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SUBCRITICAL SOLVENT EXTRACTION OF TOTAL ANTHOCYANINS FROM DRIED PURPLE WAXY CORN: INFLUENCE OF PROCESS CONDITIONS

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ABSTRACT

Total anthocyanin content in different dried parts of purple waxy corn (Zea mays L. var. ceratina) genotype Fancy Muang 111 was investigated under subcritical solvent extraction. The highest total anthocyanin content of 991.4, 1241.5 and 1552.1 µg cyanidin-3-glucoside/g dry weight of sample were obtained from dried kernel extraction (water-ethanol ratio 1:3 as solvent and sample-solvent ratio 1:20), dried cob extraction (water-ethanol ratio 1:1 as solvent and sample-solvent ratio 1:20), dried silk extraction (water-ethanol ratio 1:1 as solvent and sample-solvent ratio 1:30), respectively. The extraction was conducted at 100 ^{o}C with 15 min of N_2 purging. Additionally, the antioxidant activities assessed by DPPH, ABTS and FRAP assays showed that the dried silk extract exhibited the greatest antioxidant activity in DPPH assay (IC50=3.2 mg/mL), ABTS assay (IC50=1.3 mg/mL) and FRAP assay (634.8 mmol $FeSO_4$/100 g dry weight of sample). The extract powder had the total anthocyanin content of 632.8 µg cyanidin-3-glucoside/g powder.

PRACTICAL APPLICATIONS

The anthocyanin extraction from different dried parts of purple waxy corn was investigated by subcritical solvent extraction method. The effect of different parameters such as weight ratio of purple waxy corn samples to water, extraction temperature, extraction time, water to ethanol ratio as solvent and oxygen in the subcritical solvent extraction provided high total anthocyanin content in the extraction, and the dried silk extract containing the highest total anthocyanin content exhibited the greatest antioxidant activity. Therefore subcritical solvent extraction of total anthocyanins from purple waxy corn has been shown to be feasible and could be applicable in food processing industries. The total anthocyanin extract obtained has the potential to be developed into new health foods and to be used for the production of value added products.

INTRODUCTION

Anthocyanins show a colour spectrum from orange to blue and are highly desirable as food colourants and have also been reported to have therapeutic benefits (Smith et al. 2000; Pergola et al. 2006; Piyapanrungrueang et al. 2015). Anthocyanins are an important group of natural phenolic and hydrosoluble compounds or flavonoids and are widely found in many fruits, vegetables, cereal grains and flowers. Anthocyanins contain three phenolic rings with glycoside substitutions in the 3- and 5-positions of the flavan structure.

Purple corn (Zea mays L.) including purple waxy corn (Zea mays L. var. ceratina) is an important natural cereal crop containing anthocyanins in the grain pericarp, cob and silk (Yang et

al. 2009; Yang and Zhai 2010; Sarepoua et al. 2013) which have potential applications as natural food colourants and antioxidants. The anthocyanins found in purple corn have been characterized and the major anthocyanins detected were cyanidin-3-dimalonylglucoside, cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside, and their respective malonated counterparts (Pascual-Teresa et al. 2002; Yang and Zhai 2010).

Recently, the interest in new natural food colouring from plants including purple corn has increased due to consumer awareness of its diverse health benefits. Although data on the anthocyanins and antioxidant activity of purple corn are available, the extraction techniques applied to enhance the amount of anthocyanin extracts have not yet been examined from husk, kernel, cob and silk of purple waxy corn. Traditionally, acidified and aqueous solutions of organic solvents have been used to extract anthocyanins (Metivier et al. 1980; Ju and Howard 2003). Extraction processes use generally recognized as safe (GRAS) solvents that use water and ethanol as an extraction solvent. These processes have been investigated for their effectiveness in comparison to extractions by acids, methanol, acetone, and chloroform. As a result, extraction by water and ethanol could minimize problems of environmental impact of the process and also provide a natural means of isolating anthocyanins from food plants if extraction efficiency was improved (Metivier et al. 1980).

Subcritical water conditions have the potential to enhance extraction efficiency of anthocyanins from purple corn. Subcritical water, also known as pressurized low polarity water, is water heated above its boiling point (100 ^{o}C), but below its critical point (374 ^{o}C). These conditions allow water to remain in a liquid state due to the applied pressure. Subcritical water extraction provides advantages over conventional extraction methods, such as being "greener", faster and more efficient. Benefits of this "green" extraction technology include the reduction of energy consumption and cost. It also speeds up the extraction process. (Chemat et al. 2012). Recently, subcritical water extraction has effectively been used to recover anthocyanins from red grape pomace (Monrad et al. 2010), red cabbage (Arapitsas and Turner 2008) and red onion (Petersson et al. 2010). Even though there were many novel extraction technologies reported using superheated solvents with high pressure and safe solvents, but combination of an optimal safe solvent and temperature to extract anthocyanins and to determine the content of anthocyanins from different parts of purple waxy corn under subcritical solvent extraction has not been done yet. Therefore, the main purpose of the present study was to investigate the influence of extraction conditions under subcritical water extraction on the total anthocyanin content from four parts of dried purple waxy corn ear (husk, kernel, cob and silk). The total anthocyanin content (determined as the cyanidin-3-glucoside equivalents) and the antioxidant activity of purple waxy corn extracts were analysed.

MATERIALS AND METHODS

Sample Preparation

The dried purple waxy corns (genotype Fancy Muang 111) were obtained from Pacific Seeds (Thai) Ltd. (Saraburi, Thailand). Firstly, the husks and silks were completely removed from purple waxy corns. Then the kernels were taken off the cobs. One ear of dried purple waxy corn contained approximately 8.96, 76.17, 14.54 and 0.33 % (w/w) of dried husk, dried kernel, dried cob and dried silk, respectively. The husks, kernels, cobs and silks were put in a hot air drier (Kluaynamthai, Bangkok, Thailand) at 60 °C for 6 h to remove their storage moisture content until constant. The final moisture content of dried husk, dried kernel, dried cob and dried silk

were 4.18, 3.61, 4.58 and 4.99 g/100 g sample, respectively. The dried husks, dried cobs and dried silks were chopped up into small pieces. The average size of each of these samples was (a) 0.50 cm width x 0.75 cm length x <0.05 cm thickness (dried husk), (b) 0.50 cm width x 0.75 cm length x 0.35 cm thickness (dried cob) and (c) <0.05 cm diameter x 0.75 cm length (dried silk). For dried kernel, the average size was 0.50 cm width x 0.75 cm length x 0.35 cm thickness. Each set of experiments was conducted with the equivalent weight of each sample which corresponded to same dried weight of samples. All samples were packed into sealed bags and kept at 4±1°C until further experimentation.

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 chemicals and reagents used in the experiments were of analytical grade.

Extraction of Purple Waxy Corn Total Anthocyanins

The subcritical solvent extraction of the samples was investigated in batch reactor (75 mL volume of vessel). Each sample was weighted into the reactor and mixed with a known weight of solvent. 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. Based on previous work (Muangrat et al. 2010), the extraction time started once the desired temperature was reached, although inevitably, there would be extraction before the nominal temperature was reached.

The first experiment in the preliminary investigation was to determine the total anthocyanin content in the different parts of the purple waxy corn at a temperature of 100 ^{o}C for 15 min and a ratio of 1 part sample to 14 parts extraction water. The other factors affecting subcritical solvent extraction including weight ratio of purple waxy corn sample to water (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 nitrogen purging were investigated. At the end of each extraction experiment, the heating was stopped and the reactor was quickly removed from the heater and rapidly cooled to room temperature by an air cooling system. After 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 for further analysis.

Extract Powder Preparation

The extract sample was added with ascorbic acid 0.1%w/w to protect oxidation process and then concentrated it by evaporation using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) at 40 ^{o}C under a pressure of 20 mbar. The concentrated extract was mixed with maltodextrin (DE 10) as stabilizer to obtain 20 ^{o}Brix. This mixture was immediately placed in freezing trays and frozen at -20°C. After 24 hours, the frozen sample was dried for 48 hours at -30°C under a pressure of 130 mbar using a freeze dryer (Model 1195-7948030, Labconco, USA).

Extract Powder Qualities

The extract powder was analysed for its total anthocyanin content, total soluble solid, moisture content, water activity, solubility and colour value.

Determination of Total Anthocyanin Content

The total anthocyanin content was measured by the pH-differential method (Giusti and Wrolstad 2001). The buffer consisted of two solutions: one with potassium chloride buffer (0.025 M KCl) with pH of 1.0 and the other with sodium acetate buffer (0.4 M CH_3COONa) with pH of 4.5. The pH of buffers were adjusted to obtain final pH values of 1.0 and 4.5 with 0.1 N dilute hydrochloric acid. An aliquot of the sample (0.1 mL) was placed into a 3.0 mL cuvette and made up to the final volume with pH 1.0 buffer. Another 0.1 mL of the sample was also placed into the 3.0 mL cuvette, made up to a final volume with pH 4.5 buffer. 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. In this study the total anthocyanin content was expressed as cyanidin-3-glucoside equivalents. The total anthocyanins content was calculated using the following equation.

$$\text{Total anthocyanins content (mg cyanidin-3-glucoside/L extract)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times L} \quad (1)$$

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

Colour Coordinates

Colour of extracts was determined by CIELAB parameters using the Colour Quest XE (ColourQuest XE, HunterLab, USA), Colour measurements were expressed as tristimulus parameters, L*, a* and b*.

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). Briefly, different dilutions of the crude purple waxy corn extracts and a 60 µM solution of ethanolic DPPH solution were prepared. The initial absorbance of the DPPH in ethanol was measured at 515 nm and did not change throughout the period of assay. An aliquot (0.1 mL) of each sample (with appropriate dilution if necessary) was mixed with 3.9 mL of ethanolic DPPH solution in a cuvette and held for 30 min at room temperature in the dark. Measurements were performed in triplicate. The results were expressed as IC50 (inhibition concentration at 50% scavenging activity). The IC50 values calculated denote the concentration of the antioxidant sample required to decrease DPPH radicals to half of its initial concentration. Ascorbic acid was used as the positive control for DPPH-free radical scavenging assay.

$$\%\ \text{DPPH•\ inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad (2)$$

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 at 515 nm. 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.

Determination of Antioxidant Activity using $ABTS^{\bullet+}$ Method

The antioxidant activity of crude purple waxy corn extracts was measured in triplicate using the capacity of the extract to scavenge $ABTS^{\bullet+}$ radicals (Liyana-Pathirana and Shahidi 2006). In short, a 7.0 mM solution of ABTS in water was prepared and $ABTS^{\bullet+}$ was formed after the addition of potassium persulphate (2.45 mM) to the solution. After 16 h of 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 $ABTS^{\bullet+}$ solution, the reaction mixture was incubated for 6 min at 30 °C. The decrease in the absorbance reflected the $ABTS^{\bullet+}$ radical scavenging capacity of the antioxidant. The percentage of scavenging inhibition capacity of $ABTS^{\bullet+}$ of the extract was calculated by using the equation given below and compared with Trolox as the positive control for ABTS free radical scavenging assay. The absorbance of $ABTS^{\bullet+}$ without sample was measured as the control.

$$\%\ ABTS^{\bullet+}\ \text{inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \qquad (3)$$

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.

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 FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM ferric chloride solution in a ratio of 10:1:1 in volume. 2.85 mL of FRAP reagent was added 0.15 mL of purple waxy corn extract samples at different concentrations. The reaction mixture was incubated at 37 °C for 10 min before using. The absorbance was measured at 593 nm after 30 min using the FRAP working solution as a 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 in order 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 dry weight of sample.

Statistical Analysis

Completely Randomized Design (CRD) was used for data analysis in different experiments. All experiments were conducted 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.

RESULTS AND DISCUSSION

Total Anthocyanin Content of Different Parts of Purple Waxy Corn

The amount of total anthocyanin content from four parts of dried purple waxy corn (dried husk, dried kernel, dried cob, and dried silk) were determined at the defined extraction conditions. The total anthocyanin content of dried kernel extract was higher than those of dried husk extract, dried cob extract and dried silk extract 4.27, 1.41 and 1.34 times, respectively (Table 1). Additionally, effect of different parts of purple waxy corn on the IC50 values for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activity by the dried husk, dried kernel, dried cob and dried silk extracts are also shown in Table 1. Among the analysed extracts, the dried silk extract exhibited the highest radical scavenging activity with IC50 value of 1.9±0.2 mg/mL and 1.1±0.1 mg/mL using DPPH and ABTS assays, respectively. Using the FRAP assay, the highest reducing power was observed for the dried silk extract. The FRAP values of dried silk extract was higher than those of dried husk, dried kernel and dried cob extract 4.25, 2.25 and 2.41 times, respectively. The differences in the antioxidant activity of these extracts are possibly related to the specific composition of anthocyanin derivatives (Stintzing et al. 2002). Further work is in progress to elucidate the identification and quantification of compounds responsible for the antioxidant activity.

The dried kernel, dried cob and dried silk extracts showed significantly higher yield of total anthocyanins than the dried husk extract. Therefore, only dried kernel, dried cob and dried silk samples were selected for further investigating factors affecting extraction yield of anthocyanins.

Effect of Weight Ratio of Purple Waxy Corn Samples to Water on Total Anthocyanin Content

The effect of weight ratio of purple waxy 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, dried cob and dried silk extracts displayed the same trend of anthocyanin yield as shown in Fig. 1. For dried kernel and dried cob (Fig. 1a), increasing water in the sample-water ratio from 1:4 to 1:20 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 1:20 to 1:24. For dried silk (Fig. 1b), increasing water in the sample-water ratio from 1:4 to 1:30 resulted in an increase in the total anthocyanin content but at a sample-water ratio of 1:35 and 1:40, the total anthocyanin content slightly declined. The results are similar to those of Mohamad et al. (2013). From Fig. 1, the highest total anthocyanin content was observed, giving about 558, 558 and 988 µg cyanidin-3-glucoside/g dry weight of sample for the dried kernel extract at sample-water ratio 1:20, dried cob extract at sample-water ratio 1:20, and dried silk extract at sample-water ratio 1:30, respectively.

It has been experimentally shown that the sample-water ratio has a positive effect; in fact, when the ratio of water to sample ratio is higher, it means that the difference of the concentration between the bulk solution and the solutes becomes higher. Thus, more anthocyanin compounds

can leach out if a higher amount of water is used. A lower amount of water can lead to incomplete extraction while a higher amount can make the extraction procedure become more complete. Nevertheless, in this research the use of a more significant amount of water for extraction would take a longer heating time to raise the water up to the designed temperature. Also there would be extraction occurring before 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 suitable sample-water ratio to obtain the highest total anthocyanin content in the dried kernel, dried cob and dried silk was used for further study in subsequent sections.

Effect of Extraction Temperature on Total Anthocyanin Content

In this study, extraction of the dried kernel (ratio of dried kernel to water 1:20), dried cob (ratio of dried cob to water 1:20) and dried silk (ratio of dried silk to water 1:30) 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 Fig. 2. The total anthocyanin yield from dried kernel, dried cob and dried silk extracts significantly increased as the temperature was increased from 60 to 100 °C, then began to decrease as the temperature increased from 100 to 120 °C, which may be mainly due to the thermal degradation of anthocyanins at higher temperature (Yang et al. 2009; Cacace and Mazza 2003; Piyapanrungrueang et al. 2015). Even though a high extraction temperature leads to a higher amount of total anthocyanins, the results (Fig. 2) showed that the optimal subcritical extraction temperature was at 100 ^{o}C which resulted in the highest total anthocyanin yields extracted from all samples. In addition, the extract from the dried silk contained 1.77 times higher amount of total anthocyanins than the extracts from dried cob and dried kernel. Hence, it is clear that temperature represents a key factor in the extraction of such heat sensitive compounds. Increasing temperature helps to enhance both 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, the dissolution of impurities can also increase, and some thermally labile components such as anthocyanins can decompose. This was consistent with the reports of Yang et al. (2009), Cacace and Mazza (2003) and Piyapanrungrueang et al. (2015) who reported that higher temperature leads to the thermal degradation of the anthocyanins and a lower yield.

Effect of Extraction Time on Total Anthocyanin Content

The total anthocyanin content decreased with increasing extraction time (Fig. 3). The total amount of anthocyanins extracted from dried kernel, dried cob and dried silk at 100 ^{o}C for 15 min 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 (Fig. 2) and a sample to water ratio (Fig. 1). When the extraction time was longer than 15 min, the anthocyanin yield decreased significantly due to its degradation or loss of anthocyanins by oxidation (Yang et al. 2009; 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. The extraction time for 15 min at 100 ^{o}C yielded the highest amount of total anthocyanins. Similar to our result, Yang et al. (2009) and Piyapanrungrueang et al. (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.

Effect of Water to Ethanol Ratio on Total Anthocyanin Content

Figure 4 shows that the extraction of dried kernel (using water to ethanol ratio 1:3), dried cob (using water to ethanol ratio 1:1) and dried silk (using water to ethanol ratio 1:1) gave a high anthocyanin yield of 779.7, 850.5 and 1074.3 µg cyanidin-3-glucoside/g dry weight of sample, respectively. It was noticed that the higher amount ethanol could enhance the extraction process allowing an increase in the extracted anthocyanins when compared with that using water alone. However, the suitability of solvent depends upon the sample type. From Fig. 4, when ethanol in the water-ethanol ratio increased from 3:1 to 1:3, the total anthocyanin content from dried cob and dried silk decreased while that from dried kernel increased. With the addition of ethanol in a suitable ratio, the polarity of complex solvent will be suitable for anthocyanin solubility. Simultaneously, water could increase swelling of the sample and enhance the contact surface area between the sample matrix and the solvent leading to higher extraction efficiency.

Effect of Oxygen on Total Anthocyanin Content

The effect of oxygen on anthocyanin extraction from dried kernel, dried cob and dried silk was investigated by a comparative study involving purging and without purging nitrogen under subcritical solvent extraction. From the previous sections, the optimal extraction condition was selected for this 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 suitable conditions for anthocyanin extraction gave a total anthocyanin content of 991.4, 1241.5 and 1552.1 µg cyanidin-3-glucoside/g dry weight of sample from the dried kernel, dried cob and dried silk, respectively. Table 2 shows that the extracted sample purged with nitrogen provided the higher anthocyanin yield than this without N_2 purging 1.27, 1.46 and 1.44 times for the dried kernel, dried cob and dried silk, respectively. This could be mainly due to the decrease of oxygen which causes anthocyanins oxidation (Routray and Orsat 2011).

Antioxidant Activity of Purple Waxy Corn Extracts using DPPH, ABTS and FRAP Methods

The extracts obtained from the optimal extraction conditions of dried kernel, dried cob and dried silk were evaluated for their free radical scavenging activity using DPPH, ABTS and FRAP. The optimal extraction conditions were; dried kernel to solvent ratio 1:20 and water to ethanol ratio1:3; dried cob to solvent ratio 1:20 and water to ethanol ratio 1:1; dried silk to solvent ratio 1:30 and water to ethanol ratio 1:1. The extraction temperature of 100 ^{o}C for 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 3.2 to 38.1 mg/mL were obtained. The extract of dried silk showed the greatest scavenging effect, followed by that of dried cob and died kernel, respectively (Table 3). Using ABTS assay, the antioxidant capacity ranged from 1.3 to 11.7 mg/mL; where dried silk extract displayed the highest scavenging activity, while dried kernel extract displayed the lowest scavenging effect. For FRAP assay, the antioxidant capacity ranged from 348.8 to 634.7 mmol $FeSO_4$/100 g dry weight of sample; where the dried silk extract displayed superior antioxidant (reducing effects) compared to other extract samples.

In addition, the values of DPPH IC50, ABTS IC50 and FRAP of dried kernel, dried cob and dried silk 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, the DPPH IC50 and ABTS IC50 values of dried silk extract, dried cob extract and dried kernel 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 the lower antioxidant activity than the ascorbic acid solution (IC50=0.07 mg/mL) and the Trolox solution (IC50=0.01 mg/mL).

As shown in Table 3, it can be concluded that the dried silk and dried cob extracts consistently exhibited high antioxidant activity. High amount of total anthocyanins in the dried silk and dried cob extracts (shown in Table 2) contributed to an increase in antioxidant activity. However, the dried silk extract had the greatest total anthocyanin content and antioxidant activity to inhibit 50% of free DPPH and ABTS radicals and also gave the higher FRAP value than others.

Application in Food

In this section, the extract derived from the suitable extraction condition (ratio of dried cob to solvent 1:20 (water to ethanol 1:1), extraction temperature of 100 ^{o}C and extraction time for 15 min with purging N_2) was selected to produce as colourant powder and as an antioxidant in water ice pop product. The results showed that the total anthocyanin content of the freeze-dried extract powder was 632.8 µg cyanidin-3-glucoside/g dry weight of powder sample. Additionally, physical characteristics of the freeze-dried extract powder were analysed. It was found that the freeze-dried sample contained total soluble solid (°Brix) of 2.0, moisture content of 1.1%, water activity of 0.1 and solubility of 94.7. Its colour values measured as L*, a*, b* values were 57.3, 14.1 and 6.8, respectively.

Furthermore, the concentrated extract was further utilized to produce water ice pop product. The water ice pop product with 26.5% w/w concentrated extract had water, milk powder, stabilizer, sugar, salt and citric acid approximately 26.5, 60.5, 0.50, 0.03, 12.17, 0.05 and 0.25 %w/w, respectively. The average weight for each water ice pop product was 33.46 grams per portion. Each water ice pop product contained the total anthocyanin content of 29.4 micrograms of cyanidin 3-glucoside equivalents. According to the results it was possible to apply the dried cob extract as natural food colourant or food ingredients.

CONCLUSION

This study reported the proper extraction conditions for anthocyanins from dried kernel, dried cob and dried silk as follows: sample-solvent ratio 1:20, 1:20, and 1:30, respectively, water-ethanol ratio 1:3, 1:1 and 1:1, respectively, the extraction temperature of 100 ^{o}C and the extraction time of 15 min with N_2 purging. These extraction conditions yielded high total anthocyanin levels of 991.4, 1241.5 and 1552.1 µg cyanidin-3-glucoside/g dry weight of sample from the dried kernel, dried cob and dried silk, respectively. The extract results showed that the dried silk extract had the greatest antioxidant activity. The 50% inhibitory concentration values obtained by DPPH and ABTS assays with the dried silk extract were 3.2 and 1.3 mg/mL, respectively, and the antioxidant activity by the FRAP assay was 634.8 mmol $FeSO_4$/100 g dry weight of sample. Therefore the subcritical solvent extraction process reported here could be applicable in food processing industries. By this process, the extract of purple waxy 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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TABLE 1. TOTAL ANTHOCYANIN CONTENT AND ANTHIOXIDANT ACTIVITIES OF EXTRACTS FROM DRIED HUSK, DRIED KERNEL, DRIED COB, AND DRIED SILK (EQUIVALENT DRY WEIGHT) OF PURPLE WAXY CORN

	husk	kernel	cob	silk
total anthocyanin content (μg cyanidin-3-glucoside/g dry weight of sample)	118.8±7.0^{d}	506.9±4.8^{a}	359.6±7.2^{b}	377.1±15.9^{b}
Antioxidant activities				
DPPH$^{\bullet}$ (mg/mL, IC_{50})	8.9±0.8^{c}	44.9±3.2^{a}	26.6±4.6^{b}	1.9 ±0.2^{d}
$ABTS^{\bullet+}$ (mg/mL, IC_{50})	1.9±0.1^{c}	28.4±0.4^{a}	10.9±1.1^{b}	1.1±0.1^{c}
FRAP (mmol $FeSO_4$/100 g dry weight of sample)	65.6±1.0^{d}	123.9±6.6^{b}	115.9±42.8^{c}	279.1±4.7^{a}

Mean value ± standard deviation of triplicates (n=3). Means in the same row followed by different superscript letters are significantly different at $p \leq 0.05$.

TABLE 2. THE EFFECT OF OXYGEN ON THE TOTAL ANTHOCYANIN CONTENT EXTRACTED FROM PURPLE WAXY CORN UNDER SUBCRITICAL SOLVENT EXTRACTION

	Total Anthocyanin Content (µg cyanidin-3-glucoside/g dry weight of sample)		
	Dried kernel*	Dried cob†	Dried silkπ
with N_2 purging	991.4±9.1[a]	1241.5±7.9[a]	1552.1±22.3[a]
without N_2 purging	779.7±3.9[b]	850.5±14.3[b]	1074.3±11.2[b]

Means ± standard deviation (n=3) in the column with the different superscript letters which are significantly different at $p \leq 0.05$.

* ratio of dried kernel to solvent 1:20 (water to ethanol 1:3), extraction temperature of 100 °C and extraction time for 15 min.

† ratio of dried cob to solvent 1:20 (water to ethanol 1:1), extraction temperature of 100 °C and extraction time for 15 min.

π ratio of dried silk to solvent 1:30 (water to ethanol 1:1), extraction temperature of 100 °C and extraction time for 15 min.

TABLE 3. COMPARISON OF ANTIOXIDANT ACTIVITIES IN EXTRACTS FROM DRIED KERNEL, DRIED COB AND DRIED SILK MEASURED BY THE DPPH, ABTS AND FRAP METHODS

	Antioxidant activities of extracts						
	Dried kernel*			Dried cob†			
	DPPH• (mg/mL, IC_{50})	ABTS•+ (mg/mL, IC_{50})	FRAP (mmol $FeSO_4$/100 g dry weight of sample)	DPPH• (mg/mL, IC_{50})	ABTS•+ (mg/mL, IC_{50})	FRAP (mmol $FeSO_4$/100 g dry weight of sample)	DPP (mg/ IC_5
With N_2 purging	38.1±6.6^{ns}	11.7±0.0^{b}	358.3±14.5^{a}	3.5±0.3^{ns}	3.0±0.1^{b}	348.8±24.8^{a}	3.2±0
Without N_2 purging	31.7±3.0 ^{ns}	26.2±0.8^{a}	321.8±5.5^{b}	4.1±0.2 ^{ns}	4.8±0.3^{a}	289.2±10.3^{b}	3.4±0

Means ± standard deviation (n=3) in the column with the different superscript letters which are significantly different at $p \leq 0.05$.

ns = not significant

* ratio of dried kernel to solvent 1:20 (water to ethanol 1:3), extraction temperature of 100 °C and extraction time for 15 min.

† ratio of dried cob to solvent 1:20 (water to ethanol 1:1), extraction temperature of 100 °C and extraction time for 15 min.

π ratio of dried silk to solvent 1:30 (water to ethanol 1:1), extraction temperature of 100 °C and extraction time for 15 min.

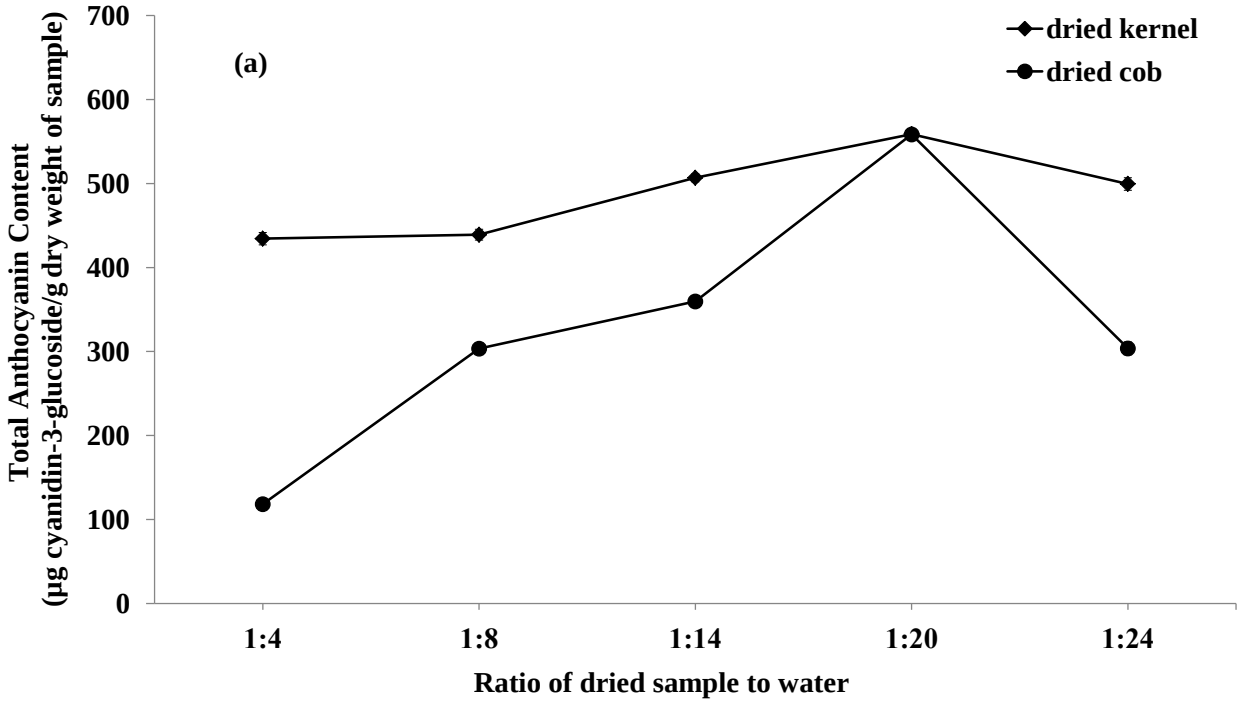
FIGURE CAPTIONS

FIG. 1. EFFECT OF WEIGHT RATIO OF PURPLE WAXY CORN SAMPLES TO WATER ON THE TOTAL ANTHOCYANIN CONTENT AT A CONSTANT EXTRACTION TEMPERATURE 100 °C AND 15 MIN

FIG. 2. THE TOTAL ANTHOCYANIN CONTENT EXTRACTED FROM DRIED KERNEL, DRIED COB AND DRIED SILK OF PURPLE WAXY CORN UNDER SUBCRITICAL WATER CONDITION OVER THE TEMPERATURE RANGE OF 60 TO 120 ^{o}C

FIG. 3. THE TOTAL ANTHOCYANIN CONTENT EXTRACTED FROM DRIED KERNEL, DRIED COB AND DRIED SILK OF PURPLE WAXY CORN UNDER SUBCRITICAL WATER CONDITION OVER THE TIME RANGE OF 15 TO 60 MIN

FIG. 4. THE TOTAL ANTHOCYANIN CONTENT EXTRACTED FROM DRIED KERNEL, DRIED COB AND DRIED SILK OF PURPLE WAXY CORN UNDER SUBCRITICAL SOLVENT CONDITION AS ADJUSTED BY WATER TO ETHANOL RATIO


(a)
dried kernel
dried cob
Total Anthocyanin Content
(µg cyanidin-3-glucoside/g dry weight of sample)
700
600
500
400
300
200
100
0
1:4
1:8
1:14
1:20
1:24
Ratio of dried sample to water

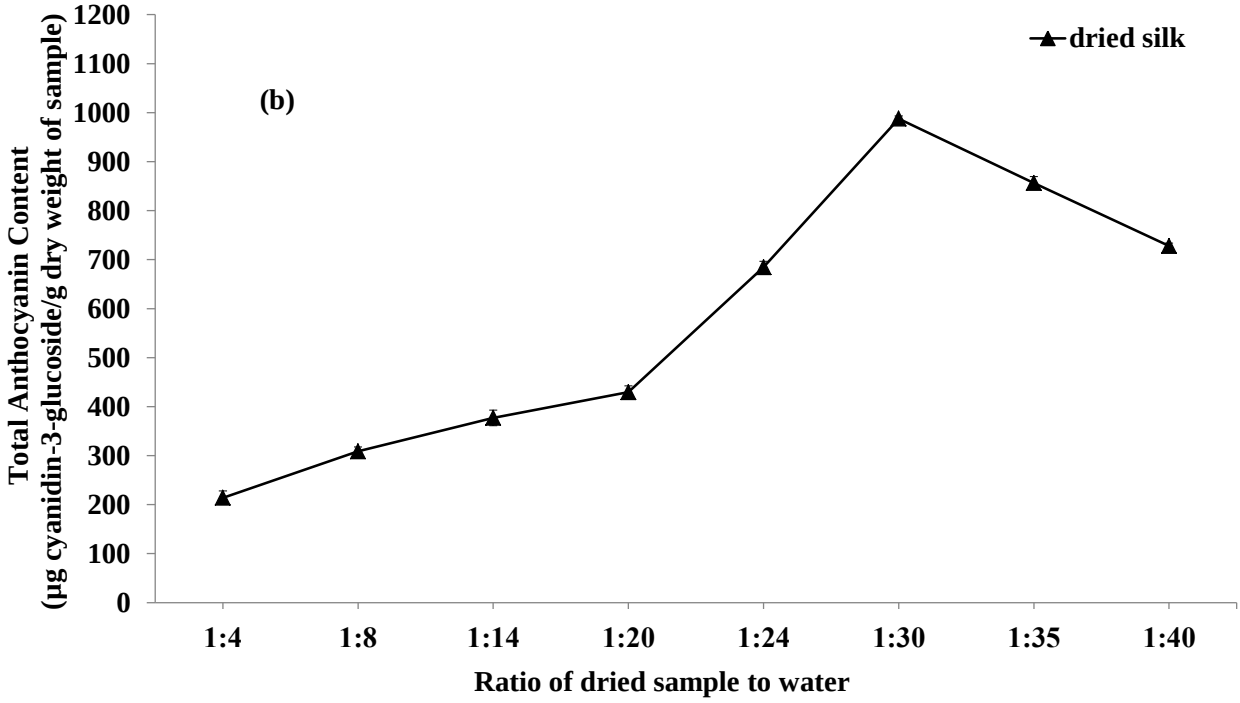

(b)
dried silk
Total Anthocyanin Content
(µg cyanidin-3-glucoside/g dry weight of sample)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1:4
1:8
1:14
1:20
1:24
1:30
1:35
1:40
Ratio of dried sample to water

FIG. 1

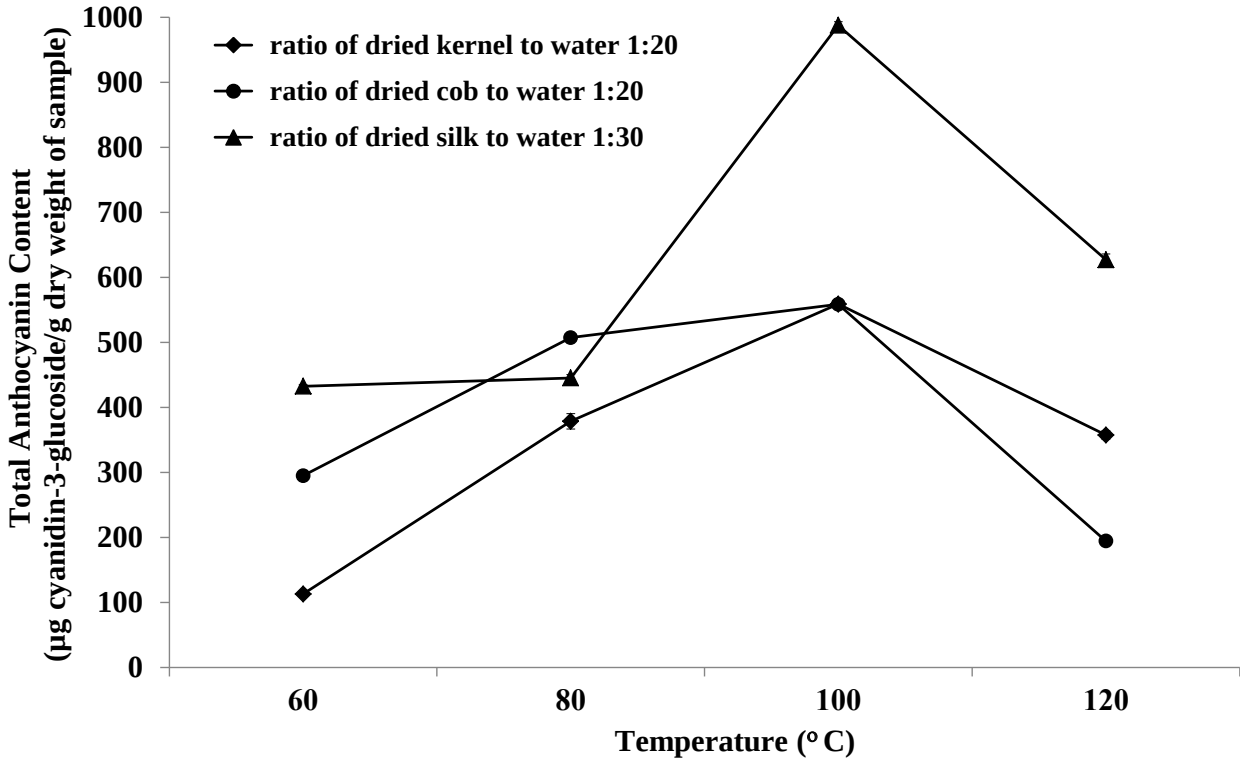

ratio of dried kernel to water 1:20
ratio of dried cob to water 1:20
ratio of dried silk to water 1:30
Total Anthocyanin Content
(µg cyanidin-3-glucoside/g dry weight of sample)
1000
900
800
700
600
500
400
300
200
100
0
60
80
100
120
Temperature (° C)

FIG. 2

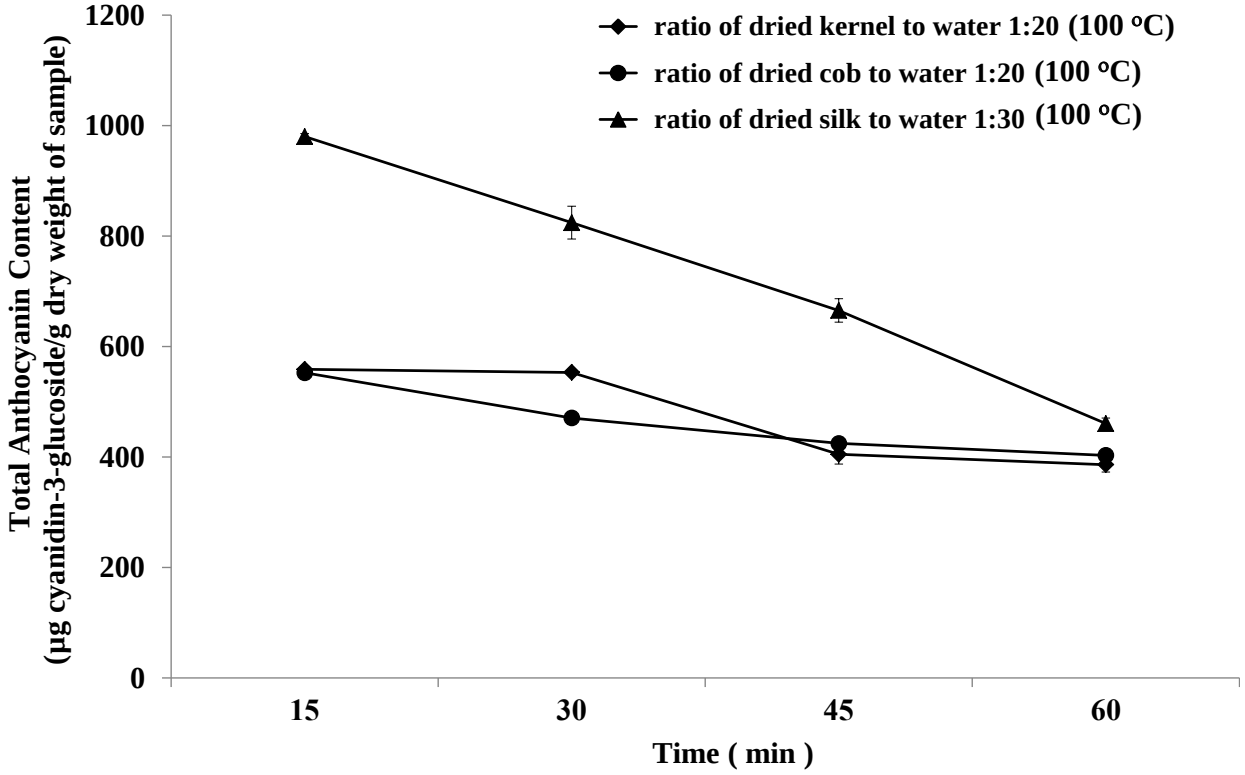

ratio of dried kernel to water 1:20 (100 °C)
ratio of dried cob to water 1:20 (100 °C)
ratio of dried silk to water 1:30 (100 °C)
Total Anthocyanin Content
(µg cyanidin-3-glucoside/g dry weight of sample)
1200
1000
800
600
400
200
0
15
30
45
60
Time (min)

FIG. 3

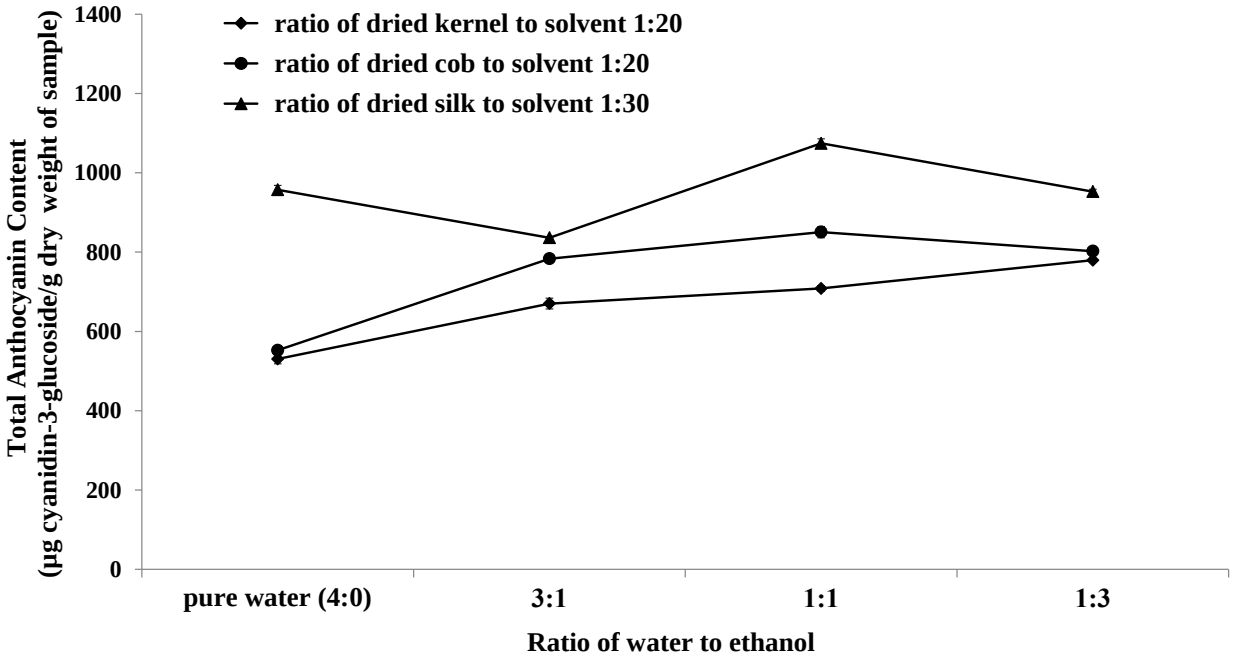

ratio of dried kernel to solvent 1:20
ratio of dried cob to solvent 1:20
ratio of dried silk to solvent 1:30
Total Anthocyanin Content
(μg cyanidin-3-glucoside/g dry weight of sample)
1400
1200
1000
800
600
400
200
0
pure water (4:0)
3:1
1:1
1:3
Ratio of water to ethanol

FIG. 4