Advanced Journal of Chemistry, Section A, 2026, 9(7), 1283-1296

Advanced Journal of Chemistry, Section A

Journal homepage: www.ajchem-a.com

Original Research Article

Optimization of Antioxidant Extraction from *Moringa oleifera* Leaves Using an Integrated OFAT and RSM–CCD Approach

Yefrida Yefrida[1]*, Deswati Deswati[1], Refilda Refilda[1], Hasifa Hayana[1], Nurjannah El Firsti[1], Adewirli Putra[2]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Science, Andalas University, Padang, Indonesia*
[2]*Department of Medical Laboratory Technology, Syedza Saintika University, Padang, Indonesia*

ARTICLEINFO

Article history

Submitted: 2025-12-17
Revised: 2026-01-20
Accepted: 2026-02-07
ID: AJCA-2512-2007
DOI: 10.48309/AJCA.2026.566928.2007

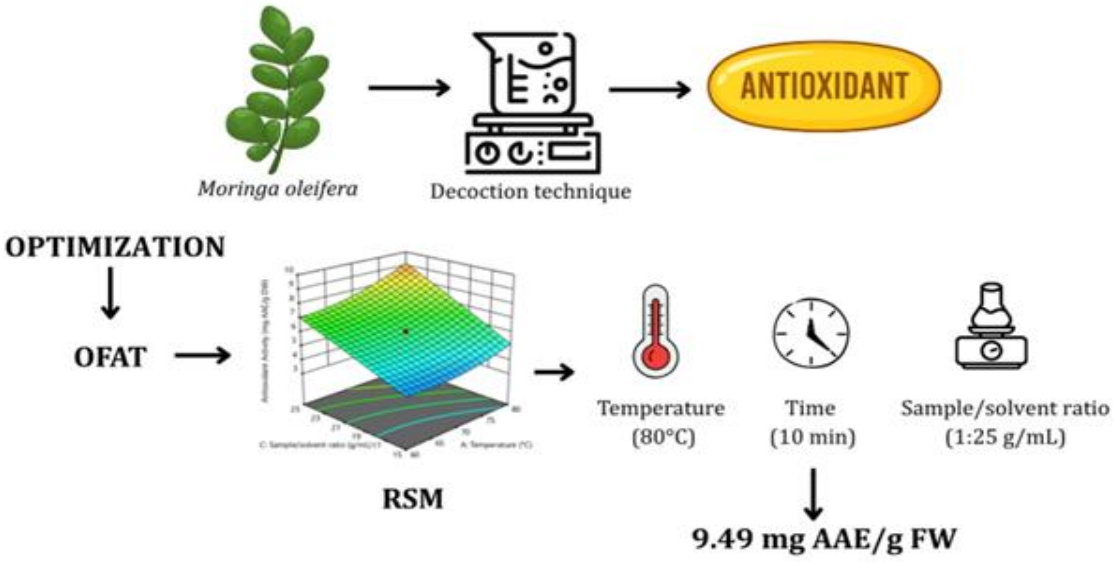
KEYWORDS

Moringa oleifera
Antioxidant extraction
Optimization
RSM-CCD
DPPH

ABSTRACT

Moringa oleifera leaves are a rich source of phenolic and flavonoid antioxidants; yet, optimisation of conventional water-based extraction is rarely modeled comprehensively. This study integrates one-factor-at-a-time (OFAT) screening with response surface methodology–central composite design (RSM-CCD) to determine optimal conditions for maximizing antioxidant yield, quantified using the DPPH assay (mg AAE/g FW). The quadratic model was highly significant ($F = 41.88$; $p < 0.0001$) with strong predictive accuracy ($R^2 = 0.9742$; adj-$R^2 = 0.9509$). The sample-to-solvent ratio was the most influential variable ($F = 287.85$; $p < 0.0001$), followed by temperature ($F = 58.76$; $p < 0.0001$), whereas extraction time showed no significant effect. Significant quadratic terms (A^2, B^2) and the AC interaction revealed curvature-driven extraction behavior. Optimal conditions 80 °C, 10 min, and 1:25 g/mL, yielded 9.49 mg AAE/g FW. This integrated OFAT–RSM approach provides novel mechanistic insight and a validated, scalable framework for efficient antioxidant extraction.

GRAPHICAL ABSTRACT



* Corresponding author: Yefrida, Yefrida
✉ E-mail: yefrida@sci.unand.ac.id

Introduction

Oxidative stress arises when the generation of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defenses, leading to oxidative damage to proteins, lipids, and DNA. This imbalance is widely recognized as a key mechanistic trigger for degenerative and metabolic disorders [1]. Antioxidants counteract free radicals through hydrogen atom donation or electron transfer, thereby mitigating oxidative injury [2]. Growing evidence indicates that the regular intake of natural antioxidant compounds contributes significantly to lowering the risk of oxidative stress–related diseases, including cancer, obesity, hypertension, and cardiovascular disorders [3]. Among botanical sources, *Moringa oleifera* is distinguished by its exceptionally high antioxidant content. Recent studies have reported that its leaves contain abundant vitamin C, vitamin A, flavonoids, carotenoids, and phenolic acids, all of which contribute to strong radical-scavenging potential [4–6]. Tyagi *et al.* further identified chlorogenic acid, rutin, quercetin glycosides, isoquercetin, kaempferol, salicylic acid, gallic acid, *p*-coumaric acid, and caffeic acid as major phenolic constituents conferring potent antioxidant capacity [7]. Metabolomic profiling has also demonstrated that the polyphenol composition of *Moringa* leaves correlates strongly with DPPH and ABTS radical-scavenging activities [8], while *in vivo* studies show that *Moringa* extract enhances antioxidant enzyme expression (SOD, GPx, and CAT), thus suppressing oxidative stress [9]. In extraction research, the one-factor-at-a-time (OFAT) approach remains a common preliminary method due to its conceptual simplicity; however, its inability to capture variable interactions and its high experimental burden limit its suitability for optimization studies [10]. Consequently, contemporary research increasingly applies response surface methodology (RSM), which can efficiently model nonlinear effects and interactions among variables while reducing experimental runs without compromising predictive accuracy [11,12]. Central composite design (CCD) is one of the most widely adopted RSM designs because it facilitates the development of second-order polynomial models to evaluate linear, quadratic, and interaction effects [13,14]. CCD has proven effective for optimizing polyphenol, flavonoid, and antioxidant extraction in numerous plant systems, such as *Origanum vulgare* [15] and *Moringa oleifera* using ultrasound-assisted extraction (UAE) [16]. Despite substantial work on *Moringa oleifera*, most optimisation studies have focused on specific extraction technologies (*e.g.,* UAE, microwave-assisted extraction) or isolated process parameters. Comprehensive modelling that integrates thermal and mass-transfer parameters under conventional solvent extraction using RSM-CCD remains limited. Moreover, few studies incorporate multi-response optimization linking DPPH-based antioxidant activity with extraction kinetics. Addressing these gaps requires a systematic modeling framework capable of elucidating inter-variable interactions and their mechanistic influence on antioxidant release. Therefore, this study aims to determine the optimal extraction conditions for *Moringa oleifera* leaves using RSM based on a CCD. The optimization focuses on three key parameters extraction temperature, extraction time, and solvent-to-material ratio to maximize antioxidant yield under conventional solvent extraction.

Experimental

Experimental rationale

The methodological framework employed in this study was designed to ensure both empirical robustness and model reliability. OFAT was first used as a screening tool to establish realistic operational limits for thermal and solvent-driven extraction behavior, which is essential when prior parameter boundaries are unavailable for

decoction-based extraction systems. While OFAT does not capture variable interactions, it provides practical starting conditions that prevent experimental bias when transitioning to multivariate optimization. The CCD within the RSM was subsequently selected because it enables efficient modeling of nonlinear responses, quantifies interactions among process variables, and reduces the number of required experiments without compromising predictive accuracy. This hybrid OFAT–CCD approach provides a rigorous optimization platform that aligns with current best practices in natural product extraction research and enhances reproducibility across laboratories.

Sampling

Fresh *Moringa oleifera* plants were collected from the Pasar Baru area, Padang, West Sumatra. Roots, stems, and leaves were transported to the Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, for taxonomic verification to ensure species authenticity.

Chemicals

Experimental materials included *Moringa oleifera* leaves, distilled water, methanol (Merck, Germany), ascorbic acid (Merck, Germany), and DPPH (1,1-diphenyl-2-picrylhydrazyl; Merck, Germany). All chemicals were of pro analysis (PA) grade to ensure analytical precision.

Sample preparation

Collected plant materials were cleaned, washed with running water, and air-dried. Leaves were separated from stems and cut into smaller pieces to enhance solvent–matrix interaction during extraction.

Sample identification

Whole plant specimens (leaves, stems, and rhizomes) were identified and authenticated at the Andalas University Herbarium. A voucher specimen was deposited under identification number 359/K-ID/AND/V/2024. The material was classified as *Moringa oleifera* (family Moringaceae).

Preliminary extraction screening using OFAT

An OFAT approach was employed as an initial screening to determine the operative range of extraction conditions. Although OFAT does not capture interaction effects, it remains widely used to establish acceptable parameter boundaries before RSM-based optimization, particularly when empirical data on thermal and solvent-dependent behavior are limited. Decoction extractions were conducted by varying temperature, extraction time, and sample-to-solvent ratio individually (Table 1). Antioxidant activity was quantified for each treatment, and the condition yielding the highest activity was selected as the CCD center point. All experiments were performed in triplicate.

Ascorbic acid standard curve preparation

Ascorbic acid standards (5–25 mg/L) were prepared, and 1 mL of each standard was mixed with 2.5 mL of 0.1 mM DPPH. After 30 min of incubation in the dark, absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The calibration curve was used as the reference for antioxidant quantification [17].

Determination of antioxidant activity

A 1 mL aliquot of each extract was diluted to 10 mL with distilled water. From this dilution, 1 mL was mixed with 2.5 mL of 0.1 mM DPPH and incubated for 30 min in the dark. Absorbance at 517 nm was recorded, and antioxidant content was calculated using the standard curve [17].

Table 1. Variation of independent variables for optimization using the OFAT method

No.	Temperature (°C)	Time (min)	Sample/solvent ratio (g/mL)
1	40	30	1:20
2	50	30	1:20
3	60	30	1:20
4	70	30	1:20
5	80	30	1:20
6	80	10	1:20
7	80	20	1:20
8	80	30	1:20
9	80	40	1:20
10	80	50	1:20
11	80	20	1:10
12	80	20	1:15
13	80	20	1:20
14	80	20	1:25
15	80	20	1:30

RSM-Based optimization using CCD

Optimization was carried out using Design-Expert software (version 13) with a CCD. CCD was selected because it allows efficient development of second-order polynomial models and quantifies interaction and curvature effects—critical for modeling complex thermal and mass transfer phenomena during plant extraction. Three independent variables—temperature, extraction time, and sample-to-solvent ratio—were coded at five levels (−α, −1, 0, +1, and +α), as shown in Table 2. Experimental runs were conducted according to the CCD matrix (Table 3), and antioxidant content was measured for each run. Model fitting, ANOVA, and generation of three-dimensional response surface plots were performed within Design-Expert. Optimal extraction conditions were determined using the numerical optimisation function.

Results and Discussion

As depicted in Figure 1, ascorbic acid concentration exhibited a clear inverse relationship with DPPH absorbance, confirming the characteristic behavior of the DPPH assay in which higher antioxidant activity results in greater radical reduction and lower absorbance values [18,19]. The calibration curve generated for ascorbic acid ($y = -0.0236x + 0.8026$) demonstrated excellent linearity, with an R^2 value of 0.9923, indicating that the model explains more than 99% of the variance—well within the IUPAC criteria for high-quality quantitative analytical methods [20]. The correlation coefficient ($r = 0.9961$) further supports strong linearity, surpassing the commonly accepted threshold ($r > 0.995$) for reliable spectrophotometric calibration curves [21,22]. These results confirm that the regression model provides robust predictive capability for determining antioxidant content based on absorbance measurements.

Determination of the RSM midpoint utilizing the OFAT method

The OFAT method was applied to assess the individual effects of temperature, extraction time, and sample-to-solvent ratio on the extraction performance. As an established preliminary screening technique, OFAT is widely used to identify dominant variables before implementing more comprehensive multivariate optimization through RSM [23].

Table 2. Experimental factors with their levels

Independent variable	Coded variable level				
	-α	-1	0	+1	+α
A, Temperature (°C)	53	60	70	80	87
B, Time (min)	3	10	20	30	37
C, Sample/solvent ratio (g/mL)	12	15	20	25	28

Table 3. Optimization design of antioxidant capacity of *Moringa oleifera*

No. Std.	Temperature (°C)	Time (min)	Sample/solvent ratio (g/mL)
1	70	20	1:20
2	80	10	1:15
3	80	30	1:25
4	87	20	1:20
5	80	30	1:15
6	80	10	1:25
7	53	20	1:20
8	70	20	1:20
9	60	10	1:25
10	70	37	1:20
11	70	20	1:20
12	60	10	1:15
13	70	20	1:12
14	70	20	1:20
15	70	20	1:28
16	70	20	1:20
17	70	20	1:20
18	60	30	1:15
19	60	30	1:25
20	70	3	1:20

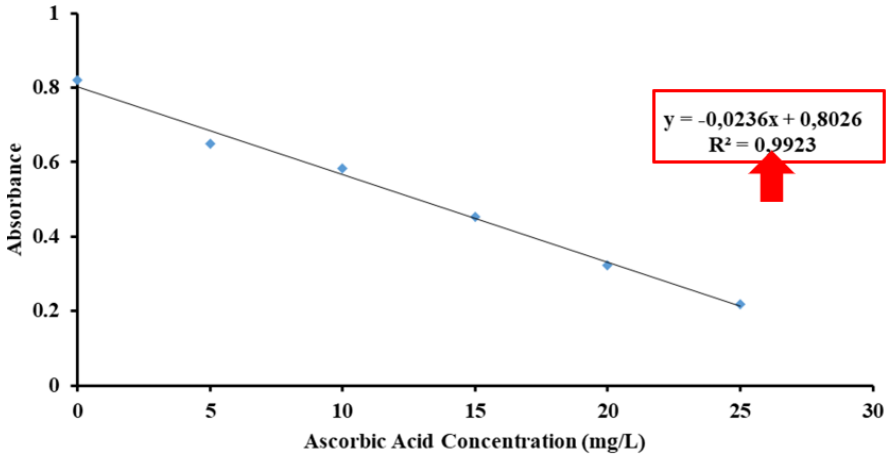


Figure 1. Standard calibration curve of ascorbic acid

In this study, the OFAT phase was designed to determine operational ranges that produce clear and measurable variations in response, ensuring that the subsequent response surface analysis is conducted within a meaningful and sensitive experimental domain [24]. The optimal conditions identified at this stage corresponded to the treatment combination yielding the highest antioxidant capacity, expressed as milligrams of ascorbic acid equivalent per gram of fresh weight (mg AAE/g FW), a standard unit widely employed in DPPH-based antioxidant evaluations [19,25].

The effect of extraction temperature

Figure 2 illustrates the influence of temperature on the antioxidant content of *Moringa oleifera* leaf extract. Antioxidant yield increased progressively with temperature when extraction time and the sample-to-solvent ratio were maintained at 30 min and 1:20 g/mL, respectively. However, temperatures above 80 °C resulted in a decline in antioxidant content, indicating thermal degradation of key secondary metabolites. This pattern aligns with previous findings showing that phenolic and flavonoid compounds are heat-labile and undergo structural deterioration when exposed to temperatures exceeding 70–80 °C [26-28]. Temperature is a critical factor in extracting antioxidant compounds from plant materials, as it directly affects solvent diffusion, solubility of bioactive compounds, cell-wall permeability, and the chemical stability of secondary metabolites. At low to moderate temperatures (40–70 °C), increasing temperature generally enhances antioxidant yield by reducing solvent viscosity, improving mass transfer, and facilitating the release of phenolic compounds. Moderate heating has been shown to accelerate the liberation of bound phenolics, thereby increasing antioxidant activities measured by DPPH, ABTS, and FRAP assays [29-31]. However, at higher temperatures (>70–90 °C), antioxidant compounds are prone to thermal degradation, leading to a significant decline in activity. Polyphenols such as quercetin, catechin, and chlorogenic acid undergo structural breakdown, and vitamin C is rapidly oxidized under excessive heat [32-34]. Studies on *Moringa oleifera* leaves, medicinal plants, and fruit extracts report a parabolic relationship, where antioxidant activity increases up to an optimum temperature but decreases sharply beyond it due to oxidation and denaturation of phenolic structures [35,36]. Therefore, optimizing temperature is essential to maximize antioxidant yield while preserving the stability of thermolabile bioactive components, typically within 55–70 °C for water- and ethanol-based extractions.

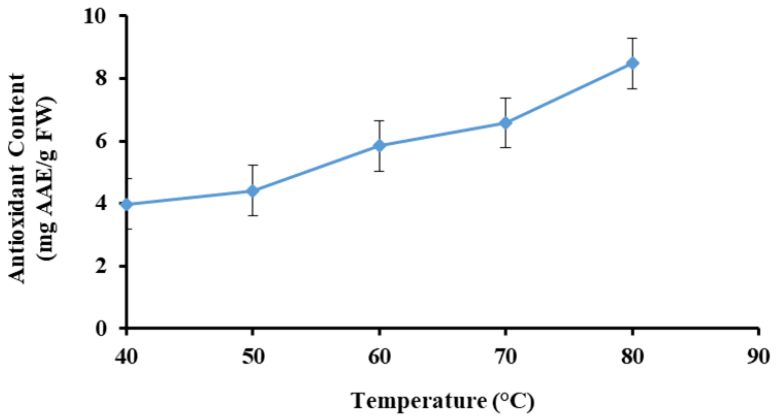


Figure 2. The effect of extraction temperature on antioxidant capacity

The effect of extraction temperature

As illustrated in Figure 3, antioxidant capacity increased sharply from 10 min, reaching a maximum at 20 min. This initial rise is attributed to optimal cell-wall softening and enhanced diffusion, which facilitate the release of phenolic compounds and other antioxidants into the solvent [37,38]. Beyond 20 min, antioxidant levels declined at 30 and 40 min due to thermal degradation, as prolonged heating compromises the structural integrity of polyphenols, flavonoids, and vitamin C, thereby reducing activity despite ongoing extraction [39,40].

From 50 to 60 min, the antioxidant content stabilized between 6.0 and 6.5 mg AAE/g FW, indicating that extraction had reached equilibrium, where the rate of compound release balanced the rate of thermal degradation [41].

Based on these observations, 20 min was identified as the optimal extraction time, corresponding to the peak antioxidant yield before significant thermal losses.

The effect of sample/solvent ratio

As shown in Figure 4, increasing the solvent volume from 50 mL (sample-to-solvent ratio 1:10) to 100 mL (1:20) enhanced the antioxidant capacity from 4.53 to 9.63 mg AAE/g FW. This improvement is attributed to increased contact between the solvent and plant material, which facilitates the dissolution and release of phenolic compounds into the solution [42,43]. The peak value at 100 mL represents the optimal solvent volume for extraction. Beyond 100 mL, antioxidant content decreased to 8.32 and 7.25 mg AAE/g FW at 125–150 mL (ratios of 1:25 to 1:30), likely due to dilution effects and an imbalanced sample-to-solvent ratio that reduces extraction efficiency despite ongoing leaching [44]. The stabilization of antioxidant levels at higher volumes indicates that extraction equilibrium had been reached, where further increases in solvent volume no longer enhance the yield [45].

RSM–CCD experimental design and antioxidant capacity response

As presented in Table 4, the center point runs (1, 8, 11, 14, 16, and 17) exhibited stable antioxidant values within ±6 mg AAE/g FW, indicating that the extraction system is consistent and suitable for quadratic modeling [46,47].

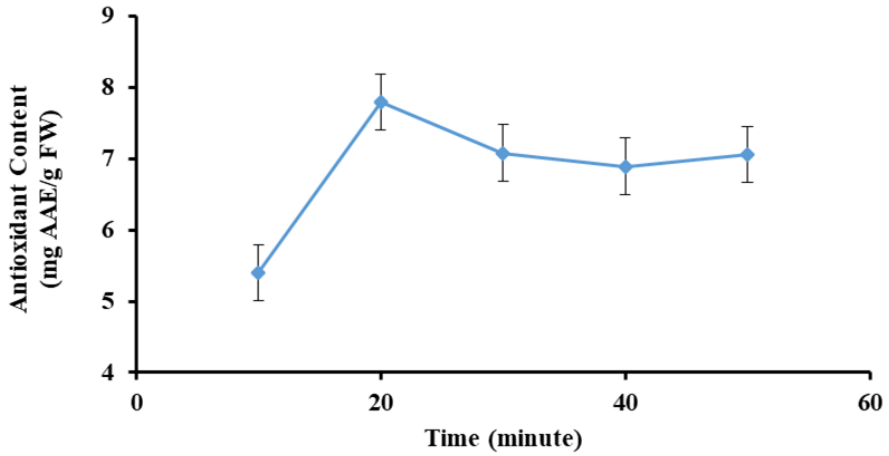


Figure 3. Effect of extraction time on antioxidant capacity

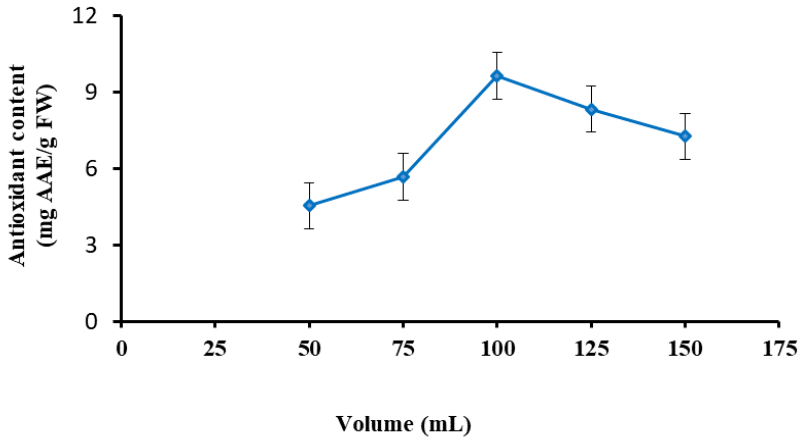


Figure 4. The effect of sample/solvent ratio on antioxidant capacity

Table 4. RSM-CCD on antioxidant capacity

No.	A (°C)	B (min)	C (g/mL)	Antioxidant content (mg AAE/g FW)
1	0	0	0	6.04
2	1	-1	-1	5.2
3	1	1	1	9.46
4	+ α	0	0	8.38
5	1	1	-1	5.5
6	1	-1	1	9.93
7	- α	0	0	5.54
8	0	0	0	6.06
9	-1	-1	1	7.29
10	0	+α	0	6.66
11	0	0	0	6.09
12	-1	-1	-1	4.43
13	0	0	- α	3.27
14	0	0	0	6.02
15	0	0	+ α	8.14
16	0	0	0	6.08
17	0	0	0	5.98
18	-1	1	-1	4.58
19	-1	1	1	8.07
20	0	- α	0	6.48

The highest responses were observed in runs 6 and 3 (9.93 and 9.46 mg AAE/g FW), corresponding to conditions with elevated temperature and sample concentration, confirming that these factors are key drivers of enhanced antioxidant capacity. This aligns with the understanding that higher temperatures and sample concentrations promote phenolic release

and accelerate solvent diffusion. In contrast, the lowest responses occurred in runs 13 and 12 (3.27 and 4.43 mg AAE/g FW), reflecting that low sample concentrations and combinations of low temperature and short extraction time are ineffective for antioxidant recovery [48]. Analysis of the axial points indicates that temperature exerts a significant quadratic effect, while extraction time has a comparatively minor influence. Overall, these results demonstrate that sample concentration (C) is the most dominant factor, followed by temperature (A), with extraction time (B) having only a moderate effect. The observed curvature further supports the suitability of a quadratic RSM model for modeling antioxidant extraction.

The ANOVA results (Table 5) indicate that the quadratic model is highly significant ($F = 41.88$; $p < 0.0001$) and consistent with the stability observed at the center points in the RSM-CCD table, suggesting that the model reliably explains the observed variation in antioxidant responses. Sample concentration (C) was identified as the most influential factor, with the highest F-value (287.85; $p < 0.0001$), corroborating the previous observation that the maximum response occurred at C = +1 and the minimum at C = −α. This finding aligns with literature reporting that increasing biomass or sample ratio directly enhances antioxidant yield [49,50].

Table 5. Quadratic RSM–CCD ANOVA model on antioxidant capacity

Source	Sum of squares	df	Mean square	F-value	*P*-value
Model	51.70	9	5.74	41.88	< 0.0001*
A, Temperature	8.06	1	8.06	58.76	< 0.0001
B, Time	0.08	1	0.08	0.63	0.4455
C, Sample/Solvent	39.48	1	39.48	287.85	< 0.0001
AB	0.14	1	0.14	1.09	0.32
AC	0.69	1	0.69	5.04	0.04
BC	0.0021	1	0.0021	0.01	0.90
A^2	2.35	1	2.35	17.11	0.002
B^2	1.01	1	1.01	7.36	0.02
C^2	0.02	1	0.02	0.18	0.68
Residual	1.37	10	0.13		
Lack of Fit	1.36	5	0.27	135.63	0.0001*
Pure Error	0.01	5	0.0020		
Cor Total	53.07	19			
$R^2 = 0.9742$	Adj $R^2 = 0.9509$			C.V %= 5.73	

Temperature (A) was also significant (F = 58.76; $p < 0.0001$), consistent with greater responses at higher temperature levels, reflecting the mechanism by which elevated temperatures increase cell permeability and accelerate the release of phenolic compounds [51,52]. In contrast, extraction time (B) was not significant ($p = 0.4455$), indicating that duration is not a dominant factor, as supported by the relatively minor variations observed across time levels in the RSM-CCD data. The A–C interaction was significant ($p = 0.04$), confirming that the combination of high temperature and high concentration produces an optimal response, whereas the other interactions (AB and BC) were negligible [53].

The quadratic terms A^2 and B^2 were significant, indicating a curved response profile with a rise to an optimum followed by a decline, while C^2 was not significant, suggesting a more linear effect of concentration. The model exhibited strong predictive capability, with $R^2 = 0.9742$ and adjusted $R^2 = 0.9509$, explaining over 95% of the response variation, and a low coefficient of variation (CV = 5.73%) indicating high precision. Although the lack-of-fit test was significant, it was influenced by minimal pure error and did not compromise the model's practical applicability [54,55].

The three response surface plots (Figure 5) exhibit patterns consistent with both the ANOVA results and the RSM-CCD data. The first plot displays a relatively flat surface along the time axis with a pronounced increase along the temperature axis, indicating that temperature (factor A) exerts a stronger influence than extraction time (factor B). This observation aligns with the ANOVA results, where factor A was significant and factor B was not, supporting the mechanism by which elevated temperatures enhance solvent diffusion and phenolic compound release [52,53].

The second plot shows a steep gradient along the temperature and sample-to-solvent ratio axes, indicating that the combination of A and C produces the greatest enhancement in antioxidant response. The diagonal contour pattern confirms a significant A×C interaction, consistent with both the ANOVA results and CCD data, where the highest responses occur at elevated temperatures and high sample concentrations [53,55]. The third plot demonstrates that the sample-to-solvent ratio (C) is the dominant factor, whereas time (B) has minimal effect, as evidenced by the steep surface in the C direction and the nearly flat surface along B. This agrees with previous literature reporting that the material-to-solvent ratio is a primary determinant of phenolic extraction efficiency [55].

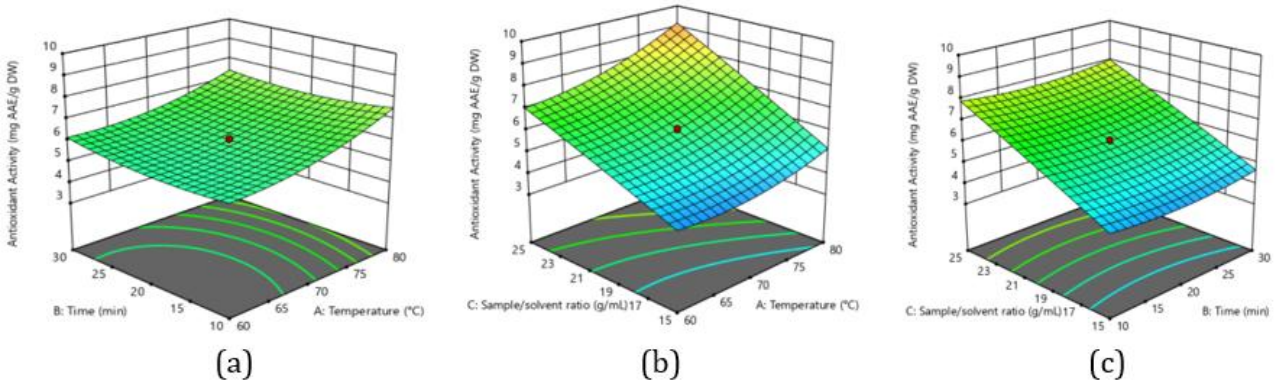


Figure 5. Response surface plots of extraction variables on antioxidant capacity (a) temperature and time, (b) temperature and sample/solvent ratio, and (c) time and sample/solvent ratio)

Collectively, these response surfaces indicate that optimal antioxidant extraction is achieved at high temperature and sample-to-solvent ratio, with a relatively short extraction time. The quadratic polynomial Equation is following:

$$Y_{respons} = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j}^{3} \beta_{ij} X_i X_j + e_i \quad (1)$$

$$\text{Antioxydant capacity} = 6{,}03 + 0{,}7686A + 0{,}0778B + 1{,}70C - 0{,}1375AB + 0{,}2925AC - 0{,}0175BC + 0{,}4023A^2 + 0{,}2645B^2 - 0{,}0414C^2$$

The fitted second-order polynomial model indicates that antioxidant capacity is predominantly governed by the linear effect of temperature (A) and, to a greater extent, the sample-to-solvent ratio (C). Among the coefficients, C (1.70) is the largest, confirming it as the most influential factor in enhancing the response, while temperature contributes significantly (0.7686) and extraction time (B) has a minimal linear effect (0.0778), consistent with the ANOVA results which indicate that time is not significant. The interaction between A and C was the only significant two-factor combination affecting antioxidant capacity, whereas AB and BC interactions were negligible. Quadratic terms A^2 and B^2 reflect curvature in the response, showing an increase to an optimum followed by a decline under extreme conditions, while C^2 was minimal, suggesting that the concentration effect is largely linear. Overall, the model confirms that the sample-to-solvent ratio is the dominant determinant of antioxidant yield, followed by temperature, while extraction time exerts only a minor influence. Maximum antioxidant capacity is achieved through the combined effect of high temperature and a high sample-to-solvent ratio.

Conclusion

This study demonstrates that extraction temperature and the sample-to-solvent ratio are the principal determinants of antioxidant yield from *Moringa oleifera* leaves, whereas extraction time indicates a negligible effect under aqueous decoction. The high predictive reliability of the RSM-CCD model (R^2 = 0.9742; adjusted-R^2 = 0.9509) confirms its suitability for modeling nonlinear thermal and mass-transfer processes, as further supported by the significant quadratic components and AC interaction. The optimized extraction conditions 80 °C, 10 min, and 1:25 g/mL, yielded 9.49 mg AAE/g FW, validating the robustness of the integrated OFAT–RSM framework. Beyond generating optimal parameters, this study's key contribution lies in providing mechanistic insight into the interplay between solubility, diffusion, and thermal stability, which has been largely overlooked in prior *Moringa* extraction research. These findings offer a reproducible and scalable foundation for developing energy-efficient and industrially viable antioxidant extraction strategies.

Acknowledgements

The authors would like to sincerely acknowledge the research team for their valuable contributions throughout the experimental design, data acquisition, and data analysis stages of this study. Special appreciation is extended to the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Andalas, for providing laboratory facilities, instrumentation support, and an academic environment that enabled the successful completion of this research. The authors would also like thank the colleagues and technical staff who contributed through constructive discussions, technical assistance, and logistical support during the course of the study. Their collective input significantly enhanced the quality, rigor, and reproducibility of the work.

Disclosure Statement

The authors declared no conflicts of interest regarding the research, authorship, or publication of this manuscript.

ORCID

Yefrida Yefrida:
https://orcid.org/0000-0002-1212-2422
Deswati Deswati:
https://orcid.org/0000-0003-2959-4861
Refilda Refilda:
https://orcid.org/0000-0002-3714-4775
Hasifa Hayana:
https://orcid.org/0009-0007-7807-3102
Adewirli Putra:
https://orcid.org/0000-0001-6500-6241

References

[1] Zhou, N.-j., Bao, W.-Q., Zhang, C.-f., Jiang, M.-L., Liang, T.-L., Ma, G.-Y., Liu, L., Pan, H.-D., Li, R.-Z. Immunometabolism and oxidative stress: Roles and therapeutic strategies in cancer and aging. *NPJ Aging*, **2025**, 11(1), 59.

[2] Ahmadinejad, F., Geir Møller, S., Hashemzadeh-Chaleshtori, M., Bidkhori, G., Jami, M.S. Molecular mechanisms behind free radical scavengers function against oxidative stress. *Antioxidants*, **2017**, 6(3), 51.

[3] Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D. Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, **2018**, 757-772.

[4] Arshad, M.T., Maqsood, S., Ikram, A., Gnedeka, K.T. Recent perspectives on the pharmacological, nutraceutical, functional, and therapeutic properties of *Moringa oleifera* plant. *Food Science & Nutrition*, **2025**, 13(4), e70134.

[5] El Ayeb, N., Lahmar, I., Bahri, H., Achour, L. Mineral and biochemical analysis, polyphenol content and antioxidant potential of *Moringa oleifera* leaves collected from two different tunisian regions. *Euro-Mediterranean Journal for Environmental Integration*, **2025**, 10(3), 2019-2031.

[6] Chhikara, N., Kaur, A., Mann, S., Garg, M., Sofi, S.A., Panghal, A. Bioactive compounds, associated health benefits and safety considerations of *Moringa oleifera* L.: An updated review. *Nutrition & Food Science*, **2021**, 51(2), 255-277.

[7] Tyagi, R., Chaudhary, A., Dangi, D., Singh, A., Yusuf, M., Chauhan, P. Application of design of experiment (doe) for optimization of multiple parameter resource constrain process: Taguchi-based fractional factorial approach. *Advanced Journal of Chemistry, Section A*, **2023**, 6(4), 391-400.

[8] Xiang, X., Song, C., Shi, Q., Tian, J., Chen, C., Huang, J., She, B., Zhao, X., Huang, R., Jin, S. Metabolomics reveals polyphenolic compounds associated with antioxidant activities in *Moringa oleifera* leaves. *Food Chem.*, 2020, 331, 127281.

[9] Mbikay, M., Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. *Frontiers in Pharmacology*, **2012**, 3, 24.

[10] Tirado-Kulieva, V.A., Sánchez-Chero, M., Yarlequé, M.V., Aguilar, G.F.V., Carrión-Barco, G., Santa Cruz, A.G.Y. An overview on the use of response surface methodology to model and optimize extraction processes in the food industry. *Current Research in Nutrition and Food Science Journal*, **2021**, 9(3), 745-754.

[11] Patra, A., Abdullah, S., Pradhan, R.C. Application of artificial neural network-genetic algorithm and response surface methodology for optimization of ultrasound-assisted extraction of phenolic compounds from cashew apple bagasse. *Journal of Food Process Engineering*, **2021**, 44(10), e13828.

[12] Dargahi, A., Samarghandi, M.R., Shabanloo, A., Mahmoudi, M.M., Nasab, H.Z. Statistical modeling of phenolic compounds adsorption onto low-cost adsorbent prepared from aloe vera leaves wastes using CCD-RSM optimization: Effect of parameters, isotherm, and kinetic studies. *Biomass Conversion and Biorefinery*, **2023**, 13(9), 7859-7873.

[13] Bahrami, M., Amiri, M.J., Bagheri, F. Optimization of the lead removal from aqueous solution using two starch-based adsorbents: Design of experiments using response surface methodology (RSM). *Journal of Environmental Chemical Engineering*, **2019**, 7(1), 102793.

[14] da Silva, B., Kupski, L., Badiale-Furlong, E. Central composite design-desirability function approach for optimum ultrasound-assisted extraction of daidzein and genistein from soybean and their antimycotoxigenic potential. *Food Analytical Methods*, **2019**, 12(1), 258-270.

[15] Brzezińska, R., Wirkowska-Wojdyła, M., Piasecka, I., Górska, A. Application of response surface methodology to optimize the extraction process of bioactive compounds obtained from coffee silverskin. *Applied Sciences*, **2023**, 13(9), 5388.

[16] Tranquilino-Rodríguez, E., Martínez-Flores, H.E. Ultrasound-assisted extraction of phenolic compounds from *Moringa oleifera* leaves by response surface methodology. *Bioactive*

Compounds in Health and Disease, **2023**, 6(11), 325-337.
[17] Gulcin, İ., Alwasel, S.H. Dpph radical scavenging assay. *Processes*, **2023**, 11(8), 2248.
[18] Brand-Williams, W., Cuvelier, M.-E., Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, **1995**, 28(1), 25-30.
[19] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H. Comparison of abts, dpph, frap, and orac assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, **2006**, 19(6-7), 669-675.
[20] Prior, R.L., Wu, X., Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, **2005**, 53(10), 4290-4302.
[21] Shrivastava, A., Gupta, V.B. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron. Young Sci*, **2011**, 2(1), 21-25.
[22] Talath, S., Hani, U. Spectrophotometric methods in pharmaceutical analysis: Principles, reagents, and applications. *International Journal of Environmental Sciences & Natural Resources*, **2024**, 34(4), 556391.
[23] Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, **2008**, 76(5), 965-977.
[24] Jovanović, A.A., Đorđević, V.B., Zdunić, G.M., Pljevljakušić, D.S., Šavikin, K.P., Gođevac, D.M., Bugarski, B.M. Optimization of the extraction process of polyphenols from Thymus serpyllum L. herb using maceration, heat-and ultrasound-assisted techniques. *Separation and Purification Technology*, **2017**,.179, 369-380.
[25] Sharma, O.P., Bhat, T.K. Dpph antioxidant assay revisited. *Food Chemistry*, **2009**, 113(4), 1202-1205.
[26] Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A., Saija, A. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chemistry*, **2005**, 89(4), 549-554.
[27] Chan, E.W.C., Lim, Y.Y., Wong, S.K., Lim, K., Tan, S., Lianto, F., Yong, M. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry*, **2009**, 113(1), 166-172.
[28] De Marchi, L., Salemi, L., Bellumori, M., Chignola, R., Mainente, F., Soto, D.V.S., Fierri, I., Ciulu, M., Zoccatelli, G. Thermal degradation of red cabbage (Brassica oleracea L. var. Capitata f. rubra) anthocyanins in a water model extract under accelerated shelf-life testing. *Food Chemistry*, **2024**, 440, 138272.
[29] Muzykiewicz-Szymańska, A., Kucharska, E., Pełech, R., Nowak, A., Jakubczyk, K., Kucharski, Ł. The optimisation of ultrasound-assisted extraction for the polyphenols content and antioxidant activity on Sanguisorba officinalis L. Aerial parts using response surface methodology. *Applied Sciences*, **2024**, 14(20), 9579.
[30] Djaoudene, O., Bachir-Bey, M., Schisano, C., Djebari, S., Tenore, G.C., Romano, A. A sustainable extraction approach of phytochemicals from date (phoenix dactylifera l.) fruit cultivars using ultrasound-assisted deep eutectic solvent: A comprehensive study on bioactivity and phenolic variability. *Antioxidants*, **2024**, 13(2), 181.
[31] Mejía, J., Giovagnoli-Vicuña, C., Jacob, C., Montenegro, G., Moreno-Switt, A.I., Giordano, A. Antioxidant, antibacterial, and bioaccessibility properties of ultrasound-extracted chilean propolis. *Antioxidants*, **2025**, 14(6), 651.
[32] Muhamad, N., Yusoff, M., Gimbun, J. Thermal degradation kinetics of nicotinic acid, pantothenic acid and catechin derived from averrhoa bilimbi fruits. *RSC Advances*, **2015**, 5(90), 74132-74137.
[33] Chen, S., Zhang, F., da Silva, A.P.G., Simal-Gandara, J., Cao, H. Vitamin c prevents myricetin degradation in boiling water by reducing ortho-quinone intermediates. *Food Chemistry*, **2025**, 481, 143926.
[34] Zhang, Y., Liu, H. Chemical and biological changes of polyphenols caused by food thermal processing. *Front. Nutr.*,**2022**, 9, 948894.
[35] Wang, Z., Tian, Y., Yang, M., Yang, J., Wang, Y., Tao, L., Sheng, J., Shi, C. Extraction of phenolic compounds from *Moringa oleifera lam*. Leaves with ultrasonic-assisted deep eutectic solvents. *Frontiers in Nutrition*, **2024**, 11, 1405128.
[36] Fombang, E.N., Nobossé, P., Mbofung, C.M., Singh, D. Impact of post-harvest treatment on antioxidant activity and phenolic profile of *Moringa oleifera* lam leaves. *Food Production, Processing and Nutrition*, **2021**, 3(1), 22.
[37] da Silva Donadone, D.B., de Castro França, I.A., Silva, D.L.G., Faria, M.G.I., Ruiz, S.P., Barros, B.C.B. Enhancement of phenolic recovery by probe-type ultrasound-assisted extraction of acerola by-product and evaluation of antioxidant and antibacterial activities. *Applied Sciences*, **2025**, 15(16), 9154.
[38] Machado-Carvalho, L., Martins, T., Aires, A., Marques, G. Optimization of phenolic compounds extraction and antioxidant activity from inonotus hispidus using ultrasound-assisted extraction technology. *Metabolites*, **2023**, 13(4), 524.
[39] Fernández-Romero, E., Chavez-Quintana, S.G., Siche, R., Castro-Alayo, E.M., Cardenas-Toro, F.P.

The kinetics of total phenolic content and monomeric flavan-3-ols during the roasting process of criollo cocoa. *Antioxidants*, **2020**, 9(2), 146.
[40] Antony, A., Farid, M. Effect of temperatures on polyphenols during extraction. *Applied Sciences*, **2022**, 12(4), 2107.
[41] Carciochi, R.A., Sologubik, C.A., Fernández, M.B., Manrique, G.D., D'Alessandro, L.G. Extraction of antioxidant phenolic compounds from brewer's spent grain: Optimization and kinetics modeling. *Antioxidants*, **2018**, 7(4), 45.
[42] Andrade, A.C., Borsoi, F.T., Saliba, A.S.M.C., de Alencar, S.M., Pastore, G.M., Arruda, H.S. Optimization of ultrasonic-assisted extraction of phenolic compounds and antioxidant activity from araticum peel using response surface methodology. *Plants*, **2024**, 13(18), 2560.
[43] Sentkowska, A., Ivanova-Petropulos, V., Pyrzynska, K. What can be done to get more—extraction of phenolic compounds from plant materials. *Food Analytical Methods*, **2024**, 17(4), 594-610.
[44] Sai-Ut, S., Kingwascharapong, P., Mazumder, M.A.R., Rawdkuen, S. Optimization of ethanolic extraction of phenolic antioxidants from lychee and longan seeds using response surface methodology. *Foods*, **2023**, 12(15), 2827.
[45] Zhang, Q.-W., Lin, L.-G., Ye, W.-C. Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine*, **2018**, 13(1), 20.
[46] Muzolf-Panek, M., Gliszczyńska-Świgło, A. Extraction optimization for the antioxidants from nigella sativa seeds using response surface methodology. *Journal of Food Measurement and Characterization*, **2022**, 16(6), 4741-4753.
[47] Balyan, U., Sarkar, B. Aqueous extraction kinetics of phenolic compounds from jamun (Syzygium cumini L.) seeds. *International Journal of Food Properties*, **2017**, 20(2), 372-389.
[48] Che Sulaiman, I.S., Basri, M., Fard Masoumi, H.R., Chee, W.J., Ashari, S.E., Ismail, M. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of clinacanthus nutans lindau leaves by response surface methodology. *Chemistry Central Journal*, **2017**, 11(1), 54.
[49] Wang, Y., Ying, L., Sun, D., Zhang, S., Zhu, Y., Xu, P. Supercritical carbon dioxide extraction of bioactive compounds from *Ampelopsis grossedentata* stems: process optimization and antioxidant activity. International. *Journal of Molecular Sciences*, **2011**, 12(10), 6856-6870.
[50] Borges, G., Degeneve, A., Mullen, W., Crozier, A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *Journal of Agricultural and Food Chemistry*, **2010**, 58(7), 3901-3909.
[51] Cacace, J., Mazza, G. Mass transfer process during extraction of phenolic compounds from milled berries. *Journal of Food Engineering*, **2003**, 59(4), 379-389.
[52] Pinelo, M., Sineiro, J., Núñez, M.a.J. Mass transfer during continuous solid–liquid extraction of antioxidants from grape byproducts. *Journal of Food Engineering*, **2006**, 77(1), 57-63.
[53] Spigno, G., Tramelli, L., De Faveri, D.M. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering*, **2007**, 81(1), 200-208.
[54] Aseibichin, C., Ulakpa, W.C., Omenogor, I., Doyah, E., Olaseinde, A.A., Anakpoha, O.C., Keke, M., Karuppannan, S. Modeling and optimization of transesterification of jatropha oil to fatty acid methyl ester: Application of response surface methodology (CCD) and taguchi orthogonal method. *RSC Advances*, **2024**, 14(17), 11784-11796.
[55] Mbisana, M., Mogopodi, D., Tshepho, R., Chibua, I., Nkoane, B. Optimization of the quechers extraction of mycotoxins in maize using response surface methodology (RSM). *Chemistry Africa*, **2024**, 7(7), 3691-3701.

HOW TO CITE THIS ARTICLE

Y. Yefrida, D. Deswati, R. Refilda , H. Hayana, N. El Firsti , A. Putra. Optimization of Antioxidant Extraction from *Moringa oleifera* Leaves Using an Integrated OFAT and RSM–CCD Approach. *Adv. J. Chem. A*, 2026, 9(7), 1283-1296.

DOI: 10.48309/AJCA.2026.566928.2007
URL: https://www.ajchem-a.com/article_240596.html