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Original Article

Phenolics, anthocyanins and antioxidant activities in waste products from different parts of purple waxy corn (*Zea mays* L.)

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Abstract

The objective of this study was to evaluate total phenolic content (TPC), total anthocyanin content (TAC), antioxidant activities (AOA) and identify the components of phenolic and anthocyanin in each type of purple waxy corn (PWC) waste, including tassel, cob, kernel pericarp and silk. The results revealed that the silk had the greatest amount of TPC related to the highest AOA, while the cob had the highest TAC. Cyanidin-3-glucoside was the dominant anthocyanin in PWC, followed by peonidin-3-glucoside and pelargonidin-3-glucoside in rank order. Protocatechuic acid was the most abundant phenolic compound in kernel pericarp and silk, while rutin was the dominant component detected in tassel and cob. Additionally, hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic and sinapic acid as well as quercetin and kaempferol were also detected in different parts of PWC.

Keywords: purple waxy corn; phenolics; anthocyanins; antioxidant activities

1. Introduction

The interest in functional and nutraceutical food has increased due to the health conscious consumers. Several studies have reported that various biological activities, such as antioxidant activities, anti-mutagenic activities, and inhibition of colorectal carcinogenesis, etc., are associated with anthocyanins and other phenolic compounds (Cevallos-Casals & Cisneros-Zevallos, 2003). Most reports on anthocyanins and phenolics in plants have focused on vegetables, fruits and wine, but those compounds are also found in cereals (Dykes & Rooney, 2007). Purple corn (*Zea mays* L.) originated from South America and it has a high concentration of anthocyanin pigments, particularly in the aleurone layer (Del Pozo-Insfran, Brenes, Saldivar, & Talcott, 2006). In Thailand, PWC has received much interest due to its health benefits. PWC kernel predominantly comprises glutinous starch and the dark purple color of its kernel pericarp is due to high contents of anthocyanins and phenolic compounds (Harakotr, Suriharn, Tangwongchai, Scott, & Lertrat, 2014). Interestingly, both types of compounds have been found in high concentrations throughout the purple corn plant, especially in flower, cob, leaf, silk, kernel pericarp, and husk (Cevallos-Casals & Cisneros-Zevallos, 2003; Fossen, Slimestad, & Andersen, 2001; Li *et al.*, 2008). Generally, tassel (a male floral organ), cob and silk are waste products of corn plant after the harvest. The corn seed at dry stage is suitable for seed production and food industry due to longer shelf-life than in the milky stage (Mohamed, Lertrat, & Suriharn, 2017), while seeds of low quality will be utilized as animal feed. However, because of

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its high content of pigments in pericarp fraction, this study also had an interest in the kernel pericarp for potentially serving as a source of phytochemical compounds. In the last few years, some publications have identified the contents of different components of anthocyanins and phenolics in PWC by-products (Simla, Boontang, & Harakotr, 2016; Thapphasaraphong, Rimdusit, Priprem, & Puthongking, 2016). Mohamed *et al.* (2017) reported that some parts of PWC (seed, cob, silk, husk and tassel at different growth stages) could be good sources of phenolics and anthocyanins as well as of AOA. Moreover, phytochemical compounds in many parts of purple corn showed various biological activities according to scientific evidence, for example preventing hyperlipidemia and cerebrovascular diseases (Zhang *et al.*, 2010b), and had protective effect against diabetic cataract (Thiraphatthanavong *et al.*, 2014). To date, information about the phytochemical compositions and concentrations in some parts of PWC is still lacking. So, further investigations are required in order to provide fundamental information about phytochemical compounds, supporting potential applications in pharmaceutical and food industries.

Therefore, the objective of this study was to investigate TPC, TAC, and AOA, and to identify some anthocyanins and phenolics using ultra-performance liquid chromatography (UPLC) in four agricultural waste products, including tassel, cob, kernel pericarp and silk of PWC.

2. Materials and Methods

2.1 Chemicals and Reagents

Folin-Ciocalteu reagent was purchased from Merck. Caffeic acid, vanillic acid, ferulic acid and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Fluka. Cyanidin-3-glucoside (Cy3G) was purchased from Chromadex®. Gallic acid, syringic acid, protocatechuic acid, hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, quercetin hydrate, rutin, kaempferol, peonidin-3-glucoside (Pn3G), pelaganidin-3-glucoside (Pg3G), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich.

2.2 Plant materials

The four parts of PWC (open-pollinated corn variety of KND), namely tassel, cob, kernel pericarp, and silk, were obtained from the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. The tassel was harvested at 10 days after flowering, whereas the silk was separated from ears at edible stage (20 days after pollination) because at these times, the tassel and silk lack effective utilization for kernel production. The kernels were removed from corn harvested at dry stage (35-40 days after pollination). The high quality kernels were used for seed production, whereas the kernel pericarp section was collected form the low quality kernel. The corn cobs were collected after corn kernels were removed.

All materials were dried in a hot air oven at 60°C for approximately 6 h until a final moisture content below 8%. After drying, the tassels were removed from their pedicels, whereas the dried silks were cut to approximately 1 cm. The cob epidermis section used was collected from the surface of corn cob. All samples were then finely ground in a grinder before passing through a 30 mesh sieve. The samples were kept in aluminum foil bags at -18°C before extraction.

2.3 Sample extraction

The samples were extracted using a modified extraction process described by Kapcum, Uriyapongson, Alli, and Phimphilai (2016). A mix of 0.1% HCl in 60% methanol was used as extraction solvent following Ju and Howard (2003). A 0.5 g aliquot of each sample was added with 10 mL of solvent and then shaken for 2 h at room temperature. After centrifugation at 3,000 rpm for 10 min, the mixture was filtered through filter paper Whatman No. 1. The residues were re-extracted twice with 5 mL of solvent following the same procedure. The three aliquots of extract were pooled together in a 25 mL volumetric flask and then the volume was made up with the 0.1% HCl in 60% methanol. The extract solutions were kept at -18°C until further analysis.

2.4 Analysis of TPC

TPC was determined using the Folin-Ciocalteu colorimetric method as described by Dewanto, Wu, Adom, & Liu (2002). The reaction mixture containing 125 µL of the extract and 250 µL of Folin-Ciocalteu reagent with an addition of 3 mL of distilled water, was mixed well and then allowed to stand for 6 min, before adding 2.5 mL of 7% sodium carbonate solution. The reaction mixture was allowed to stand for 90 min at room temperature in the dark and was then measured for absorbance at 760 nm using a spectrophotometer (Shimadzu UV-1800, Japan). TPC is expressed in mg gallic acid equivalents per 100 g sample on dry basis (mg GAE/100 g, d.b.).

2.5 Analysis of TAC

TAC was determined using the pH-differential method (Lee, Durst, & Wrolstad, 2005). Two 0.2 mL aliquots of the extract were added with 3.8 mL of 0.025 M potassium chloride buffer at pH 1.0 and with 0.4 M of sodium acetate buffer at pH 4.5, then allowed to stand for 20 min in the dark before measurement of absorbance at 520 and 700 nm. TAC is expressed in mg cyanidin-3-glucoside equivalents per 100 g sample on dry basis (mg Cy3G/100 g, d.b.) with molecular weight of 449.2 g/mol and extinction coefficient of 29,600 M^{-1} cm^{-1}.

2.6 Analysis of AOA

2.6.1 $ABTS^{\bullet+}$ scavenging assay (ABTS)

The scavenging ability was determined using the ABTS assay described by Stratil, Klejdus, and Kuban (2006) with some modifications. The stock solution was prepared by mixing 7 mM ABTS with 4.75 mM of $K_2O_8S_2$ solution in 1:1 (v/v) ratio, and was then kept for 12 h at room temperature without light exposure. Before measurement, the working solution was prepared by mixing the stock solution with phosphate buffer saline (pH 7.4) to absorbance 1.0 AU at 734 nm. A 40 µL sample of extract was reacted with 4 mL of the

working solution and allowed to stand for 10 min in the dark before measurement. AOA is expressed in mg Trolox equivalents per 100 g sample on dry basis (mg TE/100 g d.b.).

2.6.2 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging assay

Free radical scavenging activity was determined using DPPH assay as described by Leong & Shui (2002), with some modifications. A 100 µL sample of the extract was added to freshly prepared of DPPH• in methanol with absorbance 1.0 AU at 517 nm. The AOA was measured after allowing to stand at room temperature for 30 min in the dark. The activity is expressed in mg Trolox equivalents per 100 g sample on dry basis (mg TE/100 g, d.b.).

2.7 UPLC analysis

2.7.1 Purification

The extracts were purified using a C-18 Sep-Pak cartridge (20 cc., 5 g of sorbent) (Waters Associates, Milford, MA) before UPLC analysis, following the procedure described by Ju and Howard (2003) with some modifications. The cartridge was previously activated by methanol followed by 0.01% aqueous HCl. After 5 mL of the extract was loaded into the cartridge, anthocyanins and phenolics were absorbed onto the column, whereas, sugars, acids, and other water-soluble compounds were eluted by 10 mL of 0.01% aqueous HCl. Phenolic compounds were subsequently washed out with 10 mL of ethyl acetate and anthocyanins were eluted using 10 mL of methanol containing 0.01% HCl. Both phenolic and anthocyanin fractions were combined and concentrated using a rotary evaporator, then the volume was made up to 10 mL by methanol. All extracts were filtered through a 0.22 µm nylon syringe filter prior to UPLC analysis. The UPLC conditions for phenolic and anthocyanin identification followed the method described by Kapcum and Uriyapongson (2018).

2.7.2 Determination of phenolics

The twelve phenolic compounds were determined by a Waters® Acquity UPLC® system (Waters Corporation, Milford, MA, USA) equipped with a diode array detector. A 2 µL sample of the extract was loaded into the Acquity UPLC BEH C-18 column (2.1×50 mm, 1.7 µm) operated at 40°C. The detection wavelength was set at 280 nm for hydroxybenzoic acids (gallic, protocatechuic, hydroxy benzoic, vanillic and syringic acid), 320 nm for hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic and sinapic acid) and 370 nm for flavonols (rutin, quercetin and kaempferol). The phenolic compounds were eluted at 0.5 mL/min using a gradient system for two solvents: (A) acetonitrile and (B) 1% acetic acid. The gradient conditions were as follows: 0% A at 0 min, increasing to 30% A within 15 min, increasing to 80% A within 3 min and increasing to 100% A within the next 5 min, decreasing to 0% A within the next 2 min, and equilibrated before the next injection. The different phenolic compounds were quantified based upon their peak areas against determined standard calibration curves. The results are expressed in mg of each phenolic per 100 g sample (d.b.).

2.7.3 Determination of anthocyanins

A 2 µL sample of the extract was loaded in an Acquity UPLC BEH C-18 column (2.1×50 mm, 1.7 µm) with solvent flow rate at 0.6 mL/min and the column temperature was set at 40°C. The detection wavelength was set at 525 nm. The mobile phase was a gradient solvent containing 0.3% phosphoric acid (A) and acetonitrile (B). The gradient conditions were as follows: 90% A at 0 min, decreasing to 80% A within 2 min, and increasing to 90% A within the next 0.1 min, holding at 90% A for 0.9 min before equilibration at 90% A. The results are expressed as mg of each anthocyanin per 100 g sample (d.b.).

2.8 Statistical analysis

The data are presented as mean ± standard deviation from duplicate extractions. Analysis of variance (ANOVA) was run for each parameter from the completely randomized experimental design by using statistical software (SPSS 19.0, SPSS Inc., USA). The significant differences between means were assessed by Duncan's multiple range test (DMRT) at a level of $p \leq 0.05$.

3. Results and Discussion

3.1 TPC

TPC is the main active compound in cereal that protects our body against degenerative diseases, for example heart disease and cancer (Dykes & Rooney, 2007). TPC levels in different parts of PWC are presented in Table 1. The four parts of PWC were significantly different for TPC ($p \leq 0.05$). The silk showed the highest amount of TPC, followed by cob, kernel pericarp and tassel (4,262.00, 3,118.41, 2,588.47 and 2,003.94 mg GAE/100 g (d.b.), respectively). Dong *et al.* (2014) mentioned that stigma maydis (corn silk) has higher TPC than cob and husk when extracted by 50% methanol. However, the parts of corn plant in this present study were harvested at different times resulting in differences in TPC level. This might be due to the maturity stage of plant, which influences the accumulation of bioactive compounds. During the growth period of plant, there are physical and structural changes. The new biosynthesis of phenolics may be interrupted or ended in an older plant, causing decreased phenolic content. (Rahman & Rosli, 2014). Thus, it might be possible that at an older stage (dry stage) of cob and kernel pericarp these might have lower extract recovery than the silk from a younger stage (edible stage). Another reason could be that the corn silk serves as source of nutrients including phenolics to the germinating pollen (Martin, 1970). For the tassel, the part of pollen is a good source of phenolic compounds (Mohsen & Ammar, 2009), although the mass fraction of pollen in whole ground tassel is low: this might have caused the lowest amount of TPC.

In prior studies the TPC concentrations in different parts of purple corn were different from our study. However, many factors affect phenolic accumulation in purple corn. Regarding sample preparation, a longer drying time of

Table 1. Total phenolic contents (TPC), total anthocyanin contents (TAC) and antioxidant activities (AOA) in purple waxy corn waste products

Parameter	Parts of purple waxy corn			
	Tassel	Cob	Kernel pericarp	Silk
TPC*	2,003.94 ± 29.62^{d}	3,118.41 ± 14.50^{b}	2,588.47 ± 27.08^{c}	4,262.00 ± 87.48^{a}
TAC**	56.97 ± 0.84^{d}	1,082.50 ± 52.88^{a}	669.26 ± 29.22^{c}	877.84 ± 36.73^{b}
AOA***				
ABTS assay	1,753.45 ± 19.75^{d}	3,129.31 ± 65.13^{b}	2,630.37 ± 32.35^{c}	3,512.38 ± 86.49^{a}
DPPH assay	770.58 ± 4.16^{d}	1,511.34 ± 22.52^{c}	1,862.77 ± 22.58^{b}	1,974.04 ± 53.18^{a}

Mean values in the same row with different superscripts are significantly different ($p \leq 0.05$).
*: The data are expressed as mg gallic acid/100 g (dry basis).
**: The data are expressed as mg cyaniding-3-glucoside/100 g (dry basis).
***: The data are expressed as mg Trolox/100 g (dry basis).

material might reduce TPC as observed for PWC silk subjected to prolonged drying (60°C for 24 h) (9.8-12.1 mg/100g, d.b.) (Sarepoua, Tangwongchai, Suriharn, & Lertrat, 2015) compared to TPC in the silk from the present study dried at 60°C for 6 h. Jing, Noriega, Schwartz, and Giusti (2007) reported that the growing location affected phenolic content in purple corn cob. TPC in PWC cob grown in Khon Kaen University Farm, Thailand, was higher than in purple corn cob from different locations in Peru (950-1,431 mg/100 g, d.b.) (Jing *et al.*, 2007). Regarding variety, PWC tassel variety of KGW1 (commercial F1 hybrid) had TPC level 1,222 mg/100g (d.b.) (Duangpapeng *et al.*, 2018) which is lower than in PWC tassel of our study.

Based on a comparison of TPC in the parts of PWC with several berry fruits, for example bilberry, cranberry, cowberry, red raspberry and strawberry, which are considered the richest sources of phenolic compounds, in the range from 1,600 to 3,300 mg/100 g (d.b.) (Kahkonen *et al.*,1999; Kahkonen, Hopia, & Heinonen, 2001), the waste parts of PWC could potentially serve as sources of phenolic compounds.

3.2 TAC

Anthocyanins are the major flavonoid compound type found mainly in the pericarp section of pigmented cereals (Dykes & Rooney, 2007). The TAC varied among the corn parts ($p \leq 0.05$). The highest TAC was observed in epidermis section of cob, followed by silk, kernel pericarp and tassel in this order (Table 1). From the previous reports, anthocyanins are highly accumulated in all tissues of PWC, especially in kernel skin and cob (Mohamed *et al.*, 2017; Moreno, Sánchez, Hernández, & Lobato, 2005). Indeed, anthocyanins are distributed in the external plant tissue surfaces in nature for many functions in biochemistry, but these results are in conflict with the trend found in nature. While the reason for this result is unclear, it might be that the cob accumulates anthocyanins for later redistribution to different parts of the plant as needed (Cevallos-Casals & Cisneros-Zevallos, 2003).

The level of anthocyanins in purple corn varies depending on many factors. Regarding the growing location, TAC in purple corn cob grown in Peru (290-584 mg/100g, d.b.) as reported by Jing *et al.* (2007) was lower than in PWC of this present study. Regarding the extraction method, Muangrat, Pongsirikul, and Blanco (2018) studied the ultrasound assisted extraction of PWC cob with 50% ethanol as a solvent and the results showed that TAC (13.9-23.8 mg/100g, d.b.) was lower than for PWC cob in our study. This might be due to sensitivity of anthocyanins to ultrasound. Additionally, PWC tassel from variety KGW1 was studied by Duangpapeng *et al.* (2018) and had TAC (158.2 mg/100 g, d.b.), lower than in tassel of KND variety in this study.

When compared with other anthocyanin rich sources, such as red sweet potato (618 mg/100 g, d.b), blueberry (925-2,404 mg/100 g, d.b) (Cevallos-Casals & Cisneros-Zevallos, 2003), or black rice bran (1,227-5,096 mg/100 g, d.b.) (Zhang, Zhang, Zhang, & Liu, 2010a), this PWC cob and silk could be considered candidates for anthocyanin rich sources.

3.3 Identification of anthocyanins

The different anthocyanin compounds in PWC by-products are summarized in Table 2. In chromatography of anthocyanins by UPLC, six major peaks were detected for the extract, representing Cy3G, Pg3G and Pn3G, while three of the peaks were not identified (data not shown). From prior reports, three of the non-acylated anthocyanins in corn seed, cob, flower, husk and leaf were identified as Cy3G, Pg3G and Pn3G, whereas their respective malonated counterparts were identified as cyanidin-3-(6″-malonylglucoside), pelargonidin-3-(6″-malonylglucoside) and peonidin-3-(6″-malonyl glucoside) (Fossen *et al.* 2001; Li *et al.*, 2008; Yang & Zhai, 2010). Cy3G was observed as the dominant compound in almost all parts of the PWC, whereas Pn3G ranked second highest followed by Pg3G. These results are in agreement with the report of Navarro, Torres, Fernández-Aulis, & Peña (2018). Cy3G is also the major compound found in other pigmented cereals, such as rice, rye, wheat and barley (Dykes & Rooney, 2007). The highest concentration of Cy3G was found in the cob (854.31 mg/100 g, d.b.), followed by the silk (514.98 mg/100 g, d.b.). Generally, Cy3G plays a key role in various health benefits, including prevention of obesity, diabetes (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003) and cancer (Seeram, Zhang, & Nair, 2003). Pg3G and Pn3G had the highest levels in silk, at 196.14 and 244.37 mg/100 g (d.b.), respectively.

3.4 Identification of phenolics

The concentration of each phenolic compound varied significantly ($p \leq 0.05$) among the studied parts of PWC plant (Table 3). However, information on the phenolic composition in each part of PWC is still limited.

Table 2. The concentrations of some individual anthocyanins in purple waxy corn waste products

Compound* (mg/100 g)	Parts of purple waxy corn			
	Tassel	Cob	Kernel pericarp	Silk
Cy3G	1.81 ± 0.25^{d}	854.31 ± 17.94^{a}	258.54 ± 0.79^{c}	514.98 ± 20.67^{b}
Pg3G	trace	175.62 ± 12.14^{a}	138.31 ± 2.18^{b}	196.14 ± 2.35^{a}
Pn3G	1.21 ± 0.21^{d}	193.55 ± 0.57^{b}	173.84 ± 2.04^{c}	244.37 ± 3.70^{a}

Mean values in the same row with different superscripts are significantly different ($p \leq 0.05$).
*: The data are expressed as mg/100g based on dry basis.
Cy3G: cyanidin-3-glucoside, Pg3G: pelargonidin-3-glucoside, Pn3G: peonidin-3-glucoside

Table 3. The concentrations of individual phenolics and total phenolic contents in purple waxy corn waste products

Compound* (mg/100 g)	Parts of purple waxy corn			
	Tassel	Cob	Kernel pericarp	Silk
		Hydroxybenzoic acids		
Gallic acid	nd	5.93 ± 0.09^{b}	6.96 ± 0.06^{a}	3.87 ± 0.40^{c}
Protocatechuic acid	80.91 ± 6.96^{d}	126.59 ± 2.42^{c}	727.56 ± 0.28^{a}	166.54 ± 3.47^{b}
Hydroxybenzoic acid	9.07 ± 1.32^{c}	20.79 ± 1.47^{b}	90.14 ± 3.51^{a}	9.60 ± 0.60^{c}
Vanillic acid	27.35 ± 0.91^{a}	21.99 ± 1.12^{b}	18.90 ± 0.15^{c}	9.53 ± 0.10^{d}
Syringic acid	0.58 ± 0.00^{d}	45.60 ± 1.77^{a}	13.70 ± 0.95^{c}	25.31 ± 0.72^{b}
		Hydroxycinamic acids		
p-Coumaric acid	4.23 ± 0.06^{c}	14.53 ± 0.11^{a}	6.81 ± 0.14^{b}	6.66 ± 0.16^{b}
Caffeic acid	1.96 ± 0.05^{d}	3.68 ± 0.08^{c}	10.88 ± 0.07^{a}	5.16 ± 0.15^{b}
Ferulic acid	20.08 ± 0.46^{a}	11.78 ± 0.37^{b}	10.20 ± 0.05^{c}	12.18 ± 0.79^{b}
Sinapic acid	2.18 ± 0.15^{c}	14.38 ± 1.14^{b}	13.34 ± 0.95^{b}	23.50 ± 0.57^{a}
		Flavonols		
Rutin	91.88 ± 2.50^{b}	154.61 ± 5.44^{a}	64.67 ± 1.44^{c}	100.36 ± 0.33^{b}
Quercetin	3.14 ± 0.11^{d}	11.84 ± 0.25^{c}	17.78 ± 0.83^{a}	15.13 ± 1.27^{b}
Kaempferol	3.29 ± 0.01^{b}	7.82 ± 0.07^{a}	3.60 ± 0.37^{b}	1.58 ± 0.10^{c}

Mean values in the same row with different superscripts are significantly different ($p \leq 0.05$).
nd: not detected
*: The data are expressed as mg/100 g based on dry basis.

Protocatechuic acid and rutin were the most abundant phenolic acid and flavonol in PWC, respectively, ranging from 80.91-727.56 and 64.67-100.36 mg/100 g (d.b.), respectively. In general, protocatechuic acid is wildly distributed in many fruits, cereals, spices and natural medicines. It has been reported to have various numerous health related activities, such as antioxidant, anti-bacterial, anti-cancer, anti-diabetic, anti-ageing and anti-inflammatory activity (Kakkar & Bais 2014). Whereas rutin known as a member of flavonol group has many potential activities, such as antioxidant, anti-inflammatory and anti-carcinogenic (Sattanathan, Dhanapal, Umarani, & Manavalan, 2011). The cob had the highest concentrations of syringic acid, *p*-coumaric acid, rutin and kaempferol, while the kernel pericarp had the highest level of gallic acid, protocatechuic acid, hydroxybenzoic acid, caffeic acid and quercetin. Interestingly, all parts of PWC tested had detectable quercetin and kaempferol. Due to their bioactivities that reduce the risk of many chronic diseases, both of these compounds are objects of increased interest (Shahidi & Yeo, 2018). Quercetin and kaempferol are generally present in some parts of cereals. Sultana, Anwar, Asi, and Chatha (2008) reported that rice bran contains kaempferol at a low concentration (0.1 mg/100 g, d.b.) but quercetin was not detected, and moreover, these compounds were not detected in rice hull. In wheat, quercetin was found in parts of bran and husk (8.9-10.7 mg/100 g, d.b.), while kaempferol is only detected in bran (5.2 mg/100 g, d.b.). In this case, corn cob contained quercetin (10.25 mg/100 g, d.b.), but kaempferol was not detected. In case of tassel, the information on identification of phenolics is limited. Ferulic acid was mainly located in the tassel (20.08 mg/100 g, (d.b.)). According to Dong *et al.* (2014) who identified phenolics in husk, cob and silk of Chinese corn extracted by 80% ethanol, resveratrol was the highest component by 8.82-18.95 mg/100 g (d.b.), followed by gallic acid (1.84-3.15 mg/100 g, d.b.) and caffeic acid (1.34-2.99 mg/100 g, d.b.), while protocatechuic acid, chlorogenic acid, rutin, and kaempferol were identified in only trace amounts. From these findings, the various parts of PWC had high concentrations of different phenolics, which indicates that some by-products of PWC can be good sources of natural bioactive compounds providing desirable health benefits and prevention of certain diseases.

3.5 AOA

AOA analysis was performed using two methods (ABTS and DPPH assays) because these methods have been frequently used to measure AOA in plant extracts. For antioxidant analysis, measuring AOA in more than one assay

is necessary for understanding the various modes of action of antioxidants in samples (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009). The highest AOA for both assays was observed in the silk (Table 1). According to the results, AOA examined by ABTS method showed the same trend as the TPC results, while the DPPH showed different result from TPC. The ABTS assay was more strongly correlated with TPC than the DPPH assay (r=0.934 and r=0.750, respectively, $p \leq 0.01$, data not shown). Likewise, the ABTS assay exhibited high correlation with TAC, whereas the DPPH assay showed moderate correlation (r=0.914 and r= 0.760, $p \leq 0.01$, respectively, data not shown). ABTS and DPPH assays are decolorisation assays based on electron transfer, to measure the reducing capacity of antioxidants. The dissolution of $ABTS^{\bullet+}$ is done in an aqueous solution applicable to both hydrophilic and lipophilic antioxidant systems, whereas the $DPPH^{\bullet}$ assay is done in an organic solvent and is applicable to hydrophobic systems (Floegel *et al.*, 2011). However, most phenolic acids and anthocyanins, which are the major antioxidant compounds in purple corn, are likely hydrophilic (water-soluble), and the hydrophilic antioxidants can dissolve well in an aqueous medium to quench radicals better than in an organic medium. The findings of Floegel, Kim, Chung, Koo, & Chun (2011) are in a good agreement with this result, as the ABTS method showed better AOA in vegetables than the DPPH method. Moreover, they mentioned that the great difference between AOA measured by ABTS and DPPH assays was observed in high anthocyanin content foods. However, both assays of AOA in silk were related to the TPC concentration, indicating that TPC was the important contributor to AOA (Žilić, Serpen, Akıllıoğlu, Gökmen, & Vančetović, 2012). Regarding the anthocyanins, Pg3G and Pn3G had high correlations with both AOA assays, especially with the ABTS assay (r=0.974 and 0.968, $p \leq 0.01$, respectively, data not shown). However, no significant correlation was observed between Cy3G and DPPH assay, which might be because the hydrophilic anthocyanin was not properly dissolved in the organic medium of the assay. Phenolics of the hydroxybenzoic group, particularly syringic and gallic acids showed only moderate correlations with ABTS and DPPH assays (r=0.777 and r= 0.785, respectively, $p \leq 0.05$, data not shown). Additionally, sinapic acid of hydroxycinamic group gave strong positive correlation with both AOA assays (r=0.909-0.962, $p \leq 0.01$, data not shown), while quercetin of flavonol group correlated with both AOA assays, particularly with the DPPH assay (r=0.964, $p \leq 0.01$, data not shown). The chemical structures of phenolics play importance roles in their antioxidant capacities. Their aromatic ring structures have one or more hydroxyl groups with ability to donate hydrogen atoms or electrons to free radicals.

4. Conclusions

The results of this study showed that the silk possessed the highest TPC and exhibited the strongest AOA, while cob proved to be the best source of TAC. Among phenolic acids, protocatechuic acid was identified as the predominant compound, whereas rutin was the most abundant of flavonol in PWC. Cy3G was the major anthocyanin in all parts of PWC.

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