović, J., Kravić, N., Srdić, J., Stevanović, M., Filipović, M., & Anđelković, V. (2019). Micronutrient variability in maize inbred lines. *AGROFOR International Journal*, 4(2), 43-50. <https://doi.org/10.7251/AGRENG1902043M>
- Mladenović Drnić, S., Vukadinović, J., Srdić, J., Milašinović-Šeremešić, M., & Anđelković, V. (2021). Effect of cooking on the content of carotenoids and tocopherols in sweet corn. *Food and Feed Research*, 48(2), 119-129. <https://doi.org/10.5937/ffr48-31960>
- Mora, M. R., & Dando, R. (2021). The sensory properties and metabolic impact of natural and synthetic sweeteners. *Comprehensive Reviews in Food Science and Food Safety*, 20(2), 1554-1583. <https://doi.org/10.1111/1541-4337.12703>
- Nikolić, V., Božinović, S., Vančetović, J., Radosavljević, M., & Žilić, S. (2020). Grain properties of yellow and red kernel maize hybrids from Serbia. *Selekcija i semenarstvo*, 26(2), 7-14. <https://doi.org/10.5937/SelSem2002007N>
- Ortiz-Martinez, M., Otero-Pappatheodorou, J. T., Serna-Saldívar, S. O., & García-Lara, S. (2017). Antioxidant activity and characterization of protein fractions and hydrolysates from normal and quality protein maize kernels. *Journal of Cereal Science*, 76, 85-91. <https://doi.org/10.1016/j.jcs.2017.05.021>
- Parris, N., Moreau, R. A., Johnston, D. B., Singh, V., & Dickey, L. C. (2006). Protein distribution in commercial wet-and dry-milled corn germ. *Journal of Agricultural and Food Chemistry*, 54(13), 4868-4872. <https://doi.org/10.1021/jf060336d>
- Pérez-Grau, L., Cortadas, J., Puigdomènech, P., & Palau, J. (1986). Accumulation and subcellular localization of glutelin-2 transcripts during maturation of maize endosperm. *FEBS Letters*, 202(1), 145-148. [https://doi.org/10.1016/0014-5793\(86\)80666-4](https://doi.org/10.1016/0014-5793(86)80666-4)
- Pravilnik. (2018). Pravilnik o kvalitetu žita, mlinskih i pekarskih proizvoda i testenina. *Službeni glasnik RS*, 68/16, 56/18.
- Radosavljević, M., Milašinović-Šeremešić, M., Terzić, D., Todorović, G., Pajić, Z., Filipović, M., Kaitović, Ž., & Mladenović-Drnić, S. (2012). Effects of hybrid on maize grain and plant carbohydrates. *Genetika*, 44(3), 649-659. <https://doi.org/10.2298/GENSR1203649R>
- Radosavljević, M., Milašinović-Šeremešić, M., Terzić, D., Jovanović, Ž., Srdić, J., & Nikolić, V. (2020). Grain chemical composition of dents, popping maize and sweet maize genotypes. *Journal on Processing and Energy in Agriculture*, 24(2), 77-80. <https://doi.org/10.5937/jpea24-28790>
- Raffan, S., & Halford, N. G. (2019). Acrylamide in food: Progress in and prospects for genetic and agronomic solutions. *Annals of Applied Biology*, 175(3), 259-281. <https://doi.org/10.1111/aab.12536>
- Serpen, A., Gökmen, V., Pellegrini, N., Fogliano, V. (2008). Direct measurement of the total antioxidant capacity of cereal products. *Journal of Cereal Science*, 48(3), 816-820. <https://doi.org/10.1016/j.jcs.2008.06.002>
- Singh, J., Metrani, R., Shivanagoudra, S. R., Jayaprakasha, G. K., & Patil, B. S. (2019). Review on bile acids: Effects of the gut microbiome, interactions with dietary fiber, and alterations in the bioaccessibility of bioactive compounds. *Journal of Agricultural and Food Chemistry*, 67(33), 9124-9138. <https://doi.org/10.1021/acs.jafc.8b07306>
- Statista. (2022). *Global corn production in 2021/2022, by country*. <https://www.statista.com/statistics/254292/global-corn-production-by-country/>
- Tollenaar, M., Ahmadzadeh, A., & Lee, E. A. (2004). Physiological basis of heterosis for grain yield in maize. *Crop Science*, 44, 2086-2094. <https://doi.org/10.2135/cropsci2004.2086>
- Wang, J. F., Geil, P. H., Kolling, D. R., & Padua, G. W. (2003). Analysis of zein by matrix-assisted laser desorption/ionization mass spectrometry. *Journal of Agricultural and Food Chemistry*, 51, 5849-5854. <https://doi.org/10.1021/jf026133g>
- Watson, S. A. (2003). Description, development, structure, and composition of the corn kernel. In P. J. White, & L. A. Johnson (Eds.), *Corn Chemistry and Technology* (pp. 69-106). St. Paul, MN: American Association of Cereal Chemists, Inc.
- Zhao, H. L., Yao, Q. I. N., Xiao, Z. Y., Qin, S. U. N., Gong, D. M., & Qiu, F. Z. (2022). Revealing the process of storage protein rebalancing in high quality protein maize by proteomic and transcriptomic. *Journal of Integrative Agriculture*. 1-16. <https://doi.org/10.1016/j.jia.2022.08.031>
- Žilić, S., Hadži-Tašković Šukalović, V., Milašinović, M., Ignjatović-Micić, D., Maksimović, M., & Semenčenko, V. (2010). Effect of micronisation on the composition and properties of the flour from white, yellow and red maize. *Food Technology and Biotechnology*, 48(2), 198-206.
- Žilić, S., Hadži-Tašković Šukalović, V., Dodig, D., Maksimović, V., Maksimović, M., & Basić, Z. (2011). Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *Journal of Cereal Science*, 54(3), 417-424. <https://doi.org/10.1016/j.jcs.2011.08.006>
- Žilić, S., Serpen, A., Akilloğlu, G., Gökmen, V., & Vančetović, J. (2012). Phenolic compounds, carotenoids, anthocyanins, and antioxidant capacity of colored maize (*Zea mays* L.) kernels. *Journal of Agricultural and Food Chemistry*, 60(5), 1224-1231. <https://doi.org/10.1021/jf204367z>
- Žilić, S., Nikolić, V., Mogol, B. A., Hamzaloğlu, A., Tas, N. G., Kocadağlı, T., Simić, M., & Gökmen, V. (2022). Acrylamide in corn-based thermally processed foods: A review. *Journal of Agricultural and Food Chemistry*, 70(14), 4165-4181. <https://doi.org/10.1021/acs.jafc.1c07249>

NUTRITIVNI SASTAV I BIOAKTIVNA SVOJSTVA INTEGRALNOG BRAŠNA DOBIJENOG OD INBRED LINIJA KUKURUZA

Valentina V. Nikolić*, Marijana Z. Simić, Slađana M. Žilić, Natalija B. Kravić, Jelena P. Vančetović, Mile D. Sečanski, Marko G. Vasić

Institut za kukuruz „Zemun Polje“, 11185 Beograd-Zemun, Slobodana Bajića 1, Srbija

Sažetak: Cilj ovog istraživanja bio je da se odrede hemijski sastav i bioaktivna svojstva integralnog brašna dobijenog od jedanaest inbred linija kukuruza u cilju identifikacije genotipova sa povećanim potencijalom za razvoj hibrida visoke nutritivne i funkcionalne vrednosti, pogodnih za proizvodnju hrane. Linije kukuruza, uključujući sedam standardnih žutih, dve QPM i dve linije za hibrid crvenog zrna, gajene su na oglednom polju Instituta za kukuruz na lokaciji Zemun Polje, Srbija. Integralno kukuruzno brašno dobijeno je mlevenjem u laboratorijskom mlinu. Procena hemijskog sastava i sadržaja pojedinih bioaktivnih jedinjenja, kao i ukupnog antioksidativnog kapaciteta, sprovedena je primenom standardnih laboratorijskih procedura. Najviši sadržaj skroba (73,73%) utvrđen je u liniji L8, dok je linija L10 imala najveći sadržaj proteina (12,82%). Među rastvorljivim proteinima, frakcija α -zeina bila je dominantna u većini linija, u rasponu od 0,92% do 3,57%. Najveći sadržaj ukupnih vlakana (NDF) utvrđen je u zrnu crvene boje Linije 9 (15,77%). Linija L8 je bila najbogatija ukupnim karotenoidima (21,08 mg β CE/g s.m.), dok je linija L7 imala najveći ukupni antioksidativni kapacitet (34,30 mmol Trolox/kg d.m.), što se može objasniti prisustvom antocijana u crvenom zrnu. Linija L1 je imala najveći sadržaj ukupnih šećera (3,36%), a linija L4 imala je najmanji (1,44%). Svi uzorci novih inbred linija ispitivani u ovoj studiji pokazali su dobre parametre kvaliteta u pogledu hemijskog sastava i bioaktivnih svojstava. Dobijeni rezultati mogu dati neke dragocene smernice potrebne u narednim fazama oplemenjivanja kukuruza, kao i otvoriti različite mogućnosti za korišćenje integralnog kukuruznog brašna u prehrambenoj industriji.

Ključne reči: kukuruz, integralno brašno, inbred linije, nutritivni sastav, bioaktivna svojstva

Received: 22 December 2022/ **Received in revised form:** 22 January 2023/ **Accepted:** 30 January 2023

Available online: February 2023



This open-access article is licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/> or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.