EXTRACT OPTIMIZATION AND FTIR SPECTROSCOPIC OF PECTIN FROM COCOA POD HUSK VIA MICROWAVE-ASSISTED STUDY

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ABSTRACT – *Undesirable cocoa (Theobroma cacao L.) pod husk (CPH) which is typically generated during cocoa beans processing, presents challenges concerning its proper disposal as well as in adding value to such waste. Thus, this study focused on the optimization of pectin yield (%) which was extracted from the CPH via microwave-assisted extraction (MAE) under different conditions, i.e., X_1- pH of 1-3, X_2- extraction time of 5-20 minutes and X_3- power level of 180-600 watts through central composite design (CCD) evaluation and response surface methodology (RSM) analysis. The independent variables that have significantly affected the yield are pH, extraction time and their interactions with value of $p<0.05$ whilst power level remains insignificant ($p>0.05$). At the given parameters and levels, the pectin yield ranged from 3.30–23.25%. The optimum yield (18.44%) can be achieved at pH and extraction time of 0.8 and 11 mins, respectively. The final reduced model for pectin extraction by using this method is shown in coded equation, i.e., $Y = 9.0197 - 6.8716X_1 + 0.8159X_2 - 0.0335X_2X_2 + 0.2258X_1X_2$ with the F-value of 1035.23 suggested that the model was significant. High coefficient of determination (R^2) and predicted (R^2) which were recorded at 0.9931 and 0.9906, respectively, have confirmed the adequacy of regression models adjustment pertaining to the experimental data.*

Key words: microwave-assisted extraction; pectin yield; cocoa pod husk; FTIR; central composite design

INTRODUCTION

Cocoa or *Theobroma cacao* L. (family: *Sterculiaceae*) is an economically vital crop that can be found predominantly in several tropical regions, i.e., West Africa, Central America, and South America (Bhattacharjee, 2018). Cocoa bean which has been an essential part of cocoa fruit, is a main ingredient in the chocolate and cocoa beverages industries. Revenue in the cocoa segment is approximately amounts to US$20.35bn in 2023 where the market is going to be expected to grow by 4.68% annually from 2023 to 2025, according to compound annual growth rate (CAGR). Moreover, the volume of cocoa segment is likely to rise to 1,764.1M kg by 2025 in which the market has been predicted to show a volume growth of 3.0% in 2024 (Jocelyn, 2023).

In general, cocoa beans isolation from cocoa fruits generate 52–76% of wastes in the form of cocoa pod husks (Chandel *et al.*, 2022; Campos-Vega *et al.*, 2018). Cumulatively, one ton of dried beans produces ten tons of underutilized CPH (Campos-Vega *et al.*, 2018). Upon harvesting, cocoa pod husks are conventionally left as unwanted materials to decay in the cocoa farmsteads that generates challenging waste management problem due to several environmental threats. Considering the huge demand for cocoa beans in order to fulfill appealing needs for chocolates, it has been expected CPH generation will continue to rise for the next few years. In addition to the liberation of foul odors, CPH rottening could be a host of some botanical diseases especially black pod rot (Campos-Vega *et al.*, 2018).

An economical yet environmental-friendly approach on managing of CPH waste would be by converting them into pectin, linear chains of biopolymer comprising (1, 4)-linked α-d-galacturonic acid residues and methyl esters of uronic acid (Li *et al.*, 2021). Basically, pectin composition is being highly affected by phytochemical source, extraction method, and environmental elements (Roman-Benn *et al.*, 2023). For example, extracted pectin from citrus has fewer natural sugars with smaller molecular size compared to pectin extracted from apples (Juodeikiene *et al.*, 2019). In term of extraction technique, intervention of solvent system alone, namely water, citric, hydrochloric and nitric acids, have been generally executed in order to extract pectin from several biowastes, with regard to yield as well as the quality of extracted pectin (Abubakar & Haque, 2020; Su *et al.*, 2019; Wandee *et al.*, 2019). However, pertaining to toxicity issue and environment friendliness, combination of aqueous citric acid and microwave power are appealing due to safety and rapid rate of extraction process (Karbuz & Tugrul, 2020; Pandharipande, 2020).

These naturally occurring polysaccharides have been extensively applied in varieties of innovative pharmaceutical, cosmetic, and food-related products. For instance, in term of pharmaceutical industry, pectins have been used as excipients in the manufacturing of suspensions, emulsions, matrix tablets, coated tablets compression and filming-coated tablets (Li *et al.*, 2021; Akin-Ajani & Okunlola, 2021). Over the years, numerous researches have been dedicated to development of pectin from commercial sources especially apple pomace and citrus peel with various functional properties in numerous applications. However, little attention was focused on CPH pectin despite of the fact that considerable amount of CPH wastes have been generated in all cocoa producer countries. In terms of safety and rapid rate, previous study regarding MAE as a desirable pectin isolation from CPH, have approximately yielded 10.2% (Hennessey-Ramos *et al.*, 2021), 9.64% (Pangestu *et al.*, 2020) and 8.3% (Muňoz-Almagro *et al.*, 2019) of pectin. Nevertheless, none of the previous studies were focused on a pectin extraction from CPH waste that are being produced extensively in all cocoa farms as well as plantations in Malaysia. Therefore, the aim of the study is to determine the optimized conditions of pectin extraction with aqueous citric acid under microwave-assisted power as well as characterizing the optimized pectin.

MATERIALS AND METHODS

Raw Material

Dried cocoa pod husks were obtained from Bagan Datuk Cocoa Research & Development Center, Malaysian Cocoa Board in Perak. The pod husks were completely milled in a Wiley Mill 934 miller using sieves of 2-mm and 1-mm. The finished materials that passed through the 1-mm sieve, is hereafter referred as cocoa pod husk flour (CPHF) and was used in this work for pectin extraction with citric acid.

Microwave-assisted Extraction (MAE) of Pectin from CPHF

A conventional microwave oven was used during the extraction of pectin from CPHF. Twenty (20) g of CPHF were mixed with 500 mL of citric acid in a 1 L Pyrex-beaker at various pH values, irradiation times and power levels of 1-3, 5-20 mins and 180-600 watts, respectively. The solution was cooled and filtered through a juice nylon filter apparatus. Subsequently, the filtrate was soaked in an absolute ethyl alcohol at 1:1 ratio for 12 hrs for the floated pectin to be easily garnered. The collected pectin was put inside an oven at 50ºC until constantly dried. The pectin yield was calculated according to the Equation 1 below.

$$\text{Yield (\%)} = \frac{\text{weight of dried pectin (g)}}{\text{weight of CPHF (g)}}\, x\, 100 \qquad (1)$$

Experimental Design

Three independent variables (X_1, X_2 and X_3) and five levels (-α, -1, 0, 1, and + α) of central composite design (CCD) have been implemented to optimize the pectin extraction from CPH. The complete CCD design consisted of 20 experiments with 8 factorial points, 6 axial points and 6 center points were exhibited in Table 1. Six (6) replicates run at the center of the design were executed to attain a good estimation of pure error (Sin *et al.*, 2006). The independent variables studied were ethanol concentration (X_1, %), irradiation time (X_2, min) and microwave power (X_3, watts) while the response variable measured was pectin yield (Y, %). Each one of the experiments was conducted in replicate and the average values were exhibited as the response; Y. Data was analyzed by software package from Minitab version 14. Analysis of variance (ANOVA) at significant level of 0.05 was applied to assess the significant factor.

Model Verification

Second-order polynomial model of RSM was used to obtain the optimized conditions regarding pectin extraction. The appropriateness of the model equation in term of the response value prediction, have been verified by performing the extractions under the proposed optimized conditions. A numerical optimization method in this study was used to detect a point that maximized the yield. Generated set of solutions from which the solution to be used for the verification were selected according to its desirability as well as acceptability. Experimental and predicted values of the pectin yield were compared in order to obtain the validity of the model. Replication of experiments under the selected optimized conditions were made to confirm the results.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The infra-red (IR) analysis of the extract was carried out using infra-red spectrophotometer (Agilent Technologies, Inc., CT USA, Model Cary 630) between 600–4000 cm^{-1} (Nkafamiya *et al*., 2019).

RESULTS AND DISCUSSIONS

Response Surface Methodology Experiments

<u>Fitting the Model</u>

Table 1 showed the experimental and predicted values for the pectin yield from several combination of extraction conditions generated according to the experimental design. The highest yield can be achieved at the lowest point of acidic region, i.e., pH of 0.4, 12.5 mins of extraction time and power level at 390 watts, that has caused the pectin yield to increase up to 23.25% whilst the lowest yield (3.30%) was recorded at pH value, irradiation time and power level of 3, 5 minutes and 180 watts, respectively. In this case, it was noted that the pectin extraction at middle level of power but in a high acidic condition, is effective from which the penetration of extracting solvent at intense level of acidity allows the phytochemical constituents to be isolated by radiation-assisted electromagnetic (Maulion, 2019).

Table 1: Design matrix with output response for the optimization of pectin yield from the CPH

Independent Variables				Dependent Variable	
Run	X_1*	X_2**	X_3***	Y	
				A*	B**
1	2.0	12.5	390.0	13.66	13.30
2	2.0	12.5	390.0	13.58	13.30
3	3.0	5.0	600.0	3.85	4.49
4	1.0	20.0	600.0	12.78	15.12
5	1.0	5.0	180.0	11.01	12.08
6	3.0	20.0	180.0	9.52	11.07
7	2.0	24.7	390.0	13.87	10.80
8	2.0	12.5	390.0	14.24	13.30
9	0.4	12.5	390.0	23.25	20.76
10	2.0	12.5	732.9	13.44	11.47
11	2.0	0.3	390.0	6.17	5.76
12	3.6	12.5	390.0	9.27	8.28
13	2.0	12.5	47.1	10.34	8.82
14	2.0	12.5	390.0	13.90	13.30
15	1.0	20.0	180.0	12.42	13.60
16	3.0	5.0	180.0	3.30	2.78
17	3.0	20.0	600.0	8.40	9.15
18	2.0	12.5	390.0	13.80	13.30
19	2.0	12.5	390.0	10.46	13.30
20	1.0	5.0	600.0	16.97	17.24

Note:
X_1* : pH; X_2** : Irradiation Time (mins); X_3*** : Power (watts); A* : Experimental value; B** : Predicted value

Relationship between the tested independent variables and the response by multiple regression analysis application was indicated in Equation (2):

$$Y = 9.0197 - 6.8716X_1 + 0.8159X_2 - 0.0335X_2X_2 + 0.2258X_1X_2 \quad (2)$$

Where X_1 = pH; X_2 = irradiation time

ANOVA was implemented to assess the linearity and quadratic effects of the independent variables as well as their interactions. Regression coefficients on the response variable was evaluated as well to fit the response function and experimental data (Table 2). Due to the extremely low probability value (p=0.000), ANOVA of the regression model has proved that the model was significantly reliable. In this case, the R^2 value (0.948) was too close to the adjusted R^2 (0.922). The reliability of the model was also being supported by the lack of fit (p>0.05). Moreover, a small coefficient of variation (0.87) has strongly indicated that the experimental results were accurate and reliable.

Table 2: ANOVA of the second-order polynomial model for the pectin yield

Source	dF	Sum of Squares	Mean Square	*F*-value	*p*-value	
Model	9	337.805	37.534	15.23	0.000	*
Linear	3	235.095	78.365	31.79	0.000	
Quadratic	3	67.219	22.406	9.09	0.006	
Interaction	3	35.49	11.830	4.80	0.034	
Residual	8	19.719	2.465			
Lack of Fit	5	14.081	2.816	1.50	0.393	**
Pure Error	3	5.639	1.880			
Total	19	381.846				

Note:
* : Significant; ** : Not Significant

The results of multiple regression and significance of regression coefficients for the pectin yield model were exhibited in Table 3. It could be observed that the linear terms of pH (X_1) and extraction time (X_2), quadratic terms of extraction time (X_2) as well as power level (X_3) and interaction term of pH (X_1) with extraction time (X_2), had significantly affected the pectin yield (at least p<0.05). Amongst all three investigated parameters, pH was the most superior in the extraction of pectin from cocoa pod husks, followed by the extraction time.

Table 3: Estimated regression coefficients of the second-order polynomial model for the pectin yield

Regression coefficients		Pectin Yield (%)	*p*-value
Intercept	X_0	9.0197	
Linear	X_1	– 6.8716***	0.000
	X_2	0.8159***	0.007
	X_3		
Quadratic	X_1^2		
	X_2^2	– 0.0335***	0.002
	X_3^2		
Interaction	X_1X_2	0.2258**	0.016
	X_1X_3		
	X_2X_3		

<u>*Analysis of Response Surface Plot*</u>

The contour plot and three-dimensional (3D) response surface of the pectin yield which were generated from Minitab version 14 software, were shown in Figures 1 and 2, respectively. Both Figures 1 and 2 denoted the pH and extraction time effects on pectin yield at fixed power level of 390 watts. Table 3 showed the pH of citric acid exhibited a prominent effect on pectin yield particularly in linear manner (p=0.000). Its linear effect on pectin yield were negative which explained the nature of the curve (Figure 2). At the middle of extraction time (11 mins), pectin yield went up corresponsive with the intense pH (0.8) up to 18.44%, however, further increasing of extraction time has led to the reduction of pectin yield. Furthermore, extraction of pectin from CPH has been positively affected by the synergistic effect between pH and extraction time ($p<0.05$) (Table 3) where the extraction process has been mainly favored in two issues: extraction time might be short in the presence of low pH or longer extraction time might be needed in the presence of high pH. From the industrialization point of view, low pH with short extraction time would be more favorable as long extraction period rendered time consumption and economically impractical.

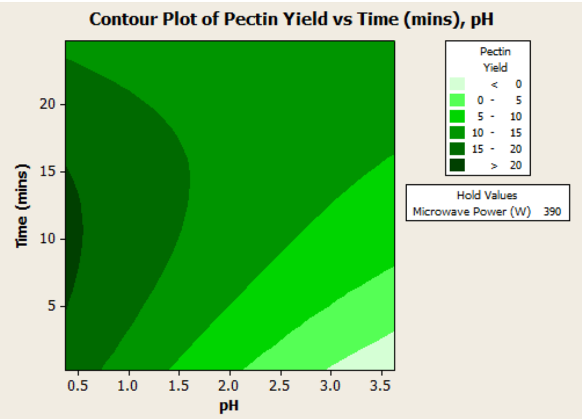


Figure 1: Contour Plot for pectin yield from CPH showing combined effects of time and pH

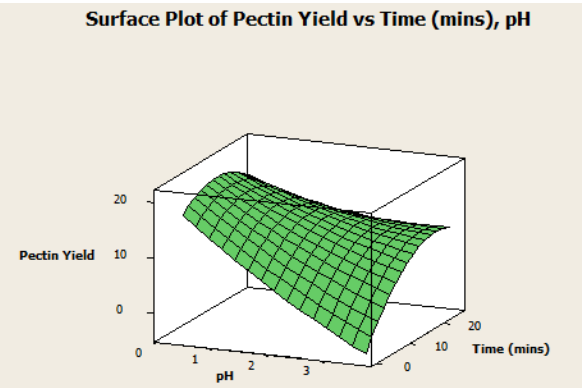


Figure 2: 3D Surface plot for pectin yield from CPH showing combined effects of time and pH

From the response surface plot, it was apparent that the pH was superior parameter in the extraction of pectin from cocoa pod husks, followed by irradiation time under microwave-assisted technique. Fast rate transfer of microwave energy heating at lower setting have been occurred in many pectin extraction studies. In most cases, elevating the microwave power to a higher level would cause a decrease in the pectin extraction due to the fragmented of its cell walls that could resulted to a decrease of pectin yield. Such problem has deactivated pectin clusters which is literally due to extensive permeation of microwave power and irradiation time (Sari *et al.*, 2018). Results showed that the extraction conducted at moderate power (390 watts) within shorter period (11 mins) was sufficient for the pectin to be isolated from the CPH. This condition was able to minimize pectin fragmentation due to high heat.

<u>*Verification of the Predictive Model*</u>

The experimental result was very close to the predicted value (Table 4). This indicated that the observed as well as experimental values predicted from the regression model, were at a high fit degree. Hence, the response surface modeling from this study could be effectively applied to predict the yield of pectin extracted from cocoa pod husks within the specific range of parameter.

Table 4: Optimum conditions, predicted as well as experimental values of the pectin yield (%)

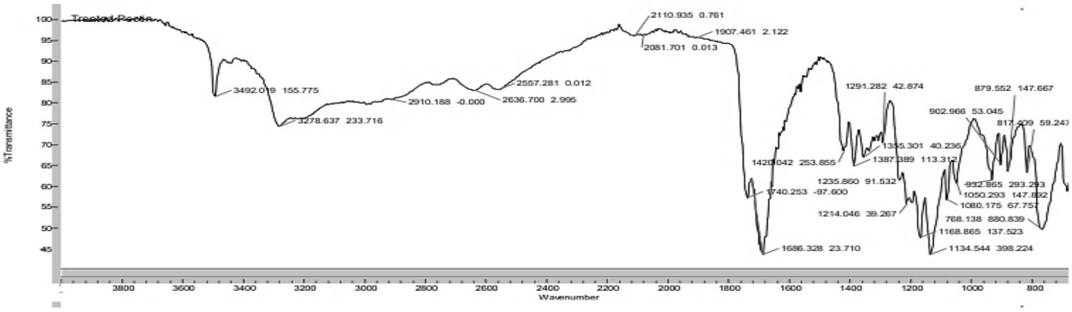
Optimum Conditions			**Pectin Yield (%)**	
X_1^*	X_2^{**}	X_3^{***}	A^*	B^{**}
0.8	11	390	19.85± 1.22	18.44

Note:
X_1^* : pH; X_2^{**} : Irradiation Time (mins); X_3^{***} : Power (watts); A^* : Experimental value; B^{**} : Predicted value

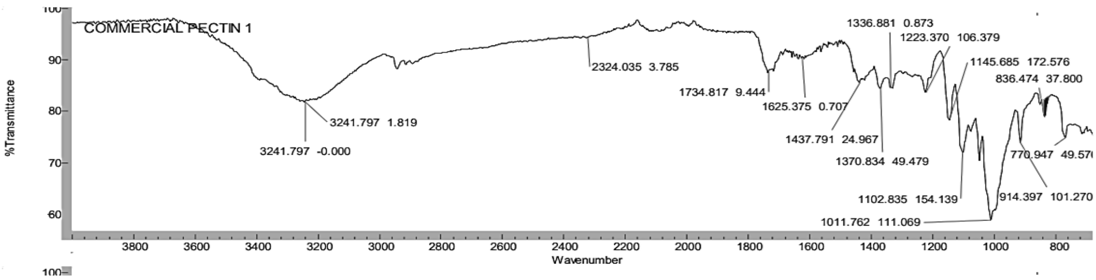
FTIR spectroscopy of the extracted pectin

The spectrum of pectin indicated that the peak at 3492 cm^{-1} was due to the stretching of –OH groups (Figure 3A) whereas the peak at 3278 cm^{-1} indicated C–H stretching vibration. The peaks at 1,420 and 1,387 cm^{-1} could be assigned to $–CH_2$ scissoring and –OH bending vibration peak, respectively. The peak at 1,134 cm^{-1} suggested –CH–O–CH– stretching. The peak at 1,168 cm^{-1} suggested the presence of –CH–OH in aliphatic cyclic secondary alcohol (Mishra *et al.*, 2008).

The bands in the CPH pectin spectrum are within this region, a major absorption peak between 1420 cm^{-1} and 1355 cm^{-1}. These two bands correspond, respectively, to asymmetrical and symmetrical stretching vibrations due to the COOH group of polygalacturonic acid. The absorbance at 1214 and 1134 cm^{-1} are the anhydrouronic acid, because all peptic polysaccharides characterized mainly by these peaks. Therefore, it is imperative to say that the pectin polysaccharide extracted from CPH (Figure 3A) shows similar pectin molecule when compared with the standard spectra, thus a good match with the spectrum of commercial pectin (Figure 3B) (Nkafamiya *et al.*, 2019).



(A)



(B)

Figure 3: FTIR spectrum of CPH (A) and commercial pectin (B)

CONCLUSIONS

Various conditions of pH, irradiation time and microwave power were properly studied for their optimized conditions from which CPH pectin was successfully isolated. The independent variables that significantly affected the pectin yield from cocoa pod husks were pH and irradiation time. Superior quality of pectin yield (18.44%) was achieved at the optimized conditions of 0.8 for pH and 11 mins of irradiation time at 390 watts of power level. The obtained results of FTIR have confirmed that the extracted polysaccharide of this current study was found to be pectin. The MAE technique of pectin extraction from the main by-product of cocoa production is indeed the true definition of circular economy where the environment is well-protected through the environmental-friendly waste disposal and there is high potential of economics being accelerated through the production of value-added pectin products from such waste.

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