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Effect of processing conditions of hot pressurized solvent extraction in batch reactor on anthocyanins of purple field corn

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Abstract: Total anthocyanin content of the dried kernel and dried cob of purple field corn was investigated under hot pressurized solvent extraction conditions. The highest total anthocyanin content of 492.51 µg cyanidin-3-glucoside g^{-1} dry weight of sample was obtained from dried kernel extraction using water-ethanol ratio 1:3 as solvent at sample-solvent ratio 1:8 and extraction temperature of 80°C. For the dried cob extraction, the highest total anthocyanin content obtained was 1890.49 µg cyanidin-3-glucoside g^{-1} dry weight of sample using water-ethanol ratio 1:1 as solvent at sample-solvent ratio 1:8, and extraction temperature of 100°C. The extraction was carried out at pressure of 0.20 MPa and 15 min with N_2 purging. Additionally, the antioxidant activities assessed by DPPH, ABTS and FRAP assays showed that the dried cob extract exhibited the greatest antioxidant activity in DPPH assay (IC50=3.83 mg mL^{-1}), ABTS assay (IC50=3.84 mg mL^{-1}) and FRAP assay (421.76 mmol $FeSO_4$ 100 g^{-1} dry weight of sample).

Keywords: anthocyanin, purple field corn, hot pressurized solvent extraction, antioxidant activities

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1 Introduction

In recent years, new natural color substances have received increasing attention in the food industries. The consumption of natural products instead of synthetic color substance dyes increases in consumers that concern with the health and safety of foods. As natural pigments, anthocyanins are highly desirable as food colorants and have also been reported to have therapeutic benefits (Pergola et al., 2006; Vayupharp and Laksanalamai, 2015). Anthocyanin extracts in powder and liquid forms are expected to be alternative choices for synthetic color substances.

Anthocyanins are responsible for the orange, red and blue color of flowers, fruits and vegetables. Anthocyanins are an important group of natural phenolic and hydrosoluble compounds or flavonoids and are widely found in tissues of plants, including leaves, stems, roots, flowers, and fruit (Vayupharp and Laksanalamai, 2015). Anthocyanins contain three phenolic rings with glycoside substitutions in the 3- and 5-positions of the flavan structure. Anthocyanins not only consist of hydrocarbons but they also have polyphenol compounds that are highly soluble in water and polarized solvents.

Purple field corn (*Zea mays* L.) was collected from an experimental field of Rajamangala University of Technology Lanna Phitsanulok (RMUTL), Thailand (Suket et al., 2014). The purple field corn has potential applications as natural food colorants and antioxidants. Suket et al. (2014) reported that the extract obtained from the purple field corn cob contained total anthocyanins glucoside as 6022 mg 100 g^{-1} by colorimetric method. The anthocyanins found in purple field corn have been characterized and the major anthocyanins detected were cyaniding-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside (Suket et al., 2014). Although data on the anthocyanins

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and antioxidant activity of purple corn are available, the extraction techniques applied to enhance the amount of anthocyanin extracts have not yet been examined for kernel, cob and silk of purple field corn. Traditionally, acidified, aqueous solutions of organic solvents have been used to extract anthocyanins (Ju and Howard, 2003; Hua et al., 2013; Vayupharp and Laksanalamai, 2015). However, it is preferable to use solvents like water and ethanol which are less toxic, particularly in food, rather than methanol, acetone and chloroform. Water and ethanol as solvents could provide a natural means of isolating anthocyanins from food plants if extraction efficiency was improved (Hua et al., 2013; Vayupharp and Laksanalamai, 2015).

The use of pressurized fluid extraction is an attractive alternative, since it allows fast extraction, small volume of solvent consumption, and automated extraction procedures. Fluid under pressure above its boiling point, but below its critical temperature, is called a hot pressurized solvent or a subcritical solvent (Chen et al., 2004). Pressure applied to the extraction has to be higher than the saturated vapor pressure of that fluid to maintain it at liquid state (Chen et al., 2004). The pressurized fluid extraction has been used for extraction of anthocyanins from various plants (Ju and Howard, 2003; Arapitsas and Turner, 2008). Recently, hot pressurized water extraction is effectively used to recover anthocyanins from red grape pomace (Vergara-Salinas et al., 2013), red cabbage (Arapitsas and Turner, 2008) and red onion (Petersson et al., 2010). Moreover, ethanol could be used as co-solvent to enhance the aqueous solubility of target compounds. Therefore water-ethanol mixtures seem to be the most suitable solvents for the extraction because of their different polarity, possibility of mixing them in any proportion and their acceptability for human consumption (Zhang et al., 2007). Therefore, the aim of this study was to evaluate the effect of extraction conditions on the content of anthocyanins obtained from dried kernel and dried cob of purple field corn using hot pressurized solvent extraction in a batch reactor. The purple field corn extracts obtained were analysed. Additionally, the antioxidant activity of purple field corn extracts was analysed.

2 Materials and methods

2.1 Raw materials and sample preparation

The dried purple field corns (open-pollinated variety) were generously supplied by the National Corn and Sorghum Research Center, Kasetsart University, Nakornratchsrima, province, Thailand. The kernels (1000 g) and cobs (1000 g) were put in a hot air drier (Kluaynamthai, Bangkok, Thailand) at 60°C for 6 h to remove their storage moisture content until constant weight. The final moisture content of dried kernel and dried cob were 15.15 and 7.85 g 100 g^{-1} sample, respectively. The dried cobs were chopped into small pieces of approximately 0.5-1.0 cm long. All samples were packed into sealed bags and kept at 4°C±1°C until further experimentation.

2.2 Chemical and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich, Germany. 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) was obtained from Fluka (Buchs, Switzerland). Ascorbic acid, potassium persulfate ($K_2S_2O_8$), sodium acetate (CH_3COONa), ferric chloride ($FeCl_3$) and ferrous sulphate ($FeSO_4$) were obtained from Loba Chemie (India). Potassium chloride (KCl) was purchased from Ajax Finechem Pty Ltd. (Australia). Ethanol (95%) and hydrochloric acid (37%) were high purity from RCI Labscan Limited (Bangkok, Thailand). All the chemicals and reagents used in the experiments were of analytical grade.

2.3 Extraction of total anthocyanins from purple field corn

The hot pressurized solvent extraction of the samples was investigated in batch reactor (75 mL volume of vessel). Each sample was weighed and then mixed with a known weight of solvent. This mixture was then put in reactor. After the reactor was sealed, it was placed into an electronic heater with temperature controller. Subsequently, the reactor was heated to 60-120°C over a holding period of 15-60 min with a constant pressure of 0.20 MPa. The extraction time started once the desired

temperature was reached, although inevitably, there would be extraction before the nominal temperature was reached (Muangrat et al., 2010).

The first experiment in the preliminary investigation was to determine the total anthocyanin content in the different parts of the purple field corn at a temperature of 100°C for 15 min and a ratio of one part sample to 14 parts extraction water. The other affecting factors investigated for hot pressurized solvent extraction were weight ratio of purple field corn sample to water (1:2, 1:4, 1:8, 1:14, 1:20 and 1:24) (equivalent dry weight), water to ethanol ratio (3:1, 1:1 and 1:3) using pure water as a control solvent and oxygen. At the end of each extraction experiment, the heating was turned off and the reactor was quickly removed from the heater and rapidly cooled to room temperature by an air-cooling system. Upon cooling to ambient temperature, the extracted sample was filtered through filter paper (Whatman paper No. 4). The filtered extracts were cooled and stored at 4±1°C in closed brown glass bottles used for analysis.

2.4 Determination of total anthocyanin content

The concentration of anthocyanins in extracts was directly determined by pH differential (Giusti and Wrolstad, 2001). Two cuvettes (3.0 mL) were added with 0.1 mL of the extracts obtained from the extraction process. The first cuvette 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. Absorbance was measured by a double-beam UV-visible spectrophotometer (PerkinElmer Instruments, Lambda 25 UV/VIS Spectrometer, Shelton, USA) at 510 and 700 nm, respectively. Total anthocyanin content was expressed as follows:

Total anthocyanins content (mg cyanidin-3-glucoside L^{-1} extract)=(A×MW×DF×1000)/(e×L) (1)

where, A is the absorbance = [(A510–A700) at pH 1.0] – [(A510–A700) at pH 4.5]; e is molar absorptivity of cyanidin-3-glucoside (26,900 L mol^{-1} cm^{-1}); L is the cell path length (1 cm); MW is the molecular weight of anthocyanin (449.2 g mol^{-1}); DF is the dilution factor. From Equation (1), the unit of the total anthocyanins content was further converted into μg cyanidin-3-glucoside equivalents g^{-1} dry weight of sample.

2.5 Determination of antioxidant activity using $DPPH^{•}$ method

The DPPH scavenging activity assay was conducted in triplicate with a method described by Brand-Williams et al. (1995). The extract samples were added with ethanol solutions at different concentrations of 0.5 to 50 mg mL^{-1}. In brief, 0.1 mL of various concentrations of anthocyanin solution was mixed with 60 μM DPPH solution in ethanol. The mixture was left in the dark at room temperature for 30 mins, and then the decrease in absorbance at 515 nm was measured. Measurements were performed in triplicate. Ascorbic acid was used as the positive control for DPPH-free radical scavenging assay. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

$$\% \text{ DPPH• inhibition} = (A_{control}-A_{sample}) \times 100/A_{control} \quad (2)$$

where, $A_{control}$ is the absorbance of the DPPH radical without any antioxidant as control, and A_{sample} is the absorbance reading of DPPH• added to sample or the positive control. Ethanol was used as a blank. The antioxidant activity of each sample was expressed as the amount of sample necessary to inhibit the initial DPPH• concentration by 50% (IC50) that was calculated graphically.

2.6 Determination of antioxidant activity using $ABTS^{•+}$ method

The antioxidant activity of crude purple field corn extracts was measured in triplicate using the capacity of the extracts to scavenge $ABTS^{•+}$ radicals (Liyana-Pathirana and Shahidi, 2006). In short, a 7.0 mM solution of ABTS in water was prepared and $ABTS^{•+}$ was formed after the addition of potassium persulphate (2.45 mM) to the solution. After 16 h incubation in darkness at room temperature, the stock solution was diluted with ethanol until the absorbance reached 0.70 ± 0.05 at 734 nm. After mixing of 10 μL sample to 190 μL of diluted $ABTS^{•+}$ solution, the reaction mixture was incubated for 6 min at 30°C. The decrease in the absorbance reflected the $ABTS^{•+}$ radical scavenging capacity of the antioxidant. The percentage of scavenging inhibition capacity of $ABTS^{•+}$ of the extract was calculated using the equation given below and comparison with Trolox was used as the positive control for ABTS free radical scavenging assay. The absorbance of $ABTS^{•+}$ without

sample was measured as the control.

$$\% \text{ ABTS}^{\bullet+} \text{ inhibition} = (A_{control}-A_{sample}) \times 100/A_{control} \quad (3)$$

where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample plus ABTS radical at t=6 min. The antioxidant activity of each sample was expressed as the amount of sample necessary to inhibit the initial $ABTS^{\bullet+}$ concentration by 50% (IC50) that was calculated graphically.

2.7 Determination of antioxidant activity using ferric reducing antioxidant power assay (FRAP)

The ferric reducing antioxidant power assay (FRAP) of each standard solution was measured in triplicate according to Benzie and Strain (1996). The ferric reducing antioxidant powder (FRAP) reagent contained 300 mM acetate buffer (pH 3.6), 10 mM 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. FRAP reagent (2.85 mL) was added with 0.15 mL of purple field corn extract samples at different concentrations. The sample was incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm, using the FRAP working solution as blank. In the FRAP assay, the antioxidant potential of extract samples was determined from a calibration curve plotted using the $FeSO_4$ solution linear regression equation to calculate the FRAP values of the extract samples. The FRAP values, derived from triplicate analyses, were calculated according to the calibration curve obtained using $FeSO_4$ in the concentration range of 0.15-5.0 mM. The calibration curve was y=0.0038x+0.0899, y=absorbance at 593 nm, x=concentration of $FeSO_4$ in mmol, R^2 =0.9979. The antioxidant capacity based on the ability to reduce ferric ions of extract samples was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents 100 g^{-1} dry weight of sample.

2.8 Statistical analysis

The Completely Randomized Design (CRD) was used for data analysis in different experiments. All the experiments were performed in triplicate. The results were expressed as mean ± standard deviation, and the mean values were considered significantly different at $p \leq 0.05$ by Duncan's multiple range tests after subjecting to an analysis of variance (ANOVA) processed with SPSS 17.0.

3 Results and discussion

3.1 Total anthocyanin content and antioxidant activities from dried kernel and cob of purple field corn

This preliminary study investigated the amount of total anthocyanin content and antioxidant activities from two parts of dried purple field corn (dried kernel and dried cob) at the different extraction conditions (extraction temperature of 100°C for 15 min using water as solvent at ratio of 1:14 (sample: solvent)). The results as shown in Table 1 revealed that the dried cob extract contained 6.67 times higher total anthocyanin content.

Table 1 Total anthocyanin content and antioxidant activities of extracts from dried kernel and dried cob (equivalent dry weight) of purple filed corn

Extract samples	Total anthocyanin content (μg cyanidin-3-glucoside g^{-1} dry weight of sample)	Antioxidant activities		
		$DPPH^{\bullet}$ (mg mL^{-1}, IC_{50})	$ABTS^{\bullet+}$ (mg mL^{-1}, IC_{50})	FRAP (mmol $FeSO_4$ 100 g^{-1} dry weight of sample)
Dried kernel	72.02±4.99^a	45.36±4.37^a	34.66±0.17^a	24.73±1.51^b
Dried cob	480.60±6.05^b	4.64±0.13^b	2.87±0.11^b	273.56±0.73^a

Note: Mean values followed by different letters in same column are significantly $p \leq 0.05$ different.

Additionally, the IC50 values for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activity by the dried kernel and dried cob extracts are also summarized in Table 1. Among the analysed extracts, the dried cob extract exhibited the highest radical scavenging activity with IC50 value of 4.64 and 2.87 mg mL^{-1} using DPPH and ABTS assays, respectively. Using the FRAP assay, the highest reducing power was observed for the dried cob extract. The FRAP values of dried cob extract was higher than that of dried kernel extract 11.1 times. The results showed in Table 1 indicated that the dried cob extract was higher in antioxidant activity than dried kernel extract due to its

higher level of total anthocyanins. However, the differences in the antioxidant activity of these extracts could possibly related to the specific composition of anthocyanin derivatives (Stintzing et al., 2002; Lee et al., 2015). Further work is in progress to elucidate the identification and quantification of compounds responsible for the antioxidant activity.

3.2 Effect of weight ratio of purple field corn samples to water on total anthocyanin content

The effect of weight ratio of purple field corn samples to water on the total anthocyanin content was determined at a constant extraction temperature of 100°C and extraction time of 15 min. The results showed that the dried kernel and dried cob extracts displayed the same trend of anthocyanin yield as shown in Figure 1. At the sample-water ratio from 1:4 to 1:24, the total anthocyanin content in the dried cob extract was significantly higher than that in the dried kernel extract. For dried kernel and dried cob (Figure 1), increasing water in the sample-water ratio from 1:2 to 1:8 led to an increase in the total anthocyanin content. However, the total anthocyanin content decreased when the water in the sample-water ratio was further increased from 1:8 to 1:24. The results were similar to those of Mohamad et al. (2013).

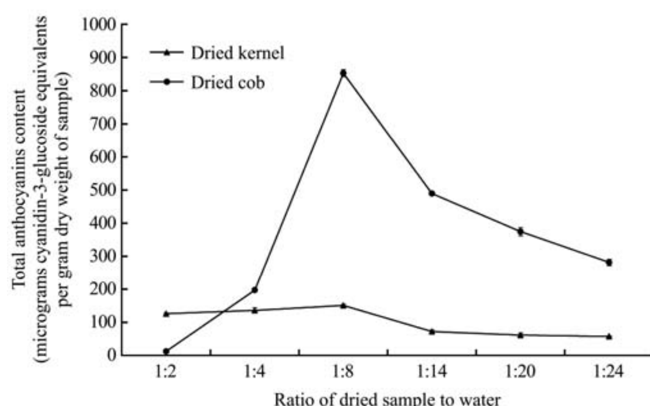


Figure 1 The effect of weight ratio of sample to water on the total anthocyanin content at a constant extraction temperature of 100°C and 15 min

From Figure 1, the highest total anthocyanin content was observed, giving approximately 150.74 and 852.93 µg cyanidin-3-glucoside g^{-1} dry weight of sample for the dried kernel extract (sample-water ratio 1:8) and dried cob extract (sample-water ratio 1:8), respectively. It was discovered that lower amount of water can lead to incomplete extraction while higher amount enhances the complete extraction (Figure 1). Nevertheless, increment of heating time to reach targeted temperatures was observed in this study when more significant amount of water for extraction used. This could be higher thermal mass of water. Consequently, there would be a number of extractions occurred prior to the desired extraction temperature was reached. This may cause a reduction in the total anthocyanin content due to heat accumulation. Therefore, a suitable water to sample ratio is preferred in order to achieve higher extraction yields. The optimal sample-to-water ratio to obtain the highest total anthocyanin content in the dried kernel and dried cob was used for further study in subsequent sections

3.3 Effect of extraction temperature on total anthocyanin content

In this study, extraction of the dried kernel (ratio of dried kernel to water 1:8) and dried cob (ratio of dried cob to water 1:8) was carried out at different temperatures (i.e. 60°C, 80°C, 100°C and 120°C) for 15 min extraction time while other extraction parameters were kept constant. The results are shown in Figure 2. The total anthocyanin yield from dried kernel extract increased as the temperature was increased from 60°C to 80°C. However, the yield was gradually decreased as the temperature increased from 80°C to 120°C. Raising of the extraction temperature from 60°C to 100°C for dried cob had resulted in a significant increase in the total anthocyanin content. However, the total anthocyanin content declined at the extraction temperature more than 100°C.

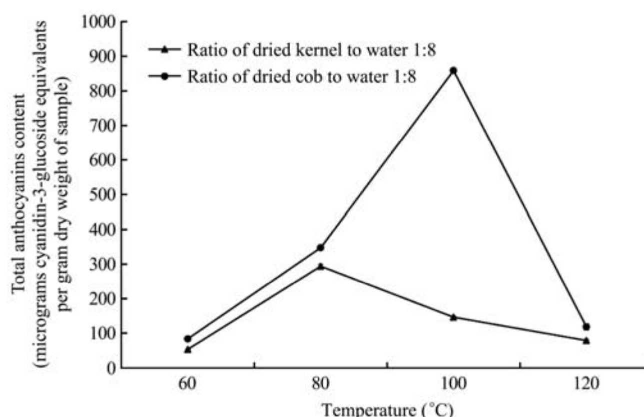


Figure 2 The effect of temperature on the extraction of total anthocyanins from dried kernel and dried cob of purple field corn under hot pressurized water extraction condition

At higher extraction temperature, the total anthocyanin content decreased which may be mainly due to the thermal degradation of anthocyanins (Vayupharp and Laksanalamai, 2015; Piyapanrungrueang et al., 2015). Even though high extraction temperature leads to a higher amount of total anthocyanins, the results (Figure 2) showed that the optimal extraction temperatures were 80°C and 100°C that resulted in the highest total anthocyanin yields extracted from dried kernel and dried cob, respectively. In addition, the extract from dried cob contained 2.92 times higher amount of total anthocyanins than that from dried kernel at the optimal hot extraction temperature.

As presented in Figure 2, it is clear that temperature represents a key factor in the extraction of such heat sensitive compounds. Higher temperature helps to enhance the solubility of solute. The extracted material might be softened by heating and the solvents easily penetrate and diffuse through the material structure. Therefore, the extraction could be improved by higher extraction temperature (Piyapanrungrueang et al., 2015). Simultaneously, dissolution of impurities can also increase, and some thermally labile components such as anthocyanins could decompose. This was consistent with the reports of Vayupharp and Laksanalamai (2015) and Piyapanrungrueang et al., (2015) who reported that higher temperature leads to the thermal degradation of the anthocyanins and a lower yield.

3.4 Effect of extraction time on total anthocyanin content

Figure 3 displays total anthocyanin content was decreased with increasing extraction time. The total amount of anthocyanins extracted from dried kernel and dried cob at 80°C and 100°C for 15 min were approximately 293.50 and 858.31 μg cyanidin-3-glucoside g^{-1} dry weight of sample, respectively, which was higher than those for 30, 45 and 60 min. These results demonstrated that extraction time duration can influence the extraction yield in addition to extraction temperature (Figure 2) and a sample to water ratio (Figure 1). The extraction time for 15 min at 80°C and 100°C yielded the highest amount of total anthocyanins extracted from dried kernel and dried cob, respectively.

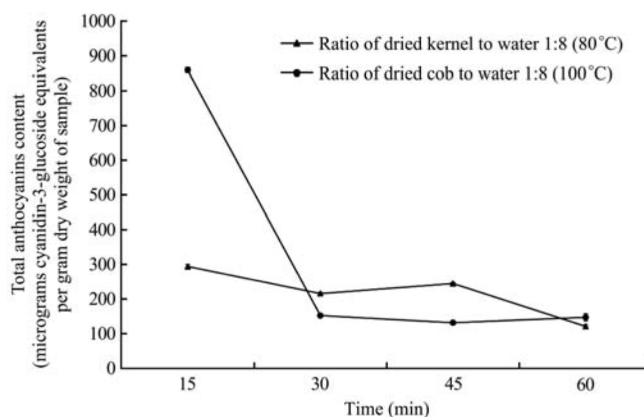


Figure 3 The effect of time on the extraction of total anthocyanins from dried kernel and dried cob of purple field corn under hot pressurized water extraction condition

Results in Figure 3 show longer extraction time than 15 min resulted significant reduction of anthocyanin yield extracted from dried kernel and dried cob due to its degradation or the loss of anthocyanins by oxidation (Vayupharp and Laksanalamai, 2015; Piyapanrungrueang et al., 2015). Although the extraction time was short at 15 min, it was sufficient to allow solvents to penetrate deeply into samples and efficiently released more anthocyanins. Consistent with this result, Vayupharp and Laksanalamai (2015) studied the effect of extraction time on the anthocyanin yield obtained from purple corn. The results revealed that the longer period of extraction time led to a significantly lower anthocyanin yield because of the anthocyanin degradation.

3.5 Effect of water to ethanol ratio on total anthocyanin content

The effect of water to ethanol ratio on anthocyanin extraction was investigated. Figure 4 shows that the extraction of dried kernel (using water to ethanol ratio 1:3) and dried cob (using water to ethanol ratio 1:1) contained a high anthocyanin yield of approximately 375.07 and 1057.27 μg cyanidin-3-glucoside g^{-1} dry weight of sample, respectively. It was noticed that higher amount ethanol could enhance the extraction process allowing an increase in the extracted anthocyanins when compared with that using water alone. When ethanol in the water-ethanol ratio increased from 3:1 to 1:1, the total anthocyanin content from dried cob significantly increased (Figure 4). The total anthocyanin content from dried cob decreased when the ethanol in the water-ethanol ratio increased from 1:1 to 1:3. For dried kernel, the total

anthocyanin content gradually increased when the ethanol in the water-ethanol ratio increased from 3:1 to 1:3. Therefore, the optimal water to ethanol ratio was 1:3 and 1:1 for total anthocyanin extraction from dried kernel and dried cob, respectively.

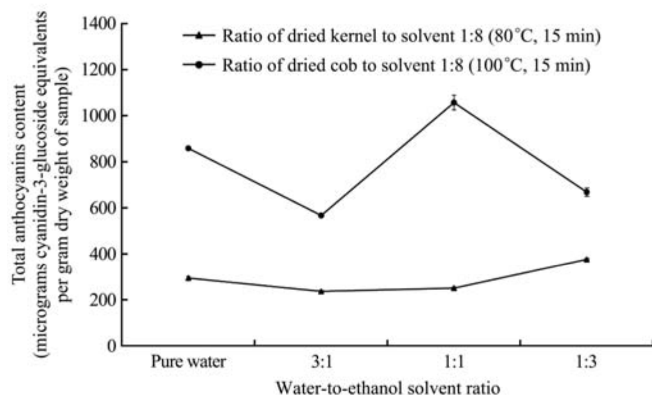


Figure 4 The effect of weight ratio of water to ethanol on the extraction of total anthocyanins from dried kernel and dried cob of purple field corn under hot pressurized solvent extraction condition

The results in Figure 4 revealed that the suitability of solvent depends upon the sample type. With the addition of ethanol in a suitable ratio, the polarity of complex solvent will be suitable for anthocyanin solubility. Simultaneously, water could increase the swelling of sample and enhance the contact surface area between the sample matrix and the solvent leading to higher extraction efficiency. The optimal sample-water ratio to obtain the highest total anthocyanin content in the dried kernel and dried cob was used for further study in subsequent sections

3.6 Effect of oxygen on total anthocyanin content

The effect of oxygen on anthocyanin extraction from dried kernel and dried cob was investigated by a comparative study involving purging and without purging of nitrogen under hot pressurized solvent extraction. From the previous studies described above, the optimal extraction condition was selected for further study. At each experimental condition in this section, the residual air in the 75 mL reactor was purged with nitrogen for 15 min before extraction. The results (Table 2) showed that these optimal conditions for anthocyanin extraction gave a total anthocyanin content of 492.51 and 1890.49 µg cyanidin-3-glucoside/g dry weight of sample from the dried kernel and dried cob, respectively.

Additionally, Table 2 shows that the extracted sample purged with nitrogen produced the higher anthocyanin yield than this without N_2 purging 1.31 and 1.79 times for the dried kernel and dried cob extracts, respectively. This could be mainly due to the decrease of oxygen which causes anthocyanins oxidation (Routray and Orsat, 2011). As shown in Table 2, the extracted sample purged with nitrogen provided higher anthocyanin yield than this without N_2 purging for both the dried kernel extract and dried cob extract. This could be mainly due to the decrease of oxygen which causes anthocyanins oxidation leading to increase the total anthocyanin yield.

Table 2 The effect of oxygen on the total anthocyanin content extracted from dried kernel and dried cob of purple field corn under hot pressurized solvent extraction

Condition	Total Anthocyanin Content (µg cyanidin-3-glucoside g^{-1} dry weight of sample)	
	Dried kernel*	Dried cob†
with N_2 purging	492.51±4.84^a	1890.49±21.31^a
without N_2 purging	375.07±3.35^b	1057.27±32.04^b

Note: Mean values followed by different letters in same column are significantly p≤0.05 different.

* ratio of dried kernel to solvent 1:8 (water to ethanol 1:3), extraction temperature of 80°C and extraction time for 15 min.

† ratio of dried cob to solvent 1:8 (water to ethanol 1:1), extraction temperature of 100°C and extraction time for 15 min.

3.7 Antioxidant activity of purple field corn extracts using DPPH, ABTS and FRAP methods

The extracts obtained from the optimal extraction conditions of dried kernel and dried cob were evaluated for their free radical scavenging activity using DPPH, ABTS and FRAP. The optimal extraction conditions were dried kernel to solvent ratio 1:8, water to ethanol ratio 1:3 and extraction temperature of 80°C; dried cob to solvent ratio 1:8, water to ethanol ratio 1:1 and extraction temperature of 100°C. The extraction time of 15 min with N_2 purging was used for all samples. The results are shown in Table 3. For DPPH assay, the antioxidant capacities ranged from 3.83 to 18.87 mg mL^{-1} were obtained. The extract of dried cob showed the greatest scavenging effect, followed by that of died kernel (Table 3). Using ABTS assay, the antioxidant capacity ranged from 3.84 to 24.28 mg mL^{-1}; where dried cob extract displayed the highest scavenging activity, while dried kernel extract displayed the lowest scavenging effect. For FRAP assay, the antioxidant capacity ranged from 73.33 to 421.76 mmol $FeSO_4$ 100 g^{-1} dry weight of sample;

where dried cob extract displayed superior antioxidant (reducing effects) compared to dried kernel extract sample.

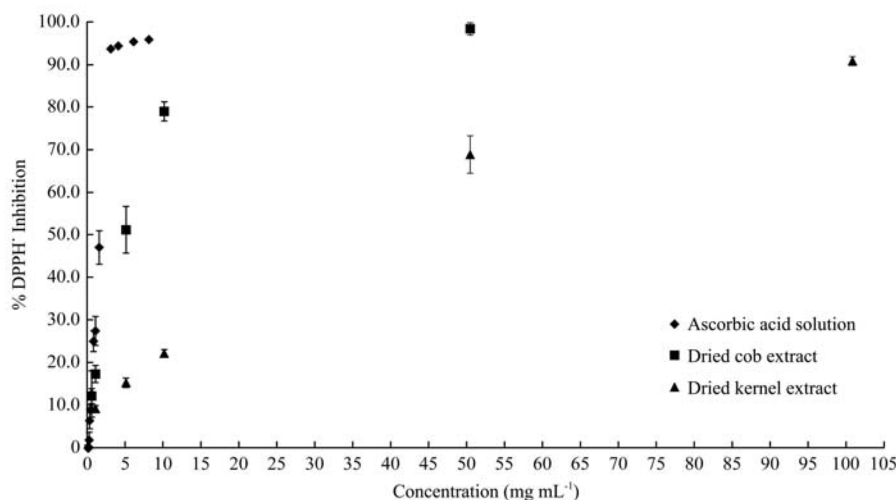
In addition, the values of DPPH IC50, ABTS IC50 and FRAP of dried kernel and dried cob extracts obtained from the optimal extraction conditions were compared with these conditions without N_2 purging. In Table 3, all experimental conditions with N_2 purging gave the lower 50% inhibitory concentration values by DPPH and ABTS assay and higher FRAP values than those without N_2 purging. Hence, the results indicated that antioxidant compounds such as anthocyanins might react with oxygen leading to reduced antioxidant activity.

Moreover, Figure 5 shows the DPPH IC50 and ABTS IC50 values of dried kernel extract and dried cob extract were compared to ascorbic acid solution and the Trolox solution. The results showed that the DPPH IC50 and ABTS IC50 values of all extract samples provided lower antioxidant activity than ascorbic acid solution (IC50=0.07 mg mL^{-1}) and the Trolox solution (IC50= 0.01 mg mL^{-1}).

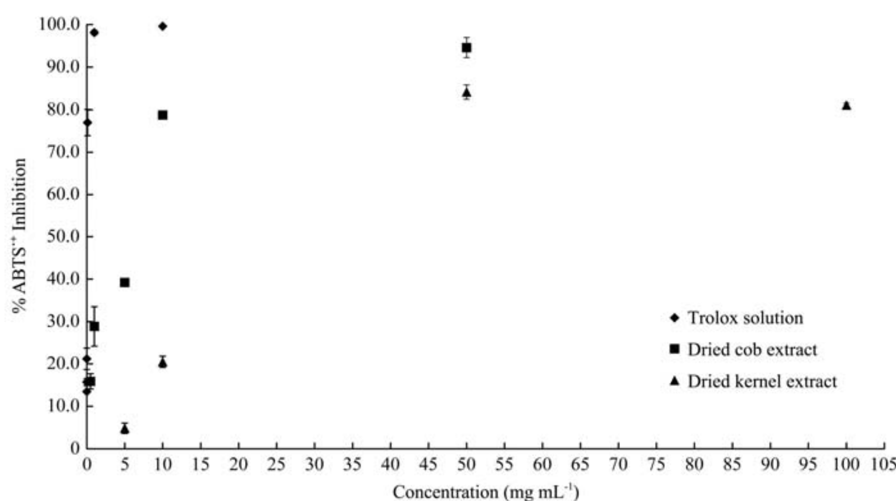
Table 3 Comparison antioxidant activities in extracts from dried kernel and dried cob measured by the DPPH, ABTS and FRAP methods

Condition	Antioxidant activity of dried kernel extract			Antioxidant activity of dried cob extract		
	$DPPH^•$ (mg mL^{-1}, IC_{50})	$ABTS^{•+}$ (mg mL^{-1}, IC_{50})	FRAP (mmol $FeSO_4$ 100 g^{-1} dry weight of sample)	$DPPH^•$ (mg mL^{-1}, IC_{50})	$ABTS^{•+}$ (mg mL^{-1}, IC_{50})	FRAP (mmol $FeSO_4$ 100 g^{-1} dry weight of sample)
With N_2 purging	$18.87±1.55^b$	$24.28±0.01^a$	$73.33±2.91^a$	$3.83±0.33^b$	$3.84±0.39^b$	$421.76±10.49^a$
Without N_2 purging	$70.95±3.84^a$	$22.48±0.02^b$	$67.63±5.01^b$	$5.84±0.21^a$	$4.87±0.19^a$	$365.54±10.65^b$

Note: Mean values followed by different letters in same column are significantly $p≤0.05$ different.



a. DPPH



b. ABTS

Figure 5 Comparisons of (a) DPPH and (b) ABTS free-radical scavenging activity of dried kernel and dried cob extracts and ascorbic acid solution and Trolox solution

The dried cob extract consistently exhibited higher antioxidant activity than the dried kernel as shown in Table 3. High amount of total anthocyanins in the dried cob extract (shown in Table 2) contributed to an increase in antioxidant activity. The results could therefore indicate that the dried cob extract had the greatest total anthocyanin content and antioxidant activity to inhibit 50% of free DPPH and ABTS radicals and also gave the highest FRAP value.

4 Conclusions

Extraction temperature, extraction time, sample-solvent ratio, water-ethanol ratio and oxygen have influenced on extraction yields of total anthocyanins from dried kernel and dried cob of purple field corn under hot pressurized solvent extraction in a batch reactor. The yields of total anthocyanins were determined as the cyanidin-3-glucoside equivalent. This study discovered the proper extraction conditions for anthocyanins from the dried kernel, and dried cob as follows: sample-solvent ratio 1:8 and 1:8, respectively, water-ethanol ratio 1:3 and 1:1, respectively, the extraction temperature of 80°C and 100°C, respectively, the extraction time of 15 min with N_2 purging and the extraction pressure of 0.20 MPa. These extraction conditions yielded high total anthocyanin levels of 492.51 and 1890.49 μg cyanidin-3-glucoside g^{-1} dry weight of sample from the dried kernel and dried cob, respectively. The extract results showed that the dried cob extract had the greatest antioxidant activity. The 50% inhibitory concentration values obtained by DPPH and ABTS assays with the dried cob extract were 3.83 and 3.84 mg mL^{-1}, respectively, and the antioxidant activity by the FRAP assay was 421.76 mmol $FeSO_4$ 100 g^{-1} dry weight of sample. Therefore, the hot pressurized solvent extraction process reported here could be applicable in food processing industries. By this process, the extract of purple field corn has the potential to be developed into new health foods and could be a natural source of antioxidants for the production of value-added products.

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