Timing and Pattern of Anthocyanin Accumulation during Grain Filling in Purple Waxy Corn (Zea mays L.) Suggest Optimal Harvest Dates

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ABSTRACT: Purple-corn kernels contain anthocyanins, a group of antioxidants proposed to be beneficial to human health. This study investigated the concentrations of anthocyanins and amino acids and the composition of fatty acids in the kernels of purple waxy corn (Zea mays L.) “Heukjinjuchal” during grain filling to determine when the grain nutritional value is at its highest. During grain filling, anthocyanin contents increased as the kernel color darkened. Among the anthocyanins measured, cyanidin-3-β-D-glucoside reached the highest contents, 57.0–409.1 mg kg\(^{-1}\) fresh weight in raw kernels and 1027.6 mg kg\(^{-1}\) in dry seeds. Pelargonidin-3-β-O-glucoside and malvidin-3-β-O-glucoside became detectable at 21 days after silking; they occurred in the second- and third-highest amounts, respectively, among anthocyanins in the purple-corn cultivars tested. The anthocyanin accumulation pattern was strongly associated with physicochemical properties and partly associated with amino acid content. Anthocyanin contents increased in a stepwise rather than linear fashion. This study showed that kernels undergo dramatic changes that affect the nutritional value of fresh corn.

1. INTRODUCTION

Waxy corn (Zea mays L.) is increasingly consumed as a fresh vegetable in Asian countries\(^1\,^2\) and purple waxy corn is preferred by consumers due to the health-promoting properties of the anthocyanin pigments in the aleurone or pericarp. In purple corn, anthocyanins are reported to have antimutagenic and anticancer properties as well as antioxidant and antiobesity activities and can scavenge free radicals.\(^3\,^4\,^5\)

Anthocyanins, a large and diverse group of water-soluble flavonoid pigments, are glycosylated polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrilium, which contains 15 carbons in a C6–C3–C6 arrangement, with two aromatic rings connected by a three-carbon bridge. Hundreds of anthocyanins have been reported\(^6\,^7\,^8\) and naturally occurring anthocyanins have various colors depending on the pH, temperature, and light intensity.\(^9\,^10\) Strack and Wray\(^11\) classified naturally occurring anthocyanins according to five functional groups: common basic structures, common methylated structures, 3-deoxy structures, rarely hydroxylated structures, and rarely methylated structures. Anthocyanins are biosynthesized from malonyl CoA and p-coumaroyl CoA by chalcone synthase. Malonyl CoA is derived from the fatty acid biosynthesis pathway and p-coumaroyl CoA from phenylalanine. The analysis of anthocyanins is complex due to their capacity to undergo structural transformation and multiplex reactions.\(^12\)

Many reports have profiled the anthocyanins of various corn cultivars and landraces. For example, Abdel-Aal et al.\(^13\) and Aoki et al.\(^14\) reported that purple corn contains the anthocyanins cyanidin-3-O-β-D-glucoside, pelargonidin-3-O-β-D-glucoside, peonidin-3-O-β-D-glucoside, cyanidin-3-O-β-D-(g-malonyl-glucoside), pelargonidin-3-O-β-D-(g-malonyl-glucoside), and peonidin-3-O-β-D-(g-malonyl-glucoside). However, the daily changes in anthocyanin profiles during seed maturation have not been previously reported. Furthermore, the relationships among anthocyanin accumulation, their precursor amino acids, and fatty acid have not yet been reported in purple waxy corn. Here, we evaluated the concentrations and profiles of anthocyanins in the kernel of the purple waxy corn “Heukjinjuchal”, one of Korea’s leading cultivars, during grain filling. Our findings suggest that
anthocyanin and amino acid contents should be considered during the production and breeding of waxy purple corn to help maximize nutritional value.

2. RESULTS AND DISCUSSION

2.1. Profiling Anthocyanins in Corn Seeds. Of 11 anthocyanin standards, cyanidin-3-β-O-glucoside (C3G), pelargonidin-3-β-O-glucoside (P3G), and malvidin-3-β-O-glucoside (M3G), were well-separated and occurred at a higher content than others in the purple-corn cultivars, whereas no anthocyanin was detected in the two yellow-corn cultivars or in B73 (Figure 1). The three specific anthocyanins were further confirmed by mass spectrometry (Supporting Information Figure S1). The purple color of the three cultivars accumulated in the aleurone but not in the pericarp, when the pericarp was filled out from a kernel. The C3G content of Heukjinjuchal, “Suwon90”, and “Miheugchal” were 1027.6 ± 26.7, 338.0 ± 13.7, and 314.2 ± 29.8 mg kg⁻¹, respectively, in the fully matured and dried kernels (Figure 1C). Heukjinjuchal had the highest anthocyanin content and was therefore selected for further anthocyanin profiling during grain filling.

2.2. Color Changes and Anthocyanin Content during Grain Filling. Colored kernels began to appear at 12 days after silking (DAS) at the middle of the cob. Pigmentation continuously increased up to 30 DAS, spreading to the proximal and distal ends of the cob (Figure 2A). As grain filling took place, the number and peak absorbance intensity also increased (Figure 2B). C3G was first detected in kernels at 16 DAS and was the most abundant anthocyanin across the developmental stages analyzed. P3G and M3G began to be

Figure 1. Anthocyanin amounts in five waxy-corn cultivars and B73. The kernels were fully matured and dried. (A) Photographs of six corn cultivars used in high-performance liquid chromatography (HPLC) analysis. Bar = 1 cm. (B) HPLC chromatograms of the six cultivars. Three major anthocyanins, cyanidin-3-β-O-glucoside (C3G), pelargonidin-3-β-O-glucoside (P3G), and malvidin-3-β-O-glucoside (M3G), are shown. The six cultivars are labeled with different colors. The retention times for three anthocyanins are indicated in the parenthesis. Standard anthocyanin chromatogram is shown in yellow. (C) Amounts of three anthocyanins in three purple-colored cultivars.

Figure 2. Anthocyanin accumulation in kernels of the purple-corn cultivar Heukjinjuchal during grain filling. (A) Representative corn cob pictures during grain filling. (B) Ultraperformance liquid chromatography chromatogram of the three major anthocyanins in kernels of purple-corn Heukjinjuchal. Cyanidin-3-β-O-glucoside (C3G), pelargonidin-3-β-O-glucoside (P3G), and malvidin-3-β-O-glucoside (M3G) are shown. (C) Changes in anthocyanin content during grain filling. Error bars represent standard deviations of three biological replicates.
detected at 21 DAS (Figure 2C). Anthocyanin content rose continuously during the grain-filling stages, and sharp increases were detected at 24 and 29 DAS. The C3G content ranged from 57.1 to 409.7 mg kg\(^{-1}\) fresh weight during grain filling. The P3G and M3G contents ranged from 48.4 to 135.9 and from 21.1 to 120.3 mg kg\(^{-1}\) of fresh weight, respectively. The graph shows that anthocyanin mass increases took place in a stepwise manner with linear accumulation (\(R^2 > 0.89\); Figure 2C). The results show that the anthocyanin accumulation in purple corn largely occurs during grain filling.

Aoki et al.\(^{15}\) showed that cyanidin, pelargonidin, and their malonolylated derivatives are present in purple-corn seeds. Another study found that C3G and P3G were the major anthocyanins, in red and pink colored corn, respectively.\(^{14}\) Salinas Moreno et al.\(^{16}\) found that cyanidin in colored corn contained important anthocyanins and that C3G is the major anthocyanin component of the purple-corn seeds. In addition, the present study showed that C3G is a major anthocyanin component during grain filling.

Lopez-Martinez et al.\(^{17}\) found that the total anthocyanin content of Mexican corn strains ranges from 15.4 to 8509.0 mg Lugo et al.\(^{18}\) measured 646.0 corn hybrid seeds. In Heukjinjuchal, C3G totaled 1027.6 mg 2C). The results show that the anthocyanin accumulation in purple corn largely occurs during grain filling.

2.3. Correlations between Anthocyanin Content and Physicochemical Properties.

We measured the physical properties of kernels during grain-filling stages (Table 1). Dry weight continuously increased from 17 to 30 DAS. Fresh weight tended to increase during grain filling, but there were several decrements related to moisture content. An analysis of the correlation among the three anthocyanins detected in Heukjinjuchal and physicochemical characteristics (Table 2) showed that the anthocyanin content displayed a strong positive correlation coefficient with fresh weight and dry weight and a negative correlation with the moisture content. We attribute two sharp C3G increments observed at 29 DAS in part to the moisture content, which was lower at that time. Increased fresh weight due to starch accumulation and decreased moisture in purple-corn kernel during grain-filling stages has previously been shown.\(^{20}\)

For the analysis of seed color, the correlation analysis of lightness, redness, and yellowness with the anthocyanin content is shown in Table 3. Lightness (L\(^{*}\)) and yellowness (b\(^{*}\)) had negative correlations with the anthocyanin content, whereas redness (a\(^{*}\)) had a positive correlation with the anthocyanin content, showing that the corn kernel color is derived from the accumulation of anthocyanins and is a good indicator of anthocyanin accumulation.

| Table 1. Physical Parameters of Purple-Corn Kernels at Different Ripening Stages\(^{4}\) |
|---------------------------------|-----------------|-----------------|-----------------|
| DAS\(^{a}\) | fresh weight (g/100 kernel) | moisture content (%) | dry weight (g/100 kernel) | dry matter accumulation\(^{b}\) |
|---|---|---|---|
| 15 | 15.0 ± 0.2 | 65.7 ± 1.4 | 5.1 ± 0.1 | 33.8 ± 1.2 |
| 16 | 16.5 ± 1.2 | 54.6 ± 3.6 | 7.4 ± 0.5 | 45.0 ± 4.6 |
| 17 | 18.5 ± 0.9 | 62.2 ± 0.9 | 6.8 ± 0.4 | 37.2 ± 3.7 |
| 18 | 17.0 ± 0.5 | 57.3 ± 1.4 | 7.2 ± 0.4 | 42.1 ± 1.4 |
| 19 | 20.1 ± 0.5 | 61.0 ± 0.9 | 8.1 ± 0.3 | 40.1 ± 2.7 |
| 20 | 20.8 ± 1.4 | 57.6 ± 2.5 | 8.8 ± 0.7 | 42.3 ± 2.7 |
| 21 | 18.7 ± 0.6 | 51.8 ± 1.3 | 8.9 ± 0.3 | 47.7 ± 2.2 |
| 22 | 19.5 ± 0.7 | 52.2 ± 0.6 | 9.2 ± 0.5 | 47.5 ± 3.5 |
| 23 | 18.6 ± 1.0 | 49.7 ± 0.8 | 9.2 ± 0.4 | 49.7 ± 0.5 |
| 24 | 19.7 ± 1.1 | 51.4 ± 2.7 | 9.5 ± 1.1 | 48.3 ± 2.6 |
| 25 | 20.9 ± 0.4 | 53.4 ± 0.8 | 9.7 ± 0.2 | 46.3 ± 1.4 |
| 26 | 19.9 ± 1.4 | 49.9 ± 1.4 | 9.8 ± 0.7 | 49.7 ± 5.4 |
| 27 | 21.4 ± 1.2 | 52.5 ± 1.1 | 9.7 ± 0.4 | 45.7 ± 4.3 |
| 28 | 22.3 ± 1.2 | 53.3 ± 1.2 | 10.1 ± 0.3 | 45.2 ± 2.6 |
| 29 | 23.1 ± 3.9 | 50.1 ± 3.1 | 11.3 ± 1.8 | 49.2 ± 4.1 |
| 30 | 25.1 ± 2.4 | 50.0 ± 0.8 | 12.3 ± 0.4 | 49.6 ± 5.4 |
| 31 | 27.9 ± 1.1 | 54.0 ± 0.9 | 12.3 ± 0.4 | 44.0 ± 0.5 |

\(^{a}\)Days after silking (DAS). \(^{b}\)Dry matter accumulation (%) = (dry weight/fresh weight) × 100. \(^{c}\)*Least significant difference (LSD) at 5% probability. \(^{d}\)*Values represent means of three independent replicates ± standard deviation.

| Table 2. Correlation between Anthocyanin Contents and Fresh Weight, Dry Weight, and Dry Matter Accumulation during Grain-Filling Stages\(^{e}\) |
|-----------------|-----------------|-----------------|-----------------|
| | FW\(^{a}\) | MC\(^{b}\) | DW\(^{c}\) | DMA\(^{d}\) |
| C3G | 0.79** | −0.63** | 0.89** | 0.52** |
| P3G | 0.74** | −0.67** | 0.86** | 0.54** |
| M3G | 0.75** | −0.53** | 0.82** | 0.43** |

\(^{a}\)Fresh weight (FW) (g/100 kernel). \(^{b}\)Moisture content (MC) (%). \(^{c}\)Dry weight (DW) (g/100 kernel). \(^{d}\)Dry matter accumulation (DMA) (%) = (dry weight/fresh weight) × 100. \(*\) and ** represent significance at \(p < 0.05\) and \(p < 0.01\), respectively.

| Table 3. Correlation among Anthocyanin Contents and Lightness, Redness, and Yellowness\(^{d}\) |
|-----------------|-----------------|-----------------|-----------------|
| L\(^{*}\) | a\(^{*}\) | b\(^{*}\) |
| C3G | −0.79** | 0.72** | −0.90** |
| P3G | −0.78** | 0.72** | −0.88** |
| M3G | −0.79** | 0.72** | −0.86** |

\(^{d}\)Lightness. \(^{a}\)*Redness. \(^{b}\)*Yellowness. \(*\) and ** represent significance at \(p < 0.05\) and \(p < 0.01\), respectively.
During grain filling, these amino acids showed a clear oscillation pattern. This pattern could be related to anthocyanin accumulation during grain filling, as cyclic decrements of these amino acids could limit the quantities available for anthocyanin biosynthesis. Correlation analysis among amino acid showed three clear clusters during grain filling. Phenylalanine and tyrosine showed one of the strongest correlations to each other and belonged to cluster I along with methionine, leucine, and isoleucine. Cluster I correlated weakly with other clusters, whereas clusters II and III had strong positive correlations with each other (Figure 3B). We also analyzed the correlations between anthocyanins and amino acids, since phenylalanine and tyrosine are precursors of anthocyanins (Figure 3C). Cysteine showed a significant positive correlation, while lysine, glycine, and aspartic acid showed significant negative correlations ($p < 0.05$). Phenylalanine and tyrosine showed a positive but nonsignificant correlation. Intermediate products should be further analyzed to elucidate the relationships among amino acids and anthocyanins.

Kernels of colored corn, including red, blue, and purple varieties, have a higher antioxidant capacity and ability to scavenge free radicals compared with light-colored corns, suggesting that those cultivars can be used as nutraceuticals. Although anthocyanins accumulated throughout grain filling in our study, the best harvest time could be 29 DAS when kernel softness and amino acid content are also taken into account. If an earlier harvest is required, timing it to occur during 24–28 DAS would not reduce the quality with regard to anthocyanin and amino acid contents. Since anthocyanin accumulation can be affected by temperature and light intensity, harvest time could be changed according to environmental conditions. More research on this topic is needed.

2.5. Changes of Fatty Acids during Grain Filling. The other precursor of the anthocyanin biosynthesis pathway is malonyl CoA, which is also a precursor of fatty acid biosynthesis. The exact contents of the fatty acids were not determined in this study; however, fluctuations among the three fatty acids measured (palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1)) showed dynamic changes in grain filling. In purple waxy-corn kernels, the fatty acid composition was stable from 15 to 22 DAS but fluctuated between stearic acid (C18:0) and oleic acid (C18:1) during 24–29 DAS (Figure 4). Oleic acid made up about half of the observed total of three fatty acids and was the highest at 24 DAS. The stearic acid composition was the highest, making up 36.2% of the total contents of the three fatty acids at 27 DAS. The palmitic acid (C16:0) showed a relatively stable composition throughout grain filling, ranging from 16.6 to 19.9% of the three fatty acids (Figure 4). Other fatty acids such as C14:0, C18:2, C18:3, C20:0, and C22:0 were not detected in any of the samples analyzed. Palmitic acid is elongated to stearic acid, which is then transformed to C18:1 unsaturated fatty acid by stearoyl-CoA desaturase. The fatty acid composition after 30 DAS could be explained by the transfer of palmitic acid to stearic acid, but stearic acid is rarely

Figure 3. Amino acid profiles in kernels of purple-corn Heukjinjuchal during grain filling. (A) Amount of each amino acid during grain filling. Error bars represent standard deviations of three biological replicates. (B) Correlations among amino acids. Cluster I, II, and III were designated with cluster analysis. (C) Correlations among amino acid and cyanidin-3-β-D-glucoside (C3G). Color scales for B and C represent Pearson’s $R$.

Figure 4. Fatty acid composition changes during grain filling. Error bars represent standard deviations of three biological replicates.
transferred to oleic acid as the corn kernel reaches the late stages of grain filling.

3. CONCLUSIONS

Commercial fresh corn is harvested at 25—30 DAS, depending on growth conditions. Nutritional value is not typically considered when determining the harvest time. This study showed that, during development, corn kernels undergo dramatic changes that can affect their nutritional value. This study showed that kernels at 26 and 29 DAS have more amino acids and anthocyanin, respectively. Taking these changes into account may help ensure high-quality fresh corn production.

4. MATERIALS AND METHODS

4.1. Preparing Kernels. Five waxy-corn cultivars, three purple and two yellow, plus B73 were used to investigate anthocyanin profiles in fully matured and dried seeds (Figure 1A). The five waxy-corn cultivars, “Ilmichal”, “Mibaek2”, Heukjinjuchal, Suwon90, and Mibeugchal, are single-cross hybrids dominating the fresh waxy-corn seed market in South Korea and were developed by the National Institute of Crop Science or by Gangwon-do Agricultural Research and Extension Services.25—28 The corn was grown in the field of National Institute of Crop Science, Suwon, Korea (37°15’47”N, 126°59’16”E). Preplant broadcast manure at a dose of 15 000 kg ha⁻¹ and basal fertilizer containing 145, 30, and 60 kg ha⁻¹ for N, P₂O₅, and K₂O, respectively, were applied for field preparation. Corn seeds were sown on May 2, 2013, at 30 and 65 cm spacing, within and between rows, respectively. During the growth period, the average of the daily lower and the higher temperature was 18.1—27.2 °C and 70% relative humidity on average. Kernels were harvested at 15 to 31 days after silking (DAS). Each day, over 20 kernels from three different cobs were weighed and immediately placed in liquid nitrogen (N₂) and stored at −72 °C. To minimize variation, kernels were sampled in the middle of the cob.

4.2. Determining Color. Kernel color values L* (0 = black, 100 = white), a* (negative = green, positive = red), and b* (negative = blue, positive = yellow) were measured using a color difference meter (Minolta Co. Ltd., Japan). Calibration was carried out on a standard white plate. Fresh corn kernels were placed in a round cuvette and measured nine times for each time point (3 technical repeats × 3 biological replications).

4.3. Chemicals and Reagents. Eleven anthocyanin standards were purchased from Extrasynthese Co. (Genay, France): 3,4’,5,7-tetrahydroxy-3’,5’-dimethoxylaviloyl chloride (malvidin chloride), 3,3’,4,5,7-pentahydroxylaviloyl chloride (cyanidin chloride), 3,3’,4,5,7,7-hexahydroxylaviloyl chloride (delphinidin chloride), 3,4’,5,7-tetrahydroxy-3’,5’-methoxylaviloyl chloride (peonidin chloride), 3,3’,4,5,7-pentahydroxy-3’,5’-methoxylaviloyl chloride (petunidin chloride), 3,5-bis(glucosylxylo)-4’,7-di-hydroxylaviloyl chloride (pelargonidin chloride), cyanidin-3-O-glucoside chloride (kurmanin chloride), pelargonidin-3-O-glucoside chloride, delphinidin-3-b-O-glucoside chloride (myrtillin chloride), malvidin-3-b-O-glucoside chloride (oenin chloride), and pelargonidin-3-O-glucoside chloride (callistephin chloride). All chemicals and regents were of analytical grade.

4.4. Extracting Samples. Anthocyanins were extracted from ground kernels according to the method described by Aoki et al.15 Approximately 2 g of powder from each type was added to a flask containing 20 mL of 0.1% hydrochloric acid (HCl) aqueous solution. This flask was shaken on a platform shaker at 150 rpm at 37 °C for 24 h. Extracts of each sample were filtered through Whatman No. 42 filter paper. The extracted solution was stored at 4 °C in darkness until analyzed.

4.5. Anthocyanin Quantification by Liquid Chromatography and Mass Spectrometry. Qualitative and quantitative anthocyanin analyses were performed with ultras-performance liquid chromatography (UPLC) with a Waters ACQUITY BEH C18 column (particle size 1.7 μm, 2.1 mm × 100 mm, Waters, MA) and with a photodiode array detector at 530 nm. The HPLC-grade solvents such as water and acetonitrile for UPLC analysis were purchased from J. T. Baker (New Jersey). Sample extracts were filtered through a 0.2 μm membrane syringe filter before injection. Mobile phase A was 0.1% formic acid in water; mobile phase B was 0.1% formic acid in acetonitrile. HPLC analysis for anthocyanin was performed using an LC system (Waters e2695 Separation Module) equipped with a PDA detector (Waters 2487 Dual λ Absorbance Detector) and with a C18 column (YMC-Pack ODS-AM). A gradient mobile phase of A (5:95, formic acid/water, v/v) and B (5:95, acetonitrile/water, v/v) were set to 90% A in 5 min, gradual decrement to 60% A until 35 min, and back to 90% A until 36 min at a flow rate of 0.7 mL min⁻¹. For mass spectrometry (MS) analysis, a tetraquadrupole detector equipped with an electrospray ionization-tandem MS/MS (Waters) was used in the positive-ion mode, voltage 30 V, capillary 4 kV, and drying N₂ gas at 800 L h⁻¹.

4.6. Analyzing Amino Acids in Kernels. Amino acid quantification was performed by the method of Kim et al.29 with some modifications. Three milligrams of lyophilized fine purple-corn powder was added to 5 mL of 6N HCl. The digestion of the corn powder was incubated for 24 h at 110 °C with flushing by nitrogen gas. The resulting hydrolyzed solution was diluted with 10 mL of pure water and filtered through Millipore 0.45 μm syringe filters (Milford). Each hydrolyzed solution was loaded onto an automatic L-8800 high-speed amino-acid analyzer (Hitachi, Japan) with an ion-exchange column no. 26225C PH. Amino acid calibration mixture solutions (Ajinomoto-Takara, Japan) were used as standards.

4.7. Analyzing Fatty Acids in Kernels. The fatty acids were analyzed as described by Garces and Mancha.30 The procedure was as follows: 0.5 g of lyophilized purple-corn powder was heated with an extraction solution containing methanol/heptane/benzene/2,2-dimethoxypropane/sulfuric acid (37:36:20:5:2, v/v). The digestion and lipid transmethylation took place at the same time at 80—85 °C and slowly cooled to room temperature. The supernatant containing the fatty acid methyl esters (FAMES) was analyzed by capillary gas chromatography (GC) using the HP 6890 GC system (HP Co.) equipped with a FID detector and an HP-Innowax capillary column (cross-linked poly(ethylen glycol), 0.25 μm × 30 m). The initial oven temperature of 150 °C was sequentially raised to the final temperature of 280 °C at a rate of 4 °C min⁻¹. Carrier gas nitrogen flowed at a rate of 10 mL min⁻¹. During the determination of the composition of fatty acids of lipids from purple-corn kernel, the temperatures of the inlet and the detector were continuously maintained at 250 and 300 °C, respectively. The standard was used in the FAME mix (C14—C22) and obtained from Supelco Co. (Bellefonte).
4.8. Statistical Analysis. Statistical analysis was performed using Excel (Microsoft Office 2016) and SAS ver. 9.3 for Windows (Statistical Analysis Systems Institute Inc.). Correlation analysis of amino acid contents was performed with the web-based tool Metaboanalyst (http://metaboanalyst.ca).31

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02099.

LS–MS confirmation of major anthocyanin detected in the Heukjinjuchal (A) LC chromatogram of 15, 20, 25, and 30 DAS kernel samples; cyanidin-3-β-O-glucoside (C3G), pelargonidin-3-β-O-glucoside (P3G), and malvidin-3-β-O-glucoside (M3G) are indicated with dashed box; and (B–D) mass spectrometry of C3G, P3G, and M3G (PDF)

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REFERENCES

(1) Choe, E.; Rocheford, T. R. Genetic and QTL analysis of pericarp thickness and ear architecture traits of Korean waxy corn germplasm. *Euphytica* 2012, 183, 243–260.
(2) Lim, S.; Yi, G. Investigating seed mineral composition in Korean landrace maize (Zea mays L.) and its kernel texture specificity. *J. Integr. Agric.* 2019, 18, 1996–2005.
(3) Fukamachi, K.; Imada, T.; Ohshima, Y.; Xu, J.; Tsuda, H. Purple corn color suppresses Ras protein level and inhibits 7, 12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in the rat. *Cancer Sci.* 2008, 99, 1841–1846.
(4) Hope Smith, S.; Tate, P. L.; Huang, G.; Magee, J. B.; Meepagala, K. M.; Wedge, D. E.; Larcom, L. L. Antimutagenic activity of berry extracts. *J. Med. Food* 2004, 7, 450–455.
(5) Tsuda, T.; Horio, F.; Uchida, K.; Aoki, H.; Osawa, T. Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J. Nutr.* 2003, 133, 2125–2130.
(6) Wang, L.-S.; Stoner, G. D. Anthocyanins and their role in cancer prevention. *Cancer Lett.* 2008, 269, 281–290.
(7) Kong, J.-M.; Chia, L.-S.; Goh, N.-K.; Chia, T.-F.; Brouillard, R. Analysis and biological activities of anthocyanins. *Phytochemistry* 2003, 64, 923–933.
(8) Nygård, A.-M.; Aksnes, D. W.; Andersen, Ø. M.; Bakken, A. K.; et al. Structure determination of 6-hydroxycyanidin- and 6-hydroxydelphinidin-3-(6′-o-a-L-rhamnopyranosyl-b-D-glucopyranosides) and other anthocyanins from Alstroemeria cultivars. *Acta Chem. Scand.* 1997, 51, 108–112.
(9) Oren-Shamir, M. Does anthocyanin degradation play a significant role in determining pigment concentration in plants? *Plant Sci.* 2009, 177, 310–316.
(10) Biddle, P.; Timberlake, C. F. Anthocyanins as natural food colours—selected aspects. *Food Chem.* 1997, 58, 103–109.
(11) Mazza, G.; Irnniah, E. Anthocyanins in Fruits, Vegetables and Grains; CRC Press, Inc., 1993.
(12) Strack, D.; Wray, V. Anthocyanins. In *Methods in Plant Biochemistry*; Elsevier, 1989; pp 325–356.
(13) Ignat, I.; Volf, I.; Popa, V. I. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.* 2011, 126, 1821–1835.
(14) Abdel-Aal, E. S. M.; Young, J. C.; Rabalski, I. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *J. Agric. Food Chem.* 2006, 54, 4696–4704.
(15) Aoki, H.; Kuze, N.; Kato, Y.; Gen, S.-E. Anthocyanins isolated from purple corn (Zea mays L.). *Foods Food Ingredients J.* 2002, 41, 41–45.
(16) Salinas Moreno, Y.; Sánchez, G. S.; Hernández, D. R.; Lobato, N. R. Characterization of anthocyanin extracts from maize kernels. *J. Chromatogr. Sci.* 2005, 43, 483–487.
(17) Lopez-Martinez, L. X.; Oliart-Ros, R. M.; Valerio-Alfaro, G.; Lee, C.-H.; Parkin, K. L.; Garcia, H. S. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. *LWT–Food Sci. Technol.* 2009, 42, 1187–1192.
(18) Unias-Lago, D. A.; Heredia, J. B.; Serna-Saldivar, S. O.; Muy-Rangel, M. D.; Valdez-Torres, J. B. Total phenolics, total anthocyanins and antioxidant capacity of native and elite blue maize hybrids (Zea mays L.). *CyTA–J. Food* 2015, 13, 336–339.
(19) Žilić, S.; Serpen, A.; Akkilloğlu, G.; Gökmen, V.; Vanchétović, J. Phenolic compounds, carotenoids, anthocyanins, and antioxidant...
capacity of colored maize (Zea mays L.) kernels. J. Agric. Food Chem. 2012, 60, 1224–1231.

(20) Hu, Q.-p.; Xu, J.-g. Profiles of carotenoids, anthocyanins, phenolics, and antioxidant activity of selected color waxy corn grains during maturation. J. Agric. Food Chem. 2011, 59, 2026–2033.

(21) Del Pozo-Infran, D.; Serna Saldivar, S. O.; Brenes, C. H.; Talcott, J. T. Polyphenolics and antioxidant capacity of white and blue corns processed into tortillas and chips. Cereal Chem. J. 2007, 84, 162–168.

(22) Yang, Z.; Zhai, W. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (Zea mays L.). Innovative Food Sci. Emerging Technol. 2010, 11, 169–176.

(23) Harakotr, B.; Sutharn, B.; Tangwongchai, R.; Scott, M. P.; Letrat, K. Anthocyanin, phenolics and antioxidant activity changes in purple waxy corn as affected by traditional cooking. Food Chem. 2014, 164, 510–517.

(24) Dey, P. M.; Harborne, J. B. Plant Biochemistry; Academic Press: New York, USA, 1997; pp 452–454.

(25) Jung, T. W.; Moon, H. G.; Son, B. Y.; Kim, S. J.; Cha, S. W.; Min, H. K.; Choi, H. J.; Ryu, I. M. A new waxy corn hybrid cultivar, Ilmichal’ with good eating quality and lodging resistance. Korean J. Breed. Sci. 2006, 38, 135–136.

(26) Jung, T. W.; Song, S.; Son, B. Y.; Kim, J. T.; Baek, S. B.; Kim, C. K.; Kim, S. L.; Kim, S. J.; Kim, S. K.; Park, K. J. A black waxy hybrid corn, ‘Heukjinjuchal’ with good eating quality. Korean J. Breed. 2009, 41, 599–602.

(27) Park, K. J.; Park, J. Y.; Ryu, S. H.; Goh, B. D.; Seo, J. S.; Min, H. K.; Jung, T. W.; Huh, C. S.; Ryu, I. M. A new waxy corn hybrid cultivar, ’Mibaek 2’ with good eating quality and lodging resistance. Korean J. Breed. 2007, 39, 108–109.

(28) Park, K. J.; Ryu, S. H.; Min, H. K.; Seo, J. S.; Park, J. Y.; Goh, B. D.; Jang, J. S.; Kim, N. S. A new black waxy corn hybrid cultivar, ‘Miheugchal’ with good eating quality and high yield. Korean J. Breed. 2007, 39, 106–107.

(29) Kim, M.-Y.; Chung, I.-M.; Lee, S.-J.; Ahn, J.-K.; Kim, E.-H.; Kim, M.-J.; Kim, S.-L.; Moon, H.-I.; Ro, H.-M.; Kang, E.-Y.; Seo, S.-H.; Song, H.-K. Comparison of free amino acids, carbohydrates concentrations in Korean edible and medicinal mushrooms. Food Chem. 2009, 113, 386–393.

(30) Garces, R.; Mancha, M. One-step lipid extraction and fatty acids methyl ester preparation from fresh plant tissues. Anal. Biochem. 1993, 211, 139–143.

(31) Chong, J.; Wishart, D. S.; Xia, J. Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. Curr. Protoc. Bioinformatics 2019, 68, No. e86.