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Variability in phytochemicals and antioxidant activity in corn at immaturity and physiological maturity stages

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Abstract

The pigmented corn is a rich source of phytochemicals and many secondary metabolites. Therefore, the objectives of this research were to evaluate the performance of corn genotypes with different colors for total anthocyanin content (TAC), total carotenoid content (TCC), total phenolic content (TPC), 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and Trolox equivalents antioxidant capacity (TEAC) at immaturity and physiological maturity stages, to study the correlation among studied traits and to identify corn genotypes with high phytochemical and antioxidant activities. Corn harvested at dry kernel stage was significant and slightly higher than corn harvest at fresh kernel stage for all parameters. The purple waxy corn genotypes had the highest TAC at both stages. Field corn had the highest TCC followed by super sweet corn. For fresh kernel stage, a super sweet corn genotype (SWWY) had the highest TPC. Purple waxy corn genotypes had the highest antioxidant activity, cyanidin-3-glucoside and pelargonidin-3-glucoside at both maturity stages. TAC was significantly correlated with TEAC, DPPH, FRAP, and TPC. TPC had positive and significant correlations with TEAC, FRAP, and DPPH. Positive and significant correlations between DPPH with TEAC and FRAP were observed, whereas FRAP had a close association with TEAC. The information of this study could be used for consumer's selection of a specialty corn, production planning, development of health food products, and pharmaceutical industries.

Introduction

Corn (*Zea mays* L.) has a wide range of kernel colors such as white, yellow, orange, purple and black. In addition to its attractive colors, pigmented corn is rich in phytochemicals and many secondary metabolites such as phenolic compounds, carotenoids and flavonoids (Žilić *et al*., 2012). These constituents are regarded as an important source of antioxidants in cereals and exist in free as well as bound form (Montilla *et al.*, 2011). Corn starch is used as food additives to improve health benefits (Lim *et al*., 2013). Moreover, corn silk is also rich in these phytochemicals and also used as food additives (Ng and Wan Rosli, 2013).

Field corn with yellow or orange kernel color of endosperm has been recognized as the major source of carotenoids (provitamin A) for animal feeds (Yang and Zhai, 2010), and normal purple corn is a rich source of anthocyanin for use as colorants and functional food ingredients (Jing and Giusti, 2007). Corn showed the potential of health benefits for its antioxidant activities (Adom and Liu, 2002). Several publications have also reported phytochemical contents in different types of corns including carotenoids (Ibrahim and Juvik, 2009; Žilić *et al*., 2012), anthocyanins (Castañeda-ovando *et al.*, 2010; Cui *et al*., 2012), phenolics compounds (Zhao *et al.*, 2008; Xu *et al.*, 2010; Montilla *et al.*, 2011), antioxidant activity in normal corn (Lopez-Martinez *et al*., 2009) and tocopherol (Ibrahim and Juvik, 2009) and antioxidant activity in super sweet corn (Midoh *et al*., 2010; Song *et al*., 2010). Waxy corn is a good source of carotenoids (Kuhnen *et al*., 2010; Hu and Xu, 2011), anthocyanins, phenolics and antioxidant activity (Hu and Xu, 2011).

Because corn contains useful phytochemicals beneficial to health, corn products are therefore regarded as functional food. However, most studies so far have focused on colored kernels, and limited information is available for other types of corn at both immaturity and physical maturity stages. Therefore, the objectives of this study were to evaluate the performance of corn genotypes for carotenoids,

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anthocyanins, phenolic compounds and antioxidant activities at immaturity and physiological maturity stages, to study the correlation among studied traits and to identify corn genotypes for high phytochemical and antioxidant activities.

Materials and Methods

Corn materials

Nine hybrids and four open pollinated varieties were used in this study. The corn varieties consisted of one field corn (orange), two super sweet corn (yellow and bi-color) and ten waxy corns (white, yellow, purple, bi-color and tri-color) (Table 1). These genotypes were grown in the dry season in 2011/2012 at the Vegetable Experimental Farm of Khon Kaen University, Thailand.

The entries were planted in four-row plots with five-meters in length and spacing of 80 cm x 25 cm. Corn ears were self or sib-pollinated using hand pollination method. Corn kernels of field corn, sweet corn and waxy corn were harvested at immaturity growth stage (R4) or fresh kernel stage and physical maturity stage or dry kernel stage. Kernels samples of each maturity stage were recorded for kernel fresh weight, the kernels were oven-dried at 105 ºC for 144 hours and kernel dry weight and moisture percentage of fresh kernels were then determined. Immature kernels were immediately frozen in liquid nitrogen to block the enzymatic activities, and stored at -20 ºC until the samples were analyzed. All samples were lyophilized and stored for further analysis. The samples were ground into fine powder in a mortar and the powder was screened through a 64 mesh sieve. The samples of powder and milling fractions were mixed thoroughly and used for extraction.

Sample extraction

Extraction method for total carotenoid was described by Jaiswal *et al.* (2010) with minor modification. Samples powder of 1 gram were taken from a mortar and 6 mL of MtOH:BHT was added to each sample and grind for 10 minutes. The samples were then taken in Falcon tubes and mixed by vortexing. The samples were incubated at 60°C in water bath for 6 minutes and mixed after 3 minutes by vortexing for 10 seconds. Care must be taken to loosen the caps during heating. Freshly prepared 120 µl of KOH (1 g KOH/mL H_2O) was added to each sample and vortexed thoroughly for 20 seconds.

Saponification was done by incubating the samples for 5 minutes at 60°C in water bath, vortexing for 10 seconds and further incubating at 60 °C for 5 minutes.

Table 1. Color of kernel and ratio of kernel color of 13 corn genotypes

Variety	Ratio of kernel color (%)			
	White	Yellow	Orange	Purple
sweet corn				
SWY[1/]	-	100	-	-
SWWY	26	74	-	-
Waxy corn				
SmallearW	100	-	-	-
SmallearY	-	100	-	-
SmallearWY	31	69	-	31
Small earWYP	26	28	46	26
WW1[1/]	100	-	-	-
WW2	100	-	-	-
WWP[1/]	31	-	-	69
WP[1/]	-	-	-	100
WP1	-	-	-	100
WP2	-	-	-	100
Field corn				
FC	-	-	100	-

[1/]Commercial variety

The samples were cooled down on ice and 4 mL of H_2O was added. The samples were further added with 3 mL of Petroleum Ether (PE): Diethyl Ether (DE) (2:1, v/v) and mixed by vortexing and centrifuging for 10 minutes at 4000 rpm. After centrifuging, the supernatant was removed by means of pipette and transferred to falcon tube.

The process of mixing of 3 mL of PE:DE (2:1, v/v), centrifuging and removing of the supernatant was repeated three times, and, finally, approximately 8-9 mL of the extract was recovered. The supernatant from three consecutive centrifugations were mixed together and PE:DE (2:1, v/v) was added into the same volume of 10 mL. The mixtures were taken immediately for photometry to minimize solvent evaporation.

The extraction methods for total anthocyanins, phenolics, and antioxidant activity were those described in Yang *et al.* (2008) with minor modification. Powder samples of 0.5 grams were ground using mortar and pestle, mixed with 25 mL of methanol containing 1% 1M citric acid in a falcon tube. The samples were vortexed and then stranded at 4ºC for 24 hours. The homogenates were then centrifuged for 15 minutes at 5000 rpm and 4ºC. After centrifugation, the supernatants or the extracts were obtained by filtering through Whatman filter papers and stored at -20ºC in the dark for total anthocyanins, phenolics, and antioxidant activity analysis.

Determination of total carotenoid content (TCC)

The TCC was determined using the spectrophotometric method as described in Jaiswal *et al.* (2010). Optical density was measured at 450 nm in a spectrophotometer with the appropriate blank (PE:DE: 2:1, v, v), and the amount of carotenoid in µg/g of dry weight was calculated using Lambert-Beer equation (as described in Rocheford's Lab protocol).

Determination of total anthocyanin content (TAC)

TAC was determined using the pH differential method as described by Giusti and Wrolstad (2001). The appropriate dilution factor was determined by diluting the sample with 0.025 M potassium chloride buffer, pH 1.0, and the other samples were diluted with sodium acetate buffer, pH 4.5, diluting each by the previously determined dilution factor. These dilutions were allowed to equilibrate for 15 minutes. The absorbance of each dilution was measured at the $\lambda_{vis\text{-}max}$ (510 nm) and at 700 nm, against a blank cell filled with distilled water. The absorbance of the diluted sample (A) was calculated as follow: A = (A $\lambda_{vis\text{-}max}$ - A 700) pH 1.0 – (A $\lambda_{vis\text{-}max}$ - A 700) pH 4.5, and the monomeric anthocyanin pigment concentration in the original sample was calculated using the following formula:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1),$$

when pigment content was calculated as cyaniding-3-glucoside, where MW is the molecular weight (MW = 449.2), DF is the dilution factor (for example, if a 0.2 mL sample is diluted to 3 mL, DF = 15), and ε = molar absorptivity (ε = 26,900).

Anthocyanin levels were showed as milligrams of cyanidin 3-glucoside equivalents (CGE) per g of dry weight (DW).

Determination of total phenolic content (TPC)

TPC was determined based on the Folin-Ciocalteu's colorimetric method as described by Xu *et al.* (2010). Briefly, a 0.5 mL sample, 2.5 mL of deionized water and 0.5 mL of 1.0 M Folin-Ciocalteu reagent were mixed in 10 mL test tube 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. Extracted 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)/g 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.* (2010). 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 minutes in dark room, and the absorbance was measured at 517 nm against a solvent blank. The scavenging rate on DPPH radicals was calculated according to the formula as follows:

$$\text{scavenging rate (\%)} = [(A_0\text{-}A_1)/A_o] \times 100,$$

where A_o is the absorbance of the control solution (0.5 mL extraction solvent in 4.5 mL of DPPH solution) and A1 is the absorbance in the presence of phenolic extracts in DPPH solution.

The reducing ability was determined by using the ferric reducing antioxidant power (FRAP) assay described by Xu *et al.* (2010). Briefly, the FRAP reagent was 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 daily and warmed to 37ºC in a water bath prior to use. Then 0.1 mL of extracts were 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 minutes at 37ºC. A standard curve was constructed using $FeSO_4$ solution (100-1000 μM). FRAP value was expressed as micromoles of Fe(II)/g 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 by Jemai *et al.* (2009) with slight modifications. Briefly, $ABTS^+$ radical cation was generated by a reaction of 7 mmol/L ABTS and 2.45 mmol/L potassium per sulfate. The reaction mixture was allowed to stand in the dark at room temperature for 16-24 hours before use and it was 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 micro-liters of the diluted extracts were mixed with 1.9 mL of diluted $ABTS^+$ solution. The assay with the mixture was carried out in triplicates, the mixture was allowed to stand for 6 minutes 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.

HPLC analysis of anthocyanin components

The extracts were filtered through a 0.2 μm filter. Individual anthocyanins were separated and quantified by high-performance liquid chromatography (HPLC), Shimadzu LC-20AC pumps (Shimadzu Co., Kyoto, Japan), SPD-M20A with a photodiode array detector. A XselectCHS C-18 column (4.6

mm x 250 mm, i.d. 5 µm) was used and operated at 30°C. The anthocyanins were eluted at 1 mL/minutes using a gradient system consisting of two solvents: (A) acidified methanol (methanol-0.1% HCl, 85:15, v/v) and (B) 10% formic acid. The gradient was programmed at 20:80 (A:B, v/v) for 0.5 minutes, then changed to 85:15 for 9 minutes, 95:5 (A:B, v/v) for 0.5 minutes, and then returned to the original solvent composition within 9.5 minutes. The separated compounds were subsequently detected and identified at 520 nm on the basis of chromatographic retention times and by co-elution with added standards. Three pure anthocyanins (cyanidin 3-glucoside [kuromanin], pelargonidin 3-glucoside [callistephin], and peonidin 3-glucoside) and one anthocyanidin (cyanidin chloride) (Extrasynthese, Genay, France) were used for calibration and quantification. The extracts with pure anthocyanins were used to assist the identification of individual anthocyanins. The stock standard solutions were prepared in acidified methanol (pH 1) by weighing exactly 200-300 µg in 200 µL; 20 µL of stock solution and then diluted to 500 µL to prepare working standard solutions. The standard anthocyanins exhibited a linear relationship with a HPLC peak area in a concentration range of 0.0-1.0 µg. The coefficient of determination (r^2) ranged from 0.9933 to 0.9998 for a mixture of pure anthocyanins, which were separated by HPLC using 5, 10, and 15 µL of working standard solution.

Statistical analysis

Combined analysis of variance according to a complete randomized design was performed separately for all characters under investigation for immaturity stage or fresh kernel stage and physiological maturity stage or dry kernel stage, and where main effect was significant ($P \leq 0.05$), least significant difference (LSD) was used to compare means. All analyses were accomplished using M-STAT-C package (Bricker, 1989). The correlations between antioxidant compounds and antioxidant activities were determined by Pearson's correlation analysis. The calculation of correlation coefficients was done in Microsoft Excel.

Results and Discussion

Immature and mature kernels

In general, kernel harvested at maturity had phytochemicals higher than kernel harvested at immature stage. Kernel harvested at maturity was significantly higher than that harvested at immature growth stage for total carotenoid content (TCC), total phenolic content (TPC) (Table 2), DPPH radical scavenging activity (DPPH), Trolox equivalent antioxidant capacity (TEAC) (Table 3). However, the highest TCC (9.7 µg/g DW) and DPPH (34.2%) were observed in dry kernels.

Table 2. Means for total anthocyanin content (TAC), total carotenoid content (TCC), total phenolic content (TPC) of fresh and dry kernels from 13 corn varieties grown in the dry season 2011/12

Genotypes	TAC (mg of C-3-G/g) DW)		TCC (µg/g DW)		TPC (mg of GAE/g) DW)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
SWY	0.08	0.05	9.6	33.3	2.5	3.5
SWWY	0.06	0.11	20.2	31.1	3.1	3.3
SmallearW	0.09	0.05	1.2	1.3	1.9	2.1
SmallearY	0.09	0.13	11.1	10.8	1.7	2.2
SmallearWY	0.11	0.16	7.5	2.9	1.9	2.1
SmallearWYP	0.19	0.16	6.2	2.7	1.8	2.0
WW1	0.00	0.11	1.1	1.4	1.9	2.2
WW2	0.00	0.35	1.6	2.0	1.3	2.2
WWP	0.08	0.35	0.9	1.2	1.9	2.1
WP	1.52	1.65	2.6	1.1	2.6	4.5
WP1	0.54	1.22	1.1	1.0	2.4	4.1
WP2	0.78	1.15	1.1	1.4	2.3	4.1
FC	0.12	0.14	23.3	35.6	1.7	2.4
Mean	0.3	0.4	6.7	9.7	2.1	2.8
LSD 0.05	0.04	0.06	0.8	0.4	0.5	0.1

Table 3. Means for DPPH radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and Trolox equivalents antioxidant capacity (TEAC) of fresh and dry seeds from 13 corn varieties grown in dry season 2011/12

Genotypes	DPPH (% reduction)		FRAP (µmolFe(II)/g DW)		TEAC (µmolTE/g DW)	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
SWY	34.5	29.9	0.12	0.09	3.6	2.7
SWWY	29.1	33.9	0.12	0.10	3.8	4.3
SmallearW	15.0	34.9	0.04	0.07	1.7	2.4
SmallearY	17.5	17.2	0.05	0.07	1.6	2.4
SmallearWY	17.6	17.4	0.04	0.08	1.8	2.7
SmallearWYP	19.6	21.6	0.04	0.08	2.1	2.7
WW1	19.0	31.2	0.06	0.09	1.9	3.2
WW2	16.1	25.8	0.06	0.01	1.8	2.0
WWP	25.4	23.9	0.08	0.19	2.1	2.5
WP	62.8	55.8	0.17	0.18	6.4	7.5
WP1	48.5	68.9	0.13	0.20	5.3	6.8
WP2	54.7	68.0	0.14	0.17	6.4	6.9
FC	13.2	15.7	0.07	0.07	1.8	2.7
Mean	28.7	34.2	0.09	0.10	3.1	3.8
LSD 0.05	5.0	17.6	0.01	0.02	0.1	0.2

In previous findings, kernel harvested at mature stage had higher TAC, TPC and antioxidant activity than that harvested at immature stage but mature kernel had lower TCC than did immature kernel of yellow corn (Xu and Hu, 2011). The results in this study supported previous findings for TAC, TPC and antioxidant activity but the results were contrasting for TCC. The difference in the results of different studies could be possibly due to a genetic background of corn materials. Different color kernels were used in this study, and the color developed until maturity. This might be the reason why TCC in this study was higher than that in previous study.

Anthocyanin content

TAC values ranged from 0.00 to 1.52 mg of cyanidin-3-glucoside/g DW for fresh kernel stage and 0.05 to 1.65 mg of cyanidin-3-glucoside/g DW for dry kernel stage, and corn genotypes were highly significantly different ($P \leq 0.01$) for this trait (Table 2). WP (purple waxy corn) had the highest TAC of 1.52 mg of cyanidin-3-glucoside/g DW for fresh kernel stage followed by WP2 (0.78 mg of cyanidin-3-glucoside/g DW) and WP1 (0.54 mg of cyanidin-3-glucoside/g DW), respectively. High TAC at immature stage is important for vegetable waxy corn as antocyanins is directly related to antioxidant activity and differences in this trait indicates that types and harvesting time have significant influences on functional properties of waxy corns (Xu and Hu, 2011). WP also had the highest TAC of 1.65 mg of cyanidin-3-glucoside/g DW for dry kernel stage followed by WP1 and WP2 (1.22 and 1.15 mg of cyanidin-3-glucoside/g DW, respectively). WP1 and WP2 are single cross hybrids derived from the same male but different female parents.

The results supported previous findings of purple waxy corn (Xu and Hu, 2011) and purple maize (Kuhnen *et al.*, 2010; Zilić *et al*., 2012). The colored genotypes (black, purple, and blue) have been found to contain higher levels of TAC than the yellow and white varieties (Lopez-martinez, 2009). The differences amount of TAC among various corn genotypes could be related to the locations of the pigments in the grains or distinct anthocyanin compositions, and pigmentation can occur in the pericarp, aleurone and starchy endosperm (Betŕan *et al*., 2001). WP is a commercial hybrid with purple kernels darker than PW1 and PW2. PW1 and PW2 are related varieties with common male parent but different female parents, and, therefore, PW1 and PW2 were similar in chemical compositions in kernels.

The transparent pericarp (outer layers of the grains) of those genotypes indicates the absence or lower concentration of anthocyanins. On the other hand, the pericarp of the purple-colored grains showed a higher concentration of pigments, while their endosperm was less pigmented (Kuhnen *et al.*, 2010). Similarly, there are several registers of the occurrence of anthocyanins on the external layers of maize grains (Li *et al.*, 2008). However, the tested purple waxy corns of this study are thought to be new and interesting sources of biomass for obtaining extracts rich in anthocyanins, and thus may have applications in the food, pharmaceutical and cosmetic industries.

Carotenoid content

TCC values ranged from 0.9 to 23.3 µg/g DW for fresh kernel stage and 1.0 to 35.6 µg/g DW for dry kernel stage and corn genotypes were significantly different for TCC (Table 2). Field corn with orange kernel color had the highest TCC (23.3 µg/g DW) followed by SWWY or bi-color sweet corn genotype (20.2 µg/g DW) for fresh kernel stage. For dry kernel stage, field corn also had the highest TCC (35.6 µg/g DW) followed by SWY (33.3 µg/g DW) and SWWY (31.1 µg/g DW).

Total carotenoids first decreased, then increased, and then decreased to minimum at maturity stage (Xu *et al.*, 2010). The β-carotene and lutein contents ranged from 0 to 2.42 mg/kg dry matter and from 0 to 13.89 mg/kg dry matter, respectively (Žilić *et al.*, 2012). The range of carotenoids was in agreement with previous findings. However, high carotenoids at dry kernel stage in this study did not agree with that reported by Xu *et al*. (2010).

It should be noted that the maize kernel color, strictly depends on conjugated double bonds and the various functional groups contained in the carotenoid molecule (Rodriguez-Amaya and Kimura, 2004). Moreover, the results supported by previous studies that, for different corn types at the same stage, the levels of carotenoids were highest in normal corn, followed closely by yellow and black corns, but lowest in the white corn (Xu and Hu, 2011). In this study, the purple landraces (SWY and SWWY) showed greater potential sources of carotenoids compared to other vegetable corns. Carotenoids are plant pigments that act as antioxidants, and are especially associated with eye health (Kean *et al*., 2008). These suggested that the possibility of producing vegetable corn varieties rich in health beneficial factors for optimum human nutrition though breeding programs.

Phenolic content

TPC values ranged from 1.3 to 3.1 mg of GAE/g DW for fresh kernel stage and 2.0 to 4.5 mg of GAE/g DW for dry kernel stage (Table 2). For fresh kernel stage, SWWY sweet corn genotype with yellow and white kernel color had the highest TPC (3.1) followed by WP, SWY, WP1 and WP2. These genotypes are waxy corn and sweet corn with purple and yellow kernels colors and intermediate TPC values (2.3 to 2.6 mg of GAE/g DW). At dry kernel stage, WP, WP1 and WP2 (All genotypes are purple waxy corn.) had the highest TPC values (4.1 to 4.5 mg of GAE/g DW).

In earlier study on waxy corn with different types of kernel colors at different stage of maturation,

Corn genotypes with purplish red waxy kernels had the highest phenolic compounds (2.55-3.88 mg of GAE/g of DW) compared to corn genotypes with white waxy (0.23-1.04 mg of GAE/g of DW) and yellow waxy (0.53-0.92 mg of GAE/g of DW) and yellow normal kernels (0.67-1.11 mg of GAE/g of DW) (Xu and Hu, 2011). In comparison to previous findings, TPC values in this study were higher at both maturation stages (immature and mature) and all color types. Corn genotypes with purplish black and blue kernels also had exceptionally high phenolic compounds compared to corn genotypes with light-colored kernels (Del Pozo-Insfram *et al*., 2007). Corn genotypes with purple kernels exhibited the greatest phenolic levels followed by corn genotypes with red and black kernels, respectively (Lopez-martinez *et al.*, 2009).

The results in this study supported previous findings and confirmed that corn genotypes with darker kernel color were associated with high total phenolic content than corn genotypes with lighter kernel color. Therefore, purple waxy corn in this study is an important source for improving phenolic content.

Antioxidant activity

Corn genotypes could be classified into high, intermediate and low groups for DPPH at both maturity stages (Table 3). The high group consisted of WP, WP1 and WP2 of purple waxy corn with DPPH values ranging from 48.5 to 62.8% for fresh kernel stage and 55.8 to 68.9% for dry kernel stage. The intermediate group comprised two genotypes of sweet corn (SWY and SWWY) with DPPH values ranging from 29.1 to 34.5% for fresh kernel stage and 29.9 to 33.9% for dry kernel stage, and the low group included other waxy corn genotypes and field corn with DPPH values ranging from 13.2 to 25.4% for fresh kernel stage and 15.7 to 34.9% for dry kernel stage. This classification was clear cut for high group but there was some overlap in intermediate and low groups.

WP, WP1 and WP2 (purple waxy corn) formed high group with FRAP values ranging from 0.13 to 0.17 µmol Fe(II)/g DW for fresh kernel stage and 0.17 to 0.20 µmol Fe(II)/g DW for dry kernel stage. SWY and SWWY formed an intermediate group with FRAP value of 0.12 for fresh kernels and 0.09 and 0.10 for dry kernels. Other genotypes were classified into low group with FRAP values ranging from 0.04 to 0.08 µmol Fe(II)/g DW for fresh kernel stage and 0.01 to 0.19 µmol Fe(II)/g DW for dry kernel stage. Again, there was some overlap between intermediate and low groups but the high group was clearly separated from intermediate group especially for fresh kernels.

Table 4. Means for cyanidin-3-glucoside and pelargonidin-3-glucoside determined by HPLC (520 nm) of fresh and dry kernels of 5 waxy corn genotypes

Genotypes	Cyanidin-3-glucoside (mg/g seeds)		Pelargonidin-3-glucoside (mg/g seeds)	
	Fresh	dry	Fresh	Dry
SmallearWYP	nf	nf	nf	nf
WWP	nf	nf	nf	nf
WP	0.07	0.09	0.65	0.57
WP1	0.06	0.08	0.72	0.29
WP2	0.06	0.10	0.71	0.53
LSD 0.05	0.01	0.02	0.08	0.05

nf, not found

Corn genotypes could be classified into three categories based on TEAC values. WP, WP1 and WP2 (purple waxy corn) were put into high group with TEAC values ranging from 5.3 to 6.4 µmol TE/g DW for fresh kernel stage and 6.8 to 7.5 µmol TE/g DW for dry kernel stage. SWY and SWWY represented the intermediate group with TEAC values of 3.6 and 3.8 for dry kernels and 2.7 and 4.3 for dry kernels. Other genotypes including waxy corn and field corn with TEAC values ranging from 1.6 to 2.1 µmol TE/g DW for fresh kernel stage and 2.0 to 2.7 µmol TE/g DW for dry kernel stage were classified into low group. There was slight overlap between low and intermediate groups especially for dry kernels, but high group was clearly separated from intermediate group.

DPPH gave the highest mean values (28.7 to 34.2), TEAC had intermediate mean values (3.1 to 3.8), and FRAP gave the lowest mean values (0.09 to 0.10). Although three methods of determining antioxidant activity provided different mean values, they produced the similar information by dividing the corn genotypes into three groups, and the data were consistent among three methods. The antioxidant activity is influenced by many factors, and there are more commonly used methods that each has their advantages and disadvantages for measuring antioxidant activity, which cannot be fully described with one single method (Prior *et al*., 2005). The results indicated that the three methods are equally effective for use in evaluation of antioxidant activity in corn.

Anthocyanin identification by HPLC method

The corn genotypes of waxy corn with tri-color (white, yellow and purple), bi-color (white and purple) and mono color (purple) could be classified into two classes based on cyanidin-3-glucoside (Table

4). WP (purple waxy corn) had the highest cdyanidin-3-glucoside at fresh kernel stage (0.07 mg/g seeds), whereas WP2 and WP had high cyanidin-3-glucoside for dry kernel stage (0.10 and 0.09 mg/g seeds, respectively). This chemical was not detected in other genotypes.

Fresh kernel stage had slightly higher values of pelargonidin-3-glucoside (mg/g seeds) than did dry kernel stage. WP1 and WP2 had the highest pelargonidin-3-glucoside at fresh kernel stage (0.72 and 0.71 mg/g seed, respectively), and WP and WP2 had the highest pelargonidin-3-glucoside at dry kernel stage (0.57 and 0.53 mg/g seed, respectively). Other genotypes showed rather low pelargonidin-3-glucoside.

Cyanidin, pelargonidin, and peonidin glycosides are the main anthocyanins present in maize kernels (Lopez-Martinez *et al.*, 2009; Žilić *et al*., 2012). The compositions of these components of anthocyanins may be different in corn genotypes and populations under study. In our study, pelargonidin-3-glucoside is a major component of anthocyanin. Pelargonidin-3-glucoside is mostly presented in purple kernel corns (WP, WP1 and WP2) (Table 4), and the chemical caused high TAC in these varieties (Table 2).

The purple waxy corn genotypes in this study had purplish red or purplish pink kernels and showed high pelargonidin-3-glucoside. A study in cereals indicated that cyanidin 3-glucoside was more abundant in black and red rice and in blue, purple, and red corns, pelargonidin 3-glucoside was high in pink corn, and delphinidin 3-glucoside was prevalent in blue wheat (Abdel-Aal *et al*., 2006). Anthocyanins increase with maturity in kernels of cereals (Abdel-Aal *et al*., 2006) and fruits of horticultural crops such as strawberry (Guiwen *et al*., 1991; Hyodo, 1971), bilberry (Jaakola *et al*., 2002) and pomegranate (Kulkarni and Aradhya, 2005). The results in this study supported previous findings and also added more information on types of kernel color with high anthocyanins.

Correlations among TAC and TCC, TPC and antioxidant activity (DPPH, FRAP and TEAC)

The correlations between TAC with TEAC ($P \leq 0.01$, $r = 0.86$), DPPH ($P \leq 0.01$, $r = 0.85$), FRAP ($P \leq 0.01$, $r = 0.80$) and TPC ($P \leq 0.01$, $r = 0.73$) were positive and significant, whereas the correlation between TAC with TCC was not significant (Table 5). The correlations between TPC with TEAC ($P \leq 0.01$, $r = 0.95$), FRAP ($P \leq 0.01$, $r = 0.94$) and DPPH ($P \leq 0.01$, $r = 0.93$) were also positive and significant. Positive and significant correlations between DPPH with TEAC ($P \leq 0.01$, $r = 0.97$) and FRAP ($P \leq 0.01$, $r = 0.94$) were observed, whereas FRAP was positively and significantly correlated with TEAC ($P \leq 0.01$, $r = 0.96$).

Table 5. Correlations among total anthocyanin content (TAC), total carotenoid content (TCC), total phenolic content (TPC), DPPH radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and Trolox equivalents antioxidant capacity (TEAC) of fresh and dry seeds from 13 corn varieties

	TAC	TCC	TPC	DPPH	FRAP
TCC	0.15ns				
TPC	0.73**	0.61**			
DPPH	0.85**	0.42**	0.93**		
FRAP	0.80**	0.48**	0.94**	0.94**	
TEAC	0.86**	0.46**	0.95**	0.97**	0.96**

ns, **non-significant and significant at 0.01 probability level, respectively.

Most correlations among TAC and TCC, TPC, DPPH, FRAP and TEAC were positive and significant except for the correlation between TAC and TCC. The results indicated that TAC, TCC and TPC contributed to antioxidant activity (DPPH, FRAP and TEAC).

TAC and TCC were not well associated because these phytochemicals are synthesized under different pathways. Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They belong to a parent class of molecules called flavonoids synthesized via thephenylpropanoid pathway,while carotenoids are organic pigments that are found in thechloroplasts and chromoplasts of plants. However, both phytochemicals are health benefit and can act as anitoxidants. Therefore, TAC and TCC were well associated with DPPH, FRAP and TEAC.

In maize, total phenolic content was directly related to the total antioxidant activity (Velioglu *et al.*, 1998), and high correlations between total phenolics with DPPH ($P \leq 0.05$, $r = 0.96$) and FRAP ($P \leq 0.05$, $r = 0.96$) were reported in maize (Xu *et al*., 2010). Another study in waxy corn indicated 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 (Hu and Xu, 2011). A high-carotenoid corn diet did in fact yield eggs containing more lutein and zeaxanthin, although the effect was greater when hens were fed a competing source of lutein from marigold petals (Burt *et al*., 2013). The results supported previous findings and also added more information on the relationships of other phytochemicals with antioxidant activity.

Conclusions

Harvest at mature growth stage increased TAC, TCC, TPC DPPH, FRAP and TEAC, and corn genotypes were significantly different for these traits. WP and (purple waxy corn) had the highest TAC at both maturity stages. Field corn (FC) with orange kernel color and sweet corn genotype had high TCC at both maturity stages. SWWY (sweet corn) had the highest TPC at fresh kernel stage. Purple waxy corn genotypes had the highest antioxidant activity (DPPH, FRAP and TEAC) at both maturity stages. HPLC analysis found that corn genotypes with purple kernel colors had the highest cyanidin-3-glucoside and pelargonidin-3-glucoside at both maturity stages. TAC was closely related with TEAC, DPPH, FRAP, and TPC, and TPC had positive and significant correlations TEAC, FRAP and DPPH. Positive and significant correlation between DPPH with TEAC and FRAP were also observed, whereas FRAP had a close relationship with TEAC. The information obtained in this study is important for development of corn for used as raw material for functional food products.

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