Crops of Waxy Purple Corn: A Valuable Source of Antioxidative Phytochemicals

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***Abstract*—**Crops of waxy purple corn (Zea mays L. ceritina Kulesh., PC), a hybrid corn variety rich in anthocyanin contents, provide abundant waste byproducts from its cobs (PCC), silks (PCS), husks (PCH), and pollens (PCP), of which phytochemicals and antioxidative activities being determined. Each byproduct was be subjected to extracted by maceration and separation from various solvents. All PC byproducts contained carbohydrates, tannins, flavonoids, and anthocyanins. Thin layer chromatography reported cyanidin-3-glucoside (C3G) as the major anthocyanins of PCC and PCH. Hydro-ethanolic extracts (HE) of PCS gave the highest level of phenolic compound of 357 mg GAE/g, while that of PCH gave the highest anthocyanin contents of 0.11 mg C3G/ g. DPPH, ABTS, and FRAP assays were used to compare all extracts of PC byproducts. HE of PCH and PCS provided the best ABTS values of 328 and 328 mg TEAC/g, respectively, and also the strongest DPPH radical scavenging of 365 mg TEAC/g. Aqueous extracts of PCC gave the highest FRAP value with 800 mg Fe(II)/g. It is concluded that these byproducts of PC could provide high yield of valuable phytochemicals with antioxidative potentials for use in health products.

***Keywords*—**Waxy purple corn cobs, Waxy purple corn silks, Anthocyanins, Antioxidant activities, Phytochemicals.

I. Introduction

ANTHOCYANINS are the natural pigment compounds, which provided red, blue, and purple color in plants and flowers; such as, raspberry, mulberry, grape, and purple corn. They are water-soluble phenolic compounds as known as their chemical structures gives highly potential in ability of free radical scavenging. Their types and antioxidant abilities depend on their chemical structures, especially the numbers of methoxyl (OCH_3) and hydroxyl (OH) substitute groups exist in their molecules [10]. Furthermore, there are many publications reported other biological activities of anthocyanin besides their antioxidant activity; for example, decreasing of blood sugar level, reducing of blood cholesterol level, and vision improvement [6].

Purple corn (*Zea mays* L.) is originated from Andes Mountain, Peru. Mostly it was used as food, and food colorant. Nowadays, purple corn had been developed breeding for survival improvement. It contains rich of anthocyanin and polyphenol contents [11,19]. A waxy purple corn variety (PC) was developed to serve the local customers' preferences, i.e. taste and texture, by the Plant Breeding Research Center for Sustainable, Faculty of Agriculture, Khon Kaen University. It appears to have dark color throughout its cops (PCC), husks (PCH), pollens (PCP) and silks (PCS), which were thought to be agricultural wastes. However, it was thought to be able to turn the wastes into valuable byproducts as the pigments of these byproducts were assumed to be anthocyanins. In addition, some studies were reported that high anthocyanin contents were also found in cobs, silks, husks, and leaves of purple corn [7,12,13]. The anthocyanin constituents in ethanolic purple corn extract were identified by using LC/ESI/MS showed the results that there were found 8 anthocyanins; there are cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin 3-glucoside, cyanidin-3-(6''-malonylglucoside), pelargonidin-3-(6''-malonylglucoside), cyanidin-3-(6''-dimalonylglucoside), peonidin-3-(6''-malonylglucoside), and cyanidin-3-O-(6''-acetylglucoside) and cyanidin-3-glucoside showed the highest amount of total anthocyanin contents [8]. Recently, many researches have been reported biological abilities and therapeutic effects of purple corn extracts. The best potential of biological activity is antioxidant activities were supported by the study of [20] reported that antioxidant determination assays (TEAC, DPPH, and FRAP assays) of anthocyanin purple corn cobs and corn seeds extracts were significantly better than butylated hydroxytoluene (BHT) ($p<0.05$). In the same time, purple corn anthocyanins extract mixed with animal diet showed decreasing of oxidative stress via SOD level increment in sheep's plasma [5]. Next, ethanolic seed extract of purple corn extract can reduce blood glucose level (FPG and OGTT) in db/db mice plasma by directly stimulate insulin secretion (%HbA1C) while it can protect the death of hamster pancreatic beta cell line (HIT- T15) occurring from using glimpiride, diabetes metallitus type 2 medicine in sulfonylurea group [4]. Furthermore cyanidin-3-glucoside and pelargodin-3-glucoside, types of anthocyanin found in PCA, showed inhibition of tumor cell from androgen-dependent cell line (LNCaP) in rat [9].

To enable the use of the under-utilized byproducts of the PC, phytochemical constituents and types of anthocyanin were qualitatively analyzed. The byproducts from the waxy purple corn, i.e. cobs, silks, husk, and pollens were selected as the abundant with potential sources. Evaluation of total phenolic compounds, total anthocyanin contents and antioxidant activities could provide fundamental information for selection for use in health products. If any of the byproduct(s) is found to be rich in valuable phytochemicals, crop harvesting of the PC could have additional values in the future.

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II. Materials and Methods

A. Materials

Byproducts (cobs, silks, husks, and pollens) of waxy purple corn was provided by the Plant Breeding Research Center for Sustainable, Faculty of Agriculture, Khon Kaen University from a harvesting crop in 2014. 2,2'-azino-bis-(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS), 2,4,6 Tris (2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy 2,5,7,8-tetramethylchromane-2 carboxylic acid (Trolox), Cyanidin-3-glucoside (C3G), Peonidin-3-glucoside (Pn3G), and Pelargonidin-3-glucoside (Pg3G) standards were purchased from Sigma-Aldrich (Missouri, USA) Gallic acid was purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent was obtained from ERBApharm (Peypin, France). All chemicals used were analytical grades.

B. Methods

a. Sample preparation

Pulverized PCC, PCH, PCP and PCS were 24-hrs macerated with stirring at 25°C in water (H), ethanol (E), methanol (M), 50% ethanol in water (HE) or 50% methanol in water (HM) at a ratio of 1:25. The filtrate was separated and the residues were repeatedly extracted. All filtrate were rotary evaporated and then freeze-dried to be crude extracts which were light-protected stored at about 4°C [19].

b. Phytochemicals

The liquid extracts of the samples were subjected to these screening for phytochemicals, as follows: Molisch's test for carbohydrates, foaming for saponins, Dragendorff's and Mayer's reagents for alkaloids, ferric chloride reagent for gallic or catecholic tannins, Shinoda's color tests lead acetate for flavonoids and Fehling's reagent for free reducing sugar. Powder samples were treated with 2M NaOH to observe blue-green color for screening of the presence of anthocyanins [1,2,16,17,18].

c. Thin Layer Chromatography (TLC) method

TLC for qualitative determination of anthocyanins was, as follows: the samples on the silica gel TLC plate (1 mm thick, Merck, Germany) were eluted with a mixture of ethyl acetate, glacial acetic acid, formic acid, and water (at a volume ratio of 100:11:11:26) was compared for reference bands of the standards of 1 mg/ml of anthocyanin standards were prepared in methanol [14].

d. Total phenolic compound (TPC)

Total phenolic compound was measured according to modified methods of [3,21]. Briefly, gallic acid standard or sample 15 µl was added to 96-well microplate to mix with 85 µl of Folin-Ciocaluteu reagent which prepared by diluting ten times with water, followed by addition of 7% sodium carbonate 100 µl, then left for 90 minutes in the dark with room temperature. The absorbance was measured at 700 nm by using microplate reader. The results were expressed as mg gallic acid equivalent (GAE)/g of sample. All samples were triplicate tested.

e. Antioxidant activities

DPPH for free radical scavenging ability was conducted by mixing equal volume of trolox or samples with 0.4 mM DPPH in methanol and allowing 30 min reaction in the dark at room temperature. Absorbance of the reactants was recorded at 517 nm using a microplate reader [20]. ABTS assay to determine antiradical activity. The $ABTS^{\bullet+}$ solution was prepared by mixing 7.0 mM ABTS with 4.95 mM potassium persulfate in a volume ratio of 1:1, which was incubated in the dark for 16 h at room temperature. An appropriate proportion of trolox or samples were reacted with the $ABTS^{\bullet+}$ solution in the dark for 30 min and the absorbance at 700 nm was measured using a microplate reader [20]. The percentage of scavenging from DPPH and ABTS was calculated. All of the results were expressed as mg trolox equivalent antioxidant capacity (TEAE)/g by comparing the concentration of the standard (trolox) with the samples at 50% scavenging of free radicals.

FRAP assay to measure reducing ability of Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ complex, using a volume ratio of 10:1:1 of acetate buffer at pH 3.6 : 20 mM Ferric chloride : 10 mM TPTZ/40 mM HCl as the reagent. 10% of ferrous sulfate standard or samples in the reagent was reacted in the dark for 30 min at room temperature and its absorbance recorded at 593 nm using a microplate reader. Triplicates were averaged and expressed as mg Fe (II)/ g sample [20].

f. 2.2.6 Total anthocyanin contents (TAC)

Total anthocyanin contents were determined by using pH-differential method according to [20] with slightly modification. Briefly, the sample 5 mg was mixed with 1 ml of 0.025 M potassium chloride buffer (pH 1.0) and another sample 5 mg was mixed with 1 ml of 0.025 M sodium acetate buffer (pH 4.5). Then, sample solutions were added into 96-well microplate 200 µl. The absorbance was measured wavelength at 510 and 700 nm by using UV-Visible microplate reader. All samples were triplicate tested. The results were expressed as mg C3G/100 g sample unit.

C. Statistical analysis

The data was analyzed by using SPSS version 11.0 (SPSS Inc., USA), which consisted of descriptive analysis and comparative analysis (one-way analysis of variance). Then, Post Hoc test were used to analyze differences between groups. The correlation was also analyzed (Pearson correlation).

III. Results and Discussion

TABLE I
THE PERCENTAGE YIELDS OF EXTRACTS WAXY PURPLE CORN CROPS WITH VARIOUS EXTRACTING SOLVENTS

Extraction Solvent	Total yield (%W/W)			
	Cob	Silk	Husk	Pollen
Water (H)	7.94	14.64	7.45	14.06
Ethanol (E)	3.24	3.35	3.41	4.95
Methanol (M)	6.68	8.68	7.43	19.17
50% ethanol (HE)	10.30	17.20	11.87	15.69
50% methanol (HM)	9.25	15.11	11.30	20.76

Table 1 compares percentage yields of the PC byproducts extracts and suggests that PCP gave the highest yields, especially HE and HM extracts. Table 2 shows the results from qualitative analysis of phytochemical screening, all extracts shows the presence of carbohydrates, tannins, flavonoids, and anthocyanin compounds. In addition TLC used to identify types of anthocyanin in each extracts. TLC results showed R_f values from every extraction solvents of PCC and PCH were consisted of C3G (0.48), Pn3G (0.53), and Pg3G (0.58), excepted aqueous extract of PCH had only C3G as same as HE and HM of PCS extracts.

TABLE II
PHYTOCHEMICAL SCREENING OF COB, SILK, HUSK, AND POLLEN FROM WAXY PURPLE CORN EXTRACTS

Part	Extraction Solvent	Test*								
		Mo[1]	D[2]	Ma	Foam	NaOH	$FeCl_3$	Feh	Shi	Pb
Cob (PCC)	H	+	-	-	-	+	++	-	+	+
	E	++	-	-	-	+	+++	-	++	++
	M	++	-	-	-	+	+++	-	++	++
	HE	++	-	-	-	+	+++	-	+++	+++
	HM	++	-	-	-	+	+++	-	+++	+++
Silk (PCS)	H	+	-	-	-	+	+++	-	+++	+++
	E	+	-	-	-	+	+	-	+	+
	M	+	-	-	-	+	+++	-	++	+
	HE	+	-	-	-	+	+++	-	+++	+++
	HM	+	-	-	-	+	+++	-	+++	+++
Husk (PCH)	H	+	-	-	-	+	+++	-	++	++
	E	+	-	-	-	+	+	-	+	+
	M	++	-	-	-	+	+++	-	++	++
	HE	+	-	-	-	+	+++	-	+++	+++
	HM	++	-	-	-	+	+++	-	+++	+++
Pollen (PCP)	H	+	-	-	-	+	+	-	+	+
	E	++	-	-	-	+	+	-	-	+
	M	++	-	-	-	+	+	-	-	+
	HE	++	-	-	-	+	++	-	++	++
	HM	++	-	-	-	+	++	-	++	++

*Molisch's test (Mo), Dragendorff's test (D), Mayer's test (M), Foam test (Foam), NaOH test (NaOH), $FeCl_3$ test ($FeCl_3$), Fehling's test (Feh), Shinoda's test (Shi), Lead acetate test (Pb)

TABLE III
DPPH, ABTS, FRAP, TPC, AND TAC VALUES OF EACH EXTRACT

Part	Extraction Solvent	Assay														
		DPPH (mg TEAC/g)			ABTS (mg TEAC/g)			FRAP (mg Fe(II)/g)			TPC (mg GAE/g)			TAC (mg C3G/100 g)		
Cob (PCC)	H	174.22	±	0.58^{a}	106.82	±	0.13^{a}	800.38	±	10.28^{a}	141.14	±	3.98^{a}	1.24	±	0.01^{a}
	E	140.41	±	0.20^{b}	97.45	±	0.84^{b}	304.07	±	4.49^{b}	160.30	±	2.20^{b}	5.01	±	0.03^{abcd}
	M	175.04	±	0.68^{a}	140.21	±	0.38^{c}	465.94	±	3.12^{c}	179.37	±	2.18^{c}	5.68	±	0.00^{b}
	HE	256.60	±	1.42^{c}	237.50	±	0.21^{d}	595.11	±	1.31^{d}	266.88	±	2.50^{d}	7.46	±	0.05^{c}
	HM	272.86	±	0.86^{d}	241.07	±	0.83^{e}	475.52	±	2.66^{c}	232.41	±	1.35^{e}	5.99	±	0.03^{d}
Silk (PCS)	H	163.55	±	1.00^{a}	124.05	±	0.49^{a}	590.4	±	3.05^{a}	253.86	±	3.51^{a}	1.74	±	0.05^{a}
	E	63.75	±	0.35^{b}	101.79	±	1.06^{b}	185.82	±	0.79^{b}	116.79	±	2.77^{b}	1.18	±	0.01^{b}
	M	124.50	±	0.21^{c}	136.42	±	0.74^{c}	275.08	±	0.59^{c}	120.80	±	2.48^{b}	4.29	±	0.04^{c}
	HE	364.45	±	1.86^{d}	328.13	±	2.28^{d}	660.50	±	1.96^{d}	356.96	±	10.84^{c}	5.17	±	0.03^{d}
	HM	312.81	±	2.86^{e}	280.63	±	1.60^{e}	626.34	±	2.30^{e}	309.66	±	7.25^{d}	5.16	±	0.05^{d}
Husk (PCH)	H	341.14	±	1.81^{a}	114.02	±	0.59^{a}	603.31	±	2.34^{a}	210.98	±	1.49^{a}	5.91	±	0.10^{a}
	E	124.97	±	0.49^{b}	111.53	±	0.56^{a}	332.97	±	1.59^{b}	121.28	±	1.57^{b}	6.57	±	0.03^{b}
	M	164.72	±	1.46^{c}	202.82	±	2.78^{b}	485.00	±	1.47^{c}	175.68	±	1.77^{c}	8.35	±	0.01^{c}
	HE	289.35	±	2.03^{d}	328.41	±	1.91^{c}	711.78	±	2.12^{d}	257.37	±	5.65^{d}	10.97	±	0.18^{d}
	HM	261.83	±	1.85^{e}	317.38	±	2.23^{d}	670.90	±	3.71^{e}	303.13	±	4.15^{e}	9.00	±	0.08^{e}
Pollen (PCP)	H	81.05	±	0.73^{a}	97.17	±	1.66^{a}	81.48	±	1.84^{a}	132.36	±	0.38^{a}	0.11	±	0.00^{abc}
	E	144.35	±	1.50^{b}	57.93	±	0.25^{b}	96.65	±	1.32^{b}	145.55	±	0.73^{b}	0.00	±	0.00^{b}
	M	96.25	±	0.40^{c}	88.47	±	2.33^{c}	107.11	±	0.18^{c}	152.71	±	3.56^{b}	0.10	±	0.01^{c}
	HE	48.46	±	0.17^{d}	63.12	±	0.61^{d}	133.33	±	1.08^{d}	197.69	±	3.06^{c}	0.50	±	0.02^{abcd}
	HM	68.18	±	1.40^{e}	67.79	±	1.35^{e}	140.11	±	0.54^{e}	254.62	±	6.22^{d}	1.08	±	0.03^{d}

Values are mean (n=3). Values followed by the same letter in the same column (of each part) are not significantly different ($p<0.05$).

TABLE IV
CORRELATION COEFFICIENTS FOR LINEAR RELATIONSHIPS BETWEEN TOTAL PHENOLIC COMPOUNDS (TPC), TOTAL ANTHOCYANIN CONTENTS (TAC), AND ANTIOXIDANT ACTIVITIES (DPPH, ABTS, AND FRAP ASSAYS) ($P<0.5$)

Tests	Correlation coefficient (r) (n=3)			
	ABTS	FRAP	TAC	TPC
DPPH	0.802*	0.815*	0.660*	0.665*
ABTS		0.794*	0.841*	0.605*
FRAP			0.713*	0.579*
TAC				0.442*

*Correlation is significant at the 0.01 level (2-tailed.)

From Table 3, total phenolic compounds (TPC) and total anthocyanin contents (TAC) of the PC byproducts showed that HE and HM extracts contained higher TPC and TAC than other solvents. PCS extracted with HE showed the highest TPC value was 356.96±10.84 mg GAE/g sample. While PCH in HE gave TAC of 10.97±0.18 mg C3G/100 g, which is in line with previous report [7]. The correlation coefficient between TAC and TPC was equal to 0.442, which indicated that naturally anthocyanins were not only phenolic compounds found in PC. The other phenolic compound influenced to increasing of TPC value while TAC value tends to be constant.

Antioxidant activities by DPPH, ABTS, FRAP assays were found that PCP presented the lowest capacity to eliminate free radical in every assays. Various part and extracted solvent of PC also effect to antioxidant ability of each samples. DPPH and ABTS assays provided similar results that HE and HM of all PC parts informed significantly higher free radical scavenging capability ($p<0.05$) compared with other extracted solvents. As well as FRAP assay, The PC extracts were extracted from H, HE, and HM gave satisfy antioxidant activities. HE extracts of PCH and PCS provided the highest free radical scavenging rates in ABTS assay of 328.41±1.91 and 328.13±2.28 mg TEAC/g sample, respectively, also, PCS HE extract showed the strongest DPPH radical scavenging significantly ($p<0.05$). While FRAP assay, the aqueous PCC extract gave the best potential of ferric ion reducing rate was equal to 800.38±10.28 mg Fe(II)/g sample. The correlation coefficients between antioxidant assays, shown in Table 4, confirmed that the byproducts of PC gave antioxidative phytochemicals

IV. CONCLUSION

All byproducts extractable from the waxy purple corn were rich resources of anthocyanins, particularly HE and HM extracts due to semi-polar characteristics of the compounds and the solvents. High potential of free radical scavenging abilities in DPPH, ABTS, and FRAP assays were correlated with the phytochemicals and TAC and TPC. PCC and PCH showed the presence of three major anthocyanin constituents, including C3G, Pn3G, and Pg3G. Considering the parts of PCS, PCH is the highest interested part because it gave results satisfactorily followed by these reasons; plenty of raw material can be provided, high yield, rich of total phenolic and anthocyanin contents, and strong antioxidant capacity in all assays. Thus, these byproducts are potential for health product development in the future and should provide value adding to the agricultural waste products.

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