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Optimization of Microwave-Assisted Extraction of Phenolics From Purple Star Apple (*Chrysophyllum cainito* L.) Peel by Response Surface Methodology

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ABSTRACT

Microwave-assisted extraction (MAE) was applied to extract phenolic compounds from purple star apple (*Chrysophyllum cainito* L.) peel. Process variables were optimized using response surface methodology based on a Box–Behnken design, considering ethanol concentration, solvent-to-solid ratio, and microwave duration as key factors. Total phenolic content (TPC) and antioxidant capacities, determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, served as response variables. The fitted models showed high significance ($R^2 > 0.99$, $p < 0.05$) and predicted the optimal conditions of 81% ethanol, 26:1 mL/g solvent-to-solid, and 64 s microwave time. Experimental outcomes under these conditions – 168.2 mg gallic acid equivalents (GAE)/g dry weight (dw) for TPC, 243.5 mg ascorbic acid equivalents (AAE)/g dw for DPPH, and 158.4 mg AAE/g dw for FRAP – were in close agreement with predictions. Compared with maceration (12 h and 24 h, 81% ethanol, 26:1 mL/g solvent-to-solid ratio), MAE achieved superior or comparable phenolic yield and antioxidant activity within a much shorter extraction time, confirming its effectiveness as a rapid and sustainable extraction approach for fruit by-products.

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1. Introduction

Star apple (*Chrysophyllum cainito* L.) is a tropical fruit of the Sapotaceae family, known for its purple or green-skinned varieties. Native to tropical America, it is now widely cultivated across Asia, including Vietnam, where the Mekong Delta provides favorable growing conditions [1]-[3]. The fruit is prized for its sweet taste, distinctive peel color, and nutritional composition, while the peel and leaves have long been used in traditional medicine to treat ailments such as cough, rheumatism, and diabetes [3]. Earlier investigations identified *C. cainito* as a rich source of polyphenols, flavonoids, alkaloids, sterols, and triterpenes, which account for its strong antioxidant and therapeutic potential [2], [4].

The peel of the star apple accounts for approximately one-third of the total fruit weight [5], representing a significant post-processing by-product. Improper disposal of this waste not only contributes to environmental pollution but also leads to the loss of valuable bioresources. Several studies have reported that the peel is particularly rich in polysaccharides, phenolic compounds, flavonoids, alkaloids, steroids, and saponins [2], [5], [6]. Among these components, phenolic compounds attract particular attention for their notable antioxidant potential, which assists in scavenging free radicals and mitigating oxidative stress-related conditions such as aging, cardiovascular disorders, and cancer [7]. Therefore, valorizing star apple peel as a natural source of antioxidants represents a sustainable strategy for waste reduction while adding both economic and functional value to this underutilized fruit by-product. Phenolic compounds were extracted from the peel, pulp, and seeds of *C. cainito* using methanol maceration at 25 °C for 7 days [8]. Among the tested parts, the methanolic extract of the peel exhibited the highest TPC. In another study, phenolics were extracted from freeze-dried *C. cainito* peel and pulp powder by maceration in either water or ethanol at 40 °C for 3 h under continuous agitation [9]. The ethanolic extract of the peel showed the highest TPC (5.2 ± 0.13 g GAE/kg), significantly higher than

that of the aqueous peel extract (4.7 ± 0.05 g GAE/kg) and both pulp extracts, confirming that the peel is the main reservoir of phenolic compounds.

Traditional solvent-based extraction techniques, such as maceration, are often limited by prolonged processing times, low extraction efficiency, and high solvent consumption, which can negatively affect both yield and extract quality. In contrast, microwave-assisted extraction (MAE) has emerged as a promising, energy-efficient alternative. This technique utilizes microwave energy to induce rapid heating within plant cells, causing pressure buildup that disrupts the cellular structure and facilitates the efficient release of intracellular bioactive compounds [10]. As a result, MAE enables faster extraction, higher efficiency, and lower solvent consumption than conventional approaches, making it particularly suitable for recovering phenolics from food by-products [11]. The technique has been effectively utilized for extracting phenolic compounds from various plant matrices, including lime peel waste [12], pomegranate peel [13], pineapple peel [14], mango peel [15] and egg-plant peel [16].

However, limited studies have explored the MAE of phenolic compounds from purple star apple peel, and the optimal extraction conditions for maximizing phenolic recovery remain undefined. Because solvent concentration, solvent-to-solid ratio, and microwave duration critically influence extraction efficiency [10], [11], a systematic optimization strategy is essential. In this study, response surface methodology (RSM) was employed to evaluate both the individual and interactive effects of the selected parameters on phenolic yield and antioxidant activity. RSM is a powerful statistical approach that facilitates process optimization while minimizing the number of experimental runs [11]. Among available RSM models, the Box–Behnken design (BBD) offers superior efficiency and simplicity [17], making it well suited for experimental optimization. Therefore, the BBD was adopted to develop predictive models describing the relationships among variables and to determine the optimal MAE conditions for phenolic recovery from *C. cainito* peel.

2. Materials and Methods

2.1. Materials

Purple star apples with purple-pink skin, uniform size, and smooth, undamaged surfaces were purchased from the Thu Duc agricultural market. The fruits were washed and drained, and the leaves, stems, and pulp were removed to obtain the peel. The peel was blanched for 10 seconds to prevent browning caused by peroxidase and polyphenol oxidase. After blanching, the peel was sliced to a thickness of approximately 1–2 mm and dried at 60 °C (Memmert UF260, Germany) for 20 h. The dried peel was then ground into a fine powder using a grinder (800Y, China), and sieved through a 0.2 mm mesh. The resulting powder (hereafter referred to as SAP powder) was kept at 5 °C until use. All chemicals and reagents employed were of analytical grade, originally sourced from the United State, India, and China, and purchased from local suppliers in Vietnam.

2.2. Extraction of phenolic compounds from SAP powder

Phenolic compounds were extracted using the MAE technique, in which SAP powder (0.67–2.00 g) was combined with 20 mL of ethanol (50–90%, v/v) in an Erlenmeyer flask, maintaining a solvent-to-solid ratio between 10:1 and 30:1 mL/g. The sealed flask was irradiated in a domestic microwave oven (Electrolux EMS3085X, Sweden) at powers ranging from 180 to 900 W for 30–150 s. To prevent overheating and solvent boiling that could cause sample loss, microwave irradiation was applied intermittently in 10 s on / 10 s off cycles. After extraction, the mixture was centrifuged at 4500 rpm for 20 min (Hermle Z366, Germany), and the supernatant was collected for further analyses.

For comparison, maceration extraction of phenolic compounds from SAP powder was conducted using the same ethanol concentration and solvent-to-solid ratio as those determined under the optimal MAE conditions. The flasks were kept in dark at room temperature without agitation for 12 h and 24 h. After extraction, the mixtures were centrifuged, and the supernatants were collected for further analyses.

2.3. Optimization procedure

Preliminary single-factor experiments were first conducted to identify the key parameters affecting extraction efficiency and to determine their effective ranges. Ethanol concentration (50–90%), solvent-to-solid ratio (10:1–30:1 mL/g), microwave power (180–900 W), and extraction time (30–150 s) were

independently examined while keeping the other factors constant. The results indicated that total phenolic content (TPC) increased with ethanol concentration up to approximately 80%, solvent-to-solid ratio up to 25:1 mL/g, and microwave power up to around 720 W, and extraction time up to about 60 s, but declined beyond these levels due to possible phenolic degradation or solvent saturation. Based on these trends, ethanol concentration, solvent-to-solid ratio, and microwave duration were selected as the main variables for further optimization using the Box–Behnken design (BBD).

Subsequently, the Box–Behnken design (BBD), integrated into response surface methodology (RSM), was utilized to quantify both the individual and interactive effects of these parameters and to optimize the extraction condition. Three independent variables were selected and coded as follows: ethanol concentration (X_1, 70–90%, v/v), solvent-to-solid ratio (X_2, 20:1–30:1 mL/g), and microwave duration (X_3, 30–90 s). Each variable was coded at three levels (−1, 0, +1) for statistical analysis. According to the BBD, a total of 15 runs were performed (Table 1) to establish second-order polynomial models describing the relationships between the process variables and the three responses (TPC, DPPH, and FRAP).

2.4. Analytical methods

2.4.1. Determination of total phenolic content (TPC)

TPC was quantified using a colorimetric assay based on the Folin–Ciocalteu method, as previously described [18]. The procedure began by mixing 2 mL of the sample extract with 5 mL of 10% (v/v) Folin–Ciocalteu reagent. Following this, 4 mL of 7.5% (w/v) Na_2CO_3 solution was introduced. The mixture was then vortexed to ensure homogeneity and kept in the dark for 1 h at room temperature. A spectrophotometer (Hitachi UH5300, Japan) was then used to record the absorbance at 765 nm. The TPC values are reported as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw).

2.4.2. Assessment of antioxidant capacity

The overall antioxidant potential was evaluated using both DPPH radical scavenging and FRAP assays [19], with minor modifications. For the DPPH test, 0.1 mL of the sample extract was combined with 3.9 mL of the 0.1 mM DPPH solution (prepared in methanol). The resulting mixture was thoroughly vortexed and then kept in the dark for 30 min at ambient temperature. The absorbance was subsequently read at 517 nm. A calibration curve was established using various concentrations of ascorbic acid, and the DPPH activity results were expressed as milligrams of ascorbic acid equivalents per gram of dry sample (mg AAE/g dw).

The FRAP procedure involved initially combining 200 µL of extract with 600 µL of 1% (w/v) $K_3Fe(CN)_6$ and 600 µL of 0.2 M phosphate buffer (pH 6.6). This reaction solution was incubated for 20 min at 50 ^oC within a shaking incubator (Jeiotech IST-3075R, Korea). To terminate the reaction, 0.6 mL of 10% trichloroacetic acid (TCA) was introduced. The resulting mixture was then treated sequentially with 2 mL of distilled water and 0.4 mL of 0.1% $FeCl_3$ for color development. After thorough agitation, the final absorbance of the mixture was monitored at 700 nm. The reducing power determined was also expressed in mg AAE/g dw.

2.5. Statistical analysis

Experiments were conducted in triplicate, and results are shown as mean ± SD. Differences among treatments were evaluated by one-way ANOVA followed by Fisher's LSD test using Minitab software (version 21) with a 95% confidence level. The design and optimization of extraction parameters was performed using the Box–Behnken design (BBD) implemented in Design-Expert software (version 11). Multi-response optimization was carried out using the desirability function approach to determine the combination of variables that provided the most favorable overall response.

3. Results and Discussion

Table 1 presents the experimental setup of the BBD applied for process optimization. In this design, the independent variables X_1, X_2 and X_3 correspond to ethanol concentration (70–90%), solvent-to-solid ratio (20–30 mL/g), and microwave duration (30–90 s), respectively. The factor ranges were determined

based on preliminary screening experiments. Across all experimental runs, the TPC varied between 125.17 to 168.01 mg GAE/g dw, while the antioxidant capacities assessed by DPPH and FRAP assays ranged from 150.92 to 242.17 mg AAE/g dw and 97.27 to 158.87 mg AAE/g dw, respectively.

Table 1. *Experimental factors and their corresponding responses (TPC, DPPH, and FRAP) in the BBD.*

No.	X_1	X_2	X_3	TPC (mg GAE/g dw)	DPPH (mg AAE/g dw)	FRAP (mg AAE/g dw)
1	90	25	90	153.34 ± 3.58	218.05 ± 4.04	143.08 ± 3.07
2	80	25	60	167.09 ± 3.86	239.35 ± 5.71	158.52 ± 4.39
3	70	20	60	125.17 ± 2.49	150.92 ± 5.25	97.27 ± 2.95
4	80	25	60	168.01± 3.05	242.17 ± 8.35	158.87 ± 4.38
5	80	30	90	148.10 ± 2.75	203.55 ± 5.87	131.38 ± 5.26
6	80	30	30	149.71 ± 3.07	188.30 ± 6.93	119.75 ± 2.19
7	80	20	30	136.83 ± 3.40	195.33 ± 6.15	107.22 ± 4.08
8	90	25	30	151.96 ± 2.97	214.73 ± 5.37	140.68 ± 3.26
9	80	25	60	166.39 ± 2.17	237.59 ± 4.00	156.65 ± 4.36
10	90	20	60	127.92 ± 4.36	177.61 ± 6.61	103.44 ± 3.33
11	90	30	60	141.97 ± 2.18	183.48 ± 6.40	125.04 ± 3.49
12	70	25	30	144.66 ± 2.95	194.76 ± 6.81	123.58 ± 3.14
13	70	30	60	133.90 ± 3.46	177.82 ± 3.98	109.26 ± 4.44
14	80	20	90	139.63 ± 3.43	178.48 ± 7.15	112.71 ± 4.30
15	70	25	90	146.49 ± 2.85	205.58 ± 7.19	136.59 ± 4.16

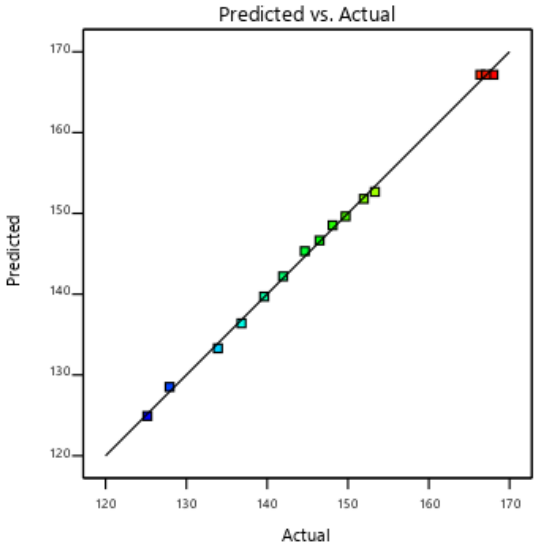
The adequacy of the models developed was evaluated using ANOVA results, as detailed in Table 2. All regression models were statistically significant ($p < 0.0001$), whereas their lack-of-fit tests were not significant ($p > 0.05$), suggesting that the models fitted the experimental data well. The coefficients of determination (R^2) exceeded 0.99, indicating that the models explained most of the variation in the responses. In addition, the predicted R^2 values (> 0.9) and Adequate Precision values (> 4) indicated acceptable model predictability and satisfactory signal-to-noise ratios. The low coefficients of variation (C.V.%), all below 2% for TPC, DPPH, and FRAP, also reflected good precision and consistency of the experimental data.

Table 2. *ANOVA results for the fitted models of TPC, DPPH, and FRAP.*

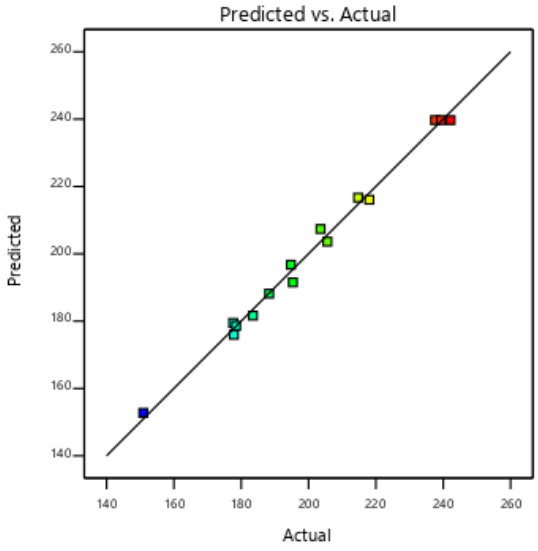
Source	DF	TPC		DPPH		FRAP	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	9	398.60	< 0.0001	77.35	< 0.0001	758.79	< 0.0001
X_1	1	112.30	0.0001	38.16	0.0016	303.35	< 0.0001
X_2	1	350.62	< 0.0001	23.45	0.0047	614.11	< 0.0001
X_3	1	3.48	0.1211	1.43	0.2856	154.80	< 0.0001
X_1X_2	1	10.17	0.0243	8.04	0.0364	27.07	0.0035
X_1X_3	1	0.07	0.7974	1.02	0.3585	32.86	0.0023
X_2X_3	1	7.02	0.0455	18.73	0.0075	11.04	0.0209
X_1^2	1	1147.24	< 0.0001	170.31	< 0.0001	1041.14	< 0.0001
X_2^2	1	2177.23	< 0.0001	474.65	< 0.0001	4920.46	< 0.0001

X_3^2	1	60.12	0.0006	10.42	0.0233	183.13	< 0.0001
Residual	5						
Lack of Fit	3	1.08	0.5138	3.62	0.2240	0.3314	0.8087
Pure Error	2						
Total	14						

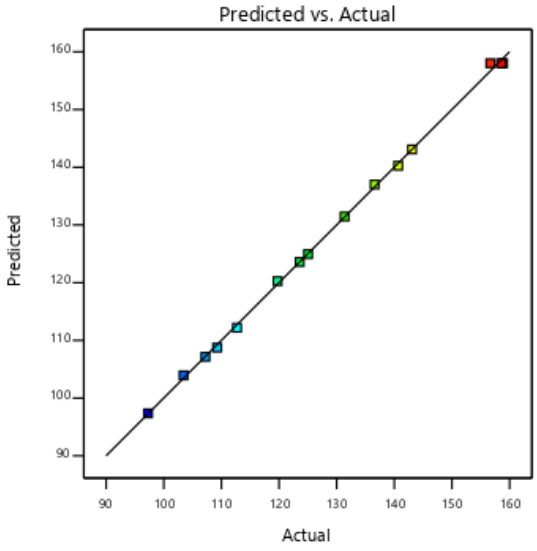
R^2	0.9986	0.9929	0.9993
Adjusted R^2	0.9961	0.9800	0.9980
Predicted R^2	0.9850	0.9012	0.9950
Adeq. Precision	62.0736	28.7122	80.3621
C.V. %	0.5678	1.85	0.7206



a) TPC



b) DPPH



c) FRAP

Figure 1. *Predicted versus actual plots for (a) TPC, (b) DPPH, and (c) FRAP.*

In addition to ANOVA, model adequacy was further examined through residual analysis. The normal probability plots of residuals for all responses (TPC, DPPH, and FRAP) showed an approximately linear pattern, suggesting that the residuals were close to normally distributed and that the models were reasonably adequate. The predicted versus experimental plots (Figure 1) also demonstrated good agreement, confirming that the quadratic models were not overfitted and adequately represented the experimental data.

The significance of each model term was subsequently evaluated according to the ANOVA results. As shown in Table 2, all factors exhibited significant effects on the responses at their quadratic levels ($p < 0.05$), indicating that the quadratic model appropriately described the curvature of the response surfaces. At the linear level, ethanol concentration (X_1) and solvent-to-solid ratio (X_2) exerted a significant influence on TPC, DPPH, and FRAP, whereas microwave duration (X_3) had a significant effect only on FRAP. Regarding the two-factor interactions, X_1X_2 and X_2X_3 significantly affected all three responses ($p < 0.05$), while X_1X_3 significantly influenced only FRAP. The second-order polynomial equations representing the effects of the independent variables (in coded form) on TPC, DPPH, and FRAP are given in Eq. (1), Eq. (2), and Eq. (3), respectively:

$$\text{TPC (mg GAE/g dw)} = 167.16 + 3.12X_1 + 5.52X_2 + 1.33X_1X_2 - 1.10X_2X_3 - 14.69\,X_1^2 - 20.23X_2^2 - 3.36X_3^2 \quad (1)$$

$$\text{DPPH (mg AAE/g dw)} = 239.70 + 8.10X_1 + 6.35X_2 - 5.26X_1X_2 + 8.03X_2X_3 - 25.19\,X_1^2 - 42.05X_2^2 - 6.23X_3^2 \quad (2)$$

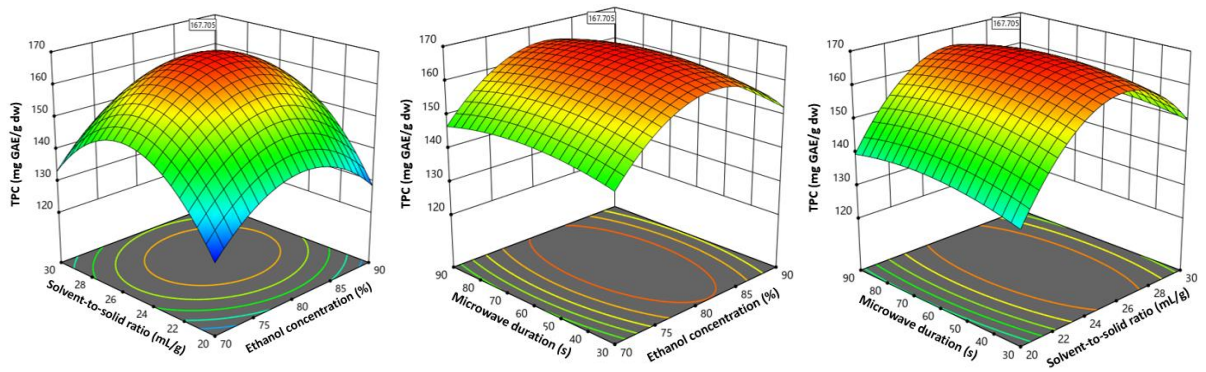
$$\text{FRAP (mg AAE/g dw)} = 158.01 + 5.69X_1 + 8.10X_2 + 4.07X_3 + 2.40X_1X_2 - 2.65X_1X_3 + 1.54X_2X_3 - 15.52\,X_1^2 - 33.74X_2^2 - 6.51X_3^2 \quad (3)$$

Response surface plots derived from the fitted quadratic models illustrated the interactive effects of the extraction variables on TPC, DPPH, and FRAP (Figure 2). Ethanol concentration (X_1) and solvent-to-solid ratio (X_2) exerted the most significant influences on all responses, consistent with the ANOVA results (Table 2). For TPC, the response increased markedly with ethanol concentration and solvent-to-solid ratio within a moderate range, reflecting an optimal balance of solvent polarity and diffusion kinetics. Moderate ethanol levels (around 80%) provided suitable polarity to dissolve both hydrophilic and moderately hydrophobic phenolics [20], [21]. Ethanol also disrupts hydrogen and hydrophobic bonds between phenolics and macromolecules, facilitating their release into the solvent phase [13]. However, at higher ethanol concentrations, excessive polarity reduction and protein denaturation may entrap phenolics within the solid matrix, thereby lowering extraction efficiency [22]. Similarly, increasing the solvent-to-solid ratio initially enhanced diffusion by enlarging the concentration gradient, but excessive solvent volumes diluted this gradient and reduced microwave absorption, leading to decreased extraction efficiency and higher energy consumption [13]. With respect to microwave time, TPC increased with microwave duration up to about 63 s, likely due to improved solvent penetration and cell rupture. Beyond this point, prolonged irradiation caused degradation of thermolabile phenolics, lowering the overall extraction yield [23], [24].

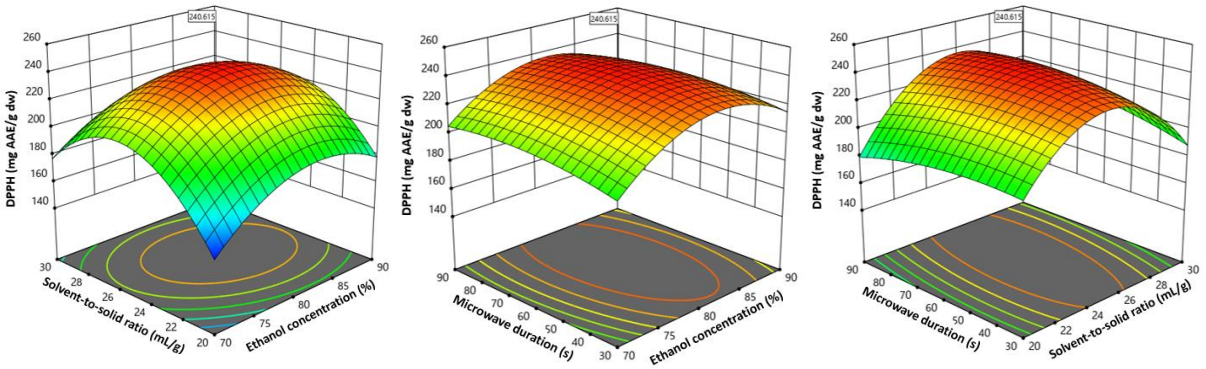
The response patterns for DPPH and FRAP followed a similar trend (Figure 2b-c), highlighting the close association between total phenolics and antioxidant capacity, as phenolic hydroxyl groups serve as efficient hydrogen and electron donors in both assays. Synergistic effects of ethanol concentration (X_1) and solvent-to-solid ratio (X_2) were again evident, with both responses reaching their maxima near the optimal levels of these factors. For FRAP, the interaction between ethanol level and microwave duration (X_1X_3) was statistically significant but less pronounced than X_1X_2, suggesting that solvent composition slightly modulated the effect of microwave energy. Moderate microwave exposure facilitated solvent penetration and phenolic release through thermal softening, whereas prolonged heating might lead to degradation of heat-sensitive compounds [22], [25], thereby reducing antioxidant capacity. Overall, ethanol concentration and the ratio of solvent to solid were the main factors governing extraction performance.

The desirability function approach was employed to identify the optimal combination of extraction parameters that maximized the overall response. Through this methodology, TPC, DPPH radical

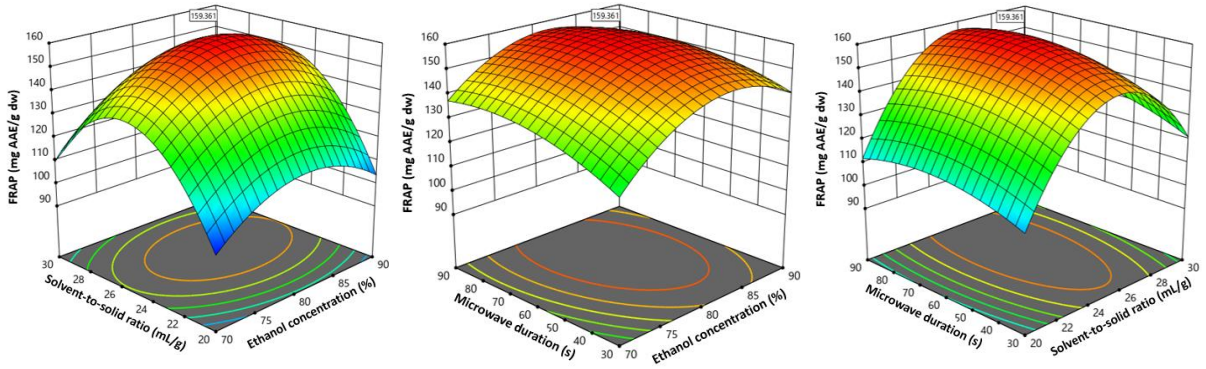
scavenging activity, and FRAP were simultaneously maximized by integrating the individual responses into a unified desirability index [26]. The resulting optimal conditions were predicted to be 81.2% ethanol, 25.5:1 mL/g solvent-to-solid ratio, and 63.5 s microwave duration, yielding an overall desirability value of 0.992. Validation experiments conducted under slightly adjusted conditions (81% ethanol concentration, 26:1 mL/g liquid-to-solid ratio and 64 s microwave time) yielded results (TPC: 168.2 mg GAE/g dw, DPPH: 243.5 mg AAE/g dw, and FRAP: 158.4 mg AAE/g dw (Table 3)) that were highly consistent with the model predictions, confirming the adequacy and reliability of the developed models.



a) TPC



b) DPPH



c) FRAP

Figure 2. *Response surfaces representing the influence of extraction conditions on: (a) TPC, (b) DPPH, and (c) FRAP.*

To assess extraction efficiency, conventional maceration was conducted under the MAE-optimized settings (81% ethanol with a solvent-to-solid proportion of 26:1 mL/g) for 12 and 24 h. As presented in Table 3, after 12 h of maceration, the TPC was markedly lower than that obtained by MAE, whereas the DPPH and FRAP values were approximately 30% and 19% lower, respectively. Extending the

extraction to 24 h produced values that did not significantly differ from those obtained by MAE ($p > 0.05$). These results indicate that MAE reached the extraction plateau much faster than maceration, achieving similar yields within only 64 seconds. The advantage of MAE, therefore, lies in its rapid extraction kinetics and energy efficiency, as microwave irradiation accelerates solvent penetration, enhances mass transfer, and promotes effective cell disruption through localized heating. Such features make MAE a powerful alternative to conventional maceration, particularly for time- and energy-sensitive applications.

Table 3. *Experimental and model-derived data for TPC, DPPH, and FRAP obtained under optimal MAE conditions and traditional maceration.*

	MAE		Conventional maceration	
	Model	**Experiment**	**12 h**	**24 h**
TPC (mg GAE/g dw)	167.7	168.2 ± 3.4^{a}	125.3 ± 5.4^{b}	175.2 ± 3.9^{a}
DPPH (mg AAE/g dw)	240.6	243.5 ± 5.5^{a}	169.5 ± 5.3^{b}	251.1 ± 4.8^{a}
FRAP (mg AAE/g dw)	159.4	158.4 ± 5.1^{a}	127.7 ± 4.7^{b}	166.5 ± 5.1^{a}

Different letters in the same row refer to significant differences among samples ($p < 0.05$).

4. Conclusion

Microwave-assisted extraction was effectively optimized to obtain phenolic-rich extracts from purple star apple (*Chrysophyllum cainito* L.) peel using response surface methodology. The optimized parameters (81% ethanol, a solvent-to-solid ratio of 26:1 mL/g, and a microwave time of 64 s) yielded the highest total phenolic content and antioxidant capacities (DPPH and FRAP). The developed models demonstrated excellent accuracy and predictive strength. Compared with conventional maceration, MAE achieved comparable or superior yields in a much shorter duration, underscoring its benefits in process efficiency and solvent utilization. Overall, MAE is an effective approach to obtain phenolics from purple star apple peel for potential use as natural antioxidants in food products.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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