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Optimizing extraction of functional compounds from Indonesian black rice using response surface methodology

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Abstract

This study aimed to determine the optimum extraction condition of anthocyanin, flavonoid, total phenolic content, and antioxidant activity of Jeliteng varieties black rice using the heat-assisted maceration method. Variations in ethanol (50-60%), citric acid (3-5%), and temperature (45-55°C) were studied using a Box–Behnken design. The regression models were statistically significant ($P < 0.001$) with determination coefficient at ≥0.900. Extraction with ethanol 56.11% and citric acid 4.42% at 49.29°C rendered an extract with 109.6 mg/100 g of anthocyanins, 25.35 mg QE/100 g of flavonoids, 295.56 mg GAE/100 g total phenolic content, and antioxidant activity reached 61.69% of inhibition. Predicted values at the optimized conditions can be confirmed with experimental values.

1. Introduction

Pigmented rice cultivars such as black rice are found and consumed mainly by people in Asian countries. Anthocyanins are the main functional compounds of black rice (Nakagawa and Maeda, 2017), and cyanidin-3-glucoside compounds are the largest anthocyanin compounds in black rice which reach 88% (Abdel-Aal *et al.*, 2006). Anthocyanins are responsible for the black colour of black rice (Lee, 2010). Other anthocyanin compounds such as cyanidin-3-rutinoside, cyanidin 3,5-glucoside, malvidin 3 glucoside, and peonidin 3 glucoside are also found in black rice and have been confirmed to have several health benefits for the human body (Chen *et al.*, 2012; Hou *et al.*, 2013). As a secondary metabolite compound, anthocyanins have high solubility. This makes the extraction process the ideal method to obtain them.

Generally, the anthocyanin extraction process uses methanol, acetone, and ethanol as solvents. Although its effectiveness is not as good as methanol and acetone, ethanol is considered safer because it is less toxic than other solvents. Ethanol concentration is the most important factor in the anthocyanin extraction process, in relation to its molecular solubility (Khazaei *et al.*, 2016). Ethanol acidified with HCl has also been reported to increase the hydrolyzed anthocyanin content of black rice (Bae *et al.*, 2017). However, the use of HCl as an acidification medium remains a concern, given its very high toxicity.

Regarding acidified ethanol solvents, citric acid has been widely reported as one of the best ethanol acidification media for anthocyanin extraction, such as anthocyanin extraction in barberry, eggplant, and red cabbage (Hosseini *et al.*, 2016), anthocyanin extraction in blueberries (Xu *et al.*, 2016), purple sweet potato (Ekaputra and Pramitasari, 2020), *Carissa carandas* fruit (Le *et al.*, 2019), black carrot (Espinosa-Acosta *et al.*, 2018), red cabbage (Shiyan *et al.*, 2018), and black rice both in the form of whole-grain and by-products (Pedro *et al.*, 2016; Halee *et al.*, 2018). Citric acid plays a role in lowering the pH value of the solvent, this is related to the stability of the anthocyanin compounds at pH < 6 (Liu *et al.*, 2018). The stability of anthocyanin compounds will increase when the solvent has a pH value of 1-3. Anthocyanins are easier to isolate, characterized by the extract having a red-blue mixed colour (do Carmo Brito *et al.*, 2017).

In addition to ethanol concentration and pH, temperature level is also the most widely reported contributing factor in the anthocyanin extraction process (Silva *et al.*, 2017). The combination of ethanol concentration, citric acid, and temperature seems to be the most promising approach to obtaining functional

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extracts from black rice. Pedro *et al*. (2016) published a study on heat-assisted maceration extraction that focused on temperature (10-50°C), extraction period (20-80 mins), and solid-liquid ratio (1:15–1:45). According to Jha *et al*. (2017) and Ryu and Koh (2018), the extraction of functional compounds from black rice and black soybeans is best done at 45-60°C. While the optimal citric acid concentration and ethanol concentration for extracting functional compounds from black rice of Jeliteng varieties have yet to be determined. Based on this information, research related to the optimization of the extraction process of black rice of Jeliteng varieties functional compounds using a combination of ethanol concentration, citric acid concentration, and temperature needs to be carried out. The extraction process uses a response surface model (RSM) approach, which is used to develop and optimize the process and product conditions with the aim that can be used to determining the best conditions in the extraction process of black rice functional compounds (Granato *et al*., 2014).

This study aimed to create an optimal model for the extraction process of functional components of Jeliteng varieties of black rice using RSM based on the concentration of ethanol, citric acid, and temperature. The result is black rice extract high in anthocyanin, total phenolic content (TPC), flavonoid, and antioxidant activity (AA).

2. Materials and methods

2.1 Materials

Black rice of Jeliteng varieties was collected from organic rice farmers in the Karanganyar region, Central Java Province, Indonesia. Other materials include food-grade citric acid from PT Gunacipta Multirasa, distilled water, ethanol reagent, $C_2H_3NaO_2$, KCl, $AlCl_3$, CH_3COOK, $C_{15}H_{10}O_7$ (quercetin), Folin–Ciocalteu Reagent, Na_2CO_3 pro analysis from Merck, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and $C_7H_6O_5$ (gallic acid) pro analysis from Sigma–Aldrich.

2.2 Maceration extraction procedure of functional compounds

The extraction of black rice anthocyanins was referred to in the study of Pedro *et al.* (2016) and Jha *et al.* (2017) with modifications. Black rice was milled to flour. Approximately 100 g of black rice flour was added with ethanol (50-60% v/v) and citric acid (3-5% w/v) with a ratio of 1:10 (w/v). The extraction process was carried out in a thermostatic water bath at a controlled temperature (45-55°C) for 120 mins with constant stirring (500 rpm). The solution was then filtered using 400 mesh filter paper. The ethanol in the extract was then evaporated using a rotary vacuum evaporator at 60°C. The viscous extract was stored in a dark glass bottle at -20°C until analysed.

2.3 Experimental design

The Box-Behnken design was used to evaluate the effect of a combination of three independent variables (ethanol concentration, citric acid concentration, and temperature) in the extraction of bioactive compounds from Indonesian black rice of Jeliteng varieties. Values for ethanol concentrations of 50, 55, and 60%, citric acid concentrations of 3, 4, and 5%, and extraction temperatures of 45, 50, and 55°C were studied. The dependent variables are anthocyanins, flavonoids, TPC, and AA. The experimental design is presented in fifteen combinations (Table 1), including three centre point replicates to confirm errors and assess the incompatibility of the proposed model. All experiments were carried out by design and triplicated.

2.4 Analyses of anthocyanins

Analysis of the anthocyanin content of the extract was done using the differential pH method (Yamuangmorn *et al*., 2018). Approximately 1 mL of the extract was put into two dark test tubes. The first test tube was added with 1 mL of potassium chloride buffer (pH 1.0), and the second test tube was added with 1 mL of sodium acetate buffer (pH 4.5). Each solution was incubated for 15 mins at room temperature (25±1°C) impermeable to light. The absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 520 nm and 700 nm. The absorbance value was obtained by subtracting the difference in absorbance at a wavelength of 520 nm and 700 nm at pH 1 with the difference in absorbance at pH 4.5. Anthocyanin content was obtained by multiplying the absorbance value by the molecular weight of cyanidin-3-glucoside (448.8 g/mol) and the amount of dilution, then divided by the coefficient of molar absorptivity of cyanidin-3-glucoside (26900 l/mol cm) and the width of the cuvette (1 cm). The anthocyanin content of black rice extract was expressed in mg/100 g.

2.5 Analyses of flavonoids

The determination of the flavonoid content was done with the modified method of Cai *et al.* (2016). A 0.5 mL extract sample was prepared in a dark tube, to which 1.5 mL of ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COOK, and 2.8 mL of distilled water were added. The solution was homogenized and incubated at room temperature (25±1°C) for 30 mins. The absorbance of the sample was measured using a UV-Vis spectrophotometer with a wavelength of 415 nm. Distilled water is used as a blank solution. The standard curve uses a solution of quercetin in distilled water with

Table 1. The Box–Behnken design and experiment data

No	Independent variables and coded			Response variables			
	Ethanol (%)	Citric Acid (%)	Temperature (°C)	Anthocyanin (mg/100 g)	Flavonoids (mg QE/100 g)	TPC (mg GAE/100 g)	AA (% of inhibition)
1	50 (-1)	3 (-1)	50 (0)	44.05±1.42	21.60±0.14	277.48±0.85	53.48±0.41
2	50 (-1)	4 (0)	55 (1)	65.57±2.12	25.43±0.19	270.28±0.85	56.16±0.25
3	50 (-1)	4 (0)	45 (-1)	59.06±0.00	21.13±0.24	299.08±0.85	56.10±0.16
4	50 (-1)	5 (1)	50 (0)	74.58±0.71	22.13±0.14	281.28±1.13	57.84±0.16
5	55 (0)	3 (-1)	45 (-1)	48.05±1.42	22.03±0.28	279.88±0.85	55.52±0.16
6	55 (0)	3 (-1)	55 (1)	50.55±0.71	26.73±0.05	304.68±1.41	55.57±0.25
7	55 (0)	5 (1)	45 (-1)	98.60±0.71	23.23±0.00	282.68±1.41	59.76±0.08
8	55 (0)	5 (1)	55 (1)	91.60±0.71	26.93±0.14	307.28±1.13	59.29±0.08
9	60 (1)	3 (-1)	50 (0)	72.58±0.71	22.63±0.09	283.88±0.28	57.38±0.33
10	60 (1)	4 (0)	45 (-1)	72.07±1.42	20.90±0.38	273.08±1.41	56.97±0.08
11	60 (1)	4 (0)	55 (1)	100.60±0.71	26.00±0.05	297.68±1.13	60.63±0.00
12	60 (1)	5 (1)	50 (0)	82.59±0.71	23.00±0.14	288.48±0.57	58.13±0.08
13	55 (0)	4 (0)	50 (0)	105.61±0.71	24.27±0.24	288.08±1.70	61.79±0.33
14	55 (0)	4 (0)	50 (0)	108.61±0.71	24.37±0.09	287.68±3.96	62.20±0.08
15	55 (0)	4 (0)	50 (0)	106.11±1.42	24.07±0.14	287.68±0.00	61.73±0.08

a concentration range of 20-100 ppm. The flavonoid content of black rice extract was expressed as mg QE/100 g.

2.6 Analyses of total phenolic content

The Folin-Ciocalteu method (Pedro *et al*., 2016), with slight modifications, was used in the analysis of TPC. The 0.5 mL of black rice extract was prepared in a dark tube and then 5 mL of Folin-Ciocalteu 10% (v/v) reagent was added. The solution was homogenized for 5 mins and added 4 mL of 7.5% Na_2CO_3 (w/v). The mixture was incubated for 60 mins at room temperature (25±1°C). Ethanol is used as a blank solution. Gallic acid in ethanol with a concentration of 100-500 ppm is used as a standard solution. Next, the absorbance was measured using a UV-Vis spectrophotometer with a wavelength of 765 nm. TPC of black rice extract was expressed as mg GAE/100 g.

2.7 Determination of free-radical scavenging activity

AA was determined using Pedro *et al*. (2016) method, with a few modifications. 1.5 mL of 0.2 mM DPPH ethanol was mixed with 0.2 mL of the sample into a test tube, and ethanol was added to a final volume of 3.5 mL. The tubes were tightly closed, homogenized, and incubated at room temperature (25±1°C) for 60 mins. The absorbance was measured at a wavelength of 517 nm. The ability of the extract to scavenge DPPH was obtained by subtracting the absorbance of the blank with the sample. The result was then compared with the absorbance of the blank and expressed in the percentage of inhibition.

2.8 Statistical analyses

Design-Expert (version 11.1.2.0) was used in determining the experimental design and data analysis. Prediction model using statistical analysis and analysis of variance (ANOVA). The suitability of the polynomial model equation is expressed by the coefficient of determination (R^2).

3. Results and discussion

3.1 Optimization and modelling of the extraction process from black rice

Optimization of the extraction process refers to the components of anthocyanins, flavonoids, TPC, and AA of black rice extract. The experimental design used the Box-Behnken design to identify different independent variables. In general, the ANOVA test (Table 2) from fifteen trials produced a very significant model ($p < 0.01$). The R^2 Adjusted values of the levels of anthocyanins, flavonoids, TPC, and AA of the extracts were 0.884, 0.981, 0.967, and 0.944, respectively, and the p-values (lack of fit) 0.052 for anthocyanins, 0.129 for flavonoids, 0.233 for TPC, and 0.054 for AA. Thus, the model is very significant and suitable as experimental data. The regression coefficient data can be used to obtain predictions of polynomial equations. The three-dimensional surface plot refers to the obtained model and is used to assess the relationship between the dependent and independent factors.

3.2 Effect of independent variables on anthocyanin contents

The linear effect had no effect on anthocyanins, but the quadratic effect had a substantial negative effect. The anthocyanin content of the extract was significantly

FULL PAPER

Table 2. Significance level of ANOVA and regression coefficient value of quadratic model

Coefficients		Estimated coefficients			
		Anthocyanins	Flavonoids	TPC	AA
Intercept		106.78	24.23	287.81	61.9
Linear	X_1 ethanol (%)	10.57**	0.28**	1.87**	1.19**
	X_2 Citric acid (%)	16.52**	0.29**	1.72**	1.63**
	X_3 Temperature (°C)	3.82	2.23**	12.85**	0.41*
Interactions	$X_1 X_2$	-5.13	-0.04	0.20	-0.90**
	X_1X_3	5.51	0.20*	-1.05	0.90**
	X_2X_3	-2.38	-0.25*	-0.05	-0.13
Quadratic	X_1^2	-18.10**	-1.63**	-6.82**	-2.64**
	X_2^2	-20.23**	-0.26*	1.78*	-2.56**
	X_3^2	-14.35**	0.76**	4.03**	-1.81**
p-value (lack of fit)		0.052	0.129	0.233	0.054
R^2		0.921	0.987	0.978	0.962
R^2 Adjusted		0.884	0.981	0.967	0.944
F value (model)		24.78**	165.07**	92.60**	53.33**

*significant at 0.05 level, **significant at 0.01 level

influenced by the linear effect of the concentration of ethanol and citric acid ($p < 0.01$). The effect from the temperature level was not significant ($p > 0.05$), although the trend was positive. This condition is believed to be related to the extraction temperature range being in the optimal range. The same condition was also reported by Ryu and Koh (2018), the extraction temperature of 50 - 60°C had no significant effect on the anthocyanin content of black soybean extract, although there was a tendency to increase. Jha *et al.* (2017) more specifically reported that the optimal anthocyanin extraction temperature was 49.46°C, while the maximum anthocyanin content was obtained in this study at 51.45° C extraction temperature.

Figure 1A-1C is the three-dimensional plot of the three variables on the anthocyanin content of the extract (44.05 – 108.61 mg/100 g). Based on estimated coefficients (Table 2), citric acid plays an important role in triggering the release of anthocyanins from the material, followed by the concentration of ethanol. Citric acid is a weak acid, the addition of an ethanol-water solvent generally results in a solution pH of about 2-3. It is not possible to adjust the pH of the extract to a lower level even if the concentration is increased. Under these conditions, anthocyanins will maintain the form of flavylium that has good stability (Yang *et al.*, 2010; Le *et al.*, 2019). Citric acid is also easier to diffuse into the plant matrix, which makes the anthocyanin hydrolysis process easier (Kurtulbaş *et al.*, 2020).

Anthocyanin levels are positively correlated to ethanol concentrations. However, when the ethanol concentration reached 57%, the anthocyanin extract decreased drastically. According to Jha *et al*. (2017) and Le *et al*. (2019), the most optimal anthocyanin solubility is at 55% ethanol concentration. In addition, the decrease in anthocyanin levels as the ethanol concentration increases is also caused by the presence of unwanted impurities being isolated from the material, which will affect the quality of anthocyanins. However, the anthocyanin content at the highest ethanol concentration (60%), was still better than at the lowest ethanol concentration (50%). Based on these data, the prediction

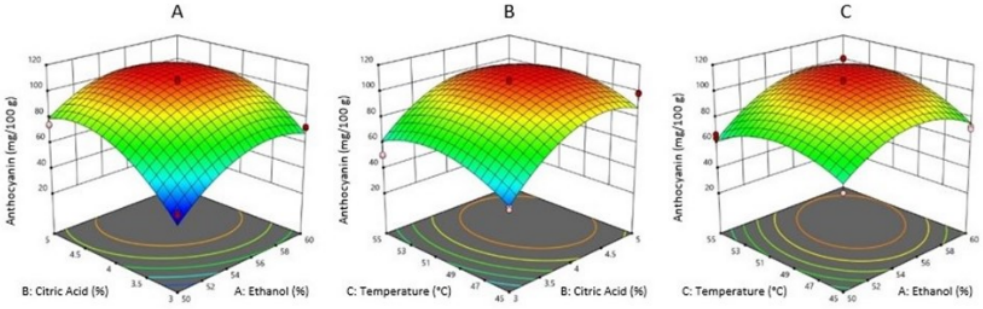

Figure 1. Response surface graphs and contour plots for the effects of (A) citric acid and ethanol concentration, (B) extraction temperature and citric acid concentration, and (C) extraction temperature and ethanol concentration on anthocyanin content of black rice extract

of the polynomial equation for the anthocyanin content of black rice extract is:

$$Y\ (anthocyanin) = 106.78 + 10.57X_1 + 16.52X_2 + 3.82X_3 - 5.13X_1X_2 + 5.51X_1X_3 - 2.38X_2X_3 - 18.10X_1^2 - 20.23X_2^2 - 14.35X_3^2$$

3.3 Effect of independent variables on flavonoids

Linear effect positively affects the flavonoids content of black rice extract, while quadratic and interaction effects, generally, negatively affect flavonoid content. Extraction temperature had the strongest positive linear effect among other variables which makes the flavonoid content of black rice extract increase significantly. Many researchers reported the effect of temperature on flavonoid extraction. Rajha *et al.* (2014) obtained the optimal temperature for solid-liquid extraction of flavonoids from grape by-products at 93°C. Meanwhile, the temperature of 94.66°C was the most optimal to obtain high flavonoid Flos populi extract (Sheng *et al.*, 2013). Increasing the extraction temperature causes a decrease in solvent viscosity, which is followed by an increase in molecular movement that causes an optimal release of bioactive compounds from black rice.

A high concentration of citric acid generally causes the pH of the solvent to decrease. Here, the concentration of citric acid is positively correlated with flavonoids, although the increase tends to be stable (Figures 3A and 3B). In previous studies, flavonoids are easier to extract at a high pH condition. The polarity of bioactive compounds at high pH will result in higher dissociation of -OH groups with a result the solubility of bioactive compounds increases. Flavonoids will be optimal when the pH of the solvent is between 4.5 – 6 (Karvela *et al.*, 2009). Recent reports support this study, flavonoids are more easily hydrolyzed at a pH of 3.24 (Soquetta *et al.*, 2019). Mai *et al.* (2020) also investigated the effect of solvent pH on the recovery of *Euonymus alatus* and suggested using a low pH (2.5 – 3.5). Each type of plant has a different amount and type of flavonoid. The position of the -OH group of each compound also affects the flavonoid content of the extract.

Different results were shown by the ethanol concentrations. Flavonoids increased until the ethanol concentration reached 55%, then began to slope and decrease rapidly when the ethanol concentration was increased further (Figure 2A and 2C). Ethanol is known to be very efficient in the extraction of flavonoids and their glycosides (Sheng *et al.*, 2013). The presence of water in the ethanol solvent has a positive impact on the extraction process, water will facilitate mass transfer between solids and liquids by increasing the permeability of the plant matrix with the result that the extraction efficiency is better. The type of flavonoid also affects the extraction process. Less polar flavonoids such as

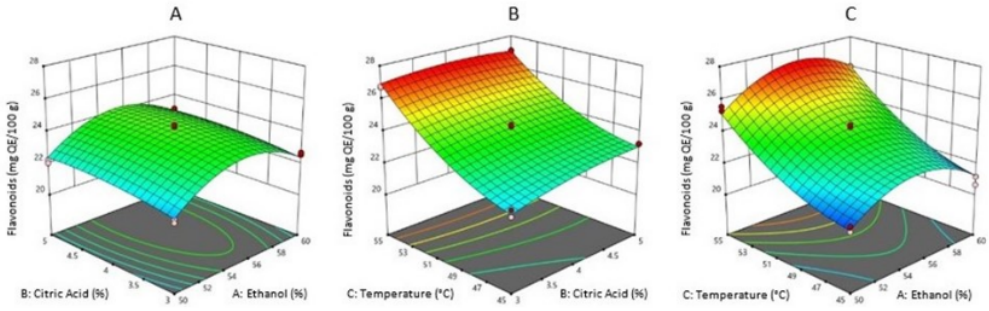

Figure 2. Response surface graphs and contour plots for the effects of (A) citric acid and ethanol concentration, (B) extraction temperature and citric acid concentration, and (C) extraction temperature and ethanol concentration on flavonoid content of black rice extract

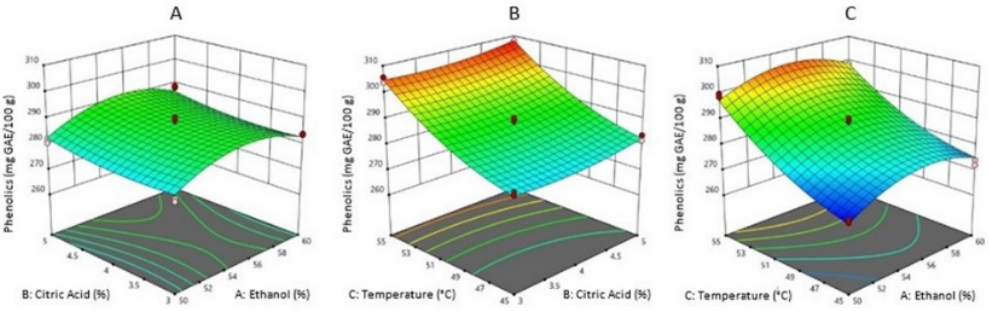

Figure 3. Response surface graphs and contour plots for the effects of (A) citric acid and ethanol concentration, (B) extraction temperature and citric acid concentration, and (C) extraction temperature and ethanol concentration on TPC of black rice

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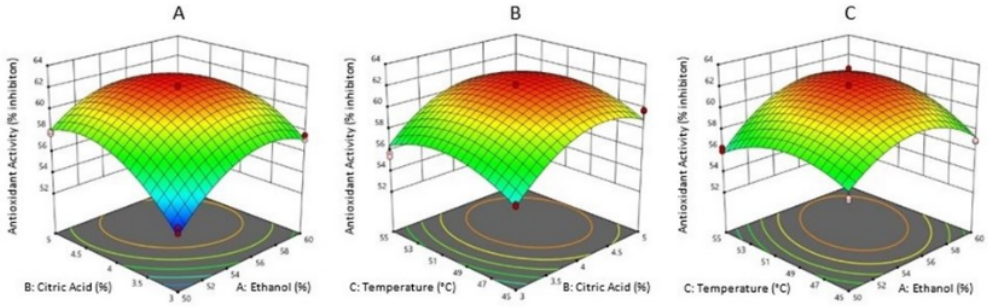

Figure 4. Response surface graphs and contour plots for the effects of (A) citric acid and ethanol concentration, (B) extraction temperature and citric acid concentration, and (C) extraction temperature and ethanol concentration on AA of black rice extract

flavonols, aglycones, and flavanones generally will optimally decompose in chloroform, acetone, ethyl acetate, and hexane solvents (Chaves *et al*., 2020). After applying the response surface regression method to black rice extract flavonoids, the polynomial equations were as follows:

$$Y\ (flavonoids) = 24.23 + 0.28X_1 + 0.29X_2 + 2.23X_3 - 0.04X_1X_2 + 0.20X_1X_3 - 0.25X_2X_3 - 1.63X_1^2 - 0.26X_2^2 + 0.76X_3^2$$

3.4 Effect of independent variables on total phenolic compounds

The integration effect does not affect TPC. However, linear and quadratic effects have a significant effect ($p < 0.05$) on TPC. Temperature is the main factor that affects the TPC of black rice extract. Increasing the temperature increased the TPC by 37 mg GAE/100 g (13.69%). Wang *et al.* (2016) also reported that increasing the extraction temperature from 50 – 70°C, succeeded in increasing the TPC of blueberries extract by 14.48% (43 mg GAE/100 g), and red pear peels extract by 23.33% (42 mg GAE/100 g). Higher temperatures can separate the TPC into smaller structures which cause the viscosity and surface tension to decrease, thereby increasing the solubility in solvents. This condition does not apply when the extraction time is increased. Long extraction time in high temperatures causes degradation of TPC (Yilmaz and Toledo, 2006).

TPC decreased when the ethanol concentration was more than 55% (Figures 4B and 4C). Ethanol concentration was reported to reduce the boiling point and polarity of the solution, resulting in a decrease in the isolation of TPC. The balanced ethanol-water mixture gives a positive contribution to the TPC. Ethanol plays a role in increasing the solubility of solutes, and the role of water is very important in the desorption process of the material matrix with the result that the extraction will be optimal (Mustafa and Turner, 2011). TPC increases slowly when the concentration of citric acid is increased, this can be seen in Figures 3A and 3B. The addition of citric acid causes the pH of the solution to become acidic, this condition will support the separation of phenolic bound to protein and carbohydrate polymers. The solubility of hydrophobic TPC in micelles will increase, due to decreased proton activity in deprotonated phenols and their ionic characteristics. Thus, the amount of phenol extracted increased with the addition of citric acid (El-Abbassi *et al*., 2014; İlbay *et al*., 2014). The polynomial equation of the total phenol extract is predicted as follows:

$$Y\ (TPC) = 287.81 + 1.87X_1 + 1.72X_2 + 12.85X_3 + 0.20X_1X_2 - 1.05X_1X_3 - 0.05X_2X_3 - 6.82X_1^2 + 1.78X_2^2 + 4.03X_3^2$$

3.5 Effect of independent variables on antioxidant activity

The concentration of ethanol and citric acid had a very significant ($p < 0.001$) positive linear effect on the AA of the extract, while the extraction temperature factor had a positive linear effect with a weak significance ($p < 0.05$). The main factors affecting the AA of the extract were the concentration of citric acid, then the concentration of ethanol, and finally the temperature. The interaction of ethanol with citric acid and citric acid with temperature is negatively correlated, while the interaction of ethanol - temperature produces a positive correlation. Based on a statistical analysis of the data obtained, the polynomial equation of AA of the extract is predicted according to the following equation:

$$Y\ (AA) = 61.90 + 1.19X_1 + 1.63X_2 + 0.41X_3 - 0.90X_1X_2 + 0.90X_1X_3 - 0.13X_2X_3 - 2.64X_1^2 - 2.56X_2^2 - 1.81X_3^2$$

The antioxidant activity of black rice extract ranged from 53.18 – 62.20% of inhibition, higher than that reported by Pedro *et al*. (2016), that is 29.13 – 51.78% of inhibition. The main antioxidant in black rice comes from anthocyanin compounds. Zhang *et al*. (2006) explain that the cyanidin-3-glucoside compound in black rice acts as an antioxidant hydrogen donor (AH_4). Its presence can disrupt the lipid autoxidation chain reaction by supplying hydrogen atoms four times (AH_4), offering

H to (R-), and then returning R- to the original lipid (RH). This will stop further adipose oxidation.

3.6 Optimization of the parameters

Table 3 shows the optimum maceration conditions for the extraction process using RSM. Ethanol with a concentration of 56.11% as a solvent, added with 4.42% citric acid as a medium for reducing pH and the maceration process at 49.29°C can produce a functional extract of black rice which is rich in anthocyanins (109.64 mg/100 g), high in flavonoids (25.35 mg QE/100 g), TPC (295.56 mg GAE/100 g), and AA (61.96% of inhibition). The hypothesis fits the experimental results obtained based on the optimum conditions using the RSM model.

Table 3. Optimum conditions of extraction for black rice

Factor		Optimal Condition
Ethanol (%)		56.11 (%)
Citric Acid (%)		4.42 (%)
Temperature (°C)		49.29 (°C)
Response	Prediction value	Experimental value
Anthocyanins (mg/100 g)	109.64	112.1
Flavonoids (mg QE/100 g)	25.35	25.43
TPC (mg GAE/100 g)	295.56	296.95
AA (% of Inhibition)	61.96	61.24

4. Conclusion

The different conditions (ethanol concentration, citric acid concentration, and extraction temperature) for functional compounds extraction revealed that ethanol concentration and citric acid concentration markedly affect the anthocyanins content, flavonoids, TPC, and AA. While extraction temperature only affects the flavonoid content, TPC, and AA. The results support black rice as an important source of functional compounds, which can be optimally extracted using a combination of ethanol solvents (56.11%), and citric acid (4.42%) with the help of controlled heat (49.29°C). This can provide new opportunities, especially for the beverage industry to develop functional drinks.

Conflict of interest

The authors declare no conflict of interest.

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