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Relationships between phytochemicals and antioxidant activity in corn silk

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Abstract

Corn silk (CS) has been used as traditional Chinese herb for centuries. However, the information on phytochemicals properties and CS production in different types of corn genotypes has not been available. The objectives of this study were to evaluate variability for the contents of phenolic compounds, flavonoid and anthocyanin and their antioxidant activities in CS and to identify corn types with high contents of the compounds and antioxidant activities (AA). Ten corn hybrids were evaluated with three replications. The contents of total phenolics (TPC), total flavonoids (TFC), total anthocyanin (TAC) and AA were determined. Variations in the compound contents were observed in different CS varieties. The results indicated that baby corn (BC) had the highest CS yield basis. Purple waxy corn (PWC) had the highest contents of TPC, TFC and TAC. In addition, PWC and BC possessed the highest AA. AA was correlated with TPC and TFC. PWC was the most promising for high all contents and AA. Therefore, PWC and BC are superior for CS production for use in the functional food, nutraceutical industries and for breeding program in the future.

Introduction

Corn silk is named of the long styles and stigmas on flower pistils. The stigmas are fine and soft, yellowish to green or purple threads of female flowers depending on varieties. The corn silks are collected for traditional herbal medicine remedy before the plant is pollinated (Maksimovic *et al.*, 2005). Corn silk has been seen as waste and by product from fresh corn production. However, corn silk has been used in many parts of the world for herb treatment of hypertension, tumor, hyperglycemia, hepatitis, cystitis, gout, kidney stones, diabetes nephritis and prostatitis (Hu and Deng, 2011). Corn silk is an excellent source of many bioactive compounds such as volatile oils, steroids, alkaloids and natural antioxidants such as flavonoids and other phenolic compounds with beneficial effects on human health (Liu *et al.*, 2011) and minerals such as Ca, K, Mg, Mn and Zn are presented in corn silk (Wan *et al.*, 2010a). Wang *et al.* (2011) reported that corn silk consisted of 9.65% moisture, 17.6% protein, 0.29% fat, 3.91% ash and 40% dietary fiber.

Health benefits of corn silk have been reported in many investigations. Corn silk extract could promote insulin production in animals, support the recovery of the injured â-cells of the pancreas (Sepehoi *et al.*, 2011) and control blood sugar level in rats (Yang and Zhai, 2010). Corn silk of some local corn varieties is ground and used as food additive and flavoring in several regions of the world. For example, corn silk powder is used as food additive to improve the content and physical characteristics of beef patties (Wan *et al.*, 2010b).

Currently, products from corn silks such as tea, powder, and cosmetics are commercial in China, Korea, Japan, USA and UK. However, corn silk is still considered as a waste from a corn processing. There are the ample opportunities to convert such a waste into value-added products from corn.

Corn silk is an excellent source of many bioactive compounds, especially for flavonoid compounds (Ren *et al.*, 2009). However, there are few reports on systematic evaluation of phytochemical profiles and antioxidant activity in corn silk and the information on corn silk production in different corn types and varieties is still lacking. Therefore, the objectives of this study were to evaluate variability for the contents of phenolic compounds, flavonoid, anthocyanin and their antioxidant activities in corn silk and to identify corn types with high antioxidant contents and activities. The information of this study would be useful for corn silk production and breeding programs aiming to develop superior corn genotypes

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for functional food products.

Materials and Methods

Plant materials

Ten corn hybrids including five waxy corns. These sweet corns and the baby corn were selected for the present study (Table 1). All corn types were grown in the Vegetable Farm, Khon Kaen University, Khon Kaen, Thailand (18° 51’ N, 98° 45’ E, 200 masl) in the rainy season during May to July 2011. A Completely Randomized Design (CRD) was used in this study. The corn were cultivated and harvested at market maturity for 3 replicates. Corn silk of waxy corn and sweet corn was harvested at 18 - 20 days after flowering (R4 growth stage), but corn silk of baby corn was harvested at 7 - 10 days after flowering (R1 growth stage). The weight of corn silk was recorded to calculate the yield per area ($kg.ha^{-1}$). The drying percentage is the term to indicate the yield of material after drying. Fresh corn silk samples were washed with distilled water, oven-dried at 60°C for 24 hours (Hu *et al.*, 2010) the final moisture content of 10 to 11%, ground into powder using a grinder, vacuum packed and stored below -20°C until analysis. The ground samples were analyzed for bioactives and antioxidant activity in triplicate.

Extraction of corn silk

Corn silk samples were extracted using a modified method of that described by Hu *et al.* (2010). Briefly, 3 g corn silk powders was mixed with 60 mL of 95% ethanol in a flask and extracted at 70°C in a water bath shaker (WB14/SV1422, Gmb HCo. KG Memmert, Shanghai, China). After 1.5 hours, the extract was filtrated through filtered paper Whatman No.1 to remove the debris. The filtrate was evaporated using a rotary flash evaporator (Eyela SB-651, Kokusan Enshinki Co., Tokyo, Japan) to remove the solvent. The residue was reconstituted with 5 mL methanol and stored at 0 - 4°C until analysis.

The extract was spectrophotometrically on a spectrophotometer (10S UV-Vis, Thermo Scientific Genesys, Australia) determined for total phenolic contents (TPC), total flavonoid contents (TFC), total anthocyanin contents (TAC) and antioxidant activity by DPPH free-radical-scavenging activity assay.

Determination of total phenolic contents

The total phenolic content of corn silk extract was determined using the Folin-Ciocalteu colorimetric method and gallic acid was used as a standard (Liu *et al*., 2011). Folin-Ciocalteu reagent was diluted with distilled water at the ratio of 1:10. The 0.5 mL sample corn silk extract was mixed with 3 mL of the diluted Folin-Ciocalteu reagent and 2.5 mL of 0.2% (w/v) Na_2CO_3 solution. The mixture was allowed to stand for 30 min at room temperature (ca. 25°C) and the absorbance of the resulting solution was read at 750 nm using a spectrophotometer. The blank consisted of all reagents and solvents, but without the sample. The total phenolic content was determined using the standard calibration curve and expressed as gallic acid equivalents per dry mass of corn silk sample (µg GAE/g dried sample).

Determination of total flavonoid contents

The total flavonoid contents of corn silk extract was determined using a modified colorimetric aluminum chloride method and rutin hydrate was used as a standard (Liu *et al*., 2011). Briefly, a dilute solution of 0.5 mL corn silk extracts in 2.5 mL methanol was mixed with 3 mL of 0.01 mol/L aluminum chloride in methanol. Then the mixture was allowed to stand for 10 min at room temperature (ca. 25°C). The absorbance of the resulting solution was read at 400 nm using a spectrophotometer. The total flavonoid content was determined using the rutin calibration curve at concentrations from 0 - 0.100 mg/mL in methanol and expressed as rutin hydrate equivalents per dry mass of corn silk sample (µg RE/g dried sample).

Total anthocyanin content determination

Total anthocyanin content was determined according to the pH-differential method (Ku *at al*., 2009). The corn silk extract from each sample made up to 6 mL with pH 1.5 buffers in 1% HCl in methanol extracted of corn silk. After extracting at room temperature (ca. 25°C) for 20 min, each solution was measured absorbance at 530 nm and 700 nm against blanks of pH 1.0 and 4.5 buffers. Total anthocyanin amount was determined by the following equation:

$$\text{Total anthocyanin (mg/L)} = (A \times MW \times 1000 \times DF) / (\varepsilon \times 1)$$

where: A was adjusted absorbance calculated from (A_{530}-A_{700}) at buffer 1.0 - (A_{520}-A_{700}) at buffer 4.5, 1000 was a converting factor from molar to ppm, DF was a dilution factor. For a quantification, external calibration for cyanidin-3-glucoside was the molecular weight (MW) of 449.2 and molar absorptivity of 1% HCl in methanol (ε) was 34,300. The results were expressed as microgram of cyanidin-3-glucoside equivalents per dry mass of corn silk sample (µg C3G/g dried sample).

Antioxidant activity assay

Antioxidant activity of the corn silk extract was measured using the DPPH free radical scavenging activity (Liu *et al*., 2011). Initially, 0.2 mL of corn silk extract was added to 1 mL of 0.2 mM freshly prepared DPPH methanol solution. The reaction mixed and allowed to stand for 30 min in the dark conditions. The control contained all reagents except the extract fraction was used as a blank. Absorbance was read against a blank at 517 nm using a spectrophotometer. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and corn silk extract to determine the ascorbic acid equivalent antioxidant concentration. The percentage of DPPH radical scavenging activity (%) of sample was calculated as follows:

$$\text{DPPH radical scavenging activity (\%Inhibition)} = (1-A_{sample}/A_{control}) \times 100$$

where, A sample is the absorbance of the extract or standard and a control is the absorbance of the control. All tests were run in duplicate and analyses of the samples were run in triplicate and averaged.

Statistical analysis

The data was analyzed of variance (ANOVA) and the mean comparison was determined by least significant difference (LSD) test at $p < 0.05$ using SPSS v19 (SPSS Inc, USA). The correlation between antioxidant compounds and antioxidant activities was determined by Pearson's correlation analysis.

Results and Discussion

The colors of corn silk were different depending on varieties and types of vegetable corn. The visualized colors of corn silk were yellow-white in waxy corn, light brown in sweet corn, purple in purple waxy corn. The corn silks were divided into two groups based on harvesting times for vegetable ears. Group 1 consisting of sweet corn and waxy corn varieties was harvested at market maturity after pollination, and sort kernels are consumed, Group 2 comprising baby corn did not have kernels because the ears are too young to produce kernels and was harvested before pollination and the silks are still pale yellow-green. These two types of corn silks are usually considered as waste product, and this study aim is to find the opportunities to use corn silks as raw material to produce value-added functional food products. Therefore, the samples of corn silk were harvested at market maturity and dried at low heat, and the method for sample treatment was similar to that used for herb processing for phytochemical extraction.

The percentage yields of corn silk and the corn silk yields per hectare presented in both wet and dry basis, including percentage drying, are shown in Table 2. The yields were different from variety to variety. Corn silk yield ranged from 123.8 to 527.0 kg ha^{-1} for fresh weight and 16.0 to 64.0 kg ha^{-1} for dry weight, and BC1 and BC2 were also the best genotypes for these traits with silk fresh yields of 527.0 and 420.1 kg ha^{-1}, respectively, and dry silk yields of 64.0 and 48.5 kg ha^{-1}, respectively. Both genotypes were baby corn. Silk dry weight followed the similar pattern, and BC1 and BC2 were also the best genotypes for silk dry weight.

Drying percentages ranging from 11.5 to 20.9% were observed among corn genotypes (Table 2). PWC1 had the highest drying percentage (20.9%) followed by WC2 (18.2 %), whereas BC1 and BC2 had the lowest drying percentages (12.1% and 11.5%, respectively). Purple waxy corn, white waxy corn and sweet corn had higher drying percentages than those of baby corn.

The corn varieties with high silk yield and high drying percentage are preferable for use as raw materials for functional food products. However, in this study, the varieties with high silk yield did not have high drying percentage. The drying percentage in corn silk production is important because it affects dried matter yield. Low drying percentage incurs high drying cost during processing.

Drying is an important unit operation to stabilize corn silk for the utilization as herbal medicine. The higher dried yield leads to the more economic production. Therefore, the varieties with high drying percentage are preferable. Although baby corn had the highest silk yield but it had low drying percentage. This probably is due to higher moisture content of corn silk. If the cost of drying is not too high depending on drying technologies, baby corn might be a good choice because of its higher silk yield.

Table 2 indicates that the corn silk yields, both fresh and dried form, were various upon genotypes. Wall and Corgen (1999) reported that the fresh to dried weight ratio (FW/DW) related to genotypes and environments. However, maturity of corn also affects the corn silk yield in this study. This information is useful for a selection of corn varieties to exploit the benefits of corn silk for production of functional food products.

The compositions of corn silk are important to determine the health benefits of corn silk. Phenolic compounds are the most important phytochemicals in corn silk which flavonoids and anthocyanins are the most common groups. There were significant differences ($p < 0.05$) in total phenolic contents, total

Table 1. The list of ten Thailand corn silks used in this study

Entry No	Variety	Types of Corn[a]	Kernel color	Origin
1	PWC 1	Purple Waxy Corn	purple	Khon Kaen University
2	PWC 2		purple	Company1
3	WC 1	White Waxy Corn	white	Khon Kaen University
4	WC 2		white	Company1
5	WC 3		white/violet	Company1.
6	SC 1	Sweet Corn	yellow	Company2
7	SC 2		yellow	Company1
8	SC 3[b]		yellow/white	Khon Kaen University
9	BC 1	Baby Corn	yellow	Company3
10	BC 2		yellow	Company1

[a]Vegetable Corn.
[b]Small ear sweet corn.

Table 2. Means of corn silk yield fresh weight and dry weight of ten corn genotypes

Variety	Yield of corn silk (kg ha^{-1})[c]		Drying
	Fresh[a]	Dry[b]	percentage
Purple waxy corn			
PWC 1	153.3	32.0	20.9
PWC 2	187.3	28.4	15.2
White waxy corn			
WC 1	144.8	22.0	15.2
WC 2	196.1	35.8	18.2
WC 3	182.4	27.7	15.2
Sweet corn			
SC 1	160.4	26.7	16.8
SC 2	283.4	44.8	15.8
SC 3	123.8	16.0	12.9
Baby corn			
BC 1	527.0	64.0	12.1
BC 2	420.1	48.5	11.5
Mean	237.9	34.6	15.4
F-test	**	**	**
C.V.%	6.31	9.48	8.32
LSD (0.05)	21.8	4.8	1.9

[a] The yield of corn silk after harvesting in fresh weight.
[b]The yield of corn silk after drying in a hot air oven at 60°C for 24 hours (final moisture content of 10 to 11%).
[c] Plant density: 50,000 plants per hectare.

flavonoid contents, total anthocyanin contents and antioxidant activity among the corn varieties (Figure 1).

Total phenolic contents ranging from 80.8 to 117.1 µg GAE/g of dry samples were observed among corn genotypes (Figure 1a). Total phenolic contents could be roughly divided into three classes: high, intermediate and low. The genotypes with high total phenolic contents (114.2 - 117.1 µg GAE/g) included two purple waxy corns (PWC1, PWC2) one white waxy corn (WC2) and two baby corn (BC1, BC2). The genotypes with intermediate total phenolic contents (102.2 - 105.7 µg GAE/g) consisted of WC1 and WC3 of waxy corns with white and mix kernel colors, respectively, whereas the genotypes with

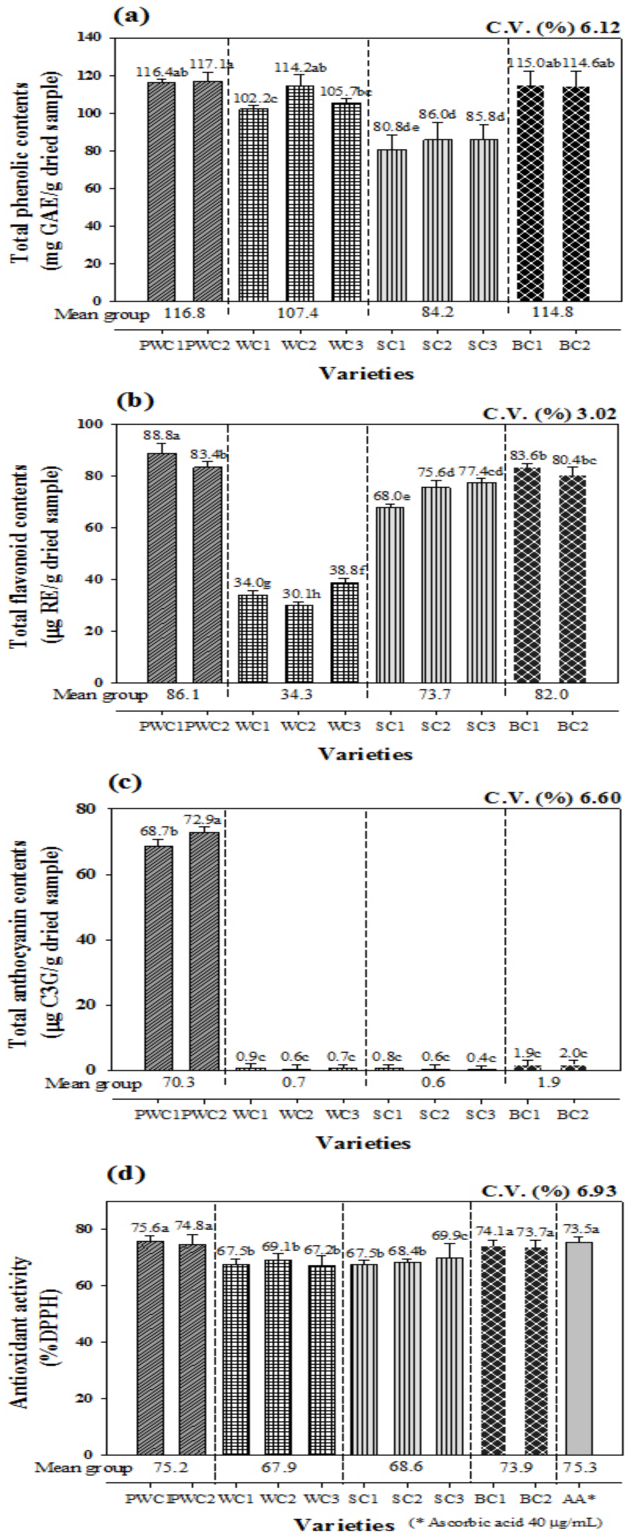

Figure 1. The contents of total phenolic (a), flavonoid (b), anthocyanin (c) and antioxidant activity (d)with different letters within the same column differed significantly ($p < 0.05$) in corn silk of ten corn hybrids

low total phenolic contents (80.8 - 86.0 µg GAE/g) comprised SC1, SC2 and SC3 of sweet corns.

The corn silk of some genotypes contains a high level of phenolics that under enzymatic oxidation to form free radical, which condense among themselves or with proteins to form brown pigments (Sukalovic *et al*., 2010). When total phenolic content alone were considered, then promising corn types would be waxy corn and baby corn.

Corn varieties were significantly different for total flavonoid content, and flavonoid contents ranging from 30.1 to 88.8 µg RE/g of dried samples were observed (Figure 1b). Because the differences in the high group ranging from 68.0 to 88.8 µg RE/g were not clearly separated, the corn genotypes were divided into high and low groups. The high group consisted of purple waxy corns (PWC1 and PWC2), sweet corns (SC1, SC2 and SC3) and baby corns (BC1 and BC2), and the low group ranging from 30.1 to 38.8 µg RE/g included all white and mix color waxy corns (WC1, WC2 and WC3).

Flavonoids are the most common and widely distributed group of phenolic compounds, occurring in corn silk parts. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Based on the results, total flavonoid contents of all fractions from corn silk were in descending orders similar to those of total phenolic contents, and flavonoids are the major phenolic compounds present in corn silk (Maksimovic *et al*., 2005)

Flavonoids were found at the highest concentration in corn silk (Hu *et al.*, 2010). Previous study found that total phenolics and total flavonoids constituted the largest portion of phytochemicals in corn silk (Liu *et al*., 2011). Moreover, upper parts of corn silk had higher total phenolics and total flavonoids than the lower parts (Alam, 2011).

Several fruits, vegetables and drinks such as salad, tomato, bell pepper, strawberry, broad bean, apple, grape, red wine and tea are the main sources of flavonoids (Flavo, 2012). Corn silk contains a medium level of flavonoids (10 - 50 µg/g or µg/ml). However, it is considered as waste product in corn industry, and it is promising for use to produce value-added products. Therefore, this information is very important for use of the alternative natural source of flavonoids form corn silk.

Significant differences among corn varieties were observed for total anthocyanin contents ranging from 0.4 to 72.9 µg C3G/g dried samples (Figure 1c). The corn genotypes could be clearly classified into high group and low group. The high group consisted of purple waxy corns (PWC1, PWC2), ranging from 68.8 to 72.9 µg C3G/g, and the low group consisted of the other genotypes with total anthocyanin contents at trace and low amounts.

Anthocyanins constitute the largest and probably the most important group of water-soluble natural pigments and these compounds are unique among flavonoids as their structures undergo reversible transformation at different pH in aqueous solution (Jian and Monica, 2010). Anthocyanins are the flavonoid compounds that are produced in corn silk colors ranging from pink and to various shades of purple, and some of anthocyanins are colorless.

The silks of corn genotypes with purple color had significantly higher anthocyanin contents than those of white waxy corns, sweet corns and baby corns. Generally, low concentrations of anthocyanins were found in waxy corn and sweet corn genotypes with the yellow color because anthocyanins are a group of well-known water-soluble pigments and found in dark colored corn (black, red or purple) (Kasim *et al*., 2011). The purple silks with darker color had higher total anthocyanin contents than those of white waxy corns, sweet corns and baby corns with lighter color. High concentrations of anthocyanin were also found in the cops (923 µg C3G/g) and kernels (558 µg C3G/g) of purple corn (Yang and Zhai, 2010). However, corn silk is the waste product from vegetable corn industry, and its possibility to be used for production of value-added products is great.

Corn genotypes were significantly different for antioxidant activity (Figure 1d). However, the variation in antioxidant activity was rather low, and DPPH values ranging from 68.4 to 75.6% were observed. Purple waxy corn genotypes (PWC1 and PWC2) had the highest antioxidant activity values of 75.6 and 74.8%, respectively, which were similar to 74.1 and 73.7% of baby corn genotypes (BC1 and BC2, respectively) (Figure 1d). However, the antioxidant activity of white waxy corn was low comparing to others corn silk genotypes. Although there were large differences for total flavonoid content and total anthocyanin content, corn genotypes had similar to antioxidant activity.

Ethanol extract from corn silk exhibited a good reducing power, and the results were comparable to those of vitamin C (Ebrahimzadeh *et al*., 2008) and corn silk was also a good bioactive source of natural antioxidants (Liu *et al*., 2011). However, the corn genotypes with darker pigment generally had higher antioxidant activity than the genotypes with lighter pigment. In previous investigation, the upper parts of corn silk (expose to the air) with greater pigment had higher total antioxidant capacity that the lower parts (not expose to the air) with low pigment (Alam, 2011). These results supported previous findings and also highlighted the importance of pigmentation as a selection criterion for high antioxidant activity in corn silk.

Total phenolic content and total flavonoid content showed good correlation ($r = 0.61$, $P \leq 0.05$) with

Table 3.The correlation coefficients between the contents of functional substances and antioxidant effects by DPPH assay from ten different corn silk extracts

	Total phenolic contents	Total flavonoid contents	Total anthocyanin contents
Total flavonoid contents	0.61*		
Total anthocyanin contents	0.28 ns	0.02 ns	
% Inhibition DPPH assay	0.71**	0.63*	0.40 ns

ns, *, ** = Non-significant and significant at the 5% and 1% probability levels, respectively.

each other, and these characters also had good correlation with antioxidant activity (DPPH) ($r = 0.71$, $P \leq 0.01$ and $r = 0.63$, $P \leq 0.05$, respectively) (Table 3). Although, anthocyanin content showed positive relationship with total phenolic content and antioxidant activity, the correlation coefficients were not significant, whereas anthocyanin content did not showed any relationship with total flavonoid content.

Phenolic compounds have considerable attention for being the main sources of antioxidant activity. The results thus confirmed that total phenolic and total flavonoid contents in corn silk possessed strong antioxidant activity.

The positive and significant correlation between total phenolic content and total flavonoid content indicated that total flavonoid content contributed to a large portion of total phenolic content. The weaker correlation between total flavonoid content and antioxidant activity compared to total phenolic content with antioxidant activity also supported this conclusion.

Generally, corn silk genotypes with high total phenolic compound, total flavonoid and total anthocyanin contents exhibit high antioxidant activity. The high phytochemicals and antioxidant activity correlated with the greater color of corn silk. Previous study found a direct relationship between phytochemicals and the levels of color pigments in fruits and vegetables (Elham *et al.*, 2006). In our study, low and non-significant correlation between anthocyanin content with total phenolic content, total flavonoid content and antioxidant activity did not support previous findings.

The correlation coefficient between anthocyanin content and antioxidant activity was not significant. The results indicated that anthocyanin content had very low contribution to antioxidant activity. In previous study, anthocyanins in purple corn were largely responsible for the antioxidant activities (Yang and Zhai, 2010). The difference between the two studies would be possibly due to materials used.

Total phenolic compound and total flavonoid contents were the best indicator of the antioxidant activity of corn silk. Phenolic compounds have attracted considerable attention for being the main sources of antioxidant activity. In previous studies, the antioxidant activity of corn silk is due primarily to phenolic and flavonoid contents (Ardestani and Yazdanparast, 2007; Ren *et al.*, 2009; Hu and Deng, 2011). Our findings supported previous findings and indicated that phenolic and flavonoid contents are useful indicators for assessing the antioxidant activity of a corn silk.

The DPPH method is a widely used to evaluate the free radical scavenging ability of various samples (Lee *et al.*, 2003). A single method of DPPH was used in this study because of its simplicity and low cost, and the results of a single assay can give only limited information about the antioxidant properties of corn silk extracts, they should be interpreted with some caution. In previous investigation in Momordica charantia fruit, DPPH method was comparable to ferric thiocyanate and thiobarbituric acid methods and the differences in antioxidant activity were mainly due to the reagents used for extraction of phytochemicals (Rezaeizadeh *et al.*, 2011). If the phytochemical compositions related to antioxidant activity in the samples were similar, a single method would be sufficient to provide useful information of antioxidant activity of the samples.

Conclusions

Utilization of corn silk for commercial production of functional food is worth-exploring to convert waste agricultural products into value-added products. The questions underlying the research project were whether there were genetic variations in phytochemicals of corn silk and what types of corn are suitable for use as raw material for production of functional food products. Variations among corn genotypes were observed for total phenolic content, total flavonoid content, total anthocyanin content and antioxidant activity in corn silk. Baby corn had the highest corn silk yields per area basis and purple waxy corn had the highest contents of total phenolics, total flavonoid and total anthocyanin. In addition, purple waxy corns and baby corns possessed the highest antioxidant activities. The antioxidant activity was significantly correlated with total phenolic content and total flavonoid content but was not correlated with total anthocyanin content. For the suitability as a raw material for production of functional food products, purple corn and baby corn would be the best choices, and breeding programs aiming to improve silk yield and silk quality can be carried out with purple waxy corn and baby corn.

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