Optimization of Microwave-Assisted Extraction of Polyphenolics from Rambutan (*Nephelium Lappaceum* L.) Peels using Response Surface Methodology and Central Composite Design

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ABSTRACT

A Response Surface Methodology (RSM) was employed to optimize the Microwave-Assisted Extraction (MAE) of polyphenolics from rambutan (*Nephelium Lappaceum L.*) peels, with three key factors, involving ethanol concentration (X_1), extraction time (X_2), and material-to-solvent ratio (X_3). These factors were initially examined using single-factor analysis, while a central composite design on three levels was subsequently used for optimization. The experimental results were fitted to a second-order polynomial model, and an Analysis Of Variance (ANOVA) was conducted to assess the significance of each factor on the extraction process. Considering the maximum extracted Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AA), the optimal conditions (X_1, X_2, X_3) for each response were (42%, 132 s, 0.002 g mL^{-1}), (46%, 133 s, 0.002 g mL^{-1}), and (40%, 121 s, 0.002 g mL^{-1}), respectively. Under the optimal conditions, the rambutan peel extract yielded 225.6 ± 6.6 mg-GAE/g (TPC), 179.7 ± 9.2 mg-RE/g (TFC), and 149.3 ± 2.1 μmol-TE/g (AA).

Keywords-polyphenolics; rambutan; microwave-assisted extraction; optimization; antioxidant activity

I. INTRODUCTION

Phenolic compounds, a diverse group of plant secondary metabolites, are widely recognized for their potent antioxidant properties [1]. These compounds play a critical role in neutralizing harmful free radicals and protecting cells from oxidative damage [2]. They have been extensively studied for their potential to act against multiple viruses and mitigate chronic diseases, like diabetes, cardiovascular disease, neurodegenerative disorders, and several types of cancer [3]. The polyphenolics family, including flavonoids, phenolic acids, tannins, lignans, and stilbenes, is well-known for its wide range of biological activities [4]. These compounds also have substantial antioxidative and anti-inflammatory effects, acting as anticancer and antibacterial agents [3]. Due to their properties, polyphenolics have found widespread applications in various industries, especially food, pharmaceuticals, nutraceuticals, and cosmetic products [5].

In order to fully exploit the potential of phenolic compounds derived from plant sources, the selection of effective extraction methodologies is of paramount importance. The efficiency of polyphenolic extraction can be significantly influenced by various factors, including the sources [6] and

characteristics of the plant material, the inherent properties of the target compounds [4], the chosen extraction technique, and the specific extraction conditions [7]. These interrelated factors exert a significant influence on the yield and quality of the extracted compounds [8], ultimately impacting their bioactivity and, consequently, their commercial value and potential applications. Researchers have developed various extraction techniques to maximize the recovery of polyphenolics from plant materials. These techniques range from traditional methods, like maceration and Soxhlet extraction [9], to more advanced approaches, such as Ultrasonic-Assisted Extraction (UAE), MAE, pressurized liquid extraction, supercritical fluid, pulsed electric field, and enzyme-assisted extraction [10]. Recent studies [9, 11] have shown that MAE is an effective green method for extracting bioactive compounds from plant materials, compared to other procedures, taking into account the environmental impacts and costs of energy, water, solvent consumption, waste release, operation time, and its performance under atmospheric conditions [12].

Rambutan (*Nephelium Lappaceum L.*), belonging to the *Sapindaceae* family, is a commercially significant tropical fruit cultivated throughout Southeast Asia, Australia, South America, and Africa [13]. This fruit is esteemed for its exotic appearance, pleasant flavor, and aroma of its translucent, whitish flesh, and it is consumed in fresh, canned, or processed forms [14]. The entire rambutan fruit (peel, pulp, and seed) is a rich source of nutrients, including carbohydrates, lipids, proteins, minerals, vitamins [15], and polyphenolic compounds possessing health-promoting properties and potential for chronic disease prevention [16]. The global rambutan production was estimated at approximately 1.4 million metric tons in 2015–2017 [17], reached 2.5 million tons in 2018, and is expected to grow by 3.6% by 2023 [18]. However, the inedible thick epicarp and seed, which correspond to 37–62% and 4–9% of the whole fruit, respectively, are typically discarded as a byproduct during consumption and processing, lacking commercial value [18]. Besides, this discarded material contributes to environmental and social problems [19]. Thus, the utilization of rambutan seed and peel as a valuable source of bioactive compounds, especially polyphenolics, has gained increasing attention in recent years.

Rambutan byproducts have great potential in agriculture, future industrial foods, pharmaceuticals, and cosmetic applications [20]. Compared to rambutan seed extracts, the rambutan peel contains a higher radical scavenging activity and phenolic content [21]. This has prompted significant research into the extraction of bioactive compounds from rambutan peels. Authors in [22] compared the conventional extraction technique with UAE to evaluate the TPC in both red and yellow rambutan peel in Taiwan using ethanol/water solutions for the extraction process. Authors in [23] explored the bioactive compounds present in red rambutan peel extracts in Panama by employing a range of extraction solvents, including acidic, alkaline, aqueous, ethanolic, and hydroethanolic, alongside both UAE and boiling extraction techniques. Authors in [18] focused on optimizing the recovery of anthocyanins from red rambutan peels in Brazil by two extraction methods, heat-assisted extraction, and UAE, using RSM.

As far as is known, there is a lack of information regarding the influence of MAE and optimization using RSM on recovering polyphenolics from rambutan peels in Vietnam. This study aims to evaluate the potential of MAE to enhance the extraction of polyphenolics from rambutan peels, leading to increased levels of TPC, TFC, and AA, and to optimize the recovery of polyphenolics from rambutan peels using RSM.

The efficiency of MAE depends on several factors, including solvent type, microwave power, irradiation time, temperature, material-to-solvent ratio, and plant matrix characteristics, such as moisture content and particle size [11, 24-25]. Authors in [9] indicated that the initial moisture content had a negligible impact on the final TPC and AA values, as MAE rapidly increased sample moisture. However, the particle size significantly influenced TPC and AA. Fine particles enhanced the solvent penetration into the inner bark layer, containing rich extractable compounds. Additionally, the reduced particle size facilitated microwave penetration, accelerating the solute diffusion to the surface and reducing the extraction time. To optimize the extraction of polyphenolics from rambutan peels, three factors were evaluated involving solvent concentration, irradiation time, and material-to-solvent ratio, while maintaining constant microwave power and temperature levels.

II. MATERIALS AND METHODS

A. Materials and Chemicals

All chemical reagents were used without further purification, including ethanol (99.5%, EtOH), Folin-Ciocalteu reagent, $AlCl_3$, gallic acid, rutin, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), as supplied from Sigma-Aldrich, Germany. Red rambutans (*Nephelium Lappaceum L.*) were collected from a local market (Ho Chi Minh City, Vietnam). After having been thoroughly washed with distilled water, the fresh rambutan peels were oven-dried at 60 °C to a constant weight, then pulverized to powder, and stored at 4 °C in a dark airtight bottle.

B. Microwave-Assisted Extraction Experiments

For MAE optimization, each experiment was conducted in triplicate, and the result was expressed as a mean value and a Standard Deviation (SD) to evaluate the effect of EtOH concentration (% v/v), irradiation time (s), and material-to-solvent ratio (g mL^{-1}) on TPC, TFC, and AA extraction efficiency.

The TPC of the extracts was determined by the Folin-Ciocalteu method and measured as Gallic Acid Equivalent (GAE) [26]. In brief, 1.0 mL of diluted extract in extraction solvent, was mixed with 5.0 mL of 10% Folin-Ciocalteu reagent; after 5 min, 4.0 mL of 7.5% sodium carbonate solution was added, and the samples were incubated in the dark at room temperature for 1 h. The absorbance of the reaction solution was measured by ultraviolet-visible spectrophotometry (Libra S22 UV-Vis Spectrophotometer, Biochrom) at 765 nm. The results were expressed as milligrams of GAE per gram of dried material (mg-GAE/g).

The TFC of the extracts was estimated using the aluminum chloride colorimetric method and measured as Rutin

Equivalent (RE) [25]. In brief, 1.0 mL of diluted extract in extraction solvent and 4.0 mL of distilled water were mixed with 0.3 mL of 5% sodium nitrate solution; 5 min later, 0.3 mL of 10% aluminum chloride solution was added, and the mixture was shaken well. After 6 min, 2.0 mL of 1.0 M sodium hydroxide was added, and the volume was made up to 10.0 mL with distilled water. The absorbance of the reaction solution was measured at 510 nm. The results were expressed as milligrams of RE per gram of dried material (mg-RE/g).

The AA of the extracts was assessed by the free radical scavenging ability using the color change of the 2,2-diphenyl-picrylhydrazyl (DPPH) radical solution [9]. In brief, 4.0 mL of 0.1 mM DPPH solution was mixed with 1.0 mL of diluted extract solution, and the mixture was incubated in the dark at room temperature for 30 min. The decrease in absorbance of the mixture was measured at a wavelength of 517 nm. The Trolox Equivalent (TE) was used as the standard solution and the results were expressed as micromole of TE per gram of dried material (μmol-TE/g).

C. Microwave-Assisted Extraction Optimization

RSM was applied to optimize the extraction conditions of polyphenolics using the MAE method. The statistical tool JMP (version 18.1.1) was used to study experimental design, data analysis, and model building [11]. A central composite design with three levels (lower, central, and higher) was utilized to determine the response pattern and to establish a model. To make the analysis, the factor levels were converted into dimensionless variables (coded values: -1, 0, and 1, respectively), as shown in Table I. These ranges were chosen based on preliminary experiments.

TABLE I. RANGE AND LEVELS OF INDEPENDENT VARIABLES FOR MAE OPTIMIZATION

Independent variables		**Uncoded values**		
Uncoded	**Coded**	**Lower (–1)**	**Central (0)**	**Higher (1)**
%EtOH (v/v)	X_1	30	40	50
Time (s)	X_2	90	120	150
Material-to-solvent (g mL^{-1})	X_3	0.001	0.002	0.003

The optimal extraction conditions were identified, and their significance was assessed using the p-value, which was generated by the software based on the regression coefficient that each term combination of the independent variables produced. For each response, the empirical polynomial model was provided by [11]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2 \quad (1)$$

where, Y is the predicted response (TPC, TFC, and AA); X_i and X_j are the independent variables; β_0 is the model intercept coefficient; β_i, β_{ij}, and β_{ii} are the regression coefficients of the linear, interaction effect, and quadratic terms, respectively, and k is the number of independent parameters (k =3).

Using ANOVA, the developed model's adequacy and the regression coefficient's statistical significance were evaluated. A p-value of less than 0.05 showed a significant expression. The relationship between the various independent factors and their corresponding effects on the response were investigated by examining the 3D RSM plots [11].

III. RESULTS AND DISCUSSION

A. Single-Factor Analysis Method

In the MAE of polyphenolics, ethanol, methanol, and water are applicable due to their capacity to absorb microwave radiation [24]. In this study, it was discovered that combining EtOH and water yielded better TPC extraction efficiency than those using methanol and water at the same solvent ratio [25]. Therefore, in this investigation, the EtOH/water combination was used to evaluate the extraction efficiency of bioactive compounds from rambutan peels.

The effect of EtOH concentration (20, 40, and 60%) on TPC, TFC, and AA extraction efficiency was examined using the irradiation time and the material-to-solvent ratio fixed at 90 s and 1:100 g mL^{-1}, respectively. The results, portrayed in Table II, depicted a similar tendency, indicating that adding EtOH to water significantly increased the recovery of polyphenolics and reached a peak at 40% (v/v), then the extraction efficiency gradually decreased when the EtOH ratio exceeded 60%. These results closely matched those in [27, 28], where the optimum concentrations were 42% and 50% EtOH concentrations, respectively. Indeed, the polarity of the solvent constitutes an important factor in MAE, as polyphenolics are polar compounds; they dissolve well in a solvent containing water and a low EtOH concentration. Furthermore, adding EtOH accelerates the mass transfer between liquid and solid, enhancing the permeability of plant tissue and aiding the disruption of the solute-plant matrix boundary [9]. However, a high EtOH content can denature proteins, which alters the extraction rate and prevents polyphenolics from dissolving [27]. Thus, the solvent containing 40% EtOH (v/v) was utilized for further studies.

Regarding the effect of irradiation time, higher extraction times generally result in more analytes being extracted; however, prolonged irradiation times also cause phenolic compounds to oxidize, which reduces the extraction yield [11]. This study investigated the recovery of polyphenolics at various extraction times (90, 120, and 150 s) with other fixed factors: 40% EtOH (v/v) and a 1:100 g mL^{-1} material-to-solvent ratio. According to Table II, TPC, TFC, and AA rose as MAE irradiation time increased at the beginning of extraction, reaching a maximum of 176.8 ± 6.3 mg-GAE/g, 65.7 ± 1.1 mg-RE/g, and 68.1 ± 0.6 μmol-TE/g, respectively, at 120 s before declining after 150 s. These results permitted the selection of irradiation time at 120 s.

Using the selected concentration of EtOH and irradiation time, the influence of the material-to-solvent ratio on the recovery of polyphenolics was investigated. Table II demonstrates that the extraction efficiency of TPC, TFC, and AA raised with the increase in the material-to-solvent ratio during extraction, and was maximized at a ratio of 1:500 g mL^{-1}. The equilibrium principle generally indicates that a larger solvent volume increases substance solubilization [11]. However, the increasing amount of solvent (1:1000 g mL^{-1}) can soak up more radiation microwaves, leaving insufficient

energy to break cell walls and release the desired phenolic compounds [29]. In contrast, using a low amount of solvent can cause saturation, restricting the capacity to dissolve more material. As a result, some of the polyphenolics are trapped inside the solid, making the extraction ineffective [25]. These results permitted the selection of a material-to-solvent ratio of 1:500 g mL^{-1}.

TABLE II. EFFECT OF SINGLE-FACTOR ON TPC, TPF, AND AA

Independent variables			Investigated responses [a]		
X_1	X_2	X_3	*TPC (mg-GAE/g)*	*TFC (mg-RE/g)*	*AA (μmol-TE/g)*
20	90	1:100	143.8 ± 5.2	43.6 ± 2.4	50.0 ± 0.5
40	90	1:100	168.3 ± 0.8	62.7 ± 1.7	67.2 ± 2.6
60	90	1:100	167.0 ± 2.0	55.7 ± 1.7	65.8 ± 1.7
40	120	1:100	176.8 ± 6.3	65.7 ± 1.1	68.1 ± 0.6
40	150	1:100	170.1 ± 2.8	61.4 ± 1.2	64.7 ± 1.6
40	120	1:200	166.0 ± 4.4	95.3 ± 2.3	116.6 ± 2.7
40	120	1:500	181.3 ± 12.6	141.2 ± 9.3	144.6 ± 4.4
40	120	1:1000	151.5 ± 12.4	92.9 ± 17.2	91.7 ± 3.9

[a] mean value ± SD (for three replicates)

B. *Response Surface Methodology Modeling and Optimization of Polyphenolics Extraction*

To achieve the maximum content of TPC, TFC, and AA extracted from rambutan peels, the RSM was utilized to examine the impact of all parameters combined: %EtOH (v/v), irradiation time (s), and material-to-solvent ratio (g mL^{-1}).

According to RSM results, illustrated in Figure 1, extracting polyphenolic compounds from rambutan peels proved to be a complex process. For TPC, the response Y_1 ranged from 121.0 to 233.9 mg-GAE/g across different experimental runs. Similarly, the TFC (Y_2) and AA (Y_3) ranges were 129.5 to 174.3 mg-RE/g and 89.8 to 144.7 μmol-TE/g, respectively. Second-order polynomial models were developed to better understand the influence of various factors on these responses. These models approximate the efficiency of the reactive extraction process and can be expressed in terms of the factors studied as:

$$Y_1 = -254.97 + 12.76X_1 + 2.05X_2 + 5.89 \times 10^4 X_3 + 0.02X_1X_2 + 6.62 \times 10^2 X_1X_3 + 90.04X_2X_3 - 0.20X_1^2 - 0.01X_2^2 - 2.22 \times 10^7 X_3^2 \quad (2)$$

$$Y_2 = 32.84 + 3.19X_1 + 0.76X_2 + 1.63 \times 10^4 X_3 + 0.003X_1X_2 + 1.77 \times 10^2 X_1X_3 + 84.29X_2X_3 - 0.04X_1^2 - 0.004X_2^2 - 8.02 \times 10^6 X_3^2 \quad (3)$$

$$Y_3 = -135.79 + 8.28X_1 + 0.83X_2 + 5.10 \times 10^4 X_3 + 0.01X_1X_2 - 231.50X_1X_3 + 48.08X_2X_3 - 0.11X_1^2 - 0.006X_2^2 - 1.02 \times 10^7 X_3^2 \quad (4)$$

Figures 1 (a,b,c) and (2) depict the interactive effects of the extraction conditions on TPC recovery from rambutan peels. The amount of TPC rose with the increasing EtOH concentration from 20% to approximately 40% and the material-to-solvent ratio from 0 to approximately 0.002 g mL^{-1}. After that, further increases in these parameters led to a decrease in TPC recovery, consistent with the findings of the single-factor experiments. As shown in Table III, the quadratic term of both EtOH concentration and material-to-solvent ratio significantly influenced ($p < 0.05$) TPC recovery. Similarly, the quadratic term of the material-to-solvent ratio significantly affected TFC recovery.

Figures 1 (d,e,f) and (3) indicate analogous trends for TFC extraction, suggesting that similar factors and interactions influence both TPC and TFC recovery. However, the quadratic effect of EtOH concentration was insignificant ($p > 0.05$) for TFC, likely due to the diverse nature of TPC compounds compared to the more specific TFC [3]. This implies that optimal extraction conditions for TFC can differ from those for TPC. Additionally, the results indicate that the extraction efficiency of both TPC and TFC from rambutan peels using MAE was not significantly affected by extraction times in a 60–180 s range. This finding emphasizes one of the primary advantages of MAE, which is the significantly reduced time required to achieve quantitative product yields [18].

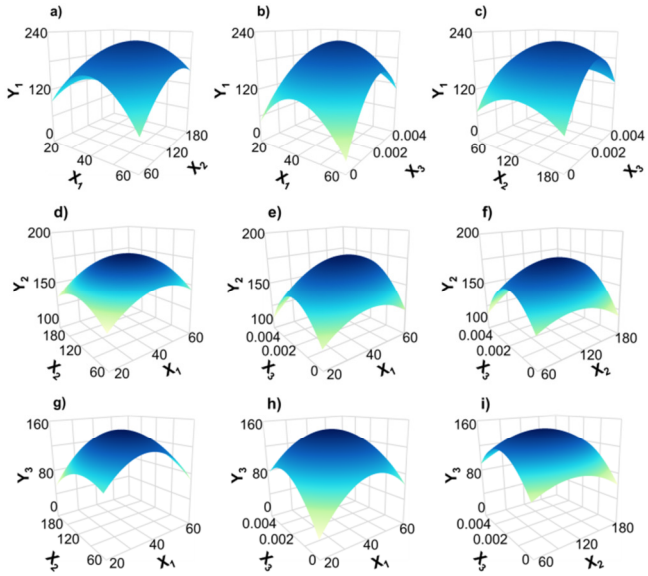

Fig. 1. RSM graphs illustrating the combined effects of the independent variables (X_1: %EtOH (v/v), X_2: irradiation time (s), and X_3: material-to-solvent ratio (g mL^{-1})) on TPC (Y_1), TFC (Y_2), and AA (Y_3).

Table IV reveals the optimal conditions for extracting phenolic compounds from rambutan peels. A material-to-solvent ratio of 0.0022 g mL^{-1} was found to be optimal. The maximum TPC was achieved at an EtOH concentration of 43.2% and an irradiation time of 132.5 s, resulting in a predicted maximum response of 222.2 mg-GAE/g. Similarly, the maximum TFC was obtained at an EtOH concentration of 46.8% and an irradiation time of 133.3 s, yielding a predicted maximum response of 176.4 mg-RE/g.

On the other hand, AA was significantly influenced by the linear term of the material-to-solvent ratio and the quadratic terms of EtOH concentration, irradiation time, and the material-to-solvent ratio ($p < 0.05$), as can be seen in Figures 1 (g,h,i) and Table IV. The optimal extraction conditions for maximizing AA were an EtOH concentration of 41.6% and an

irradiation time of 121.9 s. As shown in Table IV, the experimental results validate most of the predicted TPC, TFC, and AA values. These findings are consistent with previous single-factor surveys, but the higher polyphenolic yields highlight the effectiveness of the experimental method, as displayed in Table V. The MAE remains the preferred technique due to its efficiency, cost-effectiveness, environmental sustainability, and saving time [30]. Additionally, the extraction conditions should be refined to target specific bioactive compounds selectivity. Further comprehensive studies are essential to optimize the process and realize its potential for industrial applications.

TABLE III. CORRESPONDING P VALUES OF LINEAR, INTERACTION, AND QUADRATIC TERMS OF REGRESSION COEFFICIENTS, OBTAINED FOR SELECTED RESPONSE VARIABLES

Term	Response		
	TPC	***TFC***	***AA***
Linear			
X_1	0.3080	0.1108	0.0548
X_2	0.4294	0.4867	0.8335
X_3	0.3704	0.6350	0.0051
Interaction			
X_{12}	0.5164	0.8258	0.1287
X_{13}	0.5251	0.6455	0.3748
X_{23}	0.7937	0.5137	0.5755
Quadratic			
X_{11}	0.0237	0.1597	0.0001
X_{22}	0.1743	0.2191	0.0132
X_{33}	0.0141	0.0164	0.0003

TABLE IV. OPTIMAL CONDITIONS AND RESPONSES

Response	Optimal conditions [a]			Values [a]
	%EtOH	Time (s)	Material/solvent (g mL^{-1})	
TPC (mg-GAE/g)	42 (43.2)	132 (132.5)	0.002 (0.0022)	225.6 ± 6.6 (222.2)
TFC (mg-RE/g)	46 (46.8)	133 (133.3)	0.002 (0.0022)	179.7 ± 9.2 (176.4)
AA (µmol-TE/g)	40 (41.6)	121 (121.9)	0.002 (0.0023)	149.3 ± 2.1 (146.2)

[a] For each response variable, the predicted values are presented between brackets

TABLE V. COMPARATIVE STUDY ON RAMBUTAN PEELS

Methods	Solvents	Extraction conditions	TPC (mg-GAE/g)	References
Boiling method	HCl (10 g L^{-1})	m:s = 1:10 [a] 10 min	189	[23]
	NaOH (10 g L^{-1})		258	
	Aqueous		235	
	EtOH (96%)		233	
	Hydroethanol (600 g L^{-1})		315	
UAE	HCl (10 g L^{-1})	m:s = 1:10 20 min	231	[23]
	NaOH (10 g L^{-1})		262	
	Aqueous		280	
	EtOH (96%)		208	
	Hydroethanol (600 g L^{-1})		340	
MAE	EtOH (42%)	m:s = 0.002 132 s	225.6	This study

[a] the material-to-solvent ratio (g mL^{-1})

Based on the predicted values, Figure 2 indicates a linear relationship between AA and TPC with a strong correlation (R^2 = 0.86). This result reveals the antioxidant potential of the rambutan peel extract mainly derived from phenolics; therefore, the highest TPC permits obtaining a good AA for diverse applications.

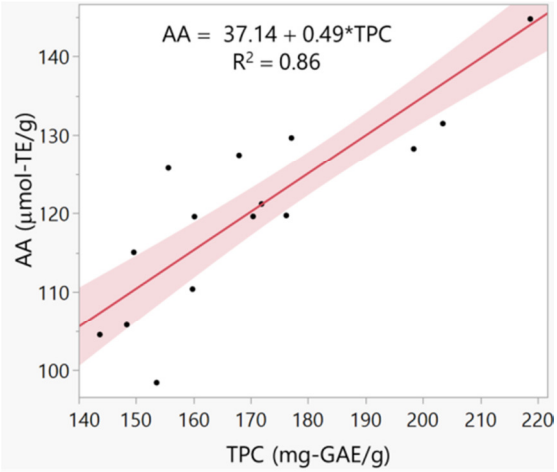

Fig. 2. Predicted relationship between TPC and AA in rambutan peels.

IV. CONCLUSION

Response Surface Methodology (RSM) was successfully applied to optimize the conditions for Microwave-Assisted Extraction (MAE) in terms of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AA) of rambutan peels. Under specific conditions, the developed model accurately predicted high levels of TPC, TFC, and AA, which were subsequently validated through experimental verification. Analysis Of Variance (ANOVA) shows that the quadratic material-to-solvent ratio significantly affected TPC, TFC, and AA. Concerning TPC, a quadratic negative effect of EtOH concentration and a linear positive effect were observed, indicating an optimal EtOH concentration for maximum TPC extraction. On the other hand, the extraction capacity of AA was influenced by different factors, including the linear material-to-solvent ratio, the quadratic EtOH concentration, and extraction time.

As an environmentally friendly process to produce phenolic-rich extracts from rambutan peels, the MAE parameters should be adjusted to target certain bioactive compounds, unlocking its possibilities for industrial usage.

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