

Comparison of polyphenols, anthocyanins and color analysis in pigmented maize from northeast Mexico

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Abstract

This work aims to evaluate and compare the chromatic characteristics, mineral concentration, and phenolic compound content (total phenols, total flavonoids, anthocyanins, and condensed tannins), as well as antioxidant capacity levels (DPPH, ABTS, and FRAP) in maize genotypes with white, yellow, orange, and blue/purple grain pigmentations. The results showed that the lightness, colour saturation, and hue angle (L^* , C^* , and h°) values ranged from 25.33 to 69.15, 1.51 to 32.22, and 32.38 to 92.07, respectively. All the maize varieties studied showed a high concentration of N, P, K and Mg. In the case of Fe, Mn, Zn and Cu, lower values were obtained with ranges of 2.18 to 3.01, 0.43 to 0.79, 1.61 to 2.43 and 0.08 to 0.42, respectively. The content of total phenols and flavonoids (free + bound) was in a range of 236.11 to 1296.16 mg GAE/100 g, and 29.20 to 221.22 mg CE/100 g, respectively, showing that the bound fractions were higher than the free fractions. Condensed tannins were only found in pigmented maize varieties in the free fraction, with values ranging between 46.58 and 411.87 mg CE/100 g, while the concentration of total anthocyanins ranged between 1.15 and 265.61 mg C3GE/100 g. Finally, the antioxidant capacity (free + bound) expressed in $\mu\text{mol TE}/100\text{ g}$ was from 870.37 to 10763.34 for DPPH, from 1396.10 to 10895.26 for ABTS, and from 634.95 to 2185.92 for FRAP, with mean values of 6.5 mg/mL for the IC50 in DPPH and ABTS. A negative correlation was found between the total phenol content and the color parameters L^* , C^* and h° , indicating that red and purple maize grains presented the highest levels of antioxidant compounds.

Keywords: antioxidant capacity; chromatic parameters; phenolic compounds; *Zea mays* L.

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Introduction

Mexico is the birthplace of maize, a plant with great diversity, comprising approximately 59 races, which represents a substantial percentage of the 220 to 300 races found in America (Rodríguez-Salinas *et al.*, 2020). The races show different grain pigmentations, with colors ranging from black to pale pink, and the most common are red and blue (Arellano-Vázquez *et al.*, 2021). The great diversity of traditional maize-based products in Mexico is linked to the wide variety of grains of local maize varieties, producing particular textures, colors and qualities to the final products (Guzzon *et al.*, 2021). Blue maize is currently highly valued in various industries globally, such as food, pharmaceutical, cosmetic, and textile, because of its distinct color derived from anthocyanins found in the grain's pericarp and aleurone layer (Rodríguez-Salinas *et al.*, 2020). Despite its nutritional and nutraceutical benefits, pigmented maize accounts for just 10% of Mexico's production, highlighting the opportunity for new products with enhanced characteristics (Sánchez-Nuño *et al.*, 2024).

Pigmented maize is attributed with unique phytochemical properties, of which there are two large families of phenolic pigments associated with maize, which include anthocyanins and carotenoids (Bello-Pérez *et al.*, 2016). Maize rich in anthocyanins is considered nutraceutical due to the preventive effect of these pigments against oxidative stress, chronic degenerative diseases and cancer (García-López *et al.*, 2023). Anthocyanins have nutraceutical and antioxidant properties that counteract free radicals responsible for causing membrane oxidation and DNA damage (Deepak *et al.*, 2022). In maize, not only the anthocyanins in the grain have an antioxidant function, there is a broader group of substances that are present in pigmented maize that are beneficial for humans. Among these phytochemical compounds there are different polyphenolic compounds, such as phenolic acids, flavonoids, anthocyanins and condensed tannins with high antioxidant power (Herrera-Sotero *et al.*, 2017; Deepak *et al.*, 2022). It is vital to generate information and record the chemical composition of maize genotypes in southeast Coahuila, Mexico to utilize their nutritional and nutraceutical properties in the human diet.

Materials and Methods

Genetic material

The grains of the nine maize genotypes with different pigmentations came from the Improvement Program of the Mexican Maize Institute and the Center for Training and Development in Seed Technology (CCDTS), of the Universidad Autónoma Agraria Antonio Narro (UAAAN). All genotypes were grown during the spring-summer 2023 agricultural cycle, in the Bajío UAAAN Experimental Field in Saltillo, Coahuila (25°21'29" N, 101°02'21" W), at an altitude of 1742 m.a.s.l., with an average annual rainfall of 350-400 mm and an average annual temperature of 19.8 °C.

Sample preparation

The grain of each genotype was separated from the cob manually, with a humidity of 12 to 13%, and then stored at 4 °C until the analysis was carried out. Figure 1 shows the characterization and description (cob, grain and flour) of the maize genotypes selected for this study.

Grain color

In a Petri dish (4.73 cm × 1.5 cm), 50 g of grain of each genotype was placed and the color was determined with a Konica Minolta reader (CR-10 Tokyo, Japan). The chromatic parameters were obtained using the CIELAB (L^* , a^* , b^*) and CIELCH (L^* , C^* , h°) color systems according to the International Commission on Food Illumination (CIE, 2004). L^* defines lightness (0 black, 100 white), a^* indicates red (positive a^*) or green value (negative a^*) and b^* indicates yellow (positive b^*) or blue value (negative b^*), C^* (chrome; h saturation level) and h° (hue angle: 0° = red, 90° = yellow, 180° = green, 270° = blue). Color

visualization was obtained using the online software ColorHexa, color converter using L^* , C^* and h° values (Color Hex, 2024).

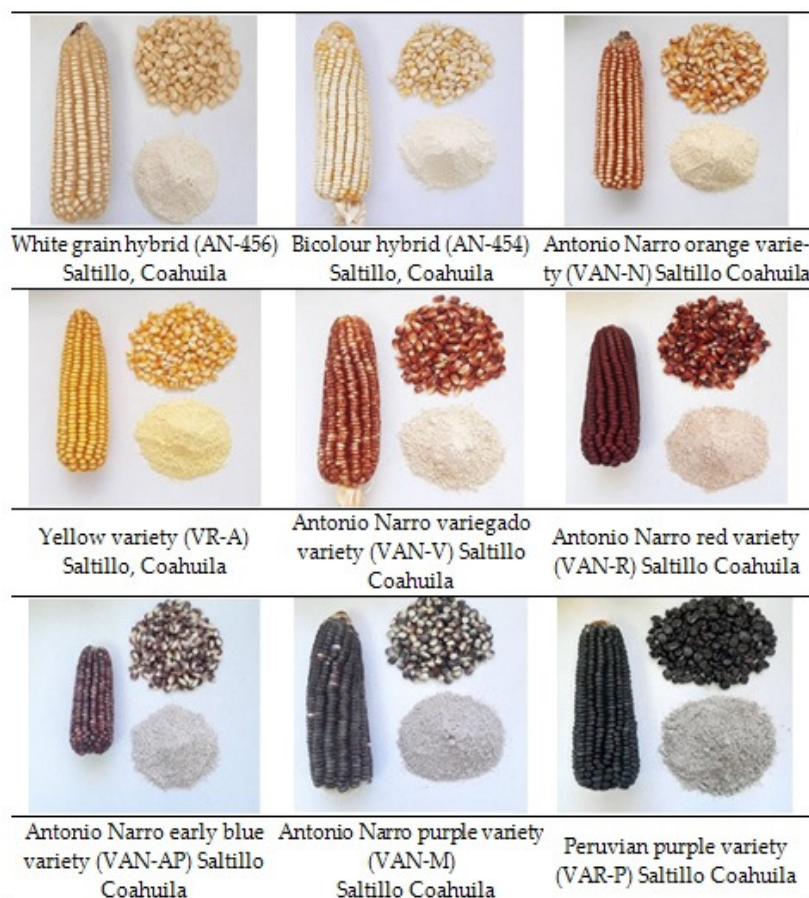


Figure 1. Pigmented maize (*Zea mays* L.) genotypes grown in southeastern Coahuila

Determination of minerals by inductively coupled plasma atomic emission spectrometry (ICP-AES)

For mineral determination, one hundred grams of grains (endosperm and bran) of each maize genotype were ground in a KRUPS GX4100 electric mill (Medford, MA, USA), then sifted to obtain particles with a <math><0.05\text{ mm}</math> size (standard mesh). Subsequently, the flour of each genotype was dried in a Yamato DX 602C oven (Yamato Scientific Co, Japan) at et al., 2020).

Extraction of free and bound phenolic compounds

The extraction of phenolic compounds was performed according to Rodríguez Salinas *et al.* (2020) by weighing 200 mg of each maize sample, which were suspended in 4 mL of 80% methanol, purged for 30 s with nitrogen and shaken for 1 h at 200 rpm, in the dark. Afterwards, the samples were centrifuged at 5000 rpm for 5 min and the supernatant was recovered and stored at

obtained from the solid residues of free phenols, adding 7 mL of 2 M NaOH and purging with nitrogen for 30 s. Afterwards, the mixture was shaken for 2 h. Subsequently, the pH was adjusted to 2.5 with concentrated HCl and the phenolics were extracted twice with 3.5 mL of ethyl acetate. The ethyl acetate extracts were combined and placed in a rotary evaporator (Yamato CF301, Yamato, Japan) to evaporate the ethyl acetate for 90 min. Once evaporated, the sample was resuspended with 4 mL of 80% methanol.

Total phenols

Total phenolics, total flavonoids, condensed tannins and antioxidant capacity assays were performed in a Thermo Spectronic BioMate3 spectrophotometer (Rochester, NY, USA), according to López-Contreras *et al.* (2015). To determine the phenol content, 0.2 mL of each extract was taken and 2.6 mL of distilled water and 0.2 mL of Folin-Ciocalteu reagent were added. After 5 min, 2 mL of 7% Na₂CO₃ were added, and the solution was stirred for 30 s, then the reaction was kept in the dark for 90 min, and the absorbance was measured at 750 nm. The concentration of phenols was reported in milligrams of gallic acid equivalent per hundred grams of sample (mg GAE/100 g), calculated from a gallic acid calibration curve from 0 to 200 mg L⁻¹.

Total flavonoids

The determination of flavonoid content was based on the reaction of the AlCl₃-NaNO₂-NaOH complex. For this, 0.2 mL of the extract were taken and 3.5 mL of distilled water were added. Subsequently, 0.15 mL of 5% NaNO₂, 0.15 mL of 10% AlCl₃ and 1 mL of 1 M NaOH were added, at intervals of 5 min each. The reaction was left for 15 min and then the absorbance was measured at 510 nm. The total flavonoid content was reported in milligrams of (+)-catechin equivalents per hundred grams of sample (mg CE/100 g), calculated from the (+)-catechin calibration curve from 0 to 200 mg L⁻¹.

Condensed tannins

The condensed tannin content was determined by the vanillin-H₂SO₄ complex reaction. From the phenolic extract, 0.25 mL were mixed with 0.65 mL of 1% vanillin solution and 0.65 mL of 25% H₂SO₄ (vanillin and H₂SO₄ previously dissolved in methanol). The reaction was carried out for 15 min, and finally, the absorbance of the samples was measured at 500 nm. The condensed tannin content was reported in milligrams equivalent of (+)-catechin per hundred grams of sample (mg CE/100 g), calculated from the calibration curve for (+)-catechin from 0 to 200 mg L⁻¹.

Total anthocyanins

The total anthocyanin content was determined based on the results of Abdel-Aal and Hucl, (1999). For this purpose, 200 mg of maize flour was homogenized with 10 mL of 1 M ethanol-HCl (85:15 v/v, pH 1, 4 °C), purged with nitrogen for 30 s, and shaken at 200 rpm for 30 min at room temperature. Samples were then centrifuged at 5750×g (4 °C, 15 min), and finally, 3 mL of the supernatant was measured at 535 nm. Anthocyanin content was reported as milligrams of cyanidin-3-glucoside (C3G) equivalents per hundred grams of sample (mg C3G/100 g) as follows: $C = (A/\epsilon) \cdot (V/1000) \cdot MW \cdot (1/\text{sample weight}) \cdot 10^6$, where: C = concentration in mg C3G/L, A = sample absorbance, ϵ = molar absorption (mg C3G = 26.695 l cm⁻¹ mol⁻¹), V = sample volume, MW = molecular weight of C3G (449.2 g mol⁻¹).

Antioxidant capacity

Antioxidant capacity assays (DPPH, ABTS and FRAP) were performed according to Rodríguez-Salinas *et al.* (2020). The antioxidant capacity was evaluated using a 60 μM DPPH (2,2-diphenyl-1-picrylhydrazyl) working solution in 80% methanol, with an absorbance adjusted to 0.7 at 517 nm. The assay was carried out

by mixing 50 μL of the phenolic extract with 1.5 mL of the DPPH working solution, the reaction was left for 30 min in the dark and the absorbance was determined.

The antioxidant capacity of ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) was carried out using a working solution obtained by mixing 1 mL of 7.4 mM ABTS and 1 mL of 2.6 mM $\text{K}_2\text{S}_2\text{O}_8$, allowing them to react for 12 h in the dark. Then, the absorbance of the working solution was adjusted to 0.7 at 734 nm by diluting with methanol. The ABTS assay was performed by mixing 50 μL of the phenolic extract with 1.5 mL of the ABTS working solution. The reaction was left for 30 min in the dark and the absorbance was measured.

The antioxidant capacity of FRAP (ferric reducing antioxidant power) was performed according to López-Contreras *et al.* (2015). FRAP was determined using a working solution obtained by mixing 300 mM $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$ (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine, in 40 mM HCl) and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in a 10:1:1 ratio. The FRAP assay was performed by mixing 50 μL of the phenolic extract with 1.5 mL of FRAP working solution. The reaction was left for 30 minutes in the dark at 37 °C, and the absorbance was measured at 593 nm.

The antioxidant capacities for DPPH, ABTS and FRAP were reported in micromoles of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent per hundred grams of sample ($\mu\text{mol TE}/100 \text{ g}$), based on a calibration curve with Trolox at concentrations ranging from 0 to 500 $\mu\text{mol L}^{-1}$. The IC₅₀ of DPPH and ABTS was calculated as the amount of the antioxidant required to inhibit 50% of the radical's oxidation. The absorbance adjusted to 0.7 in the working solutions was considered as 100% oxidation, and methanol was used as a control. The results were expressed as milligrams of maize flour per milliliter (mg mL^{-1}).

Statistical analyses

Statistical difference between samples was analyzed by analysis of variance (ANOVA), and means were compared with Tukey's test ($p \leq 0.05$) using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Results were reported as mean values of three samples \pm standard deviation.

Results


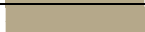







Grain color

The nine maize genotypes exhibited significant differences ($p \leq 0.05$) in the color characteristics (L^* , C^* , and h°) of the grain. Table 1 shows that L^* values varied between 25.33 and 69.15, C^* values ranged from 1.51 to 32.22, and h values ranged from 32.38 to 92.07. In the red grain genotypes, the L^* value was 31.01 and 36.04, which correspond to VAN-R and VAN-V, respectively, while the values in yellow maize ranged from 53.96 to 54.80, these values correspond to VAR-A and VAN-N, respectively. The values for purple maize were 25.33 for VAR-P, 35.14 for VAN-M and 37.27 for VAN-AP. The highest luminosity was observed in white-grained maize with values of 69.14 to 69.15 for AN-456 and AN-454, respectively (Table 1). The results of this study agree with those reported by Rodríguez-Salinas *et al.* (2020), who reported L^* values ranging from 25.13 to 63.64 in native maize genotypes from northeastern Mexico.

In the color saturation index, red maize genotypes showed values from 15.35 (VAN-R) to 19.24 (VAN-V), while in yellow maize, the values ranged from 23.02 (VAR-A) to 32.22 (VAN-N). In purple maize, C^* values were 1.51, 3.63 and 6.30 for genotypes VAR-P, VAN-M and VAN-AP, respectively (Table 1), and the values for white maize ranged from 17.12 (AN-456) to 18.17 (AN-454).

In the h angle, the values in red maize ranged from 33.17 (VAN-R) to 38.35 (VAN-V), while the h values in yellow maize ranged between 77.65 (VAN-N) to 78.90 (VAR-A), and the values of purple maize ranged between 32.38 (VAN-AP) to 65.16 (VAN-M) (Table 1). For white maize, the h values ranged from 90.84 (AN-454) to 92.07 (AN-456).

Table 1. Chromatic parameters of grains of pigmented maize genotypes from southeastern Coahuila

Genotype	Color parameters			Appearance
	<i>L*</i>	<i>C*</i>	<i>h°</i>	
AN-456	69.14 ± 3.90a	18.17 ± 1.89bc	92.07 ± 0.82a	
AN-454	69.15 ± 3.80a	17.12 ± 1.18bc	90.84 ± 1.72ab	
VAN-N	54.80 ± 1.76b	23.02 ± 4.05b	77.65 ± 4.86abc	
VAR-A	53.96 ± 1.22b	32.22 ± 8.04a	78.90 ± 1.68ab	
VAN-V	36.04 ± 1.50cd	19.24 ± 2.77bc	38.35 ± 3.00d	
VAN-R	31.01 ± 1.29d	15.35 ± 2.84c	33.17 ± 3.65d	
VAN-AP	37.27 ± 2.07c	6.30 ± 1.52d	32.38 ± 14.80d	
VAN-M	35.14 ± 2.82cd	3.63 ± 0.94d	65.16 ± 23.46bc	
VAR-P	25.33 ± 1.76e	1.51 ± 1.56d	51.74 ± 24.99cd	

*L**: lightness; *C**: color saturation; *h°*: hue angle. Values are the average of three replicates. Means (n = 3) ± standard deviation d deviation. Different letters within each column mean that the treatments were statistically different (Tukey, $p \leq 0.05$)

Concentration of macroelements

The results showed significant differences ($p \leq 0.05$) of N, P, K, Ca and Mg concentrations among the nine maize genotypes analyzed (Table 2). In the red maize genotypes, the lowest N value was found in VAN-V (1.52%) and the highest in VAN-R (2.01%), while in the yellow maize genotypes, the lowest N content was found in VAR-A (1.43%) and the highest in VAN-N (1.94%). For purple maize, the lowest N content was observed in the VAN-M genotype (1.89%), while the highest was in VAR-P (2.27%). For white maize, the lowest content was recorded in AN-456 (1.33%), with the highest in AN-454 (2.15%).

The P content in the evaluated maize genotypes ranged from 229.88 to 259.22 mg/100 g. For red maize, the lowest content was found in VAN-R (234.69 mg/100 g), and the highest in VAN-V (235.65 mg/100 g). In yellow maize, the lowest value was obtained in VAN-N (229.88 mg/100 g) and the highest in VAR-A (254.34 mg/100 g), while the P content for purple maize for the VAN-M genotype (237.87 mg/100 g) was the lowest, being the highest for VAN-AP (258.09 mg/100 g). For white maize, the lowest value was in AN-456 (234.20 mg/100 g) and the highest in AN-454 (259.22 mg/100 g).

The K content varied from 140.23 to 320.91 mg/100 g. In red maize, the lowest value was found in VAN-R (255.38 mg/100 g), and the highest in VAN-V (308.15 mg/100 g), while in yellow maize, the lowest value was obtained in VAR-A (290.28 mg/100 g) and the highest in VAN-N (305.76 mg/100 g). Purple maize genotypes had the lowest concentration in VAR-P (140.23 mg/100 g) and the highest in VAN-AP (299.57 mg/100 g), while the highest value was observed for white maize AN-454 (320.91 mg/100 g) but decreased in AN-456 (281.27 mg/100 g).

For the Ca content, the values obtained were 70.79 to 88.70 mg/100 g. In red maize, the lowest value was observed in VAN-V (76.64 mg/100 g) and the highest in VAN-R (79.60 mg/100 g), while in the yellow maize genotypes, the lowest value was obtained in VAR-A (71.81/100 g) and the highest in VAN-N (79.32 mg/100 g). The lowest Ca content for purple maize was in VAN-M (70.79 mg/100 g) and the highest in VAR-P (88.70 mg/100 g). The values for white maize varied from 75.80 in AN-454 to 82.49 for AN-456.

The Mg content among the genotypes varied from 93.65 to 109.93 mg/100 g. For the red maize genotypes, the lowest concentration was found in VAN-R (93.65 mg/100 g), and the highest in VAN-V (100.29 mg/100 g), while for yellow maize, the lowest value was found in VAN-N (93.75 mg/100 g) and the highest in VAR-A (98.73 mg/100 g). For purple maize, the lowest value was observed in VAN-M (94.31 mg/100 g) and the highest in VAN-AP (109.93 mg/100 g). For white maize, the lowest concentration of Mg was obtained in AN-456 (100.49 mg/100 g) and the highest in AN-454 (106.44 mg/100 g).

Table 2. Macronutrient content in pigmented maize genotypes grown in southeastern Coahuila

Genotype	Macronutrient (mg/100 g)				
	N %	P	K	Ca	Mg
AN-456	1.33 ± 0.10e	234.20 ± 3.21c	281.27 ± 1.59a	82.49 ± 0.77ab	100.49 ± 3.10ab
AN-454	2.15 ± 0.02ab	259.22 ± 0.57a	320.91 ± 1.271a	75.80 ± 1.20bc	106.44 ± 0.82ab
VAN-N	1.94 ± 0.06c	229.88 ± 0.49c	305.76 ± 1.82a	79.32 ± 0.98b	93.75 ± 0.85b
VAR-A	1.43 ± 0.00de	254.34 ± 2.91a	290.28 ± 1.85a	71.81 ± 2.36c	98.73 ± 0.75ab
VAN-V	1.52 ± 0.02d	235.65 ± 1.79bc	308.15 ± 2.63a	76.64 ± 1.63bc	100.29 ± 3.41ab
VAN-R	2.01 ± 0.10bc	234.69 ± 1.65c	255.38 ± 1.22ab	79.60 ± 3.381b	93.65 ± 4.05b
VAN-AP	2.03 ± 0.10bc	258.09 ± 0.41a	299.57 ± 1.29a	79.14 ± 5.80b	109.93 ± 4.86a
VAN-M	1.89 ± 0.12c	237.87 ± 0.67bc	295.03 ± 4.91a	70.79 ± 0.62c	94.31 ± 3.44b
VAR-P	2.27 ± 0.06 ^a	251.58 ± 5.30ab	140.23 ± 2.68b	88.70 ± 0.78a	102.62 ± 3.68ab

Values are the average of three replicates. Means ($n = 3$) ± standard deviation. Different letters within each column mean that treatments were statistically different (Tukey, $p \leq 0.05$)

Concentration of Microelements

The results showed significant differences ($p \leq 0.05$) in the content of Fe, Mn, Zn and Cu among the nine maize genotypes evaluated (Table 3). The lowest value was observed in the red maize genotype VAN-R (2.18 mg/100 g) and the highest in VAN-V (2.38 mg/100 g). In yellow maize, the lowest value was obtained in VAR-A (2.36 mg/100 g) and the highest in VAN-N (2.41 mg/100 g). For purple-pigmented maize, the lowest value was found in VAN-M (2.23 mg/100 g) and the highest in VAR-P (3.01 mg/100 g), and for white maize, the lowest Fe content was found in AN-456 (2.33 mg/100 g), while AN-454 (2.45 mg/100 g) had the highest value.

Table 3. Microelement content in pigmented maize genotypes grown in southeastern Coahuila

Genotype	Microelement (mg/100 g)			
	Fe	Mn	Zn	Cu
AN-456	2.33±0.043bc	0.55±0.07bc	1.61±0.22e	0.31±0.07ab
AN-454	2.45±0.035b	0.58±0.011bc	1.74±0.32d	0.35±0.02ab
VAN-N	2.41±0.047b	0.61±0.05b	1.96±0.63c	0.25±0.16b
VAR-A	2.36±0.34bc	0.53±0.02c	2.01±1.09c	0.30±0.14ab
VAN-V	2.38±0.17bc	0.58±0.08bc	1.71±0.69de	0.39±0.16ab
VAN-R	2.18±0.29c	0.56±0.05bc	1.98±0.59c	0.26±1.56b
VAN-AP	2.42±0.32b	0.63±0.10b	2.29±0.38b	0.30±0.08ab
VAN-M	2.23±0.44bc	0.43±0.02d	2.02±0.03c	0.42±0.02a
VAR-P	3.01±0.42a	0.79±0.09a	2.43±0.58a	0.08±0.22c

Values are the average of three replicates. Means ($n = 3$) ± standard deviation. Different letters within each column mean that treatments were statistically different (Tukey, $p \leq 0.05$)

For the Mn content, the values ranged between 0.43 and 0.79 mg/100 g. In red maize, the lowest value was found in VAN-R (0.56 mg/100 g) and the highest in VAN-V (0.58 mg/100 g), while in the yellow maize genotypes, the lowest value was obtained in VAR-A (0.53 mg/100 g) and the highest in VAN-N (0.61 mg/100 g). The lowest Mn content for purple maize was in VAN-M (0.43 mg/100 g) and the highest in VAR-P (0.79 mg/100 g), while the lowest value recorded for white maize was in AN-456 (0.55 mg/100 g) and the highest in AN-454 (0.58 mg/100 g).

Zn content ranged from 1.61 to 2.43 mg/100 g. In red maize, the lowest value was found in VAN-V (1.71 mg/100 g) and the highest in VAN-R (1.98 mg/100 g), while in yellow maize, the lowest value was found in VAN-N (1.96 mg/100 g) and the highest in VAR-A (2.01 mg/100 g). Purple maize genotypes had the lowest concentration in VAN-M (2.02 mg/100 g) and the highest in VAR-P (2.43 mg/100 g), while the lowest value was found for white maize in AN-456 (1.61 mg/100 g) but increased for AN-454 (1.74 mg/100 g).

The Cu content in the evaluated maize genotypes ranged from 0.08 to 0.42 mg/100 g. For red-colored maize, the lowest content was found in VAN-R (0.26 mg/100 g) and the highest in VAN-V (0.39 mg/100 g). In yellow maize, the lowest value was obtained in VAN-N (0.25 mg/100 g) and the highest in VAR-A (0.30 mg/100 g), while for the Cu content for purple maize, the VAR-P genotype (0.08 mg/100 g) had the lowest content, and VAN-M (0.42 mg/100 g) the highest. For white maize, the lowest value was in AN-456 (0.31 mg/100 g) and the highest in AN-454 (0.35 mg/100 g).

Total phenols and flavonoids

The results showed significant differences ($p \leq 0.05$) in total phenols and flavonoids among the nine maize genotypes evaluated (Table 4).

Table 4. Total phenol and flavonoid content of pigmented maize genotypes grown in southeastern Coahuila

Genotype	Total Phenols (mg GAE/100 g)			Total Flavonoids (mg CE/100 g)		
	Free	Bound	Total	Free	Bound	Total
AN-456	99.02 ± 5.97b	146.24 ± 17.65c	245.27	11.60 ± 4.00c	25.60 ± 3.46c	37.20
AN-454	110.00 ± 5.55b	126.10 ± 10.54c	236.11	14.27 ± 3.05c	19.60 ± 4.00c	33.87
VAN-N	111.89 ± 13.87b	151.40 ± 12.01c	263.29	10.93 ± 3.05c	18.26 ± 4.16c	29.20
VAR-A	135.42 ± 12.02b	267.35 ± 15.64b	402.77	32.26 ± 4.15b	30.26 ± 7.03c	62.52
VAN-V	130.68 ± 0.85b	162.70 ± 23.24c	293.39	32.26 ± 3.04b	15.59 ± 4.00c	47.85
VAN-R	130.32 ± 5.13b	268.78 ± 20.37b	399.11	43.56 ± 3.95b	92.20 ± 7.09b	135.77
VAN-AP	115.26 ± 10.65b	284.63 ± 5.48b	399.90	43.58 ± 8.00b	84.91 ± 10.12b	128.49
VAN-M	116.19 ± 14.86b	253.39 ± 11.48b	369.59	34.26 ± 6.08b	89.59 ± 9.08b	123.85
VAR-P	846.47 ± 104.78a	449.69 ± 26.48a	1296.16	97.01 ± 12.22a	124.20 ± 17.02a	221.22

Values are the average of three replicates. Means ($n = 3$) ± standard deviation. Different letters within each column mean that treatments were statistically different (Tukey, $p \leq 0.05$)

The concentration of free phenols varied from 99.02 to 846.47 mg GAE/100 g, bound phenols from 126.10 to 449.69 mg GAE/100 g, and total phenols ranged from 236.11 to 1296.16 mg GAE/100 g. In the red grain genotypes, the phenol values varied from 130.32 to 130.68 mg GAE/100 g in free, 162.70 to 268.78 mg GAE/100 g in bound and from 293.39 to 399.11 mg GAE/100 g in total phenols, while in yellow maize they varied for free phenols from 111.89 to 135.42 mg GAE/100 g, in bound phenols from 151.40 to 267.35 mg GAE/100 g and in total phenols from 263.29 to 402.77 mg GAE/100 g. In purple maize, free phenols ranged between 115.26 to 846.47 mg GAE/100 g, from 253.39 to 449.69 mg GAE/100 g for bound phenols, and from 369.59 to 1296.16 mg GAE/100 g for total phenols. In white maize, the free phenol content varied from 99.02 to 110.00 mg GAE/100 g, from 126.10 to 146.24 mg GAE/100 g for bound phenols and from 236.11 to 245.27 mg GAE/100 for total phenols.

The concentration of free flavonoids ranged from 10.93 to 97.01 mg CE/100 g, from 15.59 to 124.20 mg CE/100 g for bound flavonoids and from 29.20 to 221.22 mg CE/100 g for total flavonoids. In red maize genotypes, the values of free flavonoids ranged from 32.26 to 43.56 mg CE/100 g, from 15.59 to 92.20 mg CE/100 g for bound flavonoids, and from 47.85 to 135.77 mg CE/100 g for total flavonoids. The flavonoid results in yellow maize were from 10.93 to 32.26 mg CE/100 g for free, 18.26 to 30.26 mg CE/100 g for bound, and 29.20 to 62.52 mg CE/100 g for total flavonoids, while in purple maize genotypes, the free flavonoids varied from 34.26 to 97.01 mg CE/100 g, from 84.91 to 124.20 mg CE/100 g for bound, and from 123.85 to 221.22 mg CE/100 g for total flavonoids. In white maize, the concentration varied from 11.60 to 14.27 mg CE/100 g for free flavonoids, from 19.60 to 25.60 mg CE/100 g for bound flavonoids, and from 33.87 to 37.20 mg CE/100 g for total flavonoids.

Condensed tannins and total anthocyanins

The results showed significant differences ($p \leq 0.05$) in condensed tannins and anthocyanins (Table 5).

Table 5. Condensed tannin and total anthocyanin content of pigmented maize genotypes grown in southeastern Coahuila

Genotype	Condensed tannins (mg CE/100 g)			Anthocyanins (mg C3GE/100 g)
	Free	Bound	Total	
AN-456	ND	ND	-	3.46 ± 0.69d
AN-454	ND	ND	-	2.65 ± 0.72d
VAN-N	ND	ND	-	1.15 ± 0.52d
VAR-A	ND	ND	-	1.96 ± 0.87d
VAN-V	46.58 ± 1.17c	ND	46.58	1.73 ± 0.69d
VAN-R	59.49 ± 3.14c	ND	59.49	3.22 ± 1.05d
VAN-AP	54.80 ± 9.29c	ND	54.80	61.12 ± 5.28c
VAN-M	84.62 ± 11.76b	ND	84.62	137.24 ± 5.31b
VAR-P	411.87 ± 11.07a	ND	411.87	265.61 ± 3.25a

Values are the average of three replicates. Means ($n = 3$) ± standard deviation. Different letters within each column mean that treatments were statistically different (Tukey, $p \leq 0.05$)

The values obtained for red grain maize ranged from 46.58 to 59.49 mg CE/100 g, while in purple grain maize, the concentrations ranged between 54.80, 84.62 and 411.87 mg CE/100 g for the VAR-AP, VAN-M and VAN-P genotypes, respectively.

The values of total anthocyanins in the evaluated maize genotypes ranged from 1.15 to 265.61 mg C3GE/100 g (Table 5). In the red grain genotypes the values varied from 1.73 (VAN-V) to 3.22 (VAN-R) mg C3GE/100 g, while the lowest values were observed in yellow maize with 1.15 (VAN-N) to 1.96 (VAN-A) mg C3GE/100 g. The anthocyanin content for purple maize showed the highest values and were 61.12 (VAN-AP), 137.24 (VAN-M) and 265.61 (VAR-P) mg C3GE/100 g, respectively. The values for white maize ranged from 2.65 (AN-454) to 3.46 (AN-456) mg C3GE/100 g.

The result of the anthocyanin content showed significant negative correlations in the values of L^* and h in the grain, with correlation coefficients of -0.59 and -0.11, respectively (Table 6) which shows that as the values of L^* and h° decrease, the anthocyanin content increases.

Antioxidant Capacity

The antioxidant capacity results by DPPH in the maize genotypes varied from 310.66 to 4,267.83 for free, from 531.87 to 6,495.51 for bound, and from 870.37 to 10,763.34 $\mu\text{mol TE}/100\text{ g}$ for total phenolic compounds, while the IC50 values were from 1 to 18 mg mL^{-1} (Table 7). The values obtained for the red grain genotypes varied from 380.60 to 509.22 for free, 1,103.80 to 1,629.56 for bound, and from 1,484.41 to 2,138.78 $\mu\text{mol TE}/100\text{ g}$ for total phenols, while in yellow maize, the values varied from 310.66 to 437.23 for free, 688.44 to 1564.83 for bound, and from 999.11 to 2,002.07 $\mu\text{mol TE}/100\text{ g}$ for total phenols. For purple maize, DPPH values ranged from 601.57 to 4,267.83 for free, 1,821.18 to 6,495.51 for bound, and from 2,422.76 to 10,763.34 $\mu\text{mol TE}/100\text{ g}$ for total phenols. In white grain maize, the results ranged from 338.50 to 360.79 for free, 531.87 to 654.22 for bound, and from 870.37 to 1,015.02 $\mu\text{mol TE}/100\text{ g}$ for total phenols. The significant positive correlations between the anthocyanin content (0.89), free and bound phenols (0.99 and 0.96, respectively) and the antioxidant capacity by DPPH suggest that the high concentrations of phenolic compounds in purple and red grain maize are related to a higher antioxidant capacity (Table 6).

Table 6. Pearson correlation coefficients between phenolic compound content, color parameters (L^* , C^* , h°) and antioxidant activity

	L^* grain	C^* grain	h° grain	Free phenols	Bound phenols	Free flavonoids	Bound flavonoids	Condens ed tannins	DPPH free	DPPH Bound	ABTS free	ABTS bound	FRAP free	FRAP bound
Anthocyanins	-0.599*	0.770**	-0.115	0.853**	0.812**	0.821**	0.772**	0.916**	0.894**	0.911**	0.906**	0.896**	0.749**	0.920**
L^* grain		0.570*	0.723**	-0.479*	-0.730**	-0.767**	-0.768**	-0.748**	-0.509*	-0.644**	-0.594*	-0.530*	-0.855**	0.711**
C^* grain			0.316	-0.499*	-0.532*	-0.579*	-0.743**	-0.719**	-0.564*	-0.598*	-0.580*	-0.565*	-0.592*	-0.649**
h° grain				-0.116	-0.390	-0.375	-0.446*	-0.351	-0.123	-0.238	-0.189	-0.120	-0.485	-0.291
Free phenols					0.798**	0.850**	0.596**	0.910**	0.994**	0.961**	0.981**	0.986**	0.642**	0.892**
Bound phenols						0.920**	0.860**	0.862**	0.824**	0.915**	0.873**	0.831**	0.899**	0.902**
Free flavonoids							0.825**	0.937**	0.869**	0.942**	0.918**	0.894**	0.914**	0.918**
Bound flavonoids								0.797**	0.652**	0.761**	0.714**	0.670**	0.863**	0.794**
Condensed tannins									0.932**	0.960**	0.955**	0.939**	0.823**	0.945**
DPPH free										0.973**	0.990**	0.993**	0.679**	0.919**
DPPH bound											0.991**	0.977**	0.819**	0.958**
ABTS free												0.994**	0.763**	0.951**
ABTS bound													0.716**	0.927**
FRAP free														0.866**

Significative ($p < 0,05$); ** significative ($p < 0,01$)

ABTS

The results for ABTS ranged from 645.04 to 4,000.77 for free, from 751.06 to 6,894.49 for bound and, 1,396.10 to 10,895.26 $\mu\text{mol TE}/100\text{ g}$ for total phenols, with values of 1 to 7 mg/mL for the IC50 (Table 7). In the red grain maize, the values were from 904.77 to 1,099.54 for free, from 979.43 to 1,128.16 for bound and from 1,884.21 to 2,227.71 $\mu\text{mol TE}/100\text{ g}$ for total phenols. The ABTS results in the yellow maize genotypes ranged from 770.26 to 1,014.10 for free, from 871.60 to 1,069.42 for bound, and from 1,545.71 to 1,641.86 $\mu\text{mol TE}/100\text{ g}$ for total phenols. For purple maize, ABTS values ranged from 1,166.26 to 4,000.77 for free, 1,308.50 to 6,894.49 for bound, and 2,475.72 to 10,895.26 $\mu\text{mol TE}/100\text{ g}$ for total phenols, while for white grain maize, values ranged from 645.04 to 713.17 for free, 751.06 to 832.54 for bound, and 1,396.10 to 1,545.71 $\mu\text{mol TE}/100\text{ g}$ for total phenols.

FRAP

The values obtained for FRAP varied from 245.08 to 730.49 for free, from 389.87 to 1,455.42 for bound and from 634.95 to 2,185.92 $\mu\text{mol TE}/100\text{ g}$ for total phenols (Table 7).

In red maize, the values varied from 429.25 to 526.60 for free, 614.94 to 735.02 for bound and 1,044.20 to 1,261.63 $\mu\text{mol TE}/100\text{ g}$ for total phenols. Yellow maize produced values that ranged from 310.28 to 486.40 for free, 591.23 to 676.84 for bound and 901.52 to 1,163.24 $\mu\text{mol TE}/100\text{ g}$ for total phenols. In purple maize, FRAP values ranged from 568.66 to 730.49 for free, 820.96 to 1,455.42 for bound, and 1,367.24 to 2,185.92

$\mu\text{mol TE}/100\text{ g}$ for total phenols. White maize presented values ranging from 245.08 to 331.82 for free, 389.87 to 612.86 for bound, and 634.95 to 944.69 $\mu\text{mol TE}/100\text{ g}$ for total phenols.

Table 7. Antioxidant capacity by DPPH, ABTS and FRAP of the maize genotypes selected for the study

Genotype	DPPH ($\mu\text{mol TE}/100\text{ g}$) and IC50 (mg/mL)				ABTS ($\mu\text{mol TE}/100\text{ g}$) and IC50 (mg/mL)				FRAP ($\mu\text{mol TE}/100\text{ g}$)		
	Free	Bound	Total	IC50	Free	Bound	Total	IC50	Free	Bound	Total
AN-456	338.50 \pm 10.68 b	531.87 \pm 4.16 d	870.37	18	645.04 \pm 104.70 e	751.06 \pm 103.91 f	1396.10	7	245.08 \pm 16.62 e	389.87 \pm 10.28 e	634.95
AN-454	360.79 \pm 37.22 b	654.22 \pm 58.58 d	1015.02	16	713.17 \pm 24.60 e	832.54 \pm 23.70 ef	1545.71	6	331.82 \pm 11.28 de	612.86 \pm 11.46 d	944.69
VAN-N	310.66 \pm 51.74 b	688.44 \pm 57.95 d	999.11	18	770.26 \pm 42.72 de	871.60 \pm 24.57 ef	1641.86	6	310.28 \pm 10.00 e	591.23 \pm 15.80 d	901.52
VAR-A	437.23 \pm 43.36 b	1564.83 \pm 62.96 b	2002.07	11	1014.10 \pm 28.42 bc	1069.42 \pm 16.53 d	2083.53	4	486.40 \pm 20.10 bc	676.84 \pm 15.92 cd	1163.24
VAN-V	380.60 \pm 13.42 b	1103.80 \pm 59.85 c	1484.41	13	904.77 \pm 22.55 dc	979.43 \pm 40.26 de	1884.21	5	429.25 \pm 20.68 cd	614.94 \pm 19.43 cd	1044.20
VAN-R	509.22 \pm 13.99 b	1629.56 \pm 37.14 b	2138.78	9	1099.54 \pm 14.75 b	1128.16 \pm 21.72 dc	2227.71	4	526.60 \pm 11.54 bc	735.02 \pm 8.54 bc	1261.63
VAN-AP	601.57 \pm 17.14 b	1821.18 \pm 76.32 b	2422.76	8	1167.22 \pm 22.36 b	1308.50 \pm 13.14 bc	2475.72	4	568.66 \pm 28.63 b	820.96 \pm 17.27 b	1389.63
VAN-M	627.33 \pm 13.35 b	1885.13 \pm 71.05 b	2512.47	8	1166.26 \pm 39.05 b	1322.95 \pm 33.52 b	2489.22	4	540.76 \pm 8.26 b	826.48 \pm 8.47 b	1367.24
VAR-P	4267.83 \pm 487.70 a	6495.51 \pm 390.59 a	10763.34	1	4000.77 \pm 154.9 6a	6894.49 \pm 183.32 a	10895.26	1	730.49 \pm 100.45 a	1455.42 \pm 120.73 a	2185.92

Values are the average of three replicates. Means ($n = 3$) \pm standard deviation. Different letters within each column mean that treatments were statistically different (Tukey, $p \leq 0.05$)

Discussions

Grain color

Pigmented-grain maize (purple and red grain) has low L^* values due to the anthocyanin content in the grain pericarp (Table 5), while light-colored maize (yellow or white grain) has high L^* values due to its high carotenoid content (Colombo *et al.*, 2021). In Table 6, a negative correlation coefficient (-0.59) is observed between the L^* value and the anthocyanin content, indicating that the darker grains have the highest levels of anthocyanins.

These results agree with those reported by Harakotr *et al.* (2014), who found values from 2.9 to 29.4 in 49 maize genotypes with different color, from white to purplish black, with a high variability of polyphenolic compounds, anthocyanins and antioxidant capacity. Color parameters are related to antioxidant compounds and their activity; therefore, C^* and h° values can be used as an indirect selection of monomeric anthocyanin content, total phenolic content and antioxidant activity in maize grains (Colombo *et al.*, 2021).

The h° values define the visual perception of color, for example, the values genotypes VAR-A (78.90) and AN-456 (92.07) correspond to a visual perception of orange color, while the values of genotypes VAN-AP (32.38) and VAR-P (51.74) correspond to red tones (Salinas-Moreno *et al.*, 2012; Rodríguez-Salinas *et al.*, 2020).

Concentration of macroelements

Our results are close to the values reported by Tenorio *et al.* (2019), who reported N values of 1.34 to 1.74% in maize populations. However, the values obtained in this study are higher (1.33 to 2.27%) than the average of 1.15% N in maize grains grown in the north-central region of the USA (Tenorio *et al.*, 2019).

The P concentration found in this work agrees with other reports in grain maize (224 to 248 mg/100 g) (Bak *et al.*, 2016). In contrast, our results were lower than the range of 334.40 to 719.99 mg/100 g obtained in pigmented maize (Rodríguez-Salinas *et al.*, 2020).

Other studies have reported higher mean values of 328 to 396.0 mg/100 g in maize genotypes [1, 21]. The concentrations for Ca in this study are high compared to the average reported (7 to 32 mg/100 g) in maize varieties (Siyuan *et al.*, 2018; Rodríguez-Salinas *et al.*, 2020).

Chí-Sánchez *et al.* (2021) reported lower values than those obtained, with a range of 25.35 to 83.19 mg/100 g in native maize grown in five regions of Yucatán, Mexico. The chemical composition of maize grains has a wide variability among its different species, which depends on the environmental and topographic conditions where it is grown (Qamar *et al.*, 2017). Furthermore, the genetic information in each genotype has an important effect on the maize chemical composition (Bello-Pérez *et al.*, 2016).

Concentration of microelements

The Fe concentration agrees with the mean values reported by Chí-Sánchez *et al.* (2021) [22] and Jaradat and Goldstein (2018), who reported a concentration of 2.3 mg/100 g in maize accessions. Other studies have reported ranges from 8.20 to 11.51 mg/100 g in maize grown at the University of Agriculture, Faisalabad, Pakistan (Qamar *et al.*, 2017). The results obtained show that the Fe concentration in the evaluated maize genotypes is higher than the concentration of genotypes cultivated in different regions.

The values obtained for Mn agree with the results reported by Jaradat and Goldstein (2018), who evaluated maize genotypes obtained from a genetic improvement program, and reported values in a range of 0.32 to 0.66 mg/100 g. However, the Mn values found in this work are higher than those reported by Qamar *et al.* (2017), with concentrations of 0.24 to 0.28 mg/100 g.

The results of the Zn content were similar to those obtained by Bak *et al.* (2016), with average concentrations of 1.5 mg/100 g in different maize genotypes. However, our results were lower than those reported by Shah *et al.* (2010) and Rodríguez-Salinas *et al.* (2020) with average values of 3.6 mg/100 g.

Our results for the Cu content agree with the values reported by Rodríguez Salinas *et al.* (2020) and Chí-Sánchez *et al.* (2021) from 0.15 to 0.45 mg/100 g in different maize genotypes.

Total Phenols and flavonoids

The free phenols results are in line with the findings of Loarca-Piña *et al.* (2019) by 70%, who reported concentrations ranging from 69.40 to 212.80 mg GAE/100 g. On the contrary, Fuentes-Cardenas *et al.* (2022) reported that the concentrations of the free fractions were 8.7 to 15.6 mg GAE/100 g, concentrations that are very low compared to the values obtained in this study. In the case of bound phenols, 89% of the results obtained are within the values reported by Rodríguez-Salinas *et al.* (2020) and Fuentes-Cardenas *et al.* (2022), where they obtained concentrations of 133.50 to 339.12 mg GAE/100 g. Ferulic acid and *p*-coumaric acid have been highlighted as the main bound phenolic compounds in different maize genotypes (Zavala-López *et al.*, 2020).

Žilić *et al.* (2012) and Gálvez Ranilla *et al.* (2023) reported values similar to those obtained in this study, with ranges from 22.7 to 33.75 mg CE/100 g in free flavonoid extracts. Phenolic acids and flavonoids, as common phenolics in maize grains, exist in free, esterified (covalently bound to other molecules) and bound forms (Chen *et al.*, 2021). The aleurone layer or pericarp of maize contains primarily simple or acylated anthocyanins, which can significantly impact grain color (Cai *et al.*, 2023). In this study, a positive correlation was observed between the concentration of free and bound flavonoids (0.82 and 0.77, respectively) with total anthocyanins (Table 6). Flavonoids are a subfamily of polyphenols abundant in plant species with known beneficial effects on health, making them a promising trait for reintroduction in maize breeding programs (Wu *et al.*, 2021).

Condensed tannins and total anthocyanins

No condensed tannins were identified for the white and yellow grain genotypes, and the values obtained are higher than the results reported by Ramos-Escudero *et al.* (2012), who evaluated the INI-A601 genotype (Peru), and reported values in a range of 15 to 23 mg CE/100 g. Around 80% of the results recorded for condensed tannin content coincide with the values reported by Taylor *et al.* (2013) and Rodríguez-Salinas *et al.* (2020), who reported concentrations of 33.70 to 153 mg CE/100 g in pigmented maize.

The results reported by Gálvez-Ranilla *et al.* (2023) were lower than those obtained in this study, since in maize samples of the Granada breed (red grains), they detected values of 3.28 to 12.69 mg C3GE/100 g. On the other hand, the values obtained in this study coincide with Mendoza-Mendoza *et al.* (2017), who analyzed purple maize lines and found average values of 309 mg C3GE/100 g. In pigmented maize, flavonoid-type anthocyanins are compounds that contribute significantly to antioxidant activity (Feregrino-Pérez *et al.*, 2024). The synthesis of these compounds is influenced by genetics, maize variety, agroclimatic conditions, as well as environmental factors like light and temperature, which affect anthocyanin biosynthesis (Aguilar-Hernández *et al.*, 2019).

Antioxidant capacity

DPPH

Around 78% of the values obtained are similar to those reported by Rodríguez-Salinas *et al.* (2020), where they reported values of 1,127 to 1,865.70 $\mu\text{mol TE}/100\text{ g}$ for DPPH for total extracts in maize genotypes with yellow, red, white and purple grain color pigmentation. In maize grains, the highest proportion of phenolic compounds is found bound to the hemicellulose of the walls of the cell structure of the pericarp, whose function is to prevent chemical and physical damage to the endosperm (Cai *et al.*, 2023).

ABTS

García-López *et al.* (2023) reported lower values than those obtained in this study, with concentrations from 546.34 to 703.78 $\mu\text{mol TE}/100\text{ g}$ for free extracts, and 683.14 to 855.63 $\mu\text{mol TE}/100\text{ g}$ for bound extracts. However, the results coincided with Rodríguez-Salinas *et al.* (2020), where they obtained values between 687.90 to 940.65 in samples of free extracts, from 2,138.25 to 3,345.75 for bound extracts and from 2,826.90 to 4,263.90 $\mu\text{mol TE}/100\text{ g}$ for total extracts.

FRAP

The antioxidant capacity for FRAP in free and bound extracts in pigmented maize were similar to those reported by Oboh *et al.* (2010) and Rodríguez-Salinas *et al.* (2020), with mean values of 465 $\mu\text{mol TE}/100\text{ g}$ for free extracts and 699 $\mu\text{mol TE}/100\text{ g}$ for bound extracts. According to the antioxidant capacity results, the levels of affectation in each method were in the following order FRAP < DPPH < ABTS. The antioxidant capacity was mostly affected in ABTS and DPPH, since these methods can determine the ability to donate hydrogen atoms and liposoluble compounds that are found in soluble and bound form (polyphenols) present in the pericarp and the aleurone layer of the maize grain (Duangpapeng *et al.*, 2019; Francavilla and Joye, 2020).

Conclusions

The results indicate significant variation among maize genotypes in terms of phenolic compound content, mineral concentration, and antioxidant capacity. Phenols and flavonoids were found in higher concentrations in bound extracts of pigmented maize, whereas condensed tannins and anthocyanins were only present in free extracts of purple and red maize. The L^* and C^* color values were lower in purple and red maize, and there was a significant correlation between the values of L^* , C^* and h° , phenols, flavonoids, anthocyanins and antioxidant capacity. Pigmented maize displayed the highest values of antioxidant capacity in both free and

bound extracts. The results indicate notable genetic diversity among the evaluated maize genotypes, which can be utilized in genetic improvement initiatives to enhance their national consumption, stimulate production, conserve them, and promote their use in agroindustry.

Author Contributions

Conceptualization, JGL. and PAV.; methodology, JGL, PAV, SJM, and EHA.; software, JGL, PAV, EHA; validation JGL, PAV, EHA, and AFN; formal analysis PAV, NCM, AFN; investigation, PAV, JGL, SRB.; resources, JGL, PAV, AFN, EHA, and SRB.; data curation PAV, and JGL.; writing-original draft preparation JGL and PAV; writing-review and editing JGL and PAV; visualization JGL, PAV, and SJM; supervision JGL, PAV, AFN, and EHA; project administration JGL and PAV; funding acquisition JGL. and PAV

All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data presented in this study are contained within this article or are available upon request from the corresponding author.

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Conflicts of Interest

Authors have declared no conflict of interest.

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