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Carotenoid Content and Bioaccessibility in Commercial Maize Hybrids

Dora Zurak [1], Darko Grbeša [1], Marija Duvnjak [1], Goran Kiš [1], Tatjana Međimurec [2] and Kristina Kljak [1,*]

[1] Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia; dzurak@agr.hr (D.Z.); dgrbesa@agr.hr (D.G.); mduvnjak@agr.hr (M.D.); kis@agr.hr (G.K.)
[2] Ministry of Agriculture, Directorate for Professional Support to the Development of Agriculture and Fisheries, Bani 110, Buzin, 10010 Zagreb, Croatia; tatjana.medimurec@mps.hr
* Correspondence: kkljak@agr.hr; Tel.: +385-1-239-4022

Abstract: The objective of the present study was to determine the content and bioaccessibility of carotenoids in 104 maize hybrids available at the market. Lutein, zeaxanthin, α- and β-cryptoxanthin, and β-carotene were determined in whole grains and micelles produced during standardized INFOGEST digestibility analysis, and their bioaccessibility was calculated as the ratio of micellar and grain carotenoids. Tested hybrids varied in total carotenoid content, with 34% having total carotenoid content in the range of 15–20 μg/g dry matter (DM) and 41% in the range of 20–25 μg/g DM. The amount of bioaccessible carotenoids increased linearly ($p < 0.05$) with increasing content in the grain, and decreased among determined carotenoidsd in the order: lutein (52%) > zeaxanthin (43%) > β-carotene (43%) > α-cryptoxanthin (27%) > β-cryptoxanthin (26%). Bioaccessibility of lutein, zeaxanthin, and β-carotene decreased with increasing content in the grain ($p < 0.05$). On average, only 43% of the total carotenoids were bioaccessible in commercial maize hybrids tested, which should be considered when formulating an animal diet.

Keywords: maize grain; lutein; zeaxanthin; β-cryptoxanthin; β-carotene; in vitro digestibility

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1. Introduction

Carotenoids in foods are recognized as compounds with a variety of important biological roles in all living systems. Since humans and animals cannot synthesize them de novo, they are considered essential components of their diet. Due to their pigmenting and antioxidant properties and their function as provitamin A precursors, carotenoids have wide application as food additives and colorants. Despite their wide occurrence in nature, most industrially used carotenoids are synthesized chemically, and only a small portion is obtained by extraction from plants or algae [1]. Nowadays, great attention is attributed to food origin, and synthetic compounds tend to be replaced with natural alternatives. In recent years, efforts have been made to improve the carotenoid content in staple foods to overcome vitamin A deficiency in areas with limited access to animal products, fruits and vegetables [2]. Cereal grains provide more than 50% of the daily requirement of calories, but they often lack in essential minerals and vitamins [3]. However, yellow maize is considered the only one with an appreciable carotenoid content (11.14 μg/g dry matter (DM); [4]).

Maize (*Zea mays L.*) is a major staple food in Latin America, Asia, and Africa, whereby in other regions of the world, it is mainly used as animal feed [5,6]. Due to their recognized value, maize hybrids are biofortified in some countries—such as Brasil, Nigeria, or India—to achieve higher carotenoid content [7]. Maize grain contains provitamin A carotenoids, mainly β-carotene and β-cryptoxanthin, although the non-provitamin A carotenoids lutein and zeaxanthin dominate. However, maize genotypes differ substantially in carotenoid profile; 64 different genotypes, including varieties of different geographical origins, commercial hybrids, lines in the selection, cornflakes, popcorn, and sweet corn, had

total carotenoid content ranging from 0.50 to 68.80 μg/g DM, with ranges 0–29.70 μg/g DM for lutein, 0.30–38.20 μg/g DM for zeaxanthin, 0–5.90 μg/g DM β-cryptoxanthin, 0–3.90 μg/g DM for α-carotene and 0–2.80 μg/g DM for β-carotene [8]. These reported variable contents, as well as low concentrations of provitamin A carotenoids in grains of different genotypes are one of the main reasons why yellow maize is often unjustifiably neglected as a source of carotenoids.

The carotenoid profile of maize genotypes represents only a potential to provide humans and animals with the carotenoids contained in the grain. To exert their biological activities, carotenoids must be released from the grain matrix, solubilized in lipid emulsion droplets, and transferred into mixed micelles, and thus become bioaccessible [9]. Bioaccessibility can be defined as the food fraction released from the matrix into the gastrointestinal tract and available for intestinal absorption/assimilation [10]. The amount of the released carotenoids depends on their physicochemical properties (such as cis vs. trans isomers), deposition form within the food matrix (localization, particle size), degree of food processing (raw vs. processed foods), interaction with other food compounds (lipids, fibers), gut health, nutritional status and genotype [11,12].

Growing concerns about food safety and the negative aspects of pigment production have increased the search for natural alternatives and thus interest in the genetic manipulation of carotenoid content in cereal grains [1,13,14]. However, numerous commercial maize hybrids are available on the market with little information on their carotenoid profiles. Many commercial hybrids contain high concentrations of carotenoids, but the lack of data on their content, release from the grain matrix, and incorporation into mixed micelles does not provide good insight into their provitamin A and pigmentation potential. The current published literature contains data on the bioaccessibility and bioavailability of carotenoids primarily from maize products and includes some, but not all maize grain carotenoids [15–17]. Although domestic animals eat minimally processed (post-harvest dried and milled or pelleted) maize grain, information on carotenoid bioaccessibility would provide more appropriate insight into pigmentation potential than carotenoid content. Therefore, the objective of this study was to determine the bioaccessibility of individual and total carotenoids from commercial maize hybrids using an in vitro method and to investigate the relationship between carotenoid content and their bioaccessibility.

2. Materials and Methods

2.1. Sample Preparation

This study was conducted on 104 commercial maize hybrids (Table 1). The maize hybrids were grown in a test field in central Croatia in 2019, with each hybrid planted on a plot 6 m wide and 50 m long. A representative sample of each hybrid was taken at harvest with a total weight of 2 kg; five subsamples were taken immediately after harvest with the maize harvester and combined into one sample. Subsequently, the samples were dried at 40 °C to a moisture content below 12%. To prevent their spoilage and loss of carotenoids, samples were packed in vacuum-sealed bags and stored at −4 °C. Immediately before analysis, the samples were brought to room temperature. Then, a portion of each sample was ground in a laboratory mill with a 1 mm sieve (Cyclotec 1093, Foss Tecator, Sweden) for in vitro digestibility, and the other portion was ground in a ball grinder (MM200, Retsch, Germany) for carotenoid analysis. The moisture content of all samples was determined by drying at 103 ± 2 °C for 4 h.

Table 1. List of tested commercial maize hybrids.

Hybrid	Hybrid	Hybrid	Hybrid
Bc Institut Agram	KWS Kapitolis	Pioneer P0164	PIO Os 522
Bc Institut Alibi	KWS Kollegas	Pioneer P0200	PIO Os 3850
Bc Institut Bc 323	KWS Kolumbaris	Pioneer P0216	PIO Posavac 36
Bc Institut Bc 344	KWS Konfites	Pioneer P0217	PIO Velimir
Bc Institut Bc 415	KWS Kashmir	Pioneer P0412	RWA ES Inventive
Bc Institut Bc 418	KWS Orlando	Pioneer P0725	RWA Ajowan
Bc Institut Bc 424	KWS KxB 8386	Pioneer P9241	RWA Inclusiv
Bc Institut Bc 525	KWS KxB 8453	Pioneer P9300	RWA Persic
Bc Institut Bc 572	KWS Smaragd	Pioneer P9363	RWA Gladiator
Bc Institut Instruktor	LG 30.3115	Pioneer P9415	RWA Glumanda
Bc Institut Kekec	LG 30.315	Pioneer P9757	RWA Ulyxxe
Bc Institut Majstor	LG 31.322	Pioneer P9889	RWA Hexagon
Bc Institut Pajdaš	LG 31.377	Pioneer P9903	RWA Tweetor
Bc Institut Tesla	LG 31.545	Pioneer P9911	RWA Urbanix
Bc Institut Thriler	LG 368/08	Pioneer P9978	Syngenta Sy Andromeda
DKC 4670	LG Shannon	PIO [1] Tomasov	Syngenta Sy Atomic
DKC 4920	MAS 34B	PIO Jablan	Syngenta Sy Bilbao
DKC 4943	MAS 48L	PIO Kulak	Syngenta Sy Carioca
DKC 5031	MAS 64P	PIO Os 3114	Syngenta Sy Chorintos
DKC 5068	NS seme 3022	PIO Os 3150	Syngenta Sy Kreon
DKC 5075	NS seme 4015	PIO Os 3450	Syngenta Sy Lucius
DKC 5093	NS seme 4051	PIO Os 378	Syngenta Sy Photon
DKC 5182	NS seme 6102	PIO Os 398	Syngenta Sy Premeo
DKC 5685	NS seme 6102	PIO Os 4014	Syngenta Sy Sandoro
DKC 5830	NS seme Haris	PIO Os 4015	Syngenta Sy Senko
KWS Balasco	P0023	Os 403	Syngenta Sy Zoan

[1] PIO—Agricultural Institute Osijek.

2.2. In Vitro Digestion Procedure

The INFOGEST in vitro procedure was used to determine the bioaccessibility of maize carotenoids [18]. Although this procedure was developed to mimic digestion in the human tract, it is the most commonly used method to determine carotenoid bioaccessibility [19–22] and was selected in the present study due to the lack of a standardized digestibility procedure for animals. The potential applicability of the method to animal studies has been tested in some aspects, e.g., Egger et al. [23] compared the INFOGEST procedure with digestion in pigs and showed that protein hydrolysis determined using the method is similar to in vivo protein digestion at the gastric and intestinal endpoints.

The method was adapted to a starch-rich matrix by adding amyloglucosidase and invertase in the small intestine phase, according to Englyst et al. [24]. Additional adaptation was made to stimulate digestive behavior in the digestive tract of poultry; maize samples were ground to pass a 1 mm screen to stimulate gridding action in the gizzard [25]. All enzymes used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA, SAD). The used enzymes were from porcine origin: α-amylase (A3716, labeled activity 10 U/mg; experimentally determined 10 U/mg), pepsin (P7000, labeled activity 599 U/mg, experimentally determined: 574 U/mg), pancreatin (P7545, labeled activity 8 × USP, experimentally determined trypsin activity 9 U/mg), invertase (I4504, labeled activity ≥ 300 U/mg), amyloglucosidase (A7095, labeled activity ≥ 2 60 U/mg) and bile salts (B8631). The amount of bile salts added to the reaction mixture of enzymes and sample was calculated on the basis that porcine bile extract contains 50% bile salts with an average molecular mass of 442 g/mol [26].

Oral (SSF), gastric (SGF) and intestinal (SIF) fluids used in the in vitro digestion procedure were prepared as described by Brodkorb et al. [18]. Briefly, 1.25 g of the maize sample was mixed with 1.25 mL of ultrapure water, and 2 mL of SSF (pH 7), 0.25 mL of α-amylase solution (1500 U/mL in ultrapure water), 12.5 µL of 0.3 M $CaCl_2$, and ultrapure water to reach 5 mL were added to stimulate oral phase. After incubation for 2 min at

37 °C with horizontal shaking, 4 mL of SGF (pH 3), 3 µL of 0.3 M $CaCl_2$ and 0.5 mL of pepsin solution (40,000 U/mL in ultrapure water) were added to the mixture to simulate the gastric phase. The pH was adjusted to 3 with 6 M HCl, and the volume of 10 mL was adjusted with ultrapure water. The mixture was incubated for 2 h at 37 °C with horizontal shaking. To stimulate the intestinal phase, 4.25 mL of SIF (pH 7), 20 µL of 0.3 M $CaCl_2$, and 2.5 mL of an enzyme mixture containing pancreatin (800 U/mL), amyloglucosidase (13 U/mL) and invertase (0.6 U/mL) were added to the mixture. The pH was adjusted to 7 with 1 M NaOH, and the volume of 20 mL was adjusted with ultrapure water. The mixture was incubated for 3 h at 37 °C with horizontal shaking. At the end of intestinal incubation, the tubes were placed on ice to stop intestinal digestion.

Bioaccessibility was defined as the proportion of carotenoids (individual and total) recovered in the micellar fraction after in vitro digestion relative to the amount of carotenoids in maize samples. The micellar fraction was defined as the fraction obtained after centrifugation to remove microcrystalline carotenoid aggregates and microbial contamination [27].

2.3. Extraction of Carotenoids from Whole Maize Grain

Carotenoids from whole maize grain were extracted according to the procedure described by Kurilich and Juvik [28], using β-apo-carotenal as internal standard. Briefly, after homogenization with ethanol, the samples were saponified with 80% KOH and incubated for 10 min at 85 °C in a water bath. The test tubes were then cooled in an ice bath with the addition of deionized water. The carotenoids were extracted with hexane, which was pipetted into a separate tube after centrifugation at 2200× *g* for 10 min (Centric 322A, Tehtnica, Slovenia). The extraction procedure was repeated until the colorless upper hexane layer. The collected supernatants were evaporated using rotary vacuum concentrator (RVC 2-25CD plus, Martin Christ, Germany) and dissolved in 200 µL acetonitrile:dichloromethane:methanol (45:20:35, *v*/*v*/*v*) containing 0.1% BHT.

2.4. Extraction of Carotenoids from Micellar Fraction

After digestion, 8 mL of digesta was centrifuged for 1 h at 3200× *g* at 4 °C, and the clear supernatant was filtered through 0.22 µm nylon membrane syringe filters; 5 mL of the filtered aqueous phase was used for extraction of the bioaccessible carotenoids. The extraction started with the addition of 5 mL of ethanol, 3 mL of hexane and 100 µL of β-apo-carotenal solution as internal standard. After mixing and centrifugation (2500× *g*, 5 min, 4 °C), the upper hexane phase was collected, and the extraction procedure was repeated until colorless upper hexane layer. The combined extracts were dried using the rotary vacuum concentrator (RVC 2-25 CD), and dissolved in 200 µL of acetonitrile:methanol:methylene chloride (45:20:35, *v*/*v*/*v*) containing 0.1% BHT.2.5. Carotenoid analysis using HPLC.

Lutein, zeaxanthin, α- and β-cryptoxanthin and β-carotene in the extracts of whole maize grain and micellar fraction were quantified according to the reversed-phase HPLC method described by Kurilich and Juvik [28]. Carotenoids were separated and quantified using a SpectraSystem HPLC instrument (Thermo Separation Products, Inc., Waltham, MA, USA) equipped with a quaternary gradient pump, an autosampler and a UV–vis detector. Compounds were separated on two sequentially connected C18 reversed-phase columns Vydac 201TP54 column (5 µm, 4.6 × 150 mm; Hichrom, Reading, UK), followed by a 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, and carotenoids were monitored at 450 nm.

Carotenoids [lutein (purity 99%), zeaxanthin (purity 99%), α- and β- cryptoxanthin (purity of both 99%), and β-carotene (purity 98%)] were identified by comparing their retention times and quantified by external standardization with calibration curves using

commercially available standards (Extrasynthese, France; $r^2 \geq 0.99$ for all carotenoids). The total carotenoid content was calculated by summing the contents of the individual carotenoids. For total carotenoid content in maize, each hybrid was analyzed in triplicate, and the mean value was taken as the result. Digestion was carried out for each hybrid in triplicate on two separate days, and the mean values of micellar carotenoids, both individual and total, were taken as a result.

2.5. Statistical Analysis

Statistical analyses of the obtained results were performed using SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, USA). Based on the total carotenoid content, hybrids were classified into five following groups: G1 (<15 μg/g DM), G2 (15–20 μg/g DM), G3 (20–25 μg/g DM), G4 (25–30 μg/g DM), and G5 (>30 μg/g DM). Differences between hybrid groups were subjected to analysis of variance using the MIXED procedure. Means were defined by the least squares means statement and compared using the PDIFF option; letter groups were determined using the PDMIX macro procedure. Contents of the whole grain and micellar carotenoids were assessed using Pearson correlation as implemented in the CORR procedure. Further relationships between carotenoid fractions were assessed using the REG procedure. The threshold for statistical significance was defined as $p < 0.05$.

3. Results and Discussion

3.1. Carotenoid Content in Tested Commercial Maize Hybrids

The commercial hybrids analyzed in the present study showed a wide range of total carotenoid content (14.48 to 32.61 μg/g DM), which provided the basis for the classification into five groups. The majority of the hybrids had total carotenoid content in the range of 15 to 25 μg/g DM (34%) and 20 to 25 μg/g DM (41%), while 15% had content in the range of 25 to 30 μg/g DM (Supplementary Table S1). A content of less than 15 μg/g DM was determined in 5% of the hybrids, and the same proportion of hybrids had contents above 30 μg/g DM. Carotenoid content in maize depends on factors such as genotype, climatic conditions and agronomic factors including nitrogen fertilization [29,30]. In total, 88–97% of the variation is due to differences between genotypes in their carotenoid content [28]. To minimize effects other than genotype, the maize hybrids investigated in the present study were grown in the same test field. Compared to the hybrids used in the present study, 24 inbred lines in the study by Tiwari et al. [31] had a similar range of total carotenoids (12.20–30.10 μg/g DM), with content in more than half of the lines analyzed (54%) in the range of G3 group from the present study.

Determined high diversity in total carotenoid content indicated high genotypic variability of tested maize hybrids for all determined carotenoids. With ranges of 6.25–16.81 μg/g DM for zeaxanthin and 6.37–10.46 μg/g DM for lutein, results were consistent with previous reports on the predominance of these carotenoids over other present in maize grain (83–84% depending on the group [32]). Commercial hybrids in the present study had a small prevalence of zeaxanthin over lutein, similarly to the hybrids in studies of Egesel et al. [29] and Halilu et al. [6] and opposite to the inbred lines in studies of Weber [33] and Thakkar and Failla [16] who reported lutein as the most abundant individual carotenoid. However, Saenz et al. [34] showed that the predominance of lutein or zeaxanthin was related to grain vitreousness; in 18 temperate commercial genotypes differing in hardness, lutein negatively while zeaxanthin positively correlated with grain vitreousness. The majority of the hybrids in the present study were dent, but their vitreousness could vary [35], implying that the majority of tested hybrids had higher vitreous over floury endosperm content in the grain. The contents of the other carotenoids were comparable with the reports for different maize genotypes by Kurillich and Juvik [27], Egesel et al. [24], Kean et al. [15], Kandianis [36], and Song et al. [37]. Among the provitamin A carotenoids, obtained amounts of β-cryptoxanthin (0.89–2.64 μg/g DM) exceeded the content of β-carotene (0.26–1.36 μg/g DM), which was consistent with other studies [28,29,38,39].

In general, the content of all carotenoids increased linearly with increasing carotenoid group ($p < 0.001$; Figure 1), indicating that the content of all individual carotenoids in commercial maize hybrids increases linearly with total carotenoid content. In addition, moderate to high correlations were found between carotenoids of the same biosynthetic pathway (r = 0.63 for lutein—α-cryptoxanthin, r = 0.71 for zeaxanthin—β-cryptoxanthin, r = 0.62 for zeaxanthin—β-carotene, r = 0.61 for β-cryptoxanthin—β-carotene, $p < 0.001$ for all), while traits in different branches showed low or no correlation. The results were in agreement with previous studies in which correlation analyses showed simultaneous increases in the contents of carotenoids produced in the same pathway branch, and this observation could be used for genetic improvement of provitamin A carotenoids in maize [6,40]. Moreover, a recent study by Wang et al. [41] showed that inbred lines containing a nonfunctional *Ven1* allele exhibited a decrease in polar and an increase in nonpolar carotenoids in the amyloplasts, which provides insight into breeding vitreous grain varieties and high vitamin A content in maize.

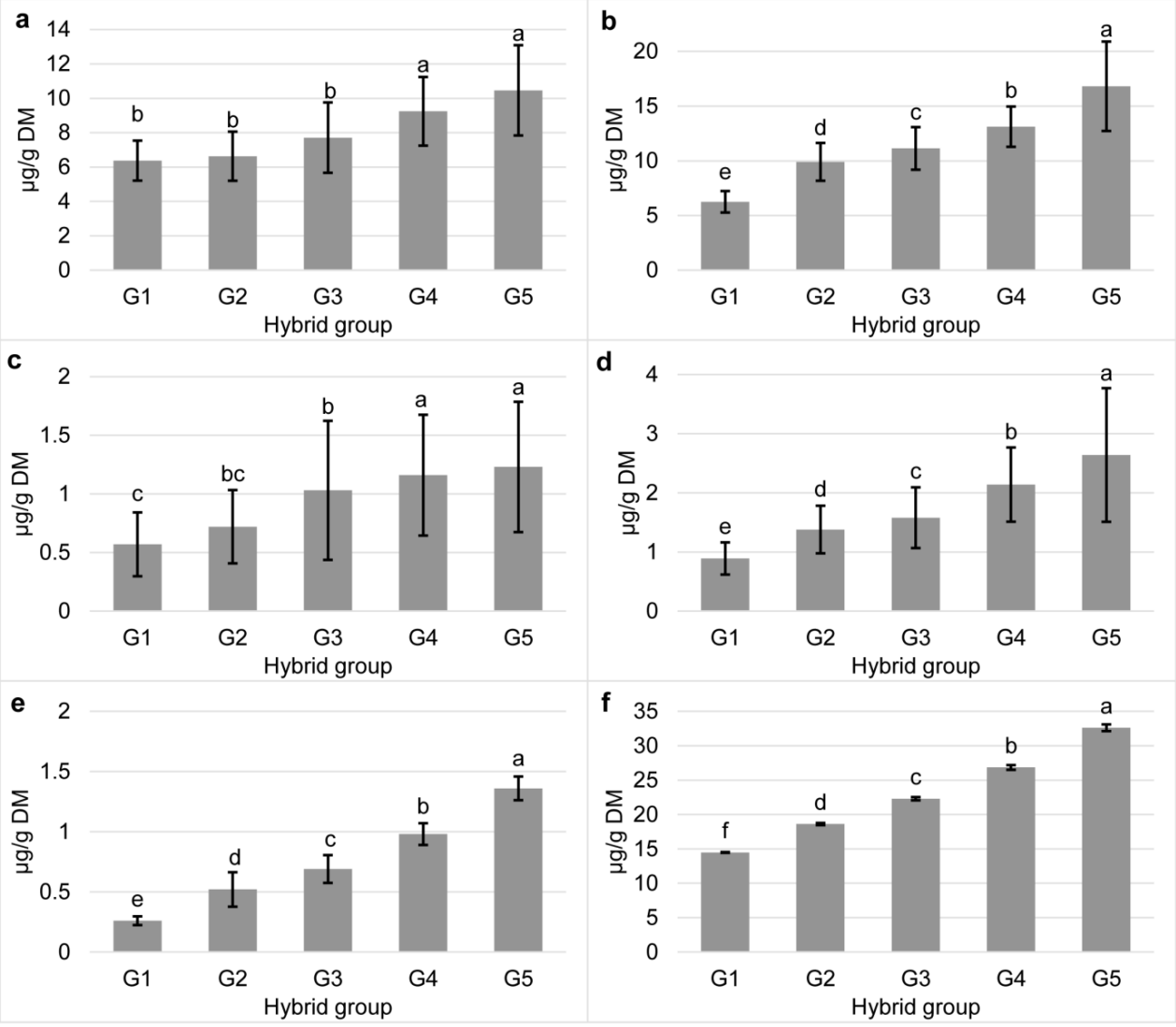

Figure 1. Content of individual (**a**), lutein; (**b**), zeaxanthin; (**c**), α-cryptoxanthin; (**d**), β-cryptoxanthin; (**e**), β-carotene) and total carotenoids (**f**) of commercial maize hybrids classified into five groups according to the total carotenoid content (G1, <15; G2, 15–20; G3, 25–25; G4, 25–30, and G5, >30 µg/g DM). Different small letters represent statistically significant differences ($p < 0.05$) between hybrid groups. Error bars represent SD.

3.2. Bioaccessibility of Carotenoids in Commercial Maize Hybrids

Micellarization efficiency is a measure of bioaccessibility and takes into account factors such as carotenoid species, food matrix, chemical state, ingested amount, and absorption modifiers [12,42]. Once carotenoids are released from the food matrix, they dissolve in the oily phase of lipid droplets, which is an important step preceding their incorporation into mixed micelles [43]. Three main factors affecting the transfer of carotenoids from emulsion lipid droplets into micelles are the type of carotenoid, pH, and concentration in bile lipids [44]. Since the above parameters were constant in this study, the micellarization efficiency of carotenoids was related to their polarity and the ratio of their concentrations in feed matrix [16,27].

In general, the amount of bioaccessible carotenoids increased with the amount of carotenoids in the maize hybrid; the correlation coefficients were 0.79 for lutein, 0.70 for zeaxanthin, 0.85 for α-cryptoxanthin, 0.78 for β-cryptoxanthin, 0.51 for β-carotene, and 0.62 for total carotenoids ($p < 0.001$, respectively). Consistent with this positive correlation, the amount of bioaccessible carotenoids increased linearly with the increasing carotenoid content in hybrid groups ($p < 0.05$). These results imply that increased carotenoid content in maize hybrid translates to increased bioaccessibility, leading to increased bioavailability when fed to animals. When the content of bioaccessible carotenoids was plotted against the content in maize grain (Figure 2), the linear regression yielded coefficients of determination between 0.26 and 0.73. Among the carotenoids detected in the tested maize hybrids, the best-fitting models were found for lutein, and α- and β-cryptoxanthin and these models were able to explain 68%, 73%, and 64% of the variability, respectively, in predicting the content of bioaccessible carotenoids from the content in the grain.

When the amount of bioaccessible carotenoids was expressed relative to the content in the grain, i.e., as bioaccessibility, the difference between groups was less pronounced, and the range of values within groups was more similar (Figure 3). Furthermore, the difference between the groups was not found for bioaccessibility of α- and β-cryptoxanthin. A proportionality was observed only for zeaxanthin, β-carotene and total carotenoids ($p < 0.05$), and this effect was opposite to the content in the maize grain or the content of bioaccessible carotenoids. This finding suggests that the bioaccessibility of those carotenoids decreased with increasing content in grain, and it is possible that competition for incorporation into micelles occurred at higher concentrations [45]. This decreasing proportionality was most pronounced for β-carotene, where the group of hybrids with <15 μg/g DM of total carotenoids had bioaccessibility of 55.9% and the group with >30 μg/g DM had 17.2%.

The average bioaccessibility of lutein, zeaxanthin, β-cryptoxanthin, and β-carotene determined in the present study exceeded the average efficiency of incorporation into synthetic micelles reported in the study by Thakkar and Faillla [16] for raw maize samples (range of 16–30%). In contrast, Dube et al. [46] reported higher average bioaccessibility of these carotenoids (62 ± 5.3%, 65 ± 4.7%, 54 ± 9.5%, and 49 ± 7.5%, respectively) for 10 maize genotypes, but the authors did not use a standardized in vitro digestibility procedure. Nonetheless, the determined broad micellarization efficiency could also be related to the maize grain matrix characteristics, particularly lipid content. Since lipids are required to incorporate carotenoids into micelles [45], the differences in lipid content among the tested maize hybrids could influence the bioaccessibility of carotenoids in the present study.

Among the carotenoids in the tested maize hybrids, the highest values of bioaccessibility were found for lutein (average 51.5%), followed by zeaxanthin (average 42.7%), β-carotene (average 34.4%), α-cryptoxanthin (average 26.5%), and β-cryptoxanthin (average 25.8%). The predominance of lutein and zeaxanthin incorporating in micelles over β-carotene agrees with previous results reported by Thakkar and Failla [16] and Dube et al. [46]. The obtained results indicate the preferential incorporation of xanthophylls in mixed micelles due to their polarity, which has been previously reported [15,16,43,47]. Due to their polar nature and location on the surface of emulsions, they can be spontaneously transferred from lipid droplets to micelles, unlike carotenes which are located in the core [43].

The bioaccessibility of β-carotene in commercial maize hybrids was higher than that of α- and β-cryptoxanthin, and this finding was surprising since β-carotene is the least polar carotenoid among those determined in the hybrids tested. Results opposite to higher bioaccessibility of β-carotene over α- and β-cryptoxanthin in the present study have been reported for raw and cooked maize [17], whole grain and degermed maize meal products [15] and maize porridge [16]. A possible reason for the high average bioaccessibility of β-carotene is its interaction with lutein. Thakkar and Failla [16] found that when the molar ratio of lutein to β-carotene is ≥7, the micellarization efficiency of β-carotene increases, which they explain by the retention of β-carotene within the core of oil droplets when lipase-mediated hydrolysis of tri-glycerides is limited, reducing the possibility of transferring hydrocarbon carotenoid into micelles. In the present study, this molar ratio was above 7 in G1, G2, G3, and G4 (23, 12, 11, and 9, respectively). The highest bioaccessibility of β-carotene was in G1 (55.86%), where possibly the enhancing effect of lutein was highest (molar ratio of lutein to β-carotene of 23). In contrast, the lowest bioaccessibility was in G5 (17.22%), where the molar ratio of lutein to β-carotene was 7.

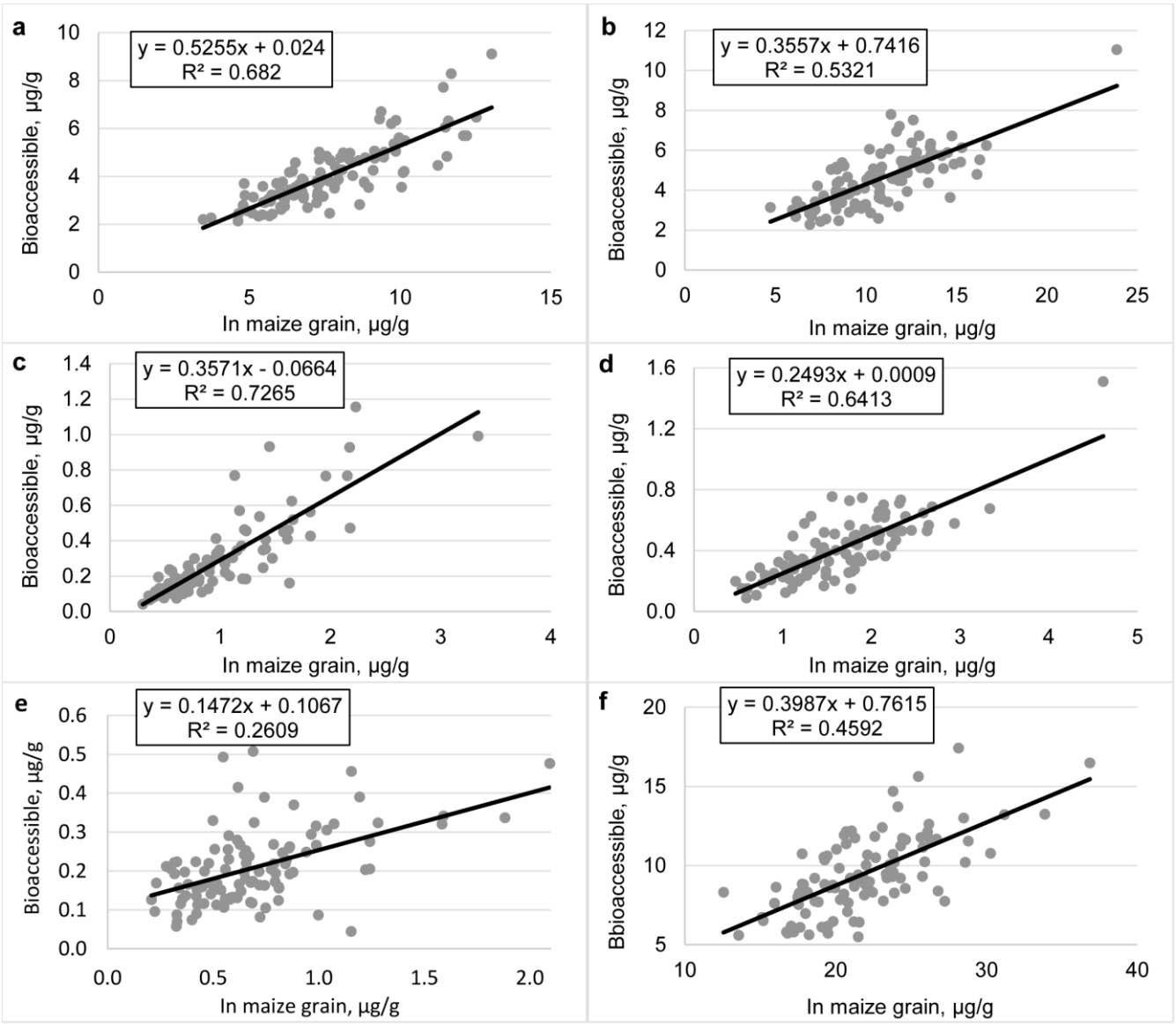

Figure 2. Linear regression of carotenoids, individual (**a**), lutein; (**b**), zeaxanthin; (**c**), α-cryptoxanthin; (**d**), β-cryptoxanthin; (**e**), β-carotene and total (**f**), in whole maize grain and bioaccessible after INFOGEST in vitro digestibility analysis.

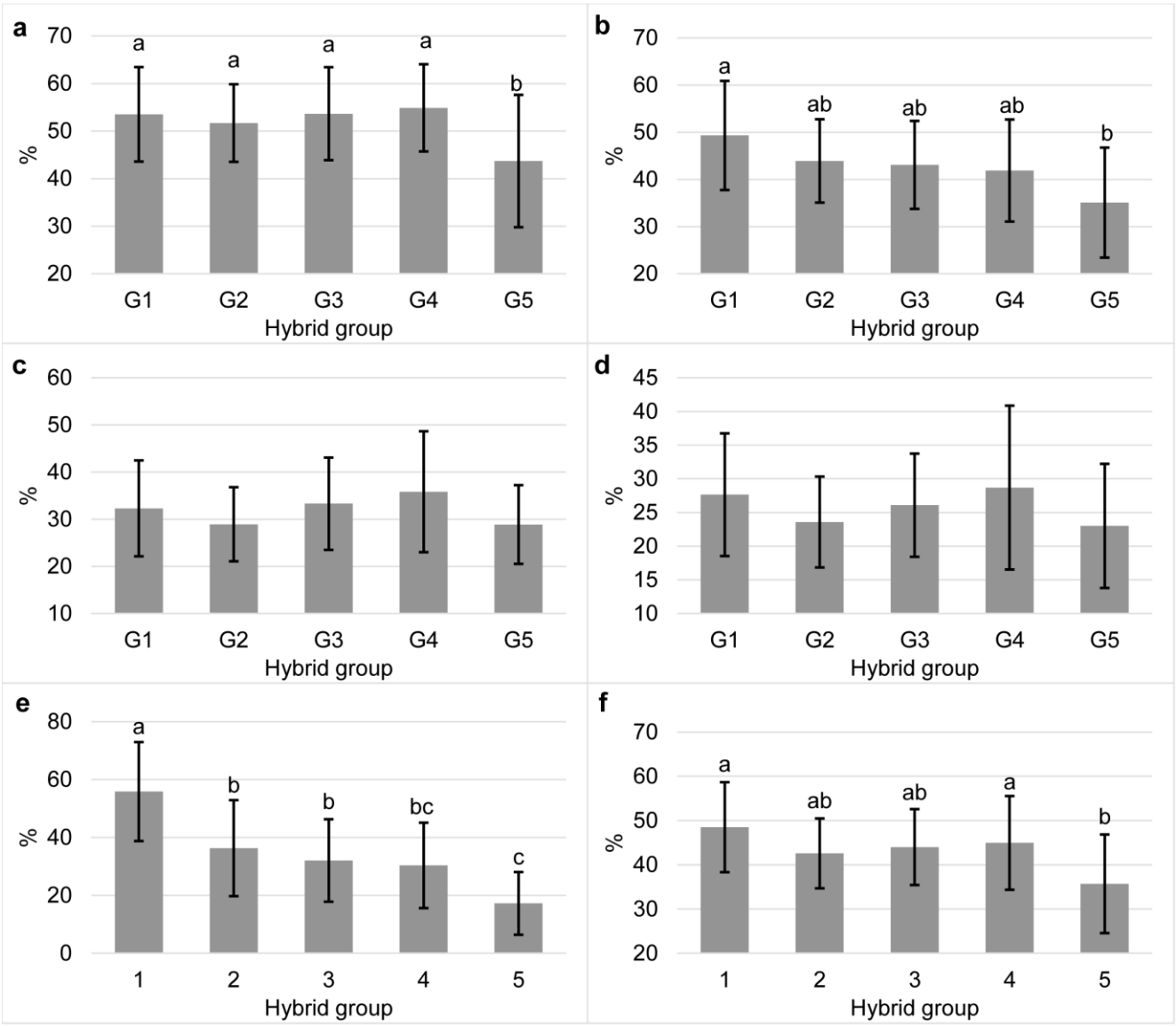

Figure 3. Bioaccessibility of individual (**a**), lutein; (**b**), zeaxanthin; (**c**), α-cryptoxanthin; (**d**), β-cryptoxanthin; (**e**), β-carotene and total carotenoids (**f**) of commercial maize hybrids classified into five groups according to the total carotenoid content (G1, <15; G2, 15–20; G3, 25–25; G4, 25–30, and G5, >30 μg/g DM). Different small letters represent statistically significant differences ($p < 0.05$) between hybrid groups. Error bars represent SD.

4. Conclusions

The results obtained in the present study show a considerable variation in the carotenoid composition of maize samples in agreement with a wide genotype variability among the commercial hybrids. The amount of bioaccessible carotenoids increased with carotenoid content in the tested hybrids, which translates to increased bioavailability when fed to animals. However, the bioaccessibility decreased with increasing content in the hybrids, suggesting that high carotenoid in grain does not imply efficient micellarization during digestion. Only 43% of the total grain carotenoids is bioaccessible and thus available for absorption by the monogastric animal. This result suggests that although maize grain is the only cereal with significant content of carotenoids, knowledge of their content is not sufficient to predict potential utilization in animals and this should be considered in the formulation of animal diets. As maize could contribute substantially to carotenoid content of animal diets due to its high proportion, further steps should be taken to evaluate factors that could increase bioaccessibility of carotenoids.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11070586/s1, Table S1: Classification of tested hybrids into groups based on total carotenoid content in the grain.

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