Microwave Assisted Extraction (MAE) and Microwave-ultrasound Assisted Extraction (MUAE) of Pectin from Pomelo Peels

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***Abstract*—In the present study, microwave assisted extraction (MAE) and microwave-ultrasound assisted extraction (MUAE) were employed to recover pectin from pomelo peel. The effects of pH, irradiation time, microwave power, sonication time (only for MUAE) were investigated using Box–Behnken design (BBD) and the extraction condition was optimized. The highest validation experimental yield were 30.24±0.97% for MAE (irradiation time = 11.97 min) and 31.57±0.77% for MUAE (irradiation time = 10.11 min, sonication time = 17.72 min). The findings are agreeable with the predicted yield of 29.37% and 31.11% respectively for MAE and MUAE. It was observed that pH and microwave power have greater effect on extraction of pectin and the microwave irradiation time has slightly been reduced if ultrasound is incorporated. Considering the yield performance, shorter extraction time and less energy intensiveness, MAE is preferred to MUAE for the extraction of pectin from pomelo peel**

***Index Terms*—pectin extraction, ultrasound, microwave, optimization**

I. Introduction

Peels of the citrus family such as orange, lemon, lime and grapefruit and etc are potential source of pectin. Pomelo (*Citrus grandis* (L.) Osbeck) as the largest citrus fruits, is also targeted for pectin extraction. Pectin is an attractive biopolymer material [1] and has widespread applications in pharmaceutical, health, cosmetic, food, and feed industries owing to its good biocompatibility, non-toxicity, and biodegradability as well as high nutritional values such as mineral binding, prebiotic effect, cholesterol regulation, and anti-cancer action. Pectin is a family of heterogeneous polysaccharides with linear backbone comprised of repeating (1 → 4)-linked-α-D-galacturonic acid units [2].

Extraction of pectin is pivotal to biotechnology which involves separation of pectin from the plant matrix. It has been reported that, an ideal extraction method should be simple, safe, reproducible, inexpensive, provide high extraction rates, time saving, non-destructive on extraction compound and suitable for industrial application [3], [4]. Pectin extracted from citrus fruits peels could add value to the citrus processing industry if pectins can be extracted effectively by applying efficient extraction technologies. Many pectin extraction methods have been investigated with the use of acids in traditional heating extraction method. On the other hand, a number of up-to-date alternatives to traditional techniques have been proposed such as ultrasound assisted and microwave assisted extraction method to improve the yield performance, the process efficiency and the quality of the extracted compound [5]. Previous study on ultrasound-microwave assisted extraction (UMAE) of pomelo peel gave satisfactory pectin yield of 38% [6] which has inspired the present investigation on the feasibility of reversing the sequence of ultrasound and microwave techniques on pectin extraction. In this study, MAE and MUAE are optimized and their performances on pectin extraction are investigated. From the comparison study, the effect of ultrasound in the combined MUAE extraction system will be examined.

II. Materials and Methods

A. Materials

Pomelo (*Citrus grandis* (L.) Osbeck) fruit was supplied by Go Chin Pomelo Nature Park, Perak, Malaysia. The peels of the fruit were cut and washed thoroughly with fresh water followed by drying in a hot air oven (Memmert 600, Schwabach, Germany) at 60 ℃ until a constant weight is attained. The peel was powdered using a blender (Faber FBG 460, Kuala Lumpur, Malaysia) and sieved into 250 μm−400 μm. The dried peel powder was stored in dry condition using an air tight container prior to use. All solvents and chemicals used in this study were obtained from R&M (Selangor, Malaysia) and distilled water was used for all extraction and analytical processes.

B. Pectin Extraction Methods

In sole microwave assisted extraction (MAE), 10 g of dried pomelo powder was mixed with 290 mL distilled water and the pH (1.7−2.3) of the mixture solution was adjusted using citric acid. The microwave treatment of the mixture solution was carried out in a microwave oven (ME711K, Suwon, South Korea) and heated under different powers (350−650 W) and irradiation times (4−12 min). After the MAE extraction, the extract was

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filtered using centrifuge (Sigma 3-15P, Osterode am Harz, Germany) operated at 4000 rpm for 10 min. The supernatant was precipitated with 250 mL of 95% (*v*/*v*) ethanol and stored in dark condition at room temperature for 24 hours to allow pectin flotation. The pectin in the sample was subsequently separated by filtration and washed using 70% (*v*/*v*) ethanol twice and then dried in hot air oven at 65 ℃ until a constant weight was attained.

In the combined microwave-ultrasound assisted extraction (MUAE) on pomelo peel, similar aforementioned method was repeated for MAE before the microwave irradiated mixture solution was transferred to an ultrasonic bath (Branson 3800, Danbury, USA) for further extraction under sonication times (12−28 min). The extract from this combined techniques will subject to the same analysis procedure as previously described for MAE.

The percentage of dried pectin yield was determined using (1):

$$\text{Pectin Yield}(\%) = \frac{weight\ of\ dried\ pectin}{weight\ of\ dried\ peel\ powder} \times 100 \quad (1)$$

C. Optimization Study

Three levels Box-Behnken response surface design was employed as shown in Table I to investigate and optimize the effect of process variables on the pectin yield using MAE and MUAE. The variables for MAE were: pH (X_1: 1.7−2.3), microwave power (X_2: 350−650 W) and irradiation time (X_3: 4−12 min). The variables for MUAE were: pH (X_1: 1.7−2.3), irradiation time (X_2: 4−12 min), microwave power (X_3: 350−650 W) and sonication time (X_4: 12−28 min).

The statistical package Design Expert 6.0.6 (State-Ease Inc., Minneapolis, USA) was used to construct the experimental design, regression analysis and numerical optimization. The performance of the process generally can be described by the second-order polynomial equation and the generalized form of the equation is:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_i \sum_{<j=2}^{k} \beta_{ij} X_i X_j \quad (2)$$

where Y represents the response variable, X_i and X_j are the independent variables affecting the response, and β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear term, quadratic term and interaction terms. The effects of process variables was analysed statistically by using analysis of variance (ANOVA) and the adequacy of the predicted optimum conditions was validated with the experimental results.

III. Results and Discussion

A. Optimization Study on the MAE and MUAE Extraction System

The pectin yield ranged from 10.48% to 29.02% for MAE and 10.59% to 30.24% for MUAE. The highest experimental yield was obtained when extraction conditions were pH of 1.7, microwave power of 650 W, irradiation time of 8 min and pH of 1.7, irradiation time of 8 min, microwave power of 650W, sonication time of 20 min.

Table II shows the analysis of variance (ANOVA) for MAE and MUAE yield of pectin with coefficient (R^2) of 0.990 and 0.917 respectively. The results indicated that the model used to fit response variables was significant ($p < 0.0001$) and adequate to represent the relationship between the responses and the independent variables.

Besides, Table II also shows that pH and microwave power exerted most effect on pectin yield for MAE and MUAE with $p < 0.0001$. The pectin yield increases significantly with decrease in pH and increase in microwave power. In MAE, an increase in irradiation time ($p < 0.01$) the pectin yield increased but not for MUAE as mild irradiation time ($p < 0.001$) was preferred.

Three-dimensional response surfaces for MAE and MUAE are shown in Fig. 1(a−c) & 1(d−i) respectively with the effects of the independent variables and their interaction on the yield of pectin. In term of yield, both MAE and MUAE preferred low pH and high microwave power within the range of investigation. MAE required longer irradiation time compare to MUAE, whereas MUAE at later prefer moderate sonication time. With regards to the total extraction time for optimized extraction condition, it is worth noting that microwave irradiation time in MUAE was shortened by mere 1.86 min as compare with the irradiation time in sole MAE but additional sonication time of 17.72 min was required. This could be explained by the effect of ultrasound on the plant surface which has enhanced the extraction performance. Sole MAE with long irradiation time may degrade pectin extracted. Hence, the combined MUAE might be an alternative for pectin extraction as microwave irradiation time would be reduced and the additional ultrasound extraction which does not involve heating will not cause thermal degradation of pectin.

The second-order polynomial equation for predicting pectin yield based on MAE and MUAE are expressed in terms of coded values as shown in Table III. An optimum pectin yield of 29.37% for MAE and 31.11% for MUAE were successfully predicted and the adequacy of the predicted optimum yield was validated. The experimental and the predicted results are very close within percentage error < 10%, indicating that the optimization was reliable.

Comparing between MAE and MUAE in term of pectin yield, there was only 1.74% increase using MUAE method. However, extra 15.86 min was needed which might not be feasible although MUAE might be an option to prevent thermal degradation of pectin as previously described.

TABLE I. Design Matrix of BBD and Pectin Extraction Yield Obtained from MAE and MUAE

Run	Microwave assisted extraction (MAE)		Microwave-ultrasound assisted extraction (MUAE)	
	Independent var.	Dependent var.	Independent var	Dependent var.

	x_1	(X_1)	x_2	(X_2)	x_3	(X_3)	Yield (%)	x_1	(X_1)	x_2	(X_2)	x_3	(X_3)	x_4	(X_4)	Yield (%)
1	0	(2.0)	-1	(350)	1	(12)	14.03	0	(2.0)	1	(12)	1	(650)	0	(20)	23.28
2	1	(2.3)	1	(650)	0	(8)	13.83	1	(2.3)	-1	(4)	0	(500)	0	(20)	11.90
3	1	(2.3)	-1	(350)	0	(8)	10.48	1	(2.3)	0	(8)	0	(500)	-1	(12)	13.46
4	1	(2.3)	0	(500)	1	(12)	13.39	0	(2.0)	1	(12)	0	(500)	-1	(12)	20.96
5	0	(2.0)	1	(650)	-1	(4)	19.24	-1	(1.7)	0	(8)	0	(500)	-1	(12)	24.14
6	0	(2.0)	0	(500)	0	(8)	15.67	1	(2.3)	0	(8)	0	(500)	1	(28)	13.02
7	-1	(1.7)	0	(500)	-1	(4)	21.12	0	(2.0)	0	(8)	0	(500)	0	(20)	21.91
8	0	(2.0)	0	(500)	0	(8)	15.11	0	(2.0)	0	(8)	0	(500)	0	(20)	22.05
9	0	(2.0)	0	(500)	0	(8)	13.78	1	(2.3)	1	(12)	0	(500)	0	(20)	14.62
10	0	(2.0)	1	(650)	1	(12)	20.22	0	(2.0)	0	(8)	-1	(350)	1	(28)	15.17
11	-1	(1.7)	-1	(350)	0	(8)	14.32	-1	(1.7)	0	(8)	1	(650)	0	(20)	30.24
12	-1	(1.7)	0	(500)	1	(12)	24.78	0	(2.0)	-1	(4)	0	(500)	-1	(12)	16.65
13	0	(2.0)	-1	(350)	-1	(4)	11.21	0	(2.0)	-1	(4)	0	(500)	1	(28)	10.59
14	1	(2.3)	0	(500)	-1	(4)	12.93	1	(2.3)	0	(8)	-1	(350)	0	(20)	16.65
15	-1	(1.7)	1	(650)	0	(8)	29.02	0	(2.0)	0	(8)	0	(500)	0	(20)	22.21
16	0	(2.0)	0	(500)	0	(8)	14.95	0	(2.0)	-1	(4)	1	(650)	0	(20)	19.45
17	0	(2.0)	0	(500)	0	(8)	15.45	-1	(1.7)	0	(8)	-1	(350)	0	(20)	20.18
18								0	(2.0)	1	(12)	-1	(350)	0	(20)	16.00
19								-1	(1.7)	0	(8)	0	(500)	1	(28)	25.26
20								1	(2.3)	0	(8)	1	(650)	0	(20)	15.59
21								0	(2.0)	0	(8)	1	(650)	1	(28)	21.19
22								-1	(1.7)	-1	(4)	0	(500)	0	(20)	16.41
23								0	(2.0)	1	(12)	0	(500)	1	(28)	19.98
24								0	(2.0)	0	(8)	0	(500)	0	(20)	19.33
25								-1	(1.7)	1	(12)	0	(500)	0	(20)	23.60
26								0	(2.0)	0	(8)	1	(650)	-1	(12)	23.00
27								0	(2.0)	0	(8)	-1	(350)	-1	(12)	13.73
28								0	(2.0)	-1	(4)	-1	(350)	0	(20)	14.91
29								0	(2.0)	0	(8)	0	(500)	0	(20)	21.22

TABLE II. ANALYSIS OF VARIANCE (ANOVA) FOR REGRESSION MODEL OF PECTIN YIELD OBTAINED FROM MAE AND MUAE

Microwave assisted extraction (MAE)						Microwave-ultrasound assisted extraction (MUAE)					
Source	SS	DF	MS	*F*	*p*	Source	SS	DF	MS	*F*	*p*
Model	382.054	9	42.45	73.58	< 0.0001	Model	545.833	14	38.99	11.01	< 0.0001
X_1-pH	186.342	1	186.34	323.00	< 0.0001	X_1-pH	248.339	1	248.34	70.12	< 0.0001
X_2-microwave power	130.169	1	130.17	225.64	< 0.0001	X_2-irradiation time	67.830	1	67.83	19.15	0.0006
X_3-irradiation time	7.841	1	7.84	13.59	0.0078	X_3-microwave power	108.661	1	108.66	30.68	< 0.0001
$X_1{}^2$	15.204	1	15.20	26.35	0.0013	X_4-sonication time	3.774	1	3.77	1.07	0.3194
$X_2{}^2$	0.002	1	0.00	0.00	0.9579	$X_1{}^2$	4.918	1	4.92	1.39	0.2583
$X_3{}^2$	5.693	1	5.69	9.87	0.0164	$X_2{}^2$	56.861	1	56.86	16.06	0.0013
X_{12}	32.206	1	32.21	55.83	0.0001	$X_3{}^2$	0.710	1	0.71	0.20	0.6613
X_{13}	2.560	1	2.56	4.44	0.0732	$X_4{}^2$	22.459	1	22.46	6.34	0.0246
X_{23}	0.846	1	0.85	1.47	0.2651	X_{12}	4.995	1	5.00	1.41	0.2547
Residual	4.038	7	0.58			X_{13}	30.914	1	30.91	8.73	0.0105
Lack of Fit	1.884	3	0.63	1.17	0.4263	X_{14}	0.608	1	0.61	0.17	0.6848

Pure Error	2.154	4	0.54
Cor Total	386.092	16	
R^2	0.990		
Adj R^2	0.976		

X_{23}	1.877	1	1.88	0.53	0.4786
X_{24}	6.452	1	6.45	1.82	0.1985
X_{34}	2.641	1	2.64	0.75	0.4024
Residual	49.583	14	3.54		
Lack of Fit	43.942	10	4.39	3.12	0.1423
Pure Error	5.640	4	1.41		
Cor Total	595.416	28			
R^2	0.917				
Adj R^2	0.833				

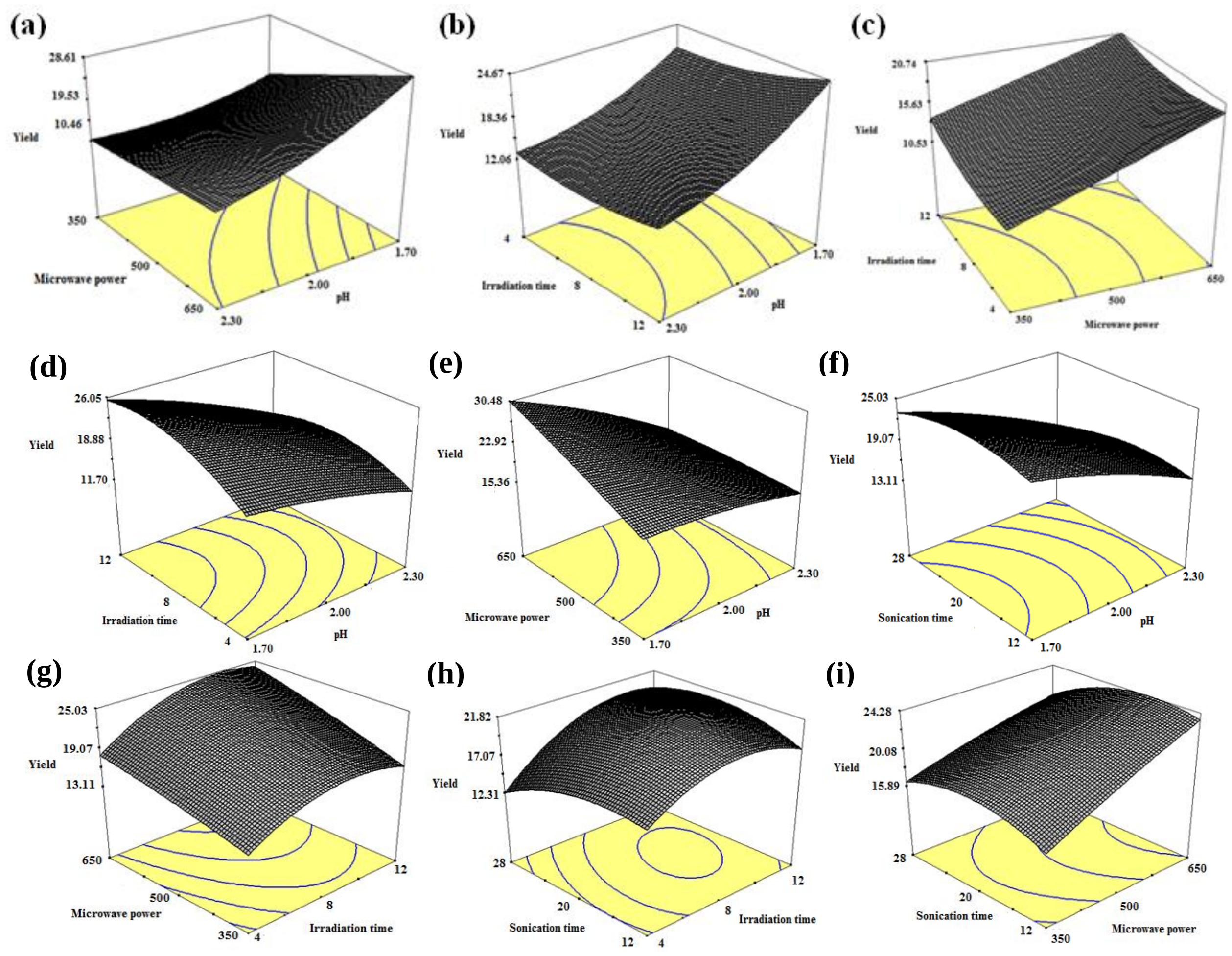

Figure 1. Response surface plots showing the effect of process variable on pectin yield, ((a)−(c)) MAE, ((d)−(i)) MUAE.

TABLE III. VALIDATION OF OPTIMUM EXTRACTION CONDITIONS

	Microwave assisted extraction (MAE)	Microwave-ultrasound assisted extraction (MUAE)
Optimum conditions	pH = 1.74, microwave power = 649.94 W, irradiation time = 11.97 .	pH = 1.73, irradiation time = 10.11 min, microwave power = 649.90 W, sonication time = 17.72 min
Equation Models	$Y = 14.99 - 4.83X_1 + 4.03X_2 + 0.99X_3 + 1.90X_1^2 + 0.02X_2^2 + 1.16X_3^2 - 2.84X_{12} - 0.80X_{13} - 0.46X_{23}$	$Y = 21.34 - 4.55X_1 + 2.38X_2 + 3.01X_3 - 0.56X_4 - 0.87X_1^2 - 2.96X_2^2 - 0.33X_3^2 - 1.86X_4^2 - 1.12X_{12} - 2.78X_{13} - 0.39X_{14} + 0.69X_{23} + 1.27X_{24} - 0.81X_{34}$
Predicted yield (%)	29.37	31.11
Experimental yield (%)	30.24±0.97	31.57±0.77
Percentage error (%)	2.88	1.47

IV. CONCLUSIONS

Optimum pectin yield of 29.37% for MAE and 31.11% for MUAE were obtained from pomelo peel extraction. In both the extraction techniques employed, pH and microwave power demonstrated highest impact on pectin yield. A slight increase in pectin yield using MUAE requires an additional 15.86 min making the combined extraction techniques not particularly suitable for pectin extraction.

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