

Optimisation of Microwave-assisted Pectin Extraction from Cocoa Pod Husk using Response Surface Methodology

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ABSTRACT

Managing the proper disposal of cocoa pod waste after beans are extracted is a very challenging process. Effective waste management requires a well-planned strategy to protect the environment from pollution, minimise disposal costs, and reduce hazardous waste. Furthermore, this study could help in turning agricultural waste into valuable products. Thus, this research aimed to extract pectin from cocoa pod fruits. The method of using microwave-assisted extraction (MAE) technique and citric acid as selected solvent extraction, as demonstrated by response surface methodology (RSM) at pH 1, 600 watts of microwave power at 5 minutes irradiation time, gave the highest yield (14.97% wt/wt). This MAE method shows great evidence in providing a high yield compared to the traditional methods. The results of DE of cocoa pectin range from 43.67-45.84%, indicating

low methoxyl pectin. Low methoxyl pectin is below 50% of DE. Pectin yield (14.1% wt/wt) was achieved at optimised pH 1 conditions at 5 minutes of irradiation time at 500 watts of microwave power. The obtained results of FTIR have confirmed that the extracted polysaccharide of this current study was found to be pectin.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an essential industrial plant that has played a significant role in the economies of numerous countries across the globe. The Ivory Coast, Ghana, Indonesia, Brazil, Nigeria, Cameroon, Ecuador, the Dominican Republic, and Papua New Guinea are all major crop cultivators (Betty et al., 2022; Salifou & Cao, 2019). Ivory Coast has historically been the top cocoa-producing country, accounting for approximately 33% of the world's supply, with Ghana following closely behind (Suwanan et al., 2021). The World Cocoa Foundation approximated that roughly 6 million farmers cultivated approximately 500 kg of cocoa beans per hectare in Asia (Beg et al., 2017). Cocoa is also grown on a smaller scale in Malaysia, Madagascar, Mexico, and the Caribbean Islands (de Souza et al., 2018; Hagiwara & Wright, 2015; Riedel et al., 2019; Verna, 2013).

Cocoa pod husk (CPH) or cocoa pod shell is an outer layer of cocoa fruits. The shape of CPH is elongated pods, and the colour of CPH slowly changes from green to yellow when ripe. The CPH is rich in bioactive compounds, such as polyphenols, pectin, dietary fibre, and fat. CPH is usually thrown as waste when cocoa beans are extracted for chocolate-making purposes, which is approximately 52-76% of CPH (Vriesmann et al., 2012). This leads to environmental pollution, such as soil and water pollution, and attracts pests and pathogens that can cause health problems for humans (Leygnima et al., 2021).

In cocoa-producing countries, such as the Ivory Coast, this cocoa waste may generate higher profits for the country by being managed properly, thus reducing environmental pollution. For sustainable cocoa production worldwide, the CPH is managed as a waste to value-added products such as CPH used as an activated carbon filter for industrial use. This includes mulching in gardens or farms to retain moisture in soil and energy generation (Dominic & Zsuzsanna, 2022).

The other method of managing CPH waste is by extracting pectin. Pectin is present in the cell walls of plants by nature, such as banana peels, apple peels, orange peels, and CPH (Satria et al., 2024). The function of pectin is to give strength and stability to the cell walls of plants. Pectin acts as an antioxidant, antitumor, and anti-inflammatory activity due to the polyphenols compound in the pectin (Maya et al., 2022; Yeison et al., 2022). Pectin is a complex carbohydrate, specifically a type of polysaccharide that is composed of long-chain monomer units, such as α -1,4-D-galacturonic acid (GalA) units. The galacturonic acid units that are partially esterified with a methyl group and acetyl ester (Musita, 2020). Pectin is a raw material that can be used in cosmetics and pharmaceutical industries (Ofosua et al., 2016), such as in creams and other products. In food industries, pectin acts as a gelling, thickening, emulsifier, and stabilising agent (Beda & Kovassi, 2012).

There are various ways to extract the pectin for example microwave-assisted extraction (MAE), ultrasound-assisted extraction (Patrathip & Rungsinee, 2018), acid extraction, enzyme-assisted extraction and the traditional way to extract the pectin is steam extraction

(Riyamol et al., 2023). These are the extraction methods to promote the pectin hence increasing the yield. Acid extraction using citric acid, hydrochloric acid, nitric acid or any solvents gives the highest yield in cocoa husk at 12.60% wt/wt, orange peel at 17.95% wt/wt, and lime peel at 15.7% wt/wt. The ultrasound technique gives a yield of mango peel 9.20% wt/wt and jackfruit peel 8.94% wt/wt while the MAE method gives a 49.83% wt/wt yield for papaya peel (Virginia & Lucia, 2020). All the extraction methods could be matched along with suitable parameters to get the higher pectin yield. Among these methods, MAE is chosen in this research because it gives higher pectin yield with the low amount of acid needed and it provides environmentally friendly.

Response surface methodology (RSM) is popularly applied in optimising pectin extraction parameters, including several factors such as temperature, pH time and solvent concentration. RSM suggests the least number of experiments required that can produce a high amount of yield with good quality (Minashree & Sunil, 2019). Central composite designs (CCD) are commonly used among researchers to construct experimental designs with various factors to fit a quadratic model.

This research was conducted to dig the information related to CPH waste by extracting various parameters and confirming the pectin compound.

MATERIALS AND METHODS

Preparation of Cocoa Pod Husk

The mixed clones of cocoa fruits in Malaysia were collected directly at the Malaysian Cocoa Board's (MCB) Cocoa Innovative and Technology Centre (CITC) in Nilai, Negeri Sembilan. The Cocoa pod was opened manually by extracting the beans. Then, the cocoa pod was sliced using a large-scale food slicer machine and dried at 40°C in the oven. The dried cocoa pod was ground into powder and sieved using a 60-mesh stainless steel sieve to get a fine powder.

Experimental Design

Three independent variables (Microwave power, pH and time) affect pectin yield extraction and degree of esterification. The selection of three independent variables was informed by previous studies that were selected based on the number of yields produced. The microwave power range was (180- 600) watts (Dao et al., 2021), pH (1-3) (Yoo et al., 2012) and time (5-20) minutes (Wang et al., 2007) exhibiting 17.01% wt/wt and 17.63% wt/wt yields on dragon fruits and jackfruit pectin, respectively. The study utilised a central composite design (CCD) to investigate the variables (Hassan et al., 2020; Mohammad et al., 2019). Each variable was assessed at five distinct levels as shown in Table 1. The central experimental condition was repeated six times as shown in Table 2. All experimental conditions were

randomly executed to reduce systematic errors. Data analysis was performed using Design Expert version 10 (Jenny et al., 2023).

The equation representing the impact of three experimental factors on the extraction of pectin yield and the degree of esterification is as follows:

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC \tag{1}$$

The dependent variable Y represents pectin yield extraction and degree of esterification. Meanwhile, variables A, B, and C are independent and represent microwave power, pH, and time, respectively; β_0 is a constant; β_1 , β_2 , and β_3 are the coefficients of linear terms and β_{12} , β_{13} , and β_{23} are the coefficients of the interaction term.

Table 1
Coded and uncoded experimental independent variables

Variables	coded	Level				
		$-\alpha$	-1	0	+1	$+\alpha$
Microwave power, watts	A	36.8	180	390	600	743.2
pH	B	0.3	1	2	3	4
Time, minutes	C	0	5	12.5	20	20.5

Note. uncoded indicates symbols and coded indicates a level of ($-\alpha$, -1, 0, +1, $+\alpha$). The 0 represents the centre point, the -1 and +1 represent the factorial points, and the $-\alpha$ and $+\alpha$ represent the axial points. A represents microwave power (watts), B represent pH, and C represent time (minutes)

Table 2
The matrix of experimental design

Run	Microwave power (watt)	pH	Time (minutes)
1	180	3	5
2	390	2	12.5
3	36.8	2	12.5
4	390	2	25.1
5	180	3	20
6	743.2	2	12.5
7	390	0.3	12.5
8	390	2	12.5
9	600	1	20
10	390	2	12.5
11	600	3	20
12	390	2	0

Table 2 (continued)

Run	Microwave power (watt)	pH	Time (minutes)
13	390	4	12.5
14	390	2	12.5
15	600	3	5
16	390	2	12.5
17	390	2	12.5
18	180	1	5
19	600	1	5
20	180	1	20

Note. This matrix represents a Central Composite Design (CCD) with 20 runs. The independent variables are Microwave power (watts), pH, and Time (minutes)

Pectin Yield Extraction using the MAE Method

Pectin extraction was performed following the procedure by Phaiphan et al. (2020) and Radityo et al. (2020) with some modifications. The cocoa pod husk (CPH) was soaked in the citric acid solutions (1g:25 mL) ratio with the pH (0.3-3) according to the experimental design lists. The mixture was extracted using a conventional microwave oven (Toshiba), at 2450 MHz working frequency, and maximum 800 W power output followed by the microwave power (watt) (36.8 – 743.2) watt and time (minutes), which were listed in the experimental design. The mixture solution in the beaker was placed in a rotating disc in the middle of the microwave. Toshiba microwave ovens typically use power levels with a maximum power of 800W and adjust the power levels of 36.8 and 743.2 watts by calculating the percentage of power level (desired wattage/Maximum wattage). After the mixture was cooled, a nylon juice strainer was used to filter the mixture. The filtrate solution was treated with 96% ethanol at (1:1) v/v ratio for 12 hours to allow precipitated pectin to occur. Afterwards, the pectin was filtered using filter paper (Whatman no. 1) and allowed to dry overnight in the oven at 40°C. Using a pestle and mortar, the dried pectin was ground into a fine powder. The weight of the pectin was then measured. The percentage of pectin yield was determined using a specific formula.

$$Pectin\ yield\ (\% \text{ wt/wt}) = \frac{\text{weight of dried pectin (g)}}{\text{weight of CPH (g)}} \times 100 \quad [2]$$

Degree of Esterification (DE)

The degree of esterification (DE) was determined using the titrimetric technique described by Phaiphan et al. (2020). A sample of 0.3 g of dried cocoa pectin was placed in a conical

flask and soaked in 2 mL of 96% ethanol, followed by dissolution in 100 mL of distilled water. Then, five drops of phenolphthalein indicator were introduced into the solution. The mixture was titrated with a 0.5 M solution of sodium hydroxide (NaOH) (initial volume, V_1) until it turned a light pink color. Afterwards, 10 mL of 0.5 M NaOH was added to the solution and left for 15 minutes. Subsequently, 10 mL of 0.5 M hydrochloric acid (HCl) and five drops of indicator were added. The mixture was titrated once more with 0.5 M NaOH (final volume, V_2) until the solution attained a light pink color. The DE was then calculated using the following formula:

$$DE (\%) = \frac{NaOH (V2)}{NaOH (V1) \pm NaOH (V2)} \times 100 \quad [3]$$

Fourier-transform Infrared Spectroscopy (FTIR) Analysis

The main functional group analysis of cocoa pectin was evaluated using the FTIR instrument. The 0.3 g cocoa pectin powder was directly placed on the crystal of the FTIR spectrophotometer (Agilent Technologies, Inc., CT USA, Model Cary 630) between 600-4,000 cm^{-1} (Jenny et al., 2023).

Statistical Analysis

All data was expressed as means \pm standard deviations. Independent analyses were performed in triplicates. Data were analysed using Design Expert version 10. The confidence level of a p -value less than 0.05 was statistically significant.

RESULTS AND DISCUSSION

Model Fitting and Analysis of Variance (ANOVA) of Pectin Yield

We use response surface methodology to assess the impact of three independent variables (microwave power, time, and pH) and their interactions with a reduced number of experiments. We opted for the central composite design (CCD) as it includes central points for calculation. The results from each run are presented in Table 4. This data is fitted to a quadratic model, which is expressed by Equation 4. Table 3 displays an analysis of variance for this quadratic model. The model F-value was 32.3, indicating an insignificant lack-of-fit relative to pure error, and this model can be utilised for predicting response. The R^2 values demonstrate the accuracy between the predicted values and the experimental results for the measured response (Radityo et al., 2020). In this research, the R^2 is 97% indicating a good fit of data to the model. The adjusted R^2 was close to the R^2 .

In Table 3, the p -value indicates which parameter significantly impacts pectin yield extraction recovery. Variables A, B, C, AC, BC, A^2 , B^2 , and C^2 had a p -value lower than

Table 3
Analysis of variance for cocoa pectin yield

Source	Sum of Squares	df	Mean Square	F-Value	p-value
Model	288.78	9	32.09	32.3	< 0.0001
A-Microwave Power	5.88	1	5.88	5.92	0.0352*
B-pH	122.57	1	122.57	123.38	< 0.0001*
C-Time	36.24	1	36.24	36.48	0.0001*
AB	2.99	1	2.99	3.01	0.1135
AC	3.47	1	3.47	3.49	0.0911
BC	16.68	1	16.68	16.79	0.0022*
A ²	6.79	1	6.79	6.84	0.0258*
B ²	50.84	1	50.84	51.17	< 0.0001*
C ²	29.59	1	29.59	29.79	0.0003*
Residual	9.93	10	0.99		
Lack of Fit	7.13	5	1.43	2.54	0.1646
Pure Error	2.81	5	0.56		
Cor Total	298.72	19			
R-Squared	0.97				
Adj R-Squared	0.94				

Note. df represents the degree of freedom. AB is the interaction between microwave power x pH, AC is the interaction between microwave power x time, and BC is the interaction between pH x time. A² is the interaction between microwave power, B² is the interaction between pH, and C² is the interaction between time. Adj-R squared indicates adjusted R squared. * indicates a significant value of $p < 0.05$

0.05, signifying that these variables significantly affect pectin yield. Although all the main effects were significant only BC as an interaction effect was significant. The modelling equation that was generated from the analysis was a second-order equation to pectin yield. Thus, equation 5 simplifies equation 4 by removing insignificant variables and is equivalent to equation 6 in uncoded terms (Wulandari et al., 2023).

$$Y = 13.50 + 0.66 A - 2.91 B + 1.63 C - 0.61 AB - 0.66 AC + 1.44 BC - 0.69 A^2 - 1.59 B^2 - 1.45 C^2 \quad [4]$$

$$Y = 13.50 + 0.66 A - 2.91 B + 1.63 C - 0.66 AC + 1.44 BC - 0.69 A^2 - 1.59 B^2 - 1.45 C^2 \quad [5]$$

$$Y = 13.50 + 0.66 (\text{microwave power}) - 2.91 (\text{pH}) + 1.63 (\text{time}) - 0.66 (\text{microwave power} \cdot \text{time}) + 1.44 (\text{pH} \cdot \text{time}) - 0.69 (\text{microwave power})^2 - 1.59 (\text{pH})^2 - 1.45 (\text{time})^2 \quad [6]$$

Table 4

The matrix of experimental design and the results of yield (% wt/wt) and DE (%)

Run	Microwave power (watt)	pH	Time (minutes)	Yield (% wt/wt)	DE (%)
1	180	3	5	3.3	44.72
2	390	2	12.5	13.66	45.79
3	36.8	2	12.5	10.34	45.55
4	390	2	25.1	13.4	45.63
5	180	3	20	9.52	45.83
6	743.2	2	12.5	13.44	45.25
7	390	0.3	12.5	13.24	44.18
8	390	2	12.5	13.58	45.81
9	600	1	20	12.78	44.66
10	390	2	12.5	14.24	45.81
11	600	3	20	8.4	45.76
12	390	2	0	6.17	45.49
13	390	4	12.5	2.23	43.52
14	390	2	12.5	13.9	45.81
15	600	3	5	3.85	43.67
16	390	2	12.5	13.8	45.81
17	390	2	12.5	12.09	45.81
18	180	1	5	11.01	45.84
19	600	1	5	14.97	45.6
20	180	1	20	12.42	43.97

Note. Yield (% wt/wt) indicates pectin yield. DE (%) indicates the degree of esterification

Analysis of Response Surface of Pectin Yield

Based on Table 4, the pectin yield from different extraction dependent variables was from 2.23% to 14.97% (wt/wt). The highest yield was recorded at high microwave power and at lower pH with a shorter time to extract the pectin. The pectin yield using the microwave extraction method (MAE) of different plant sources was apple pomace was 10.6 % (wt/wt) (Josiel et al., 2024), the pineapple was 2.27% (wt/wt) (Noorzetty et al., 2020), and red dragon fruit flesh was 17.01% (wt/wt) (Thi et al., 2020). These studies have shown that enough MAE could extract the pectin in a shorter time with a high yield.

Figures 1(a) and 1(b) depict the contour graph and response surface, showing how pH and extraction time affect pectin yield. Pectin yield declined with increasing pH, reaching its lowest point at around pH 3 (Wulandari et al., 2023). Additionally, the amount of pectin yield was affected as the extraction time was reduced. Pectin yield was much higher with a shorter extraction time and lower pH compared to higher pH, as acidic concentration facilitated pectin extraction from the cell wall.

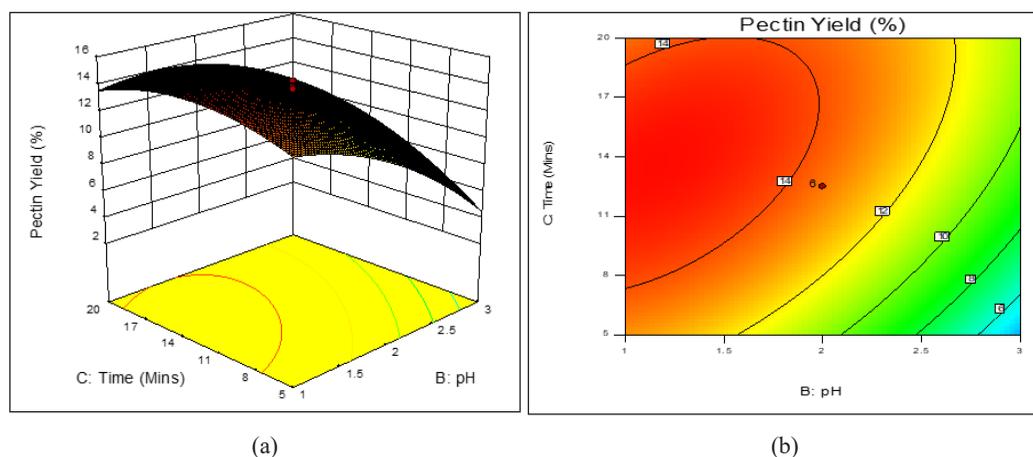


Figure 1. The three-dimensional (3D) response surface (a) and two-dimensional (2D) contour graph (b) shows the interaction of pH (B), and time (mins)

Interaction Effect of pH and Time

The data indicated that the microwave power, pH level, extraction duration, and the interplay between pH and extraction duration significantly ($p < 0.05$) impacted all the outcomes. Figures 1 (a) and 1 (b) show the production of cocoa pectin increased as the extraction duration decreased at lower pH levels, with a notable rise in yield observed at a microwave power of 600 W. At a pH of 1 and an extraction time of 5 minutes, the highest yield of 14.97% (wt/wt) was achieved. A slight decrease in yield was observed in Table 4 when the extraction duration was extended to 20 minutes under the same microwave power and pH conditions. The lowest pectin yield was 2.23% (wt/wt) at higher pH 4 and an extraction time of 12.5 minutes. The longer extraction time of pectin can lead to pectin and bioactive compounds degradation, resulting in lower yield and low quality of pectin (Dawit et al., 2023).

Theoretically, when the pH of citric acid decreases, it becomes concentrated and has high acidity. The more acidic citric acid content in the mixture of cocoa pod husk will destroy the plant cell wall with higher microwave power, and the pectin will be exposed and eluted (Siringoringo et al., 2018; Wan et al., 2021). Furthermore, this can aid in increasing the extraction efficiency between cocoa pod husk and the extracting solvent citric acid. The penetration of the extracting solvent will increase the extraction yield (Kratchanova et al., 2004). In these findings, lower pH and less extraction time produced a high yield and a good quality of cocoa pectin. Previous studies reported that increases in the pH of acid will reduce the pectin yield and reduce the quality of extracted pectin as well (Dawit et al., 2023).

Previous studies have reported that the total pectin yield depends on the extraction parameter (Happi et al., 2008). The extraction yield in this study was 14.97% (wt/wt) higher than the maximum extracted pectin of 9.64% (wt/wt) that was used in other extraction parameters (400 Watt, 1.16 pH, 15 mins) reported by Radityo et al. (2021). Other authors reported that the highest pectin yield could be obtained using citric acid as a solvent extraction (Rodríguez Rodr'iguez et al., 2022).

Model Fitting and Analysis of Variance (ANOVA) of DE

ANOVA was utilised to analyse the degree of esterification, as indicated in Table 4. The data was fitted to a quadratic model and is represented by equation 7. Table 5 presents the analysis of variance for this quadratic model. The Model F-value, denoted as 15088.49, suggests no significant lack-of-fit compared to pure error, indicating that this model is suitable for predicting the response. The R² values demonstrate the accuracy between the predicted values and the experimental results for the measured response. In this research, the R² is 99% indicating a good fit of data to the model. The adjusted R² was close to the R².

Additionally, the *p*-value displayed in Table 5 identifies which parameter has a substantial effect on the degree of esterification recovery. A, B, C, AB, AC, BC, A², B², and C² all had *p*-values less than 0.05, implying that these variables have a significant impact on the degree of esterification in equations 7 and 8.

$$Y = 45.81 - 0.086 A - 9.81 B + 0.046 C - 0.20 AB + 0.24 AC + 0.75 BC - 0.14 A^2 - 0.57 B^2 - 0.088C^2 \tag{7}$$

$$Y = 45.81 - 0.086 (\text{microwave power}) - 9.81 (\text{pH}) + 0.046 (\text{time}) - 0.20 (\text{microwave power}*\text{pH}) + 0.24 (\text{microwave power}*\text{time}) + 0.75 (\text{pH}*\text{time}) - 0.14 (\text{microwave power})^2 - 0.57 (\text{pH})^2 - 0.088(\text{time})^2 \tag{8}$$

Table 5
Analysis of variance for DE

Source	Sum of Squares	df	Mean Square	F -Value	<i>p</i> -value
Model	12.11	9	1.35	15088.49	1.85E-19
A-Microwave Power	0.10	1	0.10	1132.978	1.27E-11*
B-pH	0.0014	1	0.0013	15.64	0.002*
C-Time	0.029	1	0.030	328.21	5.63E-09*
AB	0.31	1	0.31	3455.78	4.93E-14*
AC	0.45	1	0.46	5114.62	6.97E-15*
BC	4.52	1	4.52	50640.29	7.38E-20*

Table 5 (continued)

Source	Sum of Squares	df	Mean Square	F -Value	p-value
A ²	0.29	1	0.30	3321.74	6E ⁻¹⁴ *
B ²	6.46	1	6.45	72410.63	1.24E ⁻²⁰ *
C ²	0.11	1	0.10	1232.378	8.34E ⁻¹² *
Residual	0.00089	10	8.92E ⁻⁰⁵		
Lack of Fit	0.00056	5	0.00011	1.67	0.29
Pure Error	0.00033	5	6.67E ⁻⁰⁵		
Cor Total	12.11	19			
R-Squared	0.99				
Adj R-Squared	0.99				

Note. df represents the degree of freedom. AB is the interaction between microwave power x pH, AC is the interaction between microwave power x time, and BC is the interaction between pH x time. A² is the interaction between microwave power, B² is the interaction between pH, and C² is the interaction between time. Adj-R squared indicates adjusted R-squared. * indicates a significant value of $p < 0.05$

Analysis of the Response Surface of DE

Figures 2 (a) and 2 (b) show the contour graph and response surface depicting how the degree of esterification (DE) is impacted by both pH and microwave power. Based on the response surface plot, as pH decreases from pH 3 to pH 1, DE also decreases, reaching its highest point at pH 2. The degree of esterification is also affected by microwave power, decreasing as the power decreases but peaking at 390 watts.

Figures 3 (a) and 3 (b) display the contour graph and response surface, illustrating how microwave power and time affect the outcome. The DE remained consistent regardless of changes in time. The peak DE occurs between 180 and 390 watts.

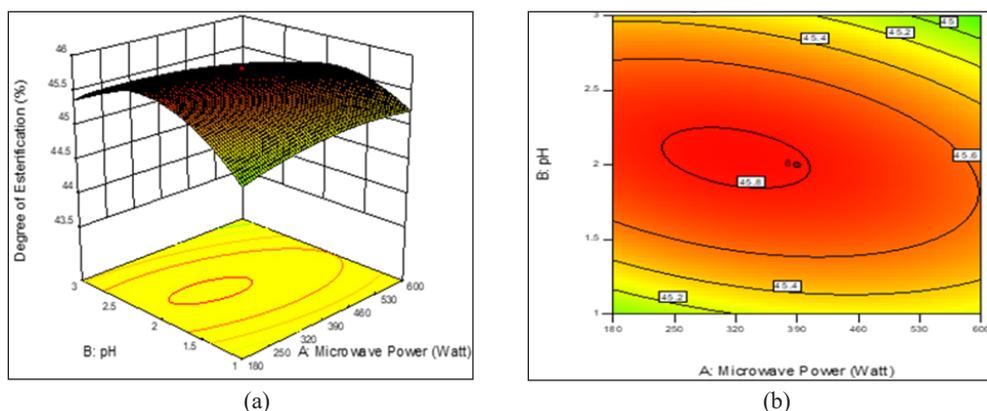


Figure 2. The three-dimensional (3D) response surface (a) and two-dimensional (2D) contour graph (b) show the interaction of microwave power (A) and pH (B) for DE at 12.5 minutes

In Figures 4 (a) and 4 (b), a contour graph and response surface illustrate the impact of pH and time. The degree of effectiveness (DE) rose as pH decreased from 2 to 1. At pH 3, the DE declined. Additionally, the DE increased as the irradiation time decreased.

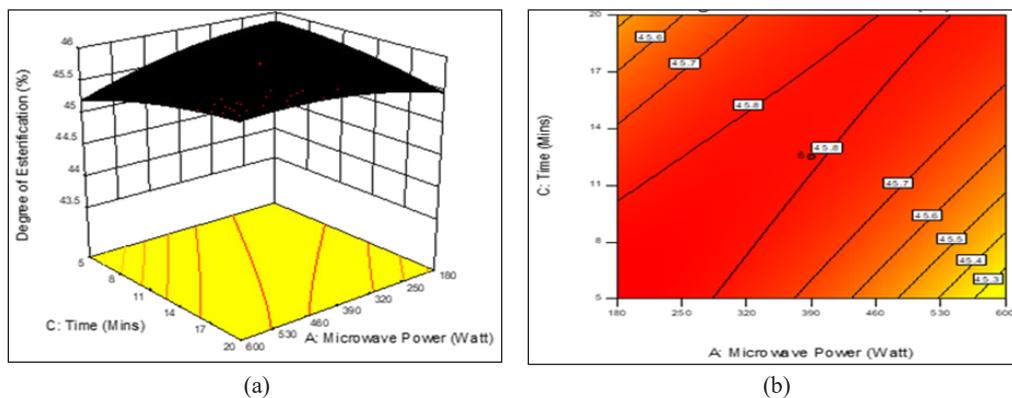


Figure 3. The three-dimensional (3D) response surface (a) and two-dimensional (2D) contour graph (b) show the interaction of microwave power (A) and time (C) for DE at pH 2

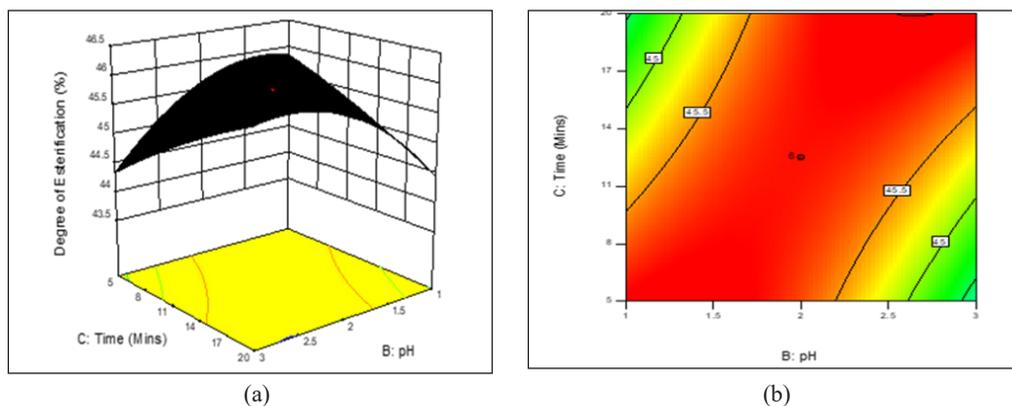


Figure 4. The three-dimensional response (3D) surface (a) and two-dimensional (2D) contour graph (b) shows the interaction of pH (B) and time (C) for DE as a function of pH at 390 watts

Interaction Effect of pH and Microwave Power

The results indicated that the microwave power, pH, extraction time, and the combined effect of pH and microwave power significantly ($p < 0.05$) impacted all the outcomes. Based on Figures 2 (a) and 2 (b), lower pH values led to higher degree of esterification (DE) values, with the most significant increase occurring at a microwave power of 180 W. A pH of 1 and an extraction time of 5 minutes resulted in the highest DE at 45.84%. When the pH was raised to 3 at the same microwave power, there was a slight decrease in DE,

but it took longer to extract the DE. Increasing the microwave power to 600 W resulted in decreased DE. The amount of DE decreased at the highest microwave power. pH, on the other hand, did not have much influence on the DE results in Table 4.

High or low microwave power indicates the ability of electromagnetic radiation to attack the cell wall of the plant and promote the release of pectin in a shorter time and low consumption of electricity. During the process of extraction, the ester bond on the D-galacturonic acid chain can be broken down due to high microwave power resulting in a low degree of esterification. At acidic pH conditions of citric acid solvent, the degree of esterification of the pectin usually produces high DE which is more than 50%. In this research, the higher DE was at higher pH 1 (45.84%) but it is considered a low degree of esterification with less than 50%. The same results were reported by Rosaria et al. (2017), the results of DE in citrus samples (orange, lemon, and grapefruit peels) were reduced at the highest microwave power of 600 W and pH 7. The previous results also support this statement where high microwave power 560 W at pH 1 the DE was 77.39%. In addition, lemon peel extracted from the MAE technique at the highest microwave power of 650 W and pH 1.4 gives a low DE of 33.2% (Pei et al., 2024).

Interaction Effect of Microwave Power and Time

The outcome of the study revealed that the microwave power, pH, extraction time, and the combined effect of microwave power and time had a significant ($p < 0.05$) impact on all the measured results. According to the data in Table 4, the DE value increased to 45.83% at 180 W when the extraction time was 20 minutes. At the same 20-minute mark, the DE values were 44.66% and 43.97%, respectively. The DE values declined as the microwave power levels changed from 600 W to 180 W. The DE values fluctuated as the extraction time varied from 5 minutes to 12.5 minutes due to the influence of microwave power applied to the cocoa pectin. Based on Figures 3 (a) and 3 (b) extraction of pectin at longer time irradiation gives higher DE compared to lower extraction time. The microwave power influenced the high rate of degree of esterification at a lower microwave power of 180 W.

Generally, high exposure of microwave irradiation to the cocoa pod husk with a shorter time could produce a good quality of cocoa pectin with high DE. The microwave power of 743.2 W and 600 W each produces de 45.25% and 45.60%, respectively. In this study, it is considered high even though the DE of cocoa pectin was low. In a grape pomace study reported by Spinei and Oroian (2022) at 560 W microwave power with shorter time extraction (120 seconds), it gives 82.29%. Other studies reported that high in DE (39%) at a high microwave power of 600 W and 75 minutes of the red-orange outer skin (Rosaria et al., 2017). When the microwave power of 650 W at 5.5 minutes produced 80.5% of DE (Pei et al., 2024).

Interaction Effect of pH and Time

The results indicate that the microwave power, pH, extraction time, and the interaction between pH and time significantly ($p < 0.05$) impacted all the outcomes. The DE values presented in Table 4 indicate that at a pH of 1 with an extraction time of 5 minutes, the DE was 45.84%, which was the highest value compared to the others. When the extraction time was also 5 minutes but with different pH levels of 3 and 1, the DE values were 44.72% and 45.6%, respectively. Based on Figures 4 (a) and 4 (b) in the response surface plot, there was a steep slope in time and pH which made a huge difference in the degree of esterification. The strong interaction of pH and time in the degree of esterification is based on the curvatures between the middle point of the plot.

Theoretically, the longer the cocoa pod husk is exposed to the microwave environment the ester bond of the pectin compound can break down and it can lead to de-esterification also pectin compound can be degraded (Hosseini et al., 2016). Longer time extraction of pectin resulting in low DE whilst shorter time for extraction could protect and produce a good quality of pectin. Besides it can produce high DE. In pH, acidic conditions help prevent hydrolysis of the ester bonds tend to have a high DE whereas in higher pH it's vice versa. The same condition with a low pH of 1.5 and a shorter time of 9 minutes produced a high DE of 49.17% in citrus (Mahmud et al., 2021). Other authors reported that pH 2 and 10 minutes showed high DE in the banana sample (Phaipan, 2019).

Degree of Esterification (DE)

The level of esterification refers to the extent to which galacturonic acid residues have been esterified to create methyl esters. The possible range for the level of esterification is 0-100%. Pectin with a degree of esterification exceeding 50% is referred to as high methoxyl (HM) pectin, while pectin with a degree of esterification lower than 50% is known as low methoxyl (LM) pectin (Morrison et al., 2000; Nanti, 2020; Wulandari et al., 2023). Based on IPPA's findings, the cocoa pectin used in this research qualifies as a low pectin ester due to mostly having a degree of esterification below 50%. The degree of esterification is a parameter that characterises pectin's ability to form a gel with a lower sugar level (Shaha et al., 2013; Wulandari et al., 2023). The glycosidic linkages of methyl esters in pectin are prone to hydrolysis, resulting in the production of galacturonic acid as microwave power increases over time, leading to a decrease in the degree of esterification. In the pectin extraction process, applying excessive microwave power to the mixture will convert pectin into pectic acid, in which the galacturonic acid molecules lack a methyl ester group. The quantity of methoxyl groups determines whether the pectin sample is categorised as high or low (Nanti, 2020; Phaipan et al., 2020; Rodsamran & Sothornvit, 2018).

Model Verification

An extra test was conducted at the midpoint to validate the model derived in equations 6 and 8. According to Table 6, the results of our model matched the experimental findings. The actual result of pectin yield and degree of esterification (DE) were close to the predicted values suggested by the experimental design. The extraction parameters microwave power (watt), pH, and time (minutes) will influence the pectin yield and the degree of esterification depends not only on the parameter conditions but also on the sources of pectin (Dawit et al., 2023). Lower microwave power will take a longer time for the citric acid solvent to penetrate the cell walls of plants resulting in yield decreases and a low degree of esterification. High microwave power was applied to the sample and easy for citric acid to break down the cell wall of the plant and release the pectin increasing the yield and degree of esterification (Noorzetty et al., 2020). Furthermore, the pH of the citric acid solvent also influenced the pectin yield and degree of esterification (Jun et al., 2019). The pectin yield and degree of esterification increase at a lower pH of 1 and 2 while pH 3 reduces its yield and the degree of esterification. For the extraction time, a shorter extraction time usually produces low yields but it prevents the pectin and bioactive compounds from degrade while the degree of esterification increases. For longer extraction time exhibits higher yields but will degrade the pectin and bioactive compounds and also it will decrease the degree of esterification (Maya et al., 2022; Vriesmann et al., 2012). Thus, the selection of

Table 6
Comparison between the predicted by the model and the actual

Microwave power, watts	pH	Time, mins	Predicted		Actual	
			Yield	DE	Yield	DE
500	1	5	13.99	45.76	14.1	45.75

Note. Yield indicates pectin yield (% wt/wt) and DE is the degree of esterification (%)

Table 7
Comparison between the optimised cocoa pectin yield and DE with the previous studies

	Yield (% wt/wt)	DE (%)	Previous studies	References
1.	14.1	-	The pectin yield% (wt/wt): Apple pomace: 10.6% Red dragon: 17.01% Pineapple: 2.27% Cocoa pod husk: 9.64%	Josiel et al., 2024 Thi et al., 2020 Noorzetty et al., 2020 Radityo et al., 2020
2.	-	45.75	The DE(%): Orange peels: 39% Citrus: 49.17% Lemon: 33.2%	Rosaria et al., 2017 Mahmud et al., 2021 Pei et al., 2021

Note. Yield indicates cocoa pectin yield (% wt/wt) and DE is the degree of esterification (%)

acid methyl ester and is derived from (COCH₃) in the polysaccharide backbone. The peaks of the esterified carbonyl group and carboxylic acid were at 1740 cm⁻¹ and 1690 cm⁻¹, respectively (Fensia et al., 2021). The ester carbonyl (C=O) stretching is particularly important as it shows the degree of esterification (DE) of the pectin. Furthermore, the carboxylic acid group peak depicted either the pectin was esterified or de-esterified. As the degree of esterification (DE) decreases, the peak becomes more prominent which has a high amount of carboxylic acid groups. Next, the C-O-C stretching peak that shown at the peak spectrum of 1135 cm⁻¹. The C-O-C stretching represents glycosidic bonds within the pectin backbone. The peak at about 1200 cm⁻¹ was a region of carbohydrates which was polysaccharide. This confirms the presence of the α -1,4-glycosidic bond between the α -1,4-D-galacturonic acid units (Chen., et al 2019). The peaks of FTIR in cocoa pectin confirmed that the α -1,4-D-galacturonic acid compound acts as a pectin compound. The previous study reported by Marsiglia et al. (2016) and Nanti (2021) showed the same band spectrum of D-galacturonic acid.

CONCLUSION

To summarise, the findings indicated that the highest yield (14.97% wt/wt) was achieved using microwave-assisted extraction at pH 1, with 600 watts of microwave power for 5 minutes. This MAE technique demonstrates strong potential for producing higher yields compared to conventional methods. The degree of esterification results for cocoa pectin ranged from 43.67% to 45.84%, indicating low methoxyl pectin, which is defined as having a degree of esterification below 50%. A pectin yield of 14.1% wt/wt was obtained under optimised conditions of 5 minutes of irradiation time at 500 watts of microwave power and pH 1. The FTIR results confirm that the extracted polysaccharide in this study was a pectin compound which was α -1,4-D-galacturonic acid. Efficient bio-waste valorisation of cocoa pod husk ensures a sustainable, eco-friendly approach to pectin extraction can create high-value products in cosmetics, pharmaceutical, and food industries while supporting economic growth and waste reduction. Furthermore, the utilisation of citric acid is both cost-effective and environmentally friendly, serving as a promising green technology for the future. Additionally, the strong solvent abilities of citric acid contribute to the high yield of pectin extraction. Thus, this study provides valuable insights into the application of MAE as an effective extraction method.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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