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Optimization of Antioxidant Extraction from Red Ginger Rhizomes (*Zingiber officinale* var. Rubrum) Using Response Surface Methodology

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ABSTRACT

The body generates endogenous antioxidants to neutralize free radicals however, when these are insufficient, antioxidants from natural sources become essential. Among these sources, the rhizome of red ginger (*Zingiber officinale* var. *rubrum*) is well known for its abundance of phenolic substances and notable antioxidant activity. This makes it a promising material for the enhancement of functional ingredients and therapeutic formulations. However, the efficiency of extraction largely depends on the experimental conditions. This study aimed to identify the optimal condition for the extraction of antioxidants from red ginger utilizing the decoction method. To achieve this, Response Surface Methodology (RSM) with a Central Composite Design (CCD) was used to test how the extraction temperature, extraction time, and sample-to-solvent ratio collectively impact the antioxidant capacity and the precision of the predictive model. The One Factor at A Time (OFAT) approach was initially used to decide the midpoint values of the parameter used in the RSM design. The antioxidant capacity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, tested utilizing a UV-visible spectrophotometer. Based on the RSM analysis, the optimal condition were established at 90°C for 50 minutes by a sample-to-solvent ratio of 1:25 g/mL. The maximum antioxidant capacity achieved was 3.7811 mg AAE/g FW, that was closely aligned with the RSM model's predicted value of 3.87587 mg AAE/g FW. The analysis of variance (ANOVA) indicated a p-value below 0.05, showing that extraction temperature, extraction time, and the sample-to-solvent ratio had a substantial impact on antioxidant capacity.

Keywords: Red ginger rhizome, Optimal condition, Antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl assay, Response Surface Methodology, Central Composite Design.

Introduction

Oxidative stress occurs when there is an imbalance among the secretion of free radicals and the availability of antioxidants. This imbalance may arise either by insufficient antioxidant intake or by an excessive generation of free radicals. Over time, this condition can lead to oxidative stress resulting in various degenerative diseases, such as heart attacks, strokes, Alzheimer's disease, and cancer[1,2,3]. Free radicals are very reactive molecules with unpaired electrons. Under normal condition, the body counteracts these reactive species by its endogenous antioxidant systems. However, when the internal defense mechanisms are insufficient, the intake of exogenous antioxidants becomes essential to maintain oxidative balance[4]. Antioxidants act by donating electrons to stabilize free radicals, thereby restraining their formation and interrupting oxidative chain reaction mechanisms.

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Antioxidants are naturally present in a large range of foods, particularly in fruits, vegetables, and various spices[5,6,7]. Ginger is recognized as a crucial natural source of antioxidants owing to its strong antioxidant capacity. It is largely used as a therapeutic spice for managing various ailments, and its rhizomes are rich in bioactive substances comprising mostly of gingerol and shogaol, that contributes to its antioxidant activity[8,9,10]. The antioxidant capacity and total flavonoid content of red ginger (*Zingiber officinale* var. *rubrum*, Jahira 1 variety) extracted by using ethanol as extracting solvent were found to be greater than those of water extract[11]. Red ginger that has undergone fermentation into kombucha demonstrates strong antioxidant potential, with an IC_{50} values varying based on the variation in fermentation period and the concentration of palm sugar used in the procedure[12]. Another study noted that the antioxidant activity of red ginger was comparable to that of Trolox, suggesting its potential as a natural therapeutic agent[13].

The antioxidant capacity of red ginger has been shown to mitigate oxidative stress, particularly during the SARS-CoV-2 pandemic[14]. Some factors can affect the antioxidant capacity of a plant extract, such as the extraction method, solvent type, temperature, extraction time, and the ratio of sample mass to solvent[15]. Common extraction techniques for red ginger include maceration, decoction, percolation, Soxhlet extraction, ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE)[16,17,18]. The solvents most commonly used in this procedure are ethanol and water, either individually or in combination[15,19]. To achieve the greatest antioxidant capacity, the extraction procedure needs to be done under optimal condition.

Optimal extraction condition can be determined utilizing the One-Factor-at-a-Time (OFAT) approach. In this method, the impact of a single parameter is tested while keeping all other variables constant. But, this approach has some limitations, as it disregards potential

interactions among factors and is often neither impactful nor efficient. Moreover, the research outcomes are generally less representative of real experimental condition[15,20]. One parameter may interact or impact another, which is often overlooked in the OFAT approach. Nevertheless, the OFAT method can still be used to decide the midpoint values used in RSM. Design of Experiment (DoE) techniques, such as RSM and multivariate factorial designs, help overcome the limitations of the OFAT method. By these approaches, researchers can simultaneously test the impacts of multiple factors and their interactions. The optimal condition derived these methods (RSM and multivariate factorial designs) are generally more accurate, reliable, and efficient[16]. Some researchers have used RSM to determine extraction condition of antioxidants by various herbal plants[21,22,23]. The effectiveness of RSM in optimizing multiple extraction parameters and enhancing the recovery of phenolic and flavonoid compounds from plant-based materials further validates its robustness as a statistical optimization tool[24].

This study aimed at optimizing the antioxidant extraction from red ginger rhizomes using the RSM approach. In this study, antioxidants were extracted using the decoction method. This method was chosen because it is commonly used by the local community for consuming herbal plant extracts. The RSM method was used to determine the optimal extraction conditions for red ginger rhizomes. The studied parameters were extraction temperature, extraction time, and the sample/solvent ratio. The midpoint of the parameters in the RSM method was determined using the OFAT method. Design Expert version 13 software was used.

Materials and Methods

Materials

Sample was sourced from Pasar Raya Padang (0°56′59″ S, 100°21′33″ E), located in Kampung Jao Subdistrict, Padang Barat District, Padang City, West Sumatra Province, Indonesia. Analytical-grade reagents supplied by Merck Indonesia were used in this study, such as ascorbic acid, methanol, and DPPH (1,1-diphenyl-2-picrylhydrazyl).

Equipment included a UV–Vis spectrophotometer (Thermo Scientific Genesys 20), an analytical balance (Mettler 200), a hot plate (Dragon Lab DLAB MS H280 Pro), and standard laboratory glassware (Pyrex). Design Expert version 13 software (Stat-Ease, Inc., Minneapolis, MN, USA) was also used.

Sample Preparation

The red ginger rhizomes were thoroughly washed to remove surface contaminants, then cut into small pieces prior to the extraction procedure.

Sample Identification

Whole red ginger plants, comprising the stems, leaves, and rhizomes, were gathered and taxonomically identified, authenticated and classified at the Herbarium of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Indonesia as *Zingiber officinale* var. rubrum (Figure 1). A voucher specimen was set and deposited in the herbarium under the identification number 7111/K-ID/ANDA/X/2024 for future reference. The red ginger plant used in this study was classified under the family "Zingiberaceae" and the genus "Zingiber"

Determination of Antioxidant Capacity

One milliliter of the sample solution was mixed with 2.5 milliliters of a 0.1 mM DPPH solution. The mixture was then incubated in the dark for 30 minutes. The absorbance was determined at a wavelength of 522 nm utilizing a UV–Vis spectrophotometer. All experiments were done in triplicate. The equivalent antioxidant concentration was determined using the standard calibration curve.

The standard calibration curve of ascorbic acid

The standard solution used was ascorbic acid. The ascorbic acid calibration curve was prepared using a series of concentrations (3, 6, 9, 12, 15 mg/L). Based on this calibration curve (Figure 2), a linear regression equation was obtained and used to determine the equivalent antioxidant concentration.

Determination of the Midpoint of RSM utilizing the OFAT Method

The One-Factor-at-a-Time (OFAT) method was used to establish the initial design and to predict the optimal condition for the extraction temperature (60, 70, 80, 90, and 100 °C), extraction time (10, 20, 30, 40, and 50 minutes), and the sample-to-solvent ratio (1:10, 1:15, 1:20, 1:25, and 1:30 g/mL). These values served as reference points for subsequent optimization utilizing RSM. The independent variables were set and adjusted following the decoction method outlined in Table 1. Subsequently, the antioxidant capacity experiment was repeated trice for each procedure to ensure the reliability of the outcomes.

Optimization of extraction indicators utilizing the RSM Method

Design-Expert software (version 13) was used to test the range of variables derived by the CCD and the OFAT approaches. In this context, the optimization procedure involved three independent variables, namely extraction temperature, extraction time, and the ratio among sample and solvent. Each independent variable was pre-decided and assigned across five coded levels, namely $-\alpha$, -1, 0, $+1$, and $+\alpha$, as illustrated in Table 2. The antioxidant capacity served as the dependent variable in this study. Subsequently, the extraction procedure followed the experimental framework presented in Table 3 to make sure consistency of the designed indicators. Afterward, the antioxidant concentration of each sample was carefully tested. The observed outcomes were documented in the response column corresponding to each treatment. Subsequently, the response data were analyzed to generate the analysis of variance (ANOVA) outcomes and to construct the three-dimensional (3D) surface plot. Finally, the optimal extraction condition were classified by the numerical optimization feature of the software.

Results and Discussion

The standard calibration curve of ascorbic acid

The antioxidant capacity was evaluated using an ascorbic acid calibration curve, which relates absorbance to concentration. The DPPH assay was selected due to its simplicity, low cost, sensitivity, and effectiveness in estimating total antioxidant content[25]. Ascorbic acid concentration was inversely proportional to absorbance, with higher concentrations producing lower absorbance values and a lighter purple color[26]. Increased electron or hydrogen donation from antioxidant compounds in red ginger extract enhanced the neutralization of DPPH radicals[27]. As the concentration of sample extract mixed with DPPH increased, the solution color faded due to reduction of DPPH through disruption of its conjugated double bonds[28]. The linear regression equation obtained was $y = -0.0339x + 0.7584$ with an R^2 value of 0.9910, indicating strong linearity (Figure 2). Previous studies used the DPPH method to determine antioxidant capacity, expressed in mg TE/g[29].

Determination of the RSM Midpoint utilizing the OFAT Method

OFAT method was used to study the impact of temperature, time, and the sample-to-solvent ratio on the overall extraction procedure[20]. The OFAT experiment was done to establish an appropriate range of condition to analyze for the RSM method[15,16]. The optimal condition in the OFAT method were classified based on the extract that exhibited the greatest antioxidant capacity across all variations. In this context, the antioxidant capacity was described as milligrams of ascorbic acid equivalent per gram of fresh weight (mg AAE/g FW)[30,31,32].

Effect of Extraction Temperature

Temperatures play a crucial role in deciding extraction efficiency, as it affects solvent penetration, solute diffusion, and the release of bioactive components by plant matrices. In general, raising the temperature helps improve mass transfer and solubility, making it easier to extract phenolic and antioxidant compounds. However, if the temperature is too high, it can cause oxidation and damage heat-sensitive compounds, reducing the extract's quality and antioxidant

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Table 1: Experimental design for OFAT optimization of extraction parameters

Variation Type	Temperature (°C)	Time (min)	Sample-to-Solvent Ratio (g/mL)
a. Temperature Effects	60	30	1:20
	70	30	1:20
	80	30	1:20
	90	30	1:20
	100	30	1:20
b. Time Effects	80	10	1:20
	80	20	1:20
	80	30	1:20
	80	40	1:20
	80	50	1:20
c. Ratio Effects	80	40	1:10
	80	40	1:15
	80	40	1:20
	80	40	1:25
	80	40	1:30

Table 2: Experimental factors and coded levels used in the RSM design

Independent Variable	−α	−1	0	+1	+α
A. Temperature (°C)	63	70	80	90	97
B. Time (min)	23	30	40	50	57
C. Ratio (g/mL)	1:12	1:15	1:20	1:25	1:28

Note: (−α) = Lower axial point; (−1) = Lower limit; (0) = Center point; (+1) = Upper limit; (+α) = Upper axial point.

Table 3: Experimental design matrix using RSM-CCD method

Run	Temperature (°C)	Time (min)	Ratio (g/mL)
1	70	30	1:15
2	63	40	1:20
3	80	40	1:20
4	80	40	1:20
5	80	40	1:12
6	80	40	1:20
7	70	50	1:15
8	80	23	1:20
9	97	40	1:20
10	90	30	1:15
11	80	40	1:28
12	90	30	1:25
13	80	40	1:20
14	70	30	1:25
15	90	50	1:15
16	80	40	1:20
17	90	50	1:25
18	80	57	1:20
19	70	50	1:25
20	80	40	1:20

A = Temperature; B = Time; C = Sample-to-solvent ratio.

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Table 4: Experimental results of antioxidant capacity from red ginger rhizome extraction using the RSM-CCD method.

Run	Temperature (°C)	Time (min)	Sample-to-Solvent Ratio (g/mL)	Antioxidant capacity (mg AAE/g FW)
1	70	30	1:15	2.2808
2	63	40	1:20	1.9416
3	80	40	1:20	2.8435
4	80	40	1:20	2.8262
5	80	40	1:12	2.5263
6	80	40	1:20	2.8973
7	70	50	1:15	2.5244
8	80	23	1:20	2.6079
9	97	40	1:20	3.4564
10	90	30	1:15	2.7431
11	80	40	1:28	3.1819
12	90	30	1:25	3.2612
13	80	40	1:20	2.8385
14	70	30	1:25	2.1256
15	90	50	1:15	2.9238
16	80	40	1:20	2.7617
17	90	50	1:25	3.8922
18	80	57	1:20	3.0637
19	70	50	1:25	2.4927
20	80	40	1:20	2.7788

Table 5: ANOVA results of the second-order (2FI) model for antioxidant capacity

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	3.76	6	0.6262	112.71	<0.0001	Significant
A (Temp)	2.59	1	2.59	465.72	<0.0001	Significant
B (Time)	0.3509	1	0.3509	63.16	<0.0001	Significant
C (Ratio)	0.4226	1	0.4226	76.06	<0.0001	Significant
AB	0.0051	1	0.0051	0.92	0.3512	Not Significant
AC	0.1057	1	0.1057	18.87	0.0007	Significant
BC	0.1515	1	0.1515	27.01	0.0002	Significant
Lack of Fit	0.0291	3	0.0097	2.12	0.1096	Not Significant
Pure Error	0.0275	6	0.0046			
Total	3.816	19				

A: Temperature, B: Time, C: Sample-to-solvent ratio.
A p-value < 0.05 indicates statistical significance, while a p-value > 0.05 indicates non-significance

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Table 6: Model fit statistics for the quadratic regression model.

Statistic	Value
Standard Deviation (Std. dev)	0.0745
Mean	2.80
Coefficient of Variation (CV, %)	2.66
R^2	0.9811
Adjusted R^2	0.9724

A low CV (2.66%) indicates high precision and reproducibility of the experimental data.
The high R^2 (0.9811) and Adj-R^2 (0.9724) confirm that the regression model explains the variability of the data with strong predictive accuracy.

Figure 1: Red ginger plant and red ginger rhizome.

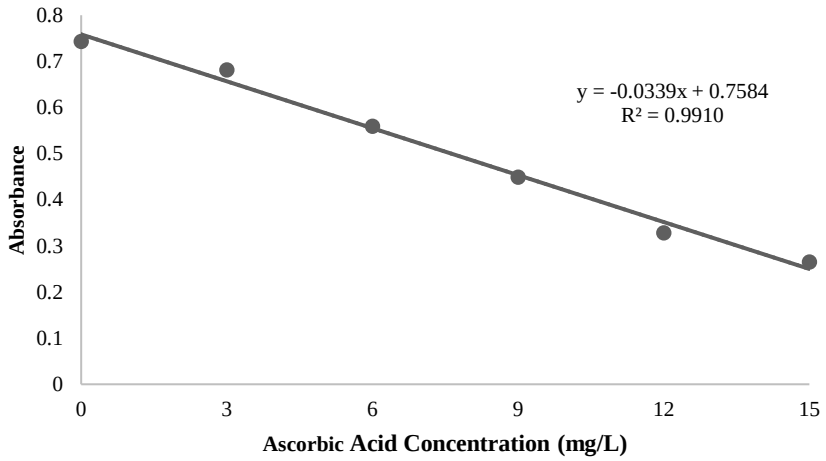

Figure 2: The Calibration Standard Curve of Ascorbic Acid

power. Therefore, keeping the temperature at the right level is crucial to get a high yield while keeping the active compounds stable[33]. Extraction temperature exhibited a significant impact on the antioxidant capacity of red ginger rhizomes ($p < 0.05$). As illustrated in Figure 3a, the antioxidant concentration improved markedly by rising temperature, reaching its peak value at 80 °C (2.44 mg AAE/g FW). Beyond this point, a further increase in temperature results in a gradual decrease in antioxidant capacity. Also, at an extraction temperature of 90 °C and 100 °C, the antioxidant capacity reduced, probably because the active

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compounds were damaged by excessive heat owing to the increased temperature.

The experimental indicators considered in this study outlined solvent concentration (0–100%), extraction temperature (30-60 °C), and extraction time (1-6 hours). Under these conditions, the optimal

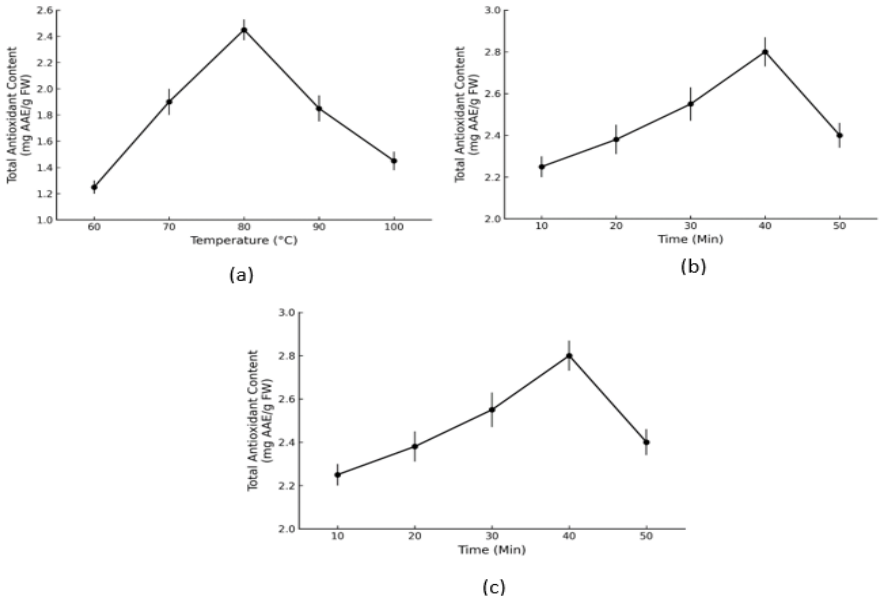

Figure 3: Effect of extraction parameters on the antioxidant capacity of red ginger rhizome determined using OFAT method: (a) extraction temperature, (b) extraction time, and (c) sample-to-solvent ratio.

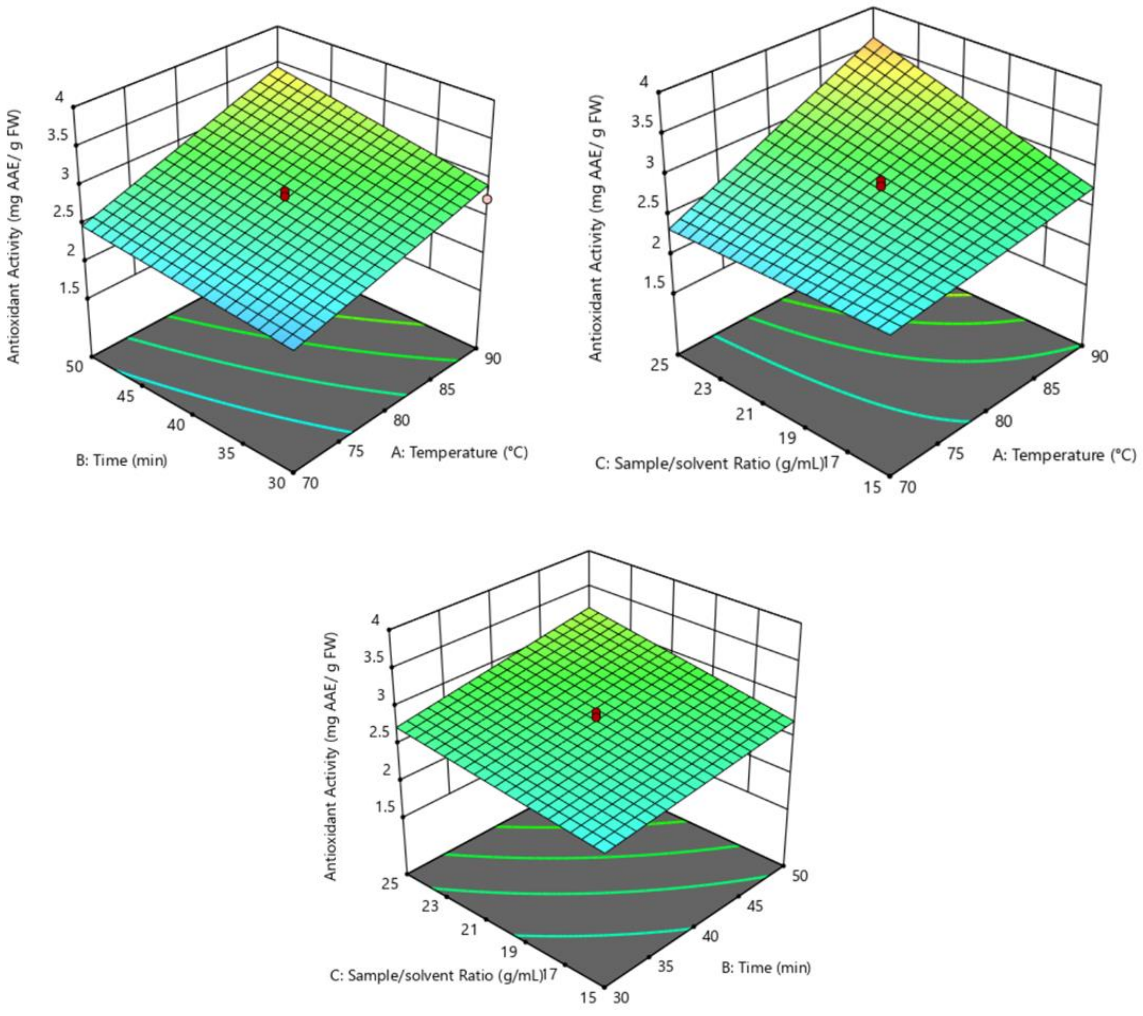

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Figure 4: Three-dimensional response surface plots showing the interaction effects of extraction temperature, extraction time, and sample-to-solvent ratio on the antioxidant capacity of red ginger rhizome.

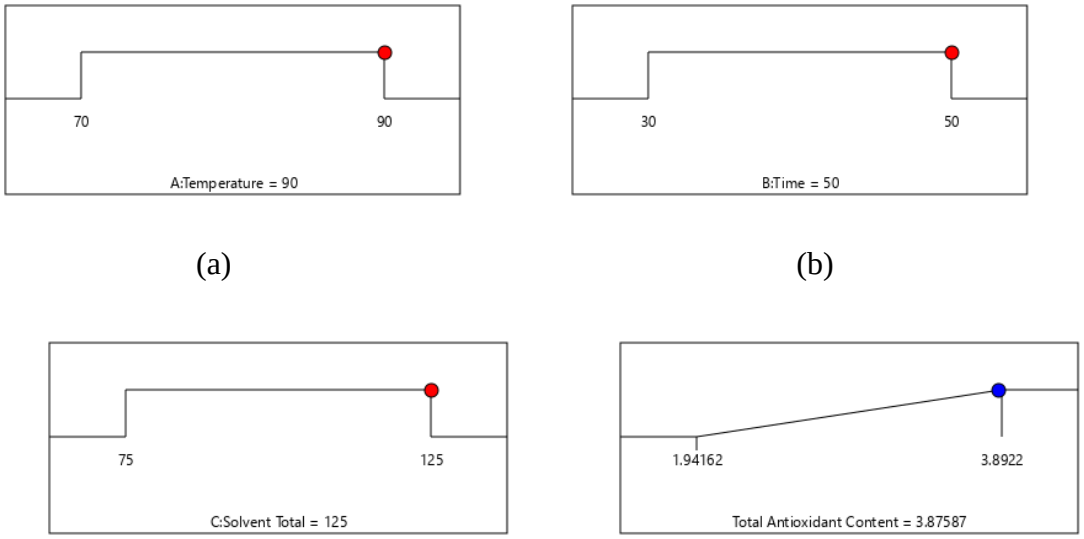

Figure 5: Optimum extraction conditions for antioxidant capacity of red ginger rhizome: (a) effect of temperature, (b) effect of time, (c) effect of sample-to-solvent ratio, and (d) predicted maximum antioxidant capacity.

extraction performance was reached utilizing a solvent concentration of 100%, an extraction temperature of 30 °C, and a time of 6 hours[34]. An extraction temperature of 85 °C has been noted to produce the greatest yield of antioxidant substances, primarily due to the improved solubility and diffusion of phenolic constituents inside the solvent matrix. However, when the temperature was further elevated to 90-100 °C, the extraction efficiency began to decrease – this was likely attributed to the thermal degradation and oxidative breakdown of heat-sensitive bioactive substances[35].

Previous study has shown the optimal temperature for enhancing the antioxidant activity of *Echinacea purpurea*. The research employed a temperature range of 25 to 75°C, and the peak antioxidant activity of 56.94 % was observed at an optimal extraction temperature of 75°C[32]. According to Reblová, antioxidant activity tends to decrease as the temperature increases within the range of 90-150 °C. Moreover, certain phenolic substances such as gallic and caffeic acids exhibit a more gradual reduction in their activity under these condition[36]. Other researchers have found the same optimal extraction temperature in the extraction of antioxidants of matcha (*Camellia sinensis*)[37], *Pinus radiata* bark[38], avocado and okra seeds[39].

A comparable trend was observed in the extraction of *Momordica charantia* leaves, where a gradual increase in temperature improved the solubility of phenolic and flavonoid substances up to an optimal point. Beyond this temperature, thermal degradation became evident at greater levels. The optimum extraction condition was noted at approximately 72°C, yielding the greatest antioxidant capacity, whereas further heating resulted in decreased stability and recovery of bioactive substances[40].

Effect of Extraction Time

Extraction time plays a crucial role in determining how much antioxidant compound can dissolve in the solvent and affects the overall efficiency. If the extraction time is too short, some antioxidants may not be extracted, giving a lower yield. On the other hand, if it takes too long, the active compounds may get damaged or broken down, reducing their quality[41,42]. Therefore, identifying the optimal extraction time is essential to ensure that the maximum antioxidant capacity can be attained.

Extraction time significantly impacted the amount of antioxidant substances released into the solvent ($p < 0.05$). In this experiment, the extraction time was varied from 10 to 50 minutes, while the temperature was kept constant at 80 °C and the sample-to-solvent ratio set at 1:20 g/mL. As illustrated in Figure 3b, the antioxidant capacity gradually improved at 10 and 40 minutes of extraction. This improvement was attributed to the extended contact among the red ginger matrix and the solvent, which promotes greater solubility of antioxidant constituents. However, as the extraction time approached 50 minutes, a decrease in antioxidant capacity was observed, most likely due to the degradation or structural damage of the antioxidant substances present in red ginger[41]. The optimal extraction time was observed to be 40 minutes, producing an antioxidant capacity of 2.7621 mg AAE/g FW. These outcomes were consistent with previous studies that noted that the extraction periods of approximately 40-42 minutes yielded the greatest antioxidant activity. At this point, solvent-solute equilibrium is reached, allowing maximum diffusion of antioxidant substances while minimizing the thermal degradation of heat-sensitive constituents[43,44]. However, Wani *et al*. [45] found an optimal extraction time of 37.02 minutes for sea buckthorn leaf extraction, while Nguyen *et al*. [46] found an optimal time of 49 minutes for *Polygonum multiflorum* Thunb. root extraction in Vietnam. The optimal extraction time of 40 minutes observed in this experiment was chosen as the central point for the RSM design. In this context, 30 minutes was designated as the lower limit (-1), while 50 minutes served as the upper limit (+1) to ensure a balanced parameter variation within the experimental range.

Effect of Sample-Solvent Ratio

To obtain the greatest antioxidant capacity the optimal sample-to-solvent ratio must be considered[47,48]. In this study, various sample-to-solvent ratios such as 1:10, 1:15, 1:20, 1:25, and 1:30 g/mL were evaluated under controlled condition. The extraction temperature was kept at 80 °C, and the time was set to 40 minutes, corresponding to the optimal indicators observed in the preceding experiments. As illustrated in Figure 3c, the antioxidant capacity improved progressively with greater sample-to-solvent ratios, reaching its maximum value at 1:20 g/mL which represents the optimal condition. This condition produced an antioxidant capacity of 2.8037 mg AAE/g FW. Increasing the solvent volume to the optimal ratio enhanced the efficiency of active compound extraction, primarily because a larger solvent volume improves the dissolution of antioxidant constituents. However, when the ratio exceeded this optimal point, a decrease in antioxidant capacity was observed. Consistent with previous outcomes, an increase in solvent volume promotes compound diffusion only up to an optimal ratio approximately 1:20 g/mL beyond this excessive dilution a reduced mass transfer efficiency give rise to diminished antioxidant activity[49].

The optimal ratio of 1:20 g/mL was also observed by Majeed in the extraction of *Origanum vulgare*[50], by Rezaei *et al*. in the extraction of apple pomace[51], and by Tourabi *et al*. in the extraction of *Mentha longifolia*[52].

Optimal Conditions for Red Ginger Rhizome Extraction Using the RSM Method

The optimal extraction condition for red ginger were determined by varying the temperature, time, and sample-to-solvent ratio[53,54]. The experimental design based on RSM is presented in Table 4. A total of twenty experimental runs were done, involving three independent variables: extraction temperature (63-97 °C), extraction time (23-57 minutes), and sample-to-solvent ratio (1:11-1:28 g/mL). The dependent variable analyzed in this study was the antioxidant capacity, described as milligrams of ascorbic acid equivalent per gram of fresh weight (mg AAE/g FW). Across all treatments, the antioxidant capacity ranged from 1.9416 to 3.8922 mg AAE/g FW. The lowest extraction yield was obtained at 63 °C for 40 minutes using a sample-to-solvent ratio of 1:20 g/mL. In contrast, the highest yield was reached at 90 °C for 50 minutes with a ratio of 1:25 g/mL. To check how accurate and reliable the model was, an analysis of variance (ANOVA) was carried out using Design-Expert software version 13.

The model's suitability was tested using several statistical indicators, including the F-value, p-value, coefficient of determination (R^2), adjusted R^2, and coefficient of variation (CV). Table 5 describes the proposed regression model for the response variable, which is antioxidant capacity. In this case, the RSM uses a two-factor interaction (2FI) regression model to identify and evaluate how the experimental factors interact with each other. The ANOVA results show that the predictive model is statistically significant and could explain the data variation, as indicated by a high F-value and a very low p-value ($p < 0.0001$). In this case, the main factors—extraction temperature, extraction time, and sample-to-solvent ratio—have a strong effect on the extraction of antioxidant compounds, with all p-values below 0.0001. In addition, variables A, B, and C represent temperature, extraction time, and the sample-to-solvent ratio respectively, showing their direct effect in the regression model. In addition, substantial interactions were found between some variables, especially between temperature and the sample-to-solvent ratio, as well as between extraction time and the sample-to-solvent ratio ($p < 0.05$). This means that certain combinations of these factors strongly affect antioxidant levels. However, the interaction between temperature and extraction time was not significant, as shown by p-values greater than 0.05[55]. This means that this combination does not affect antioxidant capacity. The Lack of Fit (LOF) value describes how well the model fits the experimental data. The LOF p-value is 0.1096 ($p > 0.05$), which means it is not significant[56].The model is consistent by the experimental data and can be used to predict ideal extraction situation.

Table 6 describes the model fit statistics for the quadratic regression model. The coefficient of variation (CV) describes how precise and consistent the model is, as it measures how much the data vary compared to the average value. In this case, a CV of 2.66% describes very little variation, meaning the model accurately represents the observed changes in antioxidant levels during extraction. The coefficient of determination (R^2) reflects the ratio of data variability that can be described by the regression model. In this study, the R^2 value fulfilled was 0.9811. A value approaching 1 suggests that the linear regression model provides a strong explanatory fit, describing that approximately 98.11% of the response variation is accounted for by the experimental factors tested[57]. The adjusted coefficient of determination (adj R^2) describes how well the added independent variables improve the model's ability to explain the results without making it too complex. In this study, the adj R^2 value of 0.9724 means that about 97.24% of the changes in the dependent variable can be described by the regression model, after considering the number of variables used in the analysis[58]. Therefore, the RSM method is helpful for finding the best extraction conditions to increase antioxidant levels in red ginger. Figure 3 describes a 3D response surface plot that explains how two independent variables relate to one dependent variable in the experiment. The third independent variable is not shown in the graph; it is kept constant at its middle value, marked by the red point in the center of the plot. This graph describes how the factors interact and how their combinations affect the result. In this case, the figure helps explain how changes in factors like extraction time, temperature, and sample-to-solvent ratio together impact the amount of antioxidants. The color patterns on the graph show changes in antioxidant levels caused by the interaction between the two independent variables. Figure 4a describes how temperature (A) and extraction time (B) relate to each other and affect antioxidant levels. Both factors seem to increase antioxidant content, but temperature has a stronger effect, shown by the steeper slope of the surface plot and the shift toward green colors. Conversely, extraction time exhibited a relatively smaller impact, as evidenced by the flatter slope and the predominance of blue tones on the surface plot. Figure 4b describes how temperature and the sample-to-solvent ratio interact, and together affect antioxidant levels. In this case, increasing both variables led to higher reaction activity. However, temperature had a stronger effect, shown by the steeper curve and the change toward yellow-green colors. In contrast, the sample-to-solvent ratio displayed a gentler slope accompanied by a green-to-blue color gradient, describing a mild impact on the response. Figure 4c illustrates the connection between extraction time and solvent volume in relation to antioxidant capacity. Both indicators show an increase in response, though by a relatively gentle slope. The predominance of green tones on the surface plot suggests a moderate level of antioxidant activity. Overall, Figure 5 describes the best conditions for the three main variables based on the RSM analysis. Figure 5a describes the optimal temperature of 90 °C, Figure 5b describes the best extraction time of 50 minutes, and Figure 4c describes the ideal sample-to-solvent ratio of 1:25 g/mL or solvent volume of 125 mL. Figure 5d describes the predicted antioxidant level, which reached 3.87587 mg AAE/g FW, as seen from the upward trend in the response curve. Under the best extraction conditions found using the RSM method, the tested antioxidant level was 3.7811 mg AAE/g FW. The relative standard deviation (RSD) between the predicted and actual values was 2.44%, which is well below the 5% limit. This small difference describes that the predicted results match the experimental data well, proving that the model is accurate and reliable in showing real observations.[57,58]. These results show that the model is quite accurate in predicting antioxidant levels.

Conclusion

The optimal extraction condition for red ginger Rhizomes were determined via the OFAT method and the optimal conditions were a temperature of 80°C, an extraction time of 40 minutes, and a sample-to-solvent ratio of 1 g per 20 mL. Under these conditions, the antioxidant capacity was 2.8037 mg AAE/g FW. In contrast, optimization utilizing the RSM described slightly different conditions, i.e. an optimal temperature of 90 °C, an extraction time of 50 minutes, and a sample-to-solvent ratio of 1:25 g/mL. These condition gave the highest antioxidant capacity, reaching 3.7811 mg AAE/g FW. The outcomes of this study reaffirm the effectiveness of RSM as a predictive model in enhancing the extraction efficiency of total antioxidant capacity of red ginger rhizomes. Furthermore, future research should emphasize on scaling-up this procedure to assess its feasibility for industrial applications, as well as the evaluation of antioxidant stability during storage. Also, a more comprehensive investigation into the bioactive compositions and biological properties of the extract may yield valuable insights into its potential utilization in food, pharmaceutical, and nutraceutical formulations.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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