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**RESEARCH ARTICLE** 



# Essential minerals and their potential bioavailability in popcorn (*Zea mays* L. subsp. *everta* (Sturtev.) Zhuk.) kernels and flakes

Vesna Dragičević<sup>1\*</sup>, Jelena Srdić<sup>1</sup>, Vojka Babić<sup>1</sup>, Jelena Vukadinović<sup>1</sup>, Milovan Stoiljković<sup>2</sup>, Snežana Jovanović<sup>1</sup>, and Milan Brankov<sup>1</sup>

<sup>1</sup> Maize Research Institute "Zemun Polje", Zemun Polje, Belgrade, Serbia.
<sup>2</sup>Vinča Institute of Nuclear Sciences, Belgrade, Serbia.
\*Corresponding author (vdragicevic@mrizp.rs)
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# ABSTRACT

Popcorn is a type of maize (*Zea mays* L. subsp. *everta* (Sturtev.) Zhuk.) characterized by its popping ability. Popcorn flakes are considered a snack, but they provide various benefits as a part of the human diet. The study sought to examine the variability of 12 popcorn hybrids in kernel yield, popping traits, essential elements, and their potential bioavailability, after microwave popping. The significant variability among genotypes in the concentration of Ca, Mg, Fe, Mn, Cu, and Zn was noticed. Microwave popping decreased phytic acid concentration in flakes of 541/1k, ZP 542/1k, ZP 657/1k, ZP 644/1k, ZP 557/1k, and ZP 686/1k (up to 11.8%), thus improving potential bioavailability of examined essential elements. The lowest molar ratio of phytic acid with essential elements in flakes of ZP 644/1k and ZP 557/1k (to 0.2 for Mg, 2.16 for Ca, 7.06 for Fe, 26.9 for Mn and 5.7 for Zn) pointed that they could be considered as a good source of Mg, Fe, and Zn, while flakes of ZP 542/1k could be a good Ca source (1.58 ratio between phytic acid and Ca). This research pointed that popcorn flakes are a rich source of highly available essential elements, and could also be successfully used for other products, such as flour, meal, etc. This implies that genotypes of popcorn maize could be introduced to the biofortification programs, reaching the significance of staple food.

Key words: Chemical composition, genotype characterization, phytic acid, popcorn quality.

# INTRODUCTION

Due to its popping ability, popcorn maize (*Zea mays* L. subsp. *everta* (Sturtev.) Zhuk.) has been gaining greater popularity over time. While the popcorn flakes have snack status in many countries worldwide, they also provide a variety of benefits as food: They are low in calories, have a low glycaemic index and at the same time they are high in dietary fibre, protein, essential minerals, vitamins, and antioxidants (Öztürk et al., 2016; Liu et al., 2023). Popcorn flakes are able to regulate excess stomach acidity and reduce feeling of hunger (Grandjean et al., 2008). Coco and Vinson (2019) and Vukadinović et al. (2024) claimed that popcorn maize and flakes are high in antioxidants, particularly bioaccessible polyphenols, promoting it as beneficial food, with the potential to be considered as a functional food. Due to its nutritional profile, popcorn flakes could be also included in the diet of malnourished children (Bathla et al., 2019).

Abebe et al. (2017) and Dada and Kutu (2022) stated that popcorn kernels could be considered as a great source of mineral elements, such as K (up to 2456 mg kg<sup>-1</sup>), Na (up to 148 mg kg<sup>-1</sup>), Mg (up to 387 mg kg<sup>-1</sup>), Ca (up to 306 mg kg<sup>-1</sup>) and P (up to 2486 mg kg<sup>-1</sup>), with lesser concentration of Fe, Mn, Zn, Cu, and Cr. The same authors pointed that the accumulation of the potentially toxic elements (Cd, As, Pb, Ni) was lower, even when popcorn maize is grown on soil fertilized from municipal solid waste compost. Moreover, the concentration of some elements increases after popping (Na, Ca, Cu, and Zn), while concentration of others (K, Mg, Cr, Mn, Fe,

Co, and Pb) decreases. However, some kernel constituents, like phytic acid, could restrain further bioavailability of mineral elements in humans and non-ruminant animals (Nkhata et al., 2018; Raboy, 2020). Thus, phytic acid concentration must be taken into account during the genotype characterization for enhanced bioavailability of mineral elements and inclusion into bio-fortification programs. Malnutrition thereby affects human health worldwide, particularly when Fe, Zn, and I were considered (Shankar, 2020), including Se, Mg, and Ca, as elements of the public health importance. From this viewpoint, molar ratio between phytic acid and mineral elements plays crucial role in genotype characterization (Raboy, 2020). Indhu et al. (2024) found high heritability and high genetic advance regarding 100 kernel weight, kernel density (mass to volume ratio), as well as concentration of phytic and inorganic P in maize kernels. Camdzija et al. (2018) found significant variability among maize genotypes in concentration of phytic and inorganic P, while the special combining ability, indicated non-additive inheritance for both traits. Nevertheless, when popcorn maize was considered, popping could reduce phytic acid concentration in flakes (Vukadinović et al., 2024), which could enhance the potential bioavailability of mineral elements. The same authors indicated that microwave popping is a safer method, due to the fact that rancidity of the oil in conventional popping was avoided. Also, greater energy efficiency over a short period of time was used in microwave ovens, with faster popping time and easier cleaning, while flakes have the lower calorie values (Mishra et al., 2014).

Cihangir and Oktem (2019), Zulkadir and İdikut (2021), Serna-Saldivar (2022) and Gopinath et al. (2024) revealed the great variability across genotypes, as well as Genotype × Environment/Location impact regarding popping traits and kernel composition. Gopinath et al. (2024) accentuated that kernel traits negatively correlated with popping traits; thus smaller (rice-type) kernels revealed better popping quality than pearl-type kernels. They also indicated that effective characterization of diverse germplasm and its incorporation is essential for efficient popcorn breeding programs.

Although popcorn traits, such as elemental composition and phytic acid concentration were well documented, there is a gap in the field between popcorn grain/flake composition and potential health benefits. Furthermore, there is a lack of information regarding ratio of phytic acid and mineral elements as a prerequisite of potential bioavailability, particularly when popcorn flakes are considered. Therefore, our study sought to examine concentration and potential bioavailability of essential elements in kernels and flakes of 12 popcorn hybrids, after microwave popping. Selection of genotypes with high potential bioavailability of essential elements could additionally promote popcorn maize as a functional food, and build background for popcorn breeders toward further enhancement of nutritional quality of popcorn genotypes.

# MATERIALS AND METHODS

#### Field trial and sample preparation

The experiments were conducted during 2021 and 2022 at the Maize Research Institute "Zemun Polje", Belgrade (44°52′ N, 20°19′ E, 81 m a.s.l.), Serbia, on a slightly calcareous Chernozem, with soil texture as silt clay loam, containing: 51.0% sand, 31.0% silt, 18.0% clay, 3.5% organic matter, 7.0 pH KCl and 7.5 pH H<sub>2</sub>O. Mineral composition revealed 68.25 mg kg<sup>-1</sup> available N, 65.00 mg kg<sup>-1</sup> available P, 105.85 mg kg<sup>-1</sup> K, 264.00 mg kg<sup>-1</sup> Mg, 2854.25 mg kg<sup>-1</sup> Ca, 10.28 mg kg<sup>-1</sup> Fe, 111.50 mg kg<sup>-1</sup> Mn, 6.23 mg kg<sup>-1</sup> Cu and 3.46 mg kg<sup>-1</sup> Zn in the 0 to 30 cm soil layer. The experiments were set up under rain-fed conditions. Preceding crop was winter wheat (*Triticum vulgare* Vill.) in both experimental years. Fertilization included incorporation of the 100 kg mono-ammonium phosphate (MAP) ha<sup>-1</sup> in the autumn in 2020 and 2021, and accordingly to the soil analysis, in spring of 2021, 250 kg urea ha<sup>-1</sup> and in spring 2022, 280 kg urea ha<sup>-1</sup> was incorporated.

Twelve popcorns (*Zea mays* L. subsp. *everta* (Sturtev.) Zhuk.) hybrids were used in the study: ZP 611k (H1), ZP 614k (H2), ZP 501k (H3), 541/1k (H4), ZP 542/1k (H5), ZP 657/1k (H6), ZP 544/1k (H7), ZP 645/1k (H8), ZP 546/1k (H9), ZP 644/1k (H10), ZP 557/1k (H11) and ZP 686/1k (H12). The elementary plot encompassed 6.2 m<sup>2</sup> with 40 plants, sown with 70 cm inter-row distance and 22 cm intra-row space, in a fully randomized block design in four replicates. Sowing was performed during the 1<sup>st</sup> week of May. Standard cultivation and cropping practices were performed in accordance with the principles of proper agricultural technology.

After harvest, kernel moisture was brought to 14% (after drying at 40 °C), and then the kernel yield (KY; t  $ha^{-1}$ ) was calculated. To determinate popcorn flakes traits, 4×100 kernels were used. Then, they were placed in a paper bag and popping was performed in a microwave oven (microwave power model 1.1 cu. Ft 1000 W;

Samsung, Suwon, South Korea) for 5 min, without any additions (oil, salt, nor sugar). Samples of the kernels and flakes were milled (laboratory mill 120, Perten, PerkinElmer, Stockholm, Sweden) (particle size < 500  $\mu$ m). The content of phytic P and essential mineral elements from popcorn kernels and flakes were determined and calculated on the dry weight (DW) basis, obtained after drying in ventilation oven (EUGE425, EU Instruments, Novo Mesto, Slovenia) to constant weight (105 °C, 4 h).

#### Meteorological conditions

Meteorological conditions during the vegetative period (May to September) in 2021 and 2022 are presented in Table 1. The both experimental years were similar with 2010 to 2020 period, regarding average temperature. Only in July 2021 average temperature was higher for 2.2 °C compared to the period 2010 to 2020. Furthermore, the precipitation amount was unequal in 2021, with 19.5, 105.5, and 38.0 mm rainfall, respectively, supplied during June, July, and August. In May and June 2022, 27.0 and 75.4 mm precipitation, respectively was delivered. Total precipitation amount for 2021 and 2022 was 252.5 and 362.7 mm, respectively.

Month	May	Jun	Jul	Aug	Sep	Sum/Average			
Year		Temperature (°C)							
2010-2020	18.2	22.6	24.5	24.7	19.5	21.9			
2021	17.9	23.8	26.7	24.3	21.9	22.9			
2022	20.2	24.3	25.6	25	18.9	22.8			
			Precipitation	amount (mm)					
2010-2020	85.6	80.4	47.4	40.7	42.8	296.9			
2021	73	19.5	105.5	38	16.5	252.5			
2022	27	75.4	66.5	66.7	127.1	362.7			

**Table 1.** Average monthly air temperature and monthly precipitation amount from April to September2021 and 2022, including average for the period 2010-2020 in Zemun Polje, Serbia.

### Determination of traits of popcorn kernels and flakes

Kernel size (KS) was expressed as a kernel number per 10 g. Popping volume (PV) was analysed according to the standard procedure metric weight volume test (MWVT), using the apparatus Cretors 2300W, official metric weight volume tester (Cretors, Wood Dale, Illinois, USA) which performs popping with oil (Srdić et al., 2017). The PV was presented as the volume of popped kernel (cm<sup>3</sup>) per weight of the fresh kernel (g) prior to popping. A standard sample of 4 × 250 g was popped and after that, the rest of un-popped kernels (UK) was determined and expressed as a percentage per weight of fresh kernel (g) prior to popping.

### Chemical analysis of popcorn kernels and flakes

Phytic P (Pphy) was determined by the method of Dragičević et al. (2011) using Wade reagent (containing 0.3 g FeCl<sub>3</sub> ×  $6H_2O$  and 3 g 5'-sulfosalicylic acid in 1 L), that develop the pink colour, upon the reaction of ferric ion and sulfosalicylic acid, and absorbance was measured on a spectrophotometer (Libra S22 UV/Vis Spectrophotometer; Biochrom, Cambridge, UK) at  $\lambda = 500$  nm.

Inductively coupled plasma optical emission spectroscopy (ICP-OES, ICP-OES, iCAP 7000 Series (dual view), Thermo Scientific, Waltham, Massachusetts, USA) was used for determination of concentration of essential mineral elements: Ca, Mg, Fe, Mn, Cu, and Zn. Samples were prepared according to the procedure described by the Association of Official Analytical Chemists (Helrich, 1990). There was no difference between kernels and flakes in the concentration of essential elements, on the dry weight basis, so their concentration was presented once for both, kernels and flakes.

#### Statistical analysis

Significant differences between treatments means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after ANOVA using two-factorial randomized complete block design,

where differences with p < 0.05 were considered significant. The molar ratio between phytic acid (Phy), Ca, Mg, Fe, Mn, Cu and Zn are presented as mean ± standard deviation (SD).

Correlation analysis (Pearson's correlation coefficients at the level of significance of 0.05) and principal component analysis (PCA) was done using MATLAB (R2011a) with PLS Toolbox software package (v.6.2.1). For the analysis, obtained data were mean-centred, auto-scaled and the singular value decomposition (SVD) algorithm was employed (95% confidence level) for Hotelling T2 limits.

## RESULTS

#### Variability of kernel yield, popping traits and chemical composition of popcorn kernels and flakes

The significant variability in all examined parameters between hybrids, years, and Hybrid × Year interaction was present for kernels and Pphy for cornflakes (Table 2). Only KY varied insignificantly at year level. The multiple differences among years were present in the concentration of Ca, Mg, and Cu in kernels.

The highest KY (Table 3), and the highest Cu concentration (Table 4) in average were obtained for H1. The kernels of H7 showed the greatest concentration Mn and the highest PV. Hybrid H3 had the highest values of KS and UK, and Pphy concentration in flakes. The Ca concentration was the highest in H5, while Mg concentration had the highest values in H11. Hybrid H10 was characterized with the highest concentrations of Fe and Zn. The highest Pphy concentration in kernels was found in H6.

**Table 2.** ANOVA for the effect of genotype, year and genotype by year interaction on kernel yield (KY), popping volume (PV), kernel size (KS), number of un-popped kernels (UK), concentration of Ca, Mg, Fe, Cu, Mn, Zn, and phytic P (Pphy), in kernels and flakes of 12 popcorn hybrids. \*Significant at 5% probability level; df: degrees of freedom; MS: mean squares.

		Replicate	Hybrid (H)	Year (Y)	Η×Υ	Error		LSD			
df	-	2	11	1	11	46	CV	(H) <sub>0.05</sub>	2021	2022	Average
Source of				MC			0/				
variation				NIS			70				
KY, t ha-1	Kernels	0.191	2.232*	0.015	0.556*	0.316	9.45	0.653	5.92	5.94	5.95
PV, cm <sup>3</sup> g <sup>-1</sup>		0.108	56.973°	21.670*	5.125*	0.137	1.00	0.430	37.96	36.45	36.99
KS, No. 10 g <sup>-1</sup>		1.556	296.196*	147.347*	21.165*	1.961	1.98	1.627	69.64	71.50	70.57
UK, %		0.413*	8.976*	0.170*	0.275*	0.149	14.69	0.565	1.67	1.46	1.566
Ca, μg g <sup>-1</sup>	Kernels/Flakes	37.427*	37152.743*	32606.262*	37548.443 <b>*</b>	24.037	2.04	7.127	155.21	323.80	239.49
Mg, μg g <sup>-1</sup>		675.375°	84022.440°	13850457.990 <sup>•</sup>	37982.179*	283.061	0.91	24.610	1301.96	2376.30	1839.1
Fe, µg g <sup>-1</sup>		6.479*	245.442*	1710.446*	175.830°	4.295	5.06	3.031	34.95	46.89	40.92
Cu, μg g <sup>-1</sup>		8.238*	274.761*	9974.264 <sup>*</sup>	264.176*	7.009	9.66	3.873	33.39	4.97	19.18
Mn, μg g <sup>-1</sup>		0.070	11.214	112.639*	6.477 <b>*</b>	1.006	8.01	1.467	11.00	14.06	12.53
Zn, μg g <sup>-1</sup>		7.669*	291.047*	5504.286*	214.977*	1.849	2.85	1.989	34.29	58.38	46.34
Pphy, mg g <sup>-1</sup>	Kernels	0.019*	0.176*	9.879*	0.128*	0.010	3.24	0.100	3.42	2.78	3.10
	Flakes	0.029*	0.406*	7.319*	0.201*	0.012	3.49	0.109	2.86	3.41	3.13

	Kernel yield	Popping volume	Kernel size	Un-popped kernels	Phytic F	(mg g <sup>-1</sup> )
Hybrid	t ha-1	cm³ g-1	No. 10 g <sup>-1</sup>	%	Kernel	Flake
H1	6.77 ± 0.14°	39.17 ± 1.18 <sup>de</sup>	65.00 ± 2.98°	1.45 ± 0.07 <sup>b</sup>	2.94 ± 0.03ª	3.30 ± 0.08e
H2	6.36 ± 0.12¢	33.92 ± 0.12 <sup>b</sup>	67.67 ± 3.10 <sup>d</sup>	3.46 ± 0.16¢	3.21 ± 0.02°	3.31 ± 0.01e
H3	6.18 ± 0.01 <sup>bc</sup>	29.00 ± 1.41ª	82.00 ± 3.76 <sup>i</sup>	4.57 ± 0.21 <sup>d</sup>	3.02 ± 0.02 <sup>ab</sup>	3.42 ± 0.08 <sup>f</sup>
H4	5.69 ± 0.09 <sup>b</sup>	38.00 ± 0.00 <sup>de</sup>	73.00 ± 3.07 <sup>f</sup>	1.17 ± 0.05 <sup>ab</sup>	3.09 ± 0.03 <sup>bc</sup>	3.03 ± 0.04°
H5	5.72 ± 0.02 <sup>bc</sup>	36.84 ± 1.65°	62.33 ± 2.86 <sup>b</sup>	0.92 ± 0.04 <sup>ab</sup>	3.12 ± 0.03 <sup>bc</sup>	2.89 ± 0.04 <sup>b</sup>
H6	6.15 ± 0.80 <sup>bc</sup>	37.25 ± 3.18 <sup>cd</sup>	60.00 ± 2.52ª	1.17 ± 0.05 <sup>ab</sup>	3.50 ± 0.04 <sup>d</sup>	3.10 ± 0.04 <sup>cd</sup>
H7	5.72 ± 0.03 <sup>bc</sup>	40.09 ± 0.12 <sup>f</sup>	77.33 ± 3.55≋	0.98 ± 0.04 <sup>ab</sup>	3.05 ± 0.02 <sup>b</sup>	3.46 ± 0.02 <sup>f</sup>
H8	5.17 ± 0.24 <sup>ab</sup>	39.00 ± 1.41 <sup>d</sup>	78.33 ± 3.29 <sup>h</sup>	0.71 ± 0.03ª	3.02 ± 0.01 <sup>ab</sup>	3.18 ± 0.04 <sup>d</sup>
H9	5.35 ± 0.05ªb	39.50 ± 3.54♭	70.33 ± 3.23e	0.73 ± 0.03ª	3.14 ± 0.01 <sup>bc</sup>	3.31 ± 0.04e
H10	4.99 ± 0.19ª	36.84 ± 1.18°	76.00 ± 3.49≋	1.03 ± 0.05 <sup>ab</sup>	3.01 ± 0.01 <sup>ab</sup>	2.78 ± 0.01ª
H11	6.69 ± 0.65℃	37.59 ± 1.29₫	72.67 ± 3.05 <sup>f</sup>	0.72 ± 0.03ª	3.15 ± 0.02℃	2.95 ± 0.05 <sup>bc</sup>
H12	6.38 ± 0.12°	39.25 ± 0.35 <sup>de</sup>	73.33 ± 3.08 <sup>f</sup>	0.66 ± 0.03ª	2.96 ± 0.02ªb	2.91 ± 0.03 <sup>b</sup>

**Table 3.** Average values for kernel yield, popping volume, kernel size, number of un-popped kernels, and phytic P concentration in kernels and flakes of 12 popcorn hybrids. Means followed by different letter are significantly different based on Fisher's least significant difference test at  $\alpha = 0.05$  level.

**Table 4.** Average values for Ca, Mg, Fe, Cu, Mn and Zn, in kernels/flakes of 12 popcorn hybrids. Means followed by different letter are significantly different based on Fisher's least significant difference test at  $\alpha = 0.05$  level.

Hybrid	Са	Mg	Fe	Cu	Mn	Zn
				—— µg g <sup>-1</sup> ———		
H1	275.0 ± 9.9 <sup>g</sup>	1761.0 ± 52.8°	33.14 ± 0.67 <sup>ab</sup>	37.32 ± 2.95°	$12.88 \pm 0.01^{bc}$	36.45 ± 1.94ª
H2	329.7 ± 21.1 <sup>i</sup>	1866.6 ± 62.2°	38.34 ± 1.04°	18.72 ± 1.22°	$12.77 \pm 1.02^{bc}$	43.73 ± 0.87°
H3	271.8 ± 13.7 <sup>g</sup>	1864.0 ± 65.8°	44.80 ± 0.74°	$26.91 \pm 1.71^{dcd}$	12.38 ± 1.61 <sup>bc</sup>	$57.91 \pm 1.14^{f}$
H4	316.1 ± 11.5 <sup>h</sup>	1822.4 ± 54.9 <sup>d</sup>	46.07 ± 0.26°	26.59 ± 1.96°	13.24 ± 0.71°	41.58 ± 0.82 <sup>b</sup>
H5	$425.2 \pm 24.1^{j}$	1751.9 ± 42.8°	31.03 ± 0.86ª	19.55 ± 1.36°	10.72 ± 0.48 <sup>ab</sup>	54.48 ± 0.88°
H6	162.8 ± 1.3 <sup>d</sup>	1879.3 ± 58.0°	36.76 ± 0.68 <sup>bc</sup>	21.86 ± 1.49°	13.64 ± 0.89°	48.19 ± 0.21 <sup>d</sup>
H7	151.3 ± 1.3°	1924.9 ± 62.8 <sup>f</sup>	44.67 ± 0.60 <sup>d</sup>	19.32 ± 1.28 <sup>b</sup>	15.62 ± 0.56 <sup>d</sup>	41.47 ± 1.50 <sup>b</sup>
H8	138.0 ± 0.9 <sup>b</sup>	1649.7 ± 68.1 <sup>b</sup>	$36.15 \pm 0.80^{bc}$	14.32 ± 0.94 <sup>b</sup>	11.00 ± 1.82ªb	33.53 ± 0.89ª
H9	201.7 ± 4.6 <sup>f</sup>	1766.6 ± 82.1°	36.83 ± 0.35 <sup>bc</sup>	13.10 ± 0.76 <sup>b</sup>	11.91 ± 2.95ªb	32.40 ± 1.14ª
H10	184.7 ± 3.8°	2068.1 ± 79.2 <sup>g</sup>	55.75 ± 0.81°	11.53 ± 0.53ª	14.61 ± 1.34°	69.15 ± 0.29 <sup>g</sup>
H11	314.3 ± 4.0 <sup>h</sup>	2094.7 ± 66.8 <sup>h</sup>	52.75 ± 0.88 <sup>e</sup>	6.79 ± 0.19 <sup>b</sup>	12.01 ± 1.33 <sup>b</sup>	49.42 ± 0.95 <sup>d</sup>
H12	103.3 ± 0.5ª	1620.4 ± 51.6ª	34.77 ± 0.86 <sup>b</sup>	14.13 ± 0.89 <sup>b</sup>	9.57 ± 1.40ª	47.71±0.17 <sup>d</sup>

# Correlation between kernel yield, popping traits, and essential elements and phytic P in popcorn kernels and flakes

According to the data present in Table 5, significantly positive correlation was observed solely between KS and Fe concentration. Negative correlation was found between KS and Pphy in kernels.

**Table 5.** Correlation between kernel yield (KY), popping volume (PV), kernel size (KS), number of un-popped kernels (UK) and examined essential elements, as well as phytic P in kernels (PphyK) and flakes (PphyFl) of 12 popcorn hybrids (Pearson correlation coefficients). \*Significant values at the level of significance of 0.05.

	Ca	Mg	Fe	Cu	Mn	Zn	PphyK	PphyFl
KY	0.269	0.035	-0.185	0.348	-0.186	-0.093	0.091	0.170
PV	-0.383	-0.228	-0.195	-0.197	0.002	-0.516	-0.088	-0.204
KS	-0.353	0.112	0.539*	-0.233	0.095	0.130	-0.592 <sup>*</sup>	0.207
UP	0.306	0.109	0.062	0.395	0.107	0.250	0.009	0.505

The PCA resulted in the four-component model (78.64% of the overall data variance), with eigenvalues 3.15 for PC1, 2.72 for PC2, 1.96 for PC3 and 1.60 for PC4, whereas axes PC1 and PC2 explained 26.30% and 22.66% of the total data variance, respectively. Mutual projections for these PCs (factor scores and loadings) are shown in Figures 1A and 1B and used for further data explanation. The PCA revealed that Mg, Fe, and Zn contributed to the first axis (PC1), with r = 0.86, r = 0.89 and r = 0.75, respectively; KY, UK, Cu, and Pphy in flakes contributed positively, while PV negatively to PC2, with r = 0.56, r = 0.85, r = 0.70, r = 0.58 and r = -0.70, respectively; Ca and Pphy in kernels contributed positively and KS negatively to PC3, with r = 0.61, r = 0.65, and r = -0.82, respectively; only Mn contributed positively to PC4, with r = 0.77. The highest variability of UK, and Cu, Pphy in flakes and lesser variability of KY were noticed for kernels of H1, H2, H8 and H9; the highest variability of PV, Ca and Phy in kernel was in H5 and H6. Greater variability of Zn, and Mg was in kernel/flakes of H11 and to a lower extent in kernels/flakes of H10. Furthermore, greater variability of KS was in H7 and Fe in H10, whereas Mn variability was recognised in H4.



**Figure 1.** Principal component analysis for kernel yield (KY), popping volume (PV), kernel size (KS), un-popped kernels (UK), Ca, Mg, Fe, Cu, Mn, Zn, and phytic P concentrations in kernels (PphyK) and flakes (PphyFl) of 12 popcorn hybrids: scores (A) and loading plot (B).

#### Ratio between phytic acid and essential elements

The molar ratio between phytic acid and essential elements revealed that the lowest values of Phy/Mg, Phy/Fe, and Phy/Zn were in the kernels and flakes of H10, whereas the lowest values of Phy/Mn molar ratio were detected in H7 kernels and H10 flakes (Table 6). The lowest Phy/Cu molar ratio was noticed in kernels and flakes of H1. At the same time, the lowest Phy/Ca was in the kernels and flakes of H5, with just as lower value obtained for Pphy/Mn in the kernels of H7 hybrid.

**Table 6.** Molar ratios between phytic acid and essential elements (Phy)/Mg, Phy/Ca, Phy/Fe, Phy/Mn and Phy/Zn in kernels and flakes of 12 popcorn hybrids. The results are mean ± standard deviation of four measurements.

	Phy/Mg	Phy/Ca	Phy/Fe	Phy/Cu	Phy/Mn	Phy/Zn				
	Kernels									
H1	0.218 ± 0.006	2.306 ± 0.015	11.60 ± 0.15	23.29 ± 0.14	29.86 ± 0.14	10.55 ± 0.16				
H2	0.225 ± 0.009	2.103 ± 0.034	10.97 ± 0.21	50.75 ± 0.33	32.92 ± 0.33	9.61 ± 0.15				
H3	0.212 ± 0.014	2.398 ± 0.134	8.82 ± 0.23	33.188 ± 0.32	31.91 ± 0.33	6.82 ± 0.14				
H4	0.221 ± 0.015	2.105 ± 0.155	8.76 ± 0.23	34.29 ± 0.52	30.47 ± 0.52	9.71 ± 0.11				
H5	0.233 ± 0.016	1.580 ± 0.150	13.13 ± 0.19	47.10 ± 0.55	38.02 ± 0.54	7.48 ± 0.17				
H6	0.243 ± 0.016	4.631 ± 0.063	$12.44 \pm 0.14$	47.28 ± 0.47	33.53 ± 0.46	9.49 ± 0.16				
H7	0.207 ± 0.023	4.345 ± 0.066	8.93 ± 0.19	46.67 ± 0.52	25.54 ± 0.52	9.62 ± 0.31				
H8	0.240 ± 0.025	4.726 ± 0.105	$10.94 \pm 0.21$	62.46 ± 0.40	35.96 ± 0.40	$11.80 \pm 0.32$				
H9	0.232 ± 0.025	3.353 ± 0.170	11.14 ± 0.22	70.75 ± 0.55	34.44 ± 0.55	12.66 ± 0.29				
H10	0.190 ± 0.027	3.514 ± 0.205	7.06 ± 0.22	77.14 ± 0.68	26.94 ± 0.69	5.69 ± 0.15				
H11	0.197 ± 0.026	2.163 ± 0.221	7.81 ± 0.26	137.14 ± 0.38	34.31 ± 0.38	8.34 ± 0.21				
H12	0.239 ± 0.007	6.188 ± 0.221	11.15 ± 0.10	62.01 ± 0.39	40.51 ± 0.39	8.13 ± 0.18				
Aver.	0.222 ± 0.017	3.284 ± 0.128	10.23 ± 0.19	57.67 ± 0.14	32.87 ± 0.44	9.16 ± 0.19				
			Flak	es						
H1	0.245 ± 0.001	2.584 ± 0.029	13.01 ± 0.15	26.12 ± 0.27	33.47 ± 0.14	11.83 ± 0.20				
H2	0.232 ± 0.002	2.163 ± 0.031	11.28 ± 0.19	52.21 ± 0.34	33.87 ± 0.25	9.89 ± 0.17				
H3	0.240 ± 0.002	2.711 ± 0.051	9.97 ± 0.16	37.53 ± 0.29	36.08 ± 0.27	7.72 ± 0.14				
H4	0.218 ± 0.002	2.068 ± 0.113	8.61 ± 0.15	33.69 ± 0.43	29.93 ± 0.34	9.53 ± 0.11				
H5	0.216 ± 0.002	1.466 ± 0.164	$12.18 \pm 0.15$	43.69 ± 0.45	35.26 ± 0.29	6.94 ± 0.17				
H6	0.216 ± 0.003	4.109 ± 0.165	11.04 ± 0.09	41.95 ± 0.61	29.75 ± 0.43	8.42 ± 0.24				
H7	0.235 ± 0.003	4.928 ± 0.069	10.13 ± 0.07	52.93 ± 0.58	28.97 ± 0.45	10.91 ± 0.21				
H8	0.252 ± 0.007	4.964 ± 0.091	11.49 ± 0.24	65.60 ± 0.64	37.77 ± 0.61	12.39 ± 0.36				
H9	0.245 ± 0.008	3.536 ± 0.121	11.75 ± 0.27	74.62 ± 0.74	36.32 ± 0.58	13.35 ± 0.38				
H10	0.176 ± 0.007	3.241 ± 0.170	6.51 ± 0.26	71.14 ± 0.53	24.85 ± 0.64	5.25 ± 0.34				
H11	$0.184 \pm 0.006$	2.027 ± 0.207	7.32 ± 0.23	128.54 ± 0.45	32.16 ± 0.74	7.82 ± 0.15				
H12	0.235 ± 0.007	6.065 ± 0.286	10.93 ± 0.25	149.99 ± 0.43	39.71 ± 0.53	7.96 ± 0.01				
Aver.	0.224 ± 0.004	3.322 ± 0.125	10.35 ± 0.18	64.83 ± 0.48	33.18 ± 0.44	9.33 ± 0.21				

# DISCUSSION

While the popcorn is mainly considered as a snack worldwide, it deserves to be an important part of human diet, due to high level of various nutrients, such as fibre, minerals, and vitamins. At the same time, it is low in calories and glycaemic index (Öztürk et al., 2016; Liu et al., 2023). Cihangir and Oktem (2019), Zulkadir and İdikut (2021), Kaplan et al. (2023), and Gopinath et al. (2024) signified that kernel yield (KY) and weight, popping volume (PV) and number of un-popped kernels (UK), as well as kernels chemical composition primarily depend on environmental conditions, including fertilization, irrigation and in lesser degree the genotype. In this research KY was primarily influenced by the genotype and year interaction, particularly precipitation amount (which was almost 100 mm lower in 2021). Gopinath et al. (2024) reported that grain yield of popcorn inbreds was 1.20-1.89 t ha<sup>-1</sup>, on average, what was few times lower to values obtained in this research (in average)

4.99-6.77 t ha<sup>-1</sup>). Also, Zulkadir and İdikut (2021) established PV of various popcorn landraces in range 8.53-23.35 cm<sup>3</sup> g<sup>-1</sup>, what was also lower to the PV achieved in this research (29.0-40.09 cm<sup>3</sup> g<sup>-1</sup>). This could be tied to the genotype yield potential and its interaction with environment, similarly to the results published by Srdić et al. (2017). In response to KY, kernel size (KS) is the main factor that determines popping volume (PV). Besides the genotype impact, PV is also significantly dependable on the environment, such as described by Kaplan et al. (2023), who achieved a gradual increase of PV in parallel to the increase of irrigation levels, from 32.76 to 34.25 cm<sup>3</sup> g<sup>-1</sup>. Nevertheless, their values are mainly lower than PV obtained in this research (PV was in the range 29.00-40.09 cm<sup>3</sup> g<sup>-1</sup>). It was confirmed that KY and PV are adversely correlated traits (Srdić et al., 2017; Castro et al., 2022), so high yield genotypes are mainly not preferred as ones with high PV and sensory characteristics.

Although the chemical composition and potential bioavailability of essential elements is well studied in standard maize genotypes (mainly dents and semi-dents) (Dragicevic et al., 2016), there is a lack of information on chemical composition and particularly bioavailability of essential elements from popcorn kernels and especially popcorn flakes. When chemical composition of kernels and flakes was considered, higher variability between years and genotypes were obtained. Greater concentrations of Cu and phytic P (Pphy) were obtained in kernels during 2021 (the experimental year with high temperature during July when anthesis and grain filling occurred), proving that accumulation of mineral elements was greater when heat stress was present (Chukwudi et al., 2022). A particularly eye-catching fact is that H1, as the genotype with the highest KY, was also the lowest in KS and Pphy in kernels. However, H3, genotype with the highest KS, had paradoxically the highest UK. The connection between kernel traits and examined essential elements indicated that greater KS was reversely connected to the Pphy in it and also the greater Fe accumulation potential. This could be of particular importance from the bio-fortification standpoint, when choosing genotypes which are lower in Pphy and thus have enhanced bioavailability of essential elements, especially Fe. Correspondingly, Zulkadir and İdikut (2021) reported a connection between some popping and quality traits, in the experiment with 35 local popcorn genotypes, indicating correspondingly the high influence of genotype. Contrary, Indhu et al. (2024) described a negative connection between seed density (mass to volume ratio) and phytic acid concentration in maize, emphasizing this trait as the preliminary selection tool for population's characterization. While great variability in the concentration of Pphy in kernels of maize genotypes is confirmed (Camdzija et al., 2018), in this research genotype influence was particularly underlined in the variability of Pphy concentration toward its decrease in flakes of H4, H5, H6, H10, H11, and H12; supporting results of Vukadinović et al. (2024) that microwave popping can reduce phytic acid concentration in popcorn flakes.

Popcorn kernels and flakes can represent a great source of mineral elements in diet (Abebe et al. 2017; Serna-Saldivar, 2022). Significant variation across genotypes in concentration of essential elements in kernels was noticed, providing great basis for breeding and development of popcorn genotypes with improved nutritional profile. Hybrid H10 was high in Fe and Zn concentration, while the highest Ca concentration was in H5 kernels, Mg concentration in H11 kernels, Cu concentration in H3 kernels and Mn concentration in H7 kernels, pointing that each of them could be considered as highly efficient genotype toward acquisition of mineral elements. Abebe et al. (2017) found that the concentration of some elements could be increased in flakes after popping (such as Na, Ca, Cu, and Zn), preliminary research indicated that after drying of kernels and flakes at 105 °C before chemical analysis, differences between them were negligible. A greater range in variations of mineral elements concentration in maze kernels was obtained by Dragičević et al. (2013), Dragicevic et al. (2016), and Kaplan et al. (2023), considering that the environment, i.e., growing conditions play crucial role in kernel composition. This statement was supported by significant variations induced by year and Year × Genotype interaction presented in this research.

Since tested genotypes were grown at the same field (same conditions), significant variability among them, in concentration of mineral elements could also present a good basis for bio-fortification (Dragičević et al., 2013; Dragicevic et al., 2016). Greater acquisition and accumulation of mineral elements are of particular importance when they were grown on soils relatively low in some essential elements, such as Fe and Zn. The same authors, including Raboy (2020), underlined relation of phytic acid and mineral elements (particularly Fe, Zn, Ca, Mg) as an important trait, for bioavailability for humans and non-ruminant animals, rather than solely the concentration of mineral elements. Regarding the high stability among genotypes in Pphy, the greater

concentration of examined essential elements, such as in kernels and flakes of H10, resulted in the lowest Phy/Mg, Phy/Fe and Phy/Zn molar ratio.

Comprehensive analysis based on PCA indicated that H1, H2, H8 and H9 are the hybrids with greater variability of UK, PphyFl, and Cu, good bioavailability of Ca and Cu from flakes. Gopinath et al. (2024) claimed that smaller kernels produce flakes with greater size (volume). Irrespective of the lowest KS, H6 kernels are high in Mg, Fe, and Zn, and also in Pphy, indicating their greater potential bioavailability, which could be achieved after processing, such as fermentation, or other procedures, that reduce concentration of phytic acid (Nkhata et al., 2018). It is important to underline that H11, as genotype high in Mg and also greater Mg bioavailability, could be a desirable candidate in bio-fortification programs.

Although popcorn maize is not as important as standard maize, its popularity as a valued food grows. This research provides valuable information about its uniqueness, supporting it as a functional food, which contains highly available essential elements. Thus, regarding the increasing demand for highly nutritious food/crops, new opportunities for popcorn maize growing and especially popcorn maize breeding have arisen, challenging popcorn maize to become a staple food.

# CONCLUSIONS

This study showed that microwave popping decreased phytic acid concentration in flakes of 541/1k, ZP 542/1k, ZP 657/1k, ZP 644/1k, ZP 557/1k, and ZP 686/1k, thus improving potential bioavailability of examined essential elements. Correspondingly, flakes of ZP 644/1k and ZP 557/1k could be considered as a good source of Mg, Fe, and Zn, and flakes of ZP 542/1k as a source of Ca. Since the popcorn flakes are a rich source of potentially highly available essential elements, popcorn kernels could be also used in various products, such as flour, meal, etc. with remarkable health benefits, due to its unique nutritional profile. Connection between kernel size, greater Fe accumulation and reduced phytic P concentration may represent important information when considering the breeding programs of popcorn for bio-fortification. Although popcorn maize is underrated as food, this research provided a novel dimension to its potential use as a staple, particularly as a biofortified crop.

#### Author contribution

Conceptualization: V.D., J.S. Methodology: V.D., J.S., J.V. Software: M.B., J.S. Validation: J.V., M.B., M.S. Investigation: J.S., V.D., J.V., M.S. Resources: J.S. Data curation: V.B., J.V. Writing-original draft: V.D., J.V. Writing-review & editing: M.B., M.S., S.J. Visualization: J.S., V.D., J.V., M.S. Supervision: M.S., M.B. Funding acquisition: M.S. All co-authors reviewed the final version and approved the manuscript before submission.

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