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Anthocyanins and antioxidant activity in coloured waxy corn at different maturation stages

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ABSTRACT

The anthocyanin content and antioxidant activity of kernels from 12 genotypes of waxy corn at two maturation stages (milk and mature) were investigated. The individual anthocyanins contained in coloured waxy corn were identified and quantified by HPLC-DAD-ESI/MS analysis. Cyanidin-3-glucoside and its derivatives were detected as being most dominant. Furthermore, acylated anthocyanins constituted 67.1–88.2% and 46.2–83.6% of the total contents at the milk and mature stages, respectively. The concentration of monomeric anthocyanin increased throughout the development of each genotype of corn. The antioxidant activity, which was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, increased with ripening. However, the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) revealed decreases in some genotypes during ripening. The kernels of a purplish black waxy corn genotype (KKU-WX111031) exhibited the greatest antioxidant activity and contained the highest level of anthocyanins among the genotypes tested at both maturation stages.

1. Introduction

Epidemiological studies have confirmed that a high dietary intake of fruits, vegetables and whole-grains is strongly associated with reduced risk of chronic diseases, such as cancer and cardiovascular disease (CVD), which are the top two causes of death in the United States and in most developing countries (Isabelle et al., 2010; Liu, 2004). These health benefits are attributed to the antioxidant compounds present in edible plants. Among these compounds, anthocyanins are naturally occurring polyphenol pigments found in many fruits, vegetables, and crops (He & Giusti, 2010). There is evidence supporting a positive association between their intake and healthy biological effects displayed *in vivo*, including anticancer, anti-inflammatory, and antioxidant characteristics (Norberto et al., 2013). Purple corn is a special cultivar of corn that is rich in anthocyanins and other functional phytochemicals, and has been regarded as a health-promoting food that is widely consumed in Peru and other Andean countries (Aoki, Kuze, & Kato,

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2002; Jones, 2005). The health benefits of anthocyanins in purple corn have been attributed to their high antioxidant activities and to other mechanisms, such as the presence of components that have been shown to potentially reduce the risk of colon cancer by inhibiting the proliferation of human colon cancer cells *in vitro* (Fukamachi, Imada, Ohshima, Xu, & Tsuda, 2001; Hagiwara et al., 2001). These compounds have also been shown to help prevent heart ischaemia-reperfusion injury, cerebrovascular diseases (Toufektsian et al., 2008), diabetes and obesity (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003).

The cultivation of waxy corn (*Zea mays* L. var. *ceratina*), which produces grain predominantly comprised of amylopectin starch, has increased in many Asian countries and has led to the development of new hybrid varieties with improved eating quality (Lertrat & Thongnarin, 2008; Perera, Lu, Sell, & Jane, 2001). This type of corn is eaten directly on the cob after cooking by boiling or steaming, similar to sweet corn. Waxy corn is harvested and consumed prior to maturity to garner the original components and to enhance its palatability. Waxy corn should ideally be harvested at various maturities according to the requirements of different foods (Hu & Xu, 2011). The phytochemical compositions and concentrations vary significantly depending on the kernel colour. Specifically, waxy corns have various kernel colours, including white, yellow, purple and black. Moreover, the pigmented corn contains more antioxidants and exhibits higher antioxidant activity than non-pigmented corn (Lopez-Martinez, Oliart-Ros, Valerio-Alfaro, Lee, & Parkin, 2009). Therefore, the interest in coloured waxy corn has increased in recent years due to consumer awareness of its diverse health benefits. Corn breeders have focused on new waxy corn hybrids containing various kernel colours to enhance the functional and antioxidant compounds found in the corn (Ji, Lee, & Yamakawa, 2010). In contrast to a dietary source with a different phytochemical composition, the corn-based phytochemicals are more easily accepted among malnourished and low-income consumers, particularly in the rural areas of developing countries (Chander, Meng, Zhang, Yan, & Li, 2008).

The individual anthocyanins of purple corn have been characterized, and these include cyanidin-3-glucoside, cyanidin-3-(6″-malonylglucoside), cyanidin-3-(3″, 6″-dimalonylglucoside), pelargonidin-3-glucoside, peonidin-3-glucoside and their malonated counterparts as the major anthocyanins (Abdel-Aal, Young, & Rabalski, 2006; Aoki et al., 2002). Furthermore, a similar anthocyanin profile was also found in corn leaves, cobs and husks (Fossen, Slimestad, & Andersen, 2001; Li et al., 2008; Zhao et al., 2008). Although data on the anthocyanins and antioxidant activity of purple corn are available, these parameters have not yet been examined in waxy corn (Mahan, Murray, Rooney, & Crosby, 2013). Hence, the anthocyanins found in coloured waxy corn were determined for the first time in this study. The main purpose of the present study was to determine the antioxidant activity exhibited by 12 genotypes of waxy corn at two maturation stages through the use of three commonly used spectrophotometric methods, namely the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, ferric ion-reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC). Moreover, the individual anthocyanins were characterized by HPLC-electrospray ionization-mass spectrometry coupled with diode array detection. The results reported in this study will be valuable to consumers, corn breeding researchers, and food and nutrition researchers.

2. Materials and methods

2.1. Chemicals and reagents

Cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, Folin-Ciocalteu's phenol reagent, 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma (St. Louis, MO, USA). All of the chemicals and reagents used in the experiments were of analytical grade.

2.2. Samples

In the present investigation, 12 waxy corn genotypes were selected based on the kernel colour, total anthocyanin concentration, and total phenolic concentration, which were determined in a preliminary study (Harakotr, B., Suriharn, B., Scott, M. P., & Lertrat, K., 2014). The Vegetable Corn Improvement Project of the Plant Breeding Research Center for Sustainable Agriculture at Khon Kaen University in Thailand (Fig. 1 and Table 1) collected these genotypes, which included commercial and landrace cultivars from various countries of Asia. These waxy corns were grown at the Vegetable Research Farm at Khon Kaen University during the dry season of 2011 (November 2011 through January 2012). Each genotype was planted in three replicates in a randomized complete block design. The recommended practices for the commercial production of corn were followed. Ears were harvested by hand at the milk stage (20 days after pollination; DAP) and the mature stage (35 DAP). For the analyses, only physiologically undamaged ears with mass between 200 and 220 g were used. At the milk stage, a length of 3 cm from the terminal tip end was removed from 10 waxy corn ears to reduce the kernel maturity variation. The kernels were then manually cut from the cob, frozen in liquid nitrogen to stop the enzymatic activity, and freeze-dried. The kernels from five mature ears were manually separated from the cob and then dried at 40 °C (moisture content ≤ 13%). All of the samples were finely ground in a sample mill, sieved through a 60-mesh screen, thoroughly mixed and stored at −20 °C until analysis.

2.3. Extraction

The anthocyanins in ground waxy corn kernels were extracted according to the method described by Rodriguez-Saona and Wrolstad (2001) and Jing, Noriega, Schwartz, and Giusti (2007) with slight modifications. Approximately 2 g of each sample were added to a flask containing 25 mL of 70% aqueous acetone acidified by the addition of HCl to 0.01% and mixed well. The flasks were shaken on a platform shaker (LabScientific Inc., Livingston, NJ, USA) at 200 rpm and room temperature for 2 h. Each sample was filtered through Whatman # 1 filter paper under vacuum using a Büchner funnel, and the slurry was

Fig. 1 – Varieties of *coloured* waxy corn genotypes at the mature stage.

Table 1 – Description, origin, total phenolic content, and monomeric anthocyanin content of waxy corn kernels at different growth stages.[a]

Pigmentation	Genotypes	Sources	Total phenolic content (mg GAE/g DW)		Monomeric anthocyanin content (μg CGE/g DW)	
			Maturation stage		Maturation stage	
			Milk	Mature	Milk	Mature
Light purple	KKU-WX211003	Thailand	8.4 ± 0.1	6.9 ± 0.1	29.2 ± 0.6	88.9 ± 0.8
	KKU-WX212005	Korea	8.0 ± 0.1	7.4 ± 0.5	19.3 ± 0.6	151.2 ± 2.1
	KKU-WX121069	China	7.8 ± 0.2	7.0 ± 0.4	11.7 ± 0.7	187.2 ± 2.3
	KKU-WX122026	Taiwan	7.7 ± 0.1	9.2 ± 0.2	22.2 ± 0.3	312.9 ± 2.9
Purple-yellow	KKU-WX211004	Thailand	7.6 ± 0.2	6.0 ± 0.1	8.0 ± 0.1	26.5 ± 1.0
Dark pink	KKU-WX212006	China	7.6 ± 0.3	7.1 ± 0.4	16.6 ± 0.7	92.6 ± 0.9
Purple-white	KKU-WX212007	Laos	7.6 ± 0.1	6.6 ± 0.1	17.2 ± 0.3	29.7 ± 0.8
	KKU-WX111035	Thailand	7.6 ± 0.1	7.4 ± 0.2	39.6 ± 2.2	83.3 ± 1.8
Purplish black	KKU-WX111031	Thailand	12.3 ± 0.2	19.7 ± 0.3	694.6 ± 0.3	1438.6 ± 7.5
Multi-coloured	KKU-WX121063	Laos	6.5 ± 0.1	6.8 ± 0.2	3.5 ± 0.1	74.8 ± 2.7
Creamy white	Big white852	Thailand	6.6 ± 0.3	6.7 ± 0.2	0.6 ± 0	0.8 ± 0
Creamy yellow	KK composite	Thailand	4.8 ± 0	6.7 ± 0.1	ND	ND

ND, not detected.

[a] The data are expressed as the means ± SD of triplicate samples.

washed with 10 mL of acidified 70% acetone. The filtrate was transferred to a separatory funnel, and 15 mL of chloroform were added. The mixture was gently mixed by turning the funnel upside down a few times. The samples were stored overnight at 4 °C or until a clear partition between the two phases was obtained. The solution was transferred to a centrifuge tube and centrifuged at 11,538 × g and 4 °C for 10 min. The upper aqueous layer containing the acetone/water mixture was collected, and the chloroform/acetone layer was carefully discarded. The residual acetone and chloroform were removed from the anthocyanin extract using a rotary evaporator at 40 °C under vacuum. The volume of the extracts was increased to 25 mL in a volumetric flask by the addition of 0.01% HCl-acidified methanol.

2.4. Determination of monomeric anthocyanin content

The total monomeric anthocyanin content was measured using the pH differential method, as described by Giusti and Wrolstad (2001). A UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA) was used to measure the absorbance at 510 and 700 nm. Anthocyanin levels were expressed as μg of cyanidin-3-glucoside equivalents per g of dry weight (μg CGE/g DW), using the reported molar extinction coefficient of 26900 M^{-1} cm^{-1} and a molecular weight of 449.2 g/mol.

2.5. Determination of total phenolic contents

The phenolic contents were determined using the Folin-Ciocalteu (F-C) method as described by Hu and Xu (2011). Briefly, the appropriate dilutions of the extracts were oxidized with F-C reagent for 90 min, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue colour was measured at 765 nm, and the phenolic content was expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

2.6. Qualitative and quantitative analyses of anthocyanins by HPLC-DAD-ESI/MS

The anthocyanins of coloured waxy corn were identified and quantified using an Agilent system (Agilent Technologies, Darmstadt, Germany) equipped with a binary pump (1100 Series) and an autosampler (1200 series). The HPLC separation was performed on a Synergi Polar-RP 80A reversed-phase column (4.6 × 250 nm I.D., 4 μm; Phenomenex, Torrance, CA, USA) with a flow rate of 1 mL/min, a detection wavelength of 250–600 nm (a representative wavelength of 525 nm), and an oven temperature of 30 °C. The mobile phases used were 5% formic acid in water (phase A) and 5% formic acid in water/acetonitrile (1:1, v/v) (phase B) (Kim et al., 2012). Gradient elution was performed as follows: a gradient of 20–50% B over a 30 min period; 50% B for 5 min; a gradient of 50–20% B for 5 min; and then a final wash with 20% B for 10 min. The MS analysis was run in positive ionization mode using electrospray ionization (ESI) source. The MS parameters were each set to a cone voltage of 300 V, a source temperature of 120 °C, a desolation temperature of 500 °C, and a desolation N_2 gas flow of 1020 L/h. The range of molecular weights was *m/z* 200–1200 in the full scan mode. The MassHunter B.02 software was used for the analysis and data collection. The data pre-processing was performed using GeneSpring MS (Agilent Technologies).

2.7. Determination of antioxidant activities

The capacity for scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by measuring the bleaching of a black-coloured methanol solution containing DPPH radicals, as described by Yao, Sang, Zhou, and Ren (2010). Trolox was used as the reference compound. The results are expressed in μmol of Trolox equivalents per g of dry weight (μmol TE/g DW). The linear range of the calibration curve was 100–1000 μM.

The reducing ability was determined using the ferric reducing antioxidant power (FRAP) assay, which was performed according to the method described by Hu and Xu (2011). The FRAP values are expressed as μmol of Fe (II) per g of dry weight (μmol Fe (II)/g DW). The linear range of the calibration curve was 10–100 μM.

The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of radical cations of ABTS by antioxidants, was conducted as described by Lopez-Martinez et al. (2009). Trolox was used as the reference compound. The results are expressed in μmol of Trolox equivalents per g of dry weight (μmol TE/g DW). The linear range of the calibration curve was 100–1000 μM.

2.8. Statistical analysis

The data are presented as the means of three replicates ± standard deviation. The results were subjected to variance analysis using the JMP Pro software (version 10, SAS Institute Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Total phenolic content (TPC) and monomeric anthocyanin content (MAC) in coloured waxy corn at different maturation stages

The total phenolic content (TPC) of waxy corn is presented in Table 1. In the milk stage, the TPC ranged from 4.8 to 12.3 mg GAE/g DW. The lower values were obtained from the creamy yellow genotype, whereas the higher values were found in the purplish black genotype. Consistent with these results, Montilla, Hillebrand, Antezana, and Winterhalter (2011) also noted a higher level of TPC in a dark-coloured genotype. However, Lopez-Martinez et al. (2009) reported that the yellow-coloured genotype contained the highest amount of TPC in their study. The mature stage revealed a trend similar to that found for the milk stage. The TPC ranged from 6.0 to 19.7 mg GAE/g DW. The lowest values were found in the KKU-WX211004 genotype and the highest values were obtained in the KKU-WX111031 genotype. However, most of the samples exhibited a decrease in TPC from the milk to the mature stages with the exception of genotypes KKU-WX122026, KKU-WX111031, KKU-121063, and KK composite, which exhibited an increase in the

TPC. Interestingly, the TPC of the purplish black-coloured genotype (KKU-WX111031) was greater than those of the other corn genotype at both maturation stages.

The monomeric anthocyanin content (MAC) of coloured waxy corn is shown in Table 1. In the milk stage, the MAC ranged from 0.6 to 694.6 μg CGE/g DW: the lowest values were obtained from the creamy white-coloured genotype, and the highest values were found in the purplish-black genotype. The MAC of corn revealed a trend contrasting that described for the phenolic content; thus, most of the corn genotypes exhibited an increase in MAC from the milk to the mature stages. In the mature stage, the MAC ranged from 0.8 to 1438.6 μg CGE/g DW, and the lowest and highest values were obtained from the genotypes that exhibited the lowest and highest MAC values in the milk stage. Moreover, KKU-WX111031 contained higher amounts of MAC at both maturation stages than those reported for pigmented corn from Bolivia (19–717 μg CGE/g DW) (Montilla et al., 2011), Mexico (721 μg CGE/g DW), and the United States (307 μg CGE/g DW) (del Pozo-Insfran, Brenes, Serna Saldivar, & Talcott, 2006), but lower than the MAC of approximately 16400 μg CGE/g DW in Andean purple corn (Cevallos-Casals & Cisneros-Zevallos, 2003). Although purplish black waxy corn has a lower anthocyanin content than Andean purple corn, waxy corns are utilized as fresh food at the milk stage or as raw materials for whole-grain foods at the mature stage. Moreover, this corn type matures earlier, exhibits a better nutrient quality, and presents improved palatability compared with normal corn (Hu & Xu, 2011). The differences in anthocyanin contents may be due to various factors, including genotypes, developmental stages, growing conditions, and even the methods used for quantification (Jing et al., 2007; Xu et al., 2010). These results support our hypothesis that the greater anthocyanin content is responsible for the observation that dark coloured genotypes containing phenolics during ripening are pigmented. Our results demonstrate that the purplish black-coloured genotype (KKU-WX111031) has high TPC and MAC and is a good natural source of antioxidant compounds with improved marketability.

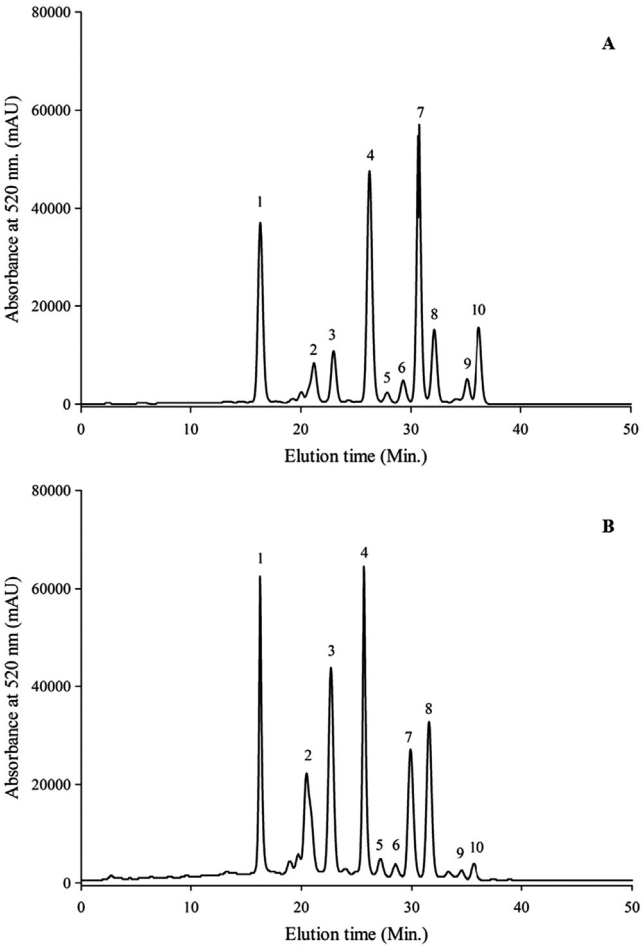

Fig. 2 – HPLC chromatograms of individual anthocyanins extracted from the waxy corn genotype "KKU-WX111031" at different maturation stages: (A) milk and (B) mature. The peak numbers show the anthocyanins that are identified in Table 2.

3.2. *Identification and quantification of anthocyanins*

The individual anthocyanins of coloured waxy corn were characterized based on the visible spectra, MS properties, and retention times of components separated by LC (Abdel-Aal et al., 2006; Aoki et al., 2002). Waxy corn showed a complex anthocyanin composition with 7–10 compounds and a maximum absorbance wavelength of approximately 520 nm (Fig. 2 and Table 2). These compounds were cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-(6″-malonylglucoside), pelargonidin-3-(6″-maloylglucoside), peonidin-3-(6″-maloylglucoside), cyanidin-3-(3″,6″-dimalonylglucoside), cyanidin-3-(6″-succinylglucoside), cyanidin-3-(3″,6″-maloylsuccinylglucoside), and peonidin-3-(6″-succinylglucoside). In addition, pelargonidin-3-(6″-maloylglucoside) was detected in the KKU-WX111031 genotype at both maturation stages and in the KKU-WX122026 genotype at the mature stage (Table 3). Moreover, peonidin-3-glucoside and cyanidin-3-(6″-succinylglucoside) were not detected in the KKU-WX211004, KKU-WX212007, and KKU-WX121063 genotypes. Significant quantitative differences were found in the anthocyanin compositions. The main anthocyanin in all of the genotypes at the milk stage was cyanidin-3-(3″,6″-dimalonylglucoside), where the main anthocyanin found at the mature stage in all of the genotypes was cyanidin-3-(6″-malonylglucoside). However, the predominant compound in the purplish black genotype (KKU-WX111031) was cyanindin-3-glucoside. These findings demonstrate that the anthocyanin constituents may be greatly affected by the specific genotypes and genetics (Cho et al., 2013). The cyanidin derivatives were the predominant compounds in all of the corn genotypes. Cyanidin derivatives constituted approximately 75.8–90.8% and 66.7–87.2% of the monomeric anthocyanin contents at the milk and mature stages, respectively. To the best of our knowledge, this is the first report on the anthocyanins present in waxy corn at both maturation stages.

The overall findings regarding the anthocyanins found in waxy corn are in accordance with those reported by other researchers. Moreno, Sanchez, Hernandez, and Lobato (2005) found that cyanidin-3-glucoside, cyanidin-3-(6″-malonylglucoside), and cyanidin-3-(3″,6″-dimalonylglucoside)

Table 2 – Massspectrometric data and identification of anthocyanins from coloured waxy corn.

Peaks	Anthocyanins[a]	m/z (amu)	
		[M] +	Fragments
1	Cyanidin-3-glucoside	449	287
2	Pelargonidin-3-glucoside	433	271
3	Peonidin-3-glucoside	463	301
4	Cyanidin-3-(6″-malonylglucoside)	535	449, 287
5	Pelargonidin-3-(6″-malonylglucoside)	519	433, 287
6	Peonidin-3-(6″-malonylglucoside)	549	463, 301
7	Cyanidin-3-(3″,6″-dimalonylglucoside)	621	449, 287
8	Cyanidin-3-(6″-succinylglucoside)	549	449, 287
9	Cyanidin-3-(3″,6″-malonylsuccinylglucoside)	635	463, 301
10	Peonidin-3-(6″-succinylglucoside)	563	463, 301

[a] These compounds are confirmed through comparisons of their retention times, peak spectra and mass-to-charge ratios to those of authentic anthocyanin standards (cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, and peonidin-3-O-glucoside).

are the major anthocyanins and that cyanidin is the main anthocyanidins accounting for 73–87% of the total anthocyanins in purplish-red corn. Montilla, Hillebrand, Antena, and Winterhalter (2011) also found cyanidin-3-glucoside and its malonated derivatives. Additionally, several dimalonylated monoglucoside of cyanidin, pelargonidin, and peonidin were found as minor constituents. Abdel-Aal et al. (2006) found pelargonidin-3-glucoside as a major anthocyanin component in pink corn. Moreover, these researchers found that the majority of anthocyanins found in pigmented corn are found in their acylated form with malonyl or succinyl moieties, and several isomers. In addition, we found cyanidin-3-glucoside, cyanidin-3-(6″-malonylglucoside), cyanidin-3-(3″,6″-dimalonylglucoside), and peonidin-3-(6″-malonylglucoside) in trace amounts in the white corn genotype (data not shown). In contrast to the results reported by Lopez-Martinez et al. (2009), anthocyanins have not been found in white- and light-coloured Mexican corn kernels. This finding was expected because these colourations indicate that carotenoids, particularly in the form of lutein and zeaxanthin, are the most abundant pigment types in light-coloured corn (Montilla et al., 2011).

Furthermore, the proportion of acylated anthocyanins in waxy corn kernels varied from 67.1% to 88.2% and from 46.2% to 83.6% of the total monomeric anthocyanin contents at the milk and mature stages, respectively. These results show that relative percentage varies among the different corn genotypes and maturation stages. The highest values of acylated anthocyanins were found in the KKU-WX212006 genotype at both maturation stages. Acylated anthocyanins are regarded as better natural food colourants because acylation is believed to confer extended stability to the anthocyanin moiety during processing and storage (Giusti & Wrolstad, 2003). Moreover, acylated anthocyanins are of interest for the development of physiologically functional foods (Matsui et al., 2001). The findings obtained in the present study indicate that coloured waxy corn at the milk stage has a higher content of acylated anthocyanins than that found at the mature stage. Therefore, immature waxy corn may be a good source of acylated anthocyanins and may provide the desirable stability for food applications.

In addition, the average anthocyanin contents at the mature stage were higher than that obtained in the milk stage, which is similar to the trend found in the analysis of the respective total monomeric anthocyanin content for each genotype. The development of all corn is characterized by the presence of a pericarp colour change varying from light- to dark-coloured. It is believed that changes in colour reflect changes in the profiles of pigments synthesized in corn, such as anthocyanins, during maturation (Hu & Xu, 2011). In general, waxy corn is consumed from corn harvested during the milk stage, when many genotypes exhibited a greater content of cyanidin derivatives, which are strong free radical scavengers, compared with some coloured rice, fruits, and vegetables (Abdel-Aal et al., 2006; Cantos, Espin, & Tomás-Barberán, 2002; Tsuda et al., 2003). Specifically, the purplish black genotype (KKU-WX111031) presented the highest content and compositions of anthocyanins at both maturation stages. These results show that waxy corns are anthocyanin-rich foods that can be consumed as a fresh food or as a whole-grain food and thus have practical applications in the functional food industry.

3.3. *Antioxidant activities in coloured waxy corn at different maturation stages*

We expected that different antioxidant activity assays would produce different rankings among the samples analyzed because no single chemical assay can accurately quantify the total antioxidant action of foods (Prior, Wu, & Schaich, 2005). Of the several methods that have been developed for the quantification of antioxidant activity, the analyses of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, ferric reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC) have been used as measures of the level of antioxidants in fruits, vegetables, cereals, and other natural sources (Cho et al., 2013). Thus, we performed DPPH, FRAP, and TEAC assays in the present study. Among the 18 genotypes of Mexican corn, pigmented genotypes showed strong antioxidant capacity with the DPPH and TEAC assays (Lopez-Martinez et al., 2009). Coloured corn contains high levels of phenolic compounds, anthocyanins, and carotenoids (Zilic, Serpen, Akillioglu, Gökmen, & Vancetovic, 2012). Moreover, black corn exhibits higher antioxidant activity than yellow and white corns (Hu & Xu, 2011). Although many studies have examined the antioxidant activity of corns, there is little informa-

Table 3 – Content of individual anthocyanins (μg per g dry weight) in waxy corn kernels at different maturation stages.[a]

Genotype	Stages	Anthocyanins									
		Cy-3-Glu	Pg-3-Glu	Pn-3-Glu	Cy-MalGlu	Pg-MalGlu	Pn-MalGlu	Cy-diMalGlu	Cy-SucGlu	Cy-MalSucGlu	Pn-SucGlu
KKU-WX211003	Milk	4.16 ± 0.01	0.45 ± 0.02	0.13 ± 0.01	7.17 ± 0.19	ND	1.99 ± 0.06	7.86 ± 0.21	0.11 ± 0.02	0.77 ± 0.03	0.52 ± 0.04
	Mature	11.26 ± 0.17	4.60 ± 0.18	1.48 ± 0.25	35.21 ± 0.90	ND	10.34 ± 0.33	11.28 ± 0.28	0.94 ± 0.16	1.63 ± 0.14	2.17 ± 0.18
KKU-WX212005	Milk	2.76 ± 0.16	2.49 ± 0.17	0.11 ± 0.02	2.90 ± 0.14	ND	0.81 ± 0.03	4.34 ± 0.18	0.48 ± 0.03	2.17 ± 0.06	0.22 ± 0.03
	Mature	16.44 ± 0.86	12.65 ± 3.14	2.62 ± 0.34	42.60 ± 0.42	ND	9.14 ± 0.95	23.59 ± 2.44	1.97 ± 0.17	3.43 ± 0.49	8.62 ± 0.96
KKU-WX121069	Milk	0.47 ± 0.06	0.39 ± 0.04	0.11 ± 0.04	1.38 ± 0.12	ND	0.30 ± 0.05	2.88 ± 0.46	0.20 ± 0.07	0.76 ± 0.18	0.20 ± 0.01
	Mature	16.49 ± 0.53	11.86 ± 0.19	3.06 ± 0.12	74.44 ± 2.07	ND	16.97 ± 1.14	42.33 ± 1.04	2.15 ± 1.05	4.07 ± 0.88	5.86 ± 1.17
KKU-WX122026	Milk	1.19 ± 0.12	0.82 ± 0.05	0.19 ± 0.07	2.68 ± 0.09	ND	0.91 ± 0.10	4.64 ± 0.21	0.29 ± 0.13	1.20 ± 0.09	0.28 ± 0.21
	Mature	29.97 ± 0.82	14.07 ± 0.74	2.64 ± 0.07	87.36 ± 2.74	Tr	25.55 ± 0.34	42.65 ± 1.15	2.57 ± 0.63	2.79 ± 0.17	5.32 ± 0.62
KKU-WX211004	Milk	0.66 ± 0.02	0.28 ± 0.01	ND	1.29 ± 0.02	ND	0.45 ± 0.03	2.01 ± 0.01	ND	0.17 ± 0.01	Tr
	Mature	2.38 ± 0.21	0.89 ± 0.07	0.28 ± 0.06	7.16 ± 0.42	ND	2.10 ± 0.21	2.40 ± 0.18	0.37 ± 0.06	0.39 ± 0.04	0.51 ± 0.08
KKU-WX212006	Milk	0.51 ± 0.04	0.66 ± 0.05	Tr	1.39 ± 0.01	ND	0.41 ± 0.02	3.59 ± 0.24	0.30 ± 0.11	3.56 ± 0.27	0.13 ± 0.02
	Mature	3.44 ± 0.16	7.81 ± 0.35	0.68 ± 0.14	17.68 ± 0.28	ND	5.78 ± 1.00	26.44 ± 1.04	2.41 ± 1.15	7.21 ± 0.30	1.21 ± 0.79
KKU-WX212007	Milk	0.91 ± 0.05	0.47 ± 0.02	ND	1.97 ± 0.08	ND	0.56 ± 0.04	4.37 ± 0.14	0.13 ± 0.05	0.51 ± 0.03	0.22 ± 0.04
	Mature	2.04 ± 0.08	1.42 ± 0.04	0.15 ± 0.05	8.07 ± 0.33	ND	2.52 ± 0.05	4.82 ± 0.19	0.20 ± 0.04	1.03 ± 0.03	0.41 ± 0.02
KKU-WX111035	Milk	4.56 ± 0.33	1.38 ± 0.09	0.59 ± 0.02	6.73 ± 0.52	ND	1.38 ± 0.07	11.83 ± 1.30	0.93 ± 0.04	1.23 ± 0.10	1.01 ± 0.03
	Mature	20.36 ± 1.34	5.81 ± 0.55	6.81 ± 1.19	22.78 ± 1.53	ND	3.32 ± 0.32	7.20 ± 0.31	5.03 ± 0.08	0.86 ± 0.28	1.10 ± 0.45
KKU-WX111031	Milk	107.41 ± 6.52	30.61 ± 0.37	32.43 ± 0.31	130.57 ± 1.53	8.62 ± 0.10	15.64 ± 0.25	182.82 ± 2.25	47.65 ± 0.71	15.36 ± 0.11	43.49 ± 0.71
	Mature	380.35 ± 3.39	98.79 ± 0.94	133.05 ± 0.01	278.96 ± 2.92	19.88 ± 0.36	15.62 ± 0.16	88.12 ± 1.16	100.68 ± 1.18	11.62 ± 3.05	11.52 ± 6.40
KKU-WX121063	Milk	0.21 ± 0.01	0.15 ± 0.05	ND	0.53 ± 0.02	ND	0.15 ± 0.01	1.08 ± 0.03	Tr	0.24 ± 0.01	Tr
	Mature	7.25 ± 76	4.54 ± 0.26	0.93 ± 0.33	24.65 ± 1.48	ND	9.39 ± 0.52	13.14 ± 0.85	0.85 ± 0.24	1.81 ± 0.15	2.25 ± 0.24

Cy, cyanidin; Glu, glucoside; Mal, malonyl; ND, not detected; Pg, pelargonidin; Pn, peonidin; Suc, succinyl; Tr, trace.

[a] The data are expressed as the means ± SD of triplicate samples.

Table 4 – Antioxidant activity of waxy corn kernels at different maturation stages determined by the DPPH, FRAP, and TEAC methods[a]

Genotypes	DPPH (μmol TE/g DW)		FRAP (μmol Fe (II)/g DW)		TEAC (μmol TE/g DW)	
	Maturation stage		Maturation stage		Maturation stage	
	Milk	Mature	Milk	Mature	Milk	Mature
KKU-WX211003	11.6 ± 0.1	13.5 ± 0.5	16.6 ± 0.3	35.5 ± 0.2	31.8 ± 0.5	57.7 ± 0
KKU-WX212005	11.2 ± 0	14.2 ± 0.2	11.7 ± 0.4	43.8 ± 0.3	32.1 ± 1.1	61.8 ± 0.4
KKU-WX121069	10.2 ± 0.3	13.9 ± 0	30.6 ± 0.1	41.6 ± 0.1	37.2 ± 0.3	18.8 ± 1.2
KKU-WX122026	10.1 ± 0.1	16.8 ± 0.4	33.0 ± 0.6	49.9 ± 0.1	48.8 ± 2.6	2.0 ± 2.8
KKU-WX211004	10.8 ± 0.1	12.1 ± 0.6	13.7 ± 0.1	23.7 ± 0.6	28.2 ± 1.5	48.9 ± 0.9
KKU-WX212006	10.3 ± 0.1	10.9 ± 0	11.6 ± 0.1	31.1 ± 0.3	29.6 ± 0.5	54.6 ± 0.6
KKU-WX212007	7.0 ± 0	8.3 ± 0.3	11.9 ± 0.3	21.2 ± 0.3	35.2 ± 1.2	53.4 ± 1.1
KKU-WX111035	9.9 ± 0	13.1 ± 0	12.6 ± 0.1	31.8 ± 0.2	35.6 ± 0.6	2.2 ± 0.6
KKU-WX111031	11.7 ± 0	21.6 ± 0	69.8 ± 0.6	159.2 ± 0.8	95.4 ± 0.2	156.8 ± 0.9
KKU-WX121063	5.4 ± 0.2	11.1 ± 0.1	22.3 ± 0.8	29.1 ± 0.2	28.9 ± 0.5	16.1 ± 2.2
Big white852	7.8 ± 0.1	10.0 ± 0.4	27.3 ± 0.1	21.6 ± 0.1	31.2 ± 1.1	4.2 ± 1.6
KK composite	0.2 ± 0.1	5.6 ± 0.1	4.3 ± 0.1	18.2 ± 0.8	8.4 ± 0.6	3.2 ± 0.9

DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity.

[a] The data are expressed as the means ± SD of triplicate samples.

tion available for the comparison of the antioxidant effects of different coloured waxy corn genotypes during ripening. The DPPHs of acidified methanol extracts from 12 waxy corn genotypes at two maturation stages are shown in Table 4. At the milk stage, the DPPH values ranged from 0.2 to 11.7 μmol TE/g DW. The lowest values were obtained from the creamy yellow genotype and the highest values were obtained from the KKU-WX111031 and KKU-WX211003 genotypes. However, the TPC and MAC in KKU-WX211003 were much lower than those found in the KKU-WX111031 genotype. Yang and Zhia (2010) reported that anthocyanins contribute mainly to the TPC and antioxidant activity. However, our results did not support this claim. It is possible that the anthocyanins in coloured corn will interfere with the DPPH radical assay, leading to an underestimation of their antioxidant activity (Yao et al., 2010). At the mature stage, the DPPH values ranged from 5.6 to 21.6 μmol TE/g DW: the lowest values were obtained from the creamy yellow genotype, whereas the highest values were found in the KKU-WX111031 genotype. All of the samples exhibited an increase in DPPH from the milk stage to the mature stage.

The ferric reducing antioxidant power (FRAP) revealed a trend similar to that described for the DPPH radical scavenging ability. The FRAP values ranged from 4.3 to 69.8 μmol Fe (II)/g DW and from 18.2 to 159.2 μmol Fe (II)/g DW at the milk and mature stages, respectively. In general, the genotypes that showed the greatest DPPH scavenging activity were also those with the greatest ferric ion-reducing activities, namely the KKU-WX111031, KKU-WX122026, KKU-WX212005, and KKU-WX121069 genotypes. Furthermore, most samples exhibited an increase in FRAP during ripening with the exception of the creamy white genotype, which presented a decrease in this measure. Consistent with this result, Hu and Xu (2011) observed that the FRAP value of white-coloured corn decreased continuously during ripening. Moreover, the present study observed that the highest reducing activity was observed in the pigmented genotypes, whereas the lowest reducing activity was found in the creamy yellow corn genotype.

The results of TEAC assay for the kernel extracts from the 12 genotypes exhibit a pattern similar to the DPPH and FRAP results. However, half of the genotypes exhibited a decrease in TEAC from the milk to the mature stage. The genotypes that showed a decrease during development also exhibited a decrease in phenolic contents, suggesting that the phenolic content may be related to the antioxidant activity. However, the phenolic content does not fully explain the observed antioxidant activities because some genotypes presented reduced phenolic contents but increased antioxidant levels. These results confirm previous research findings, which showed that the ABTS radical is more sensitive to phenolic-containing compounds than the DPPH radical (Serpen, Capuano, Fogliano, & Gökmen, 2007). The TEAC values ranged from 8.4 to 95.4 μmol TE/g DW at the milk stage and from 2.0 to 156.8 μmol TE/g DW at the mature stage. The purplish black (KKU-WX111031) genotype showed relatively higher radical scavenging activity than all the other genotypes tested. Consistent with this result, Zilic et al. (2012) reported that pigmented genotypes exhibited significantly stronger scavenging capacity than non-pigmented genotypes. According to the data obtained in the present study, it may be concluded that a higher content of anthocyanins in the corn kernel contributes to an increase in antioxidant activity.

4. Conclusions

This contribution provides information on the concentration of anthocyanins and the antioxidant activity in different genotypes of waxy corn at various maturation stages. The anthocyanin profiles exhibited qualitative and quantitative differences between genotypes and maturation stages. These observations are the first characterization of the anthocyanins in coloured waxy corn. The major anthocyanins were cyanidin-3-glucoside, cyanidin-3-(6″-malonylglucoside) and cyanidin-

3-(3″,6″-dimalonylglucoside). Most of the anthocyanins found were acylated anthocyanins, which are regarded as better candidates for food colourants because of their increased stability. In general, the purplish black genotype (KKU-WX111031) consistently exhibited the highest levels of anthocyanins and antioxidant activity among the genotypes tested. This study indicated that waxy corn, particularly the dark-coloured genotypes, is a valuable source of bioactive compounds and an effective source of natural antioxidants and thus may be of interest to various industries, e.g., dietary supplements, food additives, and cosmetics. An analysis of the antioxidant compounds and their activities in pigmented waxy corn may be of value and, in particular, may be an important factor for the development of corn breeding and nutritional foods. Further studies should be conducted to determine whether the content of antioxidants and their activities in the different parts of waxy corn, such as the cob, silk, and husk, create potentially valuable co-products for use as food additives or natural antioxidants. Additionally, the effect of cooking, processing and importantly, the applications of phytochemicals, should be studied with respect to their biological properties.

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