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Corn Husk as a Potential Source of Anthocyanins

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Anthocyanin pigments are extracted from various plants and used for diverse purposes. The overall goal of this study was to develop high-anthocyanin corn to enhance the economic efficiency of anthocyanin production. We determined and compared the anthocyanin contents from the different parts of purple corn in various breeding lines. Our results revealed that purple corn produced the anthocyanin pigment throughout the plant, especially high in the husk and cob regions, although anthocyanin levels varied significantly among different plant parts. We analyzed the 295 selected lines from the 2006 breeding population, and it showed that anthocyanin levels of husks ranged from 17.3% to 18.9% of dry weight, roughly 10 times more than the standard current purple corn kernel content, 1.78%. LC−MS/MS analysis demonstrated that the main components of purple corn husk anthocyanin were cyanidin derivatives, and the most prevalent constituents were cyanidin-3-glucoside, cyanidin-3-succinylglucoside and pelargonidin-3-(6″-malonylglucoside). The results suggested that high-anthocyanin corn will boost the purple corn pigment production far more than its current level.

KEYWORDS: Purple corn; husk; anthocyanin; breeding population

INTRODUCTION

Placing pigments into edible products significantly affects consumer selection of and attraction to those products; therefore, food colorings are fairly ubiquitous in manufactured food products. Pigments used in food manufacturing processes are classified as "natural" or "artificial" on the basis of their sources. Natural pigments have become increasingly popular with consumers because artificial pigments tend to be perceived as undesirable and harmful (*1*−*4*).

Anthocyanins are natural pigments found in at least 250 kinds of plants (*5*), usually within their seeds, roots, and fruit. Purple corn pigment, which is anthocyanin pigment extracted from purple corn, has been used for diverse purposes in the food industry (*6, 7*). A total of 10 classes of anthocyanin compounds have been identified within purple corn pigment, including cyanidin-3-glucoside(C-3-G), pelargonidin-3-glucoside (Pg-3-G), and peonidin-3-glucoside (Pn-3-G) (*8*). In addition to their applications in food coloring, recent work demonstrated that anthocyanin pigments possessed various physiological activities, such as hydroxyl and superoxide radical scavenging (*9*−*11*) and growth inhibition of colon cancer cells (*12*). C-3-G possesses authentic antioxidant and anti-inflammatory effects in vivo (*13*−*19*). There are even reports that anthocyanin mediates an antiobesity effect when consumed at high levels in an experimental diet (*6*). These studies suggest possible applications for anthocyanins that extend far beyond its current use as a food coloring.

Using mass selection and the pedigree method, we are currently developing corn varieties that express high levels of anthocyanin throughout the plant to maximize the anthocyanin production from the corn crop. The desirable varieties are not yet established, but we estimate that, upon its completion, its high color production rate will greatly enhance the economic efficiency of anthocyanin production. In this study, we investigated the anthocyanin contents from the different parts of the purple corn in the breeding lines, and food industry application possibilities for all parts of this corn plant were revealed.

MATERIALS AND METHODS

Plant material. The corn lines used in this study originated from pod corn (*Zea mays* L.) collected in North America in 2000. The corn lines were maintained by self-ing and sibbing until 2004. The breeding population of the purple corn for the anthocyanin analysis was maintained by mass selection focusing on the husk until now. In 2006, 295 lines were sampled for investigation. The sampled materials were dried at 50 °C in a drying oven and then stored at room temperature. Before being analyzed, the corn was separated into leaves, stems, and ears, the ears being further separated into husks, kernels, and cobs. These materials were powdered using home food processors.

Extraction of Anthocyanin. Anthocyanin compounds were extracted three times from the powdered samples with 10 volumes of methanol containing 1% HCl at 4 °C for 12 h per extraction. The extracts were

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Table 1. Anthocyanin Levels from Different Parts of the Purple Corn Plant

plant	anthocyanin level[a] (%)		
	husk	cob	leaf
1	13.18	4.60	0.14
2	1.25	0.74	0.75
3	1.69	0.98	0.18
4	11.28	3.21	0.79
5	12.83	2.48	0.27
6	1.49	0.49	0.12
7	11.19	2.27	0.93
8	1.27	1.36	0.62
9	1.99	1.52	0.77
10	11.34	3.88	0.14

[a] Data expressed as C-3-G equivalents.

Table 2. Anthocyanin Levels from Purple Corn Husks in the Breeding Population in 2006

anthocyanin level (%)	line
17.1−19.0	2
15.1−17.0	4
13.1−15.0	16
11.1−13.0	23
9.1−11.0	83
7.1−9.0	61
5.1−7.0	43
3.1−5.0	25
1.1−3.0	19
∼1.0	19
total	295

combined, filtered, and redissolved in 1% HCl/MeOH solvents, and their absorbance at 535 nm was measured to detect anthocyanin. Anthocyanin levels were expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of wet or dry weight (where indicated), using the reported molar extinction coefficient of 25 965 M^{-1} cm^{-1} and a molecular weight of 449 g/mol (*11*).

HPLC Analysis. Anthocyanin compounds were separated by dual online detection using diode array spectrophotometry and mass spectrometry. For HPLC, Agilent 1100 series equipment was fitted with a quaternary pump and a photodiode array detector. The column was an Eclipse XDB C_{18}, 5 μm, 4.6 mm × 150 mm or 4.6 mm × 20 mm guard column, maintained at 23 °C. The mobile phase was a gradient of solvent A (0.1% trifluoroacetic acid) to solvent B (acetonitrile, HPLC grade), and anthocyanin elution proceeded as follows: isocratic 10% B for 5 min, from 10% to 15% B over 15 min, isocratic 15% B for 5 min, from 15% to 18% B over 5 min, and from 18% to 35% B over 20 min, at a flow rate of 1 mL min^{-1}. Extracted compounds were detected spectrophotometrically at 520 nm.

Mass Spectrometry. Mass spectrometry was performed using an Agilent 3200 Q TRAP LC−MS/MS equipped with an API source, using an electrospray ionization interface. The capillary voltage and temperature were 5500 V and 700 °C, respectively, and the fragmentor was 160 V. Both the auxiliary and sheath gases were a mixture of nitrogen and helium at flow rates of 12 and 1.3 L min^{-1}, respectively, and the nebulizer pressure was set at 60 psi. Spectra were recorded in the positive ion mode.

2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Activity. The free radical scavenging capacities of the samples were determined according to a previously reported procedure employing the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) (*20*), and the results were used to obtain IC_{50} values for each sample. The IC_{50} value was defined as the concentration required for a sample to quench 50% of the free radicals in the reaction mixture under the experimental conditions.

RESULTS

Anthocyanin Levels. The purple corn lines were collected in 2000 and maintained by self-ing and sibbing until 2004. To obtain information on the anthocyanin pigments in the 2004 breeding population of the purple corn, 10 purple corn plants were sampled at random, and the different parts were analyzed for anthocyanin pigments. The results showed that anthocyanin pigments appeared in various parts of the plant, including the leaf, husk, and cob. The husk was shown to contain the highest contents of the pigments. The kernels showed various colors ranging from yellow to red brown, with no anthocyanin pigment accumulation. Therefore, the husk was focused on for the purposes of the breeding population, and from 2005, the mass selection method was used to maintain genetic diversity. In 2006, 295 lines were sampled for an analysis of the anthocyanin pigments in this population. As shown in **Tables 1** and **2**, the highest measured anthocyanin levels in the breeding population ranged from 17.3% to 18.9%. Most of the lines, 28% of the 83 total lines, ranged between 9.1% and 11.0%.

Identification of Anthocyanins in Different Plant Parts. Extracts of the different parts of the corn (husk, cob, and leaf) were prepared, and the constituents of the anthocyanin pigment in the extracts were analyzed using LC−MS/MS. By subjecting the methanol extracts to HPLC and subsequently determining the mass spectra for the peaks detected at 520 nm, the peaks were identified by comparison with the reference standards or by the LC−MS/MS data (*8*), and thereby we identified nine anthocyanins in the various parts of the purple corn (**Table 3**). The most prevalent compounds identified from the husks, leaves, and cobs were C-3-G and cyanidin derivatives.

DPPH Radical Scavenging Activity. The previous studies had identified antioxidant activity within anthocyanins; we examined the radical scavenging capabilities of purple corn husk methanol extract (CHME) and its most prevalent constituents identified in our LC−MS/MS study. For these experiments, we subjected CHME, C-3-G, Pg-3-G, Pn-3-G, cyanidin, and pelargonidin to a DPPH-based assay, using α-tocopherol as a positive control. The estimated amounts of each substance required to clear 50% of the radicals are shown in **Figures 1** and **2**. Our results indicated that the IC_{50} value of CHME was 28 μg mL^{-1}, similar to that of α-tocopherol (IC_{50} = 27.3 μg mL^{-1}). The isolated components of purple corn husk pigment displayed radical scavenging abilities as follows: C-3-G > Pn-

Table 3. Composition of MS Peaks Obtained from Different Parts of Purple Corn

peak	anthocyanin	MS/MS		relative quantity (%)		
		fragmention	molecular ion	husk	cob	leaf
1	cyanidin-3-glucoside	287	449	39.8	20.9	45.0
2	pelargonidin-3-glucoside	271	433	2.0	1.5	2.4
3	peonidin-3-glucoside	301	463	0	0	0
4	cyanidin-3-malonylglucosyl-5-glucoside	287, 535	597	0.3	6.2	0.5
5	cyanidin-3-(6″-malonylglucoside)	287, 449, 535	697	8.4	34.5	11.3
6	pelargonidin-3-(6″-malonylglucoside)	271	519	11.0	3.6	9.2
7	cyanidin-3-succinylglucoside	287, 449	549	20.8	14.8	15.8
8	cyanidin-3-(6″-(ethylmalonyl)glucoside)	287, 449	563	7.7	13.0	9.9
9	peonidin-3-(6″-malonylglucoside)	301	549	2.5	0.7	3.2
10	cyanidin derivative	287	549	7.4	4.8	2.9

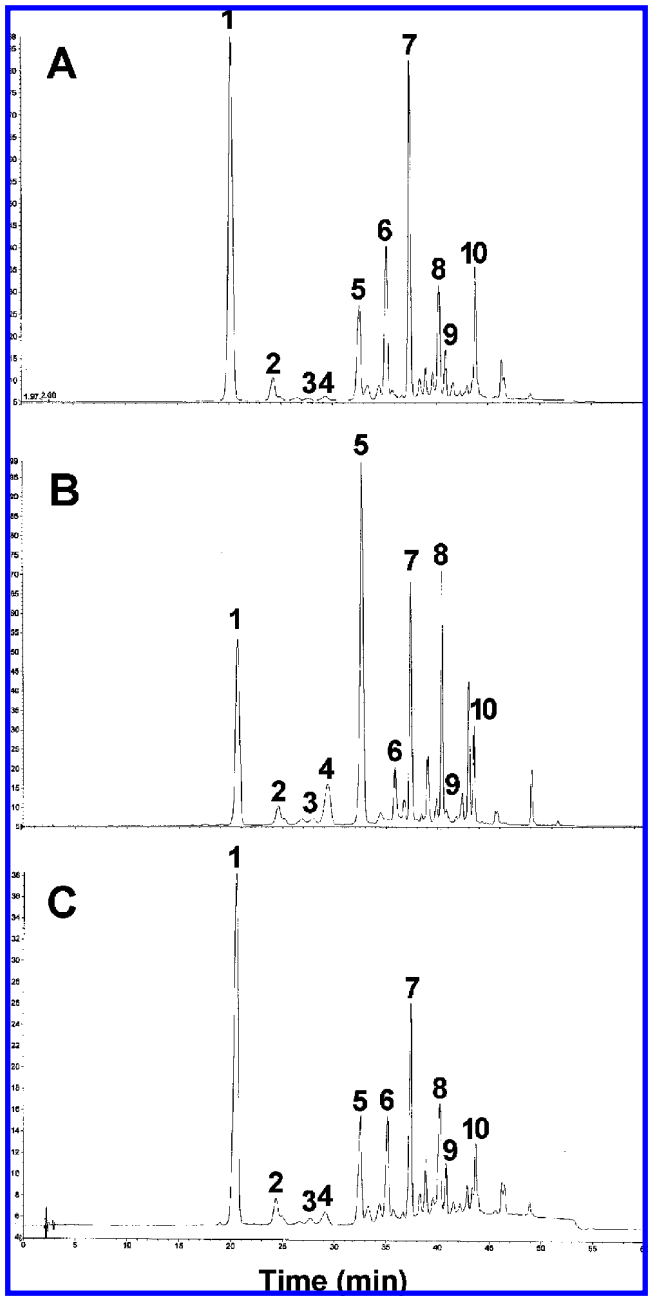

Figure 1. HPLC chromatograms of peaks detected at 520 nm for the various extracts from the different parts of purple corn: **A**, husk; **B**, cob; **C**, leaf. The peaks were identified by comparison with the reference standards or by the LC−MS/MS data (*8*). The peak numbers are shown in **Table 3**.

3-G > Pg-3-G. On the basis of our estimations, the DPPH radical scavenging ability of the anthocyanin pigment of CHME was accounted for by the radical scavenging abilities of the major constituents identified herein.

DISCUSSION

The anthocyanins are gaining recognition as natural pigments because of their safety as a food colorant and their putative nutraceutical abilities (*9*−*14*). Herein, we utilized an LC−MS/MS approach to identify the constituents of an anthocyanin from purple corn.

Around 50 tons of purple corn pigment was used in Japan, and anthocyanin pigments are also increasing in the world as food (*6*). For this reason, other groups have made various

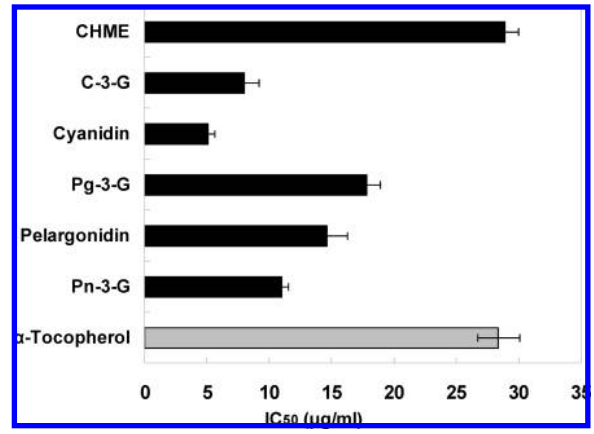

Figure 2. DPPH radical scavenging activity of anthocyanins: CHME, corn husk methanol extract; C-3-G, cyanidin-3-glucoside; Pg-3-G, pelargonidin-3-glucoside; Pn-3-G, peonidin-3-glucoside. Reported values are the mean ± SD ($N = 3$).

attempts at extracting similar pigments from black crops (e.g., black rice and purple sweet potatoes) (*11, 21*) to satisfy the expanding anthocyanin market.

Currently, purple corn pigment is extracted from the purple corn kernel and cob, which contain mostly C-3-G and has been used a food source for centuries in South America (*8*) and elsewhere. In addition to the current study, other studies have explored extracting pigment from regions of the cob other than the kernel (*22, 23*). In fact, herein we verified that anthocyanin was distributed throughout all parts of the purple corn plant, not just the kernel, and these results are supported by several other studies. One report identified C-3-G, Pg-3-G, Pn-3-G, cyanidin-3-(6″-malonylglucoside), and cyanidin-3-(3″,6″-dimalonylglucoside) in the kernel (*24*−*26*). Another isolated C-3-G, Pg-3-G, Pn-3-G, cyanidin-3-(6″-malonylglucoside), pelargonidin-3-(6″-malonylglucoside), and peonidin-3-(6″-malonylglucoside) in the cob (*8, 22*). Recently, another previous study demonstrated that C-3-G and cyanidin-3-rhamnoside and several of their derivatives were acylated with malonic acid in the leaves and flowers of the purple corn plant (*27*).

In the present study, we extracted the pigment from the husks and identified nine compounds similar to those identified in previous reports for other parts of the purple corn plant. As shown in **Table 3**, we validated that the main compound in the husks, cobs, and leaves was C-3-G. We also demonstrated that CHME possessed potent DPPH radical scavenging activity ($IC_{50} = 28\ \mu g\ mL^{-1}$) that was comparable to that of α-tocopherol ($IC_{50} = 27.3\ \mu g\ mL^{-1}$). In support of this observation, in a previous study employing a linoleic acid autoxidation system, CHME exhibited a more potent antioxidant effect than BHT, implying that CHME may be a capable food preservative (*28*). It also shows potential as a supplement to boost antioxidant levels in certain foods.

This study ascertained that the anthocyanin level in the leaves was small and the cobs contained less pigment than the husk. Reported anthocyanin levels in wheat, barley, corn, and rice ranged between 0.7 and 327.6 mg/100 g (*21*); additionally, sweet potato averaged 618 mg/100 g (dry weight) (*24*), blueberries between 925 and 2404 mg/100 g (dry weight) (*11*), purple corn kernels between 50 and 1779 mg/100 g (dry weight) (*11, 24*), and purple corncobs between 290 and 1333 mg/100 g (dry weight) (*22*); within these studies, the maximum measured anthocyanin level was 2404 mg/100 g (dry weight). Importantly, our results in **Table 2** demonstrated a maximum anthocyanin level in purple corn husk of 18 900 mg/100 g, approximately

10 times more than the maximum level measured in the kernel (1779 mg/100 g) (*11*). This phenomenon was not an isolated fluke; in fact, husks from 189 purple corn lines demonstrated anthocyanin levels well above 1779 mg/100 g, representing 68% of the total population. Since the anthocyanin levels in purple corn pigment products are around 2−20% (*7*), our data suggested that, by themselves, husks possess enough anthocyanin to be used as a food additive. Furthermore, we showed that the cobs contained around 3% anthocyanin. This value was lower than that obtained for the husks, but the pigments from the husks and cobs did possess a similar anthocyanin composition. Therefore, both the husks and cobs of purple corn represent a largely untapped resource for anthocyanins for the coloring production industry.

In this study, we used the high-anthocyanin purple corn as the main breeding material. In the silking date, we proceeded with bulk pollen control and mass selection for the breeding population of high-anthocyanin purple corn lines. We improved the populations by consistently selecting, for the next generation, plants containing more than 10% anthocyanin in the dried husk. From 2005, we conducted the pedigree system and the back-cross program together, finally achieving, in Korea and Thailand in 2007, 433 segregating lines using shuttle breeding, from which some selected lines were crossed for the preliminary yield trial.

Currently, however, the main extraction source for purple corn pigment is limited to the kernel and cob. The results of the study herein suggest that anthocyanin extraction from the husk and cob increases the higher pigment production by purple corn breeding. We are sure that these purple corn lines will boost the purple corn pigment production far more than its current level.

ABBREVIATIONS USED

C-3-G, cyanidin-3-glucoside; CHME, purple corn husk methanol extract; DPPH$^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; Pg-3-G, pelargonidin-3-glucoside; Pn-3-G, peonidin-3-glucoside.

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