

# Optimization of Ultrasonic Assisted Extraction (UAE) of Anthocyanins from Black Glutinous Rice and Evaluation of Their Antioxidant Properties

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**Abstract**—In the present study, the green technological approach - Ultrasonic Assisted Extraction (UAE) was employed for the extraction of anthocyanins, total phenolic compounds (TPC) and their antioxidant activities from black glutinous rice. The effects of ethanol concentration, amplitude and time were investigated using Box – Behnken design and extraction conditions were optimized by using response surface methodology (RSM). The optimal ethanol concentration, extraction time and amplitude were 70%, 5 min and 65% respectively. The maximum anthocyanin content, TPC and antioxidant activity were found as 50.33 mg cyn-3-glucosidase/gram fresh weight ml, 136.17 mg GAE/g, 80.14% for DPPH and 239.94mg AAE/g. Statistically, only concentration of ethanol indicated significant effect on the extraction. The ethanol concentration of 55% gave high yield of anthocyanins, TPC and antioxidant activities. In the case of amplitude and time, there was no significant difference compared to ethanol concentration. This study to a certain extent revealed important bioactive properties of black glutinous rice. i.e, the high antioxidant activity, presence of anthocyanins and TPC.

**Index Terms**—antioxidant activity, anthocyanins, Black glutinous rice, total phenolic content

## I. INTRODUCTION

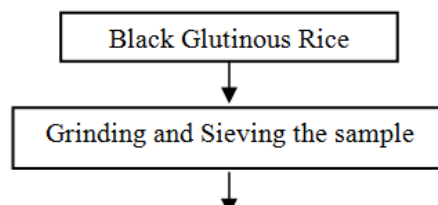
Rice (*Oryza Sativa Linn*) is an important cereal crop found in the country like Thailand, which is one of the world's largest rice exporter. According to 2001 survey, Thailand is in the ninth position for the cultivation of black glutinous rice [1]. These are found in pigmented varieties, such as black, purple and red rice which have a higher content of phenolic compounds than the white rice [2]. Black rice is one type of rice varieties, which is glutinous and have high nutritional value compared to other pigmented rice [1]. Its also known as Kings rice, Imperial rice, and Prized rice. The consumption of black glutinous rice is in the form of speciality food such as beverage drink rather than the stable food [1].

Black glutinous rice is free from gluten, cholesterol and low in sugar, salt, and fat [3]. The kernel of black glutinous rice is black because of the accumulation of anthocyanins, which is a flavonoid, an antioxidant moreover, black rice are rich in nutrients such as protein, minerals (Ca, P, Fe, and Zn) and dietary fiber contents [4]. The total anthocyanin content in the black glutinous rice ranges from 109.50 to 256.60 mg 100g<sup>-1</sup> cyanidin-3-glucoside [5]. The health benefits of anthocyanins are inhibitory effects on cancer cell proliferation, anti-inflammatory, atherosclerosis and antiviral. The high antioxidant activity of anthocyanins in sticky rice like black glutinous rice helps to prevent the harmful effects of free radicals [6].

Recently, there is an increasing interest in anthocyanins which are extracted from the plant based sources. This can be used as synthetic dyes because of their attractive color and water solubility which allow them to incorporate into the food. To overcome the serious analytical limitation, the mathematical model that gives the accurate and combined effects of different factors, is the promising approach to obtain functional extract from the black glutinous rice [7]. Thus, the objective of this study is to optimize the extraction of anthocyanins from the black glutinous rice by green technological approach – ultrasonic assisted extraction. This study also aimed at the extraction and modeling through Response Surface Methodology (RSM) to optimize the response variables such as anthocyanins content, Total Phenolic Content (TPC) and antioxidant activities of the black glutinous rice.

## II. MATERIALS AND METHODS

### A. Overall Experimental Design



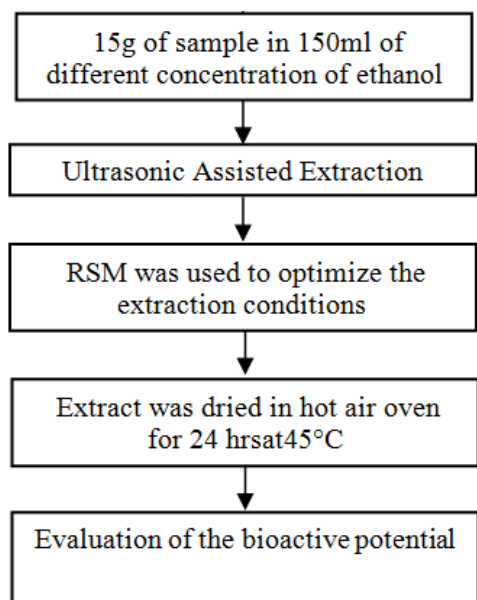


Figure 1. Flow chart showing the overall experimental design.

### B. Sample Preparation and Experimental

Black Glutinous Rice (Khaothong) was collected from the local Thai market, Talad Thai, Pathumthani, Thailand. The sample was grinded and made it into fine powder. After that, the fine powder was sieved using 40 mesh size and it was stored immediately at 8 °C for further analysis. The Fig. 1 explain the overall design of the experiment. Later, RSM was used to optimize the extraction conditions and independent variables are presented in "Table I". The anthocyanin content, total phenolic content and antioxidant activity were determined as the response variables.

TABLE I. CODED AND UNCODED LEVELS OF THE INDEPENDENT VARIABLES

Symbol	Independent variables	units	Coded levels		
			-1	0	1
X1	Ethanol concentration	%	40	55	70
X2	Amplitude	%	30	65	100
X3	Time	min	5	10	15

The 15g of black glutinous rice powder was mixed with 150 ml of different concentration of ethanol. The Ultrasonic Assisted Extractor (UAE) was used for extraction by using RSM optimization condition. After the extraction, the extract got dried in hot air oven for 24 hrs at 40 °C. From the dried extract the TPC and antioxidant activities were determined.

### C. Determination of Anthocyanin Content (Ph Differential Method)

The determination of total anthocyanin content was mainly done using the method suggested by [8]. The fresh extract of 1 ml was made up to 10 ml using a volumetric flask by adding buffer of 0.025M potassium

chloride (pH-1). Similarly, another 1 ml of sample was made up to 10 ml using a volumetric flask by adding buffer 0.4 M sodium acetate (pH-4.5). The absorbance was measured by the spectrophotometer at 510 and 700 nm with pH 1 and pH 4.5 respectively. The following equations were used to calculate the absorbance and total anthocyanins content:

$$\text{Abs} = [(A_{510} - A_{700})_{\text{pH}1.0}] - [(A_{510} - A_{700})_{\text{pH}4.5}]$$

$$\text{TAC (mg/100g)} = \frac{\text{Abs} \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times L}$$

$$\epsilon = 26,900 \text{ L mol}^{-1} \text{ cm}^{-1}$$

$$L = 1 \text{ cm}$$

$$\text{MW} = 449.2 \text{ Da}$$

$$\text{DF} = \text{dilution factor}$$

### D. Total Phenolic Content (TPC) and Antioxidant Activity

The total phenolic content of the crude extract was determined by using Folin-Ciocalteu reagent with slight modification in the method described by [9]. The calibration curve was prepared using Gallic acid as a reference standard. TPC was expressed in mg Gallic acid equivalent (GAE)/g of dry extract. The antioxidant activity of the extracts was determined by 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH) and reducing power. The DPPH radical-scavenging activity of sample was determined by using the spectrophotometric method. The following equation was used to calculate the absorbance of DPPH activity:

$$\% \text{ DPPH inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The reducing power of extract was determined according to the method used by [10]. The calibration curve was prepared using ascorbic acid as a reference standard. Reducing Power was expressed in mg ascorbic acid equivalent (AAE) /g of dry extract.

## III. RESULTS AND DISCUSSION

### A. Optimization of Ultrasound - Assisted Extraction of Bioactive Compounds from Black Glutinous Rice Extracts

For optimized extraction condition ethanol concentration, extraction time and amplitude were set as 70%, 5 min and 65% respectively. The results associated with each response factors showed that ethanol concentration was highly significant on extraction of phenolic compounds and anthocyanins. For the extraction of anthocyanins and the phenolic compounds from the plant material, the ethanol concentration was directly related. It was mainly due to the fact that aqueous ethanol is more effective in extraction than alcohol alone.

### B. Effect of Process Variables on Anthocyanins Content and Total Phenolic Content (TPC)

It can be as depicted from Fig. 2 that, when the concentration of ethanol increasing ranging from 40 to

70 %, there was an increase in the anthocyanin content from 29.13 to 47.45 mg cyn-3-glucosidase/gram fresh weight ml. Ref. [11], reported that anthocyanins are more soluble in alcoholic compounds than the water. The anthocyanin content increased with respect to time and amplitude.

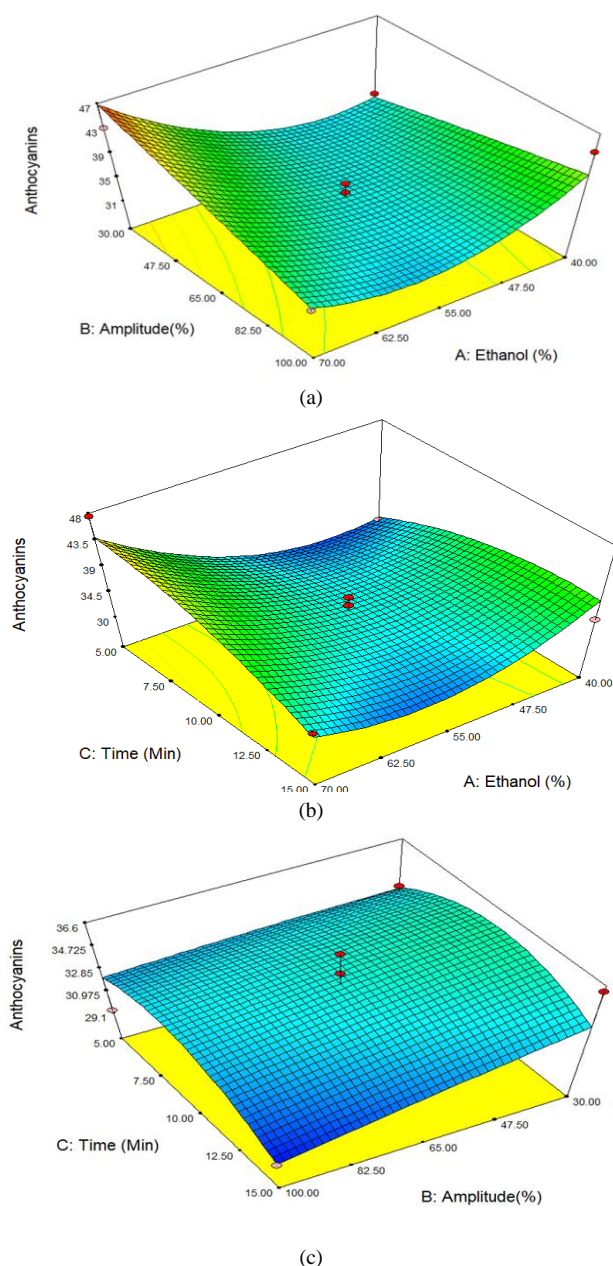


Figure 2. Response surface plots (a, b and c) showing the interactive effects of ethanol concentration, amplitude and time on anthocyanin content.

Additionally, from Fig. 3, it was observed that when the ethanol concentration was increased from 40 to 70%, there was an increase in the TPC ranging from 17.42 to 179.42 mg GAE/ g of extract. This is similar to the observation for the extraction of phenolic content from the grape, where higher yield was shown by 60% of ethanol concentration [12]. Extraction time and amplitude did not show any significant effect on the TPC and only

changed marginally which may be due to equilibrium state after certain time of extraction [8].

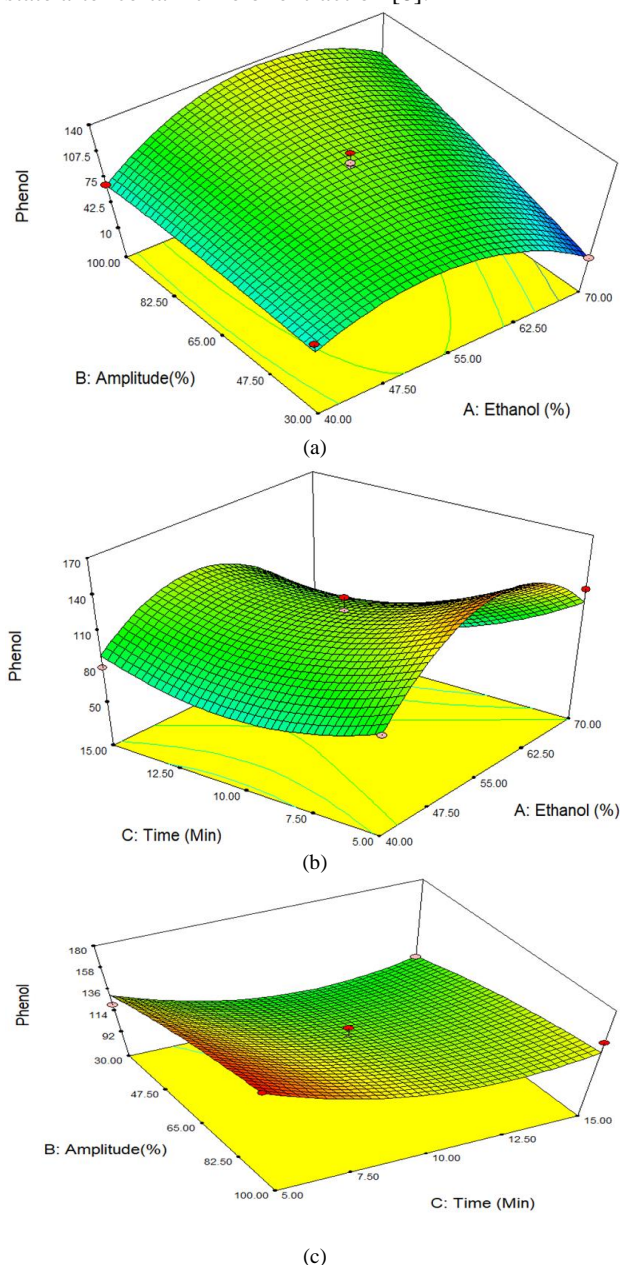
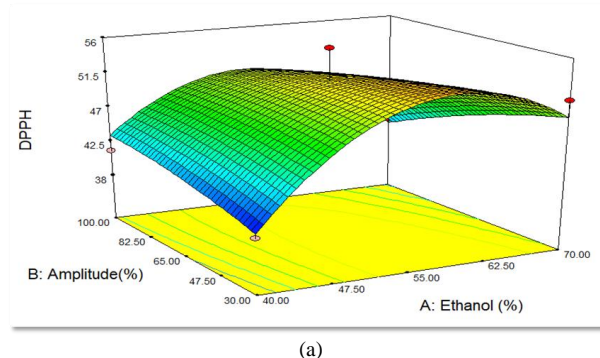


Figure 3. Response surface plots (a, b and c) showing the interactive effects of ethanol concentration, amplitude and time on total phenolic content.

### C. Effect of Process Variables on Antioxidant Activities





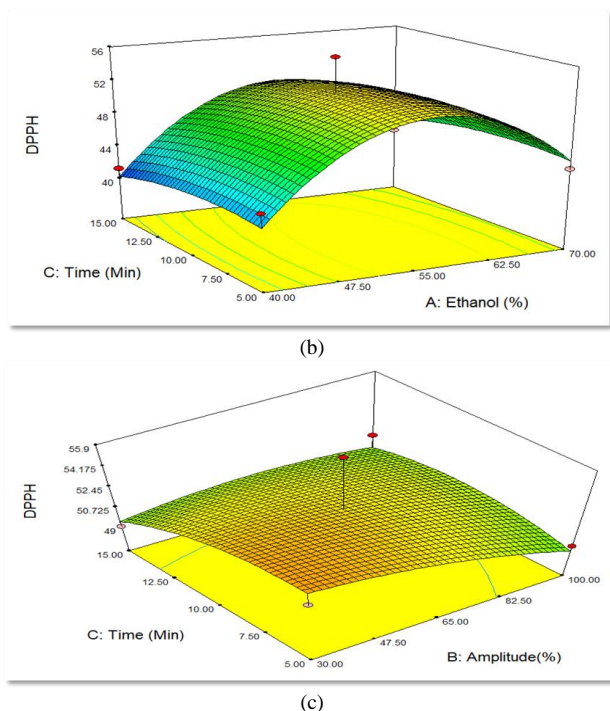


Figure 4. Response surface plots (a, b and c) showing the interactive effects of ethanol concentration, amplitude and time on the antioxidant capacity using DPPH assay.

In Fig. 4 interactive effects of ethanol concentration, amplitude and time on the antioxidant capacity using DPPH assay. The minimum and maximum value of antioxidant activity obtained for the black glutinous rice extract was observed as 38.89% and 55.9 % for DPPH inhibition. The higher inhibition activity was seen while the ethanol concentration was 60%. The extract which contain higher TPC and anthocyanins was exhibiting high antioxidant activity.

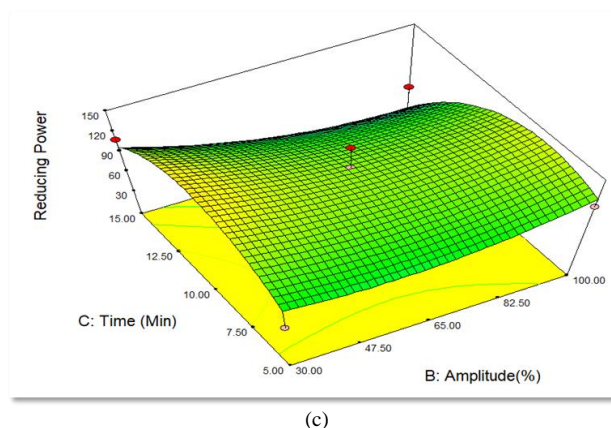
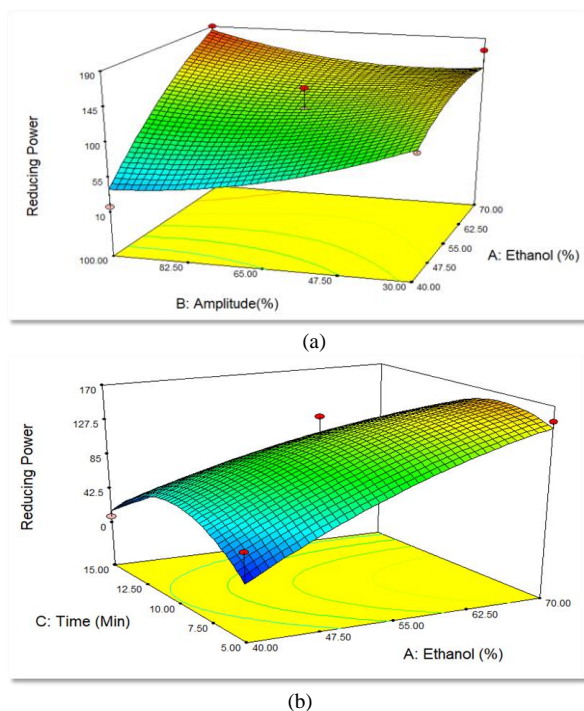


Figure 5. Response surface plots showing the interactive effects of ethanol concentration, amplitude and time on the antioxidant capacity using reducing power.

In Fig. 5 interactive effects of ethanol concentration, amplitude and time on the antioxidant capacity using reducing power. Similar effect was found in ethanol concentration on the antioxidant activity of wood apple and the association between TPC and antioxidant activity in [13]. Higher the amplitude, the DPPH activity was seen lower. It was mainly because of more cell disruptions which resulted in the production of more radicals [14].

The effect of different ethanol concentration, amplitude and time on the reducing power is shown in the Fig. 4. The maximum and minimum value for the reducing power was observed as 7.24 to 189.54 mg AAE/g of extract. It was also observed that when the concentration of ethanol was increasing, the reducing power also increased. In the case of amplitude, lower the amplitude, higher the value for reducing power and vice versa. This is due to the degradation of compounds in the higher ultrasonic extraction with respect to time[15]. The second order polynomial equation given for the predicting of response factor yield based on ultrasonic extractor are expressed in coded values as shown in "Table II".

TABLE II. EQUATION MODELS FOR THE RESPONSE FACTOR

Response factor	Model Equations
Anthocyanins	$Y_1 = + 34.26 + 1.90 * X_1 - 1.18 * X_2 - 0.67 * X_3 - 4.41 * X_1 * X_2 - 4.30 * X_1 * X_3 - 0.84 * X_2 * X_3 + 5.13 * X_1^2 - 0.10 * X_2^2 - 2.25 * X_3^2$
Total Phenolic Content	$Y_1 = + 119.48 - 4.90 * X_1 + 19.94 * X_2 - 17.15 * X_3 + 18.83 * X_1 * X_2 - 13.52 * X_1 * X_3 - 3.55 * X_2 * X_3 - 53.20 * X_1^2 - 4.74 * X_2^2 + 23.48 * X_3^2$
DPPH activity	$Y_1 = + 52.08 + 1.82 * X_1 - 0.97 * X_2 - 0.77 * X_3 - 2.93 * X_1 * X_2 + 0.12 * X_1 * X_3 + 1.07 * X_2 * X_3 - 8.20 * X_1^2 - 0.69 * X_2^2 - 1.02 * X_3^2$
Reducing Power	$Y_1 = + 121.55 + 43.92 * X_1 - 10.56 * X_2 - 21.97 * X_3 + 28.34 * X_1 * X_2 - 23.37 * X_1 * X_3 - 21.25 * X_2 * X_3 - 15.05 * X_1^2 + 16.79 * X_2^2 - 49.94 * X_3^2$

#### IV. CONCLUSION

The extraction of black glutinous rice was optimized by using the RSM methodology by varying the ethanol concentration, amplitude and time. The extract shows the higher values for the anthocyanins, phenolic compounds and antioxidant activities. Among three response variables, lower concentration of ethanol gave better results compared to higher concentration. The present study gives the information about optimized ethanol concentration to extract the bioactive compounds from the black glutinous rice powder. The presence of high anthocyanins and phenolic compounds contributes high antioxidant activities.

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