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Phenolic profiles of Andean purple corn (*Zea mays* L.)

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Abstract

Qualitative and quantitative high performance liquid chromatography with diode array detection (HPLC-DAD) was performed to characterize the presence of phenolic compounds in Andean purple corn. Phenolic compounds were analyzed by separating them in two main fractions: a water fraction (WF) and an ethyl acetate fraction (EAF). The WF rich in anthocyanins revealed the presence of cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside. The respective acylated anthocyanin glucoside forms of these compounds were also detected following alkaline hydrolysis. The EAF was composed of phenolic acids such as *p*-coumaric, vanillic acid, protocatechuic acid, flavonoids such quercetin derivatives and a hesperitin derivative. Alkaline and acid hydrolysis of the EAF revealed the presence of *p*-coumaric and ferulic acid as main components in four bound hydroxycinnamic acid forms present in the ethyl acetate fraction.

Keywords: Andean purple corn; Anthocyanins; Phenolic profiles

1. Introduction

Purple corn has been cultivated for centuries in the Andean Region. In Peru, people consume a typical drink made from purple corn called "Chicha Morada" which is believed by folklore use to improve health (Brack-Egg, 1999). Purple corn is an important source of anthocyanins, natural pigments widely distributed in the plant kingdom with peculiar features regarding coloring of foods. Anthocyanins already characterized in purple corn cobs and seeds include cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their respective malonated counterparts (Aoki, Kuze, & Kato, 2001; Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2002).

Anthocyanins are attributed with many biological activities. Antioxidant, antimutagenic and anticarcinogenic activities of anthocyanins have been extensively reported (Bomser, Madhavi, Singletary, & Smith, 1996; Kähkönen & Heinonen, 2003; Kamei et al., 1995; Katsube, Iwashita, Tsushida, Yamaki, & Kobori, 2003; Remeuf, Dorange, & Dupuy, 1983; Yoshimoto, Okuno, Kumagai, Yoshinaga, & Yamakawa, 1999; Yoshimoto, Okuno, Yamaguchi, & Yamakawa, 2001). In addition, anthocyanins have been utilized in the treatment of various blood circulation disorders resulting from capillary fragility (Wang, Cao, & Prior, 1997), in vaso-protective and anti-inflammatory studies (Lietti, Cristoni, & Picci, 1976), in the inhibition of platelet aggregation (Morazzoni & Magistretti, 1986), and in the maintenance of normal vascular permeability (Wang et al., 1997).

Beneficial health-related effects of other non-anthocyanin phenolic compounds have also been extensively reported including antioxidant (Adom & Liu, 2002; Cakir et al., 2003; Dewanto, Wu, & Liu, 2002; Friedman, 1997; Ghiselli, Nardini, Baldi, & Scaccini, 1998; Lien, Ren, Bui, & Wang, 1999; Rice-Evans, Miller, & Paganga, 1996; Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002; Wang & Lin, 2000); antimutagenic (Cardador-Martinez, Castaño-Tostado, & Loarca-Piña, 2002; Edenharper, Keller, Platt, & Unger, 2001; Edenharper, von Petersdoff, & Rauscher, 1993; Miyazawa & Hisama, 2003; Wang & Lee, 1996), anticarcinogenic activity (Ferguson, 2001; Hertog, Van Poppel,

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& Verhoeven, 1997; Iwashita, Kobori, Yamaki, & Tsushida, 2000; Katsube et al., 2003; Loo, 2003; Surh, 1999; Yang, Landau, Huang, & Newmark, 2001) and other biological properties.

In general, corn is considered an important crop worldwide. There have been some studies in the identification and quantification of phenolic compounds from yellow corn including *p*-hydroxybenzoic acid, vanillic, protocatechuic, syringic, *p*-coumaric, ferulic, caffeic and sinapic acids (Adom & Liu, 2002; Dewanto et al., 2002; Gueldner, Snook, Widstrom, & Wiseman, 1992; Hedin & Callahan, 1990; Snook et al., 1995; Sosulski, Krygier, & Hogge, 1982). However, the characterization of non-anthocyanin phenolic compounds present in purple corn has not been reported to our knowledge.

Most of the properties attributed to purple corn extracts, including coloring attributes (Duhard, Garner, & Megard, 1997; Cevallos-Casals & Cisneros-Zevallos, 2004), antioxidant (Cevallos-Casals & Cisneros-Zevallos, 2003a), antimicrobial (Cevallos-Casals & Cisneros-Zevallos, 2003b), anti-obesity activity and amelioration of hyperglycemia (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003), and anticarcinogenic properties (Hagiwara et al., 2001) were related to anthocyanins. Thus, there is need in characterizing other phenolic compounds present in purple corn in addition to anthocyanins. We believe, these phenolic compounds might be involved in health-related properties and could have been missed in previous studies in which bioactive properties were attributed to anthocyanins only.

The objectives of this study were: (1) The identification and quantification of the main anthocyanins present in Andean purple corn extracts and (2) the identification and quantification of other phenolic compounds present in Andean purple corn extracts using HPLC-DAD as a tool.

2. Materials and methods

2.1. Sample material, standards and reagents

Purple corn extract (PCE) was kindly given by Fitofarma (Lima, Peru). The powder extract was prepared by extraction of ground cobs (mesh 60) in a 60% aqueous ethanol solution at room temperature for 48 h (1 kg cobs/7L solvent). The obtained extract was filtered and spray dried (180 °C inlet and 85 °C outlet temperatures) using maltodextrins as carrier (0.5 kg maltodextrin/100 L extract). One kilogram of dried PCE contained ~40% maltodextrin.

Anthocyanin standards composed of a mixture of pelargonidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside were purchased from Polyphenols Laboratories AS (Sandnes, Norway). The anthocyanin aglycons from bilberry: cyanidin, pelargonidin, peonidin, petunidin, delphinidin and malvidin were purchased from ChromaDex™ (Santa Clara, CA, USA). Phenolic acids (vanillin, *p*-coumaric, protocatechuic, ferulic acid, gallic acid, benzoic acid), flavonols (quercetin, rutin, myricetin, kaempferol), flavones (apigenin, luteolin), flavanone (hesperitin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). For tannin determination, catechin and vanillin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sugar standards D-glucose, rhanmnose, galactose and organic acids: malonic acid, fumaric acid, quinic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

HPLC grade solvents: acetonitrile, methanol, hydrochloric acid were purchased from EM Science (a division of EM Industries Inc., Gibbstown, NJ, USA). Trifluoroacetic acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other chemicals such as sodium hydroxide, potassium hydroxide were purchased from Sigma Chemical Co, (St. Louis, MO, USA). Silica gel 60 F_{254} plates from Fisher Scientific (Fair Lawn, NJ, USA) were used in the separation and identification of sugars by thin layer chromatography. Diphenylamine and phosphoric acid were purchased from Riedel de Häen (Seelze, Germany) and aniline was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Sample preparation

A 0.5 g of purple corn extract (PCE) was partitioned in a decantation flask with 100 mL water and 400 mL of ethyl acetate. The mixture was shaken vigorously and let to stand until two phases were observed. The water fraction (WF) was freeze-dried in a freeze dryer FTS® Systems, Inc (Stone Ridge, NY, USA) at −50 °C and at 200 μm Hg of pressure. The ethyl acetate fraction (EAF) was concentrated at 240 mbar pressure in a roto-evaporator (Büchi, Switzerland) at 40 °C until dryness and then re-suspended in methanol. The dried WF and EAF represented 92% and 8% of the PCE by weight, respectively.

2.3. Tannin determination

For tannin determination the vanillic assay was carried out as recommended by Price, Van Scoyoc, and Butler (1978)

2.4. HPLC-DAD analysis of anthocyanins and other phenolic compounds

Phenolic compounds were separated using a binary Waters 515 HPLC pump system, a Waters 717 plus autosampler automated gradient controller, a SP8792 temperature controller and a Waters 996 Photodiode array detector. For peak integration a Millenium^{32} software from Waters (Milford, MA, USA) was used. An Atlantis™ C_{18} 5 μm, 4.6 mm × 150 mm column and a 4.6 mm × 20 mm guard column was used for the separation of phenolic compounds. The mobile phase was composed of solvent A (nanopure water adjusted at pH 2.3 with 2 N HCl) and solvent B (acetonitrile HPLC grade). The elution was as follows: isocratic conditions from 0 to 5 min with 85% A and 15% B. Gradient conditions from minute 5 to 30 starting with 85% A and

ending with 0%, and starting with 15% B and ending with 100%. Then, isocratic conditions from minute 30 to minute 35 with 0% A and 100% B. The flow rate was 1 mL/min and 10 μL of sample were injected (Hale, 2003). The temperature of the column was kept at 35 °C. Phenolic compounds were identified and quantified by comparing the retention time and UV–visible spectral data to known previously injected standards (1–100 ppm). Acid and alkaline hydrolysis of the samples were performed for the identification of phenolic compounds as described below.

2.5. Water fraction acid hydrolysis

The anthocyanin-rich water fraction (WF) was acid hydrolyzed according to the methodology proposed by Pascual-Teresa et al. (2002) for anthocyanins from purple corn cob. Twenty milligrams of the freeze dried WF was dissolved in 15 mL 6 M HCl and heated at 100 °C in a closed vial for 40 min. The hydrolysate was analyzed by HPLC-DAD for the identification of the anthocyanin aglycons. Retention time and UV–visible spectral data was compared with known anthocyanidin standards.

2.6. Water fraction alkaline hydrolysis

The anthocyanin-rich WF was hydrolyzed in alkali using the methodology proposed by Hong and Wrosltad (1990). Forty milligrams of the freeze dried WF was hydrolyzed (i.e., saponified) in a screw cap Falcon® tube with 10 mL 10% KOH for 8 min at room temperature in the dark after flushing the prepared tube with nitrogen. Following saponification, HCl was added to the solution until a red color appeared in the solution to stabilize anthocyanins (Pascual-Teresa et al., 2002). The hydrolysate was analyzed by HPLC-DAD for the identification of the released anthocyanin glucosides and corresponding acyl groups. Retention time and UV–visible spectral data was compared with known standards.

2.7. Ethyl acetate fraction alkaline hydrolysis

The EAF was saponified following the same protocol used by Llorach, Gil-Izquierdo, Ferreres, and Tomás-Barberán (2003). Alkaline hydrolysis was performed by adding 1 mL 4 N NaOH per each 1 mg of dried EAF and keeping it for 16 h in a screw capped Falcon® tube under a nitrogen atmosphere. Concentrated HCl was added (yellow color change, pH ~ 1.0) to the alkaline hydrolysis product and directly analyzed by HPLC-DAD. Alkaline hydrolysis served to release acyl groups from the phenolic compounds if present.

2.8. Ethyl acetate fraction acid hydrolysis

A 19.4 mg sample of the dried EAF was acid hydrolyzed using 5 mL 1.2 M HCl of a solution methanol: water (50:50) for 2 h at 90 °C as proposed by Hertog et al. (1997). The solution was adjusted to a final volume of 15 mL following cooling to room temperature. The purpose of this step was to release sugar molecules from phenolic compounds.

2.9. Study of the hydrolysis products (organic acids and sugars)

The organic acids released after hydrolysis were identified by HPLC-DAD according to the methodology proposed by Cawthray (2003). Organic acids were separated using a binary Waters 515 HPLC pump system, a Waters 717 plus autosampler automated gradient controller and a Waters 996 Photodiode array detector. Separation was performed on a Novapak C_{18} column (300 × 3.9 mm i.d.) from Waters. A guard column was used in-line prior to the analytical column. The mobile phase consisted of 93% 25 mM KH_2PO_4 adjusted to pH 2.5 with concentrated *ortho*-phosphoric acid and 7% methanol for 30 min at a flow rate of 1 mL/min. Separation was carried out at ambient temperature. Data was acquired and processed with Millenium$^{32®}$ chromatography software (Waters) with DAD acquisition from 190 to 400 nm. DAD output at 210 nm was used for identification of the organic acids.

Qualitative TLC analysis of sugars was performed using the methodology proposed by Toba and Adachi (1978). A small amount (5 μL) of concentrated sample was applied to the silica gel 60 F_{254} plates. Sugars were separated using propanol: water (85:15 v/v) as solvent. Presence of separated sugars was detected by submerging the prepared TLC plates in diphenylamine–aniline–phosphate revealing solution.

2.10. Statistical analysis

Quantitative data represent mean values with the respective standard deviation corresponding to 3 replicates.

3. Results and discussion

3.1. HPLC-DAD analysis of water fraction

Phenolic determination in the anthocyanin-rich WF was performed at 520 and 280 nm to verify the presence of anthocyanins and other phenolic compounds. The chromatographic profiles in this section correspond to the non-hydrolyzed, alkaline hydrolyzed and acid hydrolyzed water fraction at 520 nm (Fig. 1).

The chromatographic profile for the non-hydrolyzed WF indicated mainly the presence of six anthocyanins, however, small amounts of other phenolic compounds were also present. Comparison of retention times and UV–visible spectral data with known standards revealed the presence of cyanidin-3-glucoside (peak 1), pelargonidin-3-glucoside (peak 2) and peonidin-3-glucoside (peak 3). Peaks 4–6 did not match with any of the anthocyanin standards. The identified three anthocyanin glucosides in this study have been reported previously in purple corn cobs and seeds (Aoki et al., 2001; Pascual-Teresa et al., 2002).

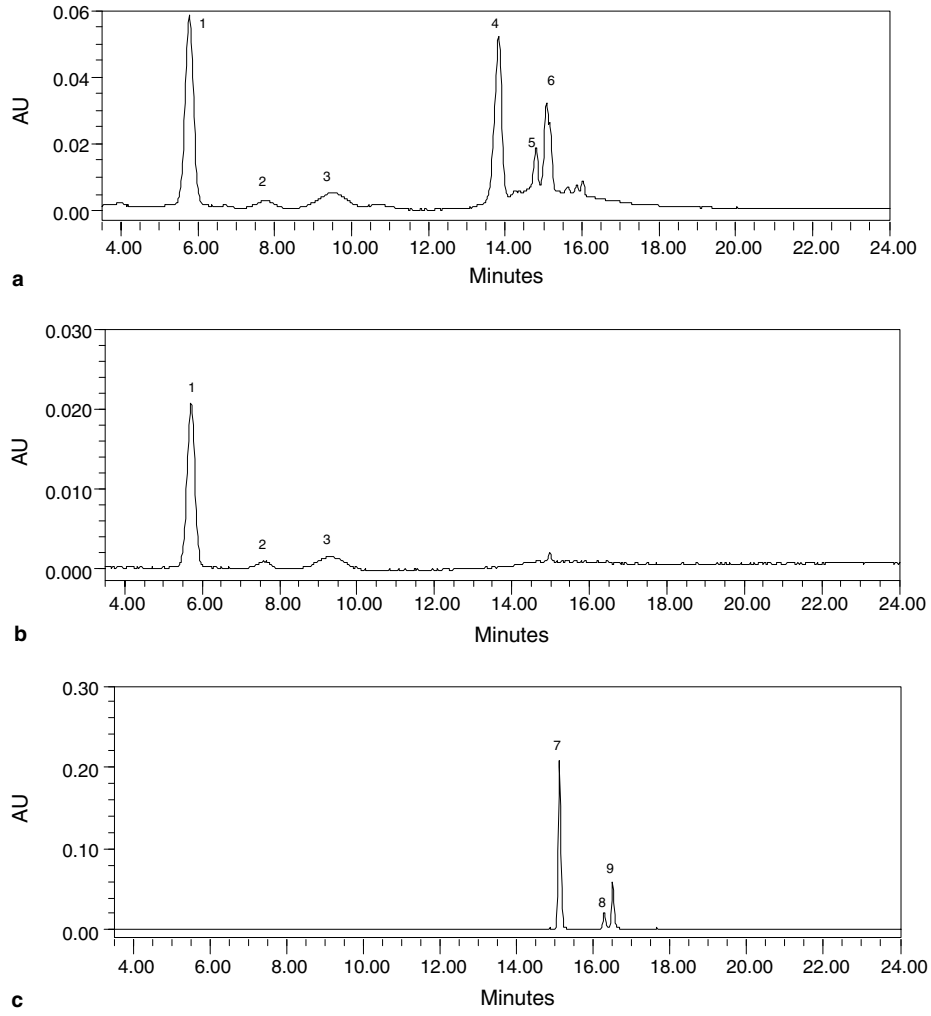

Fig. 1. Chromatographic profiles of (a) non-hydrolyzed, (b) alkaline-hydrolyzed and (c) acid hydrolyzed anthocyanin-rich water fractions (WF) at 520 nm. Samples (a) and (b) were injected at a concentration of 2.86 mg WF/mL and (c) injected at a concentration of 1.33 mg WF/mL.

After an alkaline hydrolysis, peaks 4–6 (Fig. 1(b)) yielded peaks of cyanidin-3-glucoside, pelargonidin-3-glycoside and peonidin-3-glycoside. Saponification of acylated anthocyanins produce glycosidic pigments plus the free acylating acids (Spanos & Wrolstad, 1990). Purple corn has been reported to have a significant contribution of acylated anthocyanins having as acylating group malonic acid. These anthocyanins include cyanidin-3-(6″-malonylglucoside), pelargonidin-3-(6″-malonylglucoside), and peonidin-3-(6″-malonylglucoside) as well as dimalonyl cyanidin-3-glucoside (Aoki et al., 2001; Pascual-Teresa et al., 2002). Ethyl-malonated anthocyanins have also been reported in purple corn extracts, however, it is considered to be formed during the extraction process when using 60% aqueous ethanol (1% citric acid) for 3 h at 60 °C (Pascual-Teresa et al., 2002). In this study, malonic acid could not be detected as the released acylating group since the detection of malonic acid is difficult to achieve by HPLC separation and the use of standards (Harborne & Self, 1987; Wrolstad, 2000) due to the labile nature of aliphatic acids to hydrolysis treatments.

Acid hydrolysis confirmed the presence of the three major aglycons corresponding to cyanidin (peak 7), pelargonidin (peak 8) and peonidin (peak 9) as determined with authentic anthocyanin aglycon standards and UV–visible spectral data (Fig. 1(c)). The retention time for the anthocyanin aglycons in purple corn (Fig. 1(c)) is longer than the retention times for the acylated anthocyanin glucosides found and confirmed with the alkaline hydrolysis test (Fig. 1(b)). According to Hong and Wrosltad (1990) when malonic acid is present as an acylating group in anthocyanins, it will most likely result in a decreased retention time when compared to the aglycons (Hong & Wrosltad, 1990) due to the polar nature of the malonic acid ester molecule. Similar HPLC elution pattern was observed in this study. The UV–visible spectral data of the acylated anthocyanin peaks did not show a characteristic 310 nm peak corresponding to acylation with a hydroxylated aromatic organic acid. Thus, this type of acylation was not present in anthocyanins.

In general, the presence of released glucose from the purple corn anthocyanins was confirmed with the TLC

qualitative analysis of sugars (data not shown) by previously separating the anthocyanin in a Sep-Pak cartridge.

Quantification of anthocyanins was only done in the non-hydrolyzed samples. The acylated anthocyanin glucosides are reported as their parent anthocyanin glucosides as shown in Table 1. Cyanidin-3-glucoside represented in terms of relative area at 520 nm the major type of anthocyanin in purple corn, with ~44% of the total anthocyanins, followed by the acylated cyanidin-3-glucoside which represented ~26.8%. Peonidin-3-glucoside and the corresponding acylated form represented ~9.9% and ~11%, respectively, while pelargonidin-3-glucoside and the corresponding acylated form represented smaller amounts of only ~3% and 4.6%, respectively. These relative amounts agree with those reported in the literature, except that acylated peonidin-3-glucoside in this study was slightly higher (~11% compared to 6% reported previously by Pascual-Teresa et al., 2002).

The tannin assay revealed the absence of flavan-3-ol compounds in the purple corn extract analyzed.

3.2. HPLC-DAD analysis of ethyl acetate fraction

The EAF injected in the HPLC was detected at different wave lengths (280–360 nm) to facilitate the identification of different phenolic compounds. The profiles presented on

Table 1
Quantification of anthocyanins present in the water fraction

Peak #	Retention time (min)	λ_{max} (nm)	Compound	Amount (mg/g WF)	Relative area$_{520\ nm}$
1	5.773	202.4; 279.1; 515.8	Cyanidin-3-glucoside	15.43 ± 1.59	44.37 ± 4.67
2	7.763	201.3; 275.5; 508.5	Pelargonidin-3-glucoside	2.33 ± 0.85	3.06 ± 0.58
3	9.508	198.9; 279.1; 515.8	Peonidin-3-glucoside	4.44 ± 1.09	9.97 ± 2.27
4	13.827	203.4; 279.1; 515.8	Acylated cyanidin-3-glucoside[a]	10.37 ± 3.83	26.87 ± 7.95
5	14.804	202.4; 276.7; 508.5	Acylated pelargonidin-3-glucoside[a]	2.83 ± 0.84	4.64 ± 0.85
6	15.079	202.0; 279.1; 515.8	Acylated peonidin-3-glucoside[a]	4.85 ± 0.71	11.16 ± 0.70

[a] Expressed as their anthocyanin glucoside counterparts.

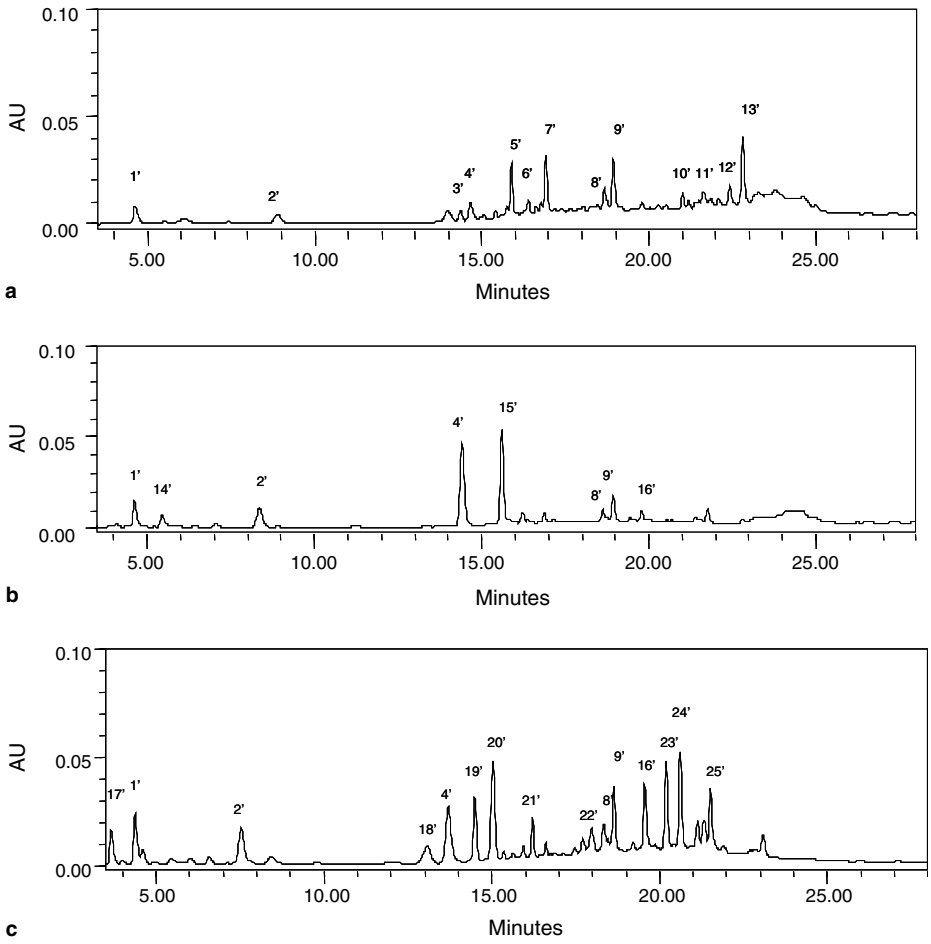

Fig. 2. Chromatografic profiles of (a) non-hydrolyzed, (b) alkaline-hydrolyzed and (c) acid-hydrolyzed ethyl acetate fractions (EAF) at 280 nm. Samples (a) and (b) were injected at a concentration of 0.42 mg EAF/mL and (c) injected at a concentration of 1.29 mg/mL.

the chromatograms in this section correspond to the non-hydrolyzed, alkaline-hydrolyzed and acid-hydrolyzed EAF at 280 nm (Fig. 2).

The affinity of flavonols for ethyl acetate due to its hydrophobic character has been reported by Krasteva, Nikolova, Danchev, and Nikolov (2004). The presence of 3,7-di-*O*-methylquercetin-5-glucoside has been found in yellow corn (Hedin & Callahan, 1990). Luteolins and other luteolin derivatives have been reported in corn silks (Gueldner et al., 1992). Vanillic acid, *p*-hydroxybenzoic acid, and protocatechuic acid has been found in yellow corn in small concentrations of 3.7, 1.3 and 3.0 μg/g, respectively (Shahidi & Naczk, 1995). The presence of kaempferol and quercetin in hydrolysates of the aleurone tissue of corn has been reported as well (Kirby & Styles, 1970).

In this study, the EAF contained a variety of different phenolic compounds such as phenolic acids and flavonols (Fig. 2). Peaks identified in non-hydrolyzed EAF correspond to protocatechuic acid (peak 1′), vanillic acid (peak 2′) and *p*-coumaric acid (peak 4′). Identification was based on retention time and UV–visible spectral data compared to known standards. The other peaks (peaks 3′, 5′–13′) could not be identified in the non-hydrolyzed EAF, thus hydrolysis treatments were used to obtain additional information.

After a 16 h alkaline hydrolysis, the chromatographic profiles of the hydrolysate lacked peaks 3′, 5′–7′, 10′–13′ (Fig. 2(b)), and yielded peaks 4′, 14′–16′. Peak 16′ corresponds to quercetin which showed a higher absorbance at 360 nm. Based on UV spectral data and the appearance of quercetin after the alkaline hydrolysis, peaks 5′–7′ in the non-hydrolyzed EAF can be classified as quercetin derivatives. Both peaks 6′ and 7′ showed maximum peak absorbance at 253.1 and 351.8 nm.

Ferulic acid (peak 15′) and an increase in *p*-coumaric acid (peak 4′) were identified in the hydrolysate. On the other hand, the UV–visible spectral data of peaks 10′–13′ in the non-hydrolyzed EAF presented similar characteristics among them indicating they may belong to the same phenolic family (UV absorption maximum at 310–326 nm). Thus, peaks 10′–13′ seem to correspond to bound forms of hydroxycinnamic acid composed mainly of ferulic or *p*-coumaric acid. The presence of hydroxycinnamic acid derivatives composed of *p*-coumaric and ferulic acids after alkaline hydrolysis has been reported previously in date palm callus with UV spectrum absorption maximum at 325 nm (Mounir & Ismail, 2004). The released peak 14′ could not be identified.

The UV spectral data of peak 8′ in non-hydrolyzed EAF matched with hesperitin (Merken, Merken, & Beecher, 2001), however, according to some authors (Justesen, Knuthsen, & Leth, 1998; Kirby & Styles, 1970) hesperitin should elute after quercetin in a reversed phase column. In this study results showed the opposite trend where quercetin (peak 16′) eluted after peak 8′. Hesperidin is the glycosylated form of hesperitin with the dissacharide rutinose composed of glucose and rhamnose. It was observed that the retention time of a standard hesperidin was shorter than the retention time for peak 8′ (Tr = 18.67 min). When hesperidin was acid hydrolyzed for 2 h at 90 °C with 1.2 M HCl, it yielded two peaks with very close retention times at 17.32 and 18.08 min. Peak 8′ would correspond to one of these two hesperitin derivatives since retention times and the UV–visible spectral data matched with these compounds obtained after the acid hydrolysis of hesperidin. Interestingly, peaks 8′ and 9′ did not disappeared under alkali or acid hydrolytic conditions (Fig. 2(a)–(c)). Peak 9′ remained unidentified.

The acid hydrolysis (Fig. 2(c)), showed the disappearance of peaks 3′, 5′–7′, 10′–13′ and the appearance of new peaks, 17′–25′. Cyanidin and pelargonidin appeared as aglycons (peaks 19′ and 21′) due to the acid hydrolysis performed (the corresponding anthocyanins were only present in the non-hydrolyzed EAF in traces). Peaks 18′ and 22′ in the hydrolyzate were tentatively identified as hydroxycinnamic acid derivatives due to their similarity to *p*-coumaric UV spectra, while peak 20′ had a UV–visible spectral data very similar to that of ferulic acid (Fig. 2(c)).

Table 2
Quantification of the phenolic compounds present in the ethyl acetate fraction

Peak #	Retention time (min)	λ_{max} (nm)	Compound	Amount (mg/g EAF)	Relative area$_{280\ nm}$
1′	4.62	204.8; 259; 293.3	Protocatechuic acid	14.61 ± 0.08	6.12 ± 0.07
2′	8.87	216.5; 259; 291	Vanillic acid	8.46 ± 0.09	6.29 ± 0.04
3′	14.36	200.1; 267.3	Unknown	6.98 ± 0.17	2.82 ± 0.11
4′	14.67	202.4; 224.8; 308.8	*p*-coumaric acid	Traces	4.00 ± 2.31
5′	15.90	202.4; 254.2; 353	Quercetin derivative	20.00 ± 0.54	11.02 ± 0.06
6′	16.40	198.9; 253.1; 351.8	Quercetin derivative	5.36 ± 0.26	2.10 ± 0.63
7′	16.92	202.4; 253.1; 351.8	Quercetin derivative	20.02 ± 1.44	14.48 ± 0.87
8′	18.67	197.8; 288.6	Hesperitin derivative	11.55 ± 1.24	6.00 ± 0.86
9′	18.95	197.8; 291; 315.9	Unknown	23.10 ± 0.65	14.02 ± 0.46
10′	21.01	198.9; 324.3	Hydroxycinnamic acid derivative	3.17 ± 0.37	3.11 ± 0.54
11′	21.64	200.1; 315.9	Hydroxycinnamic acid derivative	3.05 ± 0.86	2.94 ± 1.24
12′	22.44	198.9; 310	Hydroxycinnamic acid derivative	4.88 ± 0.03	5.55 ± 0.04
13′	22.81	197.8; 326.7	Hydroxycinnamic acid derivative	14.69 ± 0.34	19.56 ± 0.51

All the unknown compounds were quantified as protocatechuic acid. Quercetin derivatives were quantified as quercetin at 360 nm and the rest at 280 nm. Peaks 10′–13′ are bound hydroxycinnamic acid forms composed of ferulic and *p*-coumaric acid (quantified as ferulic acid at 280 nm).

We suspect peaks 18′, 20′ and 22′ were released from peaks 10′–13′, the bound forms of hydroxycinnamic acid present in the non-hydrolyzed EAF. Quercetin derivatives (peaks 5′–7′), present in the non-hydrolyzed EAF, disappeared in the hydrolysate while quercetin (peak 16′) and three quercetin derivatives (peaks 23′–25′) appeared at longer retention times (Tr > 20 min).

Flavonoids are very susceptible to hydrolysis and some loss may occur during this process (Rommel & Wrolstad, 1993; Wrolstad, 2000). In this study, the EAF acid-hydrolyzed samples had to be loaded in the HPLC system three times more concentrated to get enough absorbance to detect compounds released. Being aware that degradation can occur during acid and alkaline hydrolysis of phenolic compounds, quantification of the different compounds was done in the non-hydrolyzed EAF sample and reported in Table 2. Quercetin derivatives seem to be the major non-anthocyanin phenolic compounds present in Andean purple corn, followed by ferulic and *p*-coumaric acid derivatives. Other phenolic compounds are present in smaller amounts.

In general, the total amount of anthocyanins was ~40.25 mg/g WF (Table 1) while the total amount of non-anthocyanin phenolic compounds was ~132.85 mg/g EAF (Table 2). Since WF and EAF represented 92% and 8% of the PCE by weight, this implies that the anthocyanins and the other phenolic compounds represented ~77.7% and 22.3%, respectively, of the total phenolics present in the Andean purple corn extract.

4. Conclusion

In conclusion, HPLC-DAD analysis of purple corn extract (PCE) revealed the presence of anthocyanins and other phenolic compounds. The main anthocyanins present were identified as cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their respective acylated counterparts. Other phenolic compounds present were *p*-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperitin and quercetin and bound hydroxycinnamic acid forms composed of *p*-coumaric and ferulic acid. There are still some phenolic compounds that remained unidentified. The present study shows that non-anthocyanin phenolic compounds are diverse and present in significant amounts and should be taken into consideration when studying purple corn bioactive properties.

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