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Effects of Growing Conditions on Purple Corncob (*Zea mays* L.) Anthocyanins

PU JING,[†,§] VICTOR NORIEGA,[‡] STEVEN J. SCHWARTZ,[†] AND M. MÓNICA GIUSTI*,[†]

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, Ohio 43210-1096, and Departamento de Fitotecnia, Universidad Nacional Agraria, La Molina, Peru

Purple corn (*Zea mays* L.) has been used for centuries as a natural food colorant in South America and, more recently, in Asia and Europe. However, limited information is available on the factors affecting their anthocyanin concentration and profiles. In this study, 18 purple corn samples grown under different conditions in Peru were evaluated for quantitative and qualitative anthocyanin composition as well as total phenolics. High variability was observed on monomeric anthocyanin and phenolic contents with yields ranging from 290 to 1333 mg/100 g dry weight (DW) and from 950 to 3516 mg/100 g DW, respectively, while 30.5–47.1% of the total phenolics were anthocyanins. The major anthocyanins present were cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-maloylglucoside, pelargonidin-3-maloylglucoside, and peonidin-3-maloylglucoside, and 35.6–54.0% of the anthocyanins were acylated. Potassium sources/concentrations on the soil and seedling density did not significantly affect anthocyanin composition. The growing location affected anthocyanin levels and the percentage of anthocyanins to total phenolics ($p < 0.01$) and should be taken into account when choosing a material for color production.

KEYWORDS: Purple corncob; anthocyanins; solubility; location; processing; waste

INTRODUCTION

Anthocyanins are a class of flavonoid compounds responsible for the bright attractive orange, red, purple, and blue colors of most fruits and vegetables. There is considerable demand in the world for natural food colorants as a result of both legislative action and consumer concerns over the use of synthetic additives in their foods. Anthocyanins are a potential alternative to the use of FD&C Red No. 40, the synthetic dye with the highest consumption in the United States (*1*). However, anthocyanins usually exert low tinctorial power; therefore, large doses are needed to reproduce similar results to those obtained with small amounts of synthetic dyes, making them a comparatively expensive alternative. Identification of low-cost anthocyanin-rich sources with increased stability is very important to their practical applications in food industries.

Purple corn (*Zea mays* L.) is a rich source of anthocyanins. For centuries, purple corn has been cultivated in South America, mainly in Peru and Bolivia, and used to prepare traditional drinks and desserts. Interest in purple corn as a source of anthocyanins as colors and phytonutrients has increased over the years. Recently, anthocyanins from purple corn have been associated with possible health benefits. They have been found to have high antioxidant activities (*2*), reduce the systolic blood pressure of spontaneously hypertensive rats (*3*), and prevent obesity and diabetes in mice (*4*). A purple corn colorant was found to inhibit cell mutation induced by 2-amino-1-methyl-6-phenylimidazo pyridine (PhIP) (*5*) and to reduce chemically induced colorectal carcinogenesis (*6*).

Six major anthocyanins (cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-maloylglucoside, pelargonidin-3-maloylglucoside, and peonidin-3-maloylglucoside) were reported in commercial purple corn extracts (*7*) and purple corn kernels (*8*). In addition, cyanidin-3-dimalonylglucoside was found in purple corn kernels (*8*), and a (epi)catechin-cyanidin-3,5-glucoside was tentatively identified in commercial purple corncob colorant (*9*). Acylated anthocyanins are generally considered better food colorants since acylation is believed to confer increased stability to the anthocyanin moiety during processing and storage (*1*). However, environmental factors including visible light and UV radiation, cold temperature, drought, and water stress have been shown to induce anthocyanin accumulation in plants (*10*). In addition, the application of potassium fertilizer has shown an impact on anthocyanin synthesis in Fuji apple callus (*11*) and roselle plants (*12*).

* To whom correspondence should be addressed. Tel: 614-247-8016. Fax: 614-292-0218. E-mail: giusti.6@osu.edu.

[†] The Ohio State University.

[§] Present address: Department of Food Science, College of Food and Biotechnology, Jiangsu University, 301 Xuefu Rd., Zhenjiang, Jiangsu 212013, People's Republic of China.

[‡] Universidad Nacional Agraria.

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Table 1. Properties of 18 Samples of Purple Corncob with Monomeric Anthocyanins and Total Phenolics[a]

ID	place	seeding density	monomeric anthocyanins (mg/100 g)[c]	acylated anthocyanins (%)	total phenolics (mg/100 g)[d]	% monomeric anthocyanins to total phenolics
1	Arequipa	D1	290 (24)	36.2	950 (162)	30.3 (2.99)
2	Arequipa	D2	384 (22)	40.2	998 (19)	38.6 (3.02)
3	Cajamarca	D1	515 (2.6)	54.0	1202 (35)	47.1 (5.36)
4	Cajamarca	D2	584 (25)	49.3	1373 (90)	42.8 (4.39)
5	Canta	D1	355 (9.3)	37.1	1105 (36)	32.3 (1.76)
6	Canta	D2	506 (25)	42.3	1360 (149)	37.2 (3.99)
7	La Molina	D1	513 (25)	35.6	1420 (57)	36.2 (1.87)
8	La Molina	D2	519 (18)	36.9	1431 (87)	36.4 (1.28)
9	Pacaran	D1	519 (9)	42.7	1375 (47)	37.9 (1.27)
10	Pacaran	D2	376 (80)	39.1	1014 (29)	37.3 (1.49)

[a] D1 and D2 were two seeding densities: 50000 and 62500 plant/ha, respectively. Values are represented as means (standard error) ($n = 4$). [b] Calculation was based on 100 g of dry weight (DW). [c] Cyanidin-3-glucoside equivalent. [d] Gallic acid equivalent.

Table 2. Effect of Potassium on Levels of Anthocyanins and Total Phenolics in Purple Corncobs[a]

ID	sample	monomeric anthocyanins (mg/100 g)	total phenolics (mg/100 g)	% monomeric anthocyanins to total phenolics	% acylated anthocyanins
11	O-K	1092 (63)	3223 (215)	33.89 (2.04)	38.11
12	60-K	1114 (108)	3240 (247)	34.40 (1.43)	40.18
13	90-K	1002 (163)	2860 (160)	35.11 (1.95)	39.73
14	120-K	1011 (156)	2910 (240)	34.55 (3.77)	41.38
15	SK-60	1193 (113)	3330 (296)	35.86 (1.39)	39.71
16	SK-120	1333 (124)	3445 (260)	38.58 (3.03)	37.40
17	CK-60	1323 (57)	3516 (236)	37.58 (3.49)	38.56
18	CK-120	1153 (94)	3237 (244)	35.49 (2.10)	39.86

[a] The values 0, 60, 90, and 120 were the potassium treatment concentrations from 0, 60, and 120 kg/ha. SK and CK were potassium sulfate and potassium chloride at two concentrations. Values are represented as means (standard error) ($n = 4$).

In this study, we evaluated the anthocyanin content and profile of purple corn cultivar PM-581 (*Z. mays* L. cv. La Molina) grown in different locations and under different seeding densities. In addition, we also investigated the total phenolic content. Effects of fertilization with potassium were also evaluated. We were interested in the ratios of anthocyanins and phenolics since we have previously discussed the potential interactions of anthocyanins from purple corncob with other corncob constituents such as large molecular weight phenolics. These compounds may affect the quality of the colorant by forming insoluble complexes during colorant production (*13*). Our research should provide information to better understand anthocyanins in purple corncobs and provide fundamental knowledge for the cultivation and utilization of purple corncobs as anthocyanin-rich sources for natural food colorants and value-added ingredients.

MATERIALS AND METHODS

Materials and Reagents. A total of 18 different samples of powdered purple corncob cultivar PM-581 (*Z. mays* L. cv. La Molina) grown under different conditions in 2000 were provided by the Universidad Nacional Agraria (La Molina, Peru). Samples with IDs 1–10 were grown at five different locations in Peru: Arequipa, Cajamarca, Canta, La Molina, and Pacaran. Two seeding densities, 50000 and 62500 plant/ha, were used (**Table 1**). Samples with IDs 11–18 were grown with four different potassium levels on the soil and using two different potassium sources as fertilizers (**Table 2**).

Folin–Ciocalteu phenol reagent and the gallic acid standard (crystalline gallic acid, 98% purity) were purchased from Sigma (St. Louis, MO). All high-performance liquid chromatography (HPLC) grade solvents and other chemicals (analytic grade) were from Fisher Scientific (Fair Lawn, NJ).

Anthocyanin Extraction. About 2 g of each purple corncob powder was added to a flask containing 25 mL of 70% aqueous acetone acidified by 0.01% HCl and mixed well. All flasks were shaken on a platform shaker (LabScientific, Inc., New Jersey, USA) at 80 rpm at 10 °C for one hour. Samples were filtered through a Whatman #1 filter paper under vacuum conditions using a Büchner funnel, and the slurry was washed with 10 mL of acidified 70% acetone. The filtrate was transferred to centrifuge tubes mixing well with 15 mL of chloroform and centrifuged at 2000 rpm for 10 min at 4 °C, and the upper aqueous layer, containing the acetone/water mixture, was collected while the chloroform/acetone layer was carefully discarded. Residual acetone and chloroform were removed from the anthocyanin extract by using a rotary evaporator at 40 °C under vacuum conditions. Extracts were taken to 25 mL in a volumetric flask by 0.01% HCl-acidified water. Every treatment had three replicates.

Monomeric Anthocyanins. The total monomeric anthocyanin content was measured by the pH differential method (*14*). A Shimadzu UV–visible spectrophotometer (Shimadzu Corp., Tokyo, Japan) was used to measure the absorbance at 420, 510, and 700 nm. Monomeric anthocyanins were calculated as cyanidin-3-glucoside equivalents, using the extinction coefficient of 26900 L cm^{-1} mg^{-1} and a molecular weight of 449.2 g/L.

Total Phenolics. Total phenolics were measured using a modified Folin–Ciocalteu method (*15*). Briefly, a series of tubes were prepared with 15 mL of water and 1 mL of Folin–Ciocalteu reagent. Then, 1 mL of samples, gallic acid dilutions (standards), and water blank was added into tubes, mixed well, and left to stand at room temperature for 10 min. The 20% Na_2CO_3 solution (3 mL) was added to each test tube and mixed well before they were put in a dry-bath incubator (Fisher Scientific) at 40 °C for 20 min. After incubation, tubes were immediately cooled down in an ice bath. The absorbance of samples and standards was measured at 755 nm using a Shimadzu UV–visible spectrophotometer. Total phenolics were calculated as gallic acid equivalents based on a gallic acid standard curve.

HPLC Coupled to PDA and MS Detectors. A reverse-phase HPLC system (Shimadzu Corp.) consisted of a LC-20AD prominence liquid chromatograph (LC), a SPD-M20A prominence diode array detector, a SIL-20AC prominence autosampler at 4 °C, and a LCMS-2010EV LC–mass spectrometer. LCMS solution Version 3.30 software was used.

For the columns and mobile phase, the reversed-phase 3.5 μm Symmetry C18 column (4.6 mm × 150 mm, Waters Corp., MA) fitted

with a 4.6 mm × 22 mm Symmetry 2 microguard column (Waters Corp.) was used. Solvents and samples were filtered though 0.22 μm GE Magna* nylon membrane filters (Fisher Scientific). Separation was achieved by using a gradient mobile phase as follows: 5–25% B, 0–15 min; 25% B, 15–25 min; 25 to 5% B, 25–28 min. Solvent A was 5% (v/v) formic acid in water, and B was 100% acetonitrile. An injection volume of 15 μL with a 0.5 mL/min flow rate was used. Spectral information over the wavelength range of 254–700 nm was collected.

A 0.25 mL/min flow was diverted to a mass spectrometer. Mass spectrometry (MS) was conducted on a quadrupole ion-tunnel mass spectrometer equipped with an ESI interface (Shimadzu Corp.). Mass spectrometric analyses were performed in a positive ion mode under the tuning automode and other specific conditions as follows: nebulizing gas flow, 1.5 L/min; interface bias, +4.50 kV; block temperature, 200 °C; focus lens, −2.5 V; entrance lens, −50.0 V; prerod bias, −3.6 V; main rod bias, −3.5 V; detector voltage, 1.5 kV; and scan speed, 2000 amu/s. A full scan was performed with a mass range from 200 to 1500 *m*/*z*.

Statistical Design and Analysis. The comparison of the 18 purple corncobs was carried out as a randomized complete block design. Each block was performed as a batch that contained duplicates of 18 samples. One-way analysis of variance (ANOVA) was applied to analyze the experimental data in which factors (seeding density, culture location, type, and concentration of potassium salts) were represented. The least significance difference (LSD) test was conducted to evaluate mean differences in a general linear univariate model. All analyses were performed by SPSS (version 14.0, SPSS Inc., IL) software. For all statistics, $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Anthocyanins and Total Phenolics in Purple Corncobs. A qualitative and quantitative analysis of anthocyanin composition was done on 18 purple corn samples grown under different conditions. High variability was observed on the concentration of anthocyanins and total phenolics (**Tables 1** and **2**) among the samples. The monomeric anthocyanins content ranged from 290 to 1333 mg cyanidin 3-glucoside equivalents/100 g of dry matter, while the total phenolic content ranged from 950 to 3516 mg/100 g of dry matter as gallic acid equivalents. On the basis of these numbers, 31–47% of the total phenolics were anthocyanins. A high level of anthocyanins and a high percentage of anthocyanins over the total phenolics present would be desirable for purple corncob colorant processing and application. The levels of monomeric anthocyanins found in purple corncobs in this experiment were comparable to the levels reported for kernel pericarp (504–1473 mg/100 g) (*16*) but lower than a report of about 1640 mg anthocyanins/100 g in purple corn (*2*). Differences can be due to a variety of factors including the cultivar, growing conditions, and even the method used for anthocyanin quantitation. The report that found the highest concentration of anthocyanins in purple corn (*2*) measured anthocyanin concentration based on a single pH method (instead of the pH differential or HPLC methods) with pigment dissolved in ethanol, where anthocyanins are known to exhibit higher molar absorptivity (*17*).

Purple corncobs with ID numbers from 1 to 10 were cultivated at different locations and different densities of seeding (**Table 1**). The seeding density did not show a significant effect on the anthocyanin content in purple corncobs determined by one-way ANOVA ($p > 0.10$). Growing location—Arequipa, Cajamarca, Canta, La Molina, and Pacaran—however, had a significant impact on anthocyanins levels ($p < 0.01$). The purple corncobs that were cultured in Cajamarca, located in the northern highlands of Peru (altitude 2720 m above sea level; latitude 07.05S, longitude 78.28W), had higher levels of anthocyanins and percentages of anthocyanins to total phenolics than in other growing locations (all south of Cajamarca) determined by LSD comparison ($p = 0.02$). The purple corncobs grown in Arequipa, in southern Peru (altitude 2300 m above sea level; latitude 16.20S, longitude 71.30W) contained the lowest level of anthocyanins ($p < 0.05$). However, anthocyanin levels in purple corncobs cultivated in Canta, La Molina, and Pacaran, all in Lima (sea level; latitude 12.0S, longitude 77.0W), in the coast of Peru, were not significantly different ($p > 0.10$) among them. There were no significant differences in the percentages of anthocyanins to total phenolics among purple corncobs that were cultivated in Arequipa, Canta, La Molina, and Pacaran, except the Cajamarca where purple corncobs obtained a significantly higher ratio of anthocyanins to total phenolics (average 45.0%, $p < 0.001$). Our findings suggest that climatic conditions may have a critical impact on anthocyanin accumulation in purple corncob. The average temperature of the three locations in Lima was similar ranging from 18 to 19 °C, with little fluctuation during the day (*18*). However, the average temperatures in Cajamarca and Arequipa were lower (14–16 °C) with more marked contrasts between the temperatures between day and night. In addition, the moisture and precipitation in these areas are quite different: Cajamarca showed an average precipitation of 708 mm in the year 2000, while for the same year Arequipa had only 155 mm and Lima barely 8 mm (*18*). For this reason, Cajamarca relies mostly on natural precipitation while Arequipa and Lima use more sophisticated irrigation systems. Environmental stress factors including visible light and UV radiation, cold temperature, drought, and water stress have been shown to induce anthocyanin accumulation in plants (*10*). The stress of the plant during growth conditions in Cajamarca may have favored anthocyanin accumulation in purple corncobs. On other hand, the Peruvian Ministry of Agriculture reported (*19*) that the typical yield of corn in Lima and Arequipa is usually higher (twice as much or more) as for Cajamarca in the year 2000, suggesting that it may still be more reasonable to grow corn for pigment production in Arequipa or Lima if the pigment content of the corn is only slightly higher in Cajamarca. Therefore, conditions that favor high yields of anthocyanins in purple corncobs may be different from those that favor high yields of corn. Future research should evaluate which environmental factors have an effect of anthocyanin variation in purple corncobs and how these conditions may affect the yields of corn.

Purple corncobs whose identification numbers were from 11 to 18 were cultivated at different levels of potassium (0–120 kg/ha) or different potassium salts (potassium sulfate or potassium chloride) (**Table 2**). Potassium phosphate has been reported to enhance anthocyanin synthesis in Fuji apple callus at 10 mmol/L but decrease anthocyanin concentrations when the treatment concentration was increased (*11*). Also, the combination of fertilizer (nitrogen, phosphorous, and potassium) and growth regulator was found to increase the anthocyanin content on roselle plants (*12*). However, in our study, we did not find a significant difference on the anthocyanin concentration of purple corncobs receiving different concentrations or forms of potassium salt applied.

Anthocyanin Profiles in Purple Corns. There were 10 peaks found in purple corncobs in **Figure 1** and **Table 3**, with a maximum visible wavelength of absorbance around 520 nm. Their spectra of absorbance together with their molecular masses and fragmentation pattern information were used to determine their identities. The two major anthocyanins present were cyanidin-3-glucoside (peak 2, *m*/*z* 499), and cyanidin-3-maloylglucoside (peak 6, *m*/*z* 535) in all samples analyzed. In addition, pelargonidin-3-glucoside (peak 3, *m*/*z* 433), peonidin-3-glucoside (peak 4, *m*/*z* 463), cyanidin-3-maloylglucoside (peak

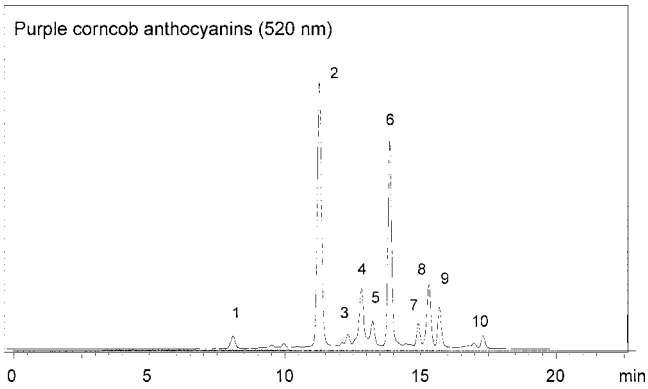

Figure 1. Anthocyanin profiles of purple corncob (*Z. mays* L.).

Table 3. Qualitative Analyses of Anthocyanins Presenting in Purple Corncobs[a]

peak	compound	λ_{max} (nm)	*m/z* (amu) [M]$^+$	*m/z* (amu) fragments
1	flavanol-anthocyanin[b]	528	899	449, 306
2	Cy 3- glc	516	449	287
3	Pg 3-glc	505	433	271
4	Pn 3-glc	516	463	303
5	Cy-3-(malonyl)glc	517	535	287
6	Cy 3-(malonyl)glc	518	535	287
7	Pg 3-(malonyl)glc	508	519	271
8	Pn 3-(malonyl)glc	520	549	301
9	Cy-3- dimalonylglc	520	621	287
10	unknown	521	635	475, 306

[a] Cy, cyanidin; Pg, pelargonidin; Pe, peonidin; and glc, glucoside. [b] Tentative identification.

5, *m/z* 535,), pelargonidin-3-maloylglucoside (peak 7, *m/z* 519), and peonidin-3-maloylglucoside (peak 8, *m/z* 549) were also found in purple corncob. These results are consistent with published literature for commercial purple corn extracts and purple corn kernels (*7, 8*). There were two isomers present in purple corncob as cyanidin-3-malonylglucoside (peaks 5 and 6). In this study, peak 9 gave the *m/z* value of 621 and was tentatively assigned as cyanidin-3-dimalonylglucoside, which was found to be present in purple corn kernels by Aoki and co-workers (*8*). To our knowledge, this is the first report on the multiacylation anthocyanin present in purple corncobs. Peak 1 had a maximum absorbance at 528 nm and gave a *m/z* of 899 and fragments of 449 and 306. This molecular mass and fragmentation pattern do not match the typical anthocyanin structures reported for purple corn or typically found in fruits and vegetables. However, the same molecular mass had been previously reported by Pascual-Teresa (*7*) and González-Paramás (*9*). The molecular mass of the fragment at 449 matches the molecular mass of cyanidin + hexose. The fragment at *m/z* 306 matches the molecular mass of flavanol (*m/z* 306). The two fragments together, plus a second sugar (hexose), would match the *m/z* of 899 and might explain the short retention time observed. Therefore, a suggested identity for this compound could be a dimer of flavanol and cyanidin-3,5-diglucoside, which was also tentatively identified as a (epi)catechin-cyanidin-3,5-glucoside to be present in commercial purple corncob colorant in a previous study (*9*). Peak 10 had a maximum absorbance at 521 nm. The *m/z* ratio was 635 and a fragmentation pattern (*m/z* 475 and 306) that did not match the *m/z* of the anthocyanins commonly found in nature. Although the smaller fragment matches the small fragment found in peak 1, we do not have enough information to propose a tentative identification for this compound. Further work would be needed to clarify the chemical structure of these compounds structurally by nuclear magnetic resonance (NMR).

The proportion of acylated anthocyanins in purple corncobs varied from 35.59 to 53.98% of total monomeric anthocyanins (**Tables 1** and **2**). The highest content of acylated anthocyanins (49–54 % of total monomeric anthocyanins) was found in purple corncobs grown in Cajamarca. Acylation of sugar substitution with aliphatic acids donates electrons to chromophores and leads to a bathochromic shift and a hyperchromic effect (*20*) and also contributes to an important stabilizing effect on anthocyanins via intermolecular interaction (*1*). Consequently, acylated anthocyanins with increased stability may impart desirable color and stability for commercial food products (*1*). Hence, not only the total monomeric anthocyanins content and the ratio of anthocyanins and total phenolics but also the percentage of acylated anthocyanins should be considered for purple corn colorants, which can provide more desirable color and stability for food products.

In conclusion, purple corncobs are rich in anthocyanins, the level of which was greatly affected by growing location. From all of the samples evaluated in this study, purple corncob from Cajamarca (Peru) contained the highest value of monomeric anthocyanins, the highest ratio of anthocyanins to total phenolics, and the highest proportion of acylated anthocyanins. However, no effects of seeding density and potassium fertilization on anthocyanin and/or phenolic content and profiles were found. Both of the ratios of anthocyanins to other phenolics (purity) and the contents of acylated anthocyanins varied greatly in purple corn. The anthocyanin concentration, the anthocyanin purity (ratio of anthocyanins to other phenolics), and the proportion of acylated anthocyanins in purple corncobs should be considered for selection of plant material most suitable for colorant production.

ABBREVIATIONS USED

DW, dry weight; HPLC, high-performance liquid chromatograph; LC, liquid chromatograph; MS, mass spectrometry; LSD, least significance difference; NMR, nuclear magnetic resonance.

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