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Microwave-assisted extraction of pectin from *Citrus maxima* peels with Box Behnken design

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Abstract: The microwave-assisted extraction of pectin from pomelo or *Citrus maxima* peels was carried out using the Box-Behnken design-based statistical modeling. During the extraction processes, the effect of the three independent variables: (1) solvent pH, (2) microwave irradiation power, and (3) microwave irradiation time on the pectin yield was studied. The results show that it follows a two-factor interaction model, and the statistical analysis showed that the model was able to explain the variability in the data and predict the relationship between the independent variables and responses. It was demonstrated that using high microwave irradiation power, long microwave irradiation times, and low solvent pH results in higher pectin extraction efficiencies. These results show that *Citrus maxima* peel is a great source of pectin and adjusting the microwave-assisted extraction parameters can greatly improve the yield.

Key Words: fruit peel; pectin; microwave-assisted extraction; yield optimization; Box Behnken design

1. INTRODUCTION

Pectin is a structural complex branched heteropolysaccharide inherently found in the cell walls of plants, distinguished for the rich presence of esterified D-galacturonic acid (GA) including homogalacturonan (HGA), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), and xylogalacturonan (XGA). The backbone of pectin, HGA is known to make up 70% of the polysaccharide linking many of its co-polymers through a series of alpha-(1→4) glycosidic linkages.(Venkatanagaraju et al., 2020) Although pectin can be sourced from various natural products including bananas, mangos, carrots, and others, pectin is mostly known to be extracted from citrus and apple pomace peels.(May & Pectin, 1990) It is typically utilized as a food ingredient as a gelling agent or stabilizer due to its colloidal nature.(Mota et al., 2020) The gelation of pectin is due to partial dehydration of the molecule to a degree in which the molecule adapted to a sol-gel intermediate form. The gel formation property is directly correlated to the degree of esterification (DE) and molecular weight of the polysaccharide.(Benassi et al., 2021)

Pectin is one of the most highly demanded food additives due to its use in jams, jellies, meat soups, confectionaries, and juice products.(Freitas et al., 2021) This high demand has led researchers to explore various extraction and refinement techniques in isolating pectin from multiple sources.

The more traditional and common techniques fundamentally involve subsequent, direct boiling and drying to extract pectic polymers from natural products successfully. Post-peel preparation, the powdered extract is exposed to hot-acid extraction solutions with long incubation periods. The extract is then precipitated using organic solvents followed by mechanical processes of pressing, drying, and grinding steps. The raw pectin extract is then washed, further purified, and filtrated using solvents. The now-separated pectin is dried, and the contaminated effluents are freeze-dried and disposed of.(Rodríguez Robledo* & Isabel Castro Vázquez, 2020) Economic analyses of traditional industrial processing of pectin-rich fruit waste have revealed a significant amount of waste by-products and high energy consumption that have been found to result in large streams of contaminated effluents, greatly limiting the scalability of industrial applications. Furthermore, studies have revealed the effects of mechanical processes on the eventual pectin yield and quality.(Ciriminna et al., 2016; Nagel et al., 2017) Although successful, the solvent-consuming and time-dependent conventions have been challenged, forcing the industry to explore more eco-friendly and less costly techniques. Microwave-assisted extraction (MAE) of natural products has proven to be a viable alternative to the more traditional methods due to the high efficiency, higher yield, and significantly less solvent waste generated in the process.(Zouambia et al., 2017) Microwave is a non-contact heat source that generates heat energy via ionic conduction between solvents and dissolved ions which eventually ruptures the cell wall and assists in the release of the natural products' constituents.(Pasandide et al., 2017)

Response surface methodology (RSM) is a collection of effective mathematical and statistical tools used to design experiments or build models involving a set of complex parameters. RSM tools allow for the evaluation of statistically predetermined combinations of each parameter and its effect on the outcome. This is achieved using a set of coefficients fitted into a mathematical model that best represents the experimental design and the analysis of the resulting response to the model.(Box et al., n.d.) Box-Behnken (BBD), a specific RSM tool, is a widely used technique for the optimization of experimental trials due to its revolving design and consistently reliable outcome.(Dong et al., 2009; J. Ganado et al., 2019) Optimization of the extraction method using a Box Behnken design would demonstrate interactions between several variables and the culminating effect on the desired yield and degree of esterification (DE). Numerous papers have been published regarding the partially water-soluble fiber and its MAE from sources such as aloe vera, citron, and mango peels.(Geng et al., 2014; Mugwagwa & Chimphango, 2019; Pasandide et al., 2018) Box-Behnken designs have allowed for the recovery of higher purity and, therefore, more biochemically beneficial pectin. This suggests that the use of BBD in eco-waste processing is a viable and fast method for determining its potential and scalability as a component in food or material manufacturing.(Matharu et al., 2016) Literature analysis has revealed that research in the extraction of pectin from pomelo or *Citrus maxima* peels is prominent, given the high yield and tunable degree of esterification in the extraction process.(Daud et al., 2019; Methacanon et al., 2014; Pasandide et al., 2017) However, optimization of the microwave-assisted procedure through means of Box Behnken designs is lacking.

This study explores the interactions between the parameters: microwave power, irradiation time, and pH in the microwave-assisted extraction of pectin as a 'greener' alternative that is highly efficient, energy-saving, and tunable. In the present work, a three-factor three-level Box Behnken design was employed, followed by a one-way analysis of variance to identify any correlations between parameters of MAE of pectic polysaccharides from CM peels (CMP).

2. METHODOLOGY

2.1 Materials

Samples of Citrus maxima were purchased from a local supermarket. The exocarp and albedo were then separated, and the mesocarp of the fruit was ground using mortar and pestle. After pulverizing the mesocarp, it was then exposed to direct sunlight to enhance evaporation and cease microbial activity. The powder was stored in bags and kept in a cool and dry environment. All chemicals and reagents were purchased from Sigma-Aldrich Pte. Ltd.

2.2 Pectin Extraction

Pectin was extracted from *Citrus maxima* peels with distilled water and a predetermined range of pH using reagent-grade 1 M H_2SO_4. Post-heating at varying microwave power and time parameters, the solution was filtered, and the pectin was precipitated using 98% ethanol. The colloid was then left to crystallize at 4°C for more than 24 hours. The resulting mixtures were filtered, and crystal residues were purified further with 98% ethanol. The final residues were then oven-dried at 50°C.

2.3 Determination of pectin yield

Post-extraction, the wet pectin was dried at 60°C in a hot air oven until its weight was constant and weight. The pectin yield was calculated from the following equation:

$$\%Yield = \left(\frac{m_0}{m}\right) x\ 100$$

where m_0 and m represent the weight of dried pectin (g) and the weight of dried pomelo peel powder, respectively.

2.4 Experimental design and statistical analysis

Correlations among extraction parameters including microwave power, irradiation time, and pH were studied as well as the corresponding effects on pectin yield and degree of esterification following a Box Behnken-designed experiment. The experimental setup was composed of three factors with three levels and two center points. The level and range of process variables are summarized in Table 1. The range of process variables was selected based on the preliminary data. The resulting data were analyzed using Design Expert Software v11 (Stat Ease Inc., Minneapolis, USA). All experimental data were analyzed using one-way analysis of variance (ANOVA) to investigate the significance of the involved factors. Statistical significance was set at $p < 0.05$.

Table 1. Range of Experimental Factors

		Factor levels		
Factors	Unit	-1	0	1
pH	-	1	2	3
Power	W	300	450	600
Time	min	2	4	6

3. RESULTS AND DISCUSSION

We investigated the roles of pH, microwave power, and heating time in terms of the pectin extraction % yield by employing a response surface method. We used a 15-run Box-Behnken design (BBD) with three factors, three levels, and three replicates at the center point. The three center point runs were done to account for the inherent variability and provide stability in the data. Table 2 shows the experimental variables, the experimental pectin yield according to the variables, and the predicted pectin yield calculated from the two-factor interaction model (equation 1):

$$\%\ yield = -10.504 + 7.313A + 0.063B + 6.413C - 0.020AB - 1.925AC - 0.002\,BC \qquad (1)$$

where A = pH, B = Power, and C = Time. The table shows that there is good agreement between the experimental yield and the predicted yield.

Table 2. Experimental design and response.

	Factors			Independent variables			Pectin yield (%)	
	A	B	C	pH	Power	Time	actual	predicted
1	1	1	0	3	600	4	13.00	12.87
2	0	1	-1	2	600	2	22.50	20.71
3	0	0	0	2	450	4	24.40	21.42
4	-1	-1	0	1	300	4	26.50	24.60
5	-1	0	1	1	450	6	39.20	37.71
6	1	0	-1	3	450	2	10.10	12.70
7	0	0	0	2	450	4	21.90	21.34
8	1	0	1	3	450	6	13.60	12.75
9	1	-1	0	3	300	4	13.10	13.39
10	0	-1	1	2	300	6	21.90	23.72
11	0	-1	-1	2	300	2	17.10	14.91
12	-1	0	-1	1	450	2	20.30	23.44
13	-1	1	0	1	600	4	38.30	34.40
14	0	1	1	2	600	6	25.50	27.45
15	0	0	0	2	450	4	24.60	21.51

The model capability was determined using the F-test and the determination coefficient R^2. Table 3 shows the analysis of variance for the regression model. The analysis illustrates that the model is highly significant (p-value < 0.01) with an F-value of

26.05. The high F-value and low p-value indicate that the responses were significantly affected by the experimental factors. Moreover, the lack of fit test (0.2524) was insignificant, and the 2FI model fitted well with the experimental data.

Table 3. Analysis of variance for the regression model.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	956.85	6	159.47	26.05	< 0.0001
A-pH	693.78	1	693.78	113.31	< 0.0001
B-Power	53.56	1	53.56	8.75	0.0182
C-Time	114	1	114	18.62	0.0026
AB	35.4	1	35.4	5.78	0.0429
AC	59.29	1	59.29	9.68	0.0144
BC	0.81	1	0.81	0.1323	0.7255
Residual	48.98	8	6.12		
Lack of Fit	44.46	6	7.41	3.27	0.2524
Pure Error	4.53	2	2.26		
Cor Total	1005.83	14			

Table 4 summarizes the statistical analysis of regression errors. The suitability of the regression model was further validated by satisfactory values of the determination coefficient ($R^2 = 0.951$), which indicates that the variability in the responses could be predicted by the model. In addition, the adjusted and predicted R^2 are in reasonable agreement, with a difference of less than 0.2. Lastly, the measure of the signal-to-noise ratio, represented by the adequate precision, was found to be 15.758, indicating an adequate signal, i.e., greater than 4. Thus, the model can predict well the design space.

Table 4. Statistical analysis of regression equation errors.

Statistical item	Values
SD	2.47
Mean	22.13
Coefficient of variation (%)	11.18
R^2	0.9513
Adjusted R^2	0.9148
Predicted R^2	0.7875
Adeq Precision	15.7584

Figure 1a-c shows the three-dimensional (3D) response surface plots and contour plots demonstrating the interaction effects between two of the variables and the pectin extraction yield, while the third variable was maintained at zero level. The optimum values of the variables at the maximum predicted responses were shown as the red areas in the contour plots. From the interaction plot between pH and power (Figure 1a), it can be observed that the variables exhibit hyperbolic behavior, and the highest yield was observed when the power was at the highest and lowest pH. From the interaction plot between pH and time (Figure 1b), it was also observed that the variables exhibit hyperbolic behavior, and the highest yield was observed when the time was the longest and lowest pH. Lastly, from the interaction plot between power and time (Figure 1c), it can be observed that the variable exhibits linear behavior, and the highest yield is obtained at the highest power and longest time.

Generally, the model shows that the highest irradiation time and longest microwave power yield the highest at the lowest pH values. Higher microwave power could help promote the loosening of the plant cell wall, which results in increased penetration of the solvent inside the CM plant matrix, and, consequently, enhances the amount of pectin liberated. The longer the microwave irradiation time, the longer the exposure of the CM plant matrix to the microwave, resulting in the further enhancement of the pectin liberated. Similar outcomes have been reported in the extraction of pectin from the citron peel and other various fruit peels, where longer treatment times would lead to higher adsorption of microwave energy, thus allowing for more pectin to dissolve in the extraction solvent.(Karbuz & Tugrul, 2021; Pasandide et al., 2018) Finally, the pectin yield can be increased further at the appropriate solvent condition. At low pH values, the breakage of the glycosidic bonds of the insoluble pectin in the cell wall is broken, causing the reduction of the molecular weight of the pectin, and it is converted to a soluble form, resulting in a higher extraction yield.(Faravash & Ashtiani, 2007) The same trend was observed in another study that investigated pectin extraction from orange peel and sugar beet pulp where a decrease in pH led to a greater dissolving capacity of the extraction solvent.(Li et al., 2012; Prakash Maran et al., 2013)

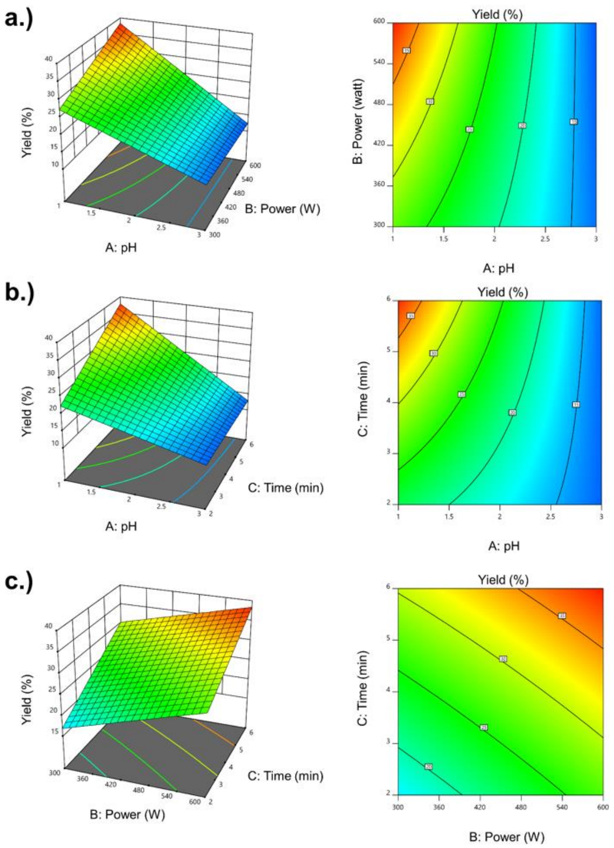

Figure 1. Response fitted surface and contour plots of pectin extraction yield versus a.) pH and power, b.) pH and irradiation time, and c.) microwave power and irradiation time.

4. CONCLUSIONS

In this study, the microwave-assisted extraction of pectin from *Citrus maxima* peels were carried out using the Box-Behnken design experiment to study the effect of (1) solvent pH, (2) microwave irradiation power, and (3) microwave irradiation time on the pectin extraction yield. The results show that the data follows a two-factor interaction model. All independent variables studied were able to explain the variability in the pectin yield, and the model could predict very well the relationship between the variables and the response. It was observed that high microwave irradiation power, long irradiation time, and low solvent pH are the most optimal for a higher pectin yield. This study presents an economical method of pectin extraction from *Citrus maxima* peels with parameters that can easily be adjusted to improve the yield.

5. ACKNOWLEDGMENTS

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