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Optimization studies on microwave assisted extraction of dragon fruit *(Hylocereus polyrhizus)* peel pectin using response surface methodology

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Abstract

Optimization of microwave assisted extraction of dragon fruit peel pectin was conducted using respond surface methodology. Effect of extraction conditions, i.e. pH value (X_1), extraction time (X_2) and solid-liquid ratio (X_3) on the extraction yield was investigated using a central composite experimental design. Optimization of microwave assisted extraction was performed and three-dimensional (3D) response surface plots were derived from the mathematical models. Analysis of variance (ANOVA) was conducted and indicated a significant interaction between extraction conditions (pH value and extraction time) and extraction yield. The optimum conditions of microwave assisted extraction were as follows: $X_1 = 2.07$; $X_2 = 65$ s and $X_3 = 66.57$. The verification test on pectin extraction was performed and revealed a perfect agreement between experimental and predicted values. The maximum predicted yield of pectin extraction was 18.53%. Overall, application of microwave assisted extraction can give rise to high quality dragon fruit peel pectin.

Introduction

Dragon fruit is the tropical fruit belongs to the Cactaceae family, known as pitaya or pitahaya. Dragon fruit is native to Mexico, Central America and South America; but now widely cultivated as fruit crops in Southeast Asian countries such as Vietnam, Taiwan, Philippines and Malaysia (Haber, 1983; Mizrahi *et al*., 1997). Based on skin and pulp color, dragon fruit can be categorized into three different species, i.e., *Hylocereus undatus* (red skin, white pulp), *Hylocereus costaricensis* (red skin and red pulp) and *Hylocereus megalanthus* (yellow peel and white pulp) (Nerd *et al.*, 2002; Hoa *et al*., 2006). Dragon fruit peel is about a quarter of the fruit mass, and it is discarded as solid waste from food industries (Jamilah *et al*., 2011). Dragon fruit peel is believed to be rich in pectin, i.e., heterogeneous structural polysaccharide contain α-1,4-linked D-galacturonic acid (D-GalA) residues (Ridley *et al.*, 2001).

In this regards, pectin extraction from dragon fruit peel is an innovative technology to minimize waste disposal and to add values to dragon fruit peel. In most cases, however, pectin extraction at acidic pH and high temperature (80–100°C) over an extended time period can cause pectin quality degradation. Therefore, alternative extraction method is required to prevent pectin quality degradation. Microwave assisted extraction (MAE) method is a reliable alternative to conventional extraction techniques, which utilize the microwave radiation to partition target components into the solvent (Eskilsson and Bjorklund, 2000). Microwave assisted extraction method could reduce extraction time, and it is far less compared to conventional extraction technique.

In recent years, microwave assisted extraction technique have been implemented in pectin extraction from apple pomace (Wang *et al*., 2007). Response surface methodology (RSM) has been proposed to model and optimize pectin extraction process. Moreover, RSM have been developed to reduce the experiment runs required for full factorial design (Ozdemir and Devres, 2000). Up to date, numerous researches have been conducted on pectin extraction from dragon fruit peel (Tang *et al*., 2011; Ismail *et al*., 2012). However, microwave assisted extraction technique have not been implemented in pectin extraction from dragon fruit peel. Therefore, in this research, microwave assisted extraction technique was implemented in pectin extraction from dragon fruit peel. Response surface methodology (RSM) was developed to optimize pectin extraction process. Thereafter, physicochemical analyses were conducted to characterize pectin extracted from

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Table 1. Independent variables and their levels for central composite design (CCD)

Independent variable	Symbol	levels				
		-2	-1	0	1	2
pH solution	X_1	1	1.5	2	2.5	3
Extraction time (s)	X_2	20	35	50	65	80
Solid-liquid ratio	X_3	30	50	70	90	110

Table 2. Chemical composition of dragon fruit peel powder (g/100 g dry sample)

Contents	Dragon fruit peel powder [a]
Moisture	5.2 ± 0.1
Ash	12.1 ± 0.2
Fat	0.8 ± 0.1
Protein	6.6 ± 0.04
Crude fiber	2.1 ± 0.1
Total carbohydrate	73.2 ± 0.1
Total dietary fiber (IDF)	70.1 ± 0.5
Insoluble dietary fiber (IDF)	56.5 ± 0.3
Soluble dietary fiber (SDF)	14.4 ± 0.4

[a] Means ± SD (n = 3).

dragon fruit peel.

Materials and Methods

Materials

The dragon fruits (Hylocereus polyrhizus) were kindly supplied by MARDI (Malaysian Agricultural Research and Development Institute). All solvents and chemicals used were analytical grade and purchased from Malaysia Sigma–Aldrich.

Preparation of dragon fruit peel

The sample preparation method was adapted from Ismail *et al,* (2012). The dragon fruits were washed and the peels were separated from the pulp. The peels were oven dried in air-circulate oven (Memmert, Germany) at 50°C until reach constant weight. The dried peels were milled in an electric grinder (Basic IKA Werke Mill MF 10, Germany). The dried powder were sieved (60-mesh size screen) and stored in a polyethylene sealed bag.

Characterization of dragon fruit peel

The moisture, ash, fat, protein (N × 6.25), crude fiber, dietary fiber and total carbohydrate content were determined by AOAC methods (AOAC, 1992; AOAC, 2000). All proximate values were expressed as g/100 g (dry basis).

Microwave-assisted extraction of dragon fruit peel pectin

The extraction procedure was adapted from Fishman and Chau (2000). The extraction process was done in microwave oven with frequency 2450 MHz (Samsung Model 71B, Malaysia). To minimize the solvent evaporation, the beaker was connected with a glass condensation device. The dried dragon fruit peel powder (1 g) was suspended in HCl solution (pH 1 to 3) at solid-liquid ratios ranged from 1:30 to 1:110 (w/v). The suspension was heated in microwave oven (800 W) at extraction times varied from 20 to 80 s. The suspension was centrifuged at 12000 rpm for 10 min, and then insoluble residue was recovered and precipitated with isopropanol (70%). The obtained pectin was filtered and then purified with isopropanol (99.6%). The purified pectin was dried in an air-circulate oven at 45°C until reach constant weight. The dried powder were sieved (60-mesh size screen) and stored for subsequent analysis. The extraction yield (Y) was calculated as follows:

$$\text{Yield of pectin (\%)} = \left({}^{w_0}/_{w}\right) \times 100 \quad (1)$$

Where, w_0 (g) is the dried weight of the final product and w (g) is the dried weight of raw material.

Optimization of experimental design

Central composite design (CCD), 20 experimental points (including six central point replicates, eight factorial points and six axial points), was chosen to evaluate the combined effect of the independent variables. The independent variables were pH of HCl solution (X_1), extraction time (X_2) and solid-liquid ratio (X_3), while the response variable was the extraction yields at 800 W microwave power levels. Each variable were adopted and coded to −2, −1, 0, 1 and 2. The factors and respective coded and uncoded levels are presented in Table 1. The second-order polynomial regression model was applied to generate predicted responses. The second-order polynomial regression model is expressed as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum\sum_{i<j} \beta_{ij} X_i \quad (2)$$

Where Y is predicted response, k is the factor number, and are regression coefficients and are the independent variables (factors). Analysis of variance (ANOVA) was conducted using Design Expert software (Stat-Ease, Inc., Minneapolis, MN, USA) to generate regression equations and regression coefficients.

Table 3. Observed and predicted extraction yields of dragon fruit peel pectin

Std	Independent variables			Observed	Predicted
	X_1	X_2	X_3		
1	1.5 (-1)	35 (-1)	1:50 (-1)	11.84	12.06
2	2.5 (1)	35 (-1)	1:50 (-1)	16.70	16.23
3	1.5(-1)	65 (1)	1:50 (-1)	15.70	16.32
4	2.5 (1)	65 (1)	1:50 (-1)	18.20	18.59
5	1.5 (-1)	35 (-1)	1:90 (1)	11.20	11.39
6	2.5 (1)	35 (-1)	1:90 (1)	13.91	13.87
7	1.5 (-1)	65 (1)	1:90 (1)	15.23	16.27
8	2.5 (1)	65 (1)	1:90 (1)	16.49	16.85
9	1 (-2)	50 (0)	1:70 (0)	12.91	12.16
10	3 (2)	50 (0)	1:70 (0)	16.74	16.91
11	2 (0)	20 (-2)	1:70 (0)	10.10	10.44
12	2 (0)	80 (2)	1:70 (0)	18.59	17.68
13	2 (0)	50 (0)	1:30 (-2)	16.10	16.01
14	2 (0)	50 (0)	1:110 (2)	14.08	13.60
15	2 (0)	50 (0)	1:70 (0)	17.35	17.40
16	2 (0)	50 (0)	1:70 (0)	17.98	17.40
17	2 (0)	50 (0)	1:70 (0)	17.57	17.40
18	2 (0)	50 (0)	1:70 (0)	16.72	17.40
19	2 (0)	50 (0)	1:70 (0)	17.10	17.40
20	2 (0)	50 (0)	1:70 (0)	18.23	17.40

X_1= pH solution, X_2= Extraction time, X_3= Solid-liquid ratio

Characterization of dragon fruit peel pectin

The moisture, ash and protein (N × 6.25) contents were determined by AOAC methods (AOAC, 2000). All proximate values were expressed as g/100 g (dry basis). The galacturonic acid (GalA) content was determined by a colorimetric assay according to Blumenkrantz and Asboe-Hansen (1973).

The degree of esterification (DE) was assessed by a titrimetric method according to the Food Chemical Codex (FCC V, 2004) with a slight modification. The dried pectin (200 mg) was moistened first with 2 ml ethanol and dissolved in 20 ml distilled water at 40°C for 2 h. The solution was titrated with 0.1 M NaOH. The titration volume was recorded as the initial titer. The solution was mixed with 0.1 M NaOH (10 ml) and allowed to stand for 15 min; and then mixed with 0.1 M HCl (10 ml). The solution was shaken until the pink color disappeared. Excess HCl was titrated with 0.1 M NaOH to a faint pink color (endpoint). The titration volume was recorded as the saponification titer (the final titer). The DE was calculated as follows:

$$DE\ (\%) = \frac{\text{The final titer}}{\text{The initial titer + the final titer}} \times 100 \qquad (3)$$

Structural characteristic was determined by the FTIR method according to Singthong *et al*. (2004). The dried pectin was encapsulated in KBr at a 1:100 ratios. The encapsulated pectin was scanned using FTIR (Perkin Elmer Spectrum GX, USA) over the spectral range 400–4000 cm^{-1} at spectral resolution 4 cm^{-1}.

Results and Discussion

Chemical composition of dragon fruit peel

The chemical composition of dragon fruit peels is shown in Table 2. Total dietary fiber (TDF) of dragon fruit peels was 70.1 %. Based on TDF, the IDF of dragon fruit peels was 56.5%, while SDF of dragon fruit peels was 14.4%. SDF of dragon fruit peel could attribute to pectin. The TDF of the dragon fruit peel was comparable to those obtained from apple (60.1% TDF, 46.3% IDF, and 13.8% SDF) (Grigelmo-Miguel and Martin-Belloso, 1999). The SDF of dragon fruit peel was comparable to those obtained from other byproducts such as apple (13.8%), pear (14.1%), orange (13.6%) and artichoke (14.3%), and much higher than those obtained from wheat bran (2.9%) and oat bran (3.6%) (Grigelmo-Miguel and Martin-Belloso, 1999).

Measurements of response functions

Experimental and predicted extraction yields of dragon fruit peel pectin are shown in Table 3. The highest extraction yield of dragon fruit peel pectin (18.59%) was achieved at pH 2, extraction time (heating time) 80 s and 1: 70 solid-liquid ratios. The lowest extraction yield of dragon fruit peel pectin (10.1%) was observed at pH 2, extraction time (heating time) 20 s and 1:70 solid-liquid ratios. The difference in extraction yield was 8.49% at extraction time 20 and 80 s. According to Bagherian *et al*. (2011), extraction yields of grapefruit pectin were significantly increased with an extraction time.

Table 4. Analysis of variance (ANOVA)

Source	Sum of square	DF	Mean square	F value	Prob > F
Yield (%)[a]					
Model	113.10	9	12.57	23.06	< 0.0001
X_1	22.54	1	22.54	41.37	< 0.0001
X_2	52.38	1	52.38	96.14	< 0.0001
X_3	5.82	1	5.82	10.68	0.0085
X_1^2	12.85	1	12.85	23.58	0.0007
X_2^2	17.52	1	17.52	32.16	0.0002
X_3^2	10.57	1	10.57	19.41	0.0013
$X_1 X_2$	1.81	1	1.81	3.33	0.0980
$X_1 X_3$	1.44	1	1.44	2.64	0.1355
$X_2 X_3$	0.20	1	0.20	0.36	0.5627
Residual	5.45	10	0.54		
Lack of fit	3.89	5	0.78	2.50	0.1691
Pure error	1.56	5	0.31		
Total	118.55	19			

[a] The coefficient of determination (R^2) = 0.9540.
X_1= pH solution, X_2= Extraction time, X_3= Solid-liquid ratio

Optimization of response functions

The results of ANOVA are presented in Table 4. The model of second-order response surface is statistically significant ($p < 0.0001$) and the residuals are randomly distributed along a straight line. The adequacy and quality of the fitted polynomial model were estimated by the coefficient of determination (R^2), which represents the proportion of the variability in the response variables. The coefficient of determination (R^2) was 0.9540, thus a regression model of second-order response surface is best fits to the actual data and only 4.60% of the respond variation is not explained by the model. Lack of fit ($p > 0.05$) had no significant effect on the response, which indicate the models demonstrate fit the data well in the experimental domain or models variations cannot be accounted for random error (Montgomery, 2001).

The change of pH (X_1) and extraction time (X_2) had significant effect on the response function ($P < 0.0001$). Linear (X_1, X_2 and X_3) and quadratic (X_1^2, X_2^2 and X_3^2) coefficients of the model were significant ($p < 0.05$). However, interaction (X_1X_2, X_1X_3 and X_2X_3) coefficients of the model were not significant ($p > 0.05$). The model of second-order response surface was derived and given in Eqs. (4). All independent variables are coded as X_1, X_2 and X_3.

$$Y = 17.40 + 1.19X_1 + 1.81X_2 - 0.60X_3 - 0.48X_1X_2 - 0.42X_1X_3 + 0.16X_2X_3 - 0.71X_1^2 - 0.83X_2^2 - 0.65X_3^2 \quad (4)$$

Where Y is the response value, X_1, X_2 and X_3 are pH, extraction time and solid-liquid ratio, respectively.

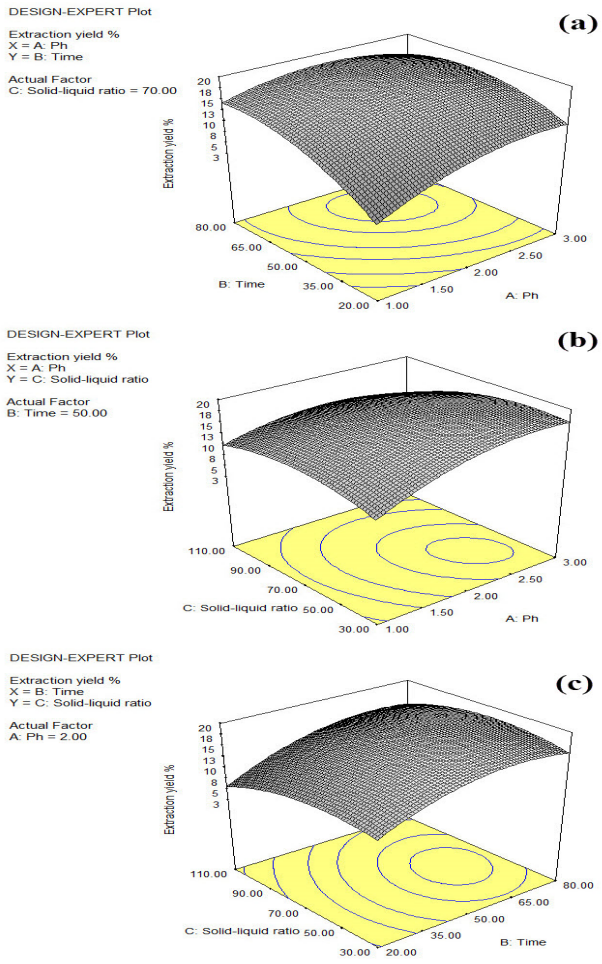

Figure 1. Response surface graphs of extraction yields: (a) effects of extraction time and pH solution at constant solid-liquid ratio (1:70); (b) effects of pH solution and solid-liquid ratio at constant extraction time (50 s); (c) effects of the extraction time and solid-liquid ratio at constant pH solution (2)

Analysis of response surface

Model of second-order response surface (Eqs. 4) is proposed to predict the effects of the three

Table 5. Predicted and experimental extraction yields of dragon fruit peel pectin obtained at optimum conditions

Optimum conditions		Predicted yield (%)	Experimental yield[a] (%)
pH value	2.07		
Extraction time (s)	65	18.53	18.50 ± 0.08
Solid-liquid ratio	66.57		

[a] means ± standard deviation of triplicate determinations.
X_1= pH value, X_2= Extraction time, X_3= Solid-liquid ratio

Table 6. Characteristic of dragon fruit peel pectin extracted at the optimized condition

Characteristics	Pectin samples	
	Dragon fruit	Citrus pectin
Moisture (%)	4.09 ± 0.10a	3.58 ± 0.08b
Ash (%)	8.67 ± 0.14a	1.37 ± 0.05b
Protein (%)	5.51 ± 0.13a	1.11 ± 0.11b
GalA (%)	67.52 ± 0.95b	85.40 ± 0.72a
Degree of esterification (%)	46.95 ± 0.97b	54.25 ± 1.78a

parameters on response function. The relationships between the experimental variables and the response are illustrated graphically in three-dimensional (3D) response surface plots (Figure. 1), i.e., one independent variable kept constant at the center point (corresponding to 0 levels in coded level), other two independent variables varied within the experimental range on the X and Y axes and response function kept constant on the Z axis.

The interactive effects of pH solution and extraction time at constant solid-liquid ratio (1:70) are shown in Figure 1a. Interactive effects of pH value and extraction time had significant effects the pectin extraction. According to Wai *et al*. (2010), the interactive effects of pH and extraction time had significant influence the pectin extraction from durian rind. Increase in pH solution from 1 to 2, and subsequent increase in extraction time from 20 to 70 s had significant increased the extraction yields. However, further increase in pH value from 2 to 3 had reduced the extraction yields. According to Pagán *et al*. (2001), increase in extraction time can significant enhanced the extraction yields. The interactive effects of pH solution and solid-liquid ratio at constant extraction (50s) are shown in Figure 1b. Increase in pH value from 1 to 2 had increased the extraction yields. On the other hand, increase in pH value from 2 to 3 had decreased the extraction yields. Also, increase in solid-liquid ratio from 1:70 to 1:110 has significantly decreased the extraction yields.

The interactive effects of extraction time and solid-liquid ratio at constant pH value (pH 2) are shown in Figure 1c. Increase in extraction time had increased the extraction yields, i.e., influence of microwave radiation can lead to higher extraction yields. Increase in the solid-liquid ratio from 1:30 to 1:70 had increased the extraction yield up to 18.5%. However, further increase in solid-liquid ratio had significant reduced extraction yields. Increase in solid-liquid ratio can cause additional burden to microwave irradiation power and thus decrease the extraction yield (Li *et al*., 2012).

Characterization of pectin obtained at optimized condition

Comparison of citrus pectin and dragon fruit peel pectin (extracted under optimal conditions) is shown in Table 6. According to FAO and EU regulation, galacturonic acid (GalA) content of high quality pectin must be at least 65% (Willats *et al*., 2006). The GalA content of dragon fruit peel pectin was 67.52%, which meet the high quality standard. Compared with other non-commercial pectin, the GalA content of dragon fruit peel pectin was higher than those obtained from carrot tissues, green tea leaves, canna edulis Ker residue and sugar beet; and comparable to those obtained from fresh orange peels and grapefruit peels (Kratchanova *et al*., 2004; Bagherian *et al*., 2011; Ele-Ekouna *et al*., 2011; Funami *et al*., 2011; Zhang et al., 2011; Ngouémazong *et al*., 2012). The degree of esterification of dragon fruit peel pectin was 46.95%, which further confirm by FTIR analysis. The degree of esterification of dragon fruit peel pectin is less than 50%, which considered as low methoxyl (LM).

FTIR analysis of dragon fruit peel pectin was performed to elucidate the main functional groups. FTIR spectra of the dragon fruit and citrus pectin were shown in Figure 2. FTIR spectra of dragon fruit peel and citrus pectin were similar, and thus confirm the extracted polysaccharide is pectin. The absorption bands in the region between 800 and 1300 cm^{-1} were corresponded to fingerprint region. The absorption bands in the region between 1000-2000 cm^{-1} were corresponded to major functional groups presented in pectin (Gnanasambandam and Proctor,

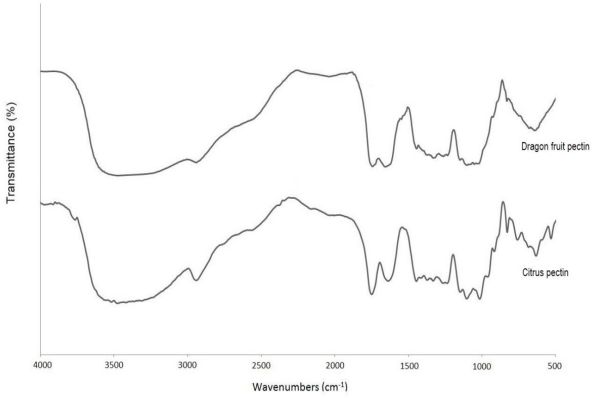

Figure 2. FTIR spectra of dragon fruit peel pectin and citrus pectin

2000). The broad absorption band in the region between 2400 and 3600 cm^{-1} was attributed to OH stretching vibration. The absorption bands in the region around 2950 cm^{-1} were assigned to the C–H stretching vibration of methyl ester in galacturonic acid (Gnanasambandam and Proctor, 2000; Monsoor, 2005). The absorption bands in the region 1745 and 1645 cm^{-1} were ascribed to methyl esterified carbonyls (C=O) and asymmetric carboxylate anions (COO-) stretching vibration. Strong absorption band in COO- couple with a weak absorption band in C=O were ascribed to low methoxyl pectin (Chatjigakis *et al*., 1998; Gannasin *et al*., 2012). Strong absorption band in COO^- couple with a weak absorption band in C=O were observed in dragon fruit peel pectin; however, it is reverse for citrus pectin. The DE was calculated as follows (Monsoor *et al.*, 2001):

Based on the FTIR analysis, the DE of dragon fruit peel and citrus pectin were 49.84% and 53.62%, respectively. The DE of dragon fruit peel and citrus pectin were comparable to DE values determined by the direct titrimetric method (pectin: 46.95%; citrus pectin: 54.25%).

Conclusion

The microwave-assisted extraction of dragon fruit peel pectin could be an efficient alternative to conventional extraction techniques. The extraction conditions of dragon fruit peel pectin i.e., pH and extraction time were shown to have significant effects on extraction yield. According to the RSM model, the optimum extraction conditions of dragon fruit peel pectin were achieved at pH solution 2.07, extraction time 65 s and solid-liquid ratio 66.57. Based on the comparison to citrus pectin, the dragon fruit peel pectin was proven to exhibit a high quality of properties and could be used in food industries.

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