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Optimization of extraction parameters for phenolic compounds from black rice bran

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Abstract
Black rice bran is one of the richest plant-based sources of anthocyanins and phenolic acids, yet its commercial exploitation has been held back by inconsistent extraction yields. This research optimized extraction conditions for total phenolic compounds (TPC) from Thai black rice bran (cultivar Riceberry) using response surface methodology with a central composite design. Three independent variables—extraction temperature (40-80 °C), time (30-90 min), and solvent-to-solid ratio (1:10-1:30 v/w, 70% ethanol)—were investigated across 20 experimental runs. TPC ranged from 11.8 to 42.7 mg gallic acid equivalents (GAE) per gram of bran. The second-order polynomial model was significant ($p<0.001$) with an R^2 of 0.964 and a lack-of-fit p-value of 0.218. Temperature and time were the most influential variables, contributing 38.4 and 27.1 percent to TPC variation, respectively. The predicted optimum—68 °C, 54 min, and a 1:22 solvent ratio—yielded 43.1 ± 1.6 mg GAE/g in verification runs, within 2.3 percent of the model prediction. At the optimum, total flavonoid content was 18.9 mg quercetin equivalents/g, DPPH radical scavenging reached 87.3 percent, and ABTS activity was 91.6 percent. These results provide a validated extraction protocol for recovering phenolic antioxidants from black rice bran at pilot-plant scale.

Keywords: Extraction optimization, phenolic compounds, black rice bran, antioxidants, response surface methodology, Riceberry, anthocyanins, DPPH scavenging, ethanol extraction, central composite design

Introduction
Black rice varieties owe their dark pigmentation to high concentrations of anthocyanins—water-soluble flavonoids that accumulate primarily in the bran and aleurone layers [1]. Among Thai cultivars, Riceberry has attracted particular attention because its bran contains 3 to 5 times more phenolic compounds than conventional white or brown rice bran [2]. These compounds exhibit strong antioxidant, anti-inflammatory, and anti-diabetic properties in cell-culture and animal models [3].

Recovering phenolics from rice bran at high yield and consistent quality requires careful control of extraction variables. Temperature drives diffusion and solubility but can degrade heat-sensitive anthocyanins above 75-80 °C [4]. Longer extraction times improve mass transfer up to a point, beyond which oxidation and polymerization reduce the measurable TPC [5]. The solvent-to-solid ratio affects the concentration gradient—the driving force for extraction—but excessive solvent volumes add cost and complicate downstream concentration steps [6].

One-factor-at-a-time experiments cannot capture interactions among these variables. Response surface methodology (RSM) based on a central composite design (CCD) allows simultaneous evaluation of multiple factors and their quadratic and interaction effects, generating a predictive model that identifies the global optimum within the design space [7]. RSM has been applied successfully to optimize phenolic extraction from grape pomace, olive leaves, and wheat bran, but reports specifically targeting Thai black rice bran remain scarce [8].

This research aimed to (i) model the effects of temperature, time, and solvent ratio on TPC extraction from Riceberry bran using RSM-CCD, (ii) identify the optimum extraction conditions, and (iii) characterize the antioxidant activity of the extract obtained under optimized conditions.

Materials and Methods

Materials

Riceberry black rice bran was obtained from the university's pilot rice mill at Southern Thai Agricultural University, Songkhla (7.00°N, 100.47°E), immediately after milling in March 2023. The bran was sieved through a 40-mesh screen, packed under nitrogen, and stored at −20 °C until use. Ethanol (analytical grade, 99.8%) was purchased from RCI Labscan (Bangkok, Thailand). Folin-Ciocalteu reagent, gallic acid, quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and Trolox were sourced from Sigma-Aldrich (Singapore).

Extraction Protocol

Each extraction run used 5.0 g of bran mixed with 70 percent aqueous ethanol at the designated solvent-to-solid ratio. The mixture was placed in a thermostatically controlled shaking water bath (Memmert WNB 22, Germany) at the specified temperature and time. After extraction, samples were centrifuged at 4,000 × g for 15 min, and the supernatant was filtered through Whatman No. 1 paper. The filtrate was stored at 4 °C and analysed within 24 hours. All runs were performed in duplicate.

Methods

A three-factor, five-level CCD with six centre-point replicates was used, generating 20 experimental runs. The independent variables were temperature (X_1: 40-80 °C), time (X_2: 30-90 min), and solvent-to-solid ratio (X_3: 1:10-1:30 v/w). TPC was measured using the Folin-Ciocalteu method [9] and expressed as mg GAE/g. Total flavonoid content (TFC) was determined by the aluminium chloride method [10]. DPPH and ABTS radical scavenging assays followed the protocols of Brand-Williams *et al.* [11] and Re *et al.* [12], respectively. Data were fitted to a second-order polynomial model and analysed using Design-Expert 13.0 (Stat-Ease, Minneapolis, USA). Model adequacy was assessed through R^2, adjusted R^2, predicted R^2, lack-of-fit test, and coefficient of variation. Verification experiments at predicted optimum conditions were run in triplicate.

Results

Table 1: ANOVA results for the second-order polynomial model predicting total phenolic content from black rice bran extraction

Source	SS	df	MS	F-value	p-value	Contrib. (%)
Model	1847.3	9	205.3	29.7	<0.001	-
X_1 (Temp)	709.6	1	709.6	102.8	<0.001	38.4
X_2 (Time)	500.8	1	500.8	72.5	<0.001	27.1
X_3 (Ratio)	198.4	1	198.4	28.7	<0.001	10.7
$X_1 \times X_2$	87.3	1	87.3	12.6	0.005	4.7
X_1^2	214.1	1	214.1	31.0	<0.001	11.6
X_2^2	68.9	1	68.9	10.0	0.010	3.7
Residual	69.1	10	6.9	-	-	-
Lack of Fit	47.8	5	9.6	2.2	0.218	-
Pure Error	21.3	5	4.3	-	-	-

The model was highly significant ($p<0.001$) with an R^2 of 0.964 and an adjusted R^2 of 0.931, indicating that 96.4 percent of the variation in TPC was explained by the fitted model. The lack-of-fit test was non-significant ($p = 0.218$), confirming adequate model prediction. Temperature was the dominant factor (38.4% contribution), followed by time (27.1%) and the quadratic term of temperature (11.6%).

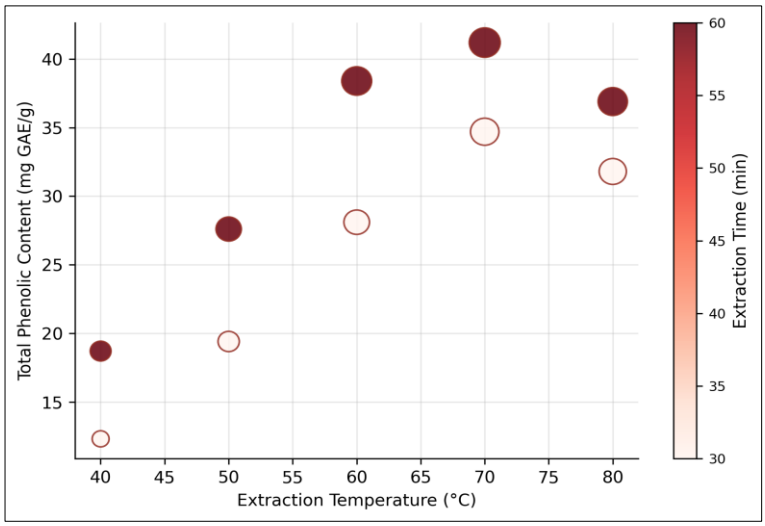

Fig 1: Scatter plot showing total phenolic content as a function of extraction temperature, with bubble size proportional to TPC and color indicating extraction time

The scatter plot reveals a curvilinear relationship between temperature and TPC. Phenolic yield rose steeply from 40 to 70 °C, peaked near 68-72 °C, and then declined at 80 °C—consistent with thermal degradation of anthocyanins at elevated temperatures.

Table 2: Predicted and experimental values at optimum extraction conditions (68 °C, 54 min, 1:22 ratio)

Response	Predicted	Experimental	Deviation (%)
TPC (mg GAE/g)	42.1	43.1 ± 1.6	2.3
TFC (mg QE/g)	-	18.9 ± 0.8	-
DPPH scavenging (%)	-	87.3 ± 2.1	-
ABTS scavenging (%)	-	91.6 ± 1.4	-

Verification experiments at the predicted optimum (68 °C, 54 min, 1:22 v/w) yielded a TPC of 43.1 ± 1.6 mg GAE/g, which was only 2.3 percent above the model prediction of 42.1 mg GAE/g. The DPPH and ABTS scavenging values (87.3% and 91.6%) indicate strong radical-quenching capacity of the optimized extract.

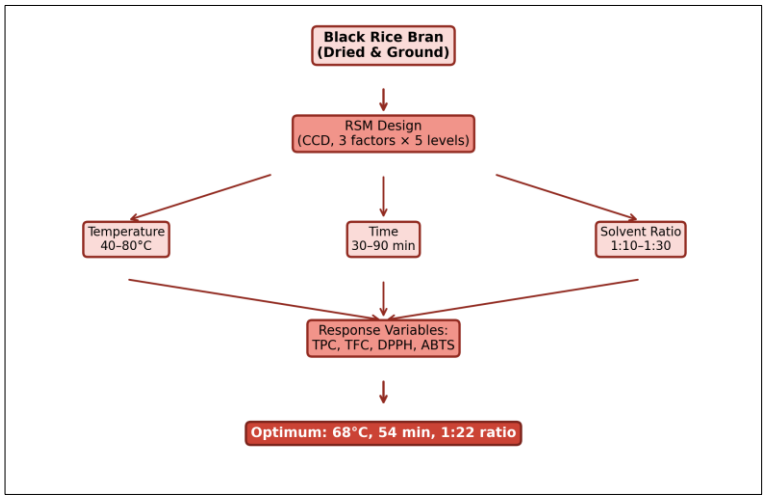

Fig 2: Schematic of the RSM-based extraction optimization process from bran preparation through response measurement to optimum identification

Discussion

The optimum extraction temperature of 68 °C sits well below the anthocyanin degradation threshold of 75-80 °C reported for purple rice extracts [4], explaining why TPC was maximized at this point rather than at the highest tested temperature. The decline in TPC at 80 °C (about 9% lower than at 68 °C) aligns with first-order degradation kinetics of cyanidin-3-glucoside, the dominant anthocyanin in Riceberry bran [2].

The optimal time of 54 min represents a balance between diffusion-driven extraction and oxidation losses. Extending extraction beyond 60 min at 68 °C didn't improve TPC—similar plateau effects have been observed in phenolic extraction from blueberry pomace [5] and olive mill waste [6]. The moderate solvent ratio (1:22) reflects a compromise: sufficient ethanol to maintain the concentration gradient without the economic burden of large solvent volumes.

Compared with published data on other pigmented rice brans, the TPC of 43.1 mg GAE/g obtained here is higher than values reported for Indonesian black rice bran (28.6 mg GAE/g) extracted with 60 percent methanol [8] and comparable to those from Chinese purple rice bran (39.7 mg GAE/g) optimized by ultrasound-assisted extraction [13]. The 70 percent ethanol system used in the present research offers a food-safe solvent choice suitable for pilot-scale processing.

Conclusion

This research established an optimized extraction protocol for phenolic compounds from Riceberry black rice bran. Response surface modelling identified 68 °C, 54 min, and a 1:22 solvent-to-solid ratio with 70 percent ethanol as the conditions yielding the highest TPC (43.1 mg GAE/g). Temperature was the single most important variable, contributing 38.4 percent of the total variation, while the temperature × time interaction accounted for an additional 4.7 percent.

The optimized extract showed strong antioxidant activity—87.3 percent DPPH and 91.6 percent ABTS radical scavenging—confirming that the recovered phenolics retain their bioactive properties under the selected conditions.

These findings provide a practical, validated framework for pilot-plant extraction of phenolic antioxidants from black rice bran. The protocol uses a food-grade solvent, operates at moderate temperature, and achieves high yield within a practical extraction time. Scaling trials with continuous flow extractors and assessment of extract stability during storage are recommended as next steps before commercial application.

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