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STARCH COMPOSITION RELATED TO PHYSICAL TRAITS IN MAIZE KERNEL

STRUKTURA SKROBA U ODNOSU NA FIZIČKE KARAKTERISTIKE ZRNA KUKURUZA

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ABSTRACT

The focus of this study is on the physical quality traits and starch composition of various maize kernel genotypes grown in Serbia. Furthermore, the aim was to determine the relationship among these quality traits. Results obtained from the Stenvert hardness test showed great variability among the maize samples. The portion of the hard endosperm fraction (HE) ranged from 53.29% to 76.28%. Test weight (TWt) and 1000-kernel weight (KWt) of 10 different ZP maize genotypes ranged from 782.69 to 907.39 kgm^{-3} and from 128.40 to 376.50 g, respectively. The specialty maize genotypes had the highest content of amylose (27.8% and 28.9%). Yellow dent genotype, ZP 606, had the lowest amylose content in the kernel (22.3%). The results suggested that the composition of starch granule differed depending on the hardness of the endosperm. The amylose content was highly correlated with the physical traits such as TWt, KWt, density and HE.

Keywords*: maize genotypes, kernel, starch composition, physical traits.*

REZIME

Cilj ovog rada je bio da se ispitaju fizički parametri kvaliteta zrna i struktura skroba različitih genotipova zrna kukuruza koji se gaje u Srbiji. Pored toga, cilj je bio da se utvrdi odnos (korelacija) između ispitivanih karakteristika. Rezultati dobijeni testom tvrdoće po Stenvert-u pokazali su veliku varijabilnost između uzoraka. Udeo frakcije tvrdog endosperma (TE) kretao se u rasponu od 53,29% do 76,28%. Hektolitarska masa (HM) i masa 1000 zrna ili apsolutna masa (AM) 10 različitih genotipova kukuruza kretale su se od 782,69 do 907,39 kgm^{-3} i od 128,40 do 376,50 g, respektivno. Specifični genotipovi kukuruza imali su najveći sadržaj amiloze (27,8% i 28,9%). Genotip žutog zubana, ZP 606, imao je najmanji sadržaj amiloze u zrnu (22,3%). Rezultati pokazuju da se struktura skrobnih granula razlikuje u zavisnosti od tvrdoće endosperma zrna. Sadržaj amiloze je bio u visokoj korelaciji sa fizičkim parametrima kvaliteta kao što su HM, AM, gustina i TE.

Ključne reči: genotip kukuruza, zrno, struktura skroba, fizičke karakteristike.

INTRODUCTION

Based on the cultivated areas and produced quantities, maize is a major field crop in Serbia. Most of the maize production in Serbia is rainfed with variable management inputs and hybrid that range from FAO 100-800. The greatest part of maize produced in both, our country and the world, is used as a processed or unprocessed product in domestic animal feeding (*Milašinović-Šeremešić et al., 2021*). Maize kernel consists of approximately 70% of starch on average, and the carbohydrate is the main chemical component of this cereal (*Milašinović et al., 2007*). The functional properties of maize starch differ depending on the granule structure and composition, which relate to the genetic background (genotype) and cultural practices. Appearance, structure and quality of starchy foods and process parameters are affected by the ratio of amylose to amylopectin. Amylose content and amylopectin branch chain length and crystallinity remarkably affect the digestibility of maize starch. Generally, the digestive rate of starch decreases with increased amylose content and branch-chain length of amylopectin (*Jane et al., 2003*). Thus, the resurgent interest has been switched to the study of individual amylose or amylopectin rather than total starch, whereas the enzymatic machinery for amylose synthesis remains elusive (*Li et al., 2018*).

Kernel hardness and vitreousness are highly associated with physical traits and significantly correlated with technological value and end usage of maize. The parameters are strongly determined by genotype (*Radosavljević et al., 2000; Milašinović-Šeremešić et al., 2019*). Kernel vitreousness, which refers to the ratio of vitreous (hard) to floury (soft) endosperm, is a major physical trait that influences hardness, post-harvest resistance to insects and fungi, rate of starch digestibility, and semolina yield for food production (*Gayral et al., 2015*). Maize genotypes may differ in their nutritional value and technological traits (millability, fermentability, etc.) due to variations in kernel hardness and chemical composition (*Milašinović et al., 2007; Semenčenko et al., 2013*). Maize kernels with a higher ratio of vitreous-to-floury endosperm resulted in higher dry matter digestibility and lower nitrogen excretion, and consequently higher production factor in 42-day-old broilers (*Benedetti et al., 2011*). In the contemporary processing of maize grain, wet and dry milling properties of grain are mainly determined by the endosperm types (*Milašinović-Šeremešić et al., 2021*). Endosperm structure of kernel e. g. the proportion of hard and soft endosperm could have a significant impact and even crucial on the process parameters (starch, gluten, fibre and bioethanol yields). A larger amount of soft endosperm fraction enables easier starch isolation in the wet-milling process as well as decomposition of the starch granules during enzymatic hydrolysis, leading to a higher starch yield, recovery and purity, that is, bioethanol yield (*Milašinović et al., 2007; Semenčenko et al., 2013*).

The focus of this study is on the physical traits and starch composition (amylose content) of various maize kernel genotypes. Furthermore, the aim of the study was to determine the relationship of these quality parameters, as well as, their effects on the amylose content. The information gathered in this paper will be useful for the selection (maize breeding) and utilization of various maize genotypes and the development of high-grade food products. Furthermore, recognizing differences in grain quality for a specific purpose from the local food industries is crucial for the development of novel maize-based products.

MATERIAL AND METHOD

For the purpose of this study, ten maize genotypes with diverse endosperm types (dent, semi-dent, and flint) were examined under the temperate condition on the slightly calcareous chernozem soil type. Selected genotypes were developed at the Maize Research Institute, Serbia (44°52'N, 20°20'E) where the field trial took place. At full maturity, maize kernels were collected from the plants that received identical management practices through the growing season at the field trial during 2016. The experiment was set up by a random block design (RCB) with 3 replications while the size of a single experimental unit was $21m^2$ (3 m x 7 m). Sowing density has amounted to 60,000 plants per ha. For the physical and chemical quality traits, two inner rows were harvested in full waxy maturity.

Physical traits

Test weight (TWt) was assessed using the AACC method 55–10.01 (AACC International, 2017). The 1000-kernel weight was estimated counting and weighting of 4×250 of kernels which are whole, unbroken.

Kernel density was analysed weighing approximately 33 g (±0.001 g) of whole kernels. Volume determinations were done using a Beckman model 930 air-comparison pycnometer (*Ignjatović et al., 2015*). The analyses were performed in three replicates.

Stenvert-Pomeranz test was used to determine the kernel hardness by milling 20 g of maize kernels in a micro hammer-mill at 3600 rpm and 2-mm sieve (*Pomeranz et al., 1985*). Results were expressed as milling response (sec) and proportion of soft and/or hard endosperm fraction (%). The milling response represents the time (s) needed for kernel grinding until the top level of the maize sample collected in a glass cylinder (125 × 25 mm) reaches the level of 17 ml. The test was performed in three replicates.

Flotation index (FI) was evaluated by counting one hundred kernels; they were placed on a beaker containing 300 mL of $NaNO_3$ solution (1.250 g/mL); the kernels and the solution were stirred and next left 1 min standing. The number of floating kernels is counted; 0-12 (FI) floated kernels - very hard kernels, 13-37 (FI) floated kernels - hard kernels, 38-62 (FI) floated kernels - intermediate kernels hardness, 63-87 (FI) and 88-100 (FI) - soft and very soft kernels (*Preciado-Ortíza et al., 2018*).

Chemical composition

Ewers polarimetric method was used for the starch determination (ISO 10520:1997). Estimation of dry matter content in the samples was done using the standard drying method in an oven at 105°C to constant mass. Protein content was estimated as the total nitrogen by the Kjeldahl method multiplied by 6.25 (AACC 2017). A rapid colorimetric method was applied for the amylose content assessment (*McGrance et al., 1998*).

Statistical analysis

The estimation of starch, amylose and protein content was performed in two replicates, and the results were statistically analysed. Program STATISTICA series 13.3 was applied to process data statistically. The correlations between the estimated quality traits were done by the determination of Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Compositional traits of maize kernel are essential for various end-uses; feed for animals, food for humans, and raw materials for industry. Maize kernel varies in compositional traits due to genetics and numerous environmental factors. Considering the fact that starch is its major nutritional and energetic component it is very important to observe differences in maize starch structure and kernel physical traits, as well as, to find the interrelationship between these quality parameters. The data for physical and chemical quality traits of the kernel of ZP maize genotypes with different genetic backgrounds are given in Table 1. Among the genotypes, there were variations in all physical and chemical traits.

Test weight (TWt) and 1000-kernel weight (KWt) of 10 different ZP maize genotypes ranged from 782.69 to 907.39 kgm^{-3} and from 128.40 to 376.50 g, respectively. The density, as an important quality trait of the kernel, ranged from 1.27 to 1.40 $g \cdot cm^{-3}$. The portion of the hard endosperm in the total milled material ranged from 53.29 to 76.28%. Great variability was found for the flotation index (FI) among the analysed samples. The parameter as an indicator of kernel hardness was below 10% in seven genotypes and below 20% in another two genotypes. In the remaining genotype FI was 42.71% (the data not presented).

Table 1. Kernel physical and chemical quality traits of various maize genotypes

	TWt	KWt	Den	FI	HE	Starch (%)	Amylose (%)	Protein (%)
Min	782.69	128.4	1.27	0	53.29	64.55	22.25	8.13
Max	907.39	376.5	1.4	42.71	76.28	68.96	28.89	11.21
SD	48.86	101.20	0.043	13.48	8.05	1.77	2.05	0.965

TWt = test weight (kgm^{-3}), KWt = 1000-kernel weight (g), Den = density (gcm^{-3}), FI = flotation index (%), HE = hard endosperm (%); Starch (%), amylose (%) and protein (%) – calculated on a dry basis; Amylose (%) – calculated on 100% of starch.

Results obtained from the Stenvert hardness test showed great variability among the maize samples. The results are in good agreement with the same trait by *Radosavljevic et al.* (2000) who found a relatively lesser proportion of hard endosperm fraction in flint maize versus dent maize kernels.

Starch is the most abundant storage carbohydrate in maize kernel. The polymer in the normal maize endosperm is approximately 25% amylose and 75% amylopectin. Starch's functional properties and applications in the food industry depend on the many properties of starch (*Alcázar-Alay and Meireles, 2015*). The amylose content determines the starch gelling and firmness, whereas the amylopectin is primarily responsible for the formation of crystalline granules and the thickening of paste (*Cornejo-Ramírez et al., 2018*). In general, high amylose improves the product texture of starch and turns it into a source of slowly digestible carbohydrate (*Raigond et al., 2019*), while low amylose corresponds to a higher peak of paste viscosity and strong resistance to retrogradation (*Van Hung et al., 2006*).

The results of the chemical composition of various maize genotypes showed differences between selected samples for all tested quality parameters. The content of starch, amylose and protein ranged from 64.6-69.0%, 22.3-28.9 and 8.1-11.2%, respectively.

The content of amylose in the starch is characteristic of the normal maize starches (approximately 25% of amylose and 75% of amylopectin). The specialty maize genotypes (popcorns) had the highest content of amylose (27.8% and 28.9%). Yellow dent genotype, ZP 606, had the lowest amylose content in the kernel (22.3%) (the data not presented).

The starch structure (amylose content) affected the kernel hardness. On the basis of correlation analysis and gained correlation coefficients (Table 2) very high dependences are noticed between the amylose content in starch and the physical traits such as TWt, KWt, Den and HE (0.912^+, -0.939^+, 0.872^+ and 0.875^+).

Table 2. Correlation matrix for kernel physical traits andamylose content for 10 maize samples

	KWt	Den	FI	HE	Amylose
TWt	-0.882^+	0.971^+	-0.654^*	0.960^+	0.912^+
KWt		-0.908^+	0.459	-0.936^+	-0.939^+
Den			-0.711^*	0.976^+	0.872^+
FI				-0.611^{**}	-0.438
HE					0.875^+

$^+$Correlation statistically significant at p<0.01 level; *Correlation statistically significant at p<0.05 level; **Correlation statistically significant at p<0.10 level; TWt = test weight (kgm^{-3}), KWt = 1000-kernel weight (g), Den = density (gcm^{-3}), FI = flotation index (%), HE = hard endosperm (%), Amylose = amylose content (%).

Protein content in maize kernel positively correlated with amylose content (0.484), and negatively correlated with starch content (-0.525) (data not presented).

On the basis of the mentioned relationships, it can be concluded that four physical quality traits, test weight, 1000-kernel weight, density and hard endosperm portion had the highest interdependence with the amylose content in starch. Maize genotypes with higher test weight, density and soft endosperm fraction, and lower 1000-kernel weight have higher amylose content in starch. Since the above parameters are indicators of kernel hardness it can be concluded the increase of hardness resulted in the increase of the amylose content in maize starch.

The results have indicated the significance of the physical parameters which are closely related to the nutritive quality and the utility value of maize kernel. The results agree with our previous findings (*Milašinović et al., 2007; and Semenčenko et al., 2013*). A recent study showed that kernel vitreousness depends more on starch-protein interactions and starch granule size and shape than on the molecular properties of starch, such as those measured through amylose content (*Kljak et al., 2018*). Small differences in kernel texture could significantly influence ruminal in situ degradability and total tract in situ–in vitro dry matter and starch digestibility (*Trináctý et al., 2016*).

Prediction of amylose content

ANOVA of FOP models exhibits the effects of the independent variables (TWt, KWt, HE and Den) on Amylose content. The analysis revealed that the linear terms of the Amylose content model (Eq. (1) was found statistically significant for most cases. The ANOVA test shows the significant effects of the independent variables on the responses (Table 3). The amylose content evaluation was mostly affected by KWt, TWt and HE in the SOP model (statistically significant at p<0.01 level), while Den was influential (p<0.10).

Table 3. ANOVA table of amylose content evaluation (sum of squares)

Term	df	Amylose
TWt	1	3.701^+
KWt	1	5.807^+
Den	1	0.284^{**}
HE	1	1.246^+
Error	5	0.327
r^2		0.991

*+Significant at p<0.01 level, **Significant at p<0.10 level, Error terms have been found statistically insignificant, df - degrees of freedom, TWt = test weight (kgm^{-3}), KWt = 1000-kernel weight (g), Den = density (gcm^{-3}), HE = hard endosperm (%), Amylose = amylose content (%).*

The FOP model had an insignificant lack of fit tests, which means that the model represented the data satisfactorily. A high coefficient of determination (r^2) is indicative that the variation was accounted for and that the data fitted satisfactorily to the proposed model. The r^2 values for observed responses were found very satisfactory and showed a good fit of the model to experimental results.

The obtained regression models for Amylose content evaluation was as follows:

Amylose content (%) =26.34+0.06xTWt-0.02xKWt-22.70xDen-0.26xHE Eq. (1)

Given that general laboratory analyses require considerable time and high cost the regression models of the amylose content can be of great practical importance due to screen quality and utility value of various maize genotypes for the food industry. In order to achieve greater reliability of the statistical models, it is necessary to continue research with a larger number of maize genotypes.

CONCLUSION

Results suggested that the composition of the starch granule i.e. the amylose content was determined by the endosperm structure. The amylose content was highly correlated with the physical traits presenting indicators of the kernel hardness. The quality parameters such as test weight, 1000-kernel weight, density and hard endosperm portion had a significant role in predicting the amylose content. The most reliable regression model for predicting the parameter is obtained through the physical traits which are easily measurable, cheap, and not time-consuming analysis.

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