OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM MALE FLOWERS (CARICA PAPAYA L.) USING RESPONSE SURFACE METHODOLOGY

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Abstract

The study was conducted with the aim of optimizing the extraction conditions for alkaloids and bioactive compounds from male papaya flowers (*Carica papaya L.*) using microwave-assisted extraction, to produce male papaya flower honey extract. The microwave power (119W – 700W) and time (60 – 120 seconds) were examined by using the experimental design employed the Response Surface Methodology (RSM) with a Central Composite Face-centered (CCF) model. The results demonstrated that the optimal extraction conditions were achieved at a microwave power of 385W and a microwave time of 80 seconds. In these conditions, alkaloid content, polyphenol content, flavonoid content, and antioxidant activity reached 0.033 ± 0.001 mg BER-H/g, 9.15 ± 0.14 mg GAE/g, 3.39 ± 0.13 mg rutin/g, 12.93 ± 0.17 mg vitamin C/g, respectively.

Keywords: optimization, RSM, papaya, male flower, bioactive compound

1. Introduction

Carica papaya L., commonly known as papaya, is a tropical plant widely recognized for its nutritional and medicinal properties. There have been many studies about the fruit, however the male flowers also drew attention for their rich phytochemical content, including alkaloids, flavonoids, and phenolic compounds. These bioactive compounds contribute to the antioxidant and antimicrobial activities, making them valuable for functional food and pharmaceutical applications (Dwivedi et al., 2020). The extraction process of these compounds is crucial for their utilization. Traditional extraction methods often took long processing times, which may lead to the degradation of sensitive compounds. The microwave-assisted extraction (MAE) has emerged as a rapid and efficient technique that utilizes microwave energy to heat solvents and plant matrices, enhancing mass transfer and reducing extraction time (Jokić, S., et al. 2012; Karami, Z., et al. 2015; Routray & Orsat, 2012). Optimization of extraction parameters such as microwave power and extraction time is essential to maximize yield and keep the activity of bioactive compounds.

Recent studies have demonstrated the efficacy of MAE in extracting bioactive compounds from various plant materials. For instance, Chew et al. (2022) optimized the extraction of rutin from male *C. papaya* leaves using MAE, achieving significant yields under specific conditions. Similarly, Le Thao My et al. (2020) employed ultrasound-assisted extraction (UAE) to optimize flavonoid extraction from Vietnamese male *C. papaya* flowers, highlighting the potential of the technique in maximizing the yield bioactive compound.

The aim of this study was to evaluate the effect of microwave-assisted extraction on the yield and the activity of bioactive compounds of papaya male flowers.

2. Materials and Methods

2.1 Materials

Male *Carica papaya* L. flowers used in this study were free from insect infestation. The flowers were dried at 55 - 60°C to a moisture content of less than 10%, subsequently ground into a fine powder, and stored at 4 – 6°C prior to use in the research.

2.2 Experimental design

An optimization experiment using a central composite face design (CCF) with 11 runs was conducted to optimize extraction factors, including microwave power (X_1) (from 119W to 700W) and time (X_1) (from 60 to120 seconds). Alkaloid content (mg BER-H /g), flavonoid content (mg rutin /g), polyphenol content (mg GAE /g), and antioxidant activity (mg vitamin C/g) were measured to evaluate the optimization process. The optimization experiment is presented in Table 2.1.

Table 2.1: Optimization treatments

Runs	Variables	
	X_1 (%)	X_2 (seconds)
1	231	60
2	539	60
3	231	100
4	539	100
5	231	80
6	539	80
7	385	60
8	385	100
9	385	80
10	385	80
11	385	80

2.3 Analytical methods

2.3.1 Alkaloid Content (TAC)

Alkaloid content was determined according to the method of Patel et al. (2015). Eight mL of the extract was pipetted into a separatory funnel, and 5 mL of Bromocresol Green (BCG) solution and 5 mL of phosphate buffer solution (pH 4.7) were added sequentially. The mixture was then vigorously shaken in the separatory funnel with 1, 2, 3, and 4 mL of chloroform. The chloroform fractions were collected and adjusted to a final volume of 10 mL with chloroform. The absorbance

of the solution was measured at a wavelength of 470 nm. A blank sample was prepared in the same manner, but without the addition of the extract. Total alkaloid content was expressed as milligrams of Berberine Chloride equivalent per gram of dry weight (mg/g dry weight, as BER-H).

2.3.2 Total Flavonoid Content (TFC)

Total flavonoid content was determined according to Shi et al. (2012). A 10 mL of the supernatant was transferred to a 25 mL volumetric flask; 2 mL of 5% $NaNO_2$ was added; after 6 minutes, 2 mL of 10% $AlCl_3$ was added; after 12 minutes, 6 mL of 1M NaOH was added, and the solution was made up to 25 mL using 70% ethanol solution. After 27 minutes, the solution was used to measure absorbance at a wavelength of 510 nm using a spectrophotometer. Rutin was used as the standard for a calibration curve. Total flavonoid content is expressed as milligrams of rutin equivalent per gram of dry weight (mg rutin/g).

2.3.3 Total phenolic content (TPC)

Polyphenolic content was determined by the Folin-Ciocalteau method based on the procedure of TCVN 9745-1:2013. 0.5 mL of the sample solution was pipetted into a test tube, followed by the addition of 2.5 mL of 10% Folin Ciocalteu reagent. The mixture was shaken well for 5 minutes. Subsequently, 2 mL of 7.5% Na_2CO_3 solution was added. The mixture was shaken well, kept in the dark for 60 minutes at room temperature, and then the absorbance (OD was determined at a wavelength of λ=765 nm. The OD values were recorded and a calibration curve was plotted to determine the polyphenol content in the extract samples. The extract samples were processed similarly to the standard polyphenol samples.

2.3.4 Antioxidant capacity (AC)

The relative DPPH radical scavenging capacity of the extract was determined by a colorimetric method using the 2,2'-diphenyl-picrylhydrazyl (DPPH) reagent (Rumpf et al., 2023). A standard vitamin C solution was used as the reference standard. To 2 mL of the sample, 2 mL of 100 ppm DPPH reagent solution was added, mixed well, and incubated in the dark for 30 minutes. The optical density was measured at a wavelength of 517 nm. The results were calculated in units of mg vitamin C (mg/g, dry matter).

2.4 Statistical analysis

Results were statistically analyzed, calculated, and plotted using Microsoft Excel. Optimization was performed using Moddel 5.0 software.

3. Results and discussion

Table 3.1: Results of optimization treatments affecting the extraction process

Runs	X_1 (%)	X_2 (seconds)	TAC (mg BER – H/g)	TPC (mg GAE/g)	TFC (mg rutin/g)	AC (mg vitamin C/g)
1	231	60	0.021	8.310	2.419	10.860

2	539	60	0.023	8.496	2.999	11.161
3	231	100	0.023	8.522	2.567	11.630
4	539	100	0.026	8.675	3.118	11.635
5	231	80	0.026	8.702	2.744	11.680
6	539	80	0.028	8.871	3.310	11.992
7	385	60	0.027	8.764	3.041	11.992
8	385	100	0.029	8.960	3.175	12.475
9	385	80	0.032	9.070	3.314	12.825
10	385	80	0.034	9.315	3.544	12.829
11	385	80	0.034	9.076	3.318	13.129

Regression analyses were performed, and polynomial equations were obtained. Ignoring non-significant factors, the second-order polynomial model for each studied factor is described as follows:

$$TAC = 0{,}0326657 + 0{,}872135 \times 10^{-3}X_1 - 3{,}11109 \times 10^{-3}X_1^2 - 2{,}62118 \times 10^{-3}\,X_2^2 + 0{,}872135 \times 10^{-3}X_2 - 0{,}0577485X_1X_2 \quad (1)$$

$$TPC = 9{,}15824 + 0{,}0640894X_1 - 0{,}21604X_1^2 - 0{,}181954X_2^2 + 0{,}0584124X_2 - 0{,}00399134X_1X_2 \quad (2)$$

$$TFC = 3{,}38886 + 0{,}21813X_1 - 0{,}203994X_1^2 - 0{,}168891X_2^2 - 0{,}0414788X_2 - 0{,}00292752X_1X_2 \quad (3)$$

$$AC = 12{,}9187 + 0{,}0744055X_1 - 0{,}536493X_1^2 - \ 0{,}453417X_2^2 + \ 0{,}152409X_2 - 0{,}010364X_1X_2 \quad (4)$$

Based on the ANOVA results, the p-values for both the first-order and second-order regression coefficients were > 0.05, indicating that the regression equation is statistically significant. Microwave power (X_1) and microwave time (X_2) have a substantial impact on the bioactive compounds in this study, particularly alkaloids. The analysis reveals that the alkaloid content is influenced by both first-order (X_1, X_2) and second-order (X_1^2, X_2^2) factors. The coefficient of determination (R^2), a measure of the model's goodness of fit for the objective functions, is close to 1 (0.978 for alkaloid content, 0.952 for polyphenol content, 0.969 for flavonoid content, and 0.956 for antioxidant activity). The adjusted R^2 values are within an acceptable range, all greater than 0.8, demonstrating good agreement and fit between the experimental data and the derived second-order polynomial equations. Furthermore, the adequacy of the model was confirmed by the Lack-of-fit test. A Lack-of-fit p-value greater than 0.05 indicates that the selected model is suitable for describing the observed data. Using the optimized protocols, the Modde 5.0 software optimization algorithm yielded the following results: the optimal conditions for efficient extraction of bioactive compounds from male papaya flowers are a microwave power of 454.58 W and a microwave time of 81.74 seconds. Under these optimal parameters, the alkaloid content is 0.032 mg/g GAE (dry weight), the polyphenol content is 9.14 mg/g GAE (dry weight), the flavonoid content is 3.45 mg/g

rutin (dry weight), the antioxidant activity is 12.82 mg/g vitamin C (dry weight), and the extraction yield is 73.16%.

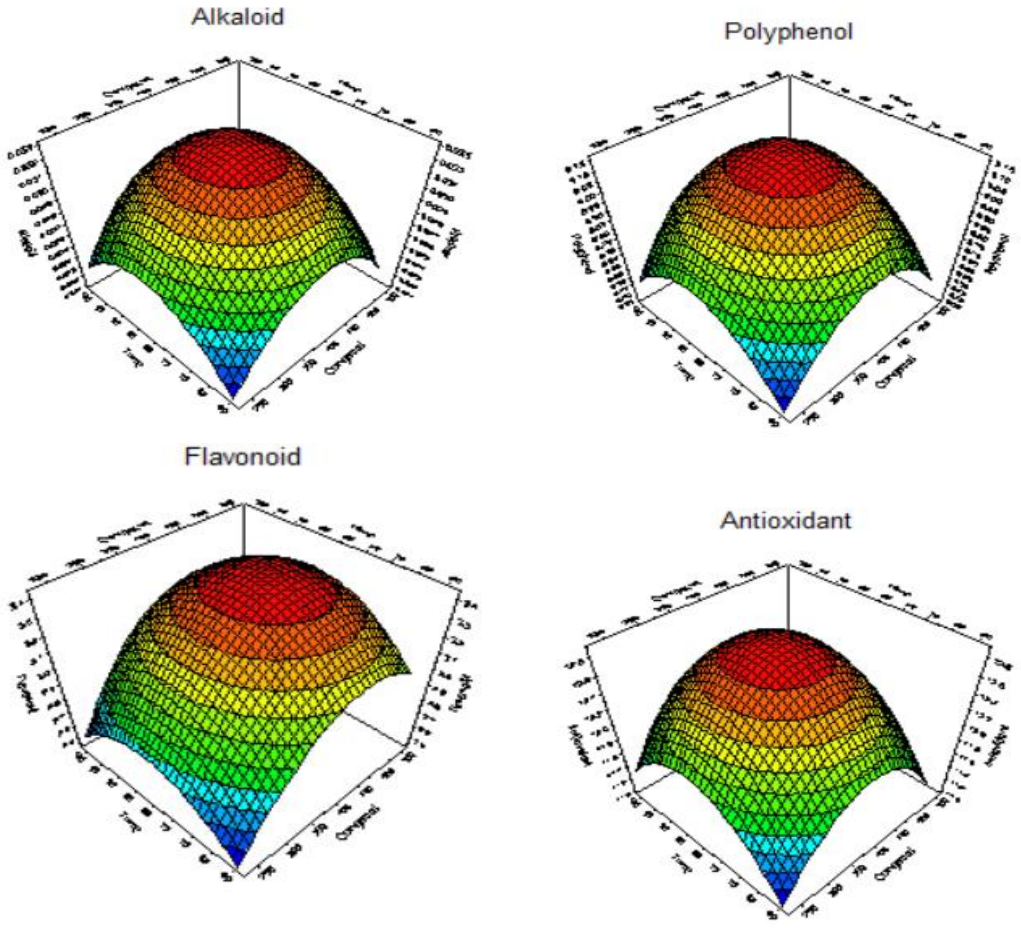

Figure 3.1: Response surface model illustrating the effect of microwave power and microwave time on alkaloid content, polyphenol content, flavonoid content, antioxidant activity

To validate the applicability of the optimized parameters, three validation experiments were conducted. Under practical conditions, the optimized parameters were adjusted as follows: microwave power 385 W and microwave time 80 seconds. The results obtained are shown in Table 3.2.

Table 3.2: Optimal parameters adjusted

Parameters	Content
TAC (mg BER -H /g)	0.033 ± 0.001
TPC (mg GAE/g)	9.15 ± 0.14
TFC (mg rutin/g)	3.39 ± 0.13
AC (mg Vitamin C/g)	12.93 ± 0.17

4. Conclusion

The extraction process of male papaya flowers (*Carica papaya* L.) using the microwave-assisted extraction method described in this study. The highest extraction efficiency was obtained at a microwave power of 385W and a microwave time of 80 seconds. The alkaloid content reached 0.033 ± 0.001 mg/g, the total polyphenol content reached 9.15 ± 0.14 mg/g, the flavonoid content reached 3.39 ± 0.13 mg/g, and the antioxidant activity reached 12.93 ± 0.17 mg/. These results provide a basis not only for further research but also for potential industrial applications to maximize the biological activities of male papaya flowers.

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