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Profiles of Carotenoids, Anthocyanins, Phenolics, and Antioxidant Activity of Selected Color Waxy Corn Grains during Maturation

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ABSTRACT: Waxy corns are becoming increasingly consumed as fresh foods or as raw materials for whole grain foods facilitating human consumption in China, so they are usually harvested before complete maturity. Unfortunately, information on functional properties of immature waxy corns is very limited. Therefore, we investigated the profiles of carotenoids, anthocyanins, phenolics, and the antioxidant activity in three types of waxy corn with different colors (white, yellow, and black) during maturation, as well as a normal corn (yellow) used as control. The results showed that black waxy corn had the highest quantity of anthocyanins, phenolics and the best antioxidant activity, yellow corn contained a relatively large amount of carotenoids, while white corn had the lowest amounts of carotenoids, anthocyanins, phenolics, and antioxidant capacity. For each type of waxy corn, the higher carotenoids were found at the M2 stage (no major difference between the M1 and M2 stages for yellow corn). The levels of anthocyanin and phenolics decreased for white and yellow corns, contrary to those for black corn during maturation. The antioxidant activity determined by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH), the ferric reducing antioxidant power (FRAP), and the Trolox equivalent antioxidant capacity (TEAC) assays increased with ripening, but no difference was found between the M2 and maturity stages for yellow and black corns. For white corn, the DPPH radical scavenging activity first increased and then decreased, while the antioxidant activity determined by TEAC and FRAP assay decreased during maturation. Differences in these parameters indicate that types and harvesting time have significant influences on functional properties of waxy corns.

KEYWORDS: carotenoids, anthocyanins, phenolics, antioxidant activity, waxy corn

INTRODUCTION

Corn (*Zea mays* L.) is one of the most important grains that provide food to much of the world's population. In addition to serving as a critical source of macro- and micronutrients,[1,2] corn is also a rich source of many phytochemicals including carotenoids,[2−6] phenolic compounds,[2,3,7,8] anthocyanins,[3,8,9] and tocopherols[2,10] in our daily diets. Several studies with these phytochemicals have shown that they have multiple functional roles, for example, as antioxidants,[3,11,12] as antimutagens,[11] and as inhibitors of colorectal carcinogenesis.[11,13]

Waxy corn (*Zea mays* L. var. *ceratina* Kulesh), a variety of corn, only contains 1% amylose and possesses better nutrient quality, palatability and digestibility than normal corn.[14] Planting and processing of waxy corn is developing rapidly especially in China. Currently, waxy corns are increasingly consumed as fresh foods or whole grain foods such as corn kernel cans, quick-frozen corn ears and so on. These foods are usually eaten or processed from waxy corn harvested before complete maturity in order to retain the original components and to enhance the palatability of foods, and waxy corns should be harvested on time according to the needs of different foods. Current research on waxy corn mainly focuses on the properties and processing of starch from ripe corns.[14−16] Some authors also reported phenolic compounds and the antioxidant activity of ripe waxy corn under different drying temperatures.[12] We have reported changes of the main nutrients, phytochemicals, and antioxidant activity in normal corn during maturation.[17] However, there are few reports to evaluate systematically profiles of phytochemicals and the antioxidant activity in waxy corn during maturation. In this study, three selected color waxy corns harvested at different stages were used to investigate changes of carotenoids, anthocyanins, phenolics, and antioxidant activity in waxy corns during maturation. Thus, we expect to provide some parameters which can contribute to harvest corns in time according to the needs of various foods, and which would greatly facilitate production of nutraceuticals that enable consumers to gain greater access to the health benefits of waxy corns.

MATERIALS AND METHODS

Corn Materials. Three types of waxy corn (*Zea mays* L. *ceratina* Kulesh) selected for the study were 'Jinxiannuo 6' (JXN6), a white-kernel type; 'Xinnuo 301' (XN301), a yellow-kernel type; 'Jinxiannuo 8' (JXN8), a black-kernel type; type Xinhuangdan 85 (XHD85), a yellow-kernel normal corn, which is preferred and commonly used by the industry, was used as control. All types of corn were planted in late April 2009 (Table 1) at Research Bases for Corns in China Xinzhou (latitude, 38°24′ N; longitude, 112°43′ E; altitude, 791 m). During the growth of the corn, the plots were fertilized with carbamide (375 kg per hectare) at 35 days after seeding (DAS) and irrigated at 40 DAS so that the corn could grow better.

Corns were picked by hand at three different stages M1, M2, and complete maturity from wax ripe stage to maturity (Table 1). At each stage, fresh weight of 100 grains was recorded, and moisture percentage of freshly harvested corn was determined using an oven-dry method. Samples were immediately frozen in liquid N_2 to block the enzymatic

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Table 1. Physical Parameters of the Corn Grains at Different Maturation Stages[a]

sowing date	maturation stage	DAS[b]	seed color	100-grain fresh wt (g)	moisture (%)	100-grain dry wt (g)	% dry matter accumulation
JXN6 (April 30)	M1[c]	86	creamy white	21.7 ± 0.8 c	61.3 ± 2.3 a	8.4 ± 1.2 c	38.7 ± 2.1 c
	M2	98	creamy yellow	31.4 ± 0.3 a	51.7 ± 0.8 b	15.2 ± 0.4 b	48.3 ± 0.4 b
	maturity	110	pure white	26.2 ± 0.2 b	18.1 ± 0.5 c	21.5 ± 0.4 a	81.9 ± 0.4 a
XN301 (April 27)	M1	96	creamy yellow	36.2 ± 1.1 b	58.2 ± 1.0 a	15.1 ± 0.2 b	41.8 ± 1.0 c
	M2	108	yellow	51.3 ± 1.4 a	49.5 ± 1.1 b	26.4 ± 0.6 a	51.5 ± 0.9 b
	maturity	120	yellow	33.5 ± 3.5 b	18.7 ± 2.6 c	27.1 ± 0.5 a	81.3 ± 3.1 a
JXN8 (April 25)	M1	106	purple	22.3 ± 0.4 c	57.4 ± 0.8 a	9.5 ± 0.3 b	42.6 ± 0.5 c
	M2	118	purplish red	42.2 ± 1.2 a	50.1 ± 0.9 b	21.1 ± 0.5 a	49.9 ± 0.8 b
	maturity	130	purplish black	26.7 ± 0.8 b	16.7 ± 2.1 c	22.2 ± 0.7 a	83.3 ± 2.8 a
XHD85 (April 22)	M1	109	creamy yellow	43.7 ± 0.2 b	51.8 ± 0.4 a	21.1 ± 0.1 c	48.2 ± 1.1 c
	M2	124	yellow	50.9 ± 0.2 a	41.2 ± 0.7 b	29.9 ± 0.8 b	58.8 ± 1.1 b
	maturity	138	golden yellow	43.9 ± 0.4 b	18.6 ± 0.5 c	35.7 ± 0.2 a	81.4 ± 2.6 a

[a] Numbers represent mean values of three independent replicates ± SD Different letters indicate statistically significant differences between the means ($P < 0.05$) for each physical parameter of the same corn type.. [b] DAS refer to the days after seeding. [c] M1 and M2 refer to the different maturation stages.

activities, and stored at −40 °C. All corn kernels were freeze-dried and ground to a fine powder in liquid nitrogen prior to extraction.

Reagents. 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) was purchased from Fluka (Switzerland). Sodium acetate, acetonitrile, and methanol, were from Merck (Germany). 2,2′-Azino-bis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salts (ABTS), lutein, zeaxanthin, β-cryptoxanthin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferulic, protocatechuic, *p*-coumaric, vanillic and gallic acids (GA) were from Sigma (USA). All other chemicals and reagent used in the experiments were of analytical grade.

Carotenoid Analysis. The content of total carotenoids was determined according to AACC method 14−50. Carotenoid analysis was carried out according to the method reported previously.[17] Briefly, 1.0 g of corn powders was transferred to a 50 mL screw-capped culture tube, and 6.0 mL of methanol containing 0.1 g of butylated hydroxytoluene/L was added. The test tubes were sealed with screw caps and placed in an 85 °C water bath for 10 min. The test tubes were then removed from the water bath, and 120 μL of 80% KOH was added to each tube. Samples were then vortexed and returned to the water bath for 10 min for saponification to occur. After the samples were saponified, the test tubes were immediately placed in an ice bath to cool, and then 3.0 mL of cold deionized water was put into each test tube, followed by 3.0 mL of hexane. The test tubes were vortexed and immediately centrifuged at 4000*g* for 10 min. The upper organic phase was collected into a 10.0 mL disposable test tube and evaporated to dryness with a universal vacuum system. The dried extract was reconstituted in 100 μL of methyl *tert*-butyl ether followed by 300 μL of methanol. Samples were stored at −20 °C under nitrogen until injected into the high-performance liquid chromatography (HPLC) column.

The carotenoid extracts were filtered through a nylon syringe filter (0.45 μm) (Filtrex Technology, Singapore) prior to HPLC analysis and analyzed in a Waters 1525 HPLC chromatograph system (Waters, Milford, MA) equipped with a Waters 2487 diode array detector (Waters, Milford, MA) and a C30 carotenoid column (Waters Corporation, 5 μm, 4.6 mm × 250 mm) using 100% methanol (containing 1.0 g of ammonium acetate/L) (solvent A) and 100% methyl *tert*-butyl ether (solvent B) as the mobile phase. The following gradient was used: 0−15 min, 100% solvent A; 15−25 min, linear gradient to 10% solvent B; 25−35 min, linear gradient to 30% solvent B; 35−55 min, linear gradient to 50% solvent B. The injection volume was 10 μL, the flow rate was 1.0 mL/min, and the monitored wavelength was 453 nm. Identification of the main carotenoids was performed by comparisons to the retention time and UV spectra of authentic standards from Sigma; then the quantitative data was calculated from their linear calibration curves under analysis conditions. All of the above experiments were replicated three times, and results for the main carotenoids were expressed as micrograms per gram dry matter basis.

Sample Extraction. Five-gram corn powders were extracted three times with 50 mL of methanol containing 1% HCl in a flask and shaking it with a rotary shaker (JB50-D; Shanghai Shengke Instruments, Shanghai, China) set at 250 rpm for 2 h at ambient temperature and in the absence of light. The homogenates were then centrifuged for 15 min by a centrifuge (Eppendorf 5417R, Germany) at 4000*g* and 4 °C. After centrifugation, the combined supernatants were reduced to dryness in vacuum at 40 °C, and the resulting precipitate was resuspended in 10 mL of 1% HCl/MeOH solvents (1 vol of 12.1 M HCl in 100 vol of methanol). The extracts of supernatant fluid were kept at −20 °C in the dark until further analysis for total anthocyanins, phenolics, and antioxidant activity.

Determination of the Total Anthocyanin Content (TAC). The TAC was determined using the spectrophotometric method as described by Abdel-Aal et al.[18] Absorbance of appropriately diluted extracts at 535 nm was immediately measured to detect anthocyanins. Anthocyanin levels were expressed as milligrams of cyanidin 3-glucoside equivalents (CGE) per 100 g of dry weight (DW), using the reported molar extinction coefficient of 25 965 M^{-1} cm^{-1} and a molecular weight of 449.2 g/mol.

Determination of the Total Phenolic Content (TPC). The TPC was determined based on the Folin−Ciocalteu colorimetric method as described by Xu et al.[17] Briefly, an aliquot (0.5 mL) of the suitable diluted extracts, 2.5 mL of deionized water and 0.5 mL of 1.0 M Folin−Ciocalteu reagent were mixed within 10 mL volumetric flasks and vortexed. After 8 min, 1.5 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured using a spectrophotometer at 765 nm wavelength after incubation for 2 h at room temperature. Extraction solvent was used as the blank, and gallic acid (GA) was used for calibration of standard curve. Phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per g of DW.

HPLC Analysis of Phenolic Components. The phenolic extracts were filtered through a nylon syringe filter (0.45 μm) prior to HPLC analysis and analyzed in a Waters 1525 HPLC chromatograph system (Waters, Milford, MA, USA) equipped with a Waters 2487 diode array detector (DAD) (Waters, Milford, MA, USA) and a reversed-phase C18 column (Alltech, Allsphere ODS-2, 5 μm, 250 mm × 4.6 mm) using a program of gradient elution. The mobile phase was composed of solvent A (0.5% acetic acid) and solvent B (acetonitrile HPLC grade). The elution was as follows: isocratic conditions from 0 to 3 min with 95% A and 5% B. A linear gradient of solvent B was used from 5 to 9% from 3 to 5 min, from 9 to 20% B from 5 to 10 min, from 20 to

40% form 10 to 30 min, with an injection of 10 μL of the sample. The flow rate was 1.0 mL/min at room temperature, and the monitored wavelength was 280 nm. The phenolic extracts and standard compounds were analyzed under the same analysis conditions. Identification of some of the main phenolic acids (ferulic, vanillic, *p*-coumaric, and protocatechuic acids) was performed by comparisons to the retention time and UV spectra of authentic standards from Sigma, and then the quantitative data was calculated from their linear calibration curves under analysis conditions. The results of the main phenolic compounds were expressed as micrograms per gram of DW.

Determination of Antioxidant Activity. DPPH radical scavenging activity of phenolics was assessed by measuring the capacity of bleaching a black colored methanol solution of DPPH radicals as described by Xu et al.[17] Briefly, 0.5 mL of phenolic extracts was mixed with 4.5 mL of 60 μM DPPH dissolved in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was measured at 517 nm against a solvent blank. The scavenging rate on DPPH radicals was calculated according to the formula scavenging rate (%) = $[1 - (A_1 - A_s)/A_o] \times 100$, where A_o is the absorbance of the control solution (0.5 mL extraction solvent in 4.5 mL of DPPH solution), A_1 is the absorbance in the presence of phenolic extracts in DPPH solution and A_s, which is used for error correction arising from unequal color of the sample solutions, is the absorbance of the extract solution without DPPH.

The reducing ability was determined by using the ferric reducing antioxidant power (FRAP) assay described by Xu et al.[17] Briefly, the FRAP reagent was freshly prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Then 0.1 mL of extracts was mixed with 1.8 mL of FRAP reagent and 3.1 mL of ultrapure water. The absorption of the reaction mixture was measured at 593 nm after incubation for 30 min at 37 °C. A standard curve was constructed using $FeSO_4$ solution (100−1000 μM). FRAP value was expressed as micromoles of Fe(II) per gram of DW.

The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of the radical cation of ABTS by antioxidants, was carried out as previously described[19] with slight modifications. Briefly, $ABTS^+$ radical cation was generated by a reaction of 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate. The reaction mixture was allowed to stand in the dark at room temperature for 16−24 h before use and used within 2 days. The $ABTS^+$ solution was diluted with methanol to an absorbance of 0.700 ± 0.050 at 734 nm. All samples were diluted appropriately to provide 20−80% inhibition of the blank absorbance. Fifty microliters of the diluted extracts was mixed with 1.9 mL of diluted $ABTS^+$ solution. The assay with the mixture was carried out in triplicate, the mixture was allowed to stand for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. Trolox solution (100−1000 μM) was used as a reference standard. The results were expressed as micromoles Trolox equivalents (TE) per gram of DW.

Statistical Analysis. All experiments were conducted three times independently, and the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to determine significant differences ($p < 0.05$) between the means by Statistical Product and Service Solutions statistics (SPSS version 13.0). Correlation coefficient and regression analyses were determined by Data Processing System (DPS, version 3.01) and EXCEL program.

RESULTS AND DISCUSSION

Physical Parameters at Different Maturation Stages. The physical characteristics of the corn grains at different maturation stages are shown in Table 1. Development of all corn grains is characterized by the presence of pericarp color change varying from creamy white to pure white for JXN6, from purple to purplish black for JXN8, and from creamy yellow to golden yellow for XN301 and XHD85. It is believed that changes of the color reflect changes in coloring matter profiles synthesized in corn grains like carotenoids, anthocyanins and so on, as well as changes in moisture content, kernel size, and density during maturation of corn. Weight of the 100 fresh grains first increased and then decreased, whereas moisture content decreased drastically from the M1 stage to the maturity stage (Table 1). For this reason the 100-grain dry weight and the rate of dry matter accumulation of corn increased significantly with ripening. These results are supported by our previous study[17] and also consistent with cereal grain ontogenesis, when grain moisture typically ceases to increase during the accumulation of storage materials (such as starch) within seeds.[20] In addition, the weight of 100 grains of different waxy corns is different and lower than that of normal corn (XHD85) at the same stage because of differences in corn types.

Carotenoids Profiles. The content of total carotenoids and main carotenoid compounds of corns at different stages are shown in Table 2. There was a significant difference in the content of total carotenoids among three stages for each corn type. The total carotenoid content first increased by 21.47% and 10.00% and then decreased by 42.33% and 41.12% for white and black corns, respectively. Total carotenoids of two types of yellow corn decreased significantly by 43.07% and 36.62% to the lowest level at the maturity stage for XHD85 and XN301, respectively. On the other hand, difference in total carotenoid content among corn types was also found. At each stage, total carotenoid concentration was highest in yellow normal corn (22.55−39.61 μg/g of DW), followed closely by yellow waxy corn (20.16−31.81 μg/g of DW) and black corn (8.55−14.52 μg/g of DW), and lowest in the white corn (1.24−2.15 μg/g of DW) as expected. Steenbock reported that the color of yellow corn grains can be attributed to the carotenoid content,[21] namely, the higher the carotenoids, the deeper the yellow color relatively. From types of corn, the conclusion agrees with the results found in this study, whereas it was differential in the corn grain color that struck our eyes during maturation of yellow corns, possibly because of the differences in content of moisture and other mediums at different maturation stages. Interestingly, white corn grain has a pale yellow at the M2 stage even though total carotenoid content was much lower than that in black corn, while black corn was purple rather than yellow during maturation, which may contribute to the greater anthocyanins contained in black corns.

The HPLC chromatogram of carotenoid extracts from mature XN301 corn grains is shown in Figure 1 (not shown for others). Main carotenoid compounds have been identified by HPLC, and the results are shown in Table 2. There was a significant difference in lutein among maturation stages and corn types, as well as in zeaxanthin and β-cryptoxanthin. For different corn types at the same stage, the levels of lutein, zeaxanthin and β-cryptoxanthin were highest in yellow normal corn, followed closely by yellow waxy and black corns, and lowest in the white corn. But no difference was found among two types of yellow corns at the M2 stage, the same at maturity. For each corn type at each stage, the content of lutein was highest, followed by zeaxanthin and then β-cryptoxanthin, which was in agreement with previous studies indicating that lutein and zeaxanthin were major carotenoid species of corn grains.[22−24] During maturation, the trend of

Table 2. Main Carotenoid Content (μg/g of DW) in Corn Grains at Different Maturation Stages[a]

type	maturation stage	lutein	zeaxanthin	β-cryptoxanthin	total carotenoids
JXN6	M1[b]	0.64 ± 0.03 bD	0.38 ± 0.02 bD	nf[c]	1.77 ± 0.07 bD
	M2	0.95 ± 0.04 aC	0.53 ± 0.02 aD	nf	2.15 ± 0.08 aD
	maturity	0.48 ± 0.01 cD	0.24 ± 0.00 cD	nf	1.24 ± 0.05 cD
XN301	M1	14.71 ± 0.72 aB	7.04 ± 0.10 aB	0.65 ± 0.07 aB	31.81 ± 0.33 aB
	M2	14.23 ± 0.55 aA	7.11 ± 0.14 aB	0.52 ± 0.05 abA	31.10 ± 0.48 aB
	maturity	9.65 ± 0.36 bA	4.14 ± 0.04 bA	0.40 ± 0.05 bA	20.16 ± 0.16 bB
JXN8	M1	5.44 ± 0.25 aC	2.59 ± 0.10 aC	0.58 ± 0.04 aB	13.20 ± 0.11 bC
	M2	5.82 ± 0.28 aB	2.35 ± 0.09 aC	0.33 ± 0.04 bB	14.52 ± 0.09 aC
	maturity	3.65 ± 0.13 bC	1.42 ± 0.04 bB	nf	8.55 ± 0.05 cC
XHD85	M1	17.52 ± 0.80 aA	10.04 ± 0.12 aA	1.12 ± 0.08 aA	39.61 ± 0.42 aA
	M2	14.38 ± 0.62 bA	8.60 ± 0.10 bA	0.56 ± 0.06 bA	34.82 ± 0.33 bA
	maturity	10.44 ± 0.54 cA	4.22 ± 0.06 cA	0.38 ± 0.02 cA	22.55 ± 0.27 cA

[a] Numbers represent mean values of three independent replicates ± SD. Mean values within a column with different lowercase letters for the same corn type at different maturation stages are significantly different at $P < 0.05$. Mean values within a column with different uppercase letters for different corn types at the same maturation stage are significantly different at $P < 0.05$. [b] M1 and M2 refer to the different maturation stages. [c] nf, not found.

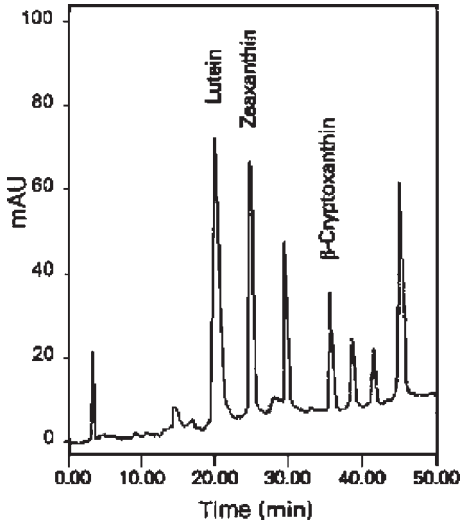

Figure 1. HPLC chromatograms of the carotenoid extracts from the mature XN301 corn grains at wavelength 453 nm.

changes in lutein, zeaxanthin, and β-cryptoxanthin was similar to the trend of changes in their respective total carotenoids for each type of corn, and these changes may result from their different degrees of conversion to metabolites respectively.[25,26] Unexpectedly, β-cryptoxanthin was not even found in white corn during maturation, which was in disagreement with the report that the content of β-cryptoxanthin in white corn is 1.27 μg/100 g of DW.[3] But these results further supported previous reports indicating large differences in carotenoids according to types[2,3] and maturation stages of corns.[17]

TAC Profiles. The TAC of different corn types at different maturation stages are presented in Table 3. The range of TAC of corns was from 0.97 to 63.58 mg of CGE/100 g of DW at the M1 stage, from 0.61 to 138.06 mg of CGE/100 g of DW at the M2 stage, and from 0.09 to 276.11 mg of CGE/100 g of DW at the maturity stage. There were significant differences in TAC among types of corn; black corn (JXN8) contained the highest TAC as compared to the other types, followed by yellow corn (no difference between XN301 and XHD85) and then white corn (JXN6) at each stage. These findings supported our hypothesis that the greater anthocyanin content was responsible for the observation that black corn containing higher carotenoid content during maturation was black rather than yellow. The TAC of different corns varied with regard to corn grains ripening (Table 3). The TAC of black corn increased continuously and significantly by 3.34-fold, on the contrary, the TAC decreased by 90.72%, 49.19%, and 35.84% from stages M1 to maturity for JXN6, XN301, and XHD85, respectively. However, normal corn XHD85 did not change in TAC between stages M1 and M2. Some pigmented corn types, such as the black, purple, and bule so on, have been found to contain higher levels than the yellow and white types.[3,12,27] This agrees with the results found in this study.

TPC Profiles. The TPC varied to different degrees between various maturation stages as well as corn types, and the results are shown in Table 3. The range of TPC of corns was from 0.67 to 2.55 mg of GAE/g of DW at the M1 stage, from 0.34 to 2.93 mg of GAE/g of DW at the M2 stage, and from 0.23 to 3.88 mg of GAE/g of DW at the maturity stage. The TPC of black corn (JXN8) was greater than that of the other corns at each stage. During maturation of corn grains, the trend of changes in TPC was similar to that in TAC for three waxy corn grains. The TPC of black corns increased continuously and significantly by 52.16%, while it decreased by 77.88% and 42.39% from the M1 to maturity stages for JXN6 and XN301 respectively, which was in agreement with previous reports that a significant decrease in the TPC was observed during maturation for corn[17] and soybean seeds.[28] Unlike waxy corns, the TPC in normal corn first increased by 65.67%, from 0.67 to 1.11 mg GAE/g of DW, and then decreased by 28.83% to 0.79 mg GAE/g of DW. Additionally, the TPC between JXN6 and XN301 showed no significant difference at the M1 stage, much lower than that in JXN8 but higher than that in normal corn (XHD85) at the same stage, however, at the M2 and maturity stages they are much lower than that in normal corn (XHD85) at the same stage.

Some free phenolic compounds have been identified by HPLC, and the HPLC chromatogram of phenolic extracts from XN301 corn grains at stage M1 is shown in Figure 2 (not shown for others). The quantitative analytical results of the phenolic compounds are shown in Table 4. It can be seen from the figure that some compounds such as protocatechuic, vanillic, *p*-coumaric, and ferulic acids were detected, and some peaks have not

Table 3. TAC and TPC in Corn Grains at Different Maturation Stages[a]

	type of corn			
maturation stage	JXN6	XN301	JXN8	XHD85
		TAC (mg of CGE/100 g of DW)		
M1[b]	0.97 ± 0.06 aB	1.24 ± 0.08 aB	63.58 ± 5.44 cA	1.73 ± 0.10 aB
M2	0.61 ± 0.04 bB	1.04 ± 0.05 bB	138.06 ± 9.85 bA	1.70 ± 0.08 aB
maturity	0.09 ± 0.01 cB	0.63 ± 0.05 cB	276.11 ± 22.62 aA	1.11 ± 0.03 bB
		TPC (mg of GAE/g of DW)		
M1[b]	1.04 ± 0.03 aB	0.92 ± 0.09 aB	2.55 ± 0.06 cA	0.67 ± 0.06 bC
M2	0.34 ± 0.04 bD	0.72 ± 0.02 bC	2.93 ± 0.07 bA	1.11 ± 0.07 aB
maturity	0.23 ± 0.04 cD	0.53 ± 0.04 cC	3.88 ± 0.16 aA	0.79 ± 0.04 bB

[a] Numbers represent mean values of three independent replicates ± SD. Mean values within a column with different lowercase letters for the same corn type at different maturation stages are significantly different at $P < 0.05$. Mean values within a line with different uppercase letters for different corn types at the same maturation stage are significantly different at $P < 0.05$. [b] M1 and M2 refer to the different maturation stages.

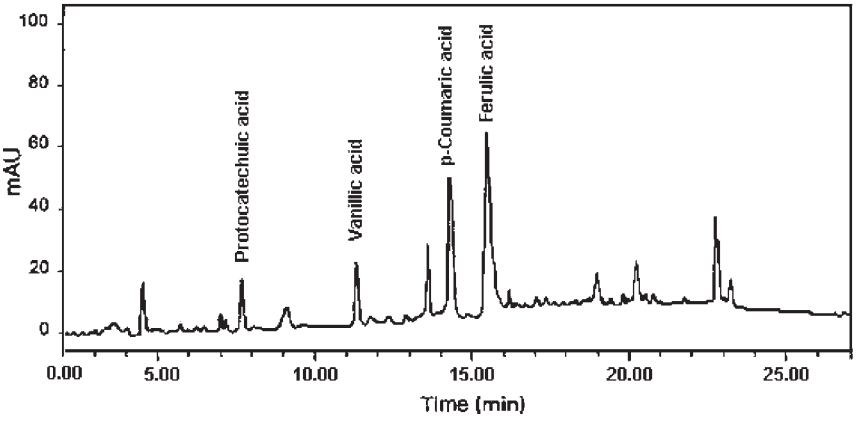

Figure 2. HPLC chromatograms of the phenolic extracts from XN301 corn grains at stage M1 at wavelength 280 nm.

Table 4. Main Free Phenolic Acids Content (μg/g of DW) in Corn Grains at Different Maturation Stages[a]

type	maturation stage	ferulic acid	vanillic acid	*p*-coumaric acid	protocatechuic acid
JXN6	M1[b]	14.52 ± 1.33 aC	2.02 ± 0.26 aB	0.54 ± 0.03 aA	1.34 ± 0.08 aC
	M2	9.85 ± 0.86 bB	0.54 ± 0.07 bD	0.40 ± 0.02 bA	0.85 ± 0.05 bC
	maturity	4.12 ± 0.52 cC	nf[c]	0.25 ± 0.03 cA	nf
XN301	M1	18.26 ± 1.20 aB	4.96 ± 0.22 aA	0.28 ± 0.02 aB	5.83 ± 0.24 aB
	M2	10.38 ± 0.58 bB	3.21 ± 0.14 bC	0.12 ± 0.03 bB	3.21 ± 0.18 bB
	maturity	6.25 ± 0.82 cB	2.45 ± 0.16 cB	nf	1.54 ± 0.11 cB
JXN8	M1	24.05 ± 1.52 aA	4.82 ± 0.18 aA	0.32 ± 0.03 aB	24.45 ± 1.28 aA
	M2	14.42 ± 0.84 bA	4.34 ± 0.26 aA	nf	10.22 ± 0.92 bA
	maturity	8.95 ± 0.84 cA	3.25 ± 0.11 bA	nf	6.02 ± 0.33 cA
XHD85	M1	16.45 ± 0.95 aBC	5.33 ± 0.25 aA	0.31 ± 0.02 aB	5.33 ± 0.43 aB
	M2	9.83 ± 0.73 bB	3.85 ± 0.12 bB	0.15 ± 0.02 bB	2.91 ± 0.14 bB
	maturity	6.54 ± 0.55 cB	3.11 ± 0.21 cA	nf	1.36 ± 0.06 cB

[a] Numbers represent mean values of three independent replicates ± SD. Mean values within a column with different lowercase letters for the same corn type at different maturation stages are significantly different at $P < 0.05$. Mean values within a column with different uppercase letters for different corn types at the same maturation stage are significantly different at $P < 0.05$. [b] M1 and M2 refer to the different maturation stages. [c] nf, not found.

been identified so far. The levels of these phenolic acids varied significantly ($p < 0.05$) among various stages of maturation. The most abundant phenolic acid was ferulic acid and the lowest *p*-coumaric acid, which was in agreement with previous studies.[3,8,29] With the development of corn growing processes, the content of vanillic, protocatechuic, *p*-coumaric, and ferulic acids decreased or disappeared significantly, indicating that they were metabolized in some way or synthesized into bound phenolics.[30,31] In the present study, the content of four free phenolic acids is not high, because most phenolic compounds in cereal grains were in

Table 5. DPPH Radicals Scavenging Activity, FRAP, and TEAC from Corn Grains Extracts at Different Maturation Stages[a]

type	maturation stage	DPPH (% reduction)	FRAP value (μmol Fe(II)/g DW)	TEAC value (μmol TE/g DW)
JXN6	M1[b]	71.83 ± 4.12 bB	20.16 ± 1.47 aA	0.99 ± 0.04 aB
	M2	84.13 ± 3.54 aB	2.22 ± 0.10 bC	0.41 ± 0.05 bC
	maturity	81.35 ± 4.60 abB	1.78 ± 0.10 bD	0.29 ± 0.04 bD
XN301	M1	55.95 ± 3.24 bC	14.97 ± 0.64 cB	0.95 ± 0.09 bB
	M2	67.94 ± 5.00 abC	20.76 ± 0.85 aB	1.18 ± 0.14 abB
	maturity	71.43 ± 4.36 aC	22.13 ± 0.96 aB	1.39 ± 0.03 aB
JXN8	M1	89.41 ± 4.11 bA	9.71 ± 0.33 bC	3.25 ± 0.65 aA
	M2	94.85 ± 3.06 abA	48.88 ± 1.48 aA	3.73 ± 0.14 aA
	maturity	98.33 ± 1.25 aA	50.46 ± 3.55 aA	3.93 ± 0.18 aA
XHD85	M1	44.38 ± 2.66 bD	15.74 ± 0.70 bB	0.90 ± 0.04 bB
	M2	62.70 ± 4.08 aC	20.37 ± 0.92 aB	1.08 ± 0.03 aB
	maturity	50.32 ± 2.57 bD	17.05 ± 0.90 bC	0.79 ± 0.01 bC

[a] Numbers represent mean values of three independent replicates ± SD. Mean values within a column with different lowercase letters for the same corn type at different maturation stages are significantly different at $P < 0.05$. Mean values within a column with different uppercase letters for different corn types at the same maturation stage are significantly different at $P < 0.05$. [b] M1 and M2 refer to the different maturation stages.

the bound form. Therefore, after phenolic extracts of corn were hydrolyzed by acid or alkaline, there would be significant increase in species of free phenolic compounds and in the content of some phenolic compounds.[8] As a secondary metabolite, the same as carotenoids and anthocyanins, the TPC in plants was also influenced by plant species and varieties.[11−13,18,27] Besides, from results mentioned previously, growth stage is also an important factor affecting the carotenoids, TAC, and TPC of corns.

Antioxidant Activity. The antioxidant activity of the corn grains is determined by DPPH, FRAP and TEAC assays, and the results are shown in Table 5. The DPPH radical scavenging activity of corn ranged from 44.38 (XHD85 at the M1 stage) to 98.33% (JXN8 at the maturity stage). For each corn type, there was difference in DPPH radical scavenging activity among different stages. The DPPH radical scavenging activity increased continuously by 27.67 and 9.07% by the end of the maturity stage for XN301 and JXN8, while it first increased by 17.12 and 41.28% and then decreased by 3.30 and 19.74% for JXN6 and XHD85 during maturation, respectively. Nevertheless, no difference in scavenging activity was investigated between the M2 and maturity stages for corn types but XHD85. For each stage, difference in DPPH radical scavenging activity among the four corn types was also significant. The DPPH radical scavenging activity in black corn was highest (89.41−98.33%), followed by white (71.83−84.13%) and yellow waxy corns (55.95−71.43%), which was higher than that in normal corn (44.38−62.70%) at the same stage.

In FRAP assay, the antioxidant activity of corn extracts ranged from 1.78 (JXN6 at the maturity stage) to 50.46 μmol Fe(II)/g of DW (JXN8 at the maturity stage) (Table 5). For each corn type, difference in FRAP value among different stages was observed. FRAP value increased continuously by 47.83% and 4.19-fold for XN301 and JXN8; contrary to that, it decreased by 91.17% by the end of the maturity stage for JXN6, while the FRAP value of extract from normal corn first increased by 29.42% and then decreased by 16.30% during maturation. At the M1 stage, the FRAP value in white corn is the highest (20.16 μmol Fe(II)/g of DW), followed by yellow corns (15.74, 14.97 μmol Fe(II)/g of DW for waxy and normal corn, respectively), and lowest in black corn (9.71 μmol Fe(II)/g of DW). Quite the opposite, FRAP of black corn was the highest, while it was the lowest in white corn at the M2 and maturity stages.

In TEAC assay, the trend of change in TEAC value was similar to FRAP value for each corn type during maturation, but the extent of change was not in keeping with the former (Table 5). TEAC value decreased by 70.71% for white corn, but increased continuously by 46.32% for yellow waxy corn. No difference in TEAC value was found for black corn among the three stages, while it first increased by 20% and then decreased by 26.85% for normal corn during maturation. At each stage, the TEAC value in black corn is the highest (3.25 μmol TE/g of DW). They are different in that no difference was detected among the other corns at the M1 stage while the TEAC value in yellow corn (no difference between XN301 and XHD85) is higher than that in white corn at the M2 and maturity stages. As a whole, the results suggested that immature waxy corns had exhibited better antioxidant activity.

Many authors reported that TAC and TPC can contributed to the antioxidant activity in some cereals or plants.[11,12,23] To further investigate the influences of TAC and TPC on the antioxidant activity of extracts from corn grains, the correlation among the antioxidant activity and TAC, TPC for each corn type during maturation was established, and correlation coefficients (R) are respectively shown in Table 6. During maturation, strong positive correlation between TAC, TPC and DPPH, FRAP, TEAC was examined for black corn, while strong negative correlation between TAC, TPC and DPPH, FRAP, TEAC was found for yellow waxy corn. There were high correlations between TAC, TPC and FRAP, TEAC, and the TAC, TPC were correlated negatively with DPPH for white corn. In yellow normal corn, the DPPH, FRAP were highly correlated to the TPC and had reverse correlation to TAC; the TEAC was correlated moderately to TAC and TPC. In addition, we also investigated correlation between the TAC and TPC with the antioxidant activity for different types of corn at the same growth stage; interestingly, the result was different from the former (Table 7). Some studies reported that there was a moderate or high correlation between total phenolics, anthocyanins and antioxidant activity of some plant products.[12,17,18,29,32−34] In another report, no correlation was observed between total phenolics and DPPH scavenging capacities.[35] These differences in correlation may be in relation to the types of materials, assessment methods for antioxidation, solvent extraction systems, and complicated extracts containing two or more antioxidant substances and so on. The results from the present investigation suggest that TAC and TPC have different influences on the antioxidant activity of different types of corn during maturation, indicating that some antioxidative

Table 6. Correlation Analysis of TAC, TPC and Antioxidant Activity for Each Corn Type during Maturation

	DPPH	FRAP	TEAC	TAC	DPPH	FRAP	TEAC	TAC
	JXN6				XN301			
TAC	−0.66[a]	0.82	0.89		−0.98	−0.99*[b]	−0.97	
TPC	−0.94	0.99	0.99*	0.88	−0.91	−0.99	−0.99*	0.98
	JXN8				XHD85			
TAC	0.99	0.99	0.92		0.16	0.20	0.76	
TPC	0.97	0.98	0.89	0.99*	0.99*	0.99*	0.79	0.21

[a] Correlation coefficient *R*. [b] (*) Significantly different ($P < 0.05$).

Table 7. Correlation Analysis of TAC, TPC and Antioxidant Activity for Different Types of Corn at the Same Growth Stage

	DPPH	FRAP	TEAC	TAC
		M1 stage		
TAC	0.81[a]	−0.85	0.99*[b]	
TPC	0.90	−0.77	0.98*	0.98*
		M2 stage		
TAC	0.76	0.69	0.97*	
TPC	0.59	0.85	0.99*	0.96*
		maturity stage		
TAC	0.76	0.91	0.96*	
TPC	0.67	0.94	0.97*	0.99*

[a] Correlation coefficient *R*. [b] (*) Significantly different ($P < 0.05$).

substances besides TAC and TPC might be contained in some types of corn, or that phenolic compounds differ greatly in different maturation stages and corn types because the antioxidant activity in extracts can be attributed to the structure of phenolic compounds beyond their content,[18,29,36] which also indicates complex change in phytochemicals and antioxidant activity in corn grain during maturation. Consequently, we should simply not judge the antioxidant activity of different types of corn at different maturation stages by one antioxidation test or one compound, especially nonprincipal antioxidant compounds. From the regression analysis, it can be concluded that changes of antioxidant activity of extracts from corns during maturation may mainly be attributed to changes of TAC and TPC for XN301, JXN8, and XHD85, different from changes of other antioxidants for JXN6, but follow-up work is needed.

In summary, profiles of carotenoids, anthocyanin, phenolics, antioxidant activity and some physical parameters, as well as the effects of TAC and TPC on antioxidant activity, were investigated in contrasting types of corn during maturation. Differences in these parameters were found among corn types and maturation stages. On the whole, black corn had the highest quantity of phytochemicals and the best antioxidant activity, followed by yellow corn, and lowest in white corn except for DPPH radical scavenging activity. Immature seeds of four types of corn studied in the present study possess high concentrations of carotenoids and antioxidant activity, and they have high contents of TAC and TPC but for black corn at the maturity stage. Therefore, the choice of the types and harvesting time of waxy corn is one of the important factors that should be considered while consuming waxy corns as a fresh food or whole grain food. Nevertheless, more studies need to be performed on anthocyanin and phenolic compounds in immature waxy corns and the effects of processing conditions on phytochemicals with biological activity.

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ABBREVIATIONS USED

GA, gallic acid; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ABTS, 2,2′-azino-bis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salts; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TPTZ, 2,4,6-tri(2-pyridyl)-*s*-triazine; GAE, gallic acid equivalents; CGE, cyanidin 3-glucoside equivalents; TAC, total anthocyanin content; TPC, total phenolic content; DW, dry weight of grains; HPLC, high-performance liquid chromatography; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; DAS, days after seeding.

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