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Antioxidant Properties and Color Stability of Anthocyanin Purified Extracts from Thai Waxy Purple Corn Cob

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Abstract The extraction, purification, and identification of extracted anthocyanins from Thai waxy purple corn cob were determined. Anthocyanins and total phenol content were prepared by 0.01% HCl- water and 0.01% HCl-ethanol extractions. The ratios of 1:20 and 1:100 (solid: solvent) for 90 mins were the proper extraction conditions to obtain the maximum amount of anthocyanin extracts and the optimum amount of total phenol content as extracted by acidified water and acidified ethanol, respectively. The antioxidant activities of the anthocyanin extract measured by DPPH radical-scavenging activities and ferric reducing antioxidant powers (FRAP) were in a range of 6.51 mM ascorbic acid /100g-6.29 mM ascorbic acid /100g, and 5.34 mM ascorbic acid /100g-4.68 mM ascorbic acid /100g, respectively. A profile of the anthocyanin extracts identified by LC-MS was composed of 7 major anthocyanins; (1) cyanidin-3-glucoside, (2) pelargonidin-3-glucoside, (3) peonidin-3-glucoside, (4) cyanidin-3-(6-malonylglucoside), (5) pelargonidin-3-(6-malonylglucoside), (6) peonidin-3-(6-malonylglucoside) and (7) cyanidin-3-(6-ethylmalonylglucoside). The color of the anthocyanin extracts was dependent upon pH which gradually changed from dark red to dark brown when pH increased from 1 to 9. An increase in the degradation rate constant (k) with a corresponding decline in the $t_{1/2}$ values was observed with the increasing temperature at pH 1 and 4. Due to the effect of pH and temperature on the stability of the anthocyanin extract from Thai waxy purple corn cob with the consequence of color changes, it is preferable to apply anthocyanin extract in acidic foods at low temperature to assure stability of color in the products.

Keywords: *anthocyanins, antioxidant properties, color stability, Thai waxy corn cob*

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1. Introduction

Synthetic color substance dyes are commonly used to make food products attractive to consumers. However, the producers must be aware of regulations and toxicity due to the considerable increase in consumers concerned with the health and safety of foods. As a consequence of social trends toward the consumption of natural products instead of synthetic ones, natural color substances have received increasing attention in the food system. At present, the growth rate of using natural color in the food industry increases 4.5% per year. As natural pigments, anthocyanins are responsible for the orange, red and blue color of flowers, fruits and vegetables. Anthocyanin extracts in powder and liquid forms are expected to be alternative choices for synthetic color substances. For example, the addition of anthocyanins extracted from beet root in milk and yogurt product has already gained popularity [8,10].

Anthocyanins are water-soluble phytochemicals. They belong to the group of flavonoids, polyphenolic molecules containing 15 carbon chains. Anthocyanins occur mainly as glycosides of anthocyanidins. Six anthocyanidins are widespread in fruits and vegetables: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. They can be found in tissues of plants, including leaves, stems, roots, flowers, and fruit. Due to their potential dietary antioxidant abilities, many researchers have studied and identified the properties of anthocyanins extracted from various plants, such as black rice (*Oryza sativa* L.) [17], purple-fleshed sweet potato [5,6], maize kernel [9], fermented purple sweet potato [4], and *Smilax aspera* L. berries [4].

In Thailand, purple waxy corn is commercially cultivated from the purple corn variety with the waxy corn variety (Zea mays ceratina) for the purpose of consumption. The mature ear of the hybrid is large, having deep purple color kernels. Thai waxy purple corn has been known as an important source of anthocyanins with peculiar features regarding natural coloring and possibly providing health benefits. Purple corn cobs should not be considered as waste products since a trace of deep purple on the cob is still noticeable. Therefore, Thai waxy purple corn cobs may be an economical alternative for natural colorants to substitute for synthetic dyes in food products. Unfortunately, there is limited information focused on anthocyanin composition and antioxidant activity of Thai waxy purple corn. The objective of this work was to study

the proper extraction methods and identify the pigment composition of a Thai variety of purple waxy corn cob. Furthermore, the antioxidant properties and thermal stability of extracted anthocyanins from corn cob in aqueous solutions with various pH values were determined.

2. Materials and Method

2.1. Sample Preparation

Purple corn cob (CNW 1127280) obtained from Chainat Research Center Station was finely ground using a lab-scale mill and dried to 12% moisture content. Dried samples were passed through the screen sieve (850 mesh), and then kept in the aluminum foil and stored at 4°C.

2.2. The Selection of Optimum Extraction Condition

The ground samples were combined with 25 ml of two different extraction solvents; 0.01% (v/v) HCl-acidified water and 0.01% (v/v) HCl-acidified ethanol. The extraction was carried out at room temperature with constant shaking at 100 rpm for 1 hour. The extract was filtered through Whatman No.1 paper, and the filter residue was re-extracted until the extract was colorless. Filtrates were combined and kept for anthocyanins content and total phenol analysis. The appropriate extraction solvent was selected according to the highest amount of anthocyanin and total phenol content obtained. Similar to the method of selecting a proper extraction solvent, the extraction ratios of 1:10, 1:15 and 1:20 (sample: solvent) and extraction time of 30 mins-2 hours were studied.

2.3. Purification of Anthocyanins

Ground corn cob (50 g) was extracted using the optimum conditions from previous study. After extraction, the sample was filtered through Whatman No.1 paper, and then evaporated by a rotary evaporator at 40°C under vacuum conditions. The concentrated sample was loaded onto an open chromatographic column C-18 chains bonded on silica. Elution was performed using three solutions with specific properties geared to proper anthocyanin purification. The sample was initially eluted with 0.01% HCL acidified distilled water to eliminate organic acid and sugar compounds, followed by ethyl acetate to exclude phenol compounds and finally by acidified ethanol (Ethanol:1% w/v citric acid, pH 2.9). The purified anthocyanin fractions were collected for further analysis

2.4. Analytical Procedure

2.4.1. Estimation of Purple Corn Cob Anthocyanins

The concentration of purple corn cob anthocyanins was directly determined by pH differential [14]. Two volumetric flasks (10 ml) were added with 2 ml of the extracts obtained from the previous extraction process. The first volumetric flask was added with 0.025 M potassium chloride buffer (pH1.0) and the other was added with 0.4 M sodium acetate (pH 4.5) and allowed to stand at room temperature for 15 mins. The absorbance was read at 510 nm and 700 nm by a spectrophotometer. Distilled water was used as the blank. Total anthocyanin content was expressed as follows:

$$\text{Total anthocyanin content } (\text{mg}/100\text{g}) = \frac{A}{eL} \times M_w \times DF \times \frac{V}{M} \times 100$$

A= $(A\lambda_{510} - A\lambda_{700})_{pH1.0} - (A\lambda_{510} - A\lambda_{700})_{pH4.5}$
DF= dilution factor (ex. The extract of 2 mL with buffer solution of 10 mL. DF =5)
e = molar absorptivity = 26900 (cyanidin-3-glucoside)
L= 1
M_w = 449.2 (cyanidin-3-glucoside)
M = dry weight (mg)
V = volume (ml).

2.4.2. Total Phenol Content

Total phenol content was analyzed by using the Folin-Ciocalteau (FC) reagent [12]. The extracts from the previous extraction process (1 ml) were diluted with 10 ml distilled water. FC reagent of 1 ml was added and the mixture allowed to stand at room temperature for 5 mins. Then 8 ml of Na_2CO_3 was added and the mixture was allowed to stand for 90 mins, after which absorbance readings at 765 nm were taken by a spectrophotometer (model 1000). Distilled water was used as the blank. Total phenol contentwas expressed as mg gallic acid /100 g.

2.4.3. HPLC-MS Analysis of Anthocyanins

For structure identification, samples were subjected to mass spectrometer (MS, Dionex Utimate 3000). Chromatographic separation was performed on a C-18 column. The purified anthocyanins were added with 0.5% HCL, and then syringe filtered (0.45µm). The injection volume was 10 µL. The flow rate was 0.8 mL/min and maintained at 35°C. The mobile phases consisted of 0.05% (v/v) trifluoroacetic acid (TFA, solvent A) in distilled water and 100% Acetonitrile (HPLC grade, solvent B). The gradient elution program was performed as follows: solvent A at 95-80% from 0 to 20 mins, at 80-60% from 20 to 50 mins. The chromatogram was then compared with the standard chromatogram using cyanidin-3-3glucoside.

2.5. Antioxidant Activity

2.5.1. DPPH Assay

The purified anthocyanin samples were added with ethanol solutions at different concentrations of 25µg/ml to 500 µg/ml. 0.5 mL of various concentrations of anthocyanin solution were mixed with 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution in ethanol. The mixture was left in the dark at room temperature for 30 mins, and then the decrease in absorbance at 517 nm was measured. Controls contained ethanol instead of the antioxidant solution while blanks contained ethanol instead of DPPH. The inhibition of DPPH radicals by the samples was calculated according to the following equation [15]:

$$\text{DPPH} - \text{Scavenging Activity } (\%) = [1 - \left(\frac{A - B}{A_0}\right)] \times 100$$

A= the absorbance in the presence of the test compound at different concentrations
B= the absorbance of blank
A_o= the absorbance of control.

2.5.2. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant powder (FRAP) reagent contained 300 mM acetate buffer (pH 3.6), 10 mM/l 2, 4, 6-tripyridy-s-triazine (TPTZ) solution, and 20 mM $FeCl_3$ $6H_2O$ at the ratio of 10:1:1, respectively, and was freshly prepared and warmed to 37°C when used. The 100 µL purified anthocyanin was added with 3-ml FRAP reagent and 300 µl distilled water. The sample was incubated at 37°C for 40 minutes. The absorbance was measured at 593 nm using a UV-visible spectrophotometer. In the blank, FRAP solution was used instead of the sample. The 1 mM/l $FeSO_4$ was used as the standard solution at the different concentrations of 100 µM to 1000 µM. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM/l $FeSO_4$. The change in absorbance between final reading selected and the blank reading was calculated for each sample and related to absorbance of a Fe standard solution [16].

$$\text{FRAP value}\ (\text{mM}/\text{l}) = \frac{0-4\,\text{min}\ \Delta A593\ \text{of test sample}}{0-4\,\text{min}\ \Delta A593\ \text{of standard}} \times \left[\text{Fe}^{2+}\right]\text{standard}\left(\text{mM}/\text{l}\right).$$

2.6. Stability of Anthocyanins

2.6.1. pH-Stability

The purified anthocyanin solution was adjusted to pH 1 and 4 by 0.1M HCL and pH 7.0 by 0.1 M NaOH at 25°C. The absorbance of all samples was measured at 400 nm to 700 nm. The assay was based on the color absorbance within 3 minutes.

2.6.2. Temperature- Stability

The 5 mL purified anthocyanin (100 µg/ml) at the stable pH as determined previously was incubated in a water bath at the controlled temperatures of 20°C, 40°C, 60°C and 80°C. Samples were periodically collected during 1 h – 8 h. Each sample was cooled in an ice bath prior to absorbance measurement for determining the anthocyanin degradation.

$$\text{Anthocyanin}\ (\%\ \text{remaining}) = \frac{A_1\ or\ A_2\ or\ A_3}{A_{0h}} \times 100$$

A_{oh}= the absorbance at time 0 h
A_1, A_2 or A_3 = the absorbance at time 1 h, 1.5 h or 2 h at different temperatures.

2.7. Statistical Analysis

All experiments were carried out in three replications and presented as mean ± standard deviation (SD). The data were statistically analyzed by one-way ANOVA, and mean comparison by DMRT. The level of statically significance was determined at $p<0.05$

3. Results and Discussion

3.1. Determine the Total Anthocyanins and Total Phenol in Corn Cob

Extraction of anthocyanins and phenol in the corn cob samples with 0.1% HCl-acidified methanol was conducted 32 times or until the clear solution was obtained. The total amount of anthocyanins and phenol were 1219.4 mg/100 g and 1840.6 mg/100 g, respectively.

3.2. Determine the Optimum Extraction Condition of Corn Cob Crude Extract

3.2.1. Anthocyanins

The range of anthocyanins was determined as 720 mg/100g - 1004 mg/100 g by using 0.01% HCl-acidified water extraction 3 times. At the ratio of 1:20 for 90 minutes, the highest amount of anthocyanins was obtained (1004 mg /100 g), yielding of 82%. Results were similar to the anthocyanins *of Hibiscud sabdariffa* extracted by water at the ratio of 1:25 (solid:solvent), giving a yield of 88% [2]. The % yield recovery of the first extraction using acidified water was 34%-50% while it dramatically decreased to 9%-12%, and 5%-11% at the second and third extractions, respectively (Table 1).

Table 1. Anthocyanins Extracted by 0.01%-HCl-Acidified Water (3 times) at Different Ratios and Extraction Times

The ratio	Time (min)	Amount of anthocyanins (mg/100mg)			
		1st extraction	2nd extraction	3rd extraction	Total amount
1:20	30	577.60±5.12	210.86±4.58	68.07±2.88	**856.53±12.58d**
	60	560.71±12.2	264.70±2.35	108.56±9.96	**933.97±24.51e**
	90	615.85±6.33	273.80±2.22	114.57±1.82	**1004.22±10.37f**
1:50	30	530.89 ±4.40	124.33 ±4.66	64.94 ±2.85	**720.16 ±11.91a**
	60	546.34 ±4.81	117.02 ±2.95	68.30 ±2.25	**731.66 ±10.01ab**
	90	530.42 ±1.73	127.91 ±1.99	95.82 ±0.78	**754.15 ±4.50b**
1:100	30	546.20±3.26	148.08 ±3.95	94.10 ±1.44	**788.43 ±8.65c**
	60	449.14 ±2.15	167.90 ±5.63	114.16 ±3.26	**731.20 ±11.04ab**
	90	**416.23 ±4.52**	**177.56 ±5.06**	**141.79 ±1.77**	**735.58 ±11.35ab**

Values with the same letters in the column are not significantly different $p<0.05$.

When using 0.01%-HCl-ethanol as solvent for extraction 3 times, the range of anthocyanins was 421 mg/100g-636 mg/100g. At the ratio of 1:100 for 90 mins, the highest amount of anthocyanins was obtained (636 mg/100g), yielding of 52%. It was noticed that when extraction time increased, the extracted amount of

anthocyanins increased at all determined ratios. Similarly, the recovery of % yield increased when the ratio of corn cob and solvent increased.Results were in agreement with those of Todaro *et al*., [13] who investigated the extraction optimization using different solvent, acid concentration, temperature, time of extraction and solvent–to–solid ratios as independent variables for recovery of anthocyanin from eggplant peel. They found that increasing extraction time from 90 mins to100 mins with the optimum ratio of solvent-to-solid (1:20-1:80) the highest amount of anthocyanins was obtained

At the first extraction time using acidified ethanol as solvent, the recovery of % yield was 15%-38% and then reduced to 7%-14%, and 3%-8% at the second and third extraction times, respectively (Table 2).

Extraction of anthocyanins is commonly conducted with water, ethanol, methanol or acetone containing a small amount of acid to obtain the flavylium cation form which is stable in highly acid media. However, it is preferable to use solvents like water and ethanol which are less toxic, particularly in food, rather than methanol and acetone. According to the amount of anthocyanins obtained from different conditions and different acidified solvents, we selected the ratio of 1:20 for 90 mins extraction time, and the ratio of 1:100 for 90 mins for acidified water and acidified ethanol, respectively to recovery of the highest amount of anthocyanins for further experiments (Table 1 and Table 2).

Table 2. Anthocyanins Extracted by 0.01%-HCl-Acidified Ethanol (3 times) at Different Ratios and Extraction Times

The ratio	Time(min)	Amount of anthocyanins (mg/100mg)			
		1st extraction	2nd extraction	3rd extraction	Total amount
1:20	30	188.09 ±2.78	137.52 ±0.82	96.34 ±1.86	**421.95 ±5.46a**
	60	195.93 ±1.17	151.43 ±0.71	101.00 ±3.89	**448.36 ±5.77b**
	90	204.57 ±1.11	173.65 ±3.68	96.18 ±3.20	**474.40 ±7.99c**
1:50	30	293.19 ±3.45	114.53 ±4.44	53.52 ±3.13	**461.24 ±11.02c**
	60	342.22±2.75	131.96 ±3.82	62.74 ±2.63	**536.92 ±9.20d**
	90	396.15 ±3.34	125.55 ±2.59	63.05 ±0.57	**584.75 ±6.50d**
1:100	30	352.09 ±4.61	119.02 ±3.11	57.71 ±2.32	**528.82 ±10.04d**
	60	383.76 ±4.49	95.83 ±2.09	46.68 ±3.65	**526.27 ±10.23d**
	90	**467.52 ±5.20**	**106.04 ±2.49**	**62.56 ±2.76**	**636.12 ±10.45e**

Values with the same letters in the column are not significantly different p<0.05.

3.2.2. Phenolic Compound

The range of phenolic compounds in corn cob as extracted by acidified water was 999 mg/100 g-1452 mg/100 g. At the solvent to solid ratio of 1:100 for 30 mins, the highest % yield of phenolic compound (79%) was obtained in the first extraction. The % yield was reduced to the range of 54%-29% and 21%-13%, for the second and third extraction, respectively. Nevertheless, at this condition, the recovery of anthocyanin content was rather low compared to the ratio of 1:20. Due to the high polarity of acidified water, it was possible that other phenolic compounds were extracted into the solvent.

When using HCl-acidified ethanol, the range of the phenolic compound was 553 mg/100 g - 872 mg/100g. The highest amount of phenolic compound was obtained when using acid ethanol at the ratio of 1:100 for 90 mins (47% yield). The % yield increased according to the high ratio and extraction time. The first extraction gave the % yield of 32%-12%, and the % yield decreased at the second and third extraction which were 11%-8% and 7%-4%, respectively. Due to the high selectivity of ethanol solvent compared to water, the solid-to-solvent ratio of 1:100 for 90 mins extraction time was the optimum condition to recover of the highest amount of anthocyanins and total phenolic compounds from anthocyanin contents (Table 3 and Table 4).

Table 3. Total Phenolic Contents of Anthocyanins Extracted from Corn Cob with HCl-Acidified Water (3 Times) at Different Ratios and Extraction times

The ratio	Time (min)	Amount of total phenol content (mg/100mg)			
		1st extraction	2nd extraction	3rd extraction	Total amount
1:20	30	543.54± 0.30	321.03±5.04	135.07±4.49	**999.63±9.84a**
	60	538.07±4.64	392.95±1.93	181.10±2.24	**1112.12±8.81b**
	90	551.70±5.06	379.76±3.97	182.57±3.53	**1114.03±12.56b**
1:50	30	974.30±5.96	262.79±2.10	132.95±2.95	**1370.04±11.01d**
	60	916.22±5.41	248.33±3.13	130.72±4.36	**1295.27±12.90c**
	90	933.28±3.11	276.70±2.31	169.55±1.67	**1379.53±7.10d**
1:100	30	1006.33±4.59	292.16±4.51	153.69±3.88	**1452.18±12.96f**
	60	881.02±5.27	304.22±3.51	192.68±3.59	**1377.92±12.37d**
	90	**905.76±5.71**	**306.08±3.06**	**221.47±4.66**	**1433.31±13.42e**

Values with the same letters in the column are not significantly different p<0.05.

According to the amount of anthocyanins and total phenolic content obtained from such conditions, and different acidified solvents, we then selected the ratio of 1:20 for 90 mins extraction time and the ratio of 1:100 for 90 mins for acidified water and acidified ethanol, respectively to recovery of the highest amount of anthocyanins and optimum amount of total phenolic contents for further experiments.

3.3. Determination of Cyanidin-3-Glucoside Content

The cyanidin-3-glucoside content of purified anthocyanin extracts from corn cob was determined by HPLC. The chromatograms obtained at 520 nm at the retention time 10.756 mins and 10.649 mins were shown to be the peak of cyanidin-3-glucoside as extracted by acidified water (1:20) and acidified ethanol (1:100)

(Figure 1 and Figure 2). The higher peak of cyanidin-3-glucoside was obtained when the standard solution of cyanidin-3-glucoside was added to the purified extract, RT at 10.562 mins (Figure 3). This confirms the retention time corresponding to cyanidin-3-glucoside.

The quantity of cyanidin-3-glucoside of two extracts by acidified water (1:20) and acidified ethanol (1:100) were compared (Table 5). The results showed slightly different quantities of cyanidin-3-glucoside in those two extracts.

Table 4. Total Phenolic Contents (mg/100g) of Anthocyanin Extracted from Corn Cob with HCl-Acidified Ethanol (3 Times) at Different Ratios and Extraction Times

The ratio	Time(min)	Amount of total phenol content (mg/100mg)			
		1st extraction	2nd extraction	3rd extraction	Total amount
1:20	30	220.73 ±3.07	193.94 ±3.18	130.12 ±3.06	**554.80 ±9.30a**
	60	247.32 ±1.35	205.17 ±1.69	128.77 ±2.66	**581.27 ±5.70b**
	90	267.11 ±2.54	212.49 ±1.05	128.69 ±1.36	**608.29 ±4.95c**
1:50	30	396.64 ±2.82	190.96 ±2.55	89.98 ±2.56	**677.58 ±7.92d**
	60	456.19 ±3.23	195.48 ±2.41	100.73 ±1.75	**752.41 ±7.39e**
	90	462.67 ±1.69	190.24 ±1.14	104.75 ±1.16	**757.65 ±3.99e**
1:100	30	501.47 ±1.44	185.62 ±2.51	90.12 ±1.80	**777.22 ±5.75f**
	60	564.47 ±2.28	163.93 ±2.50	85.06 ±3.03	**813.46 ±7.81g**
	90	**602.16 ±2.40**	**165.94 ±1.87**	**103.92 ±2.61**	**872.03 ±6.88h**

Values with the same letters in the column are not significantly different $p<0.05$.

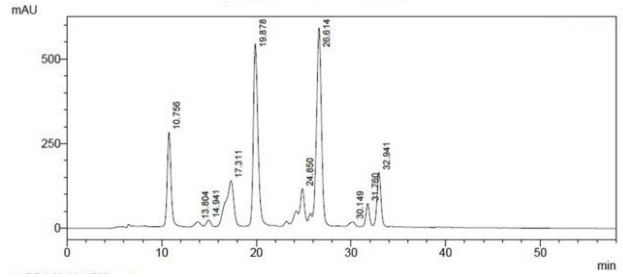

Figure 1. Chromatogram of HPLC at 520 nm of Anthocyanins Extracted by 0.01%HCl-Acidified Water

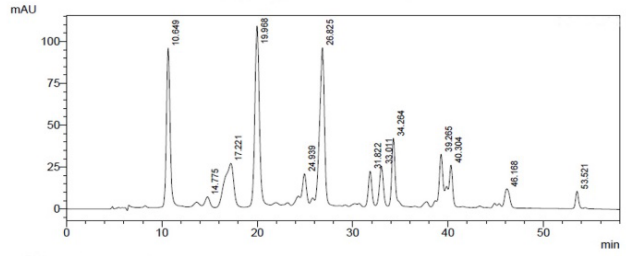

Figure 2. Chromatogram of HPLC at 520 nm of Anthocyanins Extracted by 0.01%HCl-Acidified Ethanol

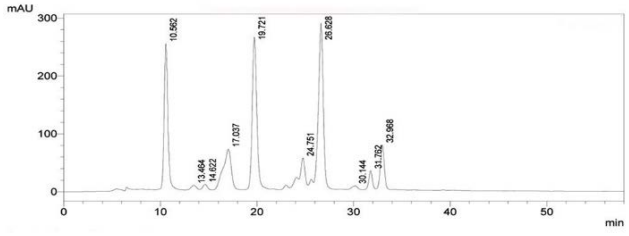

Figure 3. Chromatogram of HPLC at 520 nm of Anthocyanins Extracted by 0.01% HCl Acidified Water with the Addition of the Solution Standard of Cyanidin-3-glucoside

Table 5. Comparison of Amount of Cyanidin-3-glucoside of Anthocyanins from Corn Cob Extracted by Acidified Water and Acidified Ethanol

Type of solvent	The amount of cyanidin-3-glucoside
0.01%HCl-acidified water	272.94 ± 0.04
0.01% HCl-acidified ethanol	293.12 ± 0.02

The standard equation: $y = 1\times10^{7}x-1\times10^{6}$ ($R^2 = 0.9943$).

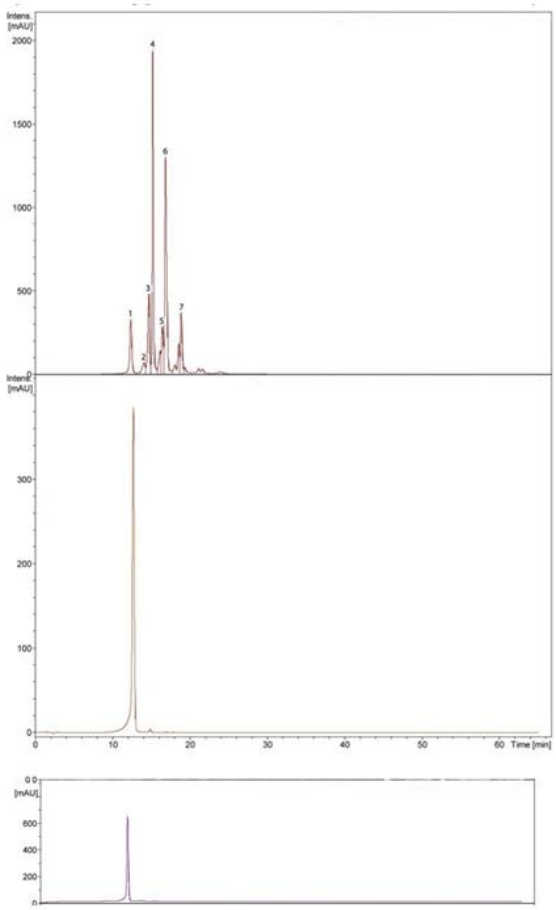

Figure 4. Chromatogram of HPLC (a) Anthocyanin Extracts from Purple Corn Cob (b) the Standard Cyanidin-3-glucoside

3.4. Identification of Anthocyanins from Purified Extracts by LC-MS

Reverse phase HPLC and MS analysis were used to identify the anthocyanins from purple corn cob extracts. Results from the chromatogram of HPLC-MS at the wavelength 520 nm indicated the cyanidin-3-glucosides eluted at peak 1 as compared to the standard cyanidin-3-glucoside. This was confirmed by retention time, spectroscopic characteristic, and fragmentation pattern

between sample extract and the cyanidin-3-glucoside standard solution (Figure 4). Peak 1 was cyanidin-3-glucoside with molecular ion $[M+H]^+$ at m/z 449 and a fragment ion $[M^+H^-162]$ at m/z 287 (Figure 5 and Figure 6).

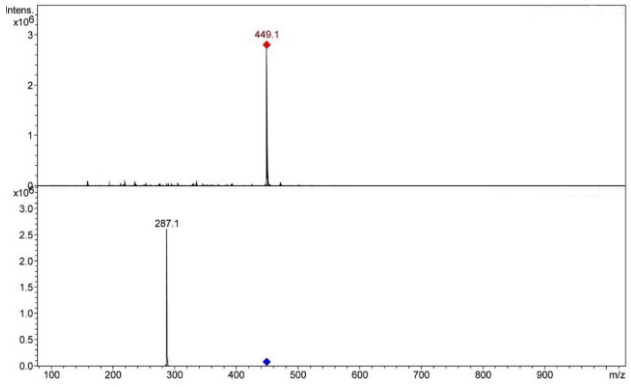

Figure 5. Chromatogram of HPLC of the Standard Solution of Cyanidin-3- glucoside, $[M]^+$ andFragment $[M+ H]^+$

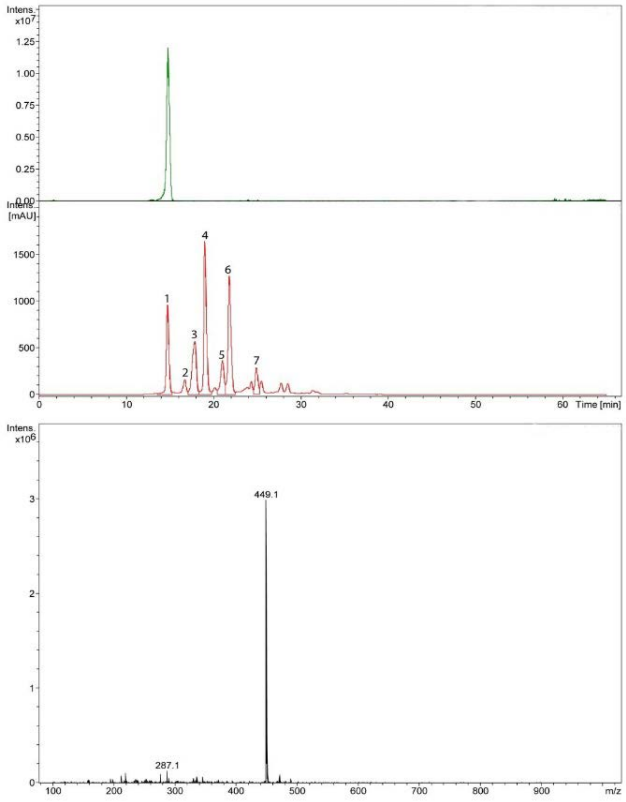

Figure 6. Chromatogram of HPLC of Cyanidin-3-glucoside and $[M]^+$ and Fragment $[M^+ H]^+$ Extracted from Purple Corn Cob

Table 6. Types of Anthocyanin Extracts in Corn Cob

Peak	Compound	m/z	
		$[M^+H]^+$ (amu)	Fragments $[M^+H]^+$
1	Cyanidin-3-glucoside	449	287
2	Pelargonidin-3-glucoside	433	271
3	Peonidin-3-glucoside	463	301
4	Cyanidin-3-(6-malonylglucoside)	35	287
5	Pelargonidin-3-(6-malonylglucoside)	519	271
6	Peonidin-3-(6-malonylglucoside)	549	301
7	Cyanidin-3-(6-ethylmalonylglucoside)	563	287

The molecular ion (M^+) and fragment $(M^+H)^+$ from LC-MS analysis indicated 7 major anthocyanins in Thai waxy corn cob which were cyanidin-3-glucoside (peak 1), pelargonidin-3-glucoside (peak 2), peonidin-3-glucoside (peak 3), cyanidin- 3-(6-malonylglucoside) (peak 4), pelargonidin-3-(6-malonylglucoside) (peak 5), peonidin-3-(6-malonylglucoside) (peak 6), and cyanidin-3-(6-ethylmalonylglucoside) (peak 7), respectively (Table 6).

The anthocyanins of purple corn (*Zea mays*) are both nonacylated and acylated. Nonacylated type anthocyanins have been identified as cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside whereas the acylated type anthocyanins are malonyl derivatives or ethylmalonyl derivatives. Due to the significant advances made in the genetic regulation of anthocyanin biosynthesis in maize, different types of anthocyanins have been found in various purple corn varieties around the world.

de Pascual-Teresa *et al*., [3] identified 9 anthocyanins from purple corn cob, namely cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, and their respective malonyl derivatives. The other three were produced during the industrial extraction process identified as the corresponding ethylmalonyl derivatives. Yang and Zhai [15] found that cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside were components in Chinese purple corn seed anthocyanins extracts, and seven kinds of anthocyanin had been detected and six kinds of anthocyanin in Chinese purple corn cob anthocyanins extracts were extracted and were identified as cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside, and their respective malonated counterparts as their anthocyanins using HPLC-MS analysis. Moreno *et al.*, [9] identified anthocyanins in the anthocyanin extracts from Mexican purple corn kernels as cyanidin-3- glucoside,pelargonidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-(6′-malonylglucoside) and cyanidin-3-(3′,6′- dimalonylglucoside).

3.5. Determination of Antioxidant Activity of Anthocyanin Extract

The antioxidant activity of anthocyanin extract was determined by DPPH assay and FRAP assay. Both assays used ascorbic acid as the standard solution. By DPPH assay, results found that anthocyanin extracts by 0.01%HCl acidified water and 0.01%HCl acidified ethanol was 6.51 mM ascorbic acid/100gand6.29 mM ascorbic acid/100g, respectively. Similarly to DPPH assay, FRAP values were5.34 mM ascorbic acid/100gand4.68 mM ascorbic acid/100gfor the acidified water extraction and acidified ethanol extraction, respectively (Table 7). It has been observed that the anthocyanins with the highest antioxidant activity were delphinidin, cyanidin, and cyanidin-3-glucoside

Table 7. Comparison of Antioxidant Activity of Anthocyanin Extracted by DPPH and FRAP Assay

Type of Solvent	DPPH assay (mM ascorbic acid/100g)*	FRAP assay (mM ascorbic acid/100g**
0.01% HCl-acidified water	6.51±0.14	5.34 ± 0.14
0.01% HCl-acidified ethanol	6.29±0.93	4.68 ± 0.11

* $y = 0.9366X - 3.4141$ ($R^2 = 0.9946$), ** $y = 0.0125X - 0.0311$ ($R^2 = 0.9955$).

3.6. The Stability of Anthocyanin Extracts in Different pH and Temperatures

The stability of anthocyanin extracts is affected by pH in terms of changing color, the characteristic of spectrum and light absorbance (Figure 7). At pH 1 the highest light absorbance of anthocyanin extracted from corn cob was found at 520 nm and then the absorbance reading decreased with increasing pH. The dark red color gradually changed to orange red with the increasing pH to 4. At pH 7 and 9, the absorbance was maximum of 570-630 nm, and color changed from light purple to dark blue (Figure 8). In an aqueous solution at pH 1-3 the flavylium cation is red colored, however, at pH 5 the resultant carbinol pseudo base is colorless and at pH 7-8 the blue purple quinoidal base is formed [1].

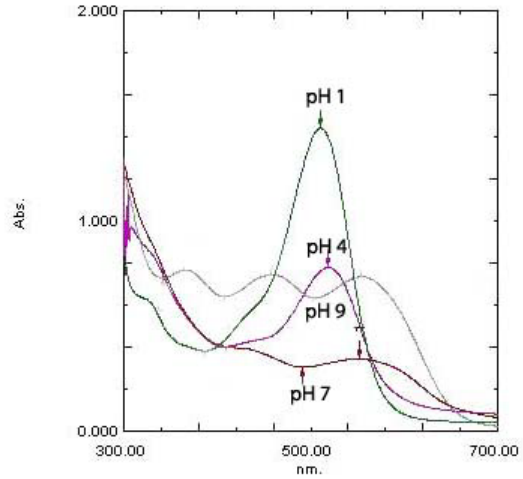

Figure 7. The Light Absorbance of Anthocyanin Extracts as Affected by pH

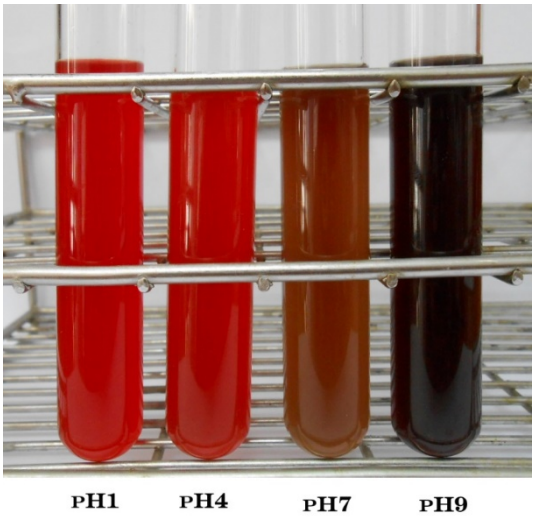

Figure 8. Changes of Color of Anthocyanin Extracts by pH

Thermal degradation of anthocyanins from purple corn cob followed the zero-order reaction kinetics at 20°-80°C in aqueous solutions with pH 1 and pH 4 (Figure 9a and b). By contrast, Jie*et al*. [6] found that thermal stability of purple-fleshed sweet potato anthocyanins in aqueous solution with various pH values followed a first – order kinetics model which may due to the different anthocyanin structure and types in such raw materials. An increase in the degradation rate constant (k) with a corresponding decline in the $t_{1/2}$ values was observed with increasing temperature at pH 1 and 4 (Table 8). Results showed that pH had significant influence on the thermal stability of anthocyanins in corn cobs. At pH 1, anthocyanin degradation was almost unremarkable (100%) with the increasing temperature from 20° to 40°C but was subjected to change (66%) at 80°C within 8 hours. However, at pH 4, the anthocyanin degradation rate was greater than at pH 1 for the heating temperature of 20° to 60°C within 8 hours. When temperature increased to 80°C, the degradation rate (60%) was not much different at pH 1 than at pH 4. Results were in agreement with the stability of anthocyanins extracted from *Kadsura cocinea* which gradully decreased from 97.9% to 88% at 20°-80°C within 3 hours [11]. Due to the effect of pH and temperature on the stabilily of anthocyanin extracts from corn cob with the consequence of color changes, it is suggested to apply the anthocyanin extract in acidic foods, with low temperature to assure a stability of color in the products.

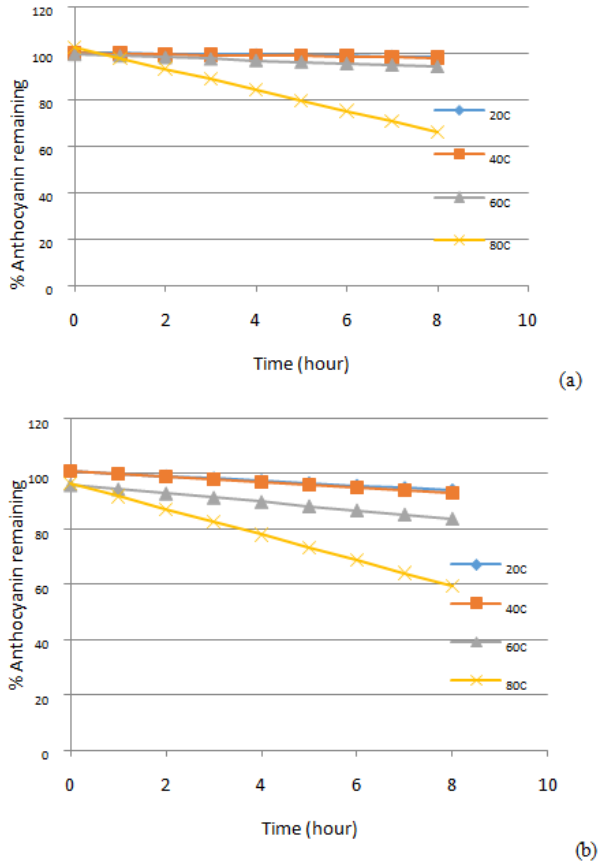

Figure 9. The Thermal Degradation of Anthocyanin Extracts at 20° to 80°C in Aqueous (a) pH 1 and (b) pH 4

Table 8. Kinetic Parameters of Degradation of Anthocyanins at Different Temperatures

pH	Temperature (c)	k (hour^{-1})	$T_{1/2}$ (h)
1	20	0.21 (0.95)[a]	240.0
	40	0.23 (0.98)	216.3
	60	0.67 (0.97)	73.8
	80	4.52 (0.99)	11.6
4	20	0.86 (0.86)	59.2
	40	0.96 (0.96)	53.0
	60	1.51 (0.91)	30.5
	80	4.65 (0.97)	10.0

[a] values in parentheses were coefficients.

4. Conclusion

Thai waxy purple corn cob is an economical alternative for anthocyanin extracts which will be substituted for synthetic dyes or fortified as antioxidant substances in food products. A profile of anthocyanins in purple corn cob is composed of both nonacylated and acylated types, with varying total amounts depending on factors of extraction. To provide the most benefit and prevent toxic residues remaining after extraction, water and ethanol are preferable to other solvents for the anthocyanin extraction. However, the limit of anthocyanin extract from purple corn cob is based on its sensitivity to pH and temperature. It is therefore suggested to apply anthocyanin extracts to acidic foods and keep the products in a low temperature in order to promote stability of color in the products.

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