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Research Article

Multi-Response Optimization of Mangosteen Peel Extraction Using Natural Deep Eutectic Solvent and Microwave Assisted Extraction for Anti-Aging

Fitroh Bawa Berkah *, Vita Paramita

Department of Industrial Technology, Vocational College, Universitas Diponegoro, Jalan Gubernur Mochtar, 50275 Semarang, Indonesia

Abstract

The peel of mangosteen (*Garcinia mangostana* L.) is rich in bioactive compounds, including xanthones, phenolics, flavonoids, anthocyanins, and vitamin C, which have been associated with antioxidant, anti-inflammatory, and anti-aging properties. This study aimed to optimize the extraction of these compounds, particularly xanthones, using Microwave-Assisted Extraction (MAE) in combination with a Natural Deep Eutectic Solvent (NADES) consisting of lactic acid, sodium acetate, and distilled water in a 3:1:2 volume ratio. The extraction process was carried out under varying microwave power levels (300, 375, and 450 Watt) and extraction times (2, 4, and 6 min). Optimization was conducted using Response Surface Methodology (RSM), and the resulting bioactive compound contents were analyzed by UV-Vis spectrophotometry. The optimal extraction conditions were determined at 300 Watt and 4.46 min, resulting in xanthone (1.40%), phenolics (42.43%), flavonoids (1.22%), anthocyanins (1.20%), and vitamin C (2.94%). These findings indicate that NADES-MAE is an effective green extraction technique for maximizing bioactive compounds from mangosteen peel, supporting its potential use in anti-aging product formulations.

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1. Introduction

Mangosteen peel contains various bioactive compounds, including xanthones, α- and β-mangostin, flavonoids, phenolics, anthocyanins, and vitamin C, which exhibit antioxidant, anti-aging, and therapeutic properties (Yuvanatemiya et al., 2023). These compounds make it a promising candidate for anti-aging applications by protecting the skin from oxidative stress caused by UV radiation and pollution. While extraction of these actives typically involves conventional solvents, recent efforts to employ green methods like Natural Deep Eutectic Solvents (NADES) combined with Microwave-Assisted Extraction (MAE) remain limited (Patra et al., 2022). Most existing studies focus on single-response optimization, thus this study addresses that gap by optimizing multiple bioactive extractions using NADES-MAE to enhance anti-aging potential.

Natural Deep Eutentic Solvent (NADES) is a natural deep eutectic solvent consisting of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). This combination creates a solvent with adjustable viscosity and polarity to enhance the extraction efficiency of bioactive compounds. The NADES consisting of lactic acid, sodium acetate, and water (3:1:2) was selected based on its previously reported effectiveness in extracting phenolic compounds (Martín et al., 2023). Sodium acetate acts as a hydrogen bond acceptor due to the presence of a carboxylate group (−COO), which can interact with the hydrogen bond donor. This group has the ability to attract protons and form strong ionic interactions with target molecules. Lactic acid acts as a hydrogen bond donor because it contains hydroxyl (−OH) and carboxyl (−COOH) groups. These groups can donate protons into the system and enhance the solubility of phenolic compounds in the solvent. The addition of water in a specific volume ratio (3:1:2) helps reduce the viscosity of the NADES mixture, facilitating the diffusion of

* Corresponding Authors.
Email: fitrohberkah@gmail.com (F.B. Berkah)

bioactive compounds (Martín et al., 2023).

Microwave Assisted Extraction (MAE) has been proven to be more efficient than conventional methods, such as percolation, Soxhlet extraction, and maceration Microwave-Assisted Extraction (MAE) is an efficient extraction method that uses microwaves to break down plant cell walls and membranes, accelerating the release of active compounds. MAE also rapidly heats the extraction system, reducing extraction time and solvent consumption. Its efficiency is influenced by factors such as extraction time, temperature, material-to-liquid ratio, and microwave power (Li et al., 2017). Studies have shown that MAE produces higher yields compared to traditional methods like Soxhlet extraction and maceration while enhancing the bioactivity of the extracted compounds (Bitwell et al., 2023). The efficiency of MAE depends on several factors, including power, time, temperature, particle size, and solvent type. Therefore, Response Surface Methodology (RSM) is used for the statistical and mathematical optimization of extraction variables.

2. Materials and Methods

2.1 Materials

The mangosteen peel (*Garcinia mangostana* L.) used in this study was obtained from Yogyakarta City, Special Region of Yogyakarta, Indonesia. Lactic acid, sodium acetate, distilled water, methanol, sodium carbonate, folin-ciocalteu reagent, ethanol, buffer solution pH 1, buffer solution pH 4.5, 10% $AlCl_3$, which were all purchased from Merck & Co., Inc. (New Jersey, US).

2.2 Instrumentation

Microwave Assisted Extraction (D-500, China), UV-Vis spectrophotometer (In Science Pro, Indonesia), digital balance (Ohaus, New Jersey USA), beaker glass (phyrex), dropper pipette, magnetic stirrer (Heidolph, Germany).

2.3 Mangosteen Peel Extraction Using MAE

The antioxidant extraction from mangosteen peel begins by preparing mangosteen peel powder and NADES (lactic acid, sodium acetate, water) in a 3:1:2 ratio, mixed using a magnetic stirrer at 50°C, 600 rpm for 45 min. The powder is then mixed with NADES (1:16 w/w) and placed into the extraction flask. The microwave extractor is turned on, and the power (300, 375, 450 Watt) and time (2, 4, 6 min) are set according to the variables. The obtained extract is filtered using filter paper, and the experiment is repeated with different variables before turning off the equipment. The extract is then analyzed for xanthone, phenolic, flavonoid, anthocyanin, and vitamin C content using UV-Vis spectrophotometry.

2.4 Determination of Xanthone Content

A total of 50 mg of extract is weighed and dissolved in 50 ml of methanol (1000 µg/ml), followed by serial dilution to 10 µg/ml. A 2.5 ml aliquot of the solution is transferred into a cuvette, and its absorbance is measured using a UV-Vis spectrophotometer at 243 nm. The sample absorbance measurements determined its xanthone content using the linear regression equation $y = 0.0571x - 0.0037$. The formula for calculating the xanthone content in mangosteen peel follows Equation (1).

$$Xanthone\ content\left(\mu g/mg\right) = \frac{C \times V \times Fp}{W} \quad (1)$$

Where C is the total xanthone concentration (µg/ml), V is the sample volume (ml), Fp is the dilution factor, and W is the dried sample weight (mg).

2.5 Determination of Total Phenolic Content

A total of 100 mg of extract is dissolved in 25 ml of distilled water. Then, 0.3 ml of the solution is pipetted, mixed with 1.5 ml of Folin-Ciocalteu reagent, stirred, and left to stand for 5 min. Subsequently, 1.2 ml of sodium carbonate is added, and the mixture is incubated for 60 min. A 2.5 ml aliquot of the solution is transferred into a cuvette, and its absorbance is measured using a UV-Vis spectrophotometer at 748.6 nm. The sample absorbance measurements determined its phenolic content using the linear regression equation $y = 0.0124x + 0.0379$. The formula for calculating the phenolic content in mangosteen peel follows Equation (2).

$$Total\ phenolic\ content\left(mgGAE/g\right) = \frac{C \times V}{W} \quad (2)$$

Where C is the total phenolic concentration (mg/ml), V is the sample volume (ml), and W is the dried sample weight (g).

2.6 Determination of Total Flavonoid Content

The flavonoid content in mangosteen peel is determined by dissolving 0.05 g of extract in 50 ml of ethanol. A 10 ml aliquot of the solution is transferred to a 50 ml volumetric flask, followed by the addition of 20 ml of distilled water, 1 ml of 10% $AlCl_3$, 1 ml of 1 M sodium acetate, and distilled water up to the marked volume. The solution is shaken and left to stand for 30 min. A 2.5 ml aliquot is placed into a cuvette, and its absorbance is measured using a UV-Vis spectrophotometer at 431 nm. The sample absorbance measurements determined its phenolic content using the linear regression equation $y = 0.0477x + 0.0068$. The formula for calculating the phenolic content in mangosteen peel follows Equation (3).

$$Total\ flavonoid\ content = \frac{C \times V}{W} \quad (3)$$

Where C is the total flavonoid concentration (mg/ml), V is the sample volume (ml), and W is the dried sample weight (mg).

2.7 Determination of Total Anthocyanin Content

The anthocyanin content in mangosteen peel is determined using the pH differential method. A pH 1 buffer solution is prepared with HCl, while a pH 4.5 buffer solution is prepared with NaOH. A 1 ml sample is dissolved in 10 ml of buffer solutions at pH 1 and pH 4.5, respectively, and its absorbance is measured using a UV-Vis spectrophotometer at 530 nm and 700 nm (Netravati et al., 2024). The formula for calculating the anthocyanin content in mangosteen peel follows Equation (4).

$$Anthocyanin\ content = \frac{A \times MW \times Df \times 1000}{\varepsilon \times 1} \quad (4)$$

Where A is the absorbance $\{(A_{530nm} - A_{700nm})pH_{1.0} - (A_{530nm} - A_{700nm})pH_{4.5}\}$, ε is the molar extinction coefficient (269,000 L/mol/cm for cyanidin-3-glucoside), MW is the molecular weight (449.2 g/mol for cyanidin-3-glucoside), Df is the dilution factor, and 1 cm is the cuvette path length.

2.8 Determination Vitamin C Content

The vitamin C content in mangosteen peel is determined by diluting 1 ml of extract in a 100 ml volumetric flask. A 2.5 ml aliquot of the solution is transferred into a cuvette, and its absorbance is measured using a UV-Vis spectrophotometer at 265 nm. The sample absorbance measurements determined its phenolic content using the linear regression equation $y = 0.0497x + 0.0222$. The formula for calculating the phenolic content in mangosteen peel follows Equation (5).

$$Vitamin\ C\ content\left(mg/100g\right) = \frac{C \times V \times Fp}{W} \quad (5)$$

Where C is the total xanthone concentration (µg/ml), V is the sample volume (ml), Fp is the dilution factor, and W is the dried sample weight (mg).

2.9 Research Design

Data analysis was performed using Minitab 2018 with the RSM for multiple response optimization, where a quadratic polynomial model was developed through the Design of Experiment (DOE) and evaluated using ANOVA, with optimization conducted using the desirability function to determine the best combination of variables; the Central Composite Design (CCD) used in this study consists of two factors with a full factorial two-level design, including 12 runs in one block without replication, featuring 4 cube points, 4 center points, and 4 axial points with α = 1, enabling efficient analysis of linear, interaction, and quadratic effects, as shown in Table 1.

Table 1. Response surface desain factor.

Factor	Low	High
Power (Watt)	300	450
Time (min)	2	6

3. Results and Discussion

3.1 Effect of Parameters on Antioxidant Activity of Mangosteen Peel Extract

Table 2 shows the extraction analysis results, indicating that the highest xanthone content was obtained at a microwave power of 300 Watt and an extraction time of 4 min, with a value of 0.7675%. The highest total phenolic content was found at a microwave power of 450 Watt and an extraction time of 4 min, with a value of 35.4774%. The highest total flavonoid content was obtained at a microwave power of 375 Watt and an extraction time of 2 min, with a value of 0.2725%. The highest total anthocyanin content was found at a microwave power of 375 Watt and an extraction time of 4 min, with a value of 0.9074%. Meanwhile, the highest vitamin C content was obtained at a microwave power of 300 Watt and

Table 2. Experimental design and the influence of independent variables on the changes in antioxidant activity of mangosteen peel extract

Run	Power (Watt)	Time (min)	A (%)	B (%)	C (%)	D (%)	E (%)
1	300	2	0.2056	18.1665	0.0741	0.3313	3.3085
2	450	2	0.1018	19.5671	0.1221	0.2201	3.0260
3	300	6	0.1020	29.9503	0.0305	0.2867	2.9572
4	450	6	0.0754	18.2168	0.1853	0.1934	3.1422
5	300	4	0.7675	17.6968	0.2071	0.1932	2.7749
6	450	4	0.0670	35.4774	0.1483	0.1406	2.9589
7	375	2	0.0574	20.4813	0.2725	0.2104	2.6701
8	375	6	0.0475	33.6742	0.0850	0.1979	2.1227
9	375	4	0.0412	20.5316	0.0545	0.1448	1.5278
10	375	4	0.0441	21.4206	0.0109	0.1832	2.1710
11	375	4	0.0379	24.3310	0.0371	0.4333	1.5280
12	375	4	0.0397	20.0535	0.0174	0.9074	2.1712

an extraction time of 2 min, with a value of 3.3085%. The increase in microwave power and extraction time affects the bioactive compound content in the extract. Higher microwave power can enhance the heating rate, accelerate the extraction of active compounds, and increase the total phenolic and flavonoid content. However, excessively high power may lead to the degradation of certain compounds, such as xanthone and anthocyanin (Djiobie Tchienou et al., 2022). While the presence of these bioactive compounds suggests potential anti-aging benefits, their functional efficacy was not confirmed in this study and should be validated through further in vitro or in vivo antioxidant assays.

3.2 *Analyze Response Surface Design*

Tables 3 and 4 show the analysis of the response surface design using ANOVA. Degrees of Freedom (DF) represent the number of independent values used to estimate the model parameters. The Adjusted Sum of Squares (Adj SS) indicates the contribution of each factor to the total variability of the response, while the Adjusted Mean Square (Adj MS), calculated by dividing Adj SS by DF, reshows the average variance explained by each factor after accounting for the influence of other variables. The F-value, obtained by dividing the Adj MS of the factor by the Adj MS of the error, indicates the relative significance of each factor in the model; higher F-values suggest a greater

Table 3. Analysis of Variance (ANOVA) of Xanthone, Phenolic, Flavonoid.

Source	DF	Xanthone (%)			Phenolic (%)			Flavonoid (%)		
		Adj SS	F- value	P-value	Adj SS	F-value	P-value	Adj SS	F-value	P-value
Model	5	0.265875	1.61	0.289	145.671	0.62	0.693	0.021673	0.46	0.791
Linear	2	0.118329	1.79	0.246	102.280	1.09	0.396	0.008149	0.44	0.665
Power	1	0.115068	3.47	0.112	9.245	0.20	0.673	0.003451	0.37	0.565
Time	1	0.003261	0.10	0.764	93.035	1.97	0.210	0.004697	0.50	0.505
Square	2	0.146055	2.20	0.191	0.265	0.00	0.997	0.010671	0.57	0.593
Power*Power	1	0.132209	3.99	0.093	0.240	0.01	0.945	0.003451	0.37	0.565
Time*Time	1	0.053890	1.63	0.249	0.097	0.00	0.965	0.003664	0.39	0.554
2-Way-Interaction	1	0.001491	0.05	0.839	43.127	0.92	0.376	0.002853	0.31	0.600
Power*Time	1	0.001491	0.05	0.839	43.127	0.92	0.376	0.002853	0.31	0.600
Error	6	0.198733			282.736			0.056007		
Lack-of-Fit	3	0.198712	9676.98	0.000	271.713	24.65	0.013	0.054834	46.75	0.005
Pure Error	3	0.000021			11.022			0.001173		
Total	11	0.464608			428.407			0.077679		

Table 4. Analysis of Variance (ANOVA) of Anthocyanin, and Vitamin C.

Source	DF	Anthocyanin (%)			Vitamin C (%)		
		Adj SS	F- value	P- value	Adj SS	F- value	P- value
Model	5	0.064418	0.18	0.961	3.54943	7.67	0.014
Linear	2	0.012193	0.08	0.920	0.10333	0.56	0.599
Power	1	0.011022	0.15	0.709	0.00124	0.01	0.911
Time	1	0.001171	0.02	0.903	0.10208	1.10	0.334
Square	2	0.052144	0.36	0.711	3.39147	18.31	0.003
Power*Power	1	0.025754	0.36	0.572	1.99411	21.54	0.004
Time*Time	1	0.009934	0.14	0.723	0.41446	4.48	0.079
2-Way-Interaction	1	0.000080	0.00	0.975	0.05463	0.59	0.472
Power*Time	1	0.000080	0.00	0.975	0.05463	0.59	0.472
Error	6	0.432162			0.55554		
Lack-of-Fit	3	0.062613	0.17	0.911	0.14177	0.34	0.799
Pure Error	3	0.369549			0.41377		
Total	11	0.496579			4.10497		

DF = Degree of Freedom, Adj SS = Adjusted Sums of Square, P-value = probability value ($p<0.05$)

Table 5. Model Equations of the responses of Xanthone, Phenolic, Flavonoid Anthocyanin, and Vitamin C.

Response (%)	Quadratic Polynomial Model Equations	Standard error	R^2
Xanton	$6.03 - 0.0320\ X_1 + 0.224\ X_2 + 0.000040\ X_1^2 - 0.0355\ X_2^2 + 0.000129\ X_1\ X_2$	0.18199	57.23%
Phenolic	$-30 + 0.144\ X_1 + 9.8\ X_2 - 0.000053\ X_1^2 + 0.05\ X_2^2 - 0.0219\ X_1\ X_2$	6.86459	34.00%
Flavonoid	$1.32 - 0.00519\ X_1 - 0.155\ X_2 + 0.000006\ X_1^2 + 0.0093\ X_2^2 + 0.000178\ X_1\ X_2$	0.09661	27.90%
Antosianin	$-2.05 + 0.0124\ X_1 + 0.104\ X_2 - 0.000017\ X_1^2 - 0.0153\ X_2^2 + 0.000030\ X_1\ X_2$	0.26837	12.97%
Vitamin C	$26.45 - 0.1182\ X_1 - 1.146\ X_2 + 0.000154\ X_1^2 + 0.0986\ X_2^2 + 0.00078\ X_1\ X_2$	0.30428	86.47%

X_1 = Power (Watt), X_2 = Time (min)

influence. The P-value is used to determine the statistical significance of each factor, where $P < 0.05$ indicates a statistically significant effect at a 95% confidence level, and $P > 0.05$ suggests the factor is not statistically significant (Paramita et al., 2022).

Furthermore, Table 5 shows the quadratic polynomial model equation used to evaluate the effects of the independent variables on each response. The resulting equation describes the influence of microwave power (X_1) and extraction time (X_2) on the xanthone content in mangosteen peel. This model was developed based on effect estimation data and evaluated using the standard error of regression (S) and the coefficient of determination (R^2). The R^2 value, which ranges from 0 to 1, indicates the proportion of response variance that can be explained by the model. Generally, an R^2 value greater than 0.8 is considered to reflect a good predictive model (Paramita et al., 2022).

3.3 *Contour Plot Overview*

Figure 1 (A–E) shows contour plots illustrating the interaction between microwave power (X_1) and extraction time (X_2) on five bioactive compounds in

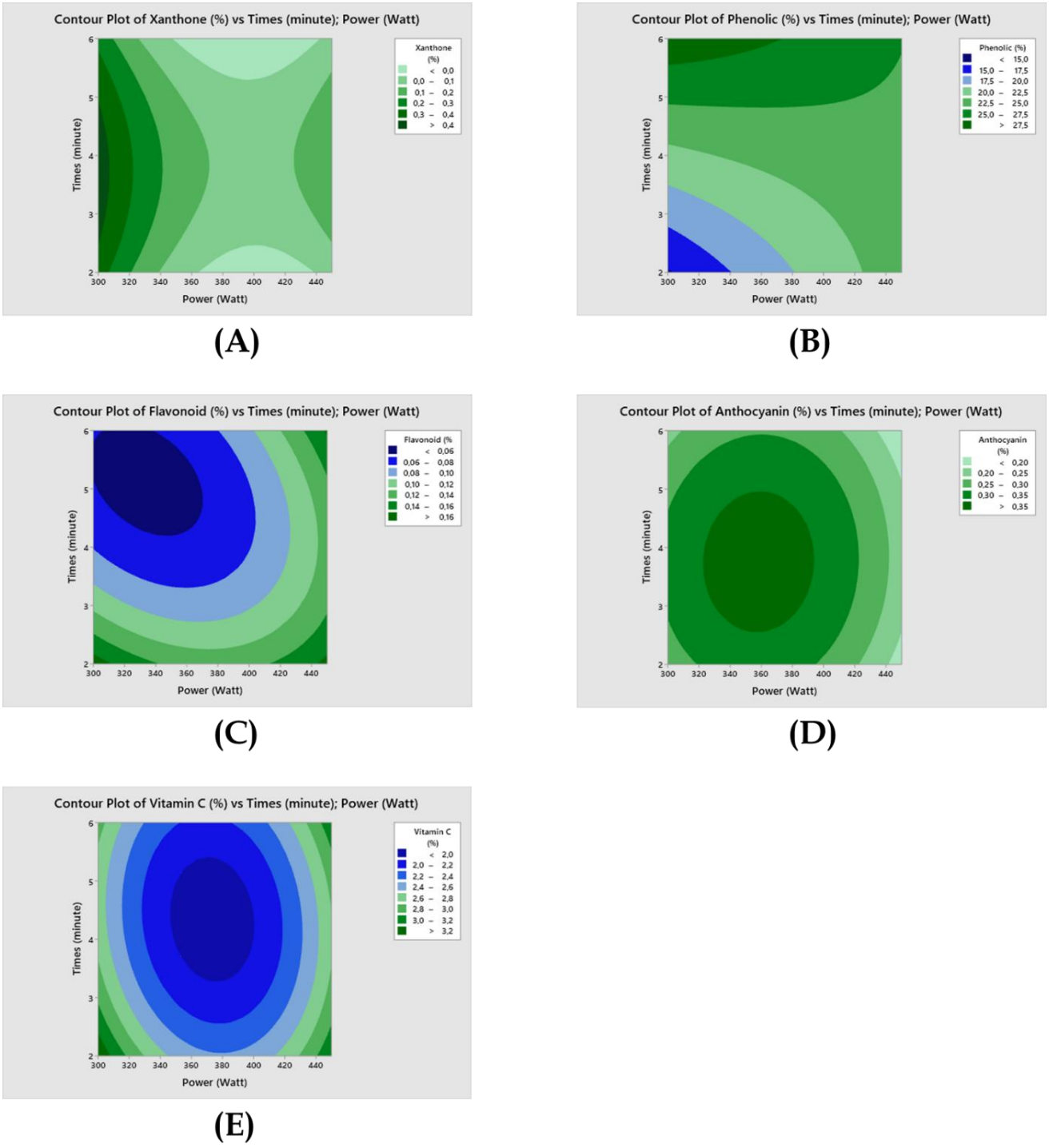

Figure 1. Contour plot of microwave power and extraction time for Xanthone (A), Phenolic (B), Flavonoid (C), Anthocyanin (D), and Vitamin C (D).

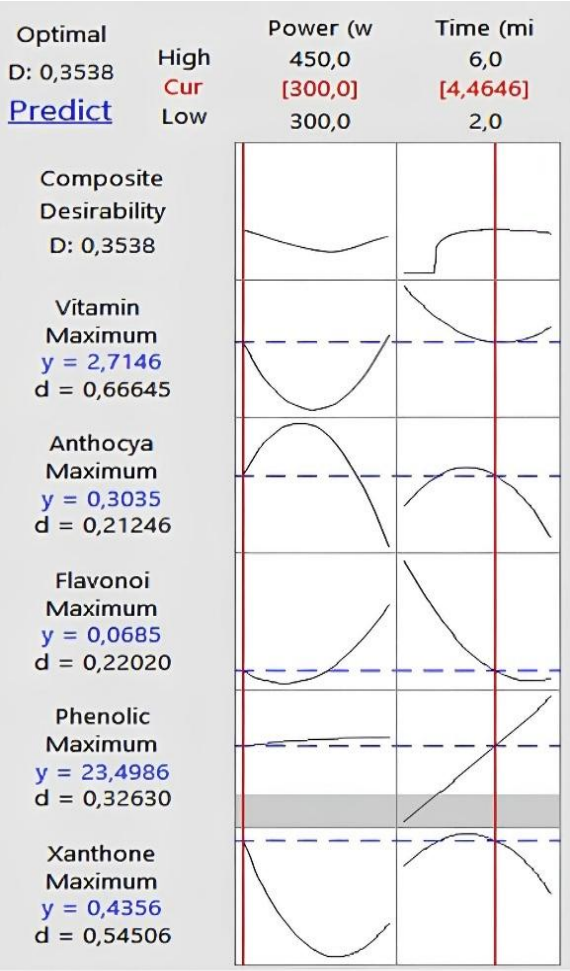

Figure 2. Multiple response prediction diagram of Xanthone, Phenolic, Flavonoid, Anthocyanin, and Vitamin C

mangosteen peel extract: xanthone, phenolics, flavonoids, anthocyanins, and vitamin C. The color gradient in the plots reshows the concentration levels of each compound, where darker shades indicate higher values (optimal response), while lighter shades correspond to lower concentrations (Rajendran et al., 2014). The contour patterns reveal that each compound responds differently to variations in power and time, with most compounds reaching optimal yields at around 300 Watt and 3–4 min of extraction time. This visualization supports the quadratic model used in the ANOVA analysis and aids in determining the most efficient processing conditions.

3.4 *Multiple Response Prediction for Xanthone, Phenolic, Flavonoid, Anthocyanin, and Vitamin C*

Figure 2 shows the multiple response prediction diagrams for Xanthone, Phenolic, Flavonoid, Anthocyanin, and Vitamin C. The optimization of mangosteen peel extraction using Microwave-Assisted Extraction (MAE) was conducted with power (300, 375, and 450 Watt) and time (2, 4, and 6 min) as variables. The response was evaluated using the desirability function (range: 0-1), where a value closer to 1 indicates a better optimization outcome (Paramita et al., 2022). The optimal process conditions for maximizing xanthone, phenolic, flavonoid, anthocyanin, and vitamin C resulted in desirability values of 0.54506, 0.32630, 0.22020, 0.21246, and 0.66645, respectively. The composite desirability (D) value of 0.3538 indicates that the best extraction conditions were achieved at 300 Watt and 4.46 min, leading to further verification steps. Each response was optimized for its maximum value to enhance the anti-aging potential of mangosteen peel extract, focusing on antioxidant and anti-inflammatory properties. Maximizing these bioactive compounds ensures a higher concentration of beneficial ingredients, improving the final product's effectiveness in combating skin aging and promoting overall skin health.

3.5 *Response Optimizer of Xanthone*

Table 6 presents the optimization results using the Multiple Response Optimizer to determine the optimal conditions for extracting bioactive compounds from mangosteen peel, which were found to be at a power of 300 Watt and an extraction time of 4.46 min. Based on the quadratic polynomial regression model, the predicted xanthone content was 0.4356%, while the actual result showed a significantly higher value of 1.4090%. Similar trends were observed for other bioactive compounds such as phenolics, flavonoids, anthocyanins, and vitamin C, where the actual values were consistently higher than the predicted ones. These differences may be attributed to raw material variability, more favorable experimental conditions, or limitations in the model's ability to account for all influencing factors. Nevertheless, the model remains useful as a guide for determining extraction conditions, although validation with real experimental data is essential to improve accuracy and process effectiveness.

4. Conclusion

This study successfully extracted bioactive compounds from mangosteen peel using NADES as a solvent combined with the efficient MAE method with minimal solvent use. UV-Vis results showed increased

Table 6. Response optimizer of Xanthone, Phenolic, Flavonoid Anthocyanin, and Vitamin C.

Comparison of Data	Power (Watt)	Time (min)	Xanthone (%)	Phenolic (%)	Flavonoid (%)	Anthocyanin (%)	Vitamin C (%)
Prediction	300	4.46	0.4356	23.4986	0.0685	0.3035	2.7146
Actual	300	4.46	1.4090	42.4303	1.2253	1.2025	2.9434

levels of xanthone (1.4090%), phenolic (42.4303%), flavonoid (1.2253%), anthocyanin (1.2025%), and vitamin C (2.9434%) under optimal conditions of 300 Watt for 4.46 min. All actual values were higher than the model predictions, indicating the influence of external factors on extraction efficiency. These findings confirm the effectiveness of the NADES-MAE method and the need to refine the predictive model.

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