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# Water Extraction Optimization of Rice Leaf Extracts via Face-Centered Composite Design (FCCD)

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ABSTRACT

Rice leaves (*Oryza sativa* L.) contain a high concentration of bioactive compounds, particularly flavonoids and phenolics, which have potential applications in functional foods. This study aims to optimize the decoction process for extracting phenolic compounds from rice leaves using Response Surface Methodology (RSM). A Face-Centered Composite Design (FCCD) was applied to evaluate the impact of extraction parameters, including time and liquid-to-solid ratio, on total phenolic content (TPC) and antioxidant activities (FRAP, DPPH, ABTS). Results showed that the pH of the extracts remained stable (6–7) regardless of extraction conditions, while colorimetric analysis indicated that a lower liquid-to-solid ratio and prolonged boiling enhanced yellowness ($b^*$) and chroma. The optimal extraction conditions determined through RSM were a boiling time of 30 minutes and a ratio of 37 mL/g, yielding maximum TPC and antioxidant activities. The model demonstrated statistical significance ($p < 0.05$), with high $R^2$ values (>0.9) and adequate precision (>4). Under these conditions, phenolic extraction efficiency improved by 3–7% compared to previous studies, while solvent usage was reduced by 7.5%. These findings confirm that optimizing decoction parameters enhances the efficiency of phenolic compound extraction while maintaining food-grade suitability, making it feasible for large-scale applications.

Keywords: Rice leaf extract, phenolic compounds, decoction, response surface methodology, antioxidant activity

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food crop in many Asian countries, and its agricultural abundance has led to extensive research on utilizing different parts of the rice plant beyond its grain. In particular, rice leaves contain a diverse range of bioactive compounds, with more than 100 flavonoids identified, primarily in young leaves during the vegetative stage [1]. These flavonoids and other phenolic compounds have demonstrated significant potential for applications in functional food production due to their antioxidant properties.

The extraction of bioactive compounds from plant materials is crucial for their effective utilization. Various techniques have been explored to maximize the recovery of phenolic compounds from rice leaves. Among them, decoction, or the boiling method, stands out as a widely used conventional approach. Decoction involves immersing plant material in water and applying heat to break down plant cell walls, thereby facilitating the release of soluble phytochemicals [2], [3]. This method is particularly advantageous for food-grade applications due to its simplicity, affordability, and suitability for large-scale production.

Given the complexity of phenolic compounds and their interactions with other bioactive components in the plant matrix, optimizing the boiling process is essential to ensure high-yield extraction while preserving the bioactivity of the compounds. Response Surface Methodology (RSM) is a powerful statistical tool that allows for the modeling and optimization of process parameters by evaluating the effects of multiple variables and their interactions [4], [5]. Unlike conventional single-factor optimization, RSM enhances process efficiency by simultaneously analyzing multiple

parameters, thereby improving extraction conditions in a systematic manner.

A previous study has been done to compare the performance of rice leaf extraction using conventional methods [6]. The results indicated that maceration yielded higher antioxidant activity. However, the decoction method remains advantageous due to its organic solvent-free nature, making it more suitable for food applications. Despite its lower efficiency in some aspects, decoction has the potential to be optimized for improved extraction yield and bioactivity retention. Therefore, this study aims to optimize the boiling conditions for extracting phenolic compounds from rice leaves using the RSM approach. A Face-Centered Composite Design (FCCD) was applied to evaluate the impact of two key extraction parameters—boiling time and liquid-to-solid ratio—on total phenolic content (TPC) and antioxidant activities (FRAP, DPPH, ABTS). These parameters were selected based on literature precedence and preliminary trials, which indicated they had the greatest influence on extraction efficiency under food-grade and water-based conditions [2], [6]. By refining the decoction process, this research seeks to develop an efficient, food-grade extraction method that is scalable for industrial production of functional rice leaf extracts.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Young rice leaves (cultivar Khao Hom Mali Khiew) used in this study were sourced from local farmers participating in the Organic Agriculture Project in Sukhothai Province, Thailand. Leaves were harvested 7 days after germination, and were thoroughly washed, spreaded on aluminum trays and sun-dried for approximately three days until the moisture content was reduced to less than 10% (wet basis). Once dried, the leaves were ground into a fine powder, vacuum-sealed, and kept at -20 °C until further analysis.

### 2.2 Experimental procedure

The decoction process was carried out using distilled water as the extraction solvent, following the methodology described in a previous study [7]. 50 mL of boiling water was prepared, followed by addition of a predetermined amount of dried rice leaf powder to make up liquid-to-solid ratios set at 20, 30, and 40 mL/g. The extraction process was performed for 10, 20, and 30 minutes. After extraction, the rice leaf extracts (RLEs) were filtered using Whatman Grade 1 qualitative filter paper, followed by centrifugation. The resulting extracts were then stored at -20 °C until further analysis.

### 2.3 Methods of analysis

#### 2.3.1 Determination of pH and chromatic properties

pH of each RLE was determined by a pH meter (Mettler Toledo, Seven Compact S220, Columbus, OH, USA). Chromatic properties were measured using a chromameter (Konica Minolta, CR-400, Tokyo, Japan), following Sabatino, et al. [8].

#### 2.3.2 Total phenolics content (TPC) and antioxidant activity (AA) analysis

Total phenolic content (TPC) was assessed using a Folin-Ciocalteu method [9]. TPC was expressed as milligrams of gallic acid equivalent per gram of dry-weight rice leaves (mg GAE/g d.b.). Antioxidant activity (AA) was evaluated using ABTS, DPPH, and FRAP assays. The ABTS assay followed the methodology described by Santarelli et al. [10], while DPPH and FRAP assays were performed according to the protocols outlined by Hamad et al. [11].For all antioxidant tests, Trolox was used as the reference standard, and results were expressed in mM TE/g d.b. Absorbance readings for all assays were obtained using a UV-VIS spectrophotometer (Lambda 25, PerkinElmer, Boston, MA, USA). Each analysis was conducted in triplicate.

#### 2.3.3 Experimental design

A two-factor-three-level face-centered central composite design (FCCD) was used to decide the optimum extraction conditions of the rice leaves. Thirteen experiments were developed using the Design Expert® software (Version 13, Stat. Ease Inc., Minneapolis, USA). Five center repetitions were carried out, with the entire experiment run unsystematically to minimize variability caused by uncontrolled factors [12]. Table 1 presents an overview of the independent variables and their coded levels.

Table 1. Summary of the independent variables and their coded levels for FCCD

| Factor Level codes | Independent variables | |
|---|---|---|
| | time (min), *A* | Ratio (mL/g), *B* |
| -1 | 10 | 20 |
| 0 | 20 | 30 |
| 1 | 30 | 40 |

### 2.3.4 Statistical analysis

The Duncan Multiple Range Test (DMRT) is employed to compared the physical properties of RLEs. Beside that, Levene's test was done before modelling was conducted with RSM. Both DMRT and Levene's test were done using IBM SPSS Statistics 22 (SPSS, Chicago, IL, USA). Following model development, an analysis of variance (ANOVA) was performed to determine the statistical significance of the independent variables and their effects on the response variables. A model ($p < 0.05$) and multiple regressions were used in interpreting the experimental data. The design was expressed by second-order polynomial regression, as shown in **Eq. (1)** where $Y$ is the response, $\beta_0$ is constant coefficient, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ represent the linear, quadratic, and interaction coefficients, respectively. Meanwhile, A and B represent the independent variables, and ε is the residual associated with the experiments.

$$Y = \beta_0 + \sum \beta_i A + \sum \beta_{ii} A^2 + \sum \beta_{ij} AB \quad (1)$$

### 2.3.5 Verification of the model

To assess the reliability of the developed model, the optimized extraction conditions obtained from the RSM were tested in actual experiment. The results were compared with the predicted values to evaluate the model's accuracy. Additionally, the residual standard error (RSE) was calculated to quantify the deviation between observed and predicted responses [12].

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of extraction conditions to properties of RLEs

**Table 2**. shows the physical characteristics of rice leaf extracts. The physical characteristics of rice leaf extracts, particularly pH and color, were influenced by the extraction parameters. Using water as the sole solvent resulted in a stable pH (6–7) with no significant differences among samples, suggesting that the extraction process maintained a neutral to slightly acidic environment. This is expected when using water as the sole solvent, as there are no strong acids or bases introduced to alter the natural pH of the plant material.

Colorimetric properties were assessed using the *CIE Lab** system, where *L** indicates lightness (0 = black to 100 = white), *a** indicates the red–green axis (positive = red, negative = green), and *b** denotes the yellow–blue axis (positive = yellow, negative = blue). All extracts showed positive *b** values, confirming yellow pigmentation. Increased *b** values under lower liquid-to-solid ratios and longer boiling times suggest enhanced yellowness. Chroma (*C**), representing color saturation, also increased under these conditions, indicating more vivid coloration. This may result from concentration effects and improved pigment release during heating [13]. However, excessive heat can degrade pigments, necessitating careful control of boiling time. Furthermore, while elevated *b** and *C** values reflect stronger visual intensity, they do not directly indicate specific compound concentrations. Therefore, total phenolic content and antioxidant activities were also evaluated.

### 3.2 Model fitting and evaluation

RSM was used with FCCD to examine the impacts of extraction variables on the TPC, FRAP, DPPH, and ABTS. **Table 3** presents the experimental design of the experiments for the response variables. The actual values of the response variables from from decoction for TPC ranged from 7.80 – 12.14 mg GAE/g d.b., and for FRAP, DPPH, and ABTS, they ranged from 16.55 – 31.05, 12.02 – 28.38, and 27.48 – 95.65 mM TE/g d.b. The model fitting was done by generating multiple regression analyses models for each response, expressed by a quadratic polynomial equation. **Table 4** represents the regression coefficients employed in the model, followed by their evaluation through ANOVA and fit statistics regression analysis.

In order to fulfil one model's adequacy, several parameters can be considered, including *p*-value model < 0.05, Lack of fit *p*–value > 0.05, $R^2$ > 0.9, and adequacy precision > 4 [4]. All p-value model for all response showed a value less than 0.05, meaning that the statistical significance of the observed difference is prominent. For instance, the result showed that model's F value and p-value for FRAP were 57.92 and <0.0001 and 104.14, respectively. It means there was only a less than 0.01% chance that F values of 57.92 could be ascribed to noise rather than data signal. For the *p*-values of terms, a value less than 0.05 indicates that model terms are significant. If there are many insignificant model terms, model term reduction may improve the model. In the case of the decoction method, the results showed significant interaction among factors in all assays ($p < 0.05$), except for ABTS. It suggested that every sample with a determined liquid-to-solid ratio will most likely behave differently at each boiling time, making it compulsory to consider the interactive effect of the two factors before the individual effects.

**Table 2.** Physical characteristics of rice leaf extracts from boiling method

| No. | Variables | | Physical Characteristics | | | | |
|---|---|---|---|---|---|---|---|
| | Ratio (mL/g) | Time (min) | pH | *L** | *a** | *b** | Chroma |
| 1 | 20 | 10 | 6.3 ± 0.1 | 91.2 ± 0.1$^{c}$ | -4.5 ± 0.1$^{a}$ | 24.4 ± 0.5$^{e}$ | 24.3 ± 0.0$^{d}$ |
| 2 | 20 | 20 | 6.4 ± 0.1 | 90.0 ± 0.2$^{b}$ | -4.4 ± 0.1$^{b}$ | 26.5 ± 0.2$^{f}$ | 26.7 ± 0.0$^{e}$ |
| 3 | 20 | 30 | 6.3 ± 0.0 | 89.1 ± 0.7$^{a}$ | -4.3 ± 0.1$^{c}$ | 29.3 ± 0.0$^{g}$ | 29.6 ± 0.0$^{f}$ |
| 4 | 30 | 10 | 6.3 ± 0.1 | 92.7 ± 0.2$^{de}$ | -4.2 ± 0.1$^{c}$ | 19.7 ± 0.5$^{c}$ | 20.7 ± 0.0$^{e}$ |
| 5 | 30 | 20 | 6.3 ± 0.2 | 92.6 ± 0.2$^{d}$ | -4.1 ± 0.0$^{d}$ | 19.2 ± 0.3$^{c}$ | 19.9 ± 0.0$^{d}$ |
| 6 | 30 | 30 | 6.3 ± 0.3 | 91.6 ± 0.0$^{c}$ | -4.2 ± 0.0$^{d}$ | 20.9 ± 0.0$^{d}$ | 21.4 ± 0.0$^{f}$ |
| 7 | 40 | 10 | 6.3 ± 0.1 | 94.6 ± 0.5$^{g}$ | -2.6 ± 0.0$^{f}$ | 9.9 ± 0.0$^{a}$ | 10.2 ± 0.0$^{a}$ |
| 8 | 40 | 20 | 6.4 ± 0.1 | 93.5 ± 0.1$^{f}$ | -3.7 ± 0.0$^{e}$ | 15.9 ± 0.4$^{b}$ | 16.7 ± 0.0$^{c}$ |
| 9 | 40 | 30 | 6.3 ± 0.2 | 93.2 ± 0.0$^{ef}$ | -3.7 ± 0.0$^{e}$ | 16.2 ± 0.0$^{b}$ | 16.6 ± 0.0$^{b}$ |

Data represents the mean ± standard deviation of three independent experiments. For each parameter Dissimilar letters in the same column indicate significantly different at $p < 0.05$ by DMRT.

**Table 3.** Face-centered composite design in terms of the coded value of independent variables with the observed responses

| Runs | Independent Variable Codes | | Responses | | | |
|---|---|---|---|---|---|---|
| | A | B | TPC | FRAP | DPPH | ABTS |
| 1 | 0 | 0 | 10.52 | 27.72 | 17.78 | 31.63 |
| 2 | -1 | -1 | 9.45 | 21.13 | 21.43 | 76.73 |
| 3 | 0 | 0 | 11.42 | 29.08 | 17.30 | 42.13 |
| 4 | 0 | 0 | 12.16 | 28.60 | 15.93 | 38.21 |
| 5 | 1 | 1 | 11.67 | 29.99 | 28.38 | 67.72 |
| 6 | 0 | -1 | 9.36 | 21.73 | 21.54 | 76.01 |
| 7 | 0 | 1 | 8.58 | 22.88 | 19.45 | 49.42 |
| 8 | 1 | -1 | 9.50 | 24.24 | 21.14 | 95.65 |
| 9 | -1 | 1 | 7.80 | 16.55 | 15.78 | 27.48 |
| 10 | 0 | 0 | 11.50 | 29.12 | 13.69 | 42.69 |
| 11 | -1 | 0 | 10.93 | 25.98 | 12.02 | 34.40 |
| 12 | 1 | 0 | 12.17 | 31.05 | 19.54 | 51.68 |
| 13 | 0 | 0 | 11.12 | 28.56 | 17.34 | 33.12 |

The Independent variables were boiling time in minute (*A*) and liquid-to-solid ratio in mL/g (*B*).
TPC was expressed as mg GAE/100 g d.b., and Trolox equivalent antioxidant activity assays were expressed as mM TE/g d.b.

The third parameter to be considered is the *p*-value of the lack of fit test. From the results, we can see that all *p*-values in this parameter have values more than 0.05. The model could accurately fit with the actual data, demonstrating that all quadratic polynomial models were steadfast and proper for denoting suitable responses [14]. The next parameter is the $R^2$ value. As it was generally believed, $R^2$ values above 0.9 may be used to indicate the adequacy of the model. However, the further assessment of $R^2$ values demonstrates the effectiveness of the model [15]. Beside the $R^2$ value, we also need to consider the value of Adjusted and Predicted $R^2$. A negative Predicted $R^2$ indicates that the general mean model may better predict response than the current model. Furthermore, The Predicted $R^2$ value that is far from the Adjusted $R^2$ may suggest an extensive block outcome or a potential problem with the model or data. In this study, each model generated had a positive predicted $R^2$ and a difference of less than 0.2 between Adjusted $R^2$ and Predicted $R^2$. The last parameter was adequacy precision. This value measures the signal-to-noise ratio. As exhibited by the results, a ratio of more than four is desirable, showing that the signal is adequate and the model can navigate the design space.

Finally, the second-order polynomial regression equations generated from the FCCD model describe the relationships between the extraction variables—boiling time (A) and liquid-to-solid ratio (B)—and the response variables: total phenolic content (TPC), and antioxidant activities (FRAP, DPPH, ABTS). The fitted models for each response are presented in **Eq. (2-5).**

Table 4. Regression coefficients and statistical parameters for the quadratic polynomial models developed for the decoction method of RLEs

| Model terms | Response | | | |
|---|---|---|---|---|
| | TPC | FRAP | DPPH | ABTS |
| $\beta_0$ | 11.28* | 28.51* | 16.20* | 37.74* |
| $\beta_i$ | 0.86* | 3.60* | 3.26* | 12.74* |
| $\beta_j$ | -0.04 | 0.39 | -0.03 | -17.29* |
| $\beta_{ij}$ | 0.96* | 2.58* | 3.22* | 5.33 |
| $\beta_{ii}$ | 0.41 | 0.27 | 0.23 | 4.86 |
| $\beta_{jj}$ | -2.17* | -5.94* | 4.99* | 24.53* |
| **ANOVA test results** | | | | |
| F-value model | | | | |
| *p*-value model | | | | |
| Lack of Fit | 0.6446 | 0.1029 | 0.7049 | 0.3907 |
| **Fit Statistics regression results** | | | | |
| $R^2$ | 0.91 | 0.98 | 0.93 | 0.96 |
| Adj. $R^2$ | 0.85 | 0.96 | 0.87 | 0.93 |
| Pred. $R^2$ | 0.65 | 0.82 | 0.74 | 0.79 |
| Adeq. Precision | 13.19 | 26.17 | 14.64 | 16.98 |

$\beta_i$ and $\beta_j$ are the values of the independent variables: extraction time (min) and liquid-to-solid ratio (mL/g), respectively for decoction.
* The term is significant at $p \leq 0.05$

$$Y_{TPC} = 11.28 + 0.86A - 0.04B + 0.96AB + 0.41A^2 - 2.17B^2 \quad (2)$$

$$Y_{FRAp} = 28.51 + 3.60A + 0.39B + 2.58AB + 0.27A^2 - 5.94B^2 \quad (3)$$

$$Y_{DPPH} = 16.20 + 3.26A - 0.03B + 3.22AB + 0.23A^2 + 4.99B^2 \quad (4)$$

$$Y_{ABTS} = 37.74 + 12.74A - 17.29B + 5.33AB + 4.86A^2 + 24.53B^2 \quad (5)$$

### 3.3 Effect of parameters

Figure 1 shows the 3D response surface to illustrate the independent variables' interactive effects on rice leaf extracts' responses. The plots were generated by plotting responses (TPC and AA) using the z-axis against each pair of independent variables of decoction method.

#### 3.3.1. Total phenolic content (TPC)

Phenolic compounds are crucial components that has an aromatic ring, carrying one or more hydroxyl groups in plant extracts that promote free radical scavenging [16]. As a basis in this study, TPC was measured using the Folin–Ciocalteu reagent in each extract. Figure 1(a) shows the response surface plot for the interactive effect of boiling time and the liquid-to-solid ratio to the TPC value in decoction. Across the range of liquid/solid reactions, extraction duration of 30 min resulted in higher TPC, up to 12.556 mg GAE/g d.b. at a constant ratio (30 mL/g). A long extraction time could improve the transfer of the phenolic compounds from plant to the environment (surrounding liquid), causing the enhancement of extractions [13].

#### 3.3.2. Antioxidant activity (AA) and its correlation to TPC

A wide variety of phytochemicals in plants has been approved to exert integrated antioxidant and anti-inflammatory actions that contribute to the health advantages of functional food [17]. Figure 1(b) – 1(d) showed the results of interactive effects of extraction parameters to antioxidant activities. Increasing the duratio of extaction times improved the antioxidant activities for all asays, similar to the trend observed for TPC values. However, when focusing on the liquid/solid ratio, two distinct trends emerged. The FRAP values showed an inward curve towards the x-y axis (which closely resembled the shape observed in the TPC values), while, other assay results had an outward curve. One possible explanation for this discrepancy is that the mechanism underlying each assay significantly impacts the results of antioxidant activity.

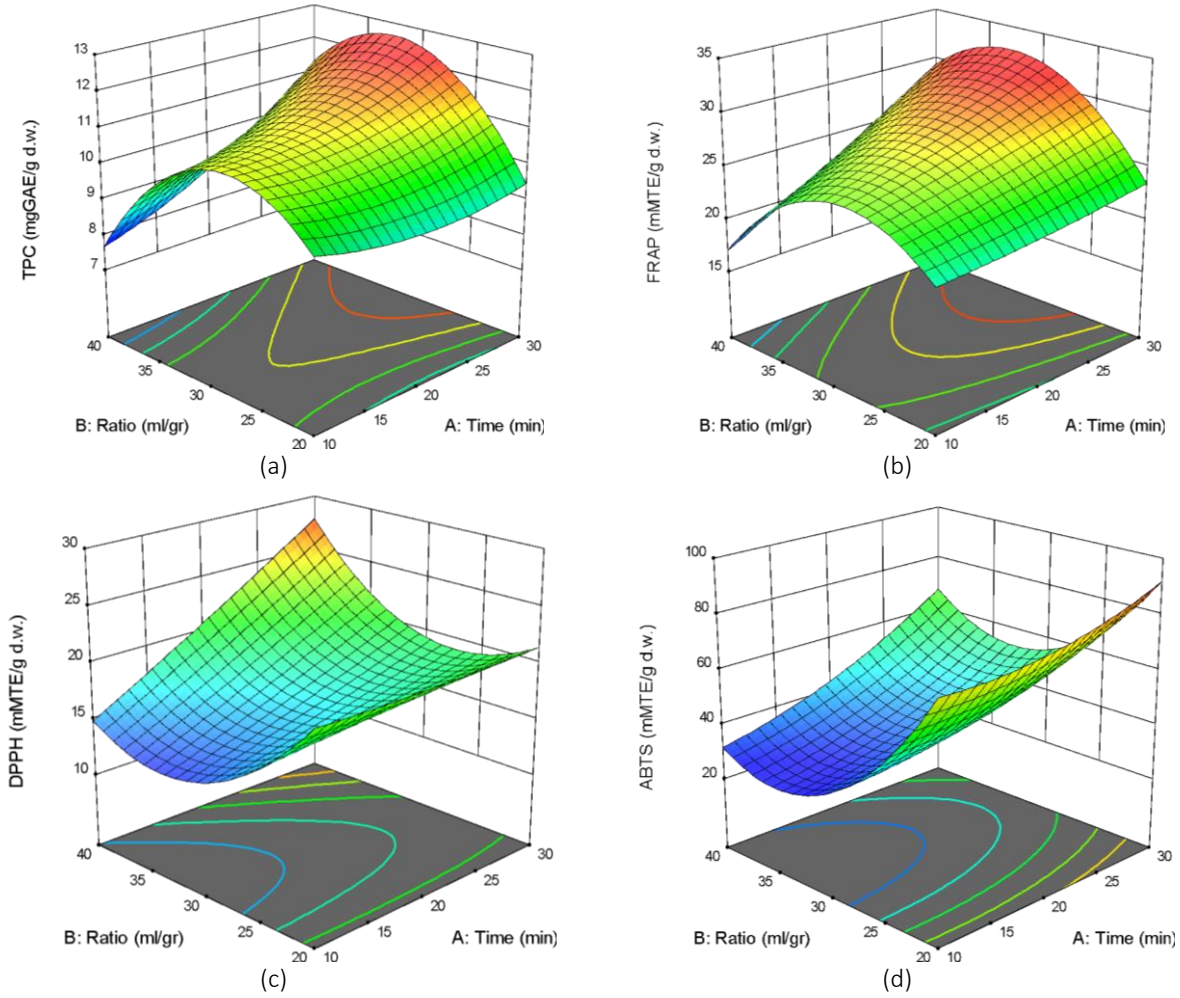


**Figure 1.** Response surface plots for the interaction effect of (a) TPC; (b) FRAP; (c) DPPH; and (d) ABTS as a function of boiling time (min) and liquid-solid ratio (mL/g) in decoction

Table 5. Predicted and actual response values for the optimized extraction parameters

| Extraction time | Ratio | Responses | Predicted Value | Experimental value | RSE (%) | Desirability |
|---|---|---|---|---|---|---|
| 30 min | 37 mL/gr | TPC (mgGAE/g d.b.) | 12.18 | 12.09 ± 1.00 | 0.75 | 0.761 |
| | | FRAP (mMTE/g d.b.) | 31.67 | 31.37 ± 1.93 | 0.94 | |
| | | DPPH (mMTE/g d.b.) | 27.88 | 30.50 ± 1.60 | 9.40 | |
| | | ABTS (mMTE/g d.b.) | 58.50 | 62.51± 0.93 | 6.86 | |

Previous studies critically reviewed analytical methods used in determining antioxidant activity [18]. Firstly, FRAP evaluated the power of antioxidants in acid conditions to reduce the complexity of ferric ions ($Fe^{3+}$) to the ($Fe^{2+}$) by a single electron transfer mechanism (SET). The SET mechanism was also the basis of the Folin-Ciocalteu method, the test for TPC. On the other hand, DPPH and ABTS both work based on utilizing mixed mode mechanism, where SET, hydrogen atom transfer (HAT), and proton-coupled electron transfer (PCET) mechanisms can contribute to differrent effect, depending on the reaction variables (i.e. liquid-to-solid ratio in this study). Similarly, other studies also found good correlations between FRAP and Folin-Ciocalteu method, e.g. for lignins [19]. Nonetheless, it is important to note that these findings cannot be universally applied but instead rely on the specific origin and structure of the sample being investigated.

### 3.4 Verification of the models

The optimal extraction conditions for maximizing TPC, FRAP, DPPH, and ABTS in the

decoction method were identified. Table 5 presents the predicted and actual response values under these optimized conditions. Based on the FCCD analysis, the optimal parameters consisted of a boiling time of 30 minutes and a liquid-to-solid ratio of 37 mL/g. As discussed previously, physical properties such as color (e.g., yellowness and chroma) were also affected by extraction conditions. Prolonged boiling can enhance pigment release and improve visual attributes, but it may also lead to the degradation of thermolabile compounds and undesirable darkening, particularly in food-grade applications. Therefore, while color contributes to product appeal, it has limitations as an optimization target. The selected extraction parameters reflect a compromise between maximizing bioactive compound recovery and maintaining acceptable physical quality.

Experimental validation demonstrated strong agreement between the predicted and actual values, with a residual standard error (RSE) of less than 10% for all measured responses [14]. Additionally, the desirability value of 0.761 confirms the suitability of these conditions for achieving optimal extraction efficiency. Comparing the result with previous study [6], it revealed that, although the decoction done in recent study still could not outperformed maceration method, but it increases the decoction results by 3 – 7% higher than previous study, and reducing the solvent consumption by 7.5%. Therefore, recent studies confirm the urgency of extraction optimization in order to obtain maximum results while maintaining the cost as low as possible, especially in food production.

## 4. CONCLUSION

The recent study successfully employed the preliminary RSM with Two-factor-three levels FCCD approach to optimize the decoction of rice leaf extracts. The optimal condition were selected based on the maximization of total phenolic content (TPC) and antioxidant activities (FRAP, DPPH, ABTS). These chemical properties were prioritized due to their relevance to the functional quality and health-promoting potential of rice leaf extracts. The second-order polynomial model is well-fitted for all the responses (Lack of fit $p$–value > 0.05, $R^2$ > 0.85, Adeq. precision > 4). Compared to the previous study, the decoction process has improved significantly, yielding 12.09 mg GAE/g d.b. from its optimum condition (extraction time 30 min and liquid-to-solid ratio of 37 mL/g) and reducing 7.5% of the required solvent. The actual response was found following the predicted values for optimized parameters (RSE < 10%), offering a potential upscale to the industrial sector of natural additives for functional food production.

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## CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

## CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

**Dwi Ayuni:**
Conceptualization, investigation, methodology, data curation, writing – original draft.
**Chaleeda Borompichaichartkul:**
Supervision, resources, writing – review and editing.

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