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Phenolic compound, anthocyanin content, and antioxidant activity in some parts of purple waxy corn across maturity stages and locations

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Abstract

The understanding on growth stage of purple waxy corn and location effect is very important for anthocyanin production systems. Therefore, the objective of this study was to evaluate phenolic compounds, anthocyanins and antioxidant activity in corn components at immaturity (22 days after pollination (DAP)) and maturity (36 DAS) stages at different growing locations. Two varieties of purple waxy corn were evaluated at two locations during the dry season 2014/2015. Total phenolic (TPC), total anthocyanin (TAC) and antioxidant activity by DPPH free-radical-scavenging assay and TEAC trolox equivalent antioxidant capacity assay were recorded. Significant differences among corn components (C) were observed for all parameters. Location (L) x Variety (V), LxC, VxC and LxVxC interactions were significant for TAC in both stages. Silk and tassel had the highest TPC at immaturity stage but husk of KND and FC111 at KK had the highest at maturity stage. Silk of KND had the highest TAC at KK at immaturity stage but cob and husk of KND had the highest at maturity stage at KK. In general, corn cob and silk had high DPPH at immaturity stage and seed, cob and husk of KND at KK had high DPPH at maturity stage. However, cob and silk had the highest TEAC at immaturity stage and cob and husk especially KND had high TEAC at maturity stage. This information is useful for use waxy corn as food, food supplement and functional food products for corn consumers and food industry.

Introduction

Research on anthocyanins content and phenolic compounds is increasing due to important bioactive properties contributing to health benefits such as antioxidative activities, even though these compounds are not nutritive (Rice-Evans and Miller, 1996; Heinonen *et al*., 1998; Setchell and Aedin, 1999). Nutrient antioxidants are compounds that should be given through foods because they cannot be produced in the human body such as anthocyanin and other phenolic compounds (Huy *et al.*, 2008). The potential health benefits of anthocyanins and other phenolic compounds have been reported in many investigations. Anthocyanins have a wide range of bioactivities conducive to human health such as super antioxidant ability, anti-diabetic capacity, proliferative quality, inflammatory effects and the effects that protect against cancer and stroke (Yang *et al.*, 2010). Previous investigations reported a significant application of anthocyanins in food industry as food natural colorants in beverage, snacks and dairy products (de Pascual-Teresa and Sanchez-Ballesta, 2008).

The phytochemicals in different parts of corn have been reported in previous studies. Corn kernel contains a wide range of colors such as white, yellow, orange, purple and black. This pigmented corn is a rich source of phytochemicals and many secondary metabolites such as anthocyanins and other phenolic compounds (Žilić *et al.*, 2012). Study showed that corn cob provides phenolic compounds and natural antioxidants, which are used for the functional food (Cevallos-Casals and Cisneros-Zevallos, 2003). Corn silk is an excellent source of flavonoid compounds and many bioactive compounds (Ren *et al*., 2009) and silk of purple waxy corn at immaturity stage has high phenolic, flavonoids, anthocyanin and antioxidant activity (Sarepoua *et al*., 2015). Corn husk of purple corn is an excellent source of anthocyanins (Li *et al*., 2008). Moreover, corn tassel included a valuable raw source of phenols and could be used to increase the shelf life of fats and oils (Mohsen and Ammar, 2009).

Waxy or glutinous corn (*Zea mays* L.var. ceratina) is a special cultivated type of corn and an important vegetable crop in East and Southeast Asia countries

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due to consumer awareness of their health benefits. Waxy corn harvested at immaturity stage is consumed as fresh food similar to sweet corn. Generally, waxy corn has shorter maturity than sweet corn. Waxy corn is also harvested at maturity stage for food industry. Furthermore, waxy corn is a good source of anthocyanins, phenolics and antioxidant activity (Hu and Xu, 2011). Kernels and cobs of purple corn possess an excellent antioxidant activity (Li *et al*., 2008). Kernels of purple waxy corn had higher anthocyanins and antioxidant activity in both milky and maturity stages than did kernels of white waxy corn, super sweet corn and field corn (Khampas *et al*., 2013). The information on systematic evaluation of anthocyanins, phenolic compounds and antioxidant activity in purple waxy corn is very limited in the literature. Furthermore, the information on the quantities of these compounds in different tissues such as kernels, cob, silk, husk and tassel in purple waxy corn is lacking and further investigations are required. Therefore, we aimed to evaluate the total content of anthocyanins, phenolic compounds and antioxidant activity in several parts of purple waxy corn at immaturity and physiological maturity stages at different locations. These information will be valuable for food researchers, who work on functional food products, and consumers, who will have benefits from the products.

Materials and Methods

Plant materials

Two varieties of purple waxy corn (FC111 and KND) were used in this study. FC111 was obtained from Pacific Seeds, Thailand. KND was obtained from Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. These two varieties were selected because the difference of purple colors in ear parts. Moreover, previous study showed that FC111 and KND had high anthocyanin in cob (Khampas *et al*., 2015).

Two corn varieties were evaluated in a randomized complete block design with three replications at two locations in Uthai thani province (UT), Thailand, and Experimental Farm of Khon Kaen University (KK), Thailand, in the dry season 2014/2015. The soil type in UT was loamy and the soil in KK was sandy. Soil pH at KK was 6.4 but soil pH at UT was 5.5. UT had organic matter of 1.4% but organic matter at KK was lower (0.7%). Both locations had similar solar radiation and rainfall at vegetative period (0-60 days), but KK had lower maximum and minimum than UT.

The agronomic practices used in the experiment followed the recommendations for commercial production of waxy corn. Briefly, the varieties were planted in six-row plots with five meters in length and spacing of 80 cm x 25 cm. Five plants from two middle rows of each variety were selected randomly and harvested at immaturity stage (22 days after pollination) and at maturity stage (36 days after pollination). Ears were manually harvested from 5 randomly selected plants, whereas tassels were cut from the same plants at pollination. Husks and silks were speared from ears, while kernels were manually separated from cobs. Each sample was fragmented into smaller fractions separately and placed in plastic bags with lock court, consisting of two different stages in two sites. The samples (tassel, husk, silk, kernel and cob) were weighed as gram and directly frozen in liquid nitrogen to stop enzymatic activity, freeze-dried and stored at -22°C until analysis.

Sample extraction

Collected plant samples were grinded through electric mixer to obtain extract powder for used as the extracts. The extraction methods for total anthocyanins, phenolic compounds and antioxidant activity were described previously (Singh *et al.*, 2013) with slight modifications. Briefly, 2 g of each sample powder was taken into a flask containing 20 mL methanol and shaken on a platform shaker (LabScientific Inc., Livingston, NJ, USA) at 200 rpm for 1 hour in room temperature. The extracts were filtrated through Whatman No.1 filter paper in order to remove the debris. The procedure was repeated two times. To remove solvent, the filtrate was evaporated using a rotary flash evaporator (BUCHI Operations India Private Limited, 394230-Surat/India) at 40°C (5-10 minutes). The residue was reconstituted with 10 mL methanol and stored at -22°C until determination of total contents.

The extracted samples were analyzed using a spectrophotomerer (10S UV-Vis, Themo Scientific Genesys, Australia) to determine total anthocyanin contents (TAC) and total phenolic contents (TPC), and antioxidant activity was assayed by two methods, DPPH assay (free-radical-scavenging activity) and TEAC assay (trolox equivalent antioxidant capacity).

Determination of total anthocyanin contents (TAC)

Total anthocyanin content was determined following the pH differential method described by Mezadri *et al.* (2008) with minor modification. In brief, 0.5 mL from each sample was mixed with 4.5 mL of KCL buffers (pH 1.0) and 4.5 mL $NaCH_3$ buffers (pH 4.5). After mixing in Vortex, all samples were stored in the dark for 30 minutes. Absorbance at 510 and 700 nm was read in a spectrophotometer and

compared against blank. The blank consisted of 0.5 mL methanol and the same amount of all chemical except for the extracted sample. The determination of anthocyanin was made as the following formula:

(A x Mw x Dilution factor x 1000)/(Ɛ x 1),

where A was absorbance calculated accordance with (A510 – A700) pH1.0 - (A510 – A700) pH4.5, 1000 was converting factor from molar to ppm, Mw was molecular weight equal 449.2 g/ mol. Ɛ was molar extinction coefficient equal 26,900 $M^{-1}cm^{-1}$. For quantification Mw and Ɛ were used for external calibration for cyaniding-3-glucoside. The anthocyanin content was expressed as microgram of cyaniding-3-glucoside equivalents per gram of sample weight (mg C3G/100 g sample).

Determination of total phenolic contents (TPC)

The total phenolic contents were determined using the Folin– Ciocalteu according to the method reported previously (Reddy *et al*., 2010) with slight modifications. Initially, 0.5 mL Folin–Ciocalteu reagent was mixed with 2.5 mL distilled water to make the ratio of 1:5. Briefly, 0.5 of each sample was mixed with 3 mL diluted Folin–Ciocalteu reagent. The mixture was allowed to stand for 3 minutes at room temperature before adding 1.5 mL Na_2CO_3. The solution was stored in the dark for 30 minutes. The absorbance of the resulting mixture with blue color was measured at 765 nm against blank, using a spectrophotometer. The total phenolic contents were determined using curve of standard calibration and expressed as microgram of gallic acid equivalents per gram of dry weight (mg GAE/100 g sample weight).

Determination of antioxidant activities

Antioxidant activities were determined using the DPPH free radical scavenging assay, which was performed according to the method described by Liu *et al.* (2011) with slight modifications. In short, 0.2 mL from extract sample was mixed with 1 ml of 0.2 mM DPPH methanol solution, which was prepared immediately. The mixture was left to stand for 30 minutes in dark conditions. The absorbance of the resulting mixture was measured at 517 nm against blank using a spectrophotometer. The blank included 0.2 mL methanol and the same amount of all reagents except for the extracted sample. The absorbance was calculated and plotted as a function of concentration of standard and the extracted sample to determine the ascorbic acid equivalent concentration of antioxidant. The percentage of DPPH activity was calculated as the following equation:

DPPH radical scavenging activity (%Inhibition) = (1-Asample/A control) x100,

where A sample is the absorbance of extract or the standard, A control is the absorbance of the control.

The Trolox equivalent antioxidant capacity (TEAC) assay measures the antioxidant capacity of a given substance compared to the standard. Trolox antioxidant capacity is measured using the ABTS Decolorization Assay, which measures the reduction of radical cations of ABTS by antioxidants. This method was used as described by Kriengsak *et al.* (2006) with minor adjustments. Briefly, 0.0192 gram from ABTS was added to 5 mL distilled water. Potassium persulphate (0.0033 gram) was added to 5 mL distilled water and then two solutions were mixed for preparing TEAC. The mixture was allowed to stand for 24 hours in dark condition. Then 1 mL of TEAC was added to 60 mL methanol for preparing the final mixture. The extracted sample (0.03 mL) was added to 3 mL of final mixture. The absorbance of the resulting mixture was read at 734 nm against blank, using a spectrophotometer. The results were expressed in µmol of Trolox equivalents per g of dry weight (µmol TE/g sample weight).

Statistical analysis

Combined analysis of variance across the locations was carried out separately in accordance with a randomized complete block design for each character under study for milky stage and maturity stage. Least significant differences (LSD) for mean comparison test were set at $P \leq 0.05$.

Results and Discussion

Sources of variation

At immaturity stage, locations (L) were significantly different ($P \leq 0.01$) for total anthocyanin content (TAC) and antioxidant activity determined by DPPH method, whereas locations were not significant different for total phenolic compound (TPC) and antioxidant activity determined by TEAC method (Table 1). However, varieties (V) were significantly different ($P \leq 0.01$) for TAC and antioxidant activity determined by TEAC method, but TPC and antioxidant activity determined by DPPH method were not significant different. The results indicated that selection of suitable purple waxy corn for TAC and antioxidant activity determined by TEAC is important.

Components (C) were significantly different ($P \leq 0.01$) for all characters. The results showed that accumulations of phytochemical were different in

Table 1. Mean square for total phenolic compound (TPC), total anthocyanin content (TAC) and antioxidant activity determined by DPPH and TEAC methods in purple waxy corn at two growth stages

d.f.	2	1	1	4	1	4	4	4	38	
Traits	Mean squares									C.V. (%)
	Block	Location (L)	Variety (V)	Component (C)	LxV	LxC	VxC	LxVxC	Pooled error	
Immaturity stage										
TPC	106.9 ns	22.6 ns	1,197.6 ns	80,533.9 **	0.4 ns	290.6 ns	219.5 ns	231.0 ns	307.7	9.3
TAC	48 ns	4,669 **	85,054 **	42,756 **	4,929 **	1,567 **	8,780 **	1,461 **	181	15.7
DPPH	30.4 ns	355.3 **	0.1 ns	1,336.3 **	2.8 ns	158.6 **	219.9 **	8.5 ns	20.3	5.8
TEAC	0.11 ns	1.24 ns	21.09 **	150.32 **	2.29 *	0.14 ns	14.04 **	0.19 ns	0.35	6.1
Maturity stage										
TPC	27 ns	1,322 *	5,114 **	43,237 **	1,557 *	4,421 **	3,608 **	1,835 **	231	9.2
TAC	140 ns	5,744 **	60,964 **	76,922 **	777 *	1,393 **	9,423 **	739 *	150	13.7
DPPH	73 ns	39 ns	484 **	2,610 **	19 ns	274 **	126 *	40 ns	35	9.9
TEAC	0.4 ns	0.7 ns	12.4 **	132.2 **	5.9 *	0.4 ns	28.9 **	1.8 ns	0.9	10.5

ns = non-significant different
d.f. = degree of freedom; C.V. = coefficient of variation
*, ** significant different at P≤ 0.05 and 0.01 probability levels, respectively

corn components. The interactions between location and variety were significant (P≤0.01) for TAC and antioxidant activity determined by TEAC method, whereas the interactions between location and component were significant (P≤0.01) for TAC and antioxidant activity determined by DPPH method. The interactions between variety and component were significant (P≤0.01) for most characters except for TPC. In contrast, the interactions among location, variety and component were significant (P≤0.01) for TAC only.

At maturity stage, locations were significant different for TPC (P≤0.01) and TAC (P≤0.05) (Table 1). The differences among varieties and components were significant (P≤0.01) for all characters. The interactions between location and variety were significant (P≤0.05) for most characters except for antioxidant activity determined by DPPH method. However, the interactions between location and component were significant (P≤0.01) for most characters except for antioxidant activity determined by TEAC. The interactions variety and component were significant (P≤0.05 and 0.01) for all characters. The interactions among location, variety and component were significant (P≤0.01 and 0.05) for TPC and TAC.

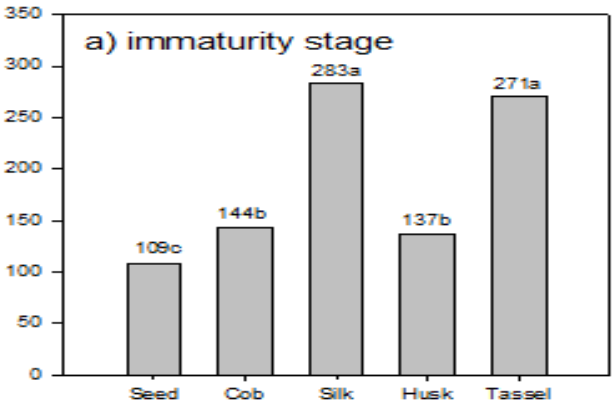
Total phenolic compound (TPC)

Locations and varieties were not significantly different for TPC at immaturity stage and their interactions were not significant (Table 1). Silk and tassel had the highest TPC from two varieties across two locations (283 and 271 mg/100 g sample, respectively) (Figure 1a). At maturity stage, husk of KND variety at UT location and KK location had the highest TPC (284 and 303 mg/100 g sample, respectively, respectively) followed by husk of FC111 variety at KK location (278 mg/100 g sample) (Figure 1b).

UT location and KK location in the dry season represented the main growing areas of purple waxy corn in Thailand, which are located in the Central Plain and the Northeast of the country. KK location had higher TPC than did UT location for husk of FC111. This can be explained by higher solar radiation (12-17 MJ m^{-2} d^{-1} for UT and 15-19 MJ m^{-2} d^{-1} for KK) and lower temperature (23-32 °C for UT and 22-28 °C for KK) at KK location during seed filling duration or phenolic accumulation (41-115 days after planting). Although, UT location had low temperature at 61-75 days after planting, the period of low temperature was very short and, lather, the temperature increased again.

Normally, waxy corn, as a vegetable corn, is consumed at immaturity stage like sweet corn. However, purple waxy corn can be used at two seed stages including immaturity and maturity stages. At maturity stage or dry stage, purple waxy corn is good for food industry because it does not spoil rapidly and has longer shelf-life than at immaturity stage.

Silk and tassel had the highest TPC at immaturity but they decreased TPC at maturity stage. Husk was only one component that increased TPC very rapidly after immaturity stage and it had the highest TPC at maturity stage. Seed did not change TPC from immaturity to maturity stage. In previous study, seed of purple waxy corn at maturity had higher TPC than at immaturity stage (Khampas *et al*., 2013). The discrepancy of the results from different studies



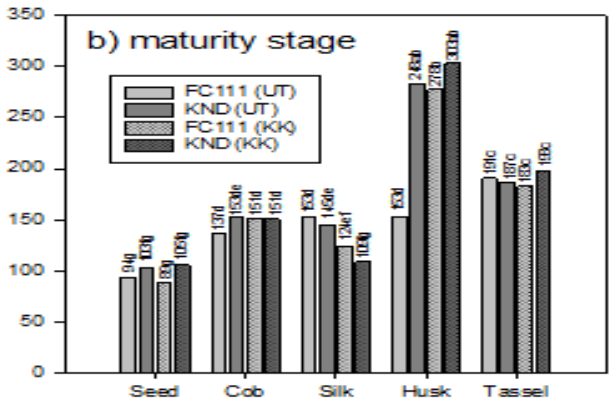
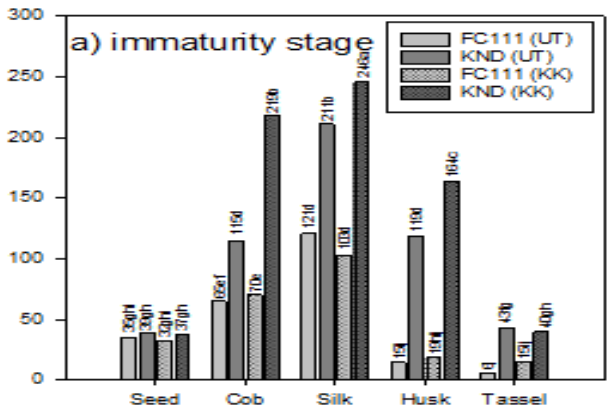


Figure 1. Total phenolic compound (TPC) (mg/100 g sample) in purple waxy corn components at two growth stages (UT is Uthai Thani and KK is Khon Kean). Means with the same letter(s) in each growth stage are not significantly different (P<0.05) by LSD.



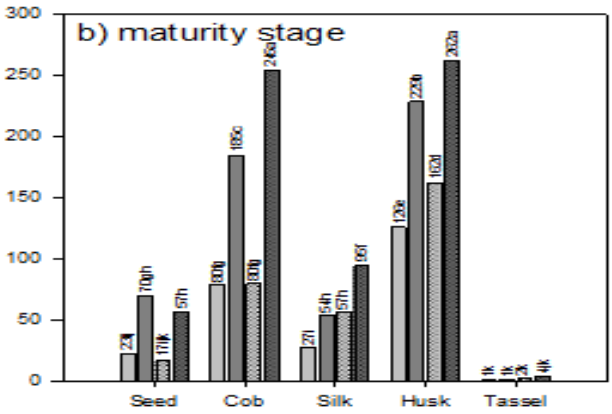


Figure 2. Total anthocyanin content (TAC) (mg/100 g sample) in purple waxy corn components at two growth stages (UT is Uthai Thani and KK is Khon Kean). Means with the same letter(s) in each growth stage are not significantly different (P<0.05) by LSD.

should be possibly due to the differences in materials used. It should be noted here that all components should be harvested at immaturity stage except for husk that it needs to be collected at maturity stage.

Total anthocyanin content (TAC)

At immaturity stage, silk of KND variety had the highest TAC at KK location (246 mg/100 g sample) followed by cob of KND variety (219 mg/100 g sample) at KK location and silk of KND variety at UT location (211 mg/100 g sample), respectively (Figure 2a). However, at maturity stage, husk and cob of KND variety at KK location had the highest TAC (262 and 254 mg/100 g sample, respectively) followed by husk and cob of KND variety at UT location (229 and 185 mg/100 g sample, respectively) (Figure 2b).

KK location had had higher TAC than did UT location at both maturity stages due to the higher solar radiation and lower temperature during anthocyanin accumulation. In previous study, low temperature might be the cause of high TAC in waxy corn cob (Khampas *et al.*, 2015). The results of two studies were rater different. In tomato tubers, phenolics and anthocyanin were increased with higher light intensity and low temperature (Reyes *et al*., 2004), but, in strawberry fruits, high temperature enhanced phenolics, flavonoids and anthocyanin (Wang and Zheng, 2001). The results of this study indicated that KK was a good location for phenolic and anthocyanin production.

Anthocyanins in corn are presented in all tissues and found at high concentrations in kernel skin and cobs (Moreno *et al*., 2005). The variation of maize and waxy corn germplasm in anthocyanins has been reported (Chander *et al.*, 2008; Harakotr *et al*., 2014). FC111 and KND are purple waxy corn varieties that were selected based on high TAC content in seed (Khampas *et al*., 2013) and in cob and good adaptation under growing conditions in Thailand (Khampas *et al*., 2015). Del Pozo-Insfram *et al.* (2007) suggested that corn genotypes with purplish black and blue kernels had very high phenolic compounds compared to corn genotypes with light-colored kernels. Moreover, corn genotypes with purple kernels showed the highest phenolic levels followed by corn genotypes with red and black kernels, respectively (Lopez-martinez *et al*., 2009).

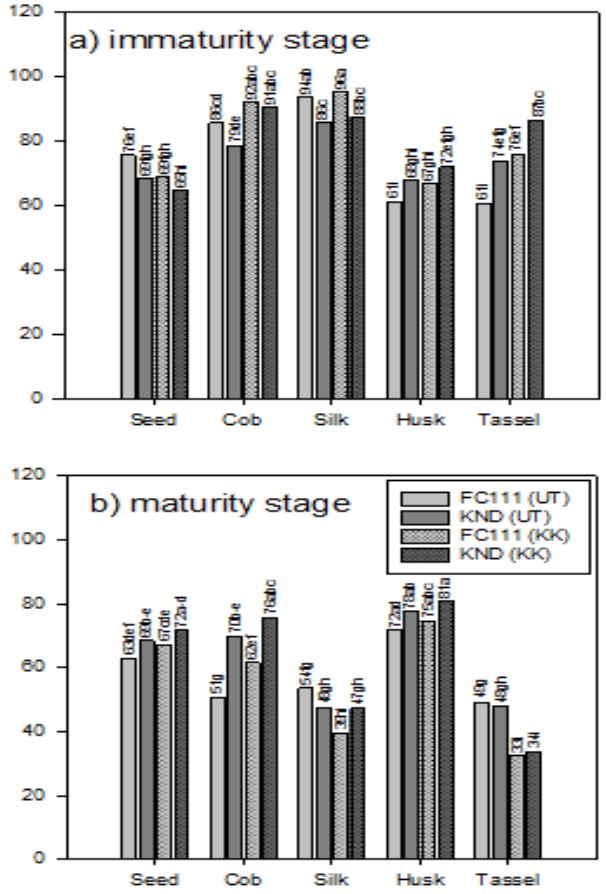


Figure 3. Antioxidant activities determination by DPPH assay (%) in purple waxy corn components at two growth stages (UT is Uthai Thani and KK is Khon Kean). Means with the same letter(s) in each growth stage are not significantly different (P<0.05) by LSD.

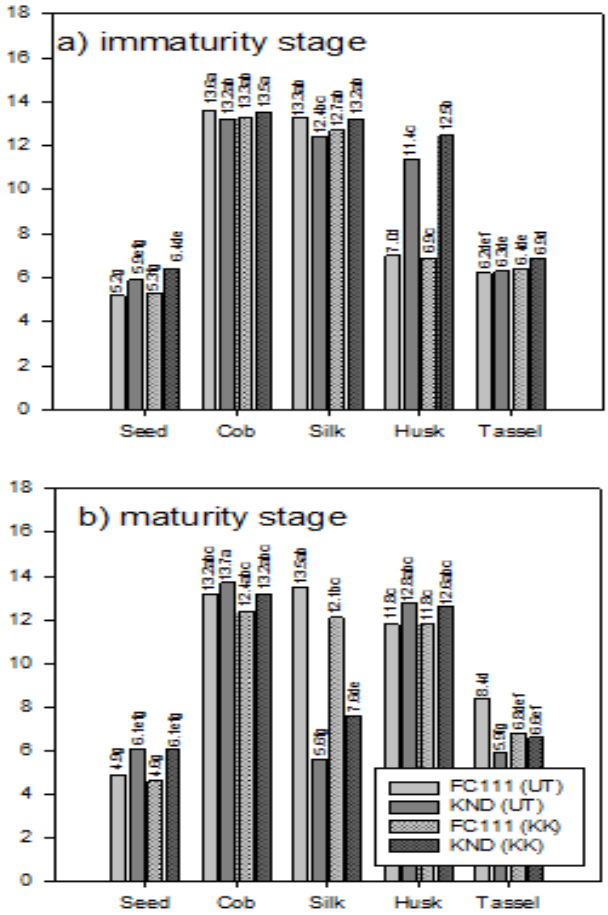


Figure 4. Antioxidant activities determination by TEAC assay (%) in purple waxy corn components at two growth stages (UT is Uthai Thani and KK is Khon Kean). Means with the same letter(s) in each growth stage are not significantly different (P<0.05) by LSD.

In this study, KND had higher TAC than did FC111 in most components except for seed at immaturity stage and tassel at maturity stage. The results were also supported by previous studies of purple waxy corn in seed (Khampas *et al.*, 2013) and cob (Khampas *et al*., 2015) at maturity stage.

Cob, silk, husk and tassel are waste products of waxy corn. In general, corn ears with seed, cob, silk and husk are sold on the market like sweet corn after that corn ears with husk or without husk are boiled or steamed. The consumers eat corn only seed and discard other components. Previous studies reported that corn seed (Khampas *et al*., 2013; Harakotr *et al.*, 2014) and cob (Khampas *et al*., 2015) were good sources of anthocyanin and antioxidant activity. Moreover, waxy corn seed is a good source of carotenoids (Kuhnen *et al*., 2010; Hu and Xu, 2011), anthocyanins, phenolics and antioxidant activity (Hu and Xu, 2011). In this study, silk and tassel were good sources of TPC at immaturity stage, but husk had the highest TPC at maturity stage.

Silk of KND variety had very high TAC at immaturity but cob and husk had high TPC at maturity stage. The results also supported by the results of Sarepoua *et al*. (2015), who found that silk of purple waxy corn genotypes at immaturity stage had higher TPC than at maturity stage. The results suggested that selection suitable corn components at the correct growing stage is very important for both TPC and TAC production.

In general, silk and tassel had high TAC at immaturity then they decreases very fast at maturity stage. However, other components including seed (for KND), cob and husk increased TAC after immaturity stage. The results supported by previous findings of Khampas *et al.* (2013) and Harakotr *et al*. (2014), who found that maturity stage had higher anthocyanin in corn seed than at immaturity stage. Accumulation of TAC in husk was very fast for almost two times compared with the husk at immaturity stage. The results suggested that silk and tassel should be harvested at immaturity stage, but other components especially husk should be collected at maturity stage.

Antioxidant activity determined by DPPH assay

At immaturity stage, silk of FC111 variety (96%),

cob of FC111 variety (92%), cob of KND variety (91%) at KK location and silk of FC111 variety (94%) at UT location had the highest DPPH followed by silk of KND variety (88%) and tassel of KND variety at KK location (87%) (Figure 3a). Husk (81%), cob (76%) and seed (72%) of KND variety, husk of FC111 variety (75%) at KK location, husk of KND variety (78%) and husk of FC111 variety (72%) at UT location had the highest DPPH at maturity stage (Figure 3b).

Location had very low effect on antioxidant activity determined by DPPH and TEAC methods except for DPPH at immaturity stage. In previous studies, low temperature enhanced antioxidant in corn cob (Khampas *et al*., 2015) and potato tuber (Lachman *et al*., 2008). The results in this study did not supported previous findings.

At maturity stage antioxidant activity determined by DPPH reduced in silk and husk but this parameter did not change in other components. The results indicated that silk and husk should be harvested for DPPH at immaturity stage.

Antioxidant activity determined by TEAC assay

Cob (13.6%), silk (13.3%) of FC111 variety, cob (13.2%), silk (12.4%) of KND variety at UT location, cob (13.5%), silk (13.2%) of KND variety, cob (13.3%) and silk of FC111 variety (12.7%) at UT had the highest TEAC followed by husk (12.5%) of KND variety at KK location and silk (12.4%) of KND variety at UT location at immaturity stage (Figure 4a). At maturity stage, cob (13.7%), husk (12.8%) of KND variety, silk (13.5%), cob (13.2%) of FC111 variety at UT location, cob (13.2%), husk (12.6%) of KND variety and cob (12.4%) of FC111 variety at KK location had the highest TEAC (Figure 4b). The results showed that at immaturity stage, KND had higher antioxidant activity determined by TEAC assay than did FC111 variety for corn husk, but FC111 variety increased TEAC until the value was similar to that of KND variety at maturity stage. The values of antioxidant activity determined by TEAC were almost the same as those determined by DPPH accept for TEAC in silk of FC111 variety, which did not decrease from immaturity to maturity stages.

Conclusion

Silk and tassel had high TPC, TAC, DPPH and TEAC at immaturity but husk had the high at maturity. KK had higher TEAC and TAC than UT. Cob, silk and husk of KND at KK location had high TAC at immaturity but cob and husk of KND were good sources at maturity. Cob and silk had high DPPH and TEAC at immaturity but cob and husk had high DPPH and TEAC at maturity.

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