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Relationship of Physical Properties and Macronutrient Composition with Carotenoid Profile in Maize Hybrids

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Abstract: Maize hybrids with higher vitreousness contain a higher carotenoid content; however, the relationship between the carotenoid profile and the physical and chemical properties related to vitreousness has not been investigated. The aim of this study was to investigate the relationship among the physical properties (kernel size, hardness, density and bulk density), macronutrient composition (crude protein and fat, starch, amylose, amylopectin and zein) and carotenoid profile (individual, total, α- and β-branch carotenoids and xanthophylls) in the grain of 15 maize hybrids. The tested hybrids displayed high variability for most analyzed traits. Three hybrids were characterized by the predominance of β-branch over α-branch carotenoids, while others showed a more uniform content of both fractions. The kernel hardness was associated with the bulk density, flotation index, kernel sphericity, crude protein and zein content. Hybrids with a higher kernel hardness and associated traits had a higher content of zeaxanthin and other β-branch carotenoids, as well as the total carotenoids. In contrast, lutein and α-branch carotenoids were related to the crude protein and amylopectin content only. The findings of the present study confirmed that kernel hardness is associated with β-branch carotenoids and provided further insight into the relationship between the carotenoid profile and commonly analyzed grain quality properties in maize hybrids. The production of higher quality maize hybrids implies a higher nutritional value of the grain due to the higher carotenoid content.

Keywords: maize; hybrid; carotenoids; hardness; bulk density; amylose; zein

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1. Introduction

Yellow maize (*Zea mays* L.) is one of the most widespread cereals in the world. It is used in various ways as food, feed and biofuel, and in many industrial and commercial products such as flour, cornmeal, grits, starch, snacks, tortillas and breakfast cereals. In domestic animal nutrition, maize is used as a primary source of energy [1,2]. In addition to its macronutrient composition, maize contributes to the diet with a variety of phytochemicals, including carotenoids, which provide desirable health benefits for humans and animals due to their antioxidant and provitamin A properties [3]. For this reason, maize is part of the staple diet of millions of people in Latin America, Asia and Africa [1]. When used as animal feed, the carotenoids from maize improve the production performance and health of animals [4], but are also deposited in animal products [5,6], which in turn serve as an important source of carotenoids in the human diet.

Yellow maize exhibits considerable natural variation in grain carotenoids (14.48–32.61 g/kg dry matter (DM)), characterized by higher concentrations of xanthophylls (lutein and zeaxanthin) compared to provitamin A carotenoids (α-cryptoxanthin, β-cryptoxanthin and β-carotene) [7,8]. Within the maize kernel, approximately 95–97% of the carotenoids are found in the endosperm, while the remaining carotenoids are distributed between the germ (2–4%) and the pericarp (1%) [9]. Maize hybrids differ in grain vitreousness, which is genetically determined and is expressed as a ratio of the vitreous endosperm, located in the outer part of the kernel, and the floury endosperm, located in the center of the kernel, with more vitreous samples being harder than less vitreous ones [10,11]. The hardness distinction between two types of endosperm has been linked to the textural and chemical properties of maize kernels [10,12], while more recent findings showed that genotypes with different kernel hardness have the ability to store specific carotenoids [8,13,14].

The carotenoid biosynthetic pathway is divided into two branches (the α- and the β-branch), with lutein and α-cryptoxanthin being the major carotenoids of the α-branch in maize, while the major carotenoids of the β-branch are zeaxanthin, β-cryptoxanthin and β-carotene [15]. Maize genotypes with harder kernels have been shown to have, on average, a higher total carotenoid content and a higher concentration of β-branch carotenoids. In comparison, higher levels of α-branch carotenoids have been observed in softer kernels [8,13]. In addition, the authors of the latter study reported a positive correlation between the total carotenoid content and the kernel vitreousness, and a negative correlation with the flotation index. In agreement, another study showed a higher provitamin A content in a genotype with higher kernel bulk density [16]. Previous studies have shown that the textural properties associated with kernel hardness are related to the physical and chemical composition [10,12]. In this context, measurements of kernel physical properties, such as size and shape, weight, density and milling resistance, can probably serve as predictors of the carotenoid composition of maize kernels.

On the other hand, the differences between endosperm types are the result of the interactions between the main storage products, i.e., proteins, starch and lipids [17]. The vitreousness reflects the compactness of the starch–protein matrix, with harder kernels generally associated with higher amylose, protein, zein and lipid content, and lower starch content than softer ones [12,18,19]. The carotenoids in maize are predominantly found in the amyloplasts and storage lipids of the endosperm in immature kernels [14], but to our knowledge, there are few data on their relationship to other kernel chemical properties. It has been reported that harder maize hybrids can store more carotenoids [8,13], which may be related to their hydrophobic interaction with protein bodies [20]. Momany et al. [21] reported that lutein is located in the core of α-zein segments with a triple helix that stabilizes its configuration. Since a more vitreous endosperm has a greater ability to store zeins [20], the different protein composition between hard and soft kernels may affect their carotenoid profile [8,13]. Nevertheless, differences in chemical composition have been linked to the in vitro carotenoid digestibility of maize kernels [22], suggesting that the chemical composition may be related to the carotenoid profile of maize hybrids.

Previous studies have shown that different maize hybrids exhibit wide genetic variation and diversity in terms of carotenoid profile and kernel physicochemical properties [7,8,10,12,19]. Although studies have shown a relationship between the kernel hardness and the specific carotenoid profile [8,13,14], there are few data on the relationship with most physical and almost all chemical properties. In this way, it would be possible to gain insight into the carotenoid profile of maize hybrids with simpler analyses. Nevertheless, hardness and hardness-related physical properties are affected by the kernel vitreousness, which is related to the carotenoid content and the chemical composition of the maize kernels [8,17,19]. Investigating the relationship between the carotenoid profile and the physical and chemical properties of the grain could, therefore, provide valuable insights into the relationship with specific properties, thus revealing the possible function of carotenoids in the kernel structure. Therefore, the aim of the present study was to investigate the potential relationship between the physicochemical properties and the carotenoid profile in the kernels of 15 commercial maize hybrids. The physical properties determined in the present study were kernel size and shape, 1000 kernel weight and volume, bulk density, Stenvert hardness, breakage susceptibility, kernel density and flotation index, whereas chemical properties consisted of the contents of ash, crude protein, crude fat, starch, amylose, amylopectin and zein.

2. Materials and Methods

2.1. Maize Hybrids

Fifteen high-yielding yellow maize hybrids (*Zea mays* L.; Table 1) belonging to various maturity groups were used in this study. Their visual appearances are shown in Figure 1, whereas images of kernels on a light box are shown in Figure 2. In addition, the color parameters according to CIE L*a*b* [23] are listed in Table 2. The tested hybrids were selected based on physicochemical properties, carotenoid profile and in vitro carotenoid bioaccessibility from 103 commercial maize hybrids from 9 seed companies (Table 1) available on the market [22]. Selection was conducted to obtain a wide range of these properties to ensure the variability of commercial maize hybrids regardless of the seed company.

The production of maize grains was described in a previously published paper [24]. Five locations were selected for each plot, representing 5 replicates for each hybrid, and 10 ears were hand-harvested from each location after physiological maturity. The ears were dried at 40 °C to approximately 120 g/kg moisture, shelled and stored at 4 °C until analysis. Whole grains were used to analyze the physical properties, while the grains for chemical and carotenoid analysis were ground in a laboratory mill (Cyclotec 1093, Foss Tocator, Hoganas, Sweden) with a 1 and 0.3 mm sieve, respectively, immediately before analysis. The moisture content of all samples was determined by drying at 102 °C for 4 (ground grains) or 24 h (whole grains) [25].

Table 1. Tested modern maize hybrids belonging to various maturity groups.

Hybrid	Abbreviation	Seed Company	Type [1]	FAO Maturity Group	Estimated Vitreousness [26] [2]
Hybrid 1	H1	Bc Institute d.d., Zagreb, Croatia	semiflint	330	70.0 ± 0.28
Hybrid 2	H2	Agricultural Institute Osijek, Osijek, Croatia	dent	350	60.5 ± 0.35
Hybrid 3	H3	Syngenta Agro d.o.o., Zagreb, Croatia	dent	350	58.1 ± 0.40
Hybrid 4	H4	KWS Sjeme d.o.o., Osijek, Croatia	dent	390	57.4 ± 0.28
Hybrid 5	H5	Corteva Agriscience Croatia d.o.o., Zagreb, Croatia	hard dent	380	58.7 ± 0.42
Hybrid 6	H6	Corteva Agriscience Croatia d.o.o., Zagreb, Croatia	dent	400	56.1 ± 0.62
Hybrid 7	H7	Agricultural Institute Osijek, Osijek, Zagreb	dent	410	65.2 ± 0.55
Hybrid 8	H8	Bayer d.o.o., Zagreb, Croatia	dent	450	60.3 ± 0.40
Hybrid 9	H9	Bc Institute d.d., Zagreb, Croatia	dent	450	56.1 ± 0.31
Hybrid 10	H10	Bc Institute d.d., Zagreb, Croatia	hard dent	460	61.6 ± 0.24
Hybrid 11	H11	Bc Institute d.d., Zagreb, Croatia	hard dent	500	68.3 ± 0.43
Hybrid 12	H12	Bc Institute d.d., Zagreb, Croatia	hard dent	510	63.6 ± 0.32
Hybrid 13	H13	Bc Institute d.d., Zagreb, Croatia	hard dent	510	60.1 ± 0.22
Hybrid 14	H14	Corteva Agriscience Croatia d.o.o., Zagreb, Croatia	dent	570	58.3 ± 0.75
Hybrid 15	H15	Syngenta Agro d.o.o., Zagreb, Croatia	dent	580	53.9 ± 0.17

[1] From seed catalog. [2] Estimated values are expressed as average ± standard error.

Table 2. Color parameters of ground kernels of tested maize hybrids (brightness (L*), redness (a*), yellowness (b*)) (*n* = 5) [1].

Hybrid	L*	a*	b*
H1	85.46 ± 0.36	−0.28 ± 0.04	34.82 ± 0.23
H2	87.56 ± 0.18	−0.38 ± 0.11	32.53 ± 0.37
H3	89.31 ± 0.22	−0.24 ± 0.06	25.21 ± 0.28
H4	89.00 ± 0.40	−1.24 ± 0.06	29.91 ± 0.37
H5	88.80 ± 0.28	−0.67 ± 0.13	30.88 ± 0.54
H6	90.01 ± 0.45	−1.24 ± 0.11	28.37 ± 0.43
H7	87.15 ± 0.49	−0.23 ± 0.08	37.70 ± 0.38
H8	88.30 ± 0.31	−0.62 ± 0.07	32.89 ± 0.23
H9	89.08 ± 0.34	−1.44 ± 0.08	30.78 ± 0.28
H10	85.52 ± 0.28	−0.20 ± 0.07	32.59 ± 0.21
H11	85.77 ± 0.41	1.11 ± 0.16	38.73 ± 0.30
H12	85.41 ± 0.55	0.97 ± 0.15	36.38 ± 0.37
H13	88.82 ± 0.30	−1.57 ± 0.13	37.10 ± 0.50
H14	89.13 ± 0.47	−1.16 ± 0.08	30.08 ± 0.71
H15	90.59 ± 0.40	−2.10 ± 0.07	32.55 ± 0.19

[1] Color parameters are expressed as average ± standard error.

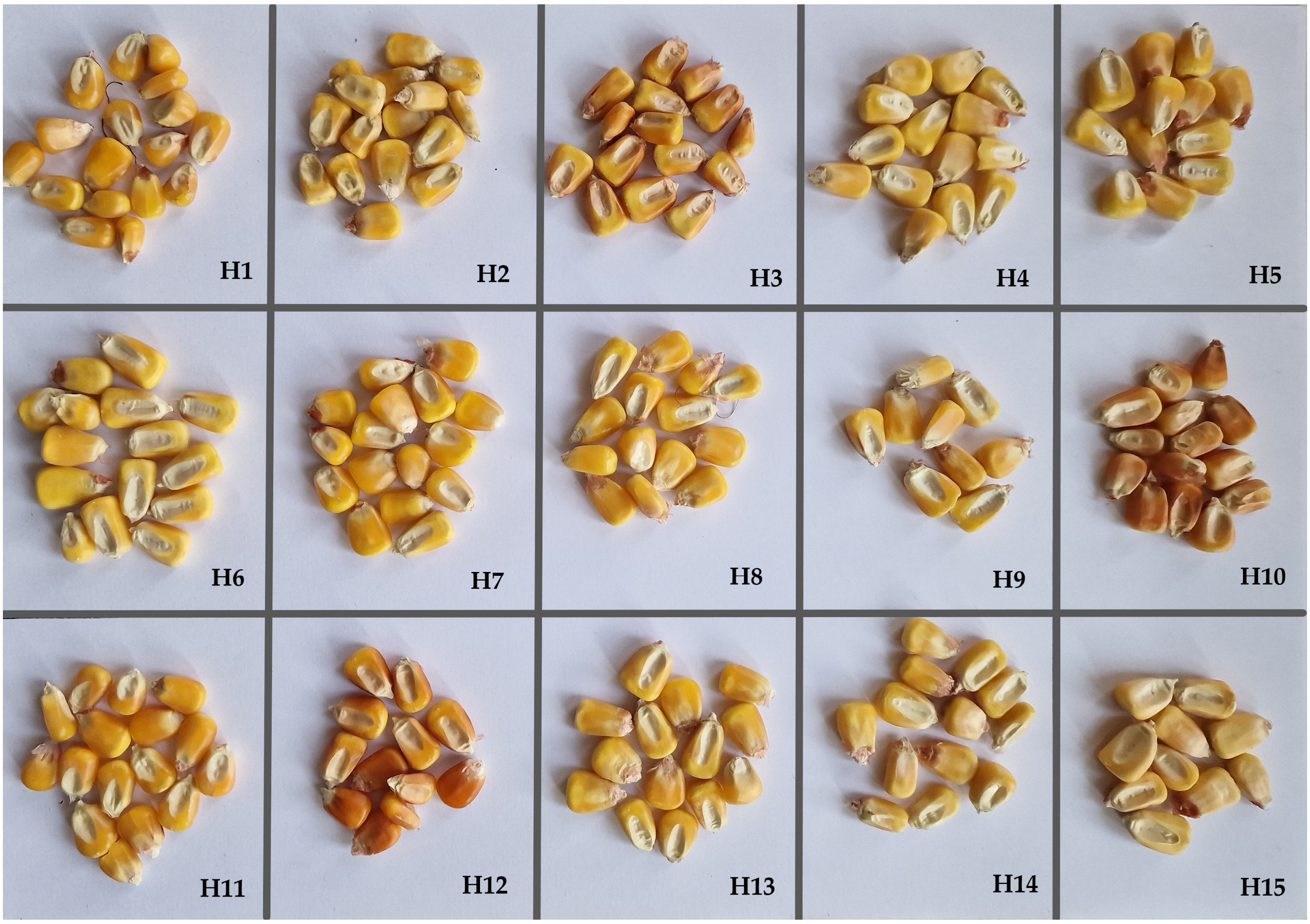

Figure 1. The visual appearance of 15 tested maize hybrids. H1–H15—the abbreviated names of the tested maize hybrids as listed in Table 1.

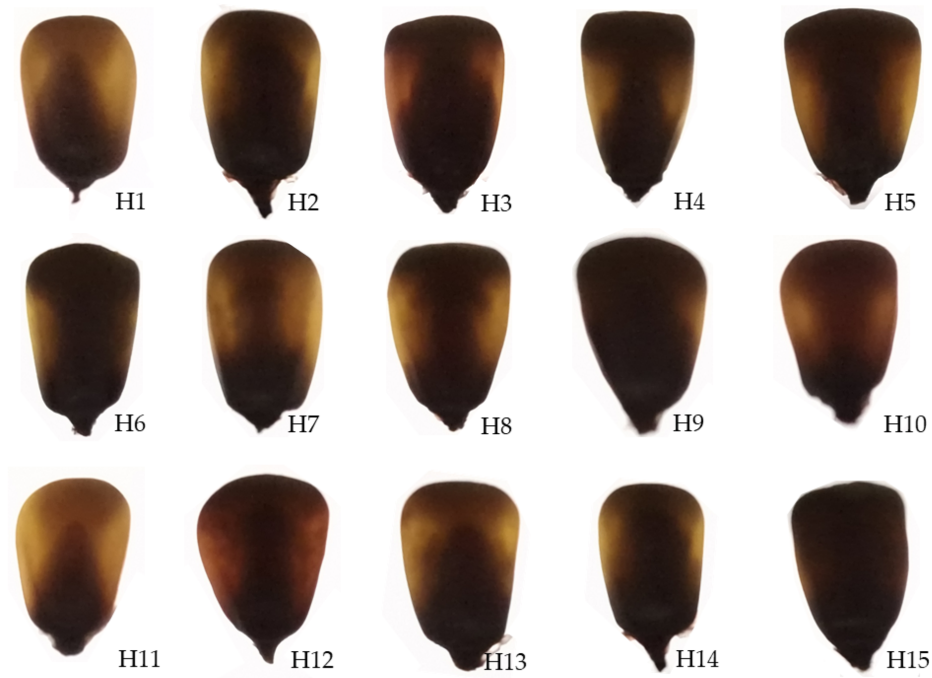

Figure 2. Kernels of tested maize hybrids on a light box. H1–H15—the abbreviated names of the tested maize hybrids as listed in Table 1.

2.2. Analyses of Physical Properties

The kernel dimensions (length, width and thickness) were measured using a digital caliper and the data were used to calculate the kernel sphericity [27]. Weight and volume of 250 g per replicate were recorded, and the test weight was calculated by dividing the weight by the volume. Kernel density was determined based on kernel weight and the volume it occupied using a pycnometer with ethanol as solvent. The flotation index was determined by stirring the maize kernels in a sodium nitrate solution with a relative density of 1.25 [11]. The breakage susceptibility was determined using the HT-I drop test apparatus, which was constructed according to the scheme described by Kim et al. [28]. Kernel hardness was determined using the Stenvert hardness test according to the method described by Pomeranz et al. [27]. The parameters of Stenvert kernel hardness were the time required to grind 17 mL of grits, the height of the grits in the grinding column and the ratio of coarse (>0.7 mm) to fine particles (<0.5 mm; C/F) in the grits; harder kernels have a longer grinding time, greater height of grits and a higher C/F. Each sample was analyzed in at least triplicate.

2.3. Analyses of Chemical Properties

The ash, crude protein (CP), crude fat (CF) and starch content of the maize samples was determined according to the standard methods. To determine ash content, the samples were ashed at 550 °C to a constant weight (ISO 5984:2022) [29]. The CP content was calculated as Kjeldahl nitrogen multiplied by a factor of 6.25 (ISO 5983-2:2009; [30]), while the CF content was determined gravimetrically using the Soxhlet extraction method (ISO 6492:1999; [31]). The total starch content was determined using a commercial enzymatic kit (Total Starch Assay Procedure, amyloglucosidase/α-amylase method, Megazyme, Ireland) according to the AOAC 996.11 method [32].

The amylose content was determined spectrophotometrically by dissolving ground maize samples with a DMSO-iodine solution. The apparent amylose content was corrected for the amylopectin content using the formula of Knutson [33]. Total zein was extracted using sodium borate buffer containing sodium dodecyl sulfate and 2-mercaptoethanol [34]. The extractions were performed three times, the supernatants were pooled and the non-zein proteins were precipitated with ethanol. The supernatant, representing the total zein fraction, was dried at 60 °C in Kjeldahl tubes and analyzed for nitrogen using the Kjeldahl method. The zein content was calculated using a conversion factor of 6.25, and the zein was expressed as content in DM and as a percentage of CP.

2.4. Analyses of Chemical Properties

Carotenoids were extracted and quantified from the maize samples according to the method described by Kurilich and Juvik [35], using β-apo-carotenal as an internal standard. Samples were sonicated (10 min; Sonorex TK 52, Bandelin, Berlin, Germany) and homogenized with ethanol containing 0.1% butylhydroxytoluene (BHT) (1 min per sample; T10 Ultra-Turaxx, IKA, Staufen, Germany). The mixtures were then saponified with 80% KOH (10 min) in a water bath at 85 °C. After cooling, the carotenoids were extracted with hexane until the upper hexane layer was colorless. The collected supernatants were evaporated (RVC 2-25CD plus, Martin Christ, Osterode am Harz, Germany) and dissolved in 200 µL of acetonitrile/methanol/methylene chloride (45:20:35, *v*/*v*/*v*) containing 0.1% BHT. Extractions were carried out under dim light, and extracts were analyzed further using HPLC on the same day.

Carotenoids were separated and quantified using the SpectraSystem HPLC instrument (Thermo Separation Products, Inc., Waltham, MA, USA) equipped with a quaternary gradient pump, an autosampler and a UV-vis detector. Carotenoids were separated on two sequentially connected C18 reversed-phase columns, Vydac 201TP54 column (5 µm, 4.6 × 150 mm; Hichrom, Reading, UK) and Zorbax RX-C18 column (5 µm, 4.6 × 150 mm; Agilent Technologies, Santa Clara, CA, USA). The separation columns were protected by a Supelguard Discovery C18 guard column (5 µm, 4 × 20 mm; Supelco, Bellefonte, PA, USA). The mobile phase consisted of acetonitrile/methanol/dichloromethane (75:25:5, *v*/*v*/*v*) containing 0.1% BHT and 0.05% triethylamine. An aliquot of 30 µL was injected, and the flow rate was 1.8 mL/min. The separations were performed at room temperature. Carotenoids were monitored on a UV-Vis detector at 450 nm.

Separated compounds were identified by comparing their retention times and quantified using external standardization with calibration curves using commercially available carotenoid standards (lutein (purity 99%), zeaxanthin (purity 99%), α- and β-cryptoxanthin (purity of both 99%) and β-carotene (purity 98%) (Extrasynthese, Genay, France); and α-carotene (purity 97%) (Supelco, Merck KGaA, Darmstadt, Germany); R2 ≥ 0.99 for all carotenoids). The total carotenoid content was calculated by summing the contents of the individual carotenoids. Each hybrid was analyzed in triplicate and the mean value was taken as the result.

The content of individual and total carotenoids in the tested hybrids was previously presented [36], and in the present study, the carotenoid profile of tested hybrids was presented as content of fractions of the following: xanthophylls (by summing the contents of lutein, zeaxanthin, α- and β-cryptoxanthin), carotenes (by summing the contents of α- and β-carotene), α-branch carotenoids (by summing the contents of lutein, α-cryptoxanthin and α-carotene) and β-branch carotenoids (by summing the contents of zeaxanthin, β-cryptoxanthin and β-carotene).

2.5. Statistical Analysis

The obtained results were analyzed using SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, USA). The experiment was conducted in a completely randomized design with five replicates. Differences between the hybrids in analyzed properties were determined using analysis of variance with hybrid as a fixed effect using the MIXED procedure. Mean values were defined using the least squares means statement and compared using the PDIFF option; letter groups were determined using the PDMIX macro procedure. The relationship between determined properties, including contents of individual and total carotenoids, was analyzed using the CORR procedure. The threshold for statistical significance was defined as $p < 0.05$.

3. Results and Discussion

The physical-chemical properties of maize kernels were determined by genotype [35,37], and although environmental conditions and agricultural practices could affect the properties [38], the maize hybrids used in the present study were grown on the same test field and using the same agricultural practices to minimize effects other than the hybrid effect.

3.1. Kernel Physical Properties

The physical properties of maize kernels have been extensively studied because they are important for industrial handling, processing and storage [39]. It has been well documented that differences in hardness between maize genotypes are closely related to chemical properties and, more recently, to carotenoid composition [8,13], which, in turn, affect the nutritional value, functionality and end use of the grains [10,12,18]. For this reason, carotenoid content could also be associated with some physical properties. The results of this study showed significant differences in the physical properties of the commercial hybrids studied (Tables 3 and 4).

Table 3. Kernel dimensions, 1000 kernel weight and bulk density of 15 tested commercial hybrids ($n = 5$).

Hybrid	Kernel Height	Kernel Length	Kernel Thickness	Sphericity	1000 Kernel Weight	1000 Kernel Volume	Bulk Density
	mm				g	mL	kg/hL
H1	11.43 i	7.87 e	5.03 ced	0.672 b	296 h	392 j	75.61 a
H2	11.54 i	7.45 f	4.74 f	0.643 def	275 i	408 i	67.45 fgh
H3	12.40 gh	8.05 de	5.05 ced	0.642 def	323 g	494 f	65.35 i
H4	13.13 bc	8.48 bc	5.09 ce	0.630 fgh	364 c	516 d	70.47 de
H5	12.85 cde	8.63 ab	4.96 ed	0.637 defg	357 cd	520 d	68.65 efg
H6	13.40 b	8.84 a	5.19 bc	0.635 efg	386 b	568 a	68.05 fgh
H7	11.70 i	8.75 a	4.90 ef	0.679 b	329 g	468 gh	70.28 de
H8	12.80 def	8.61 ab	5.28 ab	0.652 cd	391 ab	536 c	72.86 bc
H9	13.09 cd	7.99 e	5.16 bc	0.622 gh	321 g	480 g	66.80 ghi
H10	12.39 gh	8.29 cd	5.05 ced	0.648 de	352 de	500 ef	70.42 de
H11	11.65 i	8.67 ab	5.40 a	0.701 a	346 ef	460 h	75.22 a
H12	12.24 h	8.75 a	5.09 ce	0.667 bc	356 cde	480 g	74.21 ab
H13	12.55 fg	8.84 a	5.27 ab	0.666 bc	395 ab	552 b	71.52 cd
H14	12.66 efg	8.33 c	4.94 ed	0.636 defg	341 f	512 de	66.59 hi
H15	13.96 a	8.79 a	5.16 bc	0.615 h	400 a	580 a	68.89 ef
SEM	0.11	0.09	0.06	0.006	3.79	4.41	0.71
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

a–j Different letters indicate a statistically significant difference in presented physical properties between maize hybrids at $p < 0.05$. SEM—standard error of the mean.

The kernels had an average height, length and thickness of 12.52, 8.42 and 5.09 mm, respectively. The results for the determined kernel dimensions are comparable to other studies [22,38,40], in which the height of the kernels ranged between 10.56 and 15.05 mm, the length between 7.23 and 10.44 mm and the thickness between 3.51 and 5.42 mm. Smaller and rounder kernels usually belong to hybrids of higher kernel hardness [41], such that the kernel size can indirectly indicate the nutritional value of maize. Hybrid 9 showed the lowest sphericity value (0.62), while hybrid 11 showed the highest value (0.70), and this range was within the range previously reported as 0.53 to 0.77 [22,40,41]. In addition, the sphericity of the tested hybrids increased with decreasing kernel height ($r = -0.758$, $p < 0.001$) and increasing length and thickness ($r = 0.271$ and 0.352, respectively, $p < 0.05$).

Table 4. Stenvert hardness, breakage susceptibility, kernel density and flotation index of kernels of 15 tested maize hybrids (*n* = 5).

Hybrid	Time	Stenvert Height	C/F	Breakage Susceptibility	Kernel Density	Flotation Index
	s	mm		%	g/mL	%
H1	3.45 a	91.4 j	0.703 a	54.49 de	1.265 a	7.4 i
H2	2.20 ef	111.8 bc	0.502 d	50.09 f	1.155 g	99.6 a
H3	2.00 hi	112.4 abc	0.329 h	35.85 g	1.172 fg	99.6 a
H4	2.18 efg	111.2 cd	0.400 g	56.81 d	1.176 efg	87.0 bc
H5	2.09 fghi	109.2 de	0.460 f	56.68 d	1.187 ef	75.8 ef
H6	1.98 hi	112.4 abc	0.288 i	65.95 bc	1.154 g	99.4 a
H7	2.58 c	100.2 h	0.620 b	64.65 c	1.241 ab	71.0 f
H8	2.35 d	106.6 fg	0.475 ef	65.20 bc	1.216 cd	63.0 g
H9	2.06 ghi	113.8 ab	0.262 j	61.74 c	1.183 ef	78.2 de
H10	2.53 c	105.0 g	0.605 b	79.43 a	1.213 cd	63.6 g
H11	2.86 b	94.2 i	0.718 a	65.36 bc	1.263 a	9.6 i
H12	2.54 c	102.0 h	0.573 c	69.18 b	1.228 bc	39.2 h
H13	2.24 de	108.2 ef	0.495 de	75.32 a	1.197 de	60.8 g
H14	2.11 fgh	110.8 cd	0.349 h	50.41 ef	1.184 ef	91.2 b
H15	1.82 j	114.8 a	0.266 ij	77.67 a	1.178 efg	83.6 cd
SEM	0.04	0.86	0.009	1.49	0.009	2.49
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

a–j Different letters indicate a statistically significant difference in the presented physical properties between maize hybrids at $p < 0.05$. Time—time required to grind 17 mL of grits; Height—the height of the grits in the grinding column; C/F—the ratio of coarse (>0.7 mm) to fine (<0.5 mm) particles in grits from 20 g of maize grain; SEM—standard error of the mean.

The grain hardness of the hybrids was evaluated using the Stenvert test, taking into account three parameters. The time required to grind 17 mL of grits was 2.33 s on average, and the height of the ground samples varied between 91.4 and 114.8 mm. The C/F ratio showed considerable variability, ranging from 0.26 to 0.72. Regardless of this variability, the results for these three parameters were within the range of 103 commercial maize hybrids tested (1.77–3.97 s, 86–114 mm and 0.17–0.83, respectively) [22]. Bulk density is considered a simple method to indicate kernel hardness [11], and the correlations obtained in the present study (0.725, −0.755 and 0.717 for time required to grind 17 mL of grits, height of the grits in the grinding column and C/F in grits, $p < 0.001$) were in accordance with previous studies [40,42]. The breakage susceptibility ranged from 35.85 to 79.43%, with almost all hybrids showing more than 50% broken kernels when an impact energy of 0.3 J was applied. The tested hybrids were dried at 40 °C and were more susceptible to breakage when compared with other research. Kim [43] reported an average breakage susceptibility of 24.6% using the same drop tester design when grains from two hybrids were dried at 40 °C. However, this kernel property varies considerably between hybrids, as shown by the range of 22.65 to 99.76% reported for 103 commercial dent maize hybrids dried at 40 °C and tested with the same drop tester [22]. The breakage susceptibility increased with increasing kernel size (r: length—0.263, length—0.545 and thickness—0.353, $p < 0.05$) and 1000 kernel weight and volume (r = 0.591 and 0.411, respectively, $p < 0.001$). The kernel density averaged 1.20 g mL, while the tested hybrids exhibited a considerable range of flotation index (7.40 to 99.60%). The values obtained for the tested hybrids were in the range of previous studies (1.15–1.65 g/mL and 7.5–100%, respectively) [22,41]. These two parameters are indicators of kernel hardness [11], which is consistent with the results of the correlations obtained in the present study (r: 0.777 and −0.854, −0.824 and 0.865 and 0.742 and −0.787 for time required to grind 17 mL of grits, the height of the grits in the grinding column, and C/F in the grits, respectively, $p < 0.001$).

3.2. Kernel Chemical Composition

Table 5 shows the macronutrient composition of the maize hybrids tested. The ash content in the grains of the commercial hybrids tested averaged 11.09 g/kg DM, while the crude protein content varied between 81.26 (H1) and 86.69 g/kg DM (H10). The crude fat content ranged from 31.29 (H3) to 44.05 g/kg DM (H7), while the starch content averaged 698.46 g/kg DM. The obtained results were within the range reported by Zurak et al. [22] for 103 commercial maize hybrids (9.62–16.43, 71.43–110.0, 22.76–51.83 and 612–793 g/kg DM, respectively) and by Rodehutscord et al. [37] (10.9–16.1, 78.1–112, 41.7–12.3 and 660–783 g/kg DM, respectively). The tested hybrids with a higher crude protein content also had a higher crude fat content (r = 0.271, $p < 0.05$).

Table 5. Macronutrient composition of tested maize hybrids ($n = 5$).

Hybrid	Ash	Crude Protein	Crude Fat	Starch	Amylose	Amylopectin	Zein	Zein
	g/kg DM							% Crude Protein
H1	12.43 a	81.26 d	42.34 ab	662.85 h	21.63 cdef	44.66 efg	54.37 a	66.91 a
H2	11.03 cdef	83.45 bcd	37.09 efg	678.34 fgh	21.50 def	46.34 def	42.56 cdef	51.16 cde
H3	10.92 cdefg	81.85 cd	31.29 j	675.94 fgh	21.98 bcdef	45.61 ef	36.28 h	44.27 f
H4	11.74 ab	81.77 cd	35.21 gh	693.63 def	20.26 g	49.11 bcd	38.39 fgh	46.98 ef
H5	10.61 efg	81.41 d	32.46 ij	706.66 cde	22.45 abcde	48.21 bcde	37.12 gh	45.61 ef
H6	11.50 bc	81.80 cd	37.92 def	714.81 bcd	22.11 bcdef	49.36 abc	41.65 defg	50.92 cde
H7	10.97 cdefg	85.21 ab	44.05 a	747.60 a	22.70 abcd	52.06 a	46.40 bc	54.49 bcd
H8	10.67 defg	82.42 cd	34.42 hi	656.38 h	23.54 a	42.10 g	39.13 efgh	47.55 ef
H9	10.32 g	82.15 cd	40.02 cd	665.77 gh	21.11 fg	45.47 ef	40.20 efgh	48.94 def
H10	11.31 bcd	86.69 a	38.16 cdef	689.82 efg	22.02 bcdef	46.96 cdef	46.49 bc	53.63 bcd
H11	11.00 cdefg	86.68 a	40.37 bc	666.98 gh	21.23 fg	45.47 ef	49.68 b	57.36 b
H12	11.09 bcdef	83.25 bcd	38.84 cde	725.39 abc	22.75 abc	49.79 ab	45.68 bcd	54.88 bc
H13	10.90 cdefg	84.11 bc	37.76 def	735.30 ab	22.67 abcd	50.86 ab	46.28 bc	55.03 bc
H14	10.54 fg	81.38 d	37.18 efg	737.86 ab	22.85 ab	50.94 ab	43.67 cde	53.68 bcd
H15	11.30 bcde	82.62 cd	36.15 fgh	719.55 bc	21.45 efg	50.50 ab	45.27 bcd	54.78 bc
SEM	0.25	0.91	0.81	8.71	0.43	1.00	1.64	2.01
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

a–j Different letters indicate a statistically significant difference in the content of nutrients between maize hybrids at $p < 0.05$. DM—dry matter; SEM—standard error of the mean.

The crude protein and fat of the tested hybrids correlated with the Stenvert hardness parameters and the flotation index. The time required to grind 17 mL of grits and the C/F ratio in grits increased (r = 0.237 and 0.433 for crude protein and 0.542 and 0.468 for crude fat, $p < 0.05$), while the height of the grits in the grinding column and the flotation index decreased (r = −0.330 and −0.290 for crude protein and −0.509 and −0.479 for crude fat, respectively, $p < 0.05$) when the content of these two macronutrients increased. These results indicate that the tested hybrids with higher kernel hardness had higher crude protein and fat content. The relationship between kernel hardness and crude protein was consistent with the results obtained by Blandino et al. [40] in 33 samples of commercial maize genotypes. However, the significant negative relationship found between the starch content and the kernel hardness in their study was not confirmed in the present study. In contrast to the present study, Narváez-González et al. [10] found no significant correlation between the total fat content and the kernel hardness determined with a texture analyzer in 71 maize accessions from Latin America. However, the lack of this correlation could be due to the method used. On the other hand, the authors found a positive correlation between the total fat content and hard-to-soft endosperm (i.e., vitreousness).

Hybrids H4 and H8 were characterized by the lowest and highest amylose content in the kernels (20.26 and 23.54 g/kg DM, respectively), while H1 had the highest zein content (54.37 g/kg DM). The zein content in the crude protein varied between 44.27 (H3) and

66.91% (H1). The majority of hybrids tested in the present study were of dent type but showed less variation in amylose content than in zein content compared to the 103 hybrids tested in the study by Zurak et al. [22] (183.2–244.4 and 36.80–63.15 g/kg DM). These compounds are generally related to the kernel's architecture at the level from the starch granule to the endosperm and, thus, the physicochemical properties [44]. The crystallinity gradient of the starch granules is related to the amylose content, and the lower crystallinity of starch granules in a vitreous endosperm is related to the higher amylose content [45]. The starch granules are embedded in a protein matrix containing zein and the resulting structure can be densely packed, as in a vitreous endosperm, or loosely packed, as in a floury endosperm [46]. Furthermore, it is well known that the content of protein and its spatial distribution affect the texture and distribution of a vitreous endosperm in a maize kernel [47]. The content of amylose and zein is higher in a vitreous endosperm than in a floury endosperm [12,17,48,49], contributing to the differences in the structure of the two endosperms. However, Kljak et al. [19] showed that the zein content is more important for the kernel vitreousness than the amylose. Consequently, they are more important for hardness [11], which was confirmed by the correlations found between the zein content and the kernel height (r = −0.379, $p < 0.001$), sphericity (r = 0.406, $p < 0.001$), bulk density (r = 0.565, $p < 0.001$), time required to grind 17 mL of grits (r = 0.659, $p < 0.001$) and height of the ground samples in the collection column (r = −0.632, $p < 0.001$), as well as the C/F in the grits (r = 0.551, $p < 0.001$) and the flotation index (r = −0.622, $p < 0.001$) in the present study.

3.3. Carotenoid Fractions

The tested commercial hybrids showed a significant difference in the content of total xanthophylls, carotenes and α- and β-branch carotenoids (Table 6). The total xanthophyll content ranged from 16.85 (hybrid 4) to 36.90 μg/g DM (hybrid 12) and was up to 22 times higher than the carotene content within the same hybrids. It is noteworthy that the highest β-carotene content (1.78 μg/g DM) was also found in hybrid 12, while the lowest concentration was detected in hybrid 3 (0.38 μg/g DM). On average, the maize kernels contained higher levels of β- (15.98 μg/g DM) than α-branch carotenoids (10.98 μg/g DM). The concentration of α-branch carotenoids was above 10 μg/g DM in approximately half of the hybrids. In contrast, approximately 86% of the hybrids had a content of β-branch carotenoids above 10 μg/g DM. The highest α-branch carotenoid content was found in hybrid 13 (17.47 μg/g DM), while hybrid 11 had the highest β-branch carotenoid content (29.56 μg/g DM).

The predominance of xanthophylls over carotenes in maize is genetically determined, as this and earlier studies show [7,8,35,50]. However, the content of carotenes is of nutritional importance as β-carotene is most efficiently converted to retinol [51], and hybrids with a higher carotene content have a higher nutritional value for humans and animals. Consequently, hybrids H2, H5, H11 and H12 have a higher provitamin A potential than other hybrids. Since the carotenoid biosynthetic pathway is divided into two branches, the carotenoid profile of maize hybrids could have three different appearances: the predominance of the α- or the β-branch or their more uniform content. Of the hybrids tested, H1, H11 and H12 are typical examples of the predominance of β-branch carotenoids. However, a predominance of α-branch carotenoids was not found in any of the tested hybrids, and most hybrids were characterized by a similar ratio of the α- and β-branch carotenoids. These results are in agreement with the carotenoid profile for 18 genotypes investigated by Saenz et al. [8].

Table 6. Content of carotenoid fractions in tested maize hybrids (*n* = 5).

Hybrid	Xanthophylls	Carotenes	α-Branch Carotenoids	β-Branch Carotenoids
	μg/g DM			
H1	25.65 f	1.13 e	9.02 h	17.75 d
H2	31.23 c	1.70 bc	12.68 e	20.25 c
H3	18.08 i	0.38 j	10.07 g	8.39 i
H4	16.85 j	0.95 f	7.52 j	10.28 h
H5	19.40 h	1.77 ab	7.00 j	14.17 f
H6	19.43 h	0.83 g	8.19 i	12.08 g
H7	29.01 d	1.11 e	12.64 e	17.48 d
H8	25.86 ef	0.99 f	8.75 h	18.11 d
H9	24.55 g	1.07 e	11.82 f	13.81 f
H10	26.76 e	0.80 g	13.92 d	13.65 f
H11	35.06 b	1.66 c	7.15 j	29.56 a
H12	36.90 a	1.78 a	14.57 c	24.11 b
H13	31.15 c	1.33 d	17.47 a	15.02 e
H14	17.36 ij	0.51 i	8.09 i	9.78 h
H15	29.91 d	1.12 e	15.83 b	15.20 e
SEM	0.36	0.03	0.19	0.26
p	<0.001	<0.001	<0.001	<0.001

a–j Different letters indicate a statistically significant difference in the content of carotenoid fractions between maize hybrids at $p < 0.05$. DM—dry matter; SEM—standard error of the mean.

3.4. Relationship between Physicochemical Properties and Carotenoid Profile of Maize Kernels

Among the individual carotenoids, zeaxanthin and β-cryptoxanthin, and to a lesser extent β-carotene, tended to correlate most with the physical properties and macronutrients (Figure 3). The correlations with physical properties were consistent with the previously found relationship between carotenoids and kernel hardness [8,13]. Namely, a higher content of zeaxanthin and β-cryptoxanthin was found in hybrids which had a longer time required to grind 17 mL of grits (r = 0.568 and 0.481, respectively, $p < 0.001$), a lower height of ground samples in the collection column (r = −0.660 and −0.546, respectively, $p < 0.001$), higher C/F in the grits (r = 0.681 and 0.655, respectively, $p < 0.001$) and a lower flotation index (r = −0.735 and −0.543, respectively, $p < 0.001$). In addition, there were correlations with other properties indicative of kernel hardness (Section 3.1), for example, sphericity (r = 0.626 and 0.574, respectively, $p < 0.001$) and bulk density (r = 0.682 and 0.496, respectively, $p < 0.001$). Consequently, similar correlations were found between the physical properties and the β-branch carotenoid content and, to a lesser extent, xanthophylls and the total carotenoids. It should also be noted that Saenz et al. [8] investigated both flint- and dent-type maize genotypes, whereas in the present study 14 hybrids were of the dent type. This suggests that the relationship between the content of β-branch carotenoids and kernel hardness is not specific to various maize types (flint versus dent) but could also be found within the same (dent) type hybrids. Surprisingly, only for lutein and α-cryptoxanthin, and consequently for the α-branch carotenoids, a significant correlation was found for the breakage susceptibility (r = 0.470, 0.675 and 0.528, respectively, $p < 0.001$). This physical property is also related to kernel hardness, as harder kernels exhibit higher breakage susceptibility, and the extent of breakage depends on the drying temperature of the maize kernel [52].

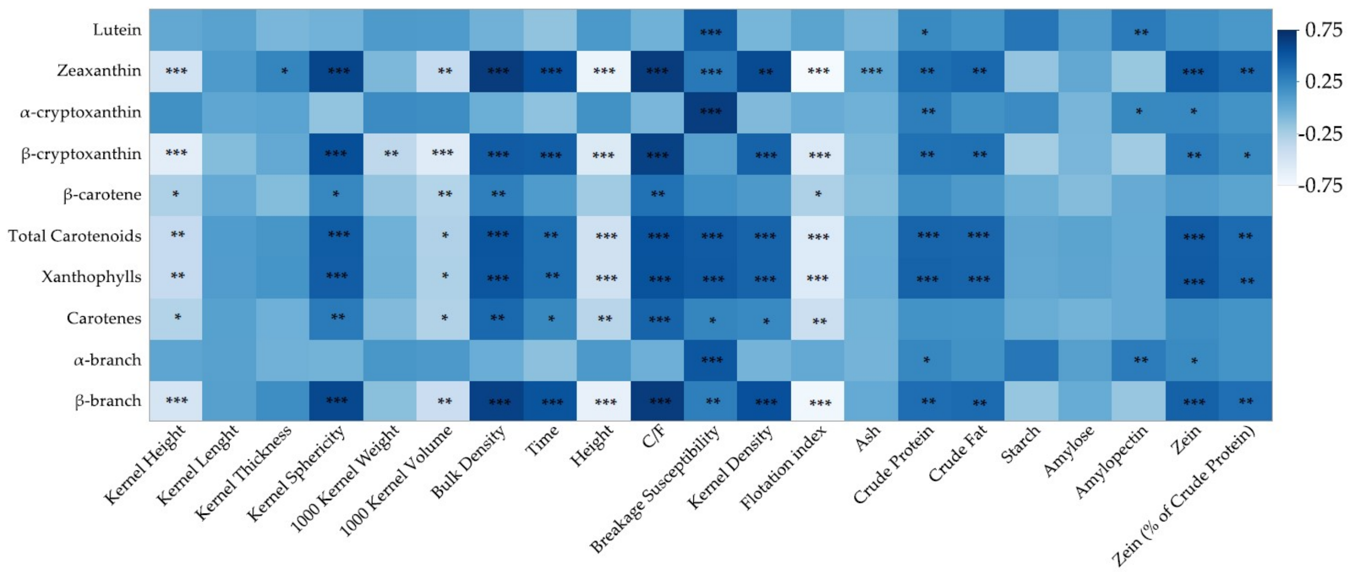

Figure 3. Correlations between physical properties, macronutrient composition and carotenoid profile in 15 tested maize hybrids. α-branch—the content of α-branch carotenoids; β-branch—the content of β-branch carotenoids; Time—time required to grind 17 mL of grits; Height—the height of the grits in the grinding column; C/F—the ratio of coarse (>0.7 mm) to fine (<0.5 mm) particles in grits from 20 g of maize grain. * p = 0.05–0.01; ** p = 0.01–0.001; *** p < 0.001.

In comparison with the physical properties, the chemical properties showed somewhat different relationships with the carotenoid profile (Figure 3). Almost all the individual carotenoids, total carotenoids and fractions were correlated with the crude protein content, which was not only observed for β-carotene and carotenes. Generally, the tested hybrids with higher crude protein content also had a higher xanthophyll and total carotenoid content (r = 0.458 and 0.445, respectively, p < 0.001). These correlations most likely reflect correlations with the zein content, as zeins are the major proteins in maize kernels [20] and are related to kernel hardness, as found in the present and previous studies [17,19,49]. Furthermore, Saenz et al. [13] suggested that carotenoids in the endosperm are distributed similarly to zeins, with the highest content in the vitreous endosperm at the periphery of the kernel and a decreasing content in the floury endosperm in the center of the kernel. Based on the correlations determined in the present study, it is possible that carotenoids contribute to the stabilization of zeins in the endosperm and, in that way, affect the physical properties of the maize grain. The zein content did not correlate with β-carotene, carotenes and, surprisingly, lutein. The latter was unexpected, as lutein is thought to be located in the core of the α-zein segments with a triple helix that stabilizes its configuration [21]. It appears that the hybrids tested in the present study, which have a higher zein content, also have a higher content of β-branch carotenoids, and these carotenoids are most likely associated with zeins [20], although the nature of this relationship is still unclear. Based on the positive correlation with crude protein and the lack of correlation with zeins, the lutein content could be related to glutelins, the proteins that account for 35% of the total nitrogen in the floury endosperm and 15% of the nitrogen in the vitreous endosperm [53]. However, this relationship should be investigated in future studies.

The amylose content did not correlate with individual carotenoids or their fractions. However, amylopectin was positively correlated with lutein, α-cryptoxanthin and α-branch carotenoids (r = 0.317, 0.238 and 0.310, respectively, p < 0.05), which was surprising. It is possible that the branched amylopectin chain could provide hydrophobic conditions for the localization of carotenoids within the starch granule. However, such correlations have not been reported previously, and the reason for this relationship should be investigated in further studies. Hybrids with a higher crude fat content also had higher contents of zeaxanthin, β-cryptoxanthin, total carotenoids, xanthophylls and β-branch carotenoids (r = 0.427, 0.383, 0.437, 0.445 and 0.416, respectively, $p < 0.001$). Although most of the crude fat of a maize kernel is in the germ, the endosperm can contain up to 1% lipids [17,54], and they are associated with the kernel vitreousness and hardness, i.e., they play an important role in starch–protein interactions that contribute to grain vitreousness [17]. Consequently, due to their polar nature, carotenoids could be related to endosperm lipids, as indirectly shown by the correlations between carotenoid and carotenoid fraction contents and hardness (Stenvert hardness test) and hardness-related physical properties (kernel density, bulk density and flotation index).

4. Conclusions

The tested hybrids differed in their physical properties, macronutrient composition and contents of xanthophylls, carotenes, and α- and β-branch carotenoids. Hybrids with a higher kernel hardness, crude protein, crude fat and zein content had higher contents of zeaxanthin, β-cryptoxanthin, total carotenoids, xanthophylls and β-branch carotenoids. However, regardless of the positive correlation between xanthophylls, total carotenoids, physical properties and nutrient composition, the contents of lutein, α-cryptoxanthin and α-branch carotenoids were only positively correlated with the crude protein content. Surprisingly, they were also correlated positively with amylopectin. The results obtained support previous findings that maize genotypes of higher hardness have a higher content of β-branch carotenoids, and it was confirmed that this relationship exists within genotypes of the same type since 14 of the tested hybrids were dent type. In addition, the results gave an insight into the relationship between the carotenoid profile and grain hardness-related properties, such as the kernel size, density, bulk density, and zein and amylose content. Specific carotenoids were associated with specific traits, leading to a separate role of α- and β-branch carotenoids and implying a different role of β-carotenoids in the hardness-associated properties of commercial maize hybrids. These hardness-associated properties also imply a higher quality of maize grain, and the production of maize hybrids with higher hardness, which can be easily determined using simple methods, implies an enhanced nutritional value for humans and animals due to the higher carotenoid content.

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