



Research article

Optimization of ultrasonic-assisted extraction of anthocyanins, flavonoids and their antioxidant activity from Doi Saket purple rice (*Oryza sativa* L.)

Sureewan Rajchasom^{a,*}, Pornsawan Sombatnan^{a,b,†}, Yardfon Tanongkankit^{b,†}, Nukrob Narkprasom^{b,†}

^a College of Integrated Science and Technology, Rajamangala University of Technology Lanna, Chiang Mai 50300, Thailand

^b Department of Agriculture and Food Engineering, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand

Article Info

Article history:

Received 2 September 2024

Revised 27 July 2025

Accepted 31 July 2025

Available 12 September 2025

Keywords:

Anthocyanin,

Antioxidant,

Product and process,

Response surface methodology,

Ultrasonic-assisted extraction

Abstract

Importance of the work: Doi Saket purple rice (*Oryza sativa* L.) is rich in anthocyanins, which are found primarily in the rice grain. However, anthocyanins are sensitive to heat, pH, light, solvents, enzymes and oxidative stress compounds. Systematic evaluation and optimization of the extraction parameters and conditions is essential to maximize the yield of bioactive compounds through ultrasonic-assisted extraction.

Objective: To investigate the optimization of various factors influencing ultrasonic-assisted extraction (UAE) of the total anthocyanin content (TAC), total flavonoid content (TFC) and hydrogen peroxide (H₂O₂) scavenging activity from Doi Saket purple rice using response surface methodology (RSM).

Materials and Methods: The Doi Saket purple rice was extracted using UAE and the optimum conditions were determined based on RSM. A Box-Behnken design was used to plan the experimental runs, resulting in 15 experimental conditions. Three factors, each at three levels were studied: solid-to-liquid ratio (1:1–1:3 weight per volume, w/v; X₁), extraction time (20–60 min; X₂) and extraction temperature (60–80°C; X₃).

Results: The optimal conditions for the UAE of TAC, TFC and H₂O₂ scavenging activity of Doi Saket purple rice were a solid-to-liquid ratio of 1:2 w/v, an extraction time of 40 min and an extraction temperature of 70°C. Under these conditions, the highest predicted values for TAC, TFC and H₂O₂ scavenging activity were 131.83 mg/g, 38.62 mg of quercetin equivalent/g rice (mg QE/g) and 83.75%, respectively. The experimental values mean (± SD) of the extracts obtained under optimal conditions were as follows: TAC, 112.77 ± 2.34 mg/g; TFC, 39.26 ± 0.35 mg QE/g and H₂O₂ scavenging activity, 75.06 ± 1.26%.

Main finding: The independent variables (solid-to-liquid ratio, extraction time and extraction temperature) were systematically identified and optimized to maximize the extraction yields of TAC, TFC and H₂O₂ scavenging activity from Doi Saket purple rice.

† Equal contribution

* Corresponding author.

E-mail address: sureewan@rmutl.ac.th (S. Rajchasom)

Introduction

Doi Saket purple rice (*Oryza sativa* L.), cultivated in northern Thailand, is rich in anthocyanins, that consist primarily of cyanidin-3-glucoside and peonidin-3-glucoside (Rerkasem et al., 2015). The dominant property of anthocyanins is their antioxidant capability (Zhang et al., 2013). Additionally, rice bran contains beneficial phytochemicals such as total flavonoids, vitamins and tocopherols (Min et al., 2012). However, anthocyanins are highly sensitive to heat, pH, light, solvents, enzymes and oxidative stress (Rein, 2005; Scalzo et al., 2008 and Castañeda-Ovando et al., 2009). Therefore, optimizing extraction parameters is crucial to preserve the stability and efficacy of the anthocyanins.

Often, conventional methods for anthocyanin extraction, such as boiling, refluxing and Soxhlet extraction, are associated with low yields, limited efficiency and long processing times. (Das et al., 2017; Srimoon and Santimalai, 2018). These drawbacks mainly result in the degradation of the anthocyanins through oxidation, hydrolysis and ionization reactions (Pratiwi and Purwestri, 2017). To overcome these limitations, ultrasonic-assisted extraction (UAE) has emerged as a promising alternative, offering improved efficiency and better preservation of phytochemicals (Cobin et al., 2015). UAE is recognized as a clean, novel and safe technique that enhances extraction by increasing diffusion rates, promoting mass transfer and disrupting plant cell walls to facilitate solvent penetration (Mason et al., 2011; Tiwary, 2015).

The key extraction parameters and conditions must be evaluated and optimized carefully to maximize the yield of bioactive compounds using UAE. Response surface methodology (RSM) is applied widely to determine optimal conditions by systematically evaluating variables such as the solid-to-liquid ratio, extraction time, temperature and solvent types. Numerous studies have demonstrated that these factors greatly influence both the yield and quality of target compounds. For example, Jiang et al. (2017) and Wang et al. (2019) used RSM to optimize anthocyanin extraction from blueberries and red rice, respectively, and found that extraction temperature, time and solid-to-liquid ratio had substantial impacts on yields, with optimal recovery being achieved under moderate temperatures and extended extraction durations. In addition, Mane et al. (2015) reported that the ultrasonic amplitude and solvent ratio substantially affected anthocyanin

yield from Purple Majesty potatoes. Although individual parameters influence extraction efficiency, simultaneous optimization of multiple variables has been demonstrated to considerably enhance the recovery of bioactive phytochemical compounds, particularly anthocyanins, from various plant sources, including purple sweet potatoes, red cabbage, mulberry and grape juice (Cocito et al., 1995; Gongjian et al., 2008; Zou et al., 2011; Demirdöven et al., 2015). However, there has been limited research on the application of RSM for optimizing the UAE of purple rice. Specifically, to date, no studies have reported the optimization of UAE conditions based on H₂O₂ scavenging activity as a response variable, particularly in the context of cosmetic or functional product applications.

Therefore, the current study aimed to investigate the effects of UAE on the total anthocyanin content (TAC), total flavonoid content (TFC) and hydrogen peroxide (H₂O₂) scavenging activity of Doi Saket purple rice extracts, using RSM to identify the optimal extraction conditions, with the solid-to-liquid ratio, extraction time and temperature considered as key variables.

Materials and Methods

Plant materials and chemicals

The Doi Saket purple rice was sourced from a local farmer in Doi-Saket, Chiang Mai province, Thailand. The rice was milled by a commercial grinding facility and stored in vacuum-sealed plastic bags at room temperature (28 ± 2°C). The analytical-grade chemicals used in this study included potassium chloride, sodium acetate trihydrate, gallic acid, aluminum chloride, hydrochloric acid (37%), 1,10-phenanthroline monohydrate, ammonium iron (II) sulfate hexahydrate, sulfuric acid (98%) and hydrogen peroxide (30%) and were purchased from Northern Chemical Co., Ltd (Chiang Mai, Thailand) and Union Science Co., Ltd (Bangkok, Thailand).

Ultrasonic-assisted extraction

The Doi Saket purple rice was extracted using an ultrasonic water bath (Model GT; Sonic-D6; Guangdong GT Ultrasonic Co., Ltd.; Shenzhen, China) operating at a frequency of 40 kHz and a power of 150 W. For each extraction run, 20 g of Doi Saket purple rice was mixed

with distilled water at solid-to-liquid ratios of 1:1, 1:2 and 1:3 weight per volume (w/v). Initially, the extraction solvent was kept at room temperature ($28 \pm 2^\circ\text{C}$) before sonication. UAE was conducted at temperatures of 60°C , 70°C or 80°C for extraction durations of 20 min, 40 min or 60 min, as defined by a Box-Behnken design (BBD), according to Yang et al. (2010) and Zhu et al. (2016). Due to the use of ultrasonic energy, the extraction temperature increased by approximately $2\text{--}3^\circ\text{C}$ above the set point and was monitored using an external thermometer. Each condition was conducted in triplicate ($n = 3$), with the independent variables shown in Table 1. After extraction, the extracts were filtered through a white cloth and the filtrate was collected for further analysis.

Table 1 Independent variables and their coded levels used in the experimental design for ultrasound-assisted extraction (UAE) of Doi Saket purple rice.

Independent variable	Level values		
	-1	0	+1
Solid-to-liquid ratio (weight per volume), X_1	1	2	3
Extraction time (minutes), X_2	20	40	60
Extraction temperature (degrees Celsius), X_3	60	70	80

Experimental design and optimization

RSM was used to optimize the impact of the UAE on the TAC, TFC and H_2O_2 scavenging activity of the Doi Saket purple rice extracts. Three independent variables

were investigated: solid-to-liquid ratio (on a w/v basis; X_1), extraction time (in minutes; X_2) and extraction temperature (in degrees Celsius; X_3). The variable levels are presented in Table 1. The BBD was utilized to structure the experimental design, consisting of 15 experimental runs with three replicates ($n = 3$), as shown in Table 2. The STATISTICA software (version 12; TIBCO Software Inc.; Palo Alto, CA, USA) was used for constructing the design matrix, graph and data analysis. A second-degree polynomial equation was fitted to the experimental data using Equation 1:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i>j}^k \beta_{ij} X_i X_j \quad (1)$$

where Y is the response (dependent variable), β_0 is the linear coefficient of the coded variables, β_i , β_{ii} and β_{ij} are the linear, quadratic and cross product regression coefficients, respectively, X_i and X_j are the independent variables and k is the number of tested variables ($k = 3$).

The statistical significance of the model was evaluated using analysis of variance, with the importance of the coefficients and variable interactions assessed based on p values. Model accuracy and validity were determined using the coefficient of determination (R^2), lack of fit and an F test ($p < 0.05$). Predictive validity was evaluated based on the coefficient of variation (CV) and a paired t test was used to compare predicted and experimental values ($p < 0.05$).

Table 2 Box-Behnken design and response variables for total anthocyanin content (TAC), total flavonoid content (TFC) and hydrogen peroxide (H_2O_2) scavenging activity of Doi Saket purple rice extracts

Run	Factor			Response variable		
	Solid-to-liquid ratio (weight per volume, X_1) (1:x)	Time (minutes, X_2)	Temperature (degrees Celsius, X_3)	TAC (mg/g)	TFC (mg QE/g)	H_2O_2 scavenging activity (%)
1	3	20	70	86.83 \pm 0.48	23.84 \pm 0.48	48.26 \pm 2.10
2	3	60	70	93.35 \pm 0.10	24.82 \pm 0.32	67.89 \pm 2.55
3	2	60	60	83.38 \pm 0.63	30.39 \pm 0.52	71.10 \pm 1.14
4	2	20	80	94.74 \pm 3.38	36.41 \pm 1.12	73.04 \pm 0.74
5	1	60	70	80.15 \pm 3.04	47.68 \pm 0.52	89.31 \pm 4.08
6	1	40	60	74.09 \pm 1.68	46.37 \pm 0.72	83.77 \pm 2.13
7	2	60	80	99.97 \pm 2.80	36.26 \pm 0.62	73.28 \pm 0.43
8	2	20	60	75.59 \pm 3.42	25.84 \pm 0.27	60.89 \pm 1.47
9	3	40	60	100.69 \pm 1.2	24.54 \pm 0.12	59.22 \pm 1.31
10	2	40	70	123.86 \pm 1.33	32.73 \pm 0.28	84.96 \pm 0.97
11	3	40	80	104.20 \pm 2.76	28.21 \pm 0.55	59.86 \pm 1.84
12	2	40	70	130.25 \pm 1.02	37.29 \pm 0.61	79.33 \pm 1.32
13	1	40	80	80.49 \pm 1.74	54.88 \pm 0.78	97.11 \pm 1.27
14	1	20	70	69.52 \pm 4.36	49.29 \pm 0.33	99.17 \pm 0.82
15	2	40	70	149.84 \pm 1.02	36.23 \pm 0.80	78.46 \pm 1.10

QE = quercetin equivalents.

Data shown as mean \pm SD values, derived from three independent experiments ($n = 3$).

Determination of total anthocyanin content

The TAC in the Doi Saket purple rice extracts was determined using a modified pH differential method (Tonutare and Moor, 2014). Extracts were diluted tenfold with distilled water and centrifuged at 3,000 rpm (approximately 1,200×g) for 15 min (Model EBA 20; Hettich Zentrifugen; Tuttlingen, Germany). Then, 200 μL of supernatant solution was adjusted to 2 mL with pH 1.0 or pH 4.5 buffer and incubated in the dark at room temperature (28 ± 2°C). The absorbance (A) was measured at 510 nm and 700 nm using an ultraviolet-visible light (UV-Vis) spectrophotometer (Model SP-UV 200; Spectrum Instruments; Shanghai, China) with distilled water as a blank. The TAC (in milligrams per gram) was calculated using Equation 2:

$$\text{TAC (mg/g)} = A_{\text{diff}} \times M_w \times D_f \times 1,000 / (\epsilon \times m) \quad (2)$$

where A_{diff} is $(A_{510\text{nm}} - A_{700\text{nm}})$ at pH 1.0 – $(A_{510\text{nm}} - A_{700\text{nm}})$ at pH 4.5, M_w is the molecular weight of anthocyanin (449.2 g/mol for cyanidin-3-glucoside), ϵ is the molar absorptivity of the anthocyanin (26,900 L/mol·cm for cyanidin-3-glucoside), m is the weight of the rice sample (in grams) and D_f is the dilution factor.

Determination of H₂O₂ scavenging activity

The H₂O₂ scavenging activity was determined using a spectrophotometric assay based on the 1,10-phenanthroline method (Mukhopadhyay et al., 2016). A 1 mM solution of 1,10-phenanthroline was prepared by dissolving 0.10 g in 100 mL of distilled water. A 1 mM ferrous ammonium sulfate solution was prepared by dissolving 40 mg in 95 mL of distilled water, followed by the addition of 5 mL of 0.01 N sulfuric acid. The assay was conducted by mixing 0.25 mL of ferrous ammonium sulfate, 1.5 mL of the test sample and 62.5 μL of 5 mM H₂O₂. The mixture was incubated in the dark at room temperature (28 ± 2°C) for 5 min before absorbance measurement. Subsequently, 1.5 mL of 1,10-phenanthroline was added to the mixture and incubated for 10 min at room temperature (28 ± 2°C). The blank solution consisted of 0.25 mL of ferrous ammonium sulfate, 1.562 mL of distilled water and 1.5 mL of 1,10-phenanthroline. Absorbance was measured at 510 nm using the UV-Vis spectrophotometer. H₂O₂ scavenging activity (%) was calculated using Equation 3:

$$\text{H}_2\text{O}_2 \text{ scavenging activity} = A_{\text{test}} / A_{\text{blank}} \times 100 \quad (3)$$

where A_{test} is the absorbance of the test sample and A_{blank} is the absorbance of the blank sample.

Determination of total flavonoid content

The TFC was determined using the aluminum chloride colorimetric assay (Chandra et al., 2014). Doi Saket purple rice extracts were diluted tenfold with distilled water and centrifuged at 3,000 rpm (approximately 1,200 ×g) for 15 min. The resulting supernatant was used for analysis. A quercetin standard stock solution (5 mg/mL in methanol) was prepared and diluted to concentrations ranging from 1.25 to 100 μg/mL. A 2% aluminum chloride solution was prepared by dissolving 2 g of aluminum chloride in 100 mL of distilled water. For the assay, 1 mL of the sample or standard solution was mixed with 1 mL of 2% aluminum chloride and incubated at room temperature (28 ± 2°C) for 1 hr. The absorbance was measured at 420 nm using the UV-Vis spectrophotometer. The TFC was calculated from the quercetin standard curve ($Y = \dots X + \dots$; $R^2 = 0.99$) and calculated based on the weight in grams of the rice sample and expressed as milligrams of quercetin equivalents per gram of rice sample (mg QE/g).

Statistical analysis

The experimental data were analyzed using the SPSS software (version 26, SPSS Inc.; USA). All measurements were performed in triplicate and reported as mean ± SD values. Statistical significance was determined using analysis of variance followed by Tukey's test with significance set at $p < 0.05$.

Results and Discussion

Response and modeling of ultrasonic-assisted extraction process

A BBD was applied to optimize the solid-to-liquid ratio, extraction time and temperature during the UAE of Doi Saket purple rice. The primary objective was to maximize the values for TAC, TFC and H₂O₂ scavenging activity. As presented in Table 2, the TAC was in the range 69.52 ± 4.36–149.84 ± 1.02 mg/g, the TFC was in the range 23.84 ± 0.48–54.88 ± 0.78 mg QE/g and the H₂O₂ scavenging activity was in the range 48.26 ± 2.10–99.17 ± 0.82%. Model performance was evaluated using values for R², F and p, as shown in Table 3. The R² values for the TAC, TFC and H₂O₂ scavenging activity were 0.93, 0.99 and 0.99, respectively. The lack-of-fit tests for all three responses were not significant ($p \geq 0.05$), confirming the adequacy of the models.

Effect of ultrasonic-assisted extraction on total anthocyanin content

The effects of the independent variables on the TAC of Doi Saket purple rice extracts were analyzed and shown in Table 3. The effect of X_1 was highly significant ($p < 0.01$), with the effects of X_1^2 , X_2^2 and X_3^2 being significant ($p < 0.05$). In contrast, the effects of X_2 , X_3 , X_1X_2 , X_1X_3 and X_2X_3 were not significant ($p \geq 0.05$). The quadratic regression model for predicting the TAC, based on the coefficients of the independent variables, is presented in Equation 4 :

$$\begin{aligned} \text{TAC} = & 131.83 + 10.10X_1 + 3.77X_2 + 5.71X_3 \\ & - 1.03X_1X_2 - 0.72X_1X_3 - 0.64X_2X_3 \\ & - 25.19X_1^2 - 26.64X_2^2 - 19.24X_3^2 \end{aligned} \quad (4)$$

The interaction effects of independent variables on the TAC in the Doi Saket purple rice extracts are illustrated in Fig. 1. In all cases, the TAC increased with rising values of the respective variables, reaching a peak of approximately 120 mg/g at optimal conditions of 70°C and 40 min and a 1:2 w/v solid-to-liquid ratio (Fig. 1A), of 70°C and a 1:2 w/v solid-to-liquid ratio (Fig. 1B) and at 70°C and 40 min (Fig. 1C). Beyond these levels, the TAC values declined. Based on these results, the extraction time, temperature and solid-to-liquid ratio significantly influenced the TAC. It was observed that lower extraction temperatures did not enhance extraction efficiency, as mild heat treatment extraction (50–80°C) contributed to the inactivation of oxidative reactions that could otherwise have degraded the total anthocyanin yield (Khoo et al., 2017). However, further increases led to anthocyanin degradation, attributed to their thermal sensitivity and susceptibility to

prolonged exposure to heat and oxygen (Mohamad et al., 2010; Chemat et al., 2016). Additionally, excessive solvent volume reduced the ultrasonic energy transfer efficiency due to the lower energy density, thereby limiting cell disruption and anthocyanin release (Pinelo et al., 2005). Similar trends have been reported in *Clitoria ternatea* (Pham et al., 2019) and purple potato; (Qi et al., 2023), as well as in black soybean (Ryu and Koh, 2019) and red rice bran; (Wang et al., 2019). These findings underlined the need to optimize extraction parameters to balance efficiency with anthocyanin stability during thermal processing.

Table 3 Analysis of variance and regression analysis of Box-Behnken design and response variables for total anthocyanin content (TAC), total flavonoid content (TFC) and hydrogen peroxide (H_2O_2) scavenging activity of Doi Saket purple rice extracts

Source	TAC <i>p</i> value	TFC <i>p</i> value	H_2O_2 scavenging activity (%) <i>p</i> value
Model	0.0193	0.0003*	0.0004*
X_1 (Solid-to-liquid ratio)	0.0356	<0.0001*	<0.0001*
X_2 (Time)	0.3351	0.4995	0.0503
X_3 (Temperature)	0.1677	0.0027	0.0157
X_1X_2	0.8450	0.5114	0.0032
X_1X_3	0.8906	0.2446	0.0716
X_2X_3	0.9032	0.2564	0.1336
X_1^2	0.0046	0.0126	0.8311
X_2^2	0.0036	0.0396	0.0171
X_3^2	0.0136	0.5906	0.0076
Lack-of-Fit	0.8605	0.8171	0.7850
R^2	0.9274	0.9879	0.9868

R^2 = coefficient of determination.

$p < 0.05$ denotes significant difference; $p < 0.01$ denotes highly significant difference.

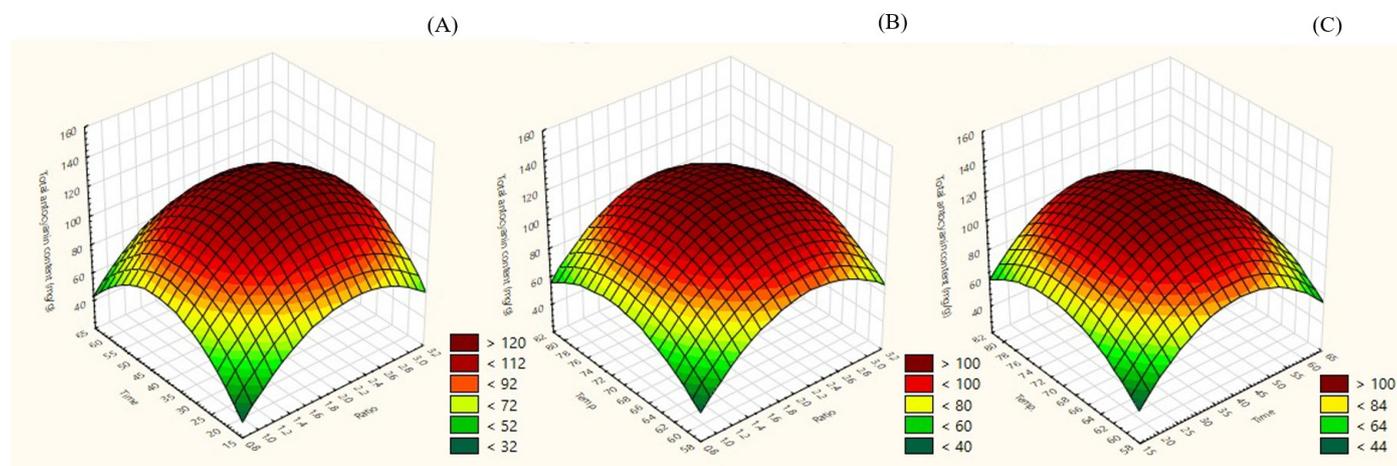


Fig. 1 Response surface plot of effects of independent variables on total anthocyanin content of Doi Saket purple rice extracts: (A) interaction between extraction time and solid-to-liquid ratio; (B) interaction between extraction temperature and solid-to-liquid ratio; (C) interaction between extraction temperature and time.

Effect of ultrasonic-assisted extraction on total flavonoid content

The effects of the independent variables on the TFC of the Doi Saket purple rice extracts were evaluated, as shown in Table 3. The quadratic term X_2^2 had a highly significant effect on TFC ($p < 0.01$) and the effects of X_1 , X_3 and X_1^2 were significant ($p < 0.05$). In contrast, the effects of X_2 , X_1X_2 , X_1X_3 , X_2X_3 and X_3^2 were not significant ($p \geq 0.05$). The corresponding quadratic regression model for predicting the TFC, based on the coefficients of the independent variables, is presented in Equation 5:

$$\begin{aligned} \text{TFC} = & 38.62 - 12.10X_1 + 0.4753X_2 + 3.58X_3 \\ & + 0.65X_1X_2 - 1.21X_1X_3 - 1.18X_2X_3 + \\ & 3.63X_1^2 - 2.64X_2^2 - 0.55X_3^2 \end{aligned} \quad (5)$$

The interaction effects of independent variables on the TFC in the Doi Saket purple rice extracts are illustrated in Fig. 2. The flavonoid content increased with prolonged extraction time and higher temperature but decreased with increasing solid-to-liquid ratio, indicating a direct effect of time and temperature and an indirect effect of the solvent ratio. As shown in Fig. 2C, the flavonoid content increased with both time and temperature, reaching a maximum of 55 mg QE/g at a 1:1 w/v solid-to-liquid ratio for 60 min at 80°C. These findings suggested that flavonoids were relatively heat-stable, consistent with reports attributing this stability to their chemical structure (Gil et al., 2002; Biesaga, 2011; Lu et al., 2018). Consistent with this observation, other studies have

demonstrated that flavonoids can be extracted effectively at temperatures in the range 60–80°C (Wang et al., 2022; Liu et al., 2025). However, degradation can occur under extreme thermal conditions (Jaakola and Hohtola, 2010; Chaaban et al., 2017; Gouot et al., 2019). The reduction in the flavonoid yield with an increasing solid-to-liquid ratio may be attributed to physical dilution effects, which reduced the efficiency of ultrasonic energy transfer, leading to less effective cell disruption (Yohannes et al., 2019). Similar patterns have been observed in cotton flower (Dong et al., 2022) and Xanthoceras sorbifolia flower extracts (Zhang et al., 2021). These results underlined the importance of optimizing extraction parameters to enhance flavonoid yield while minimizing thermal degradation and solvent-related inefficiencies.

Effect of ultrasonic-assisted extraction on hydrogen peroxide scavenging activity

Hydrogen peroxide (H_2O_2) is a strong oxidizing agent commonly used in hair bleaching due to its ability to degrade melanin (Premi and Sharma, 2016). It was used in the current study to assess the antioxidant potential of the Doi Saket purple rice extracts on H_2O_2 . H_2O_2 scavenging activity indicates the capacity of bioactive compounds, particularly anthocyanins and flavonoids, to neutralize reactive oxygen species via electron or hydrogen donation, facilitated by their polyphenolic structures (Tungmunthum et al., 2020). The effects of independent variables on the H_2O_2 scavenging activity of the Doi Saket purple rice extracts are summarized in Table 3.

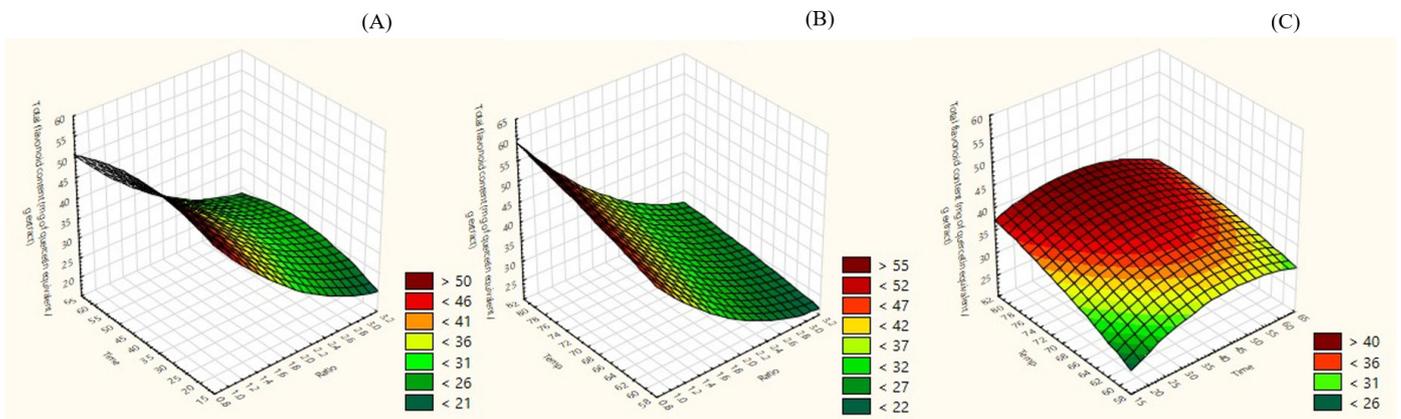


Fig. 2 Response surface plot of effects of independent variables on total flavonoid content of Doi Saket purple rice extracts: (A) interaction between extraction time and solid-to-liquid ratio; (B) interaction between extraction temperature and solid-to-liquid ratio; (C) interaction between extraction temperature and time.

The effects of X_2 , X_3 and X_2^2 on the H_2O_2 scavenging activity were highly significant ($p < 0.01$) and the effects of X_1 , X_1X_2 and X_3^2 were significant ($p < 0.05$). In contrast, the effects of X_1X_3 , X_2X_3 and X_1^2 were not significant ($p \geq 0.05$). The quadratic regression model for predicting H_2O_2 scavenging activity, based on the coefficients of the independent variables, is presented in Equation 6:

$$\begin{aligned} H_2O_2 \text{ scavenging activity (\%)} = & 83.75 - 16.77X_1 + \\ & 2.53X_2 + 3.54X_3 + 7.37X_1X_2 - 3.18X_1X_3 - 2.49X_2X_3 \\ & + 0.33X_1^2 - 5.09X_2^2 - 6.25X_3^2 \end{aligned} \quad (6)$$

The interaction effects of independent variables on the H_2O_2 scavenging activity in the Doi Saket purple rice extracts are shown in Fig. 3. Figs. 3A and 3B reveal similar trends, with the H_2O_2 scavenging activity increasing with time and temperature but decreasing with an increasing solid-to-liquid ratio. The maximum H_2O_2 scavenging activity was 65% with a 1:1 w/v solid-to-liquid ratio and an extraction time of 60 min at 70°C (Fig. 3A) and was 61% at 1:1 w/v and 70°C (Fig. 3B). In Fig. 3C, the H_2O_2 scavenging activity peaked at 58% under conditions of 20 min and 70°C and then declined beyond this point. Based on these results, the H_2O_2 scavenging activity was substantially influenced by extraction time, temperature and solid-to-liquid ratio.

Moderate solid-to-liquid ratios favored higher activity, likely due to reduced dilution, whereas excessively low or high solvent volumes may have limited the extraction efficiency by reducing or increasing the effective transmission distance during extraction (Shen et al., 2023). H_2O_2 scavenging reflects the presence and activity of bioactive compounds such as anthocyanins and flavonoids (Le et al., 2019; Beladhadi et al., 2023). Therefore, the observed trends suggested a strong correlation between H_2O_2 scavenging activity and the levels of anthocyanins and flavonoids in the extracts. This was consistent with other findings showing that the solid-to-liquid ratio affected both the flavonoid yield and H_2O_2 scavenging activity (Duan et al., 2013; Biswas et al., 2023). Furthermore, the extraction time and temperature affected the extraction efficiency of the bioactive compounds. According to this finding, moderate extraction temperatures and time improved the yield of bioactive compounds, which contributed to enhancing the H_2O_2 scavenging activity. Chatzimitakos et al. (2023) reported a similar pattern, indicating that moderate extraction temperature (50–80°C) combined with appropriate extraction durations (60–120 min) enhanced the flavonoid and phenolic contents in *Citrus limon* peel, resulting in significant H_2O_2 scavenging activity (29.59–42.46 μmol ascorbic acid equivalents/g).

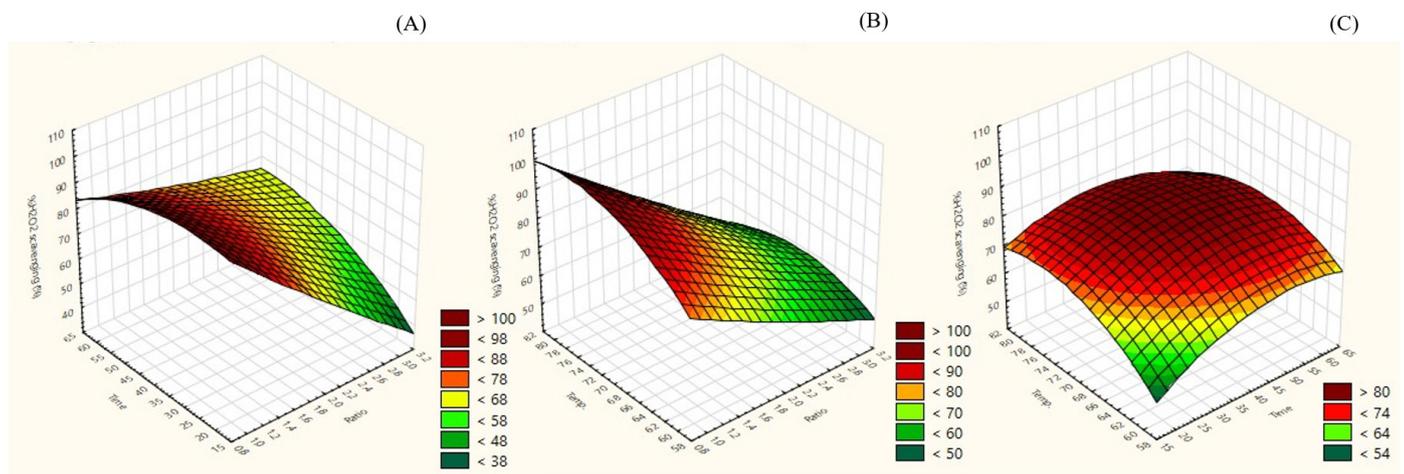


Fig. 3 Response surface plot of effects of independent variables on percentage of hydrogen peroxide scavenging activity of Doi Saket purple rice extracts: (A) interaction between extraction time and solid-to-liquid ratio; (B) interaction between extraction temperature and solid-to-liquid ratio; (C) interaction between extraction temperature and time.

Optimization and validation of extraction conditions

The optimized extraction conditions were obtained from the RSM model and are presented in Table 4. According to the analysis, the extraction factors influencing TAC, TFC and H₂O₂ scavenging activity were identified in the following order: solid-to-liquid ratio (X₁) > extraction temperature (X₃) > extraction time (X₂). Under these conditions, the predicted values were 131.83 mg/g for TAC, 38.62 mg QE/g for TFC and 83.75% for H₂O₂ scavenging activity. In order to verify the predicted value, the experiments were conducted under the suggested optimum conditions (solid-to-liquid ratio of 1:2 w/v, extraction time of 40 min and extraction temperature of 70°C). It was found that the experimental validation under these conditions yielded 112.77 ± 2.34 mg/g of TAC, 39.26 ± 0.35 mg QE/g of TFC and 75.06 ± 1.26% of H₂O₂ scavenging activity. Validation using the coefficient of variation (CV) showed values of 4.15% for TAC, 0.89% for TFC and 1.67% for H₂O₂ scavenging activity. This consistency was confirmed further by the paired t test, which revealed no significant difference between the predicted and experimental values, confirming the model's reliability for all response variables.

Conclusions

The extraction of bioactive compounds from Doi Saket purple rice using UAE was optimized using RSM. Based on the results, the solid-to-liquid ratio, extraction temperature and extraction time influenced the extraction efficiency of TAC, TFC and H₂O₂ scavenging activity, with the solid-to-liquid ratio identified as the most influential factor. The optimal extraction conditions were identified as a solid-to-liquid ratio of 1:2 w/v, extraction time of 40 min and an extraction temperature of 70°C. Under these conditions, the experimental yields were 112.77 ± 2.34 mg/g for TAC, 39.26 ± 0.35 mg QE/g for TFC and 75.06 ± 1.26% for H₂O₂ scavenging activity, consistent with the predicted values. These findings supported the potential application of Doi Saket purple rice extracts as sources of bioactive compounds for use in food and cosmetic products.

Table 4 Predicted and experimental values of response variables under the optimum conditions

Optimum conditions	Response	Predicted value	Experimental value	CV	p value
1:2 (weight per volume), 40 min, 70°C	TAC (mg/g)	131.83	112.77 ± 2.34	4.15%	0.020
	TFC (mg QE/g)	38.62	39.26 ± 0.35	0.89%	0.003
	H ₂ O ₂ scavenging activity (%)	83.75	75.06 ± 1.26	1.67%	0.015

TAC = total anthocyanin content; TFC = total flavonoid content; H₂O₂ = hydrogen peroxide; CV = coefficient of variation.

Data shown as mean ± SD values, derived from three independent experiments (n = 3).

p < 0.05 denotes significant difference; p < 0.01 denotes highly significant difference.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by a grant from Thailand Science Research and Innovation (TSRI). The Research and Development Institute and the College of Integrated Science and Technology, Rajamangala University of Technology Lanna, Thailand provided support and facilitation.

References

- Beladhadi, R.V., Shankar, K., Jayalakshmi, S.K., Sreeramulu, K. 2023. Valorization of rice biomass by a green approach to release phenolic compounds and their antioxidant activities. *Prep. Biochem. Biotechnol.* 53: 93–100. doi.org/10.1080/10826068.2022.2042821
- Biesaga, M. 2011. Influence of extraction methods on stability of flavonoids. *J. Chromatogr. A.* 1218: 2505–2512. doi.org/10.1016/j.chroma.2011.02.059
- Biswas, A., Dey, S., Xiao, A., et al. 2023. Ultrasound-assisted extraction (UAE) of antioxidant phenolics from *Corchorus olitorius* leaves: a response surface optimization. *Chem. Biol. Technol. Agric.* 10: 64. doi.org/10.1186/s40538-023-00443-2
- Castañeda-Ovando, A., Pacheco-Hernandez, M., Paez-Hernandez, M., Rodriguez, J., Galanvidal, C.A. 2009. Chemical studies of anthocyanins: A review. *Food Chem.* 113: 859–871. doi.org/10.1016/j.foodchem.2008.09.001
- Chaaban, H., Ioannou, I., Chebil, L., et al. 2017. Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *J. Food Process. Preserv.* 14: e13203. doi.org/10.1111/jfpp.13203
- Chandra, S., Khan, S., Avula, L., Lata, H., Yang, M.H., ElSohly, M.A., Khan, I.A. 2014. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. *Evid. Based Complement. Alternat. Med.* 2014: 253875. doi.org/10.1155/2014/253875

- Chemat, F., Rombaut, N., Sicaire, A.G., Meullemiestre, A., Fabiano-Tixier, A.S., Abert-Vian, M. 2016. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications – A review. *Ultrason. Sonochem.* 34: 540–560.
- Cocito, C., Gaetano, G., Delfini, C. 1995. Rapid extraction of aroma compounds in must and wine by means of ultrasound. *Food Chem.* 52: 311–320.
- Das, A.B., Goud, V.V., Das, C. 2017. Extraction of phenolic compounds and anthocyanin from black and purple rice bran (*Oryza sativa* L.) using ultrasound: A comparative analysis and phytochemical profiling. *Ind. Crops Prod.* 95: 332–341. doi.org/10.1016/j.indcrop.2016.10.041
- Demirdöven, A., Özdoğan, K., Tokatli, E. 2015. Extraction of anthocyanins from red cabbage by ultrasonic and conventional methods: optimization and evaluation. *J. Food Biochem.* 39: 491–500. doi.org/10.1111/jfbc.12153
- Dong, J., Zhou, K., Ge, X., et al. 2022. Effects of extraction technique on the content and antioxidant activity of flavonoids from *Gossypium hirsutum* Linn. flowers. *Molecules.* 27: 5627. doi.org/10.3390/molecules27175627
- Duan, M.H., Luo, M., Zhao, C.J., Wang, W., Zu, Y.G., Zhang, D.Y., Yao, X.H., Fu, Y.J. 2013. Ionic liquid-based negative pressure cavitation-assisted extraction of three main flavonoids from the pigeonpea roots and its pilot-scale application. *Sep. Purif. Technol.* 107: 26–36.
- Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Kader, A.A. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.* 50: 4976–4982. doi.org/10.1021/jf020136b
- Gongjian, F., Yonbin, H., Zhenxin, G., Deming, C. 2008. Optimizing conditions for anthocyanin extraction from purple sweet potato using response surface methodology. *LWT – Food Sci. Technol.* 41: 155–160.
- Gouot, J.C., Smith, J.P., Holzapfel, B.P., Walker, A.R., Barril, C. 2019. Grape berry flavonoids: a review of their biochemical responses to high and extreme high temperatures. *J. Exp. Bot.* 70: 397–423. doi.org/10.1093/jxb/ery392
- Jaakola, L., Hohtola, A. 2010. Effect of latitude on flavonoid biosynthesis in plants. *Plant Cell Environ.* 33: 1239–1247. doi.org/10.1111/j.1365-3040.2010.02154.x
- Jiang, H.S., Yang, J.L., Shi, Y.P. 2017. Optimization of ultrasonic cell grinder extraction of anthocyanins from blueberry using response surface methodology. *Ultrason. Sonochem.* 34: 325–331.
- Le, X., Huynh, M.T., Pham, T.N., Than, V.T., Toan, T.Q., Bach, L.G., Trung, N.Q. 2019. Optimization of total anthocyanin content, stability and antioxidant evaluation of the anthocyanin extract from Vietnamese *Carissa carandas* L. fruits. *Processes.* 7: 468. doi.org/10.3390/pr7070468
- Liu, J., Guo, X., Yang, T., Wang, X., Liang, Y. 2025. Study on the extraction and stability of total flavonoids from *Milletia speciosa* Champ. *PLoS One.* 20: e0326570. doi.org/10.1371/journal.pone.0326570
- Lu, Q., Peng, Y., Zhu, C., Pan, S. 2018. Effect of thermal treatment on carotenoids, flavonoids and ascorbic acid in juice of orange cv. Cara Cara. *Food Chem.* 265: 39–48. doi.org/10.1016/j.foodchem.2018.05.072
- Mane, S., Bremner, D.H., Tziboula-Clarke, A., Lemos, M.A. 2015. Effect of ultrasound on the extraction of total anthocyanins from Purple Majesty potato. *Ultrason. Sonochem.* 27: 509–514. doi.org/10.1016/j.ultsonch.2015.06.021
- Mason, T.J., Cobley, A.J., Graves, J.E., Morgan, D. 2011. New evidence for the inverse dependence of mechanical and chemical effects on the frequency of ultrasound. *Ultrason. Sonochem.* 18: 226–230. doi.org/10.1016/j.ultsonch.2010.05.008
- Min, B., Gu, L., McClung, A.M., Berman, C.J., Chen, M.H. 2012. Free and bound total phenolic concentrations, antioxidant capacities, and profile of proanthocyanins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. *Food Chem.* 133: 715–722. doi.org/10.1016/j.foodchem.2012.01.079
- Mohamad, M., Ali, M.W., Ahmad, A. 2010. Modelling for extraction of major phytochemical components from *Eurycoma longifolia*. *J. Appl. Sci.* 10: 2572–2577. doi.org/10.3923/jas.2010.2572.2577
- Mukhopadhyay, D., Dasgupta, P., Roy, D.S., Palchoudhuri, S., Chatterjee, I., Ali, S., Dastidar, S.G. 2016. A sensitive in vitro spectrophotometric hydrogen peroxide scavenging assay using 1,10-phenanthroline. *Free Radic. Antioxid.* 6: 123–131. doi.org/10.5530/fra.2016.1.15
- Pham, T.N., Lam, T.D., Nguyen, M.T., Le, X.T., Vo, D.N., Toan, T.Q., Vo, T.S. 2019. Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam. In: *Proceeding of 2nd International Conference on Mechanical Engineering and Applied Composite Materials*. Harbin, China. p. 012013.
- Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., Núñez, M.J. 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J. Agric. Food Chem.* 53: 2111–2117. doi.org/10.1021/jf0488110
- Pratiwi, R., Purwestri, Y.A. 2017. Black rice as a functional food in Indonesia. *FFHD.* 7: 182–194. doi.org/10.31989/ffhd.v7i3.310
- Premi, M., Sharma, H.K. 2016. Effect of extraction conditions on the bioactive compounds from *Moringa oleifera* (PKM 1) seeds and their identification using LC-MS. *J. Food Meas. Charact.* 11: 1–13.
- Qi, Z., Wang, W., Liu, Z., et al. 2023. Anthocyanin profiles in colored potato tubers at different altitudes by HPLC-MS analysis with optimized ultrasound-assisted extraction. *Foods.* 12: 4175. doi.org/10.3390/foods12224175
- Rein, M.J. 2005. Co-pigmentation reactions and color stability of berry anthocyanins. Ph.D. Thesis, Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland.
- Rerkasem, B., Jumrus, S., Yimyam, N., Prom-u-thai, C. 2015. Variation of grain nutritional quality among Thai purple rice genotypes grown at two different altitudes. *Sci. Asia.* 41: 377–385. doi.org/10.2306/scienceasia1513-1874.2015.41.377
- Ryu, D., Koh, E. 2019. Optimization of ultrasonic-assisted extraction of anthocyanins and phenolic compounds from black soybeans (*Glycine max* L.). *Food Anal. Methods.* 12: 1382–1389. doi.org/10.1007/s12161-019-01462-2.
- Scalzo, R.L., Genna, A., Branca, F., Chedin, M., Chassaigne, H. 2008. Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*B. oleracea* L. var. *capitata*) and its stability in relation to thermal treatment. *Food Chem.* 107: 136–144.

- Shen, L., Pang, S., Zhong, M., et al. 2023. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrason. Sonochem.* 101: 106646. doi.org/10.1016/j.ultsonch.2023.106646
- Srimoon, R., Santimalai, S. 2018. Extraction of anthocyanin from black plum (*Syzygium cumini* Skeels) using pulsed-electric field assisted. *KKU Sci. J.* 46: 800–811.
- Tiwary, K.B. 2015. Ultrasound: A clean, green extraction technology. *TrAC.* 71: 100–109.
- Tonutare, T., Moor, U., Szajdak, L. 2014. Strawberry anthocyanin determination by pH differential spectroscopic method—how to get true results? *Acta Sci. Pol-Hortoru.* 13: 35–47.
- Tungmunnithum, D., Drouet, S., Kabra, A., Hano, C. 2020. Enrichment in antioxidant flavonoids of stamen extracts from *Nymphaea lotus* L. using ultrasonic-assisted extraction and macroporous resin adsorption. *Antioxidants.* 9: 576. doi.org/10.3390/antiox9070576
- Wang, Y., Zhao, L., Zhang, R., Yang, X., Sun, Y., Shi, L., Xue, P. 2019. Optimization of ultrasound-assisted extraction by response surface methodology, antioxidant capacity, and tyrosinase inhibitory activity of anthocyanins from red rice bran. *Food Sci. Nutr.* 8: 921–932. doi.org/10.1002/fsn3.1371
- Wang, Z., Yang, S., Gao, Y., Huang, J. 2022. Extraction and purification of antioxidative flavonoids from *Chionanthus retusa* leaf. *Front. Bioeng. Biotechnol.* 10: 1085562.
- Yang, L., Cao, Y.L., Jiang, J.G., Lin, Q.S., Chen, J., Zhu, L. 2010. Response surface optimization of ultrasound-assisted flavonoids extraction from the flower of *Citrus aurantium* L. var. *amara* Engl. *J. Sep. Sci.* 33: 1349–1355.
- Yohannes, A., Zhang, B., Dong, B., Yao, S. 2019. Ultrasonic extraction of tropane alkaloids from *Radix physochlainae* using as extractant an ionic liquid with similar structure. *Molecules.* 24: 2897. doi.org/10.3390/molecules24162897
- Zhang, H., Wang, X., He, D., Zou, D., Zhao, R., Wang, H., Li, S., Xu, Y., Abudureheman, B. 2021. Optimization of flavonoid extraction from *Xanthoceras sorbifolia* Bunge flowers, and the antioxidant and antibacterial capacity of the extract. *Molecules.* 27: 113. doi.org/10.3390/molecules27010113
- Zhang, X., Shen, Y., Prinyawiwatkul, W., King, J.M., Xu, Z. 2013. Comparison of the activities of hydrophilic anthocyanins and lipophilic tocots in black rice bran against lipid oxidation. *Food Chem.* 141: 111–116. doi.org/10.1016/j.foodchem.2013.03.034
- Zhu, Z., Guan, Q., He, J., Liu, G., Li, S., Barba, F.J., Jaffrin, M.Y. 2016. Green ultrasound-assisted extraction of anthocyanin and phenolic compounds from purple sweet potato using response surface methodology. *Int. Agrophys.* 30: 113–122. doi.org/10.1515/intag-2015-0066
- Zou, T.B., Wang, M., Gan, R.Y., Ling, W.H. 2011. Optimization of ultrasound-assisted extraction of anthocyanins from mulberry using response surface methodology. *Int. J. Mol. Sci.* 12: 3006–3017. doi.org/10.3390/ijms12053006