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Application of the Plackett-Burman design for screening and optimisation of factors affecting the aqueous extract for total anthocyanin content of broken riceberry rice

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Abstract

The aim of the present work was to identify the key factors that influence the total anthocyanin content of the aqueous extract of broken riceberry rice (BRR). The eight-run Plackett-Burman design was used to evaluate four factors (BRR particle size, BRR powder/water ratio, extraction temperature, and time). As revealed by the results, the BRR powder/water ratio, extraction temperature, and time had a significant influence on the total anthocyanin content. As a consequence, single-factor tests were conducted using a completely randomised design (CRD), considering BRR powder/water ratio (1:2.5, 1:5, 1:7.5, and 1:10 g/mL), extraction temperature (50, 55, 60, 65, and 70°C), and time (50, 60, 70, 80, and 90 min). Based on the results, the criteria for yield optimisation were a BRR particle size of 60 mesh, BRR powder/water ratio of 1:5, extraction temperature of 60°C, and time of 70 min. With these parameters, the total anthocyanin concentration of the extract was 9.71 mg C3G/g dry material.

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Introduction

Pigmented or coloured rice (*Oryza sativa* Linn.) is an important crop in Asia that exhibits medicinal characteristics, and has been consumed for generations. Thailand grows many types of pigmented rice including red, brown, and black. Pigmented rice is considered a rich source of bioactive compounds known as antioxidants including phenolic compounds and anthocyanins (Seechamnanturakit *et al*., 2018; Thao and Niwat, 2018). As a result, pigmented rice has been reported to have a greater antioxidant capacity than non-pigmented rice.

Due to its strong antioxidant advantages, riceberry rice which is a rich purple grain is one of Thailand's most favoured rice varieties (Wang *et al.*, 2010). It is a crossbreed variant of the Khao Hom Nin rice variety which is recognised as having strong antioxidant qualities, and the Khao Dawk Mali 105 fragrant rice variety (Kongkachuichai *et al.*, 2013). When milling riceberry rice, around 20 - 30% broken riceberry rice (~1,200 - 1,800 tons per harvest season) is generated. A large amount of broken riceberry rice (BRR) is sold as raw material for animal feed, or as a component for foods and cosmetics, usually at a very low price (Luang-In *et al.*, 2018). In several reports, riceberry rice has been shown to contain total phenolic compounds, anthocyanins, vitamin E, and gamma-oryzanol that provide antioxidant power (Settapramote *et al*., 2018). Riceberry rice contains anthocyanins and phenolic compounds which are located in the pigment substances on the pericarp layer and rice kernel bran (Leardkamolkarn *et al.*, 2011; Sirichokworrakit *et al.*, 2015).

Anthocyanins are the main active chemical components found in riceberry rice. Cyanidin and peonidin are the most common anthocyanins (Maulani *et al.*, 2019). Owing to its antioxidant, anticancer, hypoglycaemic, and anti-inflammatory

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benefits, anthocyanins have been identified as beneficial dietary components (Nam *et al*., 2006; Tanaka *et al*., 2009).

To create bright colours in food items, the food colorant industry uses anthocyanins from natural sources (Ichikawa *et al*., 2001). The purification and recovery of active compounds from plant sources begin with extraction. Conventional solvent extraction using a solid-to-liquid extraction process can be used to extract anthocyanins. Solvent extraction is the most widely used process, and has proven to be a reliable and efficient method (Eliasson *et al.*, 2017; Agcam *et al*., 2017; Ongkowijoyo *et al.*, 2018). Solid-to-liquid extraction of anthocyanins is influenced by various parameters such as pH, temperatures, time, solvent types (ethanol, methanol, hot water, *etc*.), solvent concentrations, particle sizes, and shaking conditions (Cisse *et al.*, 2012; Bonfigli *et al.*, 2017; Romero-Diez *et al.*, 2019). Single-factor reviews can offer valuable information on the variations regarding the main effects on the anthocyanin components of plant sources (Peanparkdee *et al*., 2019; Le *et al.*, 2019).

The Plackett-Burman design is a recognised approach for evaluating numerous factors. It is a distinguished and extensively applied statistical approach for identifying the most important components with high significance levels for enhanced development (Plackett and Burman, 1946; Ekpenyong *et al.*, 2017). Therefore, the Plackett-Burman design simply screens the design matrix to identify the significant effects. The chosen parameters are further optimised by a completely randomised design (CRD) on each parameter. This method applies a collection of statistical techniques that uses the design of experiments to evaluate the effects of single factors, and select the optimum conditions. As a result, the aim of the present work was to determine the ideal condition for the aqueous extraction of anthocyanins, comprising BRR particle size, BRR powder/water ratio, extraction temperature, and time in order to optimise the extraction of total anthocyanin concentration from BRR.

Materials and methods

Material

Broken riceberry rice (BRR) was purchased from Allricesurin Brand, located in Surin Province, Thailand, then milled to powder, and passed through 60 and 80 mesh sieves before being gathered, packaged in plastic (HDPE) bags, and stored in cardboard boxes at -18°C until further evaluation.

Methods

Plackett-Burman design for the screening of significant independent variables

As seen in Table 1, samples of four independent factors (BRR particle size, BRR powder to water ratio, extraction temperature, and time) were tested in eight experiments and the correlating Plackett–Burman experimental design matrix (Plackett and Burman, 1946) for evaluation of the key independent factors influencing the total anthocyanin concentration.

Table 1. Conditions of the Plackett-Burman design for assessing the substantial self-determining factors influencing total anthocyanin content.

Test run no.	Independent variable and experimental level						
	A	**B**	**C**	**D**	**E**	**F**	**G**
1	+(80)	+(1:10)	+(60)	-(30)	+	-	-
2	+(80)	+(1:10)	-(30)	+(60)	-	-	+
3	+(80)	-(1:5)	+(60)	-(30)	-	+	+
4	-(60)	+(1:10)	-(30)	-(30)	+	+	+
5	+(80)	-(1:5)	-(30)	+(60)	+	+	-
6	-(60)	-(1:5)	+(60)	+(60)	+	-	+
7	-(60)	+(1:10)	+(60)	+(60)	-	+	-
8	-(60)	-(1:5)	-(30)	-(30)	-	-	-

A: BRR particle size (mesh); B: BRR powder/water ratio; C: extraction temperature (°C); D: extraction time (min), while E, F, and G: dummy factors (- signifies low level, + signifies high level).

Five grams of the BRR powders (particle size of BRR: 60 and 80 mesh) were extracted with deionised water at 1:5 and 1:10 BRR powder/water ratios. The mixtures were shaken using an orbital shaker at an extraction temperature between 30 and 60°C, and a period between 30 and 60 min based on the conditions of the designed experiments as seen in Table 1. All mixtures were filtered through a Whatman No. 1 filter paper after extraction, and vacuum-dried at 50°C in a rotary evaporator. The BRR crude extracts were stored at -18°C in brown cardboard containers until further use.

Preparation of total monomeric anthocyanin in BRR extracts

The pH differential approach was used to assess the total anthocyanin content (Giusti and Wrolstad, 2005). A mixture of 20 µL of the BRR extracts and 80 µL of potassium chloride buffer solution (pH 1.0) in the individual wells of a 96-well plate, and a mixture of 20 µL of the BRR extracts and 80 µL of sodium acetate buffer mixture (pH 4.5) were prepared. Both mixtures were given 15 min to equilibrate. At 510 and 700 nm, the absorbance of each specimen was determined. The content of total anthocyanin was calculated and then converted into milligram per gram of total anthocyanin content (TAC) by expression in mg/g of cyanidin 3-glucoside of BRR powders (mg C3G/g) using Eq. 1:

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1{,}000) / (\varepsilon \times 1) \quad \text{(Eq. 1)}$$

where, A = absorbance = $(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$, MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF = dilution factor, and ε = molar absorptivity (26,900 L/mol/cm).

Statistical analysis

To assess and identify the significant factors, the Plackett-Burman design was used. The disparity between the median of the measurements performed at the upper (+) and lower (-) values of the range covered by each variable and the response was used to determine the significant impacts of each factor on the total anthocyanin content (Table 1). The experimental results were assessed using the first-order model in Eq. 2:

$$Y = \beta_0 + \sum \beta_i x_i \quad \text{(Eq. 2)}$$

where, Y = reaction (total anthocyanin content), β_0 = model coefficient, β_i = linear coefficient, and X_i = degree of the self-determining variable. This model is intended to filter and assess the relevant elements that impact the response. However, it does not define the relationships between the components. For analysis of the experimental data, the SPSS software package was employed (Statistical Software Package for Social Sciences, SPSS Inc. and IBM Co., Chicago, IL, USA).

BRR extract preparation for single-factor experiments

The BRR powder/water ratio, extraction temperature, and duration of extraction significantly impacted the total anthocyanin concentration as shown by the preliminary results (Table 2). As a result, a CRD was employed to test the individual influence of the BRR powder/water ratio, extraction temperature, and time period. The BRR powders (5 g) with a 60-mesh particle size were extracted using various extraction criteria including the BRR powder/water ratio (1:2.5, 1:5, 1:7.5, and 1:10 g/mL), extraction temperature (50, 55, 60, 65, and 70°C), and period of time (50, 60, 70, 80, and 90 min) with shaking during the extraction process. The BRR crude extracts were obtained by centrifuging the filtrate at 50°C under vacuum and stored at -18°C for further analysis.

Table 2. *t*-values and *p*-values of the self-determining factors on total anthocyanin concentration.

Independent variable	Total anthocyanin content (mg C3G/g)		*t*-value	*p*-value
	Low level	High level		
BRR particle size	7.61 ± 0.23	8.38 ± 0.18	3.708	0.066
BRR powder/water ratio	8.46 ± 0.27	7.53 ± 0.21	-4.448	0.047[b]
Extraction temperature	6.68 ± 0.19	9.31 ± 0.26	12.556	0.006[a]
Extraction time	7.34 ± 0.12	8.65 ± 0.10	6.260	0.025[a]

[a]significant positive effect, and [b]significant negative effect at $p < 0.05$.

All treatments were conducted in triplicate. All data were analysed by one-way analysis of variance (ANOVA) with *post hoc* comparison tests (Duncan Multiple Range Test) when ANOVA revealed that the model and treatment effects were significant ($p < 0.05$) using the Statistical Software Package for Social Sciences, SPSS Inc. and IBM Co., Chicago, IL, USA.

Results and discussion

Assessment of the noteworthy independent factors using the Plackett-Burman design

Four independent variables (BRR particle size, BRR powder/water ratio, extraction temperature, and time) were assessed for the efficient accumulation of anthocyanin using the eight-run Plackett-Burman matrix. Table 2 shows the concentrations of total anthocyanin content (mg C3G/g dry basis) under the various extraction circumstances. When the computed *p*-value was less than 0.05 at $\alpha = 0.05$, the factors were judged to have a strong influence on the response (Table 2). As seen in Table 2, the particle size of BRR (low level at 60 mesh; high level at 80 mesh) did not have a significant impact on total anthocyanin concentration ($p \geq 0.05$). Similar results have been reported in previous studies on pigmented rice bran (Pajareon and Theerakulkait, 2014) which stated that the total anthocyanin content of the pigmented rice bran extract with the particle size of 60 mesh was significantly different from that of the 40 mesh, but was not significantly different from the 80-mesh particle size. Meanwhile, extraction temperature (low level at 30°C; high level at 60°C) and time (low level at 30 min; high level at 60 min) both had a highly positive impact on total anthocyanin content, whereas the solid-liquid ratio (low level at 1:5; high level at 1:10) had a considerably adverse effect ($p < 0.05$). As a result, the influence of these three parameters (BRR powder/water ratio, extraction temperature, and time) on each factor was studied further using a CRD with the given condition of the BRR particle size at 60 mesh.

Influence of BRR powder/water proportion

In several situations, a considerable amount of solvent is also needed for extraction and recovery, which might be a serious issue in terms of environmental concerns. Changing the BRR powder/water ratio from 1:2.5 to 1:5 resulted in a substantial increase in total anthocyanin content from 8.49 to 9.26 mg/g ($p < 0.05$), as seen in Figure 1.

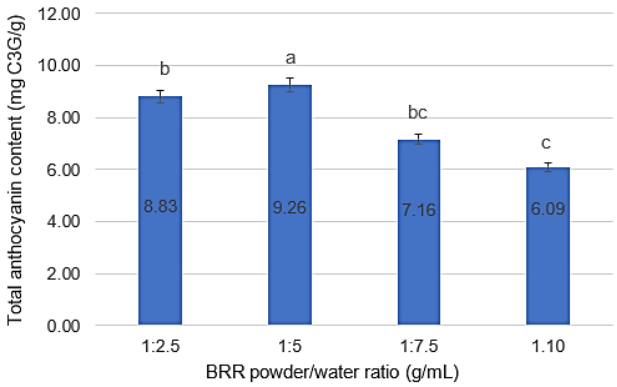

Figure 1. Effect of BRR powder/water ratio on total anthocyanin content. Data are mean values with error bars indicating ± SD. Different lowercase letters indicate significant difference (DMRT, $p < 0.05$).

However, anthocyanin yields began to drop as the ratio approached 1:5. This might be due to the fact that larger amount of solvent takes longer to heat up, thus resulting in extended exposure to light which in turn leads to anthocyanin degradation (Maran *et al.*, 2015). This can be simplified as follows. When the material/solvent ratio is inadequate to fill the medium, the hypertonic environment cannot be formed, thus resulting in the colour remaining in the material's vacuoles. Conversely, the cells expand to maximum and burst out simultaneously when the solvent/material ratio reaches a specific value (depending on the properties of the material), thus releasing the colour within the vacuoles due to the capacity to swiftly absorb water. This result agrees with Blackhall *et al.* (2018) and Le *et al.* (2019) who reported that the main factor affecting the extraction of anthocyanins in purple sweet potato and karanda fruit were the material-to-solid ratio. There is a possibility that the contact between the plant substance and the solvent will be increased by a high solid-liquid ratio, which would result in a greater anthocyanin yield (Golmakani and Moayyedi, 2016). In contrast, a solid-liquid ratio increases the extract's oxygen content, thus causing oxidation of some of the anthocyanins and subsequently, their yield will decrease (Wang *et al.*, 1997; 2022). As a result, the anthocyanin concentration peaked at 9.26 mg/g with a BRR powder/water ratio of 1:5, and this was chosen for further research.

Influence of extraction temperature

In general, a higher extraction temperature corresponds to greater solubility. This is shown by the observation that increasing the temperature reduces intermolecular interactions within the solvent, thus resulting in increased molecular mobility and increased solubility of the target chemicals in the extraction solvent. As the temperature rises, the cell matrix may be destroyed, thus leading to greater availability of constituents for extraction. However, it has also been shown that up to a particular temperature level, extraction efficiency grows with increasing temperature, and then the extraction yield starts to decline as the temperature level climbs. This varies depending on the target chemicals as well as the temperature at which each chemical degrades. When the extraction temperature was steadily increased from 50 to 60°C, the anthocyanin concentration reached 9.66 mg/g, as shown in Figure 2.

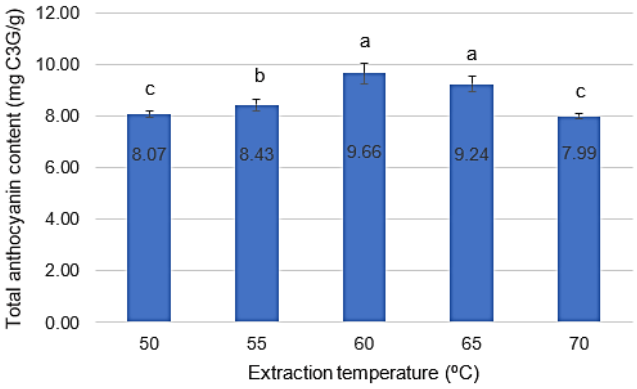

Figure 2. Effect of extraction temperature on total anthocyanin content. Data are mean values with error bars indicating ± SD. Different lowercase letters indicate significant difference (DMRT, $p < 0.05$).

The content of anthocyanin in the extract dropped slightly at 65°C ($p \geq 0.05$), and considerably at 70°C ($p < 0.05$). In addition to the temperature-induced breakdown of anthocyanin, these phenomena might be understood in term of the thermodynamic process which is enhanced by heat treatment, and results in the production of wax, resin, and mucilage in the extract, thus lowering the extraction yield (Yang *et al.*, 2010). Conversely, the anthocyanin concentration in the extract was not meaningfully altered from 60 to 65°C ($p \geq 0.05$), thus indicating that breakdown caused the content to decrease. A similar anthocyanin content in pigmented rice bran and karanda fruit have previously been reported by Pajareon and Theerakulkait (2014) and Le *et al.* (2019), respectively. The extraction temperature affected the efficacy of anthocyanin concentration. However, at temperatures above 60ºC, the rates of anthocyanin decomposition were very high. In reality, a temperature of 60°C may be used based on the composite impacts of excellent anthocyanin extraction yield.

Influence of extraction time

The amount of anthocyanin in the fluid decreased with increasing time, as illustrated in Figure 3.

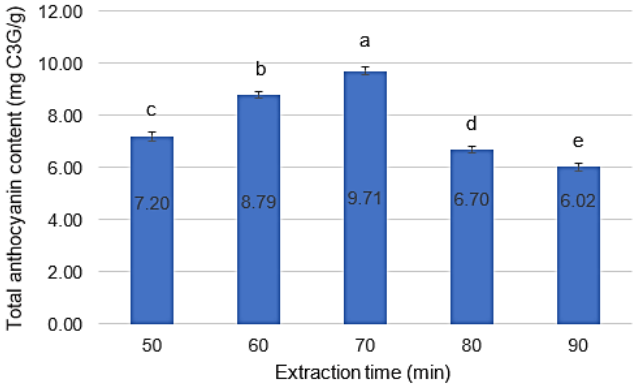

Figure 3. Effect of extraction time on the total anthocyanin content. Data are mean values with error bars indicating ± SD. Different lowercase letters indicate significant difference (DMRT, $p < 0.05$).

The extraction yield of anthocyanin from BRR increased from 7.20 to 9.71 mg/g ($p < 0.05$) as the time durations increased from 50 to 70 min, but it decreased at durations longer than 70 min, thus indicating that the removal time value greatly influenced the extraction efficiency in correlation with other factors. Anthocyanin concentration peaked at 9.71 mg/g after 70 min, but declined steadily after that, most likely owing to the anticyclonic breakdown produced by the prolonged exposure to high temperatures (Figure 3). Thao *et al.* (2015) reported that extraction time affected the potential of the total anthocyanin content of the purple rice from Vietnam. If the extraction time was excessively long, some of the anthocyanin constituents decayed. As a result, 70 min was considered the most effective time period for anthocyanin extraction yield.

Based on these findings, a BRR powder/water ratio of 1:5 g/mL, a temperature of 60°C, and a reaction time of 70 min were determined to be the optimal conditions for anthocyanin recovery from BRR.

Conclusion

Many factors influenced the total anthocyanin concentration of BRR extract in the tests. The findings of the eight-run Plackett-Burman design for assessing the relevant factors revealed that the BRR powder/water ratio, extraction temperature, and extraction duration all had a major effect on the total anthocyanin content of BRR extract. Following this, single-factor assays were carried out in order to identify the optimal conditions for all factors. High extraction yield was achieved using a BRR particle size of 60 mesh, a BRR powder/water quotient of 1:5, and an extraction temperature of 60°C, as well as an extended duration of 70 min. The maximum experimental yield of the total anthocyanin content was 9.71 mg C3G/g dry basis using these factors. The present work provided critical information for scaling up the extraction of antioxidant compounds from BRR extracts, which could be potentially applied in the pharmaceutical, cosmetic and food industries.

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