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Influence of variety and maturity on bioactive compounds and antioxidant activity of purple waxy corn (*Zea mays* L. var. ceratina)

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Abstract

Variety and maturity influence on phytochemical and its antioxidant activity. Colored corn has natural pigment, promising health benefits. Purple waxy corns were bred to improve eating quality and bioactive content of original waxy corn. The proximate chemical analysis the content of total sugar, dietary fiber, starch, total phenolic (TP), total flavonoid (TF), total anthocyanin (TA) contents as well as trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) of different varieties (KGW1 and KND) and maturity stages (eating- and mature-stage) of purple waxy corn in cob and kernel were investigated. The result showed that the kernels contained high amounts of starch, protein and dietary fiber accounting for 66-71%, 9-13% and 10-15%, respectively. Phytochemical contents and antioxidant activity in corn cob were higher than those in kernels. The highest TA content in kernels was at eating-stage of KGW1 variety (141.58±5.19 mg CGE/100g DM) and phytochemical contents in kernels significantly decreased in late harvesting ($p \leq 0.05$). Therefore, the KGW1 kernel is an alternative choice for consumers who concern about health due to its high bioactive contents and antioxidant activities. However, the bioactives were not only rich in the purple kernels, but also in the purple cobs and even richer than those in kernels. The highest level of TA content in cob was found in mature-stage of KND variety (705.64±45.84 mg CGE/100g DM). For waste utilization, the purple corn cob had a promising potential as a raw material for anthocyanin source due to high anthocyanin contents and antioxidant activities. In addition, a strong correlation between TA content and antioxidant activities was noticed. This finding indicated that KGW1 and KND varieties purple waxy corn provided high antioxidant compounds and activity which potential to be a healthy food at a proper maturation.

Introduction

Corn (*Zea mays* L.) is one of the major food crops in Asian country. It is not only a good source of carbohydrate but also natural antioxidants, such as vitamins, carotenoids, flavonoids, phenolic compounds and anthocyanins, well known for health benefits (Lopez-Martinez *et al*., 2009; Lee *et al*., 2010; Montilla *et al*., 2011). Although polished rice (*Oryza sativa* L.) is the most popular stable food, it leads to deficiencies in essential minerals, vitamins, protein, dietary fiber and other nutrients (Liu *et al.*, 2015; Zhang *et al.*, 2015). It has been reported that antioxidant compounds in colored corn and rice provide health benefits to reducing risk of chronic diseases (Chen *et al.*, 2006; Liu, 2007). Thus, corn could be an alternative for functional food due to possess of nutritive values. Normally corn could be harvested at different stages according to purpose utilization. However, its functional properties depend on varieties (Khampas *et al.*, 2015), maturity stages (Žnidarčič, 2012) and processing methods (Murador *et al*., 2014). The colored corn contains more antioxidants and exhibits higher antioxidant activity than white corn (Lopez-Martinez *et al.,* 2009; Mahan *et al.*, 2013).

Anthocyanin is a water-soluble pigment, mostly found in plant (Clifford, 2000). Anthocyanins and it derivaties are used as natural water-soluble colorants in food and beverages (Castañeda-Ovando *et al*., 2009; Lemos *et al.*, 2012). Apart from being colorant, it is also possess anti-inflammatory, anticarcinogenic

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and antioxidant activities (Norberto *et al*., 2013). It has been claimed to play roles on cardiovascular disease prevention, obesity control and diabetes alleviation properties (He and Giusti, 2010). There are many varieties of purple corns both natives and hybrids but a few researches reported on antioxidant activity, anthocyanins profile, extraction method and stability of anthocyanins in Andes purple corn (Pedreschi and Cisneros-Zevallos, 2007; Montilla *et al.,* 2011) and Chinese purple corn (Jing and Giusti, 2007; Zhao *et al*., 2008; Yang and Zhai, 2010; Liu *et al.,* 2011). Thus, corn is an alternative crop with a high potential for a further development of functional food products. However, original purple corn has high amylose content, resulting in rapid retrogradation and getting hard texture in cooked corn kernels.

Waxy corn (*Zea mays* L. var. ceratina) become more popular, especially in Asia, as healthy vegetative crop due to its soft, sticky texture and taste (Lertrat and Thongnarin, 2008; Hu and Xu, 2011). There are many varieties of waxy corns, providing different natural pigments. Purple waxy corn was bred to improve eating-quality of the cooked kernel with pleasant sweetness, soft and sticky texture. It has been reported that bioactives located in different parts of corn ear. Harakotr *et al*. (2014) reported that to DPPH-antioxidant activity of colored waxy corn increased throughout the kernels development, relating to total anthocyanin content. Sarepoua *et al.* (2015) reported that corn variety and maturity stages significantly affected on total phenolic, total flavonoid, total anthocyanin contents and antioxidant activity of corn silk ($p<0.05$). However, researches for chemical compositions and its functional properties are still needed.

Therefore, this study was aimed to determine the chemical compositions of kernels at edible and fully mature stages of two purple waxy corns (KGW1 and KND cultivars). Furthermore, total contents of phenolic, flavonoid, anthocyanin and antioxidant activities by TEAC, FRAP and ORAC assays were investigated in both kernels and cobs of purple waxy corn, including a comparison with typical anothocyanin in cereal grains, 'Hom Nin' purple rice and 'Leum Pua' black sticky rice. The results would provide important information for health benefit, application and utilization of different maturity of the two purple waxy corn ear, compared to the staple colored rice.

Materials and Methods

Chemicals

Amylose, amylopectin, gallic acid, catechin, 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (trolox), fluorescein, 2,2'-azobis-2-amidinopropane-dihydrochloride (AAPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 2,2′-azino-bis (3-ethylbenzothiazolin-6-sulfonate) diammonium salt (ABTS), were purchased from Sigma-Aldrich (St Louis, Missouri, USA). All chemicals used were analytical grade.

Plant materials and sample preparations

Purple waxy corn (KND variety) was bred between purple field and white waxy corn while KGW1 was another variety crossed bred between KND and white sweet corn. The two varieties express purple color in both corn cobs and kernels, indicating high quantity of anthocyanin claimed that occupied strong antioxidant properties. The KGW1 and KND varieties of purple waxy corn were cultivated in a demonstrated farm at the Plant breeding research center for sustainable agriculture, Khon Kaen University (KKU), Thailand, during May-July 2011 (for KGW1) and June-September 2011 (for KND). The corn ears were harvested at eating-stage and fully mature-stage of 18-20 and 30-35 days after pollination (DAP), respectively. The corn ears were transferred to the pilot plant of Food Technology department, KKU as soon as possible after harvest.

The corn ears were dehusked and separated corn silk. To control variation of kernels within an ear, 3cm from the tip and 1 cm from the stem-end of each ear were cut and discarded. Corn kernels were separated in column along the ear while corn cobs were sliced into pieces of 2 mm thickness. To minimize changes, the kernels and cobs were freeze-dried (Alpha 2-4 LD plus, Martin Christ, Germany) prior to the investigation of phytochemicals and antioxidant activity and dried in a vacuum oven at 50°C before doing other chemical analysis. All dried samples were ground using a pin mill (Lab mill 3100, Perten Instruments, Sweden) and sieved through a 50-mesh screen. The powderized samples (100 g) were vacuum packed in an aluminium foil laminated bag and kept at -18°C. The samples were thawed at ambient temperature (ca. 35±2°C) for 2-3hr prior to analysis. Black sticky (*Oryza sativa* L. var. glutinosa 'Leum Pua') and purple rice (*Oryza sativa* L. var. 'Hom Nin') from the local market were used as reference samples for phytochemicals and antioxidant activity analysis.

Proximate analysis

Analysis of moisture, protein, fat and ash contents followed the American Association of Cereal Chemists (AACC, 1995).

Total sugar content analysis

Total sugar content was measured using the phenol-sulfuric acid method. Extraction of sugar was slightly modified the method described by Chow and Landhäusser (2004). The samples (100mg) were added with 10mL of 80% ethanol, boiled in a 95°C water bath for 10 mins and the liquid was collected. Then the residue was re-extracted again. The liquid was combined and centrifuged at 10,000 × g for 10 minutes at 25°C, the supernatants was adjusted the final volume with 80% ethanol to 25 mL. The extracts were determined for sugar content by the phenol–sulfuric acid method (Dubois *et al.*, 1956). Briefly, 0.5 mL of extract was added with 0.5 mL of phenol solution (5 %w/v), mixed and then added 2.5 mL of sulfuric acid (98%). The mixture was left in the darkness for 10 minutes for color development to take place, then vortexcally shaked and cooled in an ice bath for 30 mins. The absorbance was measured at 490 nm using a UV-Visible spectrophotometer (Lamda 25, Perkin Elmer, USA). The glucose of 0, 25, 50, 75, 100 and 125mg/L were used as a standard curve. The total sugar content was expressed as gram glucose equivalents per 100grams dry matter (g GE /100g DM).

Dietary fiber analysis

Soluble and insoluble dietary fiber contents were enzymatic-gravimetrically determined using the Megazyme assay kit K-TDFR (Megazyme International, Ireland) approved by AACC (1995) and AOAC (1995). The total dietary fiber was the sum of soluble and insoluble dietary fiber contents.

Total starch, amylose and amylopectin analysis

Total starch content was investigated by amyloglucosidase/α-amylase method using the Megazyme assay kit K-TSTA (Megazyme International, Ireland) approved by AACC (1976). Amylose and amylopectin was analyzed by the iodine colorimetric determination (AACC, 1995).

Phytochemicals and antioxidant activities

The extraction was performed by a slight modification method of Hosseinian *et al.* (2008). Briefly, powdered sample (2.000±0.005 g for corn-kernels and colored rice or 0.500±0.005 g for corn-cobs) was mixed with 30 mL of 95% methanol acidified with 0.5 N HCl (a ratio of 85:15 v/v) in a centrifuged tube. The mixture was shaked in the dark condition at room temperature for 60 mins, then centrifuged at 10000 × g for 10 mins at 25°C, (Sorvall Legend Mach 1.6 R, Thermo Fisher Scientific, Germany). The residue was re-extracted again. The supernatant was pooled and filtered through a filter paper (Whatman no.1). The filtrate was collected, rotary evaporated at 45°C (Rotavapor R-124, Buchi Labortechnik AG, Switzerland) and the matrix was reconstituted in 7 mL of the acidic methanol. The extract was filtered through the Whatman no.1 paper, then through a 0.45 µm syringe filter, respectively. Finally, the extract was adjusted the volume to 10 mL with the acidic methanol and stored at -30°C in the darkness until measurements of total phenolics, flavonoids and anthocyanins contents including antioxidant activities. The analysis was performed within 2 days after the extraction.

Measurement of total phenolic content (TPC)

TPC was determined following a Folin-Ciocalteu colorimetric method (Liu *et al*., 2011). The extract was 5-times diluted with extracting solvent before analysis. The reaction cocktail, containing 0.2 mL of the diluted extract, 0.5 mL of diluted Folin-Ciocalteu's reagent : deionized water (ratio of 1:2) and 4 mL of 7.5% Na_2CO_3 (w/v), was left in the darkness for 30 mins at ambient temperature to complete the reaction. Then, the absorbance of mixture was spectrophotometrically measured at 750nm using an UV-Visible spectrophotometer. The blank was prepared from extracting solvent instead of the extract. Gallic acid of 50 to 200 mg/L was drawed a standard curve to calculate the total phenolic content in samples on term of milligram gallic acid equivalents per 100grams dry matter (mg GAE/100g DM).

Measurement of total flavonoid content (TFC)

TFC was colorimetrically performed following Shen *et al.* (2009) with a slight modification. Briefly, extracts (0.5 mL) was mixed with deionized water (2 mL) and 5% $NaNO_2$ (0.15 mL), let the reaction to take place for 5 mins, then added 10% $AlCl_3.6H_2O$ (0.15 mL), allowed to stand for another 5 mins. The cocktail was added with 1M NaOH (1 mL), mixed and kept in darkness for 15 mins. The absorbance was determined at 510 nm. The blank was prepared from extracting solvent instead of the extract. A standard curve of 25 to 200 mg/L catechin was used to estimate the TFC, expressing as mg of catechin equivalents per 100 grams dry matter (mg CE/100g DM).

Measurement of total monomeric anthocyanins content (TAC)

TAC was determined by a pH-differential method (Giusti and Wrolstad, 2001). Extract (0.5 mL) was mixed with 10 mL of 0.025M KCl buffer pH 1.0 in

a test tube and with 10 mL of 0.4 M sodium acetate buffer pH 4.5 in another tube. Both tubes were kept in darkness for 15 mins. TAC was determined by using the absorbance at 510 and 700nm, respectively. Absorbance was calculated as the following equation;

$$Abs = (A_{510} - A_{700})\ pH\ 1.0 - (A_{510} - A_{700})\ pH\ 4.5$$

TAC was calculated using the following equation;

$$TAC\ (mg/100g) = (Abs/eL) \times MW \times DF \times (V/G) \times 100$$

where Abs is the absorbance, e is the cyanindin-3-glucoside molar absorptivity (26,900), L is the cell path length (1 cm), MW is the molecular weight of anthocyanin (449.2 Da), DF is the dilution factor, V is the final volume (mL) and G is the sample powder weight in extraction step (g). TAC expressed as milligrams of cyanidin-3-glucoside equivalents per 100 grams dry matter (mg CGE/100g DM).

Trolox equivalent antioxidant capacity (TEAC) assay

TEAC assay was slightly modified from Re et al. (1999). To generate ABTS radical cation (ABTS•+ working solution), the 7 mM ABTS stock solution was reacted with 2.45 mM potassium persulfate (in the ratio 2:1) and allowed to stand in the darkness at room temperature for 12-16hr before use. The $ABTS^{\bullet+}$ solution was diluted with 5mM phosphate buffered saline (PBS) pH 7.4, to obtain a constant absorbance of 0.70±0.02 at 734 nm for 1hr. The diluted extract (ca. 10 times dilution) of 30µl was mixed with 3mL of working solution and left in darkness at ambient temperature for 15 mins. The absorbance was read at 734 nm and PBS was used as a blank. A 160 to 960 µM of trolox solutions were used to estimate the antioxidant capacity and the results are expressed in micromole of Trolox equivalents per grams dry matter (µmol TE/g DM).

Ferric reducing antioxidant power (FRAP) assay

FRAP assay, the ability of an antioxidants to reduce the yellow-orange of ferric-tripyridyl-triazine (Fe^{3+}-TPTZ) complex to the blue colored ferrous form (Fe^{2+}) was investigated following Yang and Zhai (2010) with a slight modification. The diluted extract of 0.2 mL (10 times dilution) was mixed with a 3.8 mL of freshly prepared FRAP reagent, containing 300 mM sodium acetate buffer at pH 3.6, 10 mM TPTZ and 20 mM $FeCl_3 \bullet 6H_2O$ in the ratio of 10:1:1 and left in the darkness for 30 mins. Absorbance of the mixture solution was read at 593 nm. FRAP reagent was used as a blank. The antioxidant capacity was calculated from the linear calibration curve of 80 to 480 µM Trolox solutions, expressing as micromole trolox equivalents per grams dry matter (µmol TE/g DM).

Oxygen radical absorbance capacity (ORAC) assay

ORAC assay was followed Huang *et al*. (2002). Briefly, the AAPH and fluorescein were used as a peroxyl radical generator and fluorescent probe, respectively. Potassium phosphate buffer 75 mM (pH 7.4) was a diluent for all reactants. The buffer (blank) or extract of 25 µL was mixed with 150 µL fluorescein solution (0.0816 µM) in a black 96-well micro-plate and incubated at 37°C for 10 mins before automatical injection of AAPH 25 µL (153 mM). The fluorescence intensity was measured every minute until it reached zero (excitation wavelength 485nm, emission wavelength 520 nm), using a black 96-well micro-plate reader (Varioskan Flash, Thermo scientific, Finland). The net area under curve (AUC) was calculated from a subtraction of AUCsample from AUCblank. A series of 6.25, 12.5, 25, 50, 75 and 100 µmol/L Trolox solutions was used instead of samples to draw a calibration curve. The average net AUC was plotted against Trolox concentration. The ORAC values were calculated using the regression equation of the calibration curve and reported as micromole of Trolox equivalents per gram dry matter (µmol TE/g DM).

Experimental design and statistical analysis

The 2x2 (two variety corn and two maturity stages) factorial experiments in completely randomized design were arranged. The data were presented as means±standard deviation (SD). The 'Hom Nin' purple rice and 'Leum Pua' black sticky rice were used as reference samples. The data were analyzed for variance (ANOVA) and the differences between means were analyzed by Least significant difference (LSD) at a confidence level of 95% using a statistical program, SPSS v 19.0 (SPSS Inc., USA). All treatments were carried out in duplicate at least. The correlation between attributes was performed by Pearson correlation test.

Results and Discussion

Compositions of the purple waxy corn kernels

There were significant changes in chemical composition of the two purple waxy corn kernels during maturation from eating to mature stage ($p \leq 0.05$) except for fat, ash, soluble dietary fiber and total starch contents ($p > 0.05$, Table 1). It is worth noted that moisture of the kernels of both varieties was dramatically decreased from 65.13% at eating stage to

Table 1. Compositions of KGW1 and KND purple waxy corn kernels at two maturity stages.

Compositions	KGW1 variety		KND variety	
	Eating-stage	Mature-stage	Eating-stage	Mature-stage
Moisture (% wet basis)	64.96±0.68a	49.03±0.99b	65.30±0.46a	45.35±0.34c
Protein (% w/w DM)	12.04±0.50b	13.26±0.82a	10.62±0.31c	9.16±1.50d
Fat (% w/w DM)^{ns}	6.07±0.48	5.69±0.80	5.70±0.12	4.66±0.64
Ash (% w/w DM)^{ns}	2.87±0.19	2.29±0.11	2.35±0.10	1.93±0.06
Total sugar (% w/w DM)	4.36±0.19a	1.48±0.05c	2.07±0.07b	1.47±0.12c
Total dietary fiber (% w/w DM)	10.52±1.60b	15.77±1.65a	10.46±2.05b	10.72±2.12b
Soluble dietary fiber (% w/w DM)^{ns}	2.89±1.87	2.68±2.54	0.70±0.45	0.64±0.48
Insoluble dietary fiber (% w/w DM)	7.63±1.79b	13.09±2.54a	9.76±1.66b	10.08±1.81ab
Total starch (% w/w DM)^{ns}	66.58±0.99	68.39±1.02	70.57±1.35	71.89±2.23
Amylose (% of total starch)	0.00±0.00b	0.00±0.00b	2.08±0.31a	0.00±0.00b
Amylopectin (% of total starch)	100.00±0.00a	100.00±0.00a	97.92±0.31b	100.00±0.00a

Values are mean ± SD (n=6; dietary fiber, n=4). Means within the same row with the different letters are significantly different at $p \leq 0.05$ as analyzed by LSD test. ns The data in the same row is non-significant difference ($p > 0.05$).

47.19% at mature stage (25-30% reduction). Protein contents in kernels of two variety ranged from 9.16 to 13.26 (%w/w DM). During the seed development, protein, total- and insoluble-dietary fiber contents significantly increase in KGW1 however protein content in KND decreased ($p \leq 0.05$). Total dietary fiber and insoluble fiber contents in KND kernels showed non-significant changes ($p > 0.05$). KGW1 kernels at eating-stage had total sugar content higher than that of KND kernel about 2.1 times. Total sugar contents in KGW1 and KND kernels at mature stage reduced 2.95 and 1.4 times, respectively ($p \leq 0.05$). Nevertheless, there was no significant difference in total sugar content between the two varieties at mature stage. Total starch content of purple waxy corn kernels was ranged from 66.58 to 71.89 (%w/w DM) in which 98-100% of total starch was amylopectin. This was similar to Jiranuntakul *et al.* (2011) who reported that waxy corn consisted of 98% amylopectin. Total sugar contents decreased ($p \leq 0.05$) while total starch contents trended to non-significantly increase ($p > 0.05$). This was probably due to that sugar was used in respiration and starch biosynthesis. Hai-yan *et al.* (2007) found that sucrose content gradually decreased with the concurrent increase in starch content during corn kernel development (10-40 DAP). However, starch accumulation in corn ear during the late state of maturation was low (Singh and Juliano, 1977). It was in agreement with Ketthaisong *et al*. (2013) who reported that a change in starch content of waxy corn varied by genotypes and harvesting stages. The result showed that purple waxy corn kernels could be a promising health food due to its nutritional compositions. It could be mixed with other grains to increase and improve nutritional value since different genus provides diverse quality and quantity of nutrients in grains (Chung *et al.*, 2012; Poutanen *et al.*, 2014).

Phytochemical contents and antioxidant activities of the purple waxy corn kernels

KGW1 and KND kernels as well as its cobs have purple color indicating anthocyanin existence. Table 2 shows total phenolics, flavonoid, anthocyanin contents and antioxidant activities of KGW1 and KND kernels, the edible part of corn. The data analysis showed that the interaction of main factors (variety and maturity stage) significantly affected on TPC, TAC, TEAC and ORAC in both KGW1 and KND kernels ($p \leq 0.05$) while non-significantly influenced on TFC and FRAP ($p > 0.05$). The bioactive contents and its antioxidant properties tend to decrease as maturity increased (Table 2). At eating-stage KGW1 kernels were the higher in TPC while KND kernels were greater in TFC. However, the eating-stage kernels of two varieties had no significantly different in TAC ($p > 0.05$). The purple kernels at eating-stage and mature-stage provided TPC ranging between 319.48-511.04 and 275.58-326.85 mg GAE/100g DM, TFC ranging between 45.62-51.31 and 31.84-37.17 mg CE/100g DM and TAC ranging between 140.07-141.58 and 95.52-115.05 mg CGE/100g DM respectively.

It was well-known that colored exocarp was correlated to phytochemical contents (Walter *et al.*, 2013). Black sticky rice is glutinous rice with a dark color in exocarp while purple rice is colored rice and claimed to be rich in bioactives (Tian *et al.*, 2004; Tang *et al*., 2016). Black sticky and purple rice, sorts of staple food generally consumed in southern Asian countries, were used as reference samples to compared bioactive content and its antioxidant activities. 'Leum Pua' black sticky rice provided TPC, TFC and TAC as 810.65±59.95 mg GAE/100g DM, 221.00±5.41 mg CE/100g DM and 344.37±10.41 mg CGE/100g DM, respectively. 'Hom Nin' purple rice provided TPC, TFC and TAC as 249.42±21.51 mg GAE/100g DM, 36.63±1.55 mg CE/100g DM and

Table 2. Phytochemical contents and antioxidant activities of KGW1 and KND purple waxy corn kernels at two maturities.

Samples		Total phenolic content (mg GAE/100g DM)	Total flavonoid content (mg CE/100g DM)^{ns}	Total anthocyanin content (mg CGE/100g DM)	Antioxidant activities (µmol TE/g DM)		
					TEAC assay	FRAP assay^{ns}	ORAC assay
KGW1 variety	Eating-stage	511.04±27.27a	45.62±2.80	141.58±5.19a	48.62±3.04a	31.87±2.01	48.33±3.39a
	Mature-stage	326.85±5.25b	31.85±3.19	115.05±4.35b	46.73±4.20a	26.50±2.01	44.67±3.01ab
KND variety	Eating-stage	319.48±14.75b	51.31±2.44	140.07±5.80a	47.53±4.91a	31.73±3.75	42.53±4.29b
	Mature-stage	275.58±21.39c	37.17±1.49	95.52±6.05c	38.54±1.98b	23.36±3.25	31.67±5.46c

Values are mean ± SD (n=6). Means within the same column with the different letters are significantly different at $p \leq 0.05$ as analyzed by LSD test. ns The data in the same column is non-significant difference ($p > 0.05$).

9.47±0.44 mg CGE/100g DM, respectively. The result showed that TPC, TFC and TAC contents of corn kernels at both maturities were lower than those found in 'Leum Pua' black sticky rice. However, KGW1 and KND kernels had higher contents of TPC, TFC and TAC than 'Hom Nin' purple rice, except that KGW1 at mature-stage had a lower TFC compared with 'Hom Nin' purple rice. Generally, high phenol contents were detected during the early state of maturation, since it acts as a protective substance against microorganisms (Maieves *et al*., 2015). Mature kernels in both cultivars had lower phytochemical contents than eating-stage. This was on the same line with Shao *et al*. (2014) who reported that TPC of red rice and TAC of black rice decreased significantly during development stage. This was probably due to a progressive change of phenolics to lignin during grain development with a different change rate between outer layer bran and endosperm. TPC of the purple corn kernels was comparable to strawberries (260-288 mg GAE/100g FW; Crecente-Campo *et al*., 2012), Chinese purple yam (478 mg/100g DM; Fang *et al*., 2011) and Bolivian purple corn (311.0-817.6 mg GAE/100g DM; Montilla *et al*., 2011). TFC of the purple kernels was similar to red onion bulb (72.6-110.0 mg quercetin/100g DM) and spinach (59.7-95.3 mg quercetin/100g DM; Nuutila *et al*., 2002). It was noted that TAC of the purple kernel was comparable to TAC of Chinese purple corn kernel (304.5 mg/100g of dry seeds; Zhao *et al*., 2008) and blueberry (116-224 mg/100g FM; You *et al*., 2011). Furthermore, TAC of the purple kernel was relatively higher than those of Bolivian purple corn kernel (1.9-71.7 mg/100g DM; Montilla *et al*., 2011) and red/purple colored vegetables i.e., carrot, cabbage, potato, onion, asparagus and eggplant (7.77-97.71mg/100g DM; Li *et al*., 2012).

The data analysis showed that interaction of variety and maturity stage significantly affected on antioxidant activity measured by TEAC and ORAC assays ($p \leq 0.05$) but not on FRAP assay ($p > 0.05$, Table 2). TEAC and ORAC assays of the KND kernels decreased as maturity increased ($p \leq 0.05$) while the KGW1 kernels had no significant changes during grain development ($p > 0.05$). TEAC, FRAP and ORAC of the purple kernel were 38.54-48.62, 23.36-31.87 and 31.67-48.33 µmol TE/g DM, respectively. KGW1 kernel at eating-stage showed the highest in TEAC, FRAP and ORAC of 48.62±3.04, 31.87±2.01 and 48.33±3.39 µmol TE/g DM, respectively. 'Leum Pua' black sticky rice provided antioxidant activity measured by TEAC, FRAP and ORAC as 72.95±16.10, 68.80±5.16 and 87.00±11.17µmol TE/g DM, respectively. 'Hom Nin' purple rice provided antioxidant activity measured by TEAC, FRAP and ORAC as 21.16±1.35, 13.49±0.36 and 30.83±2.32 µmol TE/g DM, respectively. The result showed that purple waxy corn kernels contained higher antioxidant activity than 'Hom Nin' purple rice, but lower than 'Leum Pua' black sticky rice. Antioxidant capacity of the purple kernels had comparable to other vegetables. Finocchiaro *et al.* (2010) reported that TEAC of spinach and black rice were 44.30 and 42.0-94.8 µmol TE/g DM, respectively. TEAC in red rice was ranged from 40.9 to 55.3 µmol TE/g DM (Mazzeo *et al*., 2011). Ou *et al*. (2002) reported FRAP in carrot and purple onion (31 µmol TE/g DM), tomato (56 µmol TE/g DM) and white cabbage (39 µmol TE/g DM). You *et al.* (2011) reported that ORAC of blueberry was ranged from 44.7 to 55.7 µmol TE/g FW. ORAC of apple and strawberry were 30.82 and 35.77 µmol TE/g, respectively (Floegel *et al*., 2011). These results indicated that purple waxy corn kernel is a potential source of a nutritional value not only a carbohydrate but also antioxidants which promote the health benefit.

Phytochemicals contents and antioxidant activities of the purple waxy corn cob

Both KGW1 and KND variety express purple color not only on kernels but also throughout their cobs, indicating that anthocyanin located both in the edible part (kernels) and non-edible part (cob). Therefore, the corn cobs were also investigated

Table 3. Phytochemical contents and antioxidant activities of KGW1 and KND purple waxy corn cobs at two maturities.

Samples		Total phenolic content (mg GAE/100g DM)	Total flavonoid content (mg CE/100g DM)	Total anthocyanin content (mg CGE/100g DM)	Antioxidant activities (μmol TE/g DM)		
					TEAC assay	FRAP assay	ORAC assay[ns]
KGW1 variety	Eating-stage	1112.86±67.11a	174.87±10.39c	472.81±14.41c	147.62±1.82b	76.06±5.31b	224.67±17.39
	Mature-stage	1132.28±109.41a	208.58±18.24b	306.76±6.17d	147.18±18.44b	75.01±5.19b	313.50±21.00
KND variety	Eating-stage	939.98±20.09b	181.14±9.88c	514.32±25.91b	150.59±7.11b	75.56±5.32b	203.50±19.66
	Mature-stage	1171.04±40.18a	294.22±26.11a	705.64±45.84a	196.52±25.85a	141.02±2.70a	267.83±43.37

Values are mean ± SD (n=6). Means within the same column with the different letters are significantly different at $p \leq 0.05$ as analyzed by LSD test. [ns] The data in column is non-significant difference ($p > 0.05$).

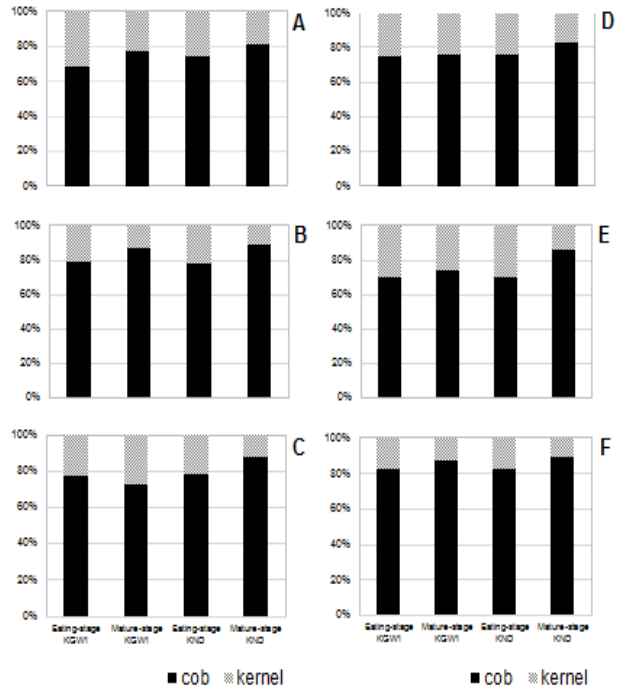

Figure 1. Relationship between phytochemical contents and antioxidant activities in two varieties at two maturity stages of purple waxy corn.
A: total phenolic content, B: total flavonoid content, C: total anthocyanin content, D: Trolox equivalent antioxidant capacity, E: ferric reducing antioxidant power, F: oxygen radical absorbance capacity.

for phytochemicals and its antioxidant properties. Table 3 showed that phytochemical contents in cob, depending on variety and maturity. The mature-stage of KND-cob had the highest in TPC, TFC and TAC ($p \leq 0.05$, Table 3). However, immature and mature-stages of KGW1 cob were not significantly different in TPC ($p > 0.05$). The purple cob at eating-stage contained TPC, TFC and TAC approximately 939.98-1112.86mg GAE/100g DM, 174.87-181.14mg CE/100g DM and 472.81-514.32 mg CGE/100g DM, respectively. The cobs at mature-stage had a little bit higher in TPC (1132.28-1171.04 mg GAE/100g DM) whereas clearly grater in TFC (208.58-294.22 mg CE/100g DM) and TAC (306.76-705.64 mg CGE/100g DM) compared to at eating-stage. The both purple cob varities had 68.5 to 80.9% TPC, 79.3 to 88.7% TFC and 72.7 to 88.0% TAC (Figure 1-A, B, C). Regardless of the corn varieties and maturity stages, it was worth noted that the TPC, TFC and TAC of cob were higher than those in kernels approximately 2.3-4.5 folds, 5.7-5.8 folds and 5.0-7.0 folds, respectively. For waste utilization, the purple waxy corn cob has potential as a source of phenolics, flavonoids and anthocyanins since it contained higher phytochemical levels than the kernels. However, TPC of the purple cob was three times lower than that reported in Andes purple cob (950-3516 mg GAE/100g DM; Jing *et al*., 2007). TAC of the purple cob was comparable to those of Andes purple corn cob (290-1333 mg/100g DM; Jing *et al.*, 2007) and fresh black raspberry (1113.1mg/100g berry; Hager *et al*., 2008). Quantities of phytochemicals varied to several factors i.e., genotype, growing condition, accumulation rate, interaction or form of phenolic acids that bound to cell structures (Jing *et al*., 2007; Shao *et al.*, 2014).

Anthocyanins are flavonoid compound. It was noticed that TFC of the purple cobs and kernels at eating- and mature-stage was lower than the TAC. This was due to the different reactant used in the spectrophotometrical protocol and calculating equation to determine TFC and TAC. It was agreed with Lewis *et al.* (1998) who reported that anthocyanin contents in colored-potatoes were higher than the flavonoid contents. This was explained that precursor in biosynthesis pathway in the tuber mainly tracked into an anthocyanins pathway rather than flavonoid pathway. Analytical method also affected on the content obtained. Chang *et al.* (2002) noted that TFC investigated by colorimetric method might be better represented total flavonoid content when performed in both of aluminum chloride reaction and 2,4-dinitrophenylhydrazine reactions. This because of aluminum chloride reaction was specific with flavones and flavonols, while 2,4-dinitrophenylhydrazine reaction was specific with flavanones and flavanonols.

Table 4. Correlations of phytochemical contents and antioxidant activities of purple waxy corn.

Correlations coefficient (*r*)	TPC	TFC	TAC	TEAC	FRAP
Corn kernel					
TFC	0.290^{ns}				
TAC	0.675**	0.687**			
TEAC	0.487*	0.224^{ns}	0.734**		
FRAP	0.549**	0.488**	0.758**	0.671**	
ORAC	0.618**	0.230^{ns}	0.639**	0.509*	0.597**
Corn cob					
TFC	0.541**				
TAC	0.128^{ns}	0.612**			
TEAC	0.393^{ns}	0.814**	0.668**		
FRAP	0.498*	0.920**	0.835**	0.798**	
ORAC	0.593**	0.206^{ns}	-0.343^{ns}	-0.007^{ns}	0.135^{ns}

Values were duplicate determinations of two varieties and two maturity stages of purple waxy corn. Total phenolic content (TPC); Total flavonoid content (TFC); Total anthocyanin content (TAC); Trolox equivalent antioxidant capacity (TEAC); Ferric reducing antioxidant power (FRAP); oxygen radical absorbance capacity (ORAC).

^{ns} The data is non-significant difference ($p>0.05$).

*,** The correlation is significant at $p\leq0.05$ or $p\leq0.01$, respectively.

The mature-stage KND cob provided the highest activities of TEAC and FRAP ($p\leq0.05$, Table 3). There were no significant differences in ORAC in the purple cobs ($p>0.05$). TEAC, FRAP and ORAC of the purple cob at eating-stage of the two varieties was 147.62-150.59, 75.56-76.06 and 203.50-224.67 µmol TE/g DM, respectively ($p>0.05$). The mature-stage of the two cultivars were significant difference in TEAC and FRAP ($p\leq0.05$) which in a range of 147.18-196.52 and 75.01-141.02µmol TE/g DM, respectively. This indicated that the both purple cobs varieties were a major source of antioxidant activities in corn ears, accounting for 75.2 to 83.6%, 70.4 to 85.7% and 82.3 to 89.4% for TEAC, FRAP and ORAC, respectively (Figure 1-D, E, F). Regardless to the corn varieties and maturity stages, the cob had higher TEAC, FRAP and ORAC than the kernel for 4.0-5.0 folds, 4.4-4.8 folds and 2.8-6.5 folds, respectively. The antioxidant capacity of the purple cob was similar to ORAC values of fresh black raspberry (192.2 µmol TE/g; Hager *et al.*, 2008). Furthermore, antioxidant capacities of purple cobs and kernels were higher than TEAC values of pumpkin, asparagus, celery, green cucumber carrot and white rice (Finocchiaro *et al.,* 2010; Mazzeo *et al.*, 2011; Deng *et al*., 2013) and higher than ORAC values of peach, mango, watermelon, broccoli, red cabbage, potato, spinach and tomato (Floegel *et al*., 2011). It has no contradiction that the antioxidant activities were closely related to phytochemicals in both kernel and cob of the purple corn.

Correlations of phytochemical and antioxidant activity

Phenolic compounds have a major contribution to antioxidant capacity (Oh *et al*., 2013). The linear correlation coefficient (r) between phytochemical contents (TPC, TFC, TAC) and antioxidant activities (TEAC, FRAP, ORAC assay) of purple waxy corn kernel and cob are presented in Table 4. The data analysis showed that there were no significant correlations between TPC and TFC, TFC and TEAC as well as TFC and ORAC of the purple kernels ($p>0.05$). This indicated that most of TPC (ca. 70%) in the purple kernels were non-flavonoids compounds. TAC showed high significant correlation ($p\leq0.01$) to TPC and TFC with r = 0.68 and 0.69, respectively, indicating that TAC was a majority in TPC and TFC categories. TAC played an important role in antioxidant activity (TEAC, FRAP and ORAC) of the purple kernels with r = 0.73, 0.76 and 0.64, respectively while TPC mostly contributed on ORAC with r = 0.62. Antioxidant activity of TFC in the purple kernels had moderate correlation with FRAP (r = 0.49, $p\leq0.01$). In the purple cobs, there were no significant correlation between TPC and TAC, as well as TPC and TEAC. There were poor correlations among ORAC and TFC, TAC, TEAC, FRAP ($p>0.05$). The statistical analysis showed strong correlation between TFC, TAC and TEAC (r = 0.81, 0.67, respectively) as well as FRAP (r = 0.92, 0.84, respectively). However, TAC in the purple kernels showed strong correlation with ORAC (r = 0.64) but not in the cobs (r = -0.34). A poor correlation coefficient between values suggested that the measured antioxidant activities were not strongly dependent on the compounds concentration (Flanigan and Niemeyer, 2014). It was probably due to the third variables involving in relationship or the phenomena were probably not a linear relationship. Nevertheless, our result suggested that TPC, TFC and TAC in kernels and cobs at different maturity and variety might have different configurations of structures influencing the antioxidant activity. Yang and Zhai (2010) reported that there were some derivatives malonated counterparts of the anthocyanins found in purple corn cobs but not in the kernels. Furthermore, the antioxidants in both parts of corn ear were composed of phenolic and non-phenolic compounds. Thus, more than one principles of assay were performed to investigate antioxidant activity since a bioactive could react in different action modes (Hu and Xu, 2011). Sarepoua *et al.* (2013) found that TPC and TFC of corn silk showed

significant and high correlation with antioxidant activity by DPPH assay ($r = 0.71$, $p \leq 0.01$ and $r = 0.63$, $p \leq 0.05$, respectively). According to Jin et al. (2016), there was a highly significant correlation coefficient between TPC and antioxidant capacity of herbal teas ($p < 0.001$). TPC in the herbal tea was highly positively correlated with DPPH ($r = 0.97$), ABTS ($r = 0.95$) and FRAP ($r = 0.97$). Therefore, TAC in the purple waxy corn played a major role in antioxidant activity because of its high correlation observed in both kernel and cob, especially by TEAC and FRAP. It indicated that bioactives in the purple corns exhibited antioxidant capacity of H-atom and electron-donating mechanism.

Conclusion

This study provided information of nutritive compositions, phytochemical contents (TPC, TFC and TAC) and antioxidant activities (TEAC, FRAP and ORAC) in the purple waxy corn for KGW1 and KND varieties. The purple kernels could be an alternative health food since they provided not only a good source of carbohydrates in the form of starch and dietary fiber but also protein, phytochemicals and antioxidant activities. The purple cobs also showed a potential of a riched-anthocyanin source, leading to a utilization of waste and value-added. The KND cobs had the highest contents of phenolics, flavonoids and anthocyanins at mature-stage. However, the highest phenolic and anthocyanins contents in kernel were found in eating-stage of KGW1. The KND cob has a potential to be a source of natural colorant for apply to use in functional food products which could be value-added of the purple waxy corn by-product, while the KGW1 kernel is suggested to consume as fresh food. This work provides useful information for farmer to harvest the purple waxy ear at an appropriate maturation and for a food scientist and technologist to fortify and develop a new purple waxy corn based product as a functional food product.

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