

Combination of Plackett-Burman and Box–Behnken designs in optimization for integrated enzyme- and microwave-assisted co-extraction of polysaccharides and polyphenols from mangosteen peels

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Abstract

Mangosteen peels, a waste constituting more than 50% of the fruit weight, contain high amounts of functional compounds such as polysaccharides and polyphenols. Their recovery could provide added value for the plant. Hence, this study investigated the optimization of an integrated enzyme- and microwave-assisted process for the simultaneous extraction of these two components from mangosteen peels (*Garcinia mangostana* Linn). The study employed a two-step optimization approach, where significant factors were first identified using Plackett–Burman design (PBD) with five factors, and the optimal levels of the significant factors were then determined using Box–Behnken design (BBD). The results found that three factors had significant effects, and their optimal conditions included a pH of 5.8, a buffer-to-solid ratio of 71 mL/g, and a microwave time of 80 s. Under these optimized conditions, the yields of polysaccharides and polyphenols were 30.95% and 85.96 mg gallic acid equivalent (GAE)/g, respectively. The results demonstrated that the integrated microwave- and enzyme-assisted extraction method is an efficient and environmentally friendly method for the extraction of polysaccharides and polyphenols from mangosteen peels. Moreover, the combination of PBD and BBD formed a powerful optimization tool that led to time-efficient and effective process optimization.

1. Introduction

Mangosteen (*Garcinia mangostana* Linn) belongs to the family of Guttiferae, is widely grown in countries with tropical climates (Pedraza-Chaverri *et al.*, 2008). During the ripening process, the color of the rinds begins to change from green and purple to a deep purple-red. The outer rind of the fruit is thick and hard; the inner rind is spongy and has a red wine color. The outer and inner skins, which account for about 60% of the fruit weight, are of great interest because they contain numerous compounds with high applications in many industries such as textiles, cosmetics, medicine and food (Ovalle-Magallanes, Eugenio-Pérez and Pedraza-Chaverri, 2017).

Numerous bioactive substances present in mangosteen peels, such as phenolic acids, tannins, xanthenes, and anthocyanins, possess the potential to serve as therapeutic agents or additives in functional foods (Suttirak and Manurakchinakorn, 2014). These compounds positively exhibit antioxidant, antibacterial,

and anti-inflammatory activities. In addition, mangosteen peels were reported to be one of the richest sources of polysaccharides (Wathoni *et al.*, 2019). Anderson *et al.* (2009) and Li and Komarek (2017) mentioned that polysaccharides extracted from mangosteen peels have the potential to lower the likelihood of specific cancers, safeguard against heart diseases, strokes, hypertension, diabetes, elevated blood cholesterol levels, obesity, certain gastrointestinal ailments, enhance gut microflora, and shorten food transit time through the digestive system. Also, they were claimed to have good antioxidant activity (Liu *et al.*, 2021).

Extraction is the most crucial step for the isolation and identification of polysaccharides and phenolic compounds. Organic solvents such as acids or methanol are used mostly in the studies of separating polysaccharides and polyphenols from mangosteen peels, respectively (Gan and Latiff, 2011; Cheok *et al.*, 2012). However, the large amount of solvent used and the multiple extraction times are the major disadvantages of

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this extraction method. Besides, reducing the evaporation of organic solvents and paying attention to the protection of personal health must always be paid attention to. Therefore, microwave- and enzyme-assisted extraction method has more potential to solve the mentioned problems because of their efficiency, being less expensive and more environmentally friendly than the organic solvent extraction method. Nadar *et al.* (2018) demonstrated that the combination of microwaves with enzymes is more versatile for the recovery of bioactive compounds from plant and their by-products because this integrated strategy has the potential to achieve higher yields of compounds from raw materials in shorter extraction times. Therefore, this project was performed by applying the integrated microwave-assisted extraction (MAE) and enzyme-assisted extraction (EAE) on the extraction of phenolic compounds and polysaccharides from mangosteen peels.

In order to extract phenolic compounds and polysaccharides by the integrated microwave- and enzyme treatment, pH, solid-to-liquid ratio, enzyme concentration, microwave time, and microwave power are important parameters for extraction yields. Plackett-Burman design (PBD) was applied to screen the factors that would significantly influence the extraction yields. PBD was claimed to reduce the number of runs, thereby economically detecting large main effects on response (McGrath *et al.*, 2023). In order to determine the optimal extraction conditions with a limited number of experiments, response surface methodology (RSM) was employed to optimize the extraction process. RSM entails employing mathematical and statistical methods to establish a suitable functional connection between a desired outcome and various independent variables (Lee *et al.*, 2013). Among many types of RSM designs, the Box Behnken design (BBD) has been widely used due to its simple structure and high efficiency (Tu *et al.*, 2005). The quadratic polynomial equation derived from BBD illustrates how test variables influence responses, identifies the correlations between test variables, and encapsulates the combined impact of all test variables on any given response. Thus, BBD is a suitable method that allows researchers to reach an appropriate result in an efficient way.

To the best of our knowledge, there is limited research on the co-extraction of polysaccharides and polyphenols from mangosteen peels. The objective of this study, therefore, was to optimize the conditions of an integrated microwave- and enzyme-assisted extraction of these two groups of compounds. Plackett-Burman design (PBD) was first employed to screen the most significant factors. Response surface methodology (RSM) in combination with Box-Behnken design (BBD) was

subsequently applied to obtain the optimal conditions for the extraction.

2. Materials and methods

2.1 Materials and chemicals

Dried mangosteen peels were purchased from a traditional medicine pharmacy in Ho Chi Minh City, Vietnam. The moisture content was less than 10%. The dried peels were ground into powder and sieved through a 500 μm sieve. The uniform powder was collected, sealed into small bags (20 g each bag) and stored in a desiccator for further use. The commercial enzyme Cellulase Onozuka R-10 (1 U/mg) was purchased from Merck. Other chemicals, including Folin-Ciocalteu's reagent, sodium acetate buffer, sodium carbonate, ethanol and acetic acid were of the analytical grade.

2.2 Sequential enzyme- and microwave-assisted extraction

The enzyme-assisted extraction of polysaccharides and polyphenols was performed as follows. An Erlenmeyer flask containing 2 g of mangosteen peel powder was filled with 20-50 mL sodium acetate buffer at pH 4-6, containing an enzyme amount of 0.4-1.2 U/g peel. It was then capped and placed in an incubator (KS 4000 ICC, IKA), shaken at 50°C, 135 rpm for 120 mins. After that, the extraction was further processed by using a microwave (Sharp R-21A1(S)VN) at 80-560 W for 30-90 s. After extraction, the mixture was cooled to room temperature and centrifuged at 4°C, 4000 rpm for 15 mins. Then, the supernatant was used to precipitate with 96% ethanol. The precipitated polysaccharides and the remaining phenolic solution were collected for later measurement.

2.3 Experimental design

The Plackett-Burman design (PBD) was employed to screen five factors of the extraction process as listed in Table 1. Each factor has two coded levels of -1 and +1. The factors with confidence levels of more than 95% ($p < 0.05$) were considered to have a significant effect on responses and were used for further optimization using

Table 1. Levels of the factors tested in the Plackett-Burman design.

Factors	Symbol	Units	Experimental value	
			Low (-1)	High (+1)
pH	A		4	6
Buffer-to-solid ratio	B	mL/g	20	50
Enzyme concentration	C	U/g	0.4	1.2
Microwave time	D	s	30	90
Microwave power	E	W	80	560

Box-Behnken design (BBD).

2.4 Analytical methods

2.4.1 Determination of polysaccharide yield

After precipitating polysaccharides by using ethanol 96%, the precipitate was collected by filtering the mixture using a filter paper and then dried in an oven at 60°C until a constant weight. The extraction yield of polysaccharides was determined as the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of crude polysaccharide (g)}}{\text{Weight of mangosteen peel powder (g)}} \times 100 \quad (1)$$

2.4.2 Determination of total phenolic content

The extraction yield of phenolics was evaluated through the total phenolic content (TPC), which was determined by Folin-Ciocalteu's assay (Le *et al.* 2021). In detail, the mixture was prepared by mixing 1mL of each sample supernatant, 2 mL of 10% (v/v) Folin-Ciocalteu's reagent and 1.5 mL of 20% sodium carbonate solution. The mixture was vortexed thoroughly and incubated at room temperature for 1 hour in the dark. Then, the absorbance of each mixture was read at 765 nm by using a spectrophotometer. The TPC was expressed as mg of gallic acid equivalents per gram of peel powder (mg GAE/g).

2.5 Statistical analysis

The experiments were conducted three times for each condition. Mean values and standard deviations were calculated for every treatment. An analysis of variance (ANOVA) was performed to identify any statistically significant differences ($p < 0.05$). Minitab and Expert Design software were employed for PBD and BBD.

3. Results and discussion

3.1 Screening of significant factors using Plackett–Burman design

PBD serves as a cost-effective and efficient screening method as it allows for the disregard of

interactions while still enabling the calculation of main effects with fewer experiments. This approach lays the groundwork for subsequent optimization efforts (Das and Dewanjee, 2018). Twelve runs with five factors at two levels in PBD are presented in Table 2. PS yield varied from 6.26 to 28.85% while the range of TPC was 36.44–57.36 mg GAE/g.

Table 2. Plackett–Burman design of factors (in coded levels) with PS yield and TPC as the responses.

No.	Coded variables					Responses	
	A	B	C	D	E	PS yield (%)	TPC (mg GAE/g)
1	1	-1	1	-1	-1	11.38±0.50	37.10±2.15
2	1	1	-1	1	-1	25.37±0.87	48.97±2.79
3	-1	1	1	-1	1	7.32±0.46	57.36±1.93
4	1	-1	1	1	-1	14.51±0.87	36.44±1.99
5	1	1	-1	1	1	28.85±1.20	51.97±1.89
6	1	1	1	-1	1	17.83±0.45	47.59±1.69
7	-1	1	1	1	-1	14.94±0.74	55.51±1.41
8	-1	-1	1	1	1	7.79±0.35	49.57±4.13
9	-1	-1	-1	1	1	6.26±0.28	46.81±1.55
10	1	-1	-1	-1	1	10.39±0.60	41.18±1.27
11	-1	1	-1	-1	-1	8.06±0.47	55.99±3.23
12	-1	-1	-1	-1	-1	6.95±0.09	47.59±1.90

The Pareto chart, a tool to identify the most significant factors (Modi and Bharadia, 2023) that contributed to the PS yield and TPC, is illustrated in Figure 1. The top 20% of the factors on the chart are typically responsible for 80% of the variation in the responses, and this cut-off point was indicated by a red vertical line, which was around 2.45 of the standardized effect. Two of the five factors, namely buffer-to-solid ratio (B) and pH (A), with their magnitudes of the effects, represented by the bars, exceeded the line for both PS yield and TPC, so they were considered to have significant contributions. In addition, factor D, which was the microwave time, had a significant effect on PS yield, but not on TPC. To optimize the condition for co-extraction of PS and polyphenols, therefore, three significant factors of pH, buffer-to-solid ratio and

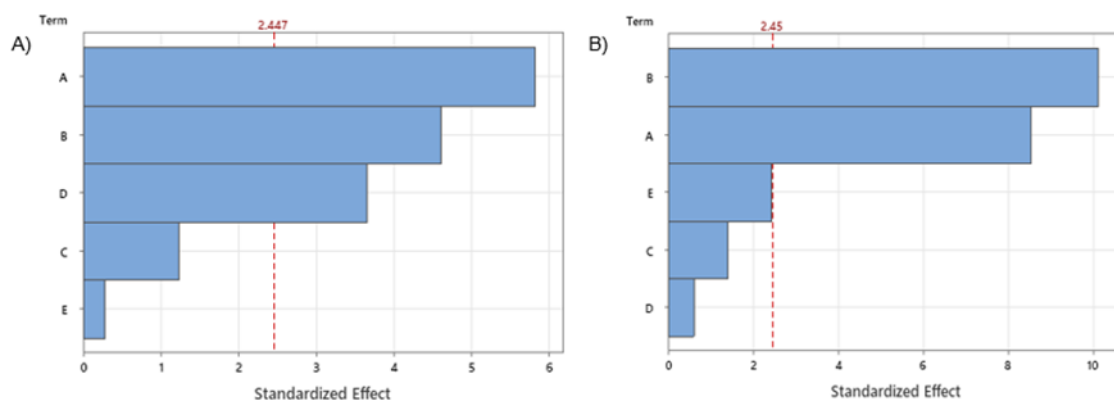


Figure 1. Pareto chart of standardized effects for A) PS yield and B) TPC.

microwave time would be selected for further investigation with BBD.

3.1 Optimization using Box-Behnken design

Table 3 presents the run matrix of Box–Behnken design for the optimization, where X₁, X₂ and X₃ are abbreviated for pH (4-7), buffer-to-solid ratio (60-80 mL/g) and microwave time (50-110 s), respectively. The range of each factor was selected based on preliminary screening. The results indicate that the PS yield varied from 15.3 to 30.3% while the TPC ranged from 57.3 to 88.5 mg GAE/g.

Table 3. Box–Behnken design of factors with the PS yield and TPC as the responses.

No.	X ₁	X ₂	X ₃	PS yield (%)	TPC (mg GAE/g)
1	4	60	80	15.30±0.43	69.20±3.08
2	7	60	80	25.86±1.93	57.29±3.67
3	4	80	80	16.03±0.60	81.16±3.31
4	7	80	80	25.61±1.65	69.60±1.43
5	4	70	50	18.87±0.82	77.26±4.11
6	7	70	50	29.79±1.27	69.09±4.05
7	4	70	110	20.06±0.94	74.86±5.45
8	7	70	110	26.22±1.48	66.48±3.43
9	5.5	60	50	23.27±0.89	64.61±1.85
10	5.5	80	50	16.17±0.87	73.35±3.17
11	5.5	60	110	19.05±0.55	63.28±1.52
12	5.5	80	110	22.22±0.79	73.09±2.94
13	5.5	70	80	28.93±1.73	88.54±2.73
14	5.5	70	80	30.34±0.30	87.62±2.27
15	5.5	70	80	29.33±1.74	88.00±4.13

Table 4 presents the ANOVA analysis to evaluate the adequacy of the models. The regression models were highly significant ($p < 0.0005$) with the insignificant lack of fit values ($p > 0.05$) indicating the adequacy of pure error. The values of the coefficient of determination (R^2) were found to be larger than 0.98, indicating that more than 98% of the variability could be explained by the selected models. Moreover, the high adjusted R^2 values of larger than 0.95, an acceptable predicted R^2 of approximately 0.8 and desirable Adequate Precision values of larger than 4 could imply adequate signals for the models. Table 4 also indicates that all factors had significant effects on both responses at their quadratic levels (X_2). Meanwhile, at the linear level, only X_1 significantly influenced the PS yield, while X_1 and X_2 had significant impacts on TPC. Among the interactions between two factors, the interactions of X_2X_3 and X_1X_3 on the PS yield were significant at $p < 0.05$ and $p < 0.1$, respectively. Second-order polynomial models describing the effects of factors (with the coded levels) on PS yield and TPC follow Eq. (2) and Eq. (3).

Table 4. ANOVA analysis for the models of PS yield and TPC.

Source	DF	PS yield		TPC	
		F-Value	P-Value	F-Value	P-Value
Model	9	31.52	0.0007	57.92	0.0002
X ₁	1	125.97	< 0.0001	83.66	0.0003
X ₂	1	1.08	0.3458	95.78	0.0002
X ₃	1	0.0275	0.8748	2.28	0.1918
X ₁ X ₂	1	0.1747	0.6933	0.0128	0.9143
X ₁ X ₃	1	4.12	0.0981	0.0046	0.9485
X ₂ X ₃	1	19.18	0.0072	0.1196	0.7435
X ₁ ²	1	18.69	0.0075	91.49	0.0002
X ₂ ²	1	103.1	0.0002	188.07	< 0.0001
X ₃ ²	1	26.83	0.0035	109.66	0.0001
Error	5				
Lack of Fit	3	3.67	0.2214	17.99	0.0531
Pure Error	2				
Total	14				
R ²			0.9827		0.9905
Adjusted R ²			0.9515		0.9734
Predicted R ²			0.7912		0.8533
Adeq. Precision			14.4		23.11
C.V. %			5.07		2.1

$$PS \text{ yield } (\%) = 29.53 + 4.65X_1 - 1.19X_1X_3 + 2.57X_2X_3 - 2.64X_1^2 - 6.20X_2^2 - 3.16X_3^2 \quad (2)$$

$$TPC \text{ (mg GAE/g)} = 88.05 - 5.0X_1 + 5.35X_2 - 7.7X_1^2 - 11.04X_2^2 - 8.43X_3^2 \quad (3)$$

Using surface response plots of the polynomial models, the relationships between the process conditions and the responses (PS yield and TPC) could be better understood by holding one variable constant at its central level while studying the relationship between the other two variables in the experimental range under investigation. The response surface plots are illustrated in Figure 2. It was noticed that the mutual impact of any two factors was directly proportional to the responses until reaching the best values, but it became inversely proportional to the responses after passing these optimal points.

By using the desirability ramp, we could quickly identify the combination of conditions that would produce the most desirable outcome. This was especially useful as there were multiple response variables (PS yield and TPC) that needed to be optimized simultaneously in this study. This was because the desirability ramp could balance the trade-offs between different variables and find the settings that produced the best overall outcome (Rao *et al.*, 2022). With the desirability of 0.975, the optimal condition included a pH of 5.76, buffer-to-solid ratio of 70.90 mL/g and a microwave time of 79.12 s. Experiments under the

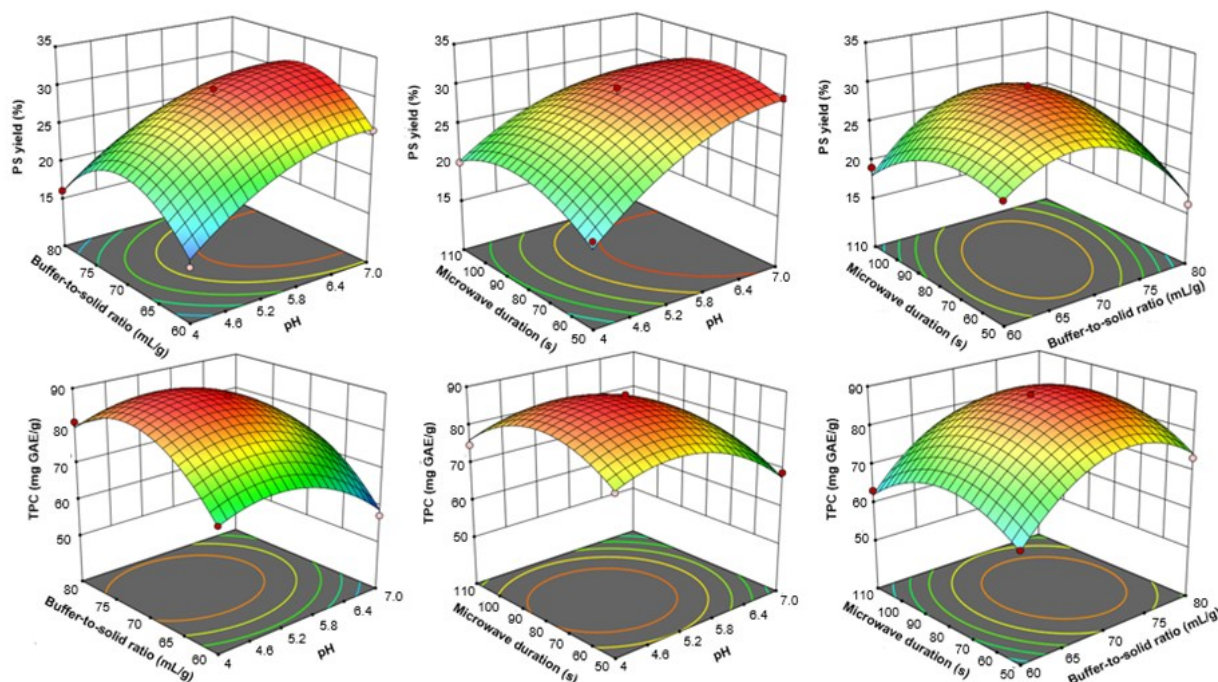


Figure 2. Response surface plots indicating the effects of the process variables on the PS yield (upper row) and TPC (lower row).

optimal conditions were conducted to validate the compatibility of the models. The experimental data were 30.95% for PS yield and 85.96 mg GAE/g for TPC, which were in good agreement with the predicted values. These results confirmed the adequacy and significance of the models.

4. Conclusion

The primary goal of this study was to determine important factors influencing the PS yield and TPC from mangosteen peels under the co-extraction with the aid of enzyme and microwave, and to achieve the maximum values of these responses. Among the five factors of the study, the Plackett-Burman design identified pH, buffer-to-solid ratio and microwave time as the significant factors. Moreover, by using a three-variable, three-level Box–Behnken design based on the RSM, the optimal extraction conditions which were determined to obtain the highest PS yield (30.95%) and TPC (85.96 mg GAE/g) were as follows: 5.8, 71 mL/g, and 80 seconds for pH, buffer-to-solid ratio, and microwave time, respectively. Additionally, it was observed that the experimental values of responses were closely related to the predicted values, which was a good indication that the models were a good fit for the data and were able to make accurate predictions.

Conflict of interest

The authors declare no conflict of interest.

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