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Revalorization of Colombian purple corn *Zea mays* L. by-products using two-step column chromatography

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ABSTRACT

Colombian purple corn *Zea mays* L. by-products have been chemically characterized. To achieve this, after Amberlite XAD-7 purification and Sephadex LH-20 fractionation, the accurate anthocyanin and flavonol profile using UHPL-DAD-ESI-MS, total monomeric anthocyanin (TMA), polyphenols using Folin-Ciocalteau reduction capacity (FCRC), and antioxidant activity (DPPH and TEAC) of each fraction were performed. Cob and leaves illustrated a more complete flavonoid profile and a higher content of anthocyanins and flavonols, strongly related to the highest reducing power and radical scavenging activity compared to grains. Furthermore, the most antioxidant fractions corresponded to the higher molecular weight compounds. The cob and leaves were enriched in cyanidin-3-*O*-glucoside, cyanidin-3-*O*-malonyl-hexoside, and peonidin-3-*O*-glucoside, peonidin-3-*O*-(6''-malonyl-glucoside). The purification and fractionation allowed us to establish the chemical and antioxidant characterization, and the resulting revalorization, of purple corn by-products for the first time, and to have available pure fractions of *Zea mays* L. for a wide diversity of industries.

1. Introduction

Purple corn (*Zea mays* L.) is originally cultivated in Latin America, mainly in Peru, Mexico, and Bolivia (Ramos-Escudero, Muñoz, Alvarado-Ortíz, Alvarado, & Yáñez, 2012), and provides remarkable antioxidant capacity due to its large amounts of secondary metabolites, such as phenolic compounds, flavonoids, and carotenoids (Monroy, Rodrigues, Sartoratto, & Cabral, 2016). Derived from its remarkable flavonoid content, purple corn has received increasing interest from consumers as a natural food additive. Nowadays, Colombia is one of the international producers of purple corn, grown mainly in the departments of Cauca and Nariño, where it reaches an approximate production of 21.065 tons/year (Ministerio de Agricultura, Perú). Along with corn kernels, used for the production of edible flour, leaves, and cob residues are generated, which are normally discarded, or used for animal feed. Nevertheless, their use could represent a good alternative in terms of the revalorization of industrial residues, which entails a reduction of the environmental impact by the circular production of by-products and an economic benefit derived from the reuse of products with added value (Maier, Schieber, Kammerer, & Carle, 2009).

In terms of potential health-enhancing properties, some antioxidant and biological activities, commonly related to the pigment profile, have been ascribed to *Zea mays* L. Among them, it is noteworthy the positive therapeutic effects in diseases caused by the oxidation of free radicals with active oxygen, the anti-inflammatory activity attributed to its phenolic compounds (Moreira, Stanquevis, Amaral, Lajolo, & Hassimotto, 2021), antimutagenic and antioxidant activities (Lao, Sigurdson, & Giusti, 2017), oxidative stress (Ramos-Escudero et al., 2012), anti-obesity (Chaiittianan, Sutthanut, & Rattanathongkom, 2017), mammary carcinogenesis (Fukamachi, Imada, Ohshima, Xu, & Tsuda, 2008), and anti-diabetes (Kang, Lim, Lee, Yeo, & Kang, 2013). Additional health-promoted properties were described by Hosoda et al. (2012), who demonstrated an increase in the antioxidant activity of sheep plasma after the intake of the purple pigments of *Zea mays* L., which are even transferred through milk to lactating dairy goats (Tian et al., 2019).

Anthocyanins are water-soluble flavonoids that, depending on the pH, are responsible for the blue, purple, or red tonalities of plants (Remini et al., 2018). Among the anthocyanins present in purple corn,

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accumulated mainly in the aleurone and pericarp layer, derivatives of cyanidin, peonidin, and pelargonidin have been described. Among them, their 3-glucoside derivatives have been described as major compounds, although petunidin-3-glucoside, cyanidin-3-dimalonylglucoside, their malonated and dimalonated derivatives (Suriano, Balconi, Valoti, & Redaelli, 2021; Zhang et al., 2019; Lao & Giusti, 2016; Urias-Lugo et al., 2015; González-Manzano et al., 2008) and succinic derivatives of cyanidin (Urias-Lugo et al., 2015) have also previously been identified in purple corn. In the same framework, different flavonols, viz. diverse derivatives of quercetin and kaempferol (Ramos-Escudero et al., 2012) have been reported in purple corn. Related to purple corn by-products, although some general studies have been performed on the cob (Lao & Giusti, 2018; Monroy et al., 2016), there is no published in-depth scientific study on accurate identification of anthocyanins and flavonols related to the antioxidant capacity of the cob, and, to the best of our knowledge, no reports on the leaves have been conducted to date.

Additionally, the implementation of purification and fractionation techniques allows to improve the purity of the extracts and to dispose of different bioactive fractions, which could be of great interest for other industries (agro-alimentary, cosmetics, etc.). In that sense, Amberlite and Sephadex are useful tools for purification by removing nondesirable compounds and fractionation (Sánchez-González, Jaime-Fonseca, San Martin-Martinez, & Zepeda, 2013; Rosero, Cruz, Osorio, & Hurtado, 2019). However, scarce scientific literature about obtaining separated fractions of flavonoids in *Zea mays* L. has been conducted, which could contribute to an in-depth characterization of its phenolic profile and their availability for further analyses.

Overall, the present work deals with the accurate chemical characterization of by-products (leaves and cob) of Colombian purple corn *Zea mays* L. to revalue them as sources of several substances of interest, compared to edible grains. To achieve this, a bioguided purification and fractionation methodology based on two-stage column chromatography with Amberlite XAD-7 and Sephadex LH-20 was carried out. Accurate identification of anthocyanins and flavonols was performed using UHPLC-DAD-ESI-MS and evaluation of the antioxidant activity and Folin-Ciocalteau reduction capacity of the obtained fractions, not previously performed to date, was undergone to assess the different parts of *Zea mays* L. purple corn as a potential source of health-related compounds, which could be the target market of a wide variety of industries.

2. Materials and methods

2.1. Chemical reagents and solvents

All solvents (Folin Ciocalteau reagent, and HPLC grade solvents), standards (cyanidin-3-*O*-glucoside, gallic acid, and Trolox), acetic acid, sodium bicarbonate, and ethanol were provided by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant material

Zea mays L. samples were collected in La Florida village, located approximately 17 km south of the city of San Juan Pasto (Nariño, Colombia) at 2240 m above sea level (1° 3′04′'N–77° 41′46′'O). The seeds were planted in May and the grains were harvested 125 days later (September). The samples were collected on the basis of the morphological characteristics: color (grains and leaves covering the cob), shape (conical and well-formed grains), and consistency (fleshy), suitable for consumption. An exemplary of the plant is stored in the herbarium of the Universidad de Nariño (code number 27140272). Using a simple random sampling model, in 12 plants, a representative sample of *Zea mays* L., with an average weight of 400 g, was collected. Purple grains, cob, and leaves covering the cob were separated, and kept at −4° C until analysis.

2.3. Preparation of the crude extracts

Crude extracts of cob (CEC), leaves (CEL), and grains (CEG) of purple corn were obtained (Hurtado, Morales, González-Miret, Escudero-Gilete, & Heredia, 2009). Briefly, small pieces (1 cm^2) of the samples were cut and then extracted with 0.5 L of methanol:acetic acid (19:1) for 24 h at 21 °C (1.7 mL/g sample, chemical maceration) until the complete discoloration of the plant material. After vacuum filtration (Whatman filter No 1), the organic solvent was evaporated at 35 °C using a rotary evaporator (Heidolph, Schwabach, Germany), and the extract was redissolved with distilled water (1 g/mL) and lyophilized at −80 °C and −0.012 mbar for 48 h (Labconco, MO, USA). Lyophilized samples were stored at 4 °C until analysis. The extraction yield (g/100 g of fresh sample) was calculated.

2.4. Purification with Amberlite XAD-7 column

To obtain polyphenol-enriched extracts, separated portions of CEC, CEL, and CEG (2.5 g diluted in 2 mL distilled water) were added to a column (42.5 × 3.7 cm id) containing a non-ionic polymeric absorbent (Amberlite XAD-7) as stationary phase (Rohm and Haas, Darmstadt, Germany). Adsorbed pigments were washed with 0.4 L of distilled water until no sugars were found (monitored with a refractometer (Mettler Toledo, USA)), and eluted with 600 mL of a mixture of methanol:acetic acid (19:1 v/v) (Hurtado & Charfuelan, 2019). The eluent was concentrated under vacuum (40 °C) and lyophilized, obtaining purified extracts of cob (PEC), leaves (PEL), and grains (PEG). The extraction yield (g/100 g of fresh sample) was calculated.

2.5. Fractionation by size exclusion chromatography

First, 0.25 g of each PEC, PEL, and PEG was dissolved in 2 mL water/methanol (70:30, v/v), and further subjected to size exclusion chromatography using a Sephadex LH-20 column (28.5 × 2 cm) (Rosero et al., 2019). The least retained compounds were eluted with 250 mL of water/methanol (70:30, v/v) (M-30), and those with intermediate weight were eluted with 150 mL of water/methanol (40:60, v/v) (M-60). Finally, the fraction most retained, consisting primarily of condensed tannins, was eluted with 200 mL of acetone/water (60:40, v/v) (A-60). In all cases, aliquots were collected every 10 mL, combining those that showed the same UV absorption profile. The three fractions of each purified extract were concentrated under vacuum (40 °C), lyophilized, and stored at –4°C for further analysis. Analyses were carried out in triplicate.

2.6. Spectrophotometric quantification of total monomeric anthocyanins (TMA)

Crude and Amberlite-purified samples were separately diluted with pH 1.0 and 4.5 aqueous buffers (Liu, Yang, Zhou, Wen, & Dong, 2019). Absorbance measurements were recorded at 520 and 700 nm after 15 min (Merck, Spectroquant® Pharo 300, USA). The content of anthocyanins was determined as follows: $[A]\ (mg/g) = ((Abs_{520} - Abs_{700})_{pH1} - (Abs_{520} - Abs_{700})_{pH4.5})(DF)(V)(MW)/(\varepsilon)(L)(W)$, where DF is the dilution factor, V is the volume (mL) of the extracts, MW is the molecular weight, ε is the molar extinction coefficient of cyanidin-3-*O*-glucoside (449.2 g/mol and 26,900 L/(mol cm) in H_2O), L is the optical path length (1 cm), and W is the weight of the dry sample (g). Results were expressed as mg cyanidin-3-*O*-glucoside per 100 g of dry extract. The analyses were conducted in triplicate.

2.7. Folin-Ciocalteau Reduction Capacity (FCRC)

Methanolic extract (100 μL) was added to 900 μL of Folin-Ciocalteu reagent in triplicate. Once the solution was maintained for 5 min at 25 °C, 750 μL of sodium bicarbonate solution was added. After stirring and standing for 90 min at 25 °C, the absorbance at 765 nm was

measured with a spectrophotometer (Merck, Spectroquant® Pharo 300, USA) (Rosero et al., 2019). Results were expressed as mg gallic acid equivalents per g of dry weight.

2.8. Anthocyanin and flavonol analysis using UHPLC-DAD-ESI-MS/MS

Separation and identification of anthocyanins and flavonols were performed in triplicate in a UHPLC Dionex Ultimate 3000 RS chromatographic system (Thermo Fisher Scientific™), equipped with a quaternary pump, and a UV–vis diode-array detector coupled to ChemStation software (Palo Alto, USA). An Xbridge BEH C_{18} column (100 × 2.1 mm, 2.5 µm particle size) was thermostatted at 25 °C, and 0.1% TFA in water (eluent A) and acetonitrile (eluent B), at a flow rate of 0.8 mL/min were used (Solarte, Cejudo-Bastante, Hurtado, & Heredia, 2022). The injection volume was 50 µL. Anthocyanins and flavonols were monitored at 520 and 360 nm, respectively, and quantification was performed using the standard curves for cyanidin-3-*O*-glucoside and quercetin, respectively. The mass spectra were obtained using an Absciex 3200 Q-trap electrospray ionisation mass spectrometry (ESI/MS^n) system (Foster City, CA, USA) equipped with a triple quadrupole linear ion trap mode, in positive ion mode using a scan range from *m/z* 50 to 1000. Nitrogen was used at 40 mL/min and 700 °C of drying temperature. Mass spectra were acquired in SIM mode, and the mass data of the molecular ions were processed through the software AB Sciex Analyst® (version 1.6.2).

2.9. Determination of the antioxidant capacity equivalent to Trolox (TEAC)

The $ABTS^+$ solution (7 Mm) was diluted with 2.45 mM of phosphate-buffered saline (PBS) in water, and stored at dark and 25 °C. Once the absorbance was reached approximately 0.70 ± 0.02 at 734 nm, after approximately 16 h, it was adjusted to pH 7.4. Subsequently, 30 µL of the sample was added in triplicate to 3 mL of the diluted solution of $ABTS^+$. After stirring and waiting for 6 min, the absorbance at 734 nm was measured (UV–Vis Pharo, Merck, Germany) (Rosero et al., 2019). The results were expressed as mmol Trolox equivalents per g of dry weight.

2.10. Antioxidant activity using the analysis of 2,2-diphenyl-1-picryl hydracyl (DPPH)

First, 3.0 mL of 0.1 mM ethanolic solution of DPPH was mixed with an aliquot (0.1 mL) of the sample. Discoloration was measured at 517 nm (UV–Vis Spectroquant® Pharo, Merck, Germany) after incubation for 30 min at 30 °C in dark conditions (Magaña-Cerino et al., 2020). The % inhibition was calculated as follows: % inhibition = $(Ac - As) \times 100/Ac$, where *Ac* and *As* are the absorbances of the control and sample, respectively. The radical scavenger activity was expressed as EC_{50} values, defined as the sample concentration (µg/mL) required to decrease the DPPH initial concentration by 50%. Analyses were conducted in triplicate.

2.11. Statistical analysis

Univariate analysis of variance (ANOVA) procedure ($p < 0.05$) was applied. To achieve summarized and synthesized information from a large set of compounds and to better understand the distribution of the samples, principal component analysis (PCA) was applied. Statgraphics Centurion 16.1.15 software was used.

3. Results and discussion

3.1. Identification and quantification of anthocyanins and flavonols after purification with Amberlite XAD-7

Table 1 summarizes the anthocyanins and flavonols identified in PEC, PEL, and PEG. Fig. 1 shows the peak assignment of PEC, as representative of the fraction with the highest extraction yield, FCRC, and TEAC (Table 2).

Anthocyanins. Seventeen anthocyanin compounds were tentatively identified in the different parts of *Zea mays* L. As evidenced in Table 1, compound **4** was the major anthocyanin quantified in all purified fractions (PEC, PEL, and PEG), and displayed pseudomolecular ion $[M]^+$ at 449 *m/z* unit and a fragmentation ion at 287 *m/z* units, due to the loss of a fragment of glucose with 162 *m/z*. Therefore, it was ascribed as cyanidin-3-*O*-glucoside, and accounted for 30–32 %, in agreement with Monroy et al. (2016) and González-Manzano et al. (2008). However, other major anthocyanins corresponded to different compounds depending on the part of purple corn. Therefore, the other major anthocyanins in PEG were compound **1**, which accounted for 22.78 % of the total area and displayed a pseudomolecular ion $[M]^+$ at 899 *m/z* unit and fragmentation ions at 737, 575, and 287 *m/z* units. Thus, it has been attributed as (epi)catechin(4–8)-cyanidin-3,5-diglucoside, due to the loss of two fragments with 162 *m/z* (two moieties of glucose), and 288 *m/z* ((epi)catechin unit). These compounds have been previously identified in the pericarp and endosperm of three varieties of purple corn (Rice, Peruvian, & Purépecha; Lao & Giusti, 2016; González-Manzano et al., 2008). Compounds **3** and **9** represented 26 % of the total area (12.09 and 14.51 %, respectively) in PEG. They displayed pseudomolecular ions $[M]^+$ at 913 and 535 *m/z* units and fragmentation ions at 751 and 589, and 287 *m/z* units, respectively. Taking into account the loss of fragments with 162 and 248 *m/z*, corresponding to two glucose moieties and one glucose $[M\text{-}324]^+$ and malonic acid moieties $[M\text{-}162\text{-}86]^+$, the compounds were attributed to (epi)catechin(4–8)-peonidin-3,5-diglucoside and cyanidin-3-*O*-malonyl-hexoside, respectively.

Regarding PEL and PEC, apart from the major anthocyanins cyanidin-3-*O*-glucoside and cyanidin-3-*O*-malonyl-hexoside (compounds 4 and 9), in agreement with González-Manzano et al. (2008), which accounted for approximately 49 % of the total area, compounds **7** and **14** represented 29 % and 21 % of the total area in PEL and PEC, respectively. In the light of the mass spectra ($[M]^+$ at 463 *m/z* and a product ion at 301 *m/z* units due to the loss of a glucose unit, $[M\text{-}162]^+$), the compound 7 was assigned to peonidin-3-*O*-glucoside. Furthermore, compound 14 had mass spectra of $[M]^+$ at 549 *m/z* and product ions at 463 and 301 *m/z* units due to the loss of a malonyl and glucose units, $[M\text{-}86]^+$ and $[M\text{-}162]^+$), assigning it as peonidin-3-*O*-(6″-malonyl-glucoside). Among the anthocyanins with an intermediate contribution to the total area, compounds **5, 8**, **13**, and **15** were highlighted (16–18 %). The product ion at 287 *m/z* unit of compounds 8 and 15 corresponded to the cyanidin aglycone, and the $[M]^+$ ions at 535 and 621 *m/z* units made ascribed them as cyanidin-3-*O*-malonyl-glucoside and cyanidin-3-*O*-(3″,6″-dimalonyl-hexoside), respectively, due to the breakdown of glucose ($[M\text{-}162]^+$) and two units of malic acid ($[M\text{-}172]^+$. Similar pattern fragmentation as compound 14 was detected in compound 13, assigned it as an isomer of peonidin-3-*O*-(6″-malonyl-hexoside). Further anthocyanin aglycone was identified as pelargonidin (product ion at 271 *m/z* unit) with a similar breakdown of compounds 4 and 7 ($[M\text{-}162]^+$), attributing it as pelargonidin-3-*O*-glucoside (compound 5). The minor anthocyanin compounds in PEL and PEC were ascribed to compounds **2**, **3**, **6**, **10**, **11**, **12**, **16,** and **17** whose identification was undergone based on the aforementioned data of $[M]^+$ and product ions (Table 1). The product ion of compound 11 (303 *m/z* unit) corresponded to delphinidin and, taking into account the $[M]^+$ at 465 *m/z* unit, was ascribed to delphinidin-3-*O*-glucoside. It is highlighted that the purification technique with Amberlite, scarcely reported in *Zea mays* L., has contributed to a large identification of anthocyanins compared to other

Table 1
Peak assignment, identification, retention times (R_t), mass spectral, mean concentration (mg/g), and standard deviations of the anthocyanins and flavonols identified in the flavonoid enriched fractions of *Zea mays* L. by UHPLC-DAD-ESI-MS.

Peak No.	Compound	R_t (min)	Mass spectra		Mean concentration (mg/g)		
					PEC	PEL	PEG
Anthocyanins			**[M]** $^+$	**Product ion**			
1	(Epi)catechin(4–8)-cyanidin-3,5-diglucoside	8.31	899	737, 575, 287	1.72 ± 0.28[c]	0.17 ± 0.03[b]	0.93 ± 0.26[a]
2	(Epi)catechin(4–8)-pelargonidin-3,5-diglucoside	11.46	883	721, 559	0.09 ± 0.01[a]	–	0.04 ± 0.01[a]
3	(Epi)catechin(4–8)-peonidin-3,5-diglucoside	13.03	913	751, 589	0.67 ± 0.07[c]	0.04 ± 0.01[b]	0.49 ± 0.13[a]
4	Cyanidin-3-*O*-glucoside	17.67	449	287	37.3 ± 3.55[c]	15.59 ± 3.4[b]	1.25 ± 0.20[a]
5	Pelargonidin-3-*O*-glucoside	21.62	433	271	5.29 ± 0.97[b]	2.17 ± 0.68[a]	–
6	Cyanidin-3-*O*-malonyl-hexoside	22.53	535	287	0.17 ± 0.03[b]	0.22 ± 0.08[a]	0.06 ± 0.01[a]
7	Peonidin-3-*O*-glucoside	23.84	463	301	14.68 ± 1.8[c]	9.14 ± 2.25[b]	0.23 ± 0.03[a]
8	Cyanidin-3-*O*-malonyl-glucoside	25.03	535	287	5.18 ± 0.57[b]	1.75 ± 0.44[a]	–
9	Cyanidin-3-*O*-malonyl-hexoside	28.06	535	287	27.53 ± 4.3[c]	13.55 ± 3.4[b]	0.59 ± 0.15[a]
10	Pelargonidin-3-*O*-(6''-malonyl-hexoside)	29.36	519	271, 433	0.84 ± 0.10[b]	0.13 ± 0.01[a]	0.11 ± 0.02[a]
11	Delphinidin-3-*O*-glucoside	30.05	465	303	0.32 ± 0.05[b]	0.05 ± 0.01[a]	–
12	Pelargonidin-3-*O*-(6''-malonyl-glucoside)	30.80	519	271, 433	0.53 ± 0.09[a]	0.65 ± 0.04[a]	–
13	Peonidin-3-*O*-(6''-malonyl-hexoside)	32.28	549	463, 301	5.53 ± 0.82[b]	2.80 ± 0.65[a]	–
14	Peonidin-3-*O*-(6''-malonyl-glucoside)	33.64	549	463, 301	10.50 ± 1.3[c]	8.03 ± 1.94[b]	0.23 ± 0.07[a]
15	Cyanidin-3-*O*-(3'',6''-dimalonyl-hexoside)	34.07	621	449, 287	5.90 ± 0.68[c]	2.70 ± 0.66[b]	0.13 ± 0.02[a]
16	Pelargonidin-3-*O*-(3'',6''-dimalonyl-hexoside)	37.66	605	561, 271	1.22 ± 0.15[b]	0.69 ± 0.08[a]	–
17	Peonidin-3-*O*-(3'',6''-dimalonyl-hexoside)	38.57	635	591, 301	2.21 ± 0.29[a]	1.49 ± 0.39[a]	–
Flavonols			**[M]** $^-$	**Product ion**			
18	Quercetin-3-*O*-rutinoside	29.23	609	301	4.07 ± 0.47[b]	7.51 ± 1.56[b]	1.31 ± 0.23[a]
19	Quercetin-3-*O*-glucoside	31.11	463	301	3.29 ± 0.17[a]	10.75 ± 2.2[a]	0.89 ± 0.25[a]
20	Isorhamnetin-3-*O*-rutinoside	34.92	623	315	0.65 ± 0.06[a]	2.77 ± 0.62[a]	–
21	Kaempferol-3-*O*-hexoside	35.51	447	285	3.59 ± 0.32[b]	9.38 ± 2.38[b]	0.41 ± 0.08[a]
22	Isorhamnetin-3-*O*-glucoside	36.12	491	315	0.85 ± 0.02[a]	1.96 ± 0.45[a]	–
23	Kaempferol-3-*O*-hexoside	37.23	447	285	2.31 ± 0.34[a]	6.06 ± 1.01[b]	–
24	Quercetin	49.03	301	[a]	1.33 ± 0.12[a]	–	–

[a]Fragmentation was not achieved. PEC, purified extract of cob. PEL, purified extract of leaves. PEG, purified extract of grain. Different superscripts in the same row denote significant differences ($p < 0.05$) by ANOVA test.

techniques (Lao, & Giusti, 2016, 2018; Monroy et al., 2016; Mora-Rochín et al., 2016; Suriano et al., 2021; Moreira et al., 2021; Urias-Lugo et al., 2015). In fact, compounds **2**, **3**, and **11** have previously been reported in *Zea mays* L. only by Paucar-Menacho, Martínez-Villaluenga, Dueñas, Frias, and Peñas (2017).

PEC and PEL presented a more complex anthocyanin profile compared to PEG, showing significantly ($p < 0.05$) higher content of almost all anthocyanins, but displaying more enrichment in peonidin derivatives (compounds 7, 13, 14, 17) and pelargonidin derivatives (compounds 5, 10, 12, and 16) (Table 1). Furthermore, although the anthocyanin profile of PEL and PEC was similar, a significantly ($p < 0.05$) higher amount of almost all anthocyanins were observed in PEC. Anyway, the high percentage of flavonoids in purple corn by-products, some of them not present either in the grains, evidences the possibility of revalorization of leaves and cob as bioactive sources.

Flavonols. Seven flavonol compounds were tentatively identified in the different parts of *Zea mays* L. The fragmentation pattern of compounds **18**, **19,** and **24** was similar, i.e. and ion at 301 *m/z* unit, which corresponded to the quercetin aglycone. Compound 19 exhibited a pseudomolecular ion $[M]^-$ 463 *m/z* unit and a fragmentation ion at 301 *m/z* unit, due to the loss of a fragment with 162 *m/z* corresponding to the breakdown of an *O*-hexoside bond. Compound 19 was assigned as quercetin-3-*O*-glucoside when compared to the retention time of the commercial standard and the mass data. The pseudomolecular ion $[M]^-$ 609 *m/z* units, with a fragmentation ion at 301 *m/z* unit, made attribute the compounds 18 as quercetin-3-*O*-rutinoside. The loss of 308 *m/z* units $[M\text{-}162{-}146]^-$ corresponded to an *O*-glycosidic bonds breakdown of a rhamnose unit (146u) and glucose (162u) (Table 1). The product ions of 315 and 285 *m/z* units were assigned as isorhamnetin and kaempferol aglycons, respectively. Taking into account the pseudomolecular ion $[M]^-$, the fragmentation patterns were similar to the aforementioned described, i.e. $[M\text{-}162{-}146]^-$ and $[M\text{-}162]^-$, attributing compounds **20** and **22** as 3-*O*-rutinoside and 3-*O*-glucoside of isorhamnetin, respectively, and compounds **21** and **23** as the isomers kaempferol-3-*O*-hexoside. The presence of these compounds in *Zea mays* L. is in agreement with Moreira et al. (2021), Zhang et al. (2019), and Ranilla et al. (2019).

PEG exhibited a less qualitative flavonol profile, limited to the presence of quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, and isorhamnetin-3-*O*-rutinoside, and a significantly ($p < 0.05$) lower content compared to that of PEL, followed by PEC (Table 1). Moreover, PEL and PEC depicted a similar flavonol profile, both qualitative and quantitative. In PEL and PEC, 3-*O*-rutinoside, and 3-*O*-glucoside of quercetin, together with the two isomers of kaempferol-3-*O*-hexoside, were the major flavonols, covering approximately 82% of the total concentration of the flavonol.

Based on the results obtained from ANOVA, non-supervised pattern recognition statistical analysis (Principal Component Analysis) was applied to the data of the anthocyanins and flavonols of the three parts of the purple corn. Five main significant principal components (PCs) were arisen according to Kaiser's criterion (eigenvalues > 1), which explained 100% of the total variance. The first PC, PC1, which explained 61.2% of the total variance, mainly contains the anthocyanins, except diglucoside derivatives, and quercetin with a negative sign. In the case of PC2, which explained 37.8% of the total variance, glycosylated flavonols, with a positive sign, and (epi)catechin(4–8)-anthocyanin-3,5-diglucoside derivatives with a negative sign, were the main contributors. Fig. 2 shows the samples to the plane defined by these two PCs, which explained 99.0% of the total variance. As can be seen, a separation by parts of the purple corn was achieved. PEL displayed the highest content of glycosylated flavonols and diglucoside-anthocyanins, while PEC showed a higher content of non-diglucoside anthocyanins and quercetin. The PEG exhibited the lowest values of non-diglucoside anthocyanins.

3.2. Extraction yield, TMA, FCRC, and antioxidant activity

CEC, CEL, and CEG accounted for an average of 18.3, 11.4, and 2.5 % of each sample's weight, respectively (Table 2), and a lower extraction

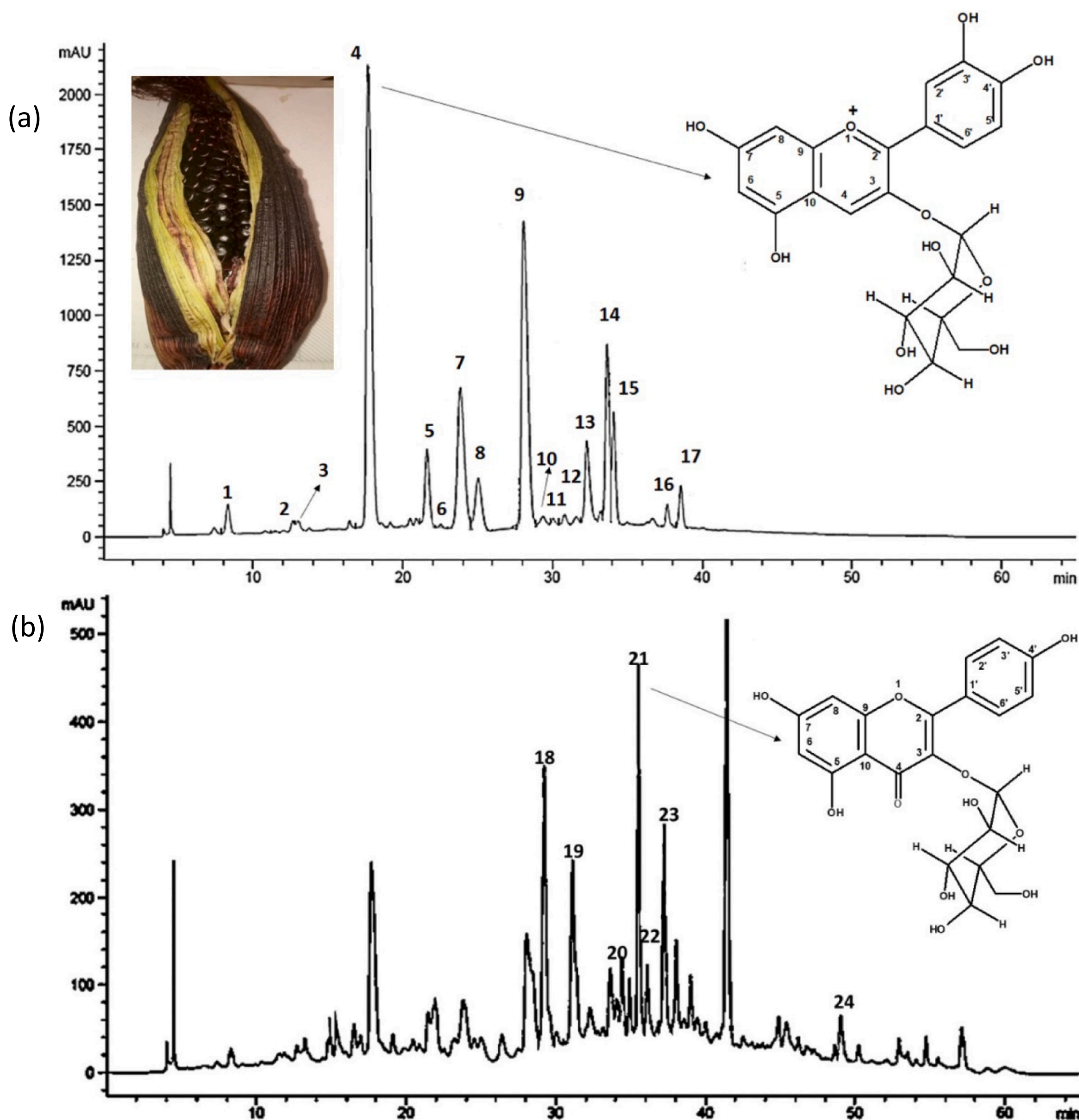

Fig. 1. UHPLC-DAD chromatogram of the purified extract of the cob (PCE): (a) anthocyanins at 520 nm, and (b) flavonols at 360 nm.

yield was obtained after purification. Regardless of crude or purified fractions, the extraction yield of the cob was significantly ($p < 0.05$) higher than that of the leaves, followed by the grains. TMA and FCRC, together with the antioxidant activity by TEAC and DPPH, of the separated crude and purified fractions, were evaluated in different parts of *Zea mays* L. for the first time using selective retention with Amberlite XAD-7. According to ANOVA, the purification process caused an enrichment of TMA, FCRC, and antioxidant activity by TEAC in grains and by-products, in the light of the significantly ($p < 0.05$) higher content in PEC, PEL, and PEG compared to CEC, CEL, and CEG, respectively, representing the effectiveness of the purification process. Furthermore, because a higher number of hydroxyl groups in the B-ring is closely related to the increase in antioxidant activity (Janeiro & Brett, 2007), the fact that the anthocyanin profile of PEC is mainly based on cyanidin derivatives (with two hydroxyl groups in the B-ring), and the additional presence of peonidin and pelargonidin (with one hydroxyl group in the B-ring) in PEL and PEG, could be consistent with the higher antioxidant capacity by TEAC of PEC (Table 2). In addition, the presence of three quercetin derivatives (with two hydroxyl groups in the B-ring) in PEC could be also contributed to its higher antioxidant activity (Semwal, Semwal, Combrinck, & Viljoen, 2016). On the contrary, the lower antioxidant activity of PEG could be attributable to the lack of many anthocyanins and flavonols. Similar behavior was found when EC_{50} was calculated (Table 2), taking into account that the lower the EC_{50} value, the higher the free radical scavenging activity, similarly to other authors (Betancourt, Cejudo-Bastante, Heredia, & Hurtado, 2017; Rosero et al., 2019). Univariate linear correlation showed the consistency between FCRC and the anti-radical activity (TEAC), observing a strong correlation between both parameters ($R^2 = 0.933$).

To scrutinize the portion of the purified samples with the highest contribution to the antioxidant capacity, less complex fractions were obtained by molecular size fractionation for each part of the purple corn (Table 3), observing an increase in FCRC and antioxidant activity compared to purified extracts. M-30 fractions had the significantly ($p < 0.05$) lowest antioxidant capacity and FCRC, being the most highly retained fractions (M-60 and A-60) the most bioactive. This behavior could be owing to the presence of a high number of phenolic rings and hydroxyl groups in elevated molecular weight compounds, which

Table 2
Mean values and standard deviation ($n = 3$) of the extraction yield, total monomeric anthocyanins (TMA), Folin-Ciocalteu reduction capacity (FCRC) and antioxidant activity by TEAC and DPPH of each crude and purified extracts isolated from *Zea mays* L.

Sample	Extraction yield (%)	TMA (mg/100 g)	FCRC (mg/g)	TEAC (mmol/g)	DPPH-EC_{50} (μg/mL)
CEC	18.28 ± 2.26^e	63.66 ± 2.44^e	37.53 ± 3.20^c	2.24 ± 0.13^c	61.28 ± 0.64^d
PEC	5.57 ± 0.45^c	173.86 ± 0.02^f	318.09 ± 4.30^f	4.04 ± 0.20^e	17.43 ± 0.46^a
CEL	11.42 ± 0.68^d	36.98 ± 2.43^b	25.80 ± 4.52^b	2.14 ± 0.30^b	63.56 ± 2.56^e
PEL	2.86 ± 0.23^b	46.54 ± 5.51^d	260.2 ± 2.73^e	3.88 ± 0.12^d	39.79 ± 0.62^b
CEG	2.58 ± 0.22^b	24.41 ± 0.99^a	21.38 ± 4.24^a	1.36 ± 0.10^a	142.42 ± 4.01^f
PEG	0.80 ± 0.06^a	41.71 ± 2.92^c	239.74 ± 6.97^d	3.61 ± 0.26^d	46.66 ± 0.19^c

CEC, CEL, CEG, crude extracts of the cob, leaves, and grains, respectively; PEC, PEL, PEG, purified extracts of the cob, leaves, and grains, respectively. Different letters in the same column denote significant differences ($p < 0.05$) by ANOVA test.

contribute to improving the antioxidant capacity (Craft, Kerrihard, Amarowicz, & Pegg, 2012). Moreover, it was noticed that the A-60 fraction of the by-products (cob and leaves) illustrated the significantly ($p < 0.05$) highest values of extraction yield, FCRC, and antioxidant activity. Furthermore, the strong consistency among FCRC and TEAC previously described was still maintained after fractionation (R^2 = 0.949, 0.947, and 0.956 in cob, leaves, and grains, respectively).

4. Conclusions

Considering the remarkable amount of anthocyanins and flavonols related to antioxidant capacity in the purple corn by-products, it is evident that the industry could consider the usage of these normally-discarded residues. Concretely, the purple corn cob and leaves represented a more complete flavonoid profile than the grains, evidenced by almost the double number of anthocyanins and flavonols identified, many of them not previously described in these residues. Moreover, taking into account their remarkable content, purple corn by-products could be an excellent source of anthocyanins and flavonols, especially cyanidin, peonidin, and pelargonidin derivatives, as well as quercetin and kaempferol derivatives. Purification and fractionation techniques allow not only this accurate identification of flavonoids but also the disposal of pure and highly antioxidant extracts and fractions for further analysis or for direct industrial use. Therefore, this work could entail not only a reduction in the waste management for the purple corn processing industry, but also an economic benefit for using by-products as food additives in agri-foods, pharmaceuticals, and cosmetics, among others.

CRediT authorship contribution statement

Estefania J. Carrera: Investigation, Writing – original draft. **María Jesús Cejudo-Bastante:** Investigation, Writing – review & editing. **Nelson Hurtado:** Conceptualization, Methodology, Resources, Writing – original draft. **Francisco J. Heredia:** Conceptualization, Supervision. **M. Lourdes González-Miret:** Conceptualization, Supervision.

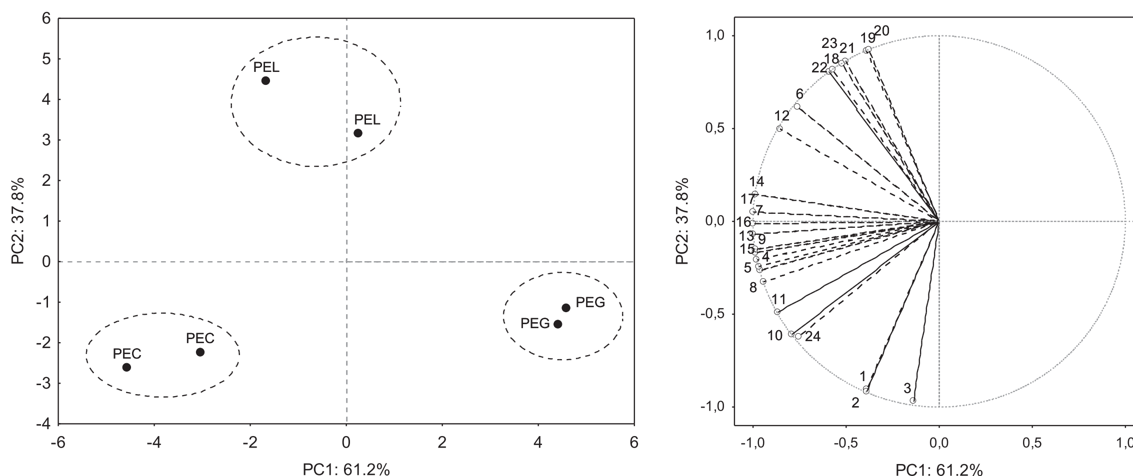

Fig. 2. Distribution of samples in the plane defined by the two first discriminate functions by parts of the purple corn *Zea mays* L., and projection of the variables (anthocyanins and flavonols) on the factor-plane extracted by PCA. Numbers correspond to anthocyanins and flavonols according to the first column of Table 1.

Table 3
Characteristics of the Sephadex fractions of *Zea mays* L. based on the extraction yield, Folin-Ciocalteu reduction capacity (FCRC), and antioxidant activity by TEAC.

Sample	M-30			M-60			A-60		
	Extraction yield %	FCRC (mg/100 g)	TEAC (mmol/g)	Extraction yield %	FCRC (mg/100 g)	TEAC (mmol/g)	Extraction yield %	FCRC (mg/100 g)	TEAC (mmol/g)
PEC	0.20 ± 0.02^a	194.48 ± 1.60^a	3.14 ± 0.05^a	0.19 ± 0.02^a	241.00 ± 4.03^a	3.91 ± 0.09^a	0.35 ± 0.02^a	295.29 ± 1.52^a	3.97 ± 0.15^a
PEL	0.13 ± 0.04^b	145.19 ± 3.94^b	2.70 ± 0.04^b	0.13 ± 0.05^b	229.07 ± 2.49^b	3.88 ± 0.04^b	0.20 ± 0.07^b	253.47 ± 3.11^b	3.86 ± 0.06^b
PEG	0.04 ± 0.01^c	104.79 ± 6.55^c	2.59 ± 0.06^c	0.04 ± 0.02^c	195.57 ± 3.79^c	3.60 ± 0.43^c	0.07 ± 0.02^c	216.78 ± 3.82^c	3.52 ± 0.21^c

PEC, purified extract of cob. PEL, purified extract of leaves. PEG, purified extract of grain. M-30, fraction eluted with water/methanol (70:30, v/v). M-60, fraction eluted with water/methanol (40:60, v/v). A-60, fraction eluted with acetone/water (60:40, v/v). Different letters in the same column denote significant differences ($p < 0.05$) by ANOVA test.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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References

Betancourt, C., Cejudo-Bastante, M. J., Heredia, F. J., & Hurtado, N. (2017). Pigment composition and antioxidant capacity of betacyanins and betaxanthins fractions of *Opuntia dillenii* (Ker Gawl) Haw cactus fruit. *Food Research International, 101*, 173–179. https://doi.org/10.1016/j.foodres.2017.09.007

Chaiittianan, R., Sutthanut, K., & Rattanathongkom, A. (2017). Purple corn silk: A potential anti-obesity agent with inhibition on adipogenesis and induction on lipolysis and apoptosis in adipocytes. *Journal of Ethnopharmacology, 201*, 9–16. https://doi.org/10.1016/j.jep.2017.02.044

Craft, B. D., Kerrihard, A. L., Amarowicz, R., & Pegg, R. B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety, 11*(2), 148–173. https://doi.org/10.1111/j.1541-4337.2011.00173.x

González-Manzano, S., Pérez-Alonso, J. J., Salinas-Moreno, Y., Mateus, N., Silva, A. M. S., Freitas, V., & Santos-Buelga, C. (2008). Flavanol-anthocyanin pigments in corn: NMR characterisation and presence in different purple corn varieties. *Journal of Food Composition and Analysis, 21*, 521–526.

Fukamachi, K., Imada, T., Ohshima, Y., Xu, J., & Tsuda, H. (2008). Purple corn color suppresses Ras protein level and inhibits 7,12-dimethylbenz *a* anthracene-induced mammary carcinogenesis in the rat. *Cancer Science., 99*(9), 1841–1846. https://doi.org/10.1111/j.1349-7006.2008.00895.x

Hosoda, K., Miyaji, M., Matsuyama, H., Haga, S., Ishizaki, H., & Nonaka, K. (2012). Effect of supplementation of purple pigment from anthocyanin-rich corn (*Zea mays* L.) on blood antioxidant activity and oxidation resistance in sheep. *Livestock Science, 145* (1–3), 266–270. https://doi.org/10.1016/j.livsci.2011.12.001

Hurtado, N. H., & Charfuelan, C. (2019). Contribución a la caracterización y evaluación de la actividad antioxidante de las antocianinas del fruto de ivilan (Monnina Obtusifolia H.B.K). *Información Tecnológica, 30*(5), 81–90. https://doi.org/10.4067/S0718-07642019000500081

Hurtado, N. H., Morales, A. L., González-Miret, M. L., Escudero-Gilete, M. L., & Heredia, F. J. (2009). Colour, pH stability and antioxidant activity of anthocyanin rutinosides isolated from tamarillo fruit (*Solanum betaceum* Cav.). *Food Chemistry, 117*(1), 88–93. https://doi.org/10.1016/j.foodchem.2009.03.081

Janeiro, P., & Brett, A. M. O. (2007). Redox behavior of anthocyanins present in *Vitis vinifera* L. *Electroanalysis, 19*(17), 1779–1786. https://doi.org/10.1002/elan.200703941

Kang, M.-K., Lim, S. S., Lee, J.-Y., Yeo, K. M., & Kang, Y.-H. (2013). Anthocyanin-rich purple corn extract inhibit diabetes-associated glomerular angiogenesis. *PLoS ONE, 8* (11), e79823.

Lao, F., & Giusti, M. M. (2016). Quantification of purple corn (*Zea mays* L.) anthocyanins using spectrophotometric and HPLC approaches: Method comparison and correlation. *Food Analytical Methods, 9*(5), 1367–1380. https://doi.org/10.1007/s12161-015-0318-0

Lao, F., & Giusti, M. M. (2018). Extraction of purple corn (*Zea mays* L.) cob pigments and phenolic compounds using food-friendly solvents. *Journal of Cereal Science, 80*, 87–93. https://doi.org/10.1016/j.jcs.2018.01.001

Lao, F., Sigurdson, G. T., & Giusti, M. M. (2017). Health benefits of purple corn (*Zea mays* L.) phenolic compounds. *Comprehensive Reviews in Food Science and Food Safety, 16* (2), 234–246. https://doi.org/10.1111/1541-4337.12249

Liu, W., Yang, C., Zhou, C., Wen, Z., & Dong, X. (2019). An improved microwave-assisted extraction of anthocyanins from purple sweet potato in favor of subsequent comprehensive utilization of pomace. *Food and Bioproducts Processing, 115*. https://doi.org/10.1016/j.fbp.2019.02.003

Magaña-Cerino, J. M., Tiessen, A., Soto-Luna, I. C., Peniche-Pavía, H. A., Vargas-Guerrero, B., Domínguez-Rosales, J. A., … Gurrola-Díaz, C. M. (2020). Consumption of nixtamal from a new variety of hybrid blue maize ameliorates liver oxidative stress and inflammation in a high-fat diet rat model. *Journal of Functional Foods, 72*. https://doi.org/10.1016/j.jff.2020.104075

Maier, T., Schieber, A., Kammerer, D. R., & Carle, R. (2009). Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants. *Food Chemistry, 112*(3), 551–559. https://doi.org/10.1016/j.foodchem.2008.06.005

Monroy, Y. M., Rodrigues, R. A. F., Sartoratto, A., & Cabral, F. A. (2016). Optimization of the extraction of phenolic compounds from purple corn cob (*Zea mays* L.) by sequential extraction using supercritical carbon dioxide, ethanol and water as solvents. *The Journal of Supercritical Fluids, 116*, 10–16. https://doi.org/10.1016/j.supflu.2016.04.011

Mora-Rochín, S., Gaxiola-Cuevas, N., Gutiérrez-Uribe, J. A., Milán-Carrillo, J., Milán-Noris, E. M., Reyes-Moreno, C., … Cuevas-Rodríguez, E. O. (2016). Effect of traditional nixtamalization on anthocyanin content and profile in Mexican blue maize (*Zea mays* L.) landraces. *LWT - Food Science and Technology, 68*, 563–569. https://doi.org/10.1016/j.lwt.2016.01.009

Moreira, V., Stanquevis, R., Amaral, E. P., Lajolo, F. M., & Hassimotto, N. M. A. (2021). Anthocyanins from purple maize (*Zea mays* L.) downregulate lipopolysaccharide-induced peritonitis in mice by modulating the MyD88 signaling pathway. *PharmaNutrition, 16*, Article 100265. https://doi.org/10.1016/j.phanu.2021.100265

Paucar-Menacho, L. M., Martínez-Villaluenga, C., Dueñas, M., Frias, J., & Peñas, E. (2017). Optimization of germination time and temperature to maximize the content of bioactive compounds and the antioxidant activity of purple corn (*Zea mays* L.) by response surface methodology. *LWT - Food Science and Technology, 76*, 236–244. https://doi.org/10.1016/j.lwt.2016.07.064

Ramos-Escudero, F., Muñoz, A. M., Alvarado-Ortíz, C., Alvarado, Á., & Yáñez, J. A. (2012). Purple corn (*Zea mays* L.) phenolic compounds profile and its assessment as an agent against oxidative stress in isolated mouse organs. *Journal of Medicinal Food, 15*(2), 206–215. https://doi.org/10.1089/jmf.2010.0342

Ranilla, L. G., Huamán-Alvino, C., Flores-Báez, O., Aquino-Méndez, E. M., Chirinos, R., Campos, D., … Shetty, K. (2019). Evaluation of phenolic antioxidant-linked in vitro bioactivity of Peruvian corn (*Zea mays* L.) diversity targeting for potential management of hyperglycemia and obesity. *Journal of Food Science and Technology, 56*(6), 2909–2924. https://doi.org/10.1007/s13197-019-03748-z

Remini, H., Dahmoune, F., Sahraoui, Y., Madani, K., Kapranov, V. N., & Kiselev, E. F. (2018). Recent advances on stability of anthocyanins. *RUDN Journal of Agronomy and Animal Industries, 13*(4), 257–286. https://doi.org/10.22363/2312-797X-2018-13-4-257-286

Rosero, J. C., Cruz, S., Osorio, C., & Hurtado, N. (2019). Analysis of phenolic composition of byproducts (seeds and peels) of avocado (*Persea americana* Mill.) cultivated in Colombia. *Molecules, 24*(17), 3209. https://doi.org/10.3390/molecules24173209

Sánchez-González, N., Jaime-Fonseca, M. R., San Martin-Martinez, E., & Zepeda, L. G. (2013). Extraction, stability, and separation of betalains from opuntia joconostle cv. Using response surface methodology. *Journal of Agricultural and Food Chemistry, 61* (49), 11995–12004. https://doi.org/10.1021/jf401705h

Semwal, D., Semwal, R., Combrinck, S., & Viljoen, A. (2016). Myricetin: A dietary molecule with diverse biological activities. *Nutrients, 8*(2), nu8020090. https://doi.org/10.3390/nu8020090

Solarte, N., Cejudo-Bastante, M. J., Hurtado, N., & Heredia, F. J. (2022). First accurate profiling of antioxidant anthocyanins and flavonols of *Tibouchina urvilleana* and *T. mollis* edible flowers aided by fractionation with Amberlite XAD-7. *International Journal of Food Science and Technology, 57*(4), 2416–2423. https://doi.org/10.1111/ijfs.15600

Suriano, S., Balconi, C., Valoti, P., & Redaelli, R. (2021). Comparison of total polyphenols, profile anthocyanins, color analysis, carotenoids and tocols in pigmented maize. *LWT, 144*, Article 111257. https://doi.org/10.1016/j.lwt.2021.111257

Tian, X. Z., Paengkoum, P., Paengkoum, S., Chumpawadee, S., Ban, C., & Thongpea, S. (2019). Short communication: Purple corn (*Zea mays* L.) stover silage with abundant anthocyanins transferring anthocyanin composition to the milk and increasing antioxidant status of lactating dairy goats. *Journal of Dairy Science, 102*(1), 413–418. https://doi.org/10.3168/jds.2018-15423

Urias-Lugo, D. A., Heredia, J. B., Muy-Rangel, M. D., Valdez-Torres, J. B., Serna-Saldívar, S. O., & Gutiérrez-Uribe, J. A. (2015). Anthocyanins and phenolic acids of hybrid and native blue maize (*Zea mays* L.) extracts and their antiproliferative activity in mammary (MCF7), liver (HepG2), colon (Caco2 and HT29) and prostate (PC3) cancer cells. *Plant Foods for Human Nutrition, 70*(2), 193–199. https://doi.org/10.1007/s11130-015-0479-4

Zhang, Q., Gonzalez de Mejia, E., Luna-Vital, D., Tao, T., Chandrasekaran, S., Chatham, L., … Kumar, D. (2019). Relationship of phenolic composition of selected purple maize (*Zea mays* L.) genotypes with their anti-inflammatory, anti-adipogenic and anti-diabetic potential. *Food Chemistry, 289*, 739–750. https://doi.org/10.1016/j.foodchem.2019.03.116