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Corn Tassel: A New Source of Phytochemicals and Antioxidants Potential for Value-added Products Development in Agro-industry

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ABSTRACT

Corn tassel is a by-product from hybrid corn seed production and a new source of phytochemicals including compounds with antioxidant activity. Four tassel development stages were evaluated in eight commercial corn varieties. Corn varieties and tassel developmental stages showed significant variations ($P \leq 0.01$) for all parameters. Total phenolic content and antioxidant activity were highest in field corn. KGW1, a purple waxy variety, had the highest anthocyanin content and carotenoid content at tassel development stages at 50% and 75% of pollen shed, whereas the tassel developmental stages at the 1st day of pollen shed and 50% of pollen shed had the highest of anthocyanin yield and carotenoid yield. The most suitable time for tassel harvest should be between the 1st day of pollen shed to 50% of pollen shed. Phytochemicals and antioxidants that are extracted from corn tassel can be used as a functional food supplement, natural pharmaceuticals and cosmetic products.

Keywords: *Zea may* L., bioactive compound, floral corn, by-products, bee pollen

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1. Introduction

Corn (*Zea may* L.) is an important cultivated cereal crop in the world. Corn kernels are a harvested product and a good source of nutrition and bioactive compounds. They are used as a raw material for food, animal feed, and bio-fuel. The use of by-products from corn production is a challenging research area that may increase corn production efficiency. Moreover, waste from corn production is also a good source of phytochemicals such as phenolic compounds, flavonoids and anthocyanins in corn silk [1], anthocyanins in corn husk [2], phenolic compounds and flavonoids in corn husk, cob and silk [3], phenolic compounds and anthocyanins in corn husk, cob, silk and pollen [4]. These phytochemicals are natural pigments in plant parts, and they function as antioxidants that are beneficial to health. Their health benefits include anti- apoptosis, anti- aging, anti- carcinogen, anti- inflammation, anti-atherosclerosis and cardiovascular protection [5].

Corn is a wind-pollinating species. The tassel of the top of plant functions as a male floral organ that produces pollen being carried by the wind to female flowers called silk, leading to fertilization and kernel production. On average, a tassel can produces 25 million pollen grains [6]. The pollen grains after shedding are viable for 60-240 minutes and can move further than 300 meters [7]. An ear of corn typically produces fewer than 1,000 filled kernels and, therefore, each tassel produces excess pollen to pollinate the ear. In hybrid seed production, a male row is sufficient to pollinate 2-3 female rows, and the ratios of 1-4 or 1-6 of male row to female rows do not affect seed setting [8]. In order to prevent self pollination of the female plant, the female rows are detasselled prior to pollen shed and the tassel is considered to be a waste product.

Pollen has high potential for development of health food products. Sweet corn pollen is an excellent source of antioxidants and high nutrients such as sugar, protein and oils [9]. Bee pollen is commercially available because it is an excellent source of carbohydrate, protein,

lipid, vitamin, carotenoid and bioactive compounds [10]. Bee pollen is used for cosmetics, dietary supplements and functional foods. However, the production of bee pollen is low, time consuming and not practical for large-scale production.

Direct collection of corn tassel is a viable method for industrial use and increases corn production efficiencies as waste from corn production is transformed into value-added products because corn tassel has high total phenolic content and antioxidant activity [11]. The pigmentations in glume, anther and pollen grains are the important sources of phytochemicals in corn tassel. However, tassel architecture and development stage affect phytochemical yield per production area. In various plants, phytochemical accumulation in plant flowers such as rose [12], tea [13], safflower [14], and cactus [15] has been shown to be dependent on development stage.

The information on phytochemicals and antioxidant property in corn tassel at different development stages is limited. The objective of this study was to investigate the phenolics, anthocyanins, carotenoids and antioxidant activity at four development stages of corn tassel. The information obtained from this study is useful for production of corn tassels in order to obtain the highest yield and quality.

2. Materials and Methods

2.1 Plant material and sample preparation

Eight commercial F_1-hybrids in Thailand including three field corns, two sweet corns, and three waxy corns were used in this experiment (Table 1). These varieties were selected because of their differences in glume and anther colors (Figure 1). Five uniform tassels were collected at four development stages, including VT tassel stage (The last branch of the tassel emerged from the whorl.), 1st day of pollen shed, 50% of pollen shed and 75% of pollen shed (Figure 2).

Tassel development stages were considered as factor A, and corn varieties were considered as factor B in a 4×8 factorial experiment. The corn varieties were planted in a randomized complete block design (RCBD) with three replications in the dry season (October-December, 2015) at the Vegetable Research Station, Khon Kaen University, Thailand. Five whole tassel samples were harvested in each plot, cut into small pieces of 2-5 cm, dipped in liquid nitrogen for stopping enzymatic activity and freeze-dried. The samples were further homogenized and milled into fine powder by an electric grinder. The particles were screened through 40-mesh sieve and stored at -20 °C until analysis.

Table 1. Source of varieties, corn type and tassel corn color characteristics.

Entry no.	Varieties	Type	Glume color	Anther color	Origin
1	PAC339	Field corn	Red-green	Yellow-pink	Pacific Seeds (Thai) Co., Ltd.
2	P4546	Field corn	Green	Pink	Pioneer Hi-Bred (Thailand) Co., Ltd.
3	S6248	Field corn	Red-green	Pink	Syngenta Seed Co., Ltd.
4	Hibrix3	Sweet corn	Green	Yellow	Pacific Seeds (Thai) Co., Ltd.
5	Sugar75	Sweet corn	Green	Yellow	Syngenta Seed Co., Ltd.
6	Sweet violet	Waxy corn	Green	Light green	East West Seed Co., Ltd.
7	Muang tam	Waxy corn	Red-green	Green-pink	Syngenta Seed Co., Ltd.
8	KGW1	Waxy corn	Purple-green	Purple	Khon Kaen University

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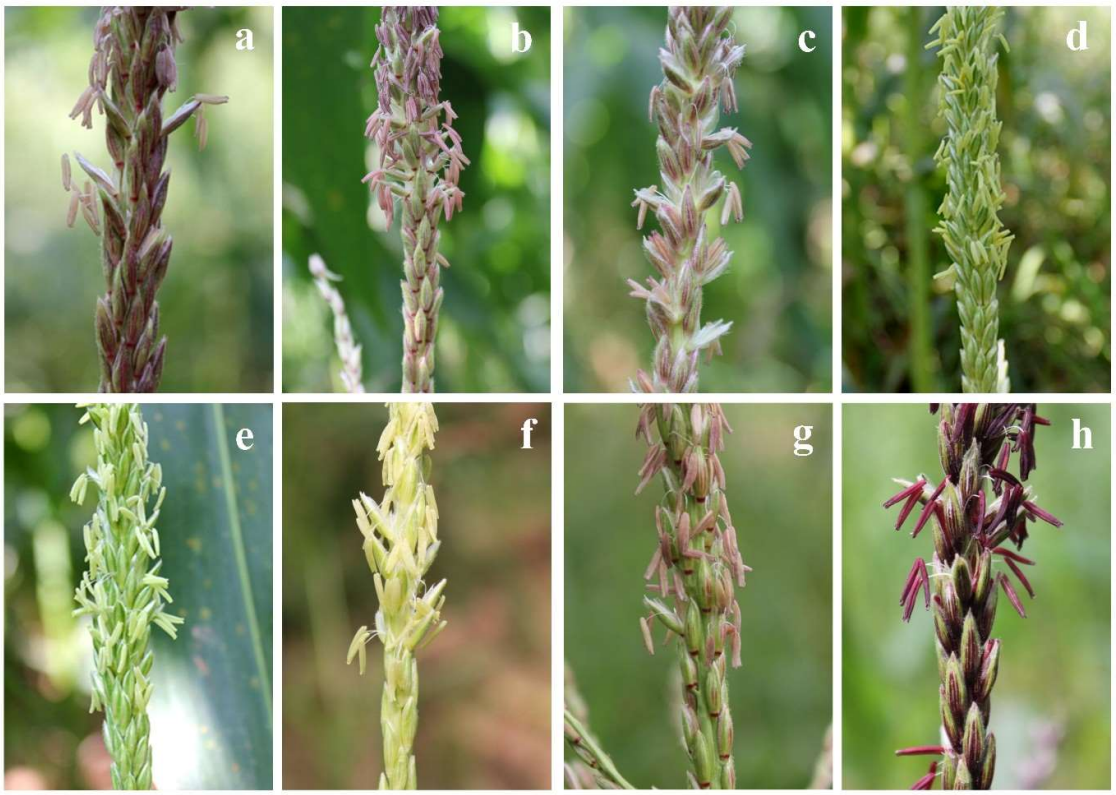

Figure 1. Close-up photographs of the main spike of the tassel of eight commercial hybrids: PAC339 (a), P4546 (b), S6248 (c), Hibrix3 (d), Sugar75 (e), Sweet violet (f), Muang tam (g) and KGW1 (h).

Figure 2. Four stages of corn tassel development: VT tassel (a), 1st day of pollen shed (b), 50% of pollen shed (c) and 75% of pollen shed (d).

2.2 Chemicals and reagents

All chemicals and reagents were of analytical grade. Citric acid, gallic acid, potassium chloride, sodium acetate trihydrate, potassium hydroxide and methanol were purchased from Ajax Finechem Pty Ltd., New South Wales, Australia. Petroleum ether, diethyl ether and ethanol were purchased from LCI Labscan Co., Ltd., Bangkok, Thailand. Potassium persulfate, butylated hydroxytoluene (BHT) and Folin-Ciocalteu reagent were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Co., Missouri, USA.

2.3 Sample extraction

The methods of sample extraction for phenolics, anthocyanins and antioxidant properties were described previously [16] with minor modification. Briefly, 0.5 g of the sample was added into 10 mL of 1% citric acid (CA) in 80% methanol (MeOH) and the mixed sample was incubated at 4 °C for 24 h. The sample was further centrifuged at 5,000 rpm for 15 min. The supernatant was filtered through Whatman No. 1 filter. The final volume of the filtrates were adjusted to 10 mL through extraction solvent and stored at -20 °C until analysis.

Sample extraction for carotenoids was carried out according to the method described by Alfieri, et al. [17]. Six mL of 0.1% butylated hydroxytoluene (BHT) in ethanol was added into 0.5 g sample. The sample was incubated at 85 °C for 5 min and saponified. One hundred and twenty µL of fresh potassium hydroxide (KOH) 17.8 M was added to the sample and incubated at 85 °C for 10 min. The sample was mixed well, put in ice bath and 4 mL of cold distilled water was added. Then, 3 mL of 2:1 (v/v) petroleum ether: diethyl ether (PE:DE) was added into the sample, thoroughly mixed and centrifuged at 2,000 rpm for 10 min. After centrifugation, the upper layer of the supernatant was collected and the remainder of corn tassel

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sample was re-extracted for 3 times. The total volume of the supernatant was combined, and adjusted to 10 mL with 2:1 PE:DE (v/v).

2.4 Determination of total phenolic content (TPC)

TPC was measured by Folin–Ciocalteu phenol reagent method [18], and it was slightly modified according to the method described by Hu and Xu [19]. Briefly, 0.5 mL of corn tassel extract described above, 2.5 mL de-ionized water and 0.5 of 1 M Folin-Ciocalteu reagent were mixed with a vortex mixer. The mixture was incubated at room temperature for 8 min. After incubation, 1.5 mL of 7.5% sodium carbonate was added into the mixture and mixed well using a vortex mixer. The reaction was stored at room temperature for 2 h. The absorbance was measured using UV-vis spectrophotometer at 765 nm. Gallic acid (GA) solution (10-100 µg/mL) was used for calibration of a standard curve. The TPC was expressed as milligram GA equivalent per gram dry weight of corn tassel sample (mg GAE/g DW sample) and was converted to total phenolic yield (TPY) per area.

2.5 Determination of total anthocyanin content (TAC)

TAC was measured by the pH differential method [20]. Corn tassel extract was separated into two parts, mixed with pH 1.0 and 4.5 buffer, vortexed and incubated under dark condition for 15 min. After incubation, TAC was measured using UV-vis spectrophotometer at 510 and 700 nm wavelengths. TAC was calculated followed formula;

$$TAC = \frac{A \times MW \times DF \times 1{,}000}{\varepsilon \times 1},$$

where A was the absorbance of the diluted sample, calculated from $A = (A_{510}-A_{700})_{pH1.0} - (A_{510}-A_{700})_{pH4.5}$, MW was molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF was dilution factor, 1,000 was a conversion unit of molar to ppm and the molar absorptivity (ε) of 26,900 $M^{-1}cm^{-1}$. TAC was expressed as microgram cyanidin-3-glucoside equivalent per gram dry weight of corn tassel sample (µg CGE/g DW sample), and total anthocyanin yield (TAY) per area was calculated.

2.6 Determination of total carotenoid content (TCC)

TCC was determined according to the method described previously [21]. The supernatant from extracted solution was used for determination of TCC using UV- vis spectrophotometer at 450 nm wavelength. The TCC was calculated using the following equation;

$$TCC = \frac{Abs \times 10^4 \times V}{A_{1cm}^{1\%} \times W},$$

where *Abs* was absorbance measured at 450 nm, *V* was final volume (mL), 10^4 was conversion factor to obtain concentration in µg, $A_{1cm}^{1\%}$ was absorption coefficient, mean value of 2,500 was used and *W* was sample weight (g). TCC value was expressed as microgram per gram dry weight of corn tassel sample (µg/g DW sample) and total carotenoids yield (TCY) per area was calculated.

2.7 Determination antioxidant capacity

DPPH$^{\bullet}$ radical scavenging assay was analysed by using the original method [22] and Trolox equivalent antioxidant capacity (TEAC) was analysed by using the method described previously [23] with minor modification [19]. In DPPH$^{\bullet}$ assay, 0.5 mL of the extracted sample with appropriate dilution factor was added with 4.5 mL of 60 µM DPPH$^{\bullet}$, which was dissolved in methanol. The sample was mixed well and set aside for 30 min in dark conditions. The absorbance was measured at 517 nm and the solvent was used as a blank. DPPH$^{\bullet}$ radical was calculated by using the following equation;

DPPH$^{\bullet}$ radical scavenging rate (%) = [1-(Abs_{sample}/$Abs_{control}$)] × 100,

where Abs_{sample} was absorbance of the sample extracts and $Abs_{control}$ was absorbance of the blank.

In the TEAC assay, the chemicals were mixed with 7 mmol/L of ABTS and 2.45 mmol/L potassium persulfate for preparation of ABTS$^{\bullet+}$ radical cation. The reaction was mixed well and set aside for 16-24 h in dark conditions at room temperature before analysis, and this

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solution was used within 2 days. Then, the $ABTS^{\bullet+}$ solution was diluted with methanol and the absorbance of 0.700±0.05 nm was determined at 734 nm. All sample extracts were diluted to provide 20-80% inhibition of blank absorbance. 50 μL of diluted sample was mixed with 1.9 mL of diluted $ABTS^{\bullet+}$ solution and mixed well. The final mixture was set aside for 6 min at room temperature. A UV-vis spectrophotometer was used to measure the optical absorbance values at 734 nm. Trolox solution was used as the standard curve. The results were expressed as micromole Trolox equivalent per gram dry weight of corn tassel sample (μmol TE/g DW sample).

2.8 Statistical analysis

Analysis of variance (ANOVA) was performed for each parameter according to a 4×8 factorial experiment with RCBD arrangement of the treatments [24]. The statistical model is; $Y_{ijk} = m + B_i + S_j + V_k + SV_{jk} + e_{ijk}$, where Y_{ijk} was the value of the observational data at the tassel development stage j in variety k and block i, m was the grand mean of the experiment for each parameter, B_i was block effects, S_j was tassel development stage effects, V_k was variety effects, SV_{jk} was the interaction effect between tassel development stage and variety, and e_{ijk} was pooled error effects. Least significant difference (LSD) was used to compare means at $p \leq 0.05$. The results were expressed as mean±standard deviation (SD).

3. Results and Discussion

3.1 Variation in tassel weight, phytochemicals and antioxidant activity

The variations observed among tassel development stages and corn varieties were significant ($p \leq 0.01$) for all parameters (Table S1). In general, variation in varieties contributed to the largest portions of total variations in most traits (51.9-95.0%) except in tassel fresh weight in which tassel development had the largest portion (41.3%). The interactions between development stage (S) and variety (V) were highly significant ($p \leq 0.01$) for TAC, TCC, TAY,

TCY and antioxidant activity determined by TEAC assay. However, the S×V interactions gave very small portions of total variations in these traits.

The results indicated that the effect of variety was the main source of variations in the phytochemicals and antioxidant capacity. The variation in varietal glume and anther colors ranging from light green to purple (Figure 1) was the most important factor determining genetic variability for these phytochemical traits. The pigmentation in plant tissue, especially floral pigments is a source of many phytochemicals e.g. chlorophyll, carotenoids, flavonoids and anthocyanin [25]. The information is important for corn breeders to develop special corn varieties and produce phytochemicals for food product processors and manufacturers to use this by-product to develop the value-added products.

3.2 Effect of development stages on tassel weight and phenolic compound

VT tassel and 1st day of pollen shed had the highest tassel fresh weight of 28.2 and 29.4 g, respectively, whereas 1st day of pollen shed had the highest tassel dry weight of 9.4 g (Figure S1). These results were similar to those in a previous study. Hybrid corn varieties increased tassel dry weight from tassel emergence to the first pollen shed. The tassel weight was highest before pollen shed and, then, it gradually reduced until pollen shed was completed [26]. The tassel weight continued to decrease after pollen shed. The reduction in tassel weight would be possibly due to the loss of pollen grains. However, the quantity of pollen production in corn tassel was depended on tassel structure in different corn varieties such as a number of tassel branches, tassel length, length of anthers, number of spikelets and tassel area index. The number of tassel branches and number of spikelets are important traits of tassel architecture as these traits were correlated with pollen production [27].

Corn varieties were significantly different ($P \leq 0.01$) for tassel fresh weight. Hibrix3 had the highest tassel fresh weight (31.2 g) followed by Sugar75 and KGW1 (29.2 and 28.8 g, respectively) (Table 2). These varieties also had high tassel dry weight. Tassel fresh weight

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and tassel dry weight across development stages had the similar pattern. Sweet corn had the highest tassel weight followed by waxy corn and field corn. Modern field corn hybrids have smaller tassels than did older corn hybrids and the smaller tassels reduce light interception and improve grain production efficiency at a high plant density [28]. In addition, smaller tassels offer less competition with the ear for resources.

Differences among development stages were observed for TPC, ranging from 11.1 to 13.0 mg GAE/g DW sample. The 1st day of pollen shed had the highest TPC of 13.0 mg GAE/g DW sample, followed by VT tassel and 50% pollen shed. Based on yield per area, the TPY ranged from 4.18 to 6.13 kg GAE/ha, showing a similar trend to the TPC (Figure S2). P4546 of field corn had the highest TPC of 15.8 mg GAE/g DW sample followed by Hibrix3 of sweet corn, S6248 and PAC339 of field corn (13.4, 12.9 and 12.8 mg GAE/g DW sample, respectively) (Table 2). On average across four developmental stages, field corn had the highest TPC followed by sweet corn and waxy corn, respectively, whereas sweet corn had the highest TPY. Hibrix3 had the highest TPY of 6.78 kg GAE/ha because this sweet corn had big tassels and high tassel weight.

Modern filed corn breeding has focused on the improvement of adaptability to climate change, drought tolerance, disease resistance and insect resistance. These characters may affect phenolic accumulation in field corn because phenolic acids play a key role as defense compounds in response to environmental stresses such as water stress, low temperature, high solar radiation, pathogenic infection and nutrient deficiency [29].

Plant phenolics are the most widely distributed natural secondary metabolites in plant phyla. Flavonoids are very large sub-groups of the phenolic compounds and play an important role in functional pollen development. The synthetic pathway of plant phenolics is controlled by development and environmental signals [30]. In general, environmental stress is the major factor of plant phenolic accumulation. Oxidative stress and production of relative oxygen

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species are induced by stresses and these factors affect proteins, DNA and lipid of plant cell. The lipid peroxidation has a harmful and damaging effect on cell membrane. Simultaneously, lipid peroxidation also plays a role as a signaling molecule through proline-linked pentose phosphate pathway for biosynthesis of secondary metabolites, and the resulting secondary metabolites are important to protect cell membrane from the oxidative stress [31].

Table 2. Means for tassel fresh weight, tassel dry weight, total phenolic content and total phenolic yield in tassel of eight corn varieties.

Varieties	Tassel weight		Phenolic compound	
	Fresh (g)	Dry (g)	Total phenolic content (mg GAE/g DW sample)	Total phenolic yield (kg GAE/ha)
Field corn				
PAC339	26.5±1.9 c	8.7±0.6 b	12.8±0.7 bc	5.59±0.7 b
P4546	23.4±0.7 d	7.3±0.2 d	15.8±1.1 a	5.80±0.5 b
S6248	24.2±0.9 d	8.2±0.3 bc	12.9±1.4 bc	5.34±0.4 b
mean	24.7	8.1	13.8	5.58
Sweet corn				
Hibrix3	31.2±2.4 a	10.1±0.6 a	13.4±1.2 bc	6.78±1.0 a
Sugar75	29.2±1.7 b	9.5±0.4 a	11.0±0.6 d	5.25±0.1 b
mean	30.2	9.8	12.2	6.01
Waxy corn				
Sweet violet	24.4±0.2 d	7.2±0.1 d	8.4±0.6 f	3.02±0.2 d
Muang tam	24.9±0.9 cd	7.7±0.2 cd	9.7±0.9 e	3.75±0.4 c
KGW1	28.8±0.3 b	9.5±0.5 a	12.2±1.8 c	5.86±0.9 b
mean	26.0	8.2	10.1	4.21

Data are expressed as the mean ± SD of three replicate.

Means with the same letter(s) in the same column are not significantly different ($P \leq 0.05$) determined by LSD.

3.3 Anthocyanin in corn tassel

TAC in corn tassel increased consistently from VT tassel stage to the later stages of flower development. The stage of 75% of pollen shed had the highest TAC of 343.1 μg CGE/g DW sample, followed by 50% of pollen shed and 1st day of pollen shed stages, whereas 1st

day of pollen shed and 50% of pollen shed stages had the highest TAY of 155.0 and 142.6 g CGE/ha, respectively (Figure S2). On average across development stages and varieties, TAC values ranged from 11.1 to 1,864.6 µg CGE/g DW sample. KGW1 had the highest TAC of 1,864.6 µg CGE/g DW sample at 75% of pollen shed followed by 50% of pollen shed, 1st day of pollen shed and VT tassel stages (1,716.6, 1,647.2 and 1,099.3 µg CGE/g DW sample, respectively). TAY was closely related to tassel dry weight, and KGW1 of purple waxy corn was a superior variety for TAY as it maintained the highest TAY at 50% of pollen shed and 1st day of pollen shed stages (898.7 and 873.4 g CGE/ha, respectively) (Table 3).

The aims of this research project were to find suitable times for corn tassel harvest suitable corn types and varieties for production of corn pollen with high anthocyanin in tassel that are appropriate for use as raw material for functional food products. The hypothesis underlying the research project is that anthocyanin level in corn tassels is related to purple color in different corn types, varieties and development stage of corn tassel. In our earlier work in other corn parts, TAC increased from the milky stage to maturity stage in waxy corn kernel and was highest in purplish black-colored genotypes [32]. Similar increase in anthocyanin in purple corn silk was also observed from silking stage to milky stage, but it slightly decreased at maturity stage [1]. Moreover, anthocyanin concentrations in purple corn was affected by severals factors such as genotype, growing location, extraction solvent and determination method. These factors should be considered for production of anthocyanin from corn tassel for industrial use.

Table 3. Means for total anthocyanin content and total anthocyanin yield in tassel of eight corn varieties evaluated at four tassel development stages.

Varieties	Tassel development stages				
	VT tassel	1st day of pollen shed	50% of pollen shed	75% of pollen shed	Mean
Total anthocyanin content (µg CGE/g DW sample)					
Field corn					
PAC339	76.0±4.2 kl	84.2±5.5 j-l	219.3±17.2 fg	208.0±2.5 g	**146.9±4.6 C**
P4546	59.8±6.6 lm	244.6±43.6 f	338.9±46.5 e	325.0±22.9 e	**242.1±26.6.7 B**
S6248	93.9±28.3 jk	132.5±49.5 i	128.1±8.8 i	176.5±4.8 h	**132.7±20.4 C**
mean	76.6	153.8	228.8	236.5	**173.9**
Sweet corn					
Hibrix3	26.4±2.5 no	18.1±6.4 no	17.4±8.1 no	16.7±9.8 no	**19.6±6.7 E**
Sugar75	12.7±6.4 no	39.1±2.9 m-o	23.1±1.4 no	27.4±6.5 no	**25.6±2.1 E**
mean	19.6	28.6	20.3	22.1	**22.6**
Waxy corn					
Sweet violet	11.5±2.5 no	11.1±0.8 o	18.7±2.7 no	17.9±3.6 no	**14.8±2.0 E**
Muang tam	42.3±15.3 mn	80.3±6.7 j-k	127.9±6.5 i	108.9±7.5 ij	**89.9±5.7 D**
KGW1	1,099.3±44.0 d	1,647.2±25.1 c	1,716.6±35.5 b	1,864.6±49.3 a	**1,582.0±38.5 A**
mean	384.4	579.5	621.1	663.8	**562.2**
Total anthocyanin yield (g CGE/ha)					
Field corn					
PAC339	33.6±1.5 h-k	39.5±1.7 h-k	88.6±11.5 d-g	86.6±10.5 d-g	**62.1±4.2 C**
P4546	22.5±1.9 h-k	99.9±21.3 d-f	121.2±8.8 d	105.4±17.2 de	**87.2±11.0 B**
S6248	40.4±10.9 h-k	58.1±23.4 f-j	52.6±6.8 g-j	64.8±5.6 e-h	**54.0±9.0 CD**
mean	32.2	65.8	87.5	85.6	**67.8**
Sweet corn					
Hibrix3	13.3±2.1 jk	10.1±4.3 jk	9.3±4.8 jk	7.2±4.4 k	**10.0±3.9 E**
Sugar75	6.0±2.8 k	20.7±1.8 h-k	11.6±1.8 jk	10.7±2.4 jk	**12.2± 0.5 E**
mean	9.6	15.4	10.4	9.0	**11.1**
Waxy corn					
Sweet violet	3.9±0.7 k	4.5±0.3 k	6.7±1.2 k	6.0±0.7 k	**5.3±0.6 E**
Muang tam	16.6±7.5 i-k	34.5±3.4 h-k	51.6±6.4 g-j	35.9±3.5 h-k	**34.7±2.5 D**
KGW1	506.1±61.1 c	873.4±114.5 a	898.7±50.4 a	733.7±56.8 b	**753.0±48.1 A**
mean	175.5	304.1	319.0	258.6	**264.3**

CGE Cyanidin 3-glucoside equivalent

Data are expressed as the mean ± SD of three replicates.

Means with the same letter(s) in the same column are not significantly different ($P \leq 0.05$) determined by LSD.

3.4 Carotenoids in corn tassel

TCC among development stages ranged from 39.9 to 54.1 μg/g DW sample. The stages of 50% of pollen shed and 75% of pollen shed had the highest TCC of 51.7 and 54.1 μg/g DW sample, respectively, whereas the stage of 50% of pollen shed had the highest TCY of 22.9 g/ha followed by the stage of 75% of pollen shed, 1st day of pollen shed and VT tassel stage (20.3, 19.7 and 17.0 g/ha, respectively) (Figure S2). These results represented that the pollen shedding was affected to tassel weight and lead to decreased carotenoids yield in the late of tassel developmental stage.

Variations in TCC and TAC followed a similar pattern across stages of tassel development and corn varieties. TCC ranged from 26.1 to 88.4 μg/g DW sample. KGW1 had the highest TCC of 88.4 and 85.7 μg/g DW sample at 50% of pollen shed and 75% of pollen shed, respectively. TCY ranged from 8.2 to 46.3 g/ha across stages of tassel development and corn varieties, and KGW1 had the highest TCY of 46.3 g/ha at 50% of pollen shed followed by 1st day of pollen shed (35.8 g/ha) and 75% of pollen shed (33.6 g/ha) (Table 4).

In general, pollen, petals and fruits have high levels of carotenoids. Pollen was an important source of carotenoids in several crops such as calendula, marigold and chamomile [33]. According to [34], beta carotene was the main carotenoid compound in pollen with the contents ranging from 10 to 200 μg/g sample and carotenoids of 17% in pollen was recorded. Total carotenoids in corn tassel in this study were higher than those reported previously in kernels of yellow-orange corn from Nigeria [35], Italy [17] and central Malawi [36]. The results indicated that there is the possibility to use corn pollen as a new source of carotenoids for functional food supplement. Collection of corn flower pollen is more convenient and productive than collection of bee pollen.

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Table 4. Means for total carotenoids content and total carotenoids yield in tassel of eight corn varieties evaluated at four tassel development stages.

Varieties	Tassel development stages				
	VT tassel	1st day of pollen shed	50% of pollen shed	75% of pollen shed	Mean
Total carotenoids content (μg/g DW sample)					
Field corn					
PAC339	32.0±5.8 o-r	31.9±4.2 o-r	49.3±10.6 e-j	50.6±5.7 d-g	**41.0±4.5 D**
P4546	23.7±5.2 s	31.3±8.4 o-s	34.6±8.2 m-q	38.5±5.8 k-m	**32.0±6.6 E**
S6248	33.8±7.4 n-r	29.8±5.5 p-s	36.4±4.6 l-p	41.6±5.9 j-n	**35.4±5.4 E**
mean	29.8	31.0	40.1	43.6	**36.1**
Sweet corn					
Hibrix3	26.8±5.9 q-s	26.1±5.4 rs	43.9±10.0 g-l	41.7±3.4 i-n	**34.6±6.1 E**
Sugar75	47.8±1.8 f-j	46.9±9.9 f-j	46.5±5.5 f-k	56.4±2.7 c-e	**49.4±3.6 C**
mean	37.3	36.5	45.2	49.1	**42.0**
Waxy corn					
Sweet violet	54.3±3.4 c-f	50.3±3.4 e-h	59.5±3.7 bc	61.4±7.8 bc	**56.4±3.3 B**
Muang tam	42.4±6.3 h-m	49.8±6.8 e-i	54.5±2.7 c-f	56.5±5.3 c-e	**50.8±4.6 C**
KGW1	58.5±7.6 cd	66.9±6.3 b	88.4±4.3 a	85.7±8.0 a	**74.9±6.2 A**
mean	51.7	55.7	67.5	67.9	**60.7**
Total carotenoid yield (g/ha)					
Field corn					
PAC339	14.0±1.4 j-m	15.1±2.6 h-l	19.8±4.4 d-i	21.2±4.8 d-g	**17.5±2.3 D**
P4546	8.9±2.0 m	12.7±3.6 k-m	12.5±3.2 lm	12.6±3.1 l-m	**11.7±2.8 E**
S6248	14.6±2.8 i-l	13.0±3.1 k-m	15.0±2.4 h-l	15.2±1.2 h-l	**14.4±2.1 E**
mean	12.5	13.6	15.8	16.3	**14.6**
Sweet corn					
Hibrix3	13.5±3.9 j-m	14.4±3.5 j-l	23.6±7.4 c-e	18.0±2.4 f-k	**17.4±4.3 D**
Sugar75	22.7±2.0 c-f	24.8±4.8 cd	23.2±3.2 c-f	22.0±0.6 c-f	**23.2±2.2 B**
mean	18.1	23.4	23.4	20.0	**20.3**
Waxy corn					
Sweet violet	18.5±2.1 e-j	21.2±1.5 d-h	21.2±1.2 d-g	20.9±3.9 d-g	**20.2±1.1 C**
Muang tam	16.2±2.7 g-l	21.9±3.4 d-g	21.9±1.6 d-f	18.7±2.7 e-j	**19.6±1.8 CD**
KGW1	27.2±6.1 c	35.8±7.7 b	46.3±3.7 a	33.6±2.5 b	**35.7±4.3 A**
mean	20.6	29.8	29.8	24.4	**25.2**

Data are expressed as the mean ± SD of three replicates.

Means with the same letter(s) in the same column are not significantly different ($P \leq 0.05$) determined by LSD.

3.5 Antioxidant capacity in corn tassel

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The results from the DPPH• and TEAC methods provided similar information about antioxidant activity. The pollen harvested at 75% of pollen shed had the lowest antioxidant capacity. Antioxidant capacity values determined by DPPH• method ranged from 75.4 to 81.8% (Figure S3). P4546 was also highest for DPPH• radical scavenging activity (92.4%) followed by PAC339 (87.3%), S6248 (82.0%) and KGW1 (78.3%), respectively (Figure S4).

Field corn had the highest antioxidant capacity measured by DPPH• assay followed by sweet corn and waxy corn, respectively. Antioxidant capacity was closely related to TPC especially for field corn as illustrated by the results for P4546. The results in this study were in agreement with those in a previous report in cobs, husks and silks of corn. TPC was also closely related to antioxidant capacity determined by DPPH• method and $ABTS^{\bullet+}$ method ($r=0.709$ and $r=0.871$, respectively) [3]. The range of DPPH• radical scavenging assay in this study was comparable to those of a previous report using different extraction solvents including ethanol (83.0-85.2%), methanol (69.9-83.7%) and acetone (69.8-80.4%) [11]. Moreover, the values of antioxidant capacity in corn tassel determined by DPPH• method ranging from 54.9 to 78.3% were similar in purple waxy and super sweet corn silk, but they were higher than those in white waxy corn silk [1].

The antioxidant capacity determined by TEAC method ranged from 54.1 to 61.2 μmol TE/g DW (Figure S3). P4546 was highest for TEAC at 1st day of pollen shed, 50% and 75% of pollen shed (79.1, 79.3 and 76.2 μmol TE/g DW sample, respectively), KGW1 was also highest for TEAC at 1st day of pollen shed and 50% of pollen shed (81.3 and 74.9 μmol TE/g DW sample), and PAC339 was highest at 50% of pollen shed (77.2 μmol TE/g DW sample) (Table 5). However, the antioxidant levels obtained from this research were lower than those obtained in purple corn kernel [32] and fresh corn pollen determined by TEAC assay [9].

The results indicated that the most antioxidant activity in tassel existed in pollen. According to [37], the major components in pollen consisted of carbohydrates, crude fibers,

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proteins and lipids and minor components included minerals, trace elements, vitamins, carotenoids, phenolic compounds, flavonoids, sterols, terpenes and amino acids. Although these components have antioxidant activity, phenolics are the most important compounds contributing to antioxidant activity in pollen. Thus, the late tassel developmental stage with the lowest pollen grains had the lowest pollen fresh weigth and pollen dry weight, resulting in the reduction in antioxidant activity.

Table 5. Means for TEAC radical scavenging capacity in tassel of eight corn varieties evaluated at four tassel development stages.

Varieties	TEAC (µmol TE/g DW sample)				
	VT tassel	1st day of pollen shed	50% of pollen shed	75% of pollen shed	Mean
Field corn					
PAC339	61.2±9.9 d-g	62.3±10.5 d-f	77.2±3.9 a-c	59.0±7.3 e-h	**64.9±6.3 C**
P4546	69.8±11.9 b-d	79.1±10.3 ab	79.3±1.8 ab	76.2±4.7 a-c	**76.1±6.0 A**
S6248	64.9±8.3 d-f	68.6±3.9 c-e	57.4±4.7 f-i	58.7±3.9 f-h	**62.4±3.8 C**
mean	65.3	70.0	71.3	64.6	**67.8**
Sweet corn					
Hibrix3	70.3±0.3 b-d	68.8±1.2 cd	55.5±9.3 f-i	59.1±10.8 e-g	**63.4±5.2 C**
Sugar75	52.3±3.6 g-j	44.2±9.4 jk	57.2±7.7 f-i	49.3±0.3 h-j	**50.8±1.8D**
mean	61.3	56.5	56.3	54.2	**57.1**
Waxy corn					
Sweet violet	47.9±10.0 ij	36.8±10.6 k-m	44.3±0.1 jk	34.3±5.4 lm	**40.8±6.4 E**
Muang tam	37.4±10.6 k-m	42.8±9.3 j-l	43.6±2.2 j-l	33.4±4.4 m	**39.3±6.4 E**
KGW1	63.0±7.2 d-f	81.3±7.6 a	74.9±9.1 a-c	62.5±11.1 d-f	**70.4±5.8 B**
mean	49.4	53.6	54.3	43.4	**50.2**

TEAC Trolox equivalent antioxidant capacity, *TE* Trolox equivalent, *DW* Dry weight

Data are expressed as the mean ± SD of three replicates.

Means with the same letter(s) in the same column are not significantly different ($P \leq 0.05$) determined by LSD.

3.6 Implications for value-added development

The natural bioactive compounds play an important role in improving the health of various populations. Fruits and vegetables are well- known souces of these compounds.

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However, some agricultural by-products normally considered as waste have high contents of phytochemicals that are potentially useful for industrial applications. Corn tassel with pollen is a by-product from corn production field and it might be a new source for agro-industry utilizations.

Corn pollen from bee-collected pollen is a super food [9,10]. Corn tassel extract had antioxidant capacity [11] and high ability to inhibit the proliferation of MGC80-3 gastric cancer cells [38]. Tasselin A extracted from sweet corn tassel has a role in inhibiting melanin production and it is used as an ingredient in skin care whitener [39]. Thus, corn tassel has a significant bioactive property and may be used as a raw material to produce many value-added products at commercial scale.

However, more downstream research is required for beter utilization of corn by-products. This includes the reliable and effective extraction methods to recover phytochemicals, the components of phytochemicals and the benefits in treating specific illness. In comparison with vegetables and fruits that have higher contents of phytochemicals than corn by-products, corn by-products should be pracitcal for its lower cost.

The by-products from field corn production are more practical for use as a raw material than the by-products from hybrid seed production as the amount of the by-products is sufficient for commercial scale and it is important to verify the appropriate time for harvest. In this study, the most appropriate times for pollen harvest would be from the 1st day to 50% of pollen shed. Although the corn tassel has lower bioactive content than some plants such as some fruits and vegetables, corn tessel is still interesting because corn is produced in large scale and large amount of corn tassel is available.

Actually, harvesting 50% of tassel and leaving the rest of the tassel in the field does not affect seed setting in corn grain production. Thus, tassel from grain production field is interesting for use as a raw material for production of several value-added products. Corn tassel

can generate additional income to corn growers and also help reduce production cost. As corn waste is reduced, corn production should be more effective and sustainable. However, the further research should focus on the environmental effects, harvest methods and the effects of post-harvest on bioactive properties.

4. Conclusions

Field corn tassels had the highest phenolic content and antioxidant activity. However, the highest phenolic yield was found in sweet corn as this type of corn had larger tassels that produced a larger amount of pollen. The development stage affected the accumulation of anthocyanin and carotenoids in tassel. The appropriate time for extracting anthocyanins and carotenoids in corn should be during the 1st day to 50% of pollen shed stages. KGW1 of purple waxy corn was the best variety for the anthocyanins and carotenoids in corn tassel. Corn tassel is a potential source of natural phenolic, anthocyanin, carotenoid and antioxidant activity, and it can be used as functional food supplement, an ingredient in natural pharmaceuticals and cosmetic products.

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