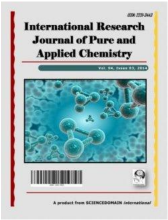

International Research Journal of Pure & Applied Chemistry

17(3): 1-10, 2018; Article no.IRJPAC.46089
ISSN: 2231-3443, NLM ID: 101647669

Microwave Assisted Extraction: A Technological Alternative for Valorization of Orange Peel for Pectin Extraction

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2018/46089
Editor(s):
(1) Dr. Hao-Yang Wang, Department of Analytical, Shanghai Institute of Organic Chemistry, Shanghai Mass Spectrometry Center, China.
Reviewers:
(1) Takeshi Nagai, Graduate School of Yamagata University, Japan.
(2) Kadja Amani Brice, Université Nangui Abrogoua, Côte d'Ivoire.
(3) Arvind Kumar, CSIR-CSMCRI, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/28180

Original Research Article

Received 11 October 2018
Accepted 21 December 2018
Published 09 January 2019

ABSTRACT

Pectin, a component of the plant cells, exists in the primary cell wall along with the middle lamella of plant tissues and is found mostly in citrus fruit peels. Peels of orange is an important source of pectin which is utilized as an emulsifying, gelling and thickening agent in varied food products. Currently, extraction of pectin is done through hot water at low pH for hours which is a time-consuming process. However, microwave extraction has verified to boost the pectin yield in lesser time. This study highlights the ability of microwave assistance in extraction of pectin from orange peels. Extraction of pectin was done with the varied combinations of microwave power (160, 320, and 480 W), microwave time (1, 2 and 3 min) and pH of citric acid solution (pH: 1, 1.5, 2). To analyze the outcome of microwave power, microwave time and pH on pectin yield, Box Behnken design of Response Surface Methodology was used. The optimized conditions, as per desired

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function test, determined for the peak pectin yield as 24.00% were; microwave power of 478 W, microwave time of 2.2 min and pH of 1.05. The validation test conducted on pectin extraction was found in acceptance with the optimized conditions.

Keywords: Pectin; orange peel; microwave assisted extraction.

1. INTRODUCTION

In citrus fruits, mostly orange, the peel represents the major waste part discarded as by-product of juice production industry, hampering the environment and economy substantially [1]. Therefore, either finding a substitute approach to utilize the peels into beneficial products or to dispose them off is the main goal for seeking a positive environmental impact [2]. Citrus peel comprises of 25–30% of pectin, thus being the prime source for viable food applications [3].

Orange peel is abundant in pectin and henceforth considered as a vital raw material for developing value-added products [4]. It is an essential functional food ingredient used mostly as gelling, thickening and stabilizing in varied foodstuff which include fruit juice, jams, jellies and confectionery [5]. Citrus peel and apple pomace are the key sources of agro wastes used to produce pectin commercially [6]. It is important polysaccharides that are largely extracted from agro wastes. Pectin, a complex polysaccharide, is chiefly composed of α-1,4-linked-*D*-galacturonic acid (Gal A) chains in which the carboxyl groups of the Gal A can be free, or methyl esterified [7]. It has a good property of gelling, emulsification and absorption, along with the function of anticancer and treating diabetes, and because of these attributes pectin is widely utilized in the field of cuisine, nutrition, pharmacy, cosmetic and the water quality disposal [8].

Current pectin processing industries mainly focus on fruit peels and by-products from various juicing industries which process loads of citrus fruits (orange, lemon and lime) globally every year [9]. Processing of pectin involves pre-treatments i.e., washing, drying and extraction at very high temperature by acid hydrolysis. Extraction is carried out using two categories of solvents i.e., inorganic acid solvents like hydrochloric, nitric or sulfuric acid and organic acid solvent for example acetic acid, oxalic, tartaric or citric acid [10]. Commercially pectin is extracted by help of hot water (60–100°C) and the pH (1.5 to 3.0) for hours giving limited yield [11]. However, microwave extraction proves to be efficient by restraining the time of extraction and producing enhanced pectin yield with minimum solvent consumption. Pectin extracted utilizing microwave and tartaric acid as solvent from passion fruit gave higher yield, also saved energy [12].

Recognizing the potential of microwave assistance in extraction, this study emphasizes on pectin extraction from orange peels by help of citric acid solution as solvent. The key goal of this investigation was to develop an effective approach that would provide an improved comprehension of the interrelations between the variables i.e., microwave power, microwave time and pH of citric acid solution and the response i.e., pectin yield and to obtain optimal conditions for maximizing the extraction of pectin from orange fruit peel. The study expects an effective microwave extraction technique, for the enhanced yield of pectin from orange peels, which may have further implementation in the food processing industry.

2. MATERIALS AND METHODS

2.1 Materials

Orange peels, byproduct of juice (citrus fruits) extraction, were obtained from juice extraction unit of local market Pantnagar (29.0222° N, 79.4908° E), Uttarakhand (India). Analytical grade chemicals were used for the experiment. Citric acid was mixed in varied proportions with deionized water in preparing the acidic solvent of different pH for pectin extraction.

2.2 Methods

2.2.1 Pretreatment of raw material

Orange peels were cleaned and washed to eradicate any foreign material. Washed and conditioned orange peels were dried in a solar drier at 45°C for 8 h followed by milling using hammer mill to 1 mm size powdered orange peel, making it convenient for pectin extraction. This orange peel powder as the raw material was packed in zip lock bags and stored in the refrigerator for further experiments to be done for pectin extraction.

2.2.2 Pectin extraction using microwave assisted extraction

Pectin was extracted with different combinations of microwave power (160,320,480 W), pH of citric acid solution (pH: 1, 1.5, 2) and microwave time (1, 2, 3 min). Three different solutions of citric acid of pH: 1, 1.5 and 2 were prepared by adding citric acid to the deionized water till the required pH is attained. In each experiment, orange peel powder (10 g) was mixed with fixed quantity of citric acid solution (200ml) and pH for solution was kept as 1, 1.5 and 2. It was then treated in the microwave oven (LG, MC-7148 MS) at working frequency of 2450MHz for 1, 2, and 3 minutes at the power 160, 320 and 480 W. After microwave treatment, the mixture of peel powder and citric acid solution was cooled to room temperature and then filtered through cheese cloth. The filtrate thus recovered after filtration was then centrifuged for 10 min at 8000 rpm [13]. Propanol was utilized to precipitate the supernatant and then left untouched for an hour with the intention of letting the pectin flotation [14]. The floating pectin was then isolated by filtration and washed with propanol. The jelly type of material thus recovered is the wet pectin and this wet pectin was then dried in tray drier at 45°C. After drying, the dry pectin mass was milled to powdered pectin form and stored for analysis and other usage in varied food products. The yield of pectin was calculated by the following formula:

$$\text{Yield (\%)} = (X / Y) \times 100 \qquad (1)$$

Where,

X is the mass of pectin extracted from the sample in gram (g) and

Y is the mass of orange peel powder sample taken up for extraction in gram (g).

2.3 Experimental Design

Response Surface Methodology is an assembly of mathematical and statistical methods that can be utilized to portray the relations between the independent and response variables. It also describes the outcome of the independent variables or combination of variables on the various procedures. Box-Behnken design was used in this study to develop the experimental design. Box-Behnken Designs is a set of rotatable or nearly rotatable second-order designs built on three-level incomplete factorial designs [15]. The number of experiments (N) for the commencement of BBD is defined as:

$$N = 2K (K-1) + C \qquad (2)$$

Where K is number of factors and C is the number of central points. For 3 factors at three levels, BBD had 17 experiments with 5 experimental runs at central points.

In this research, evaluation of the independent variables for extraction of pectin from orange peel was executed with the support of Design Expert 10 (Stat-Ease Inc, USA) Software. Box-Behnken design was implemented for assessing the consequence of microwave power (A), microwave time (B) and pH (C) on the pectin extraction from orange peel. Pectin yield was estimated as the response variables. Table 1 represents the values of independent parameters, Table 2 represents the constant parameters and Table 3 shows the whole experimental design which consisted of 17 runs.

Table 1. Independent parameters and their levels

Independent parameter	**Coded value**	**Value of levels**		
		-1	**0**	**+1**
Microwave Power (Watt)	A	160	320	480
Microwave Time (minute)	B	1	2	3
pH of Citric acid solution	C	1	1.5	2

Table 2. Values of constant parameters

Parameter	**Value**
Solvent volume per experiment	200 ml
Sample particle Size	1 mm
Sample weight per experiment	10 g

Table 3. Experimental design table

Exp no.	Coded levels			Actual levels		
	A	B	C	Microwave power (W)	Microwave time (min)	pH of citric acid solution
1	-1	0	1	160	2	2
2	0	0	0	320	2	1.5
3	-1	-1	0	160	1	1.5
4	0	1	1	320	3	2
5	0	0	0	320	2	1.5
6	-1	1	0	160	3	1.5
7	0	0	0	320	2	1.5
8	0	0	0	320	2	1.5
9	0	-1	1	320	1	2
10	-1	0	-1	160	2	1
11	0	0	0	320	2	1.5
12	0	1	-1	320	3	1
13	0	-1	-1	320	1	1
14	1	1	0	480	3	1.5
15	1	0	1	480	2	2
16	1	-1	0	480	1	1.5
17	1	0	-1	480	2	1

2.4 Data Analysis and Adequacy of Model

The model development was done by help of response surface methodology using Design Expert software. Complete second order model was set to the data and the model competence was verified using fisher's F-test and R^2 (coefficient of multiple determination). The parametric effect on several responses was done through the study of developed models. Regarding three independent variables, a second order response function has the following general form

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2 \quad (3)$$

Where, β0, βi, βij are constants Xi, Xj are the values of independent variables.

Response function was developed through multiple regression analysis which was utilized to verify the experimental data and obtain variable parameters optimized corresponding to best result. The standards of connected statistics and model coefficients in correlations of P-value and lack of fit were acquired through the program. The value of P signifies the chance of significance. A peak F-value confirmed the inadequacy of the model as the lack of fit is significant. A model with lower values of P is considered better. The models having P-value lower than 0.1 (indicating the lack of fit is unimportant at 90% confidence level) were acknowledged. The outcomes of regression analysis are obtained in terms of ANOVA, regression coefficient and associated statistics, standard deviation, coefficient of determination (R^2), lack of fit, etc. These determine adequacy of predictive model and effect of independent parameters on the response.

2.5 Optimization and Validation

Optimization was done using numerical optimization method. Design Expert software presented an appropriate arrangement of microwave power, time and pH to have an optimal value for the pectin yield. All the independent variables were set within the range and pectin yield was set for maximization. The desirability function method was incorporated in the optimization process. This numerical optimization method estimates a point that amplifies the desirability function. After optimization, appropriateness of the model equation was confirmed with experimental outcomes for expecting the optimal response. The polynomial model generated through response surface methodology was confirmed by performing 3 trials at the numerically optimized point having maximum desirability.

3. RESULTS AND DISCUSSION

3.1 Experimental Design and Statistical Analysis

To expand the microwave assisted extraction process for enhanced pectin yield, parameters studied were microwave power (160, 320 and 480 W), microwave time (1, 2 and 3 min) and pH of citric acid solution (1, 1.5 and 2). The quantity of pectin obtained during microwave assisted extraction is shown in Table 4. The pectin yield ranged from 11.03 % to 23.72 % from 10 g sample amount over entire experimental conditions.

The experimental data was studied to witness the consequences of the process parameters on the pectin yield. The outcomes of study of variance for pectin yield are given in Table 5. F-value was used to depict the significance of linear, quadratic and interactive terms. F_{cal} value for model was superior to F_{tab}, which implies that model was significant ($p < 0.01$). The result of independent variables on pectin yield was found significant at linear terms at 1% level of significance ($F_{cal} > F_{tab}$), quadratic along with the interactive terms.

Table 4. Pectin yield as affected by experimental conditions

Exp. no	Microwave power (W)	Microwave time (min)	pH of citric acid solution	Pectin yield (%)		
				Actual	Predicted	RD (%)
1	160	2	2	11.03	11.33	-2.71
2	320	2	1.5	20.15	20.51	-1.78
3	160	1	1.5	11.44	11.44	0.00
4	320	3	2	14.33	14.05	1.95
5	320	2	1.5	20.33	20.51	-0.88
6	160	3	1.5	14.11	14.08	0.21
7	320	2	1.5	20.53	20.51	0.09
8	320	2	1.5	20.73	20.51	1.07
9	320	1	2	11.69	11.39	2.56
10	160	2	1	21.86	21.58	1.28
11	320	2	1.5	20.85	20.51	1.63
12	320	3	1	22.36	22.66	-1.34
13	320	1	1	16.18	16.45	-1.66
14	480	3	1.5	21.13	21.13	-0.85
15	480	2	2	19.73	20.00	-1.36
16	480	1	1.5	14.88	14.90	-0.13
17	480	2	1	23.72	23.42	1.26

**Relative deviation (RD) = [(actual value – predicted value)/actual value] x 100*

Table 5. Analysis of variance (ANOVA) for pectin yield

Source	Sum of squares	DF	Mean square	F-value	p-value
Model	283.48	9	31.50	222.75	< 0.0001*
A-Microwave Power	55.23	1	55.23	390.58	< 0.0001*
B-Microwave Time	39.34	1	39.34	278.20	< 0.0001*
C-pH	93.43	1	93.43	660.76	< 0.0001*
AB	3.20	1	3.20	22.66	0.0021*
AC	11.70	1	11.70	82.72	< 0.0001*
BC	3.13	1	3.13	22.16	0.0022*
A^2	5.02	1	5.02	35.47	0.0006*
B^2	68.60	1	68.60	485.16	< 0.0001*
C^2	0.49	1	0.49	3.47	0.1047
Residual	0.99	7	0.14		
Lack of Fit	0.66	3	0.22	2.71	0.1795
Pure Error	0.33	4	0.082		
Cor Total	284.47	16			
R^2	0.9965				
Adj R^2	0.9920				
Pred R^2	0.9609				

** significant at 1% level of significance respectively.*

Experimental data was fitted to the second order model using multiple regression analysis and the results are given in the Table 5. The statistical analysis displays that the projected model was sufficient, showing significant fit having very reasonable values of R^2 for pectin yield. The R^2 value for the yield of pectin was 99.65 %, which shows that the model could account for 99.65 % data. Closer the value of R^2 to unity, the improved the empirical model fits the actual data. Insignificant value of lack of fit verified that the model developed is valid. The second order polynomial equation was developed which represents response, pectin yield as functions of microwave power, pH of citric acid solution and microwave time. An empirical relationship among the responses and input variables in coded form can be stated by the following equation:

$$Y = 20.52 + 2.63A + 2.22B - 3.42C + 0.89AB + 1.71AC - 0.88BC - 1.09A^2 - 4.04B^2 - 0.34C^2 \quad (4)$$

Where, Y is pectin yield, A, B and C are the coded values of of microwave power, microwave time and pH of citric acid solution respectively.

Different diagnostic plots were used to analyse the adequacy of the model for example normal % probability, predicted versus actual and internally studentized residuals are shown in Fig. 1. The predicted values and the experimental values lie almost contiguous to the straight line which indicated the reasonable agreement with real data (Fig 1a). Fig 1b shows the normal % probability plot of residuals for response was normally distributed, lying nearby to the straight line and shows no deviation of the variance. Fig. 1c shows the internally studentized residuals plot which proves the adequacy of the fitted model as all the data points lie within the limits.

3.2 Effect of Process Parameters on Pectin Yield

Model of second-order response surface (Equation 4) is proposed to project the outcomes of the three parameters on response. The relationships between the variables and the response are illustrated graphically in three-dimensional (3D) response surface plots (Figs. 2a, 2b, and 2c).

3.2.1 Effect of microwave power

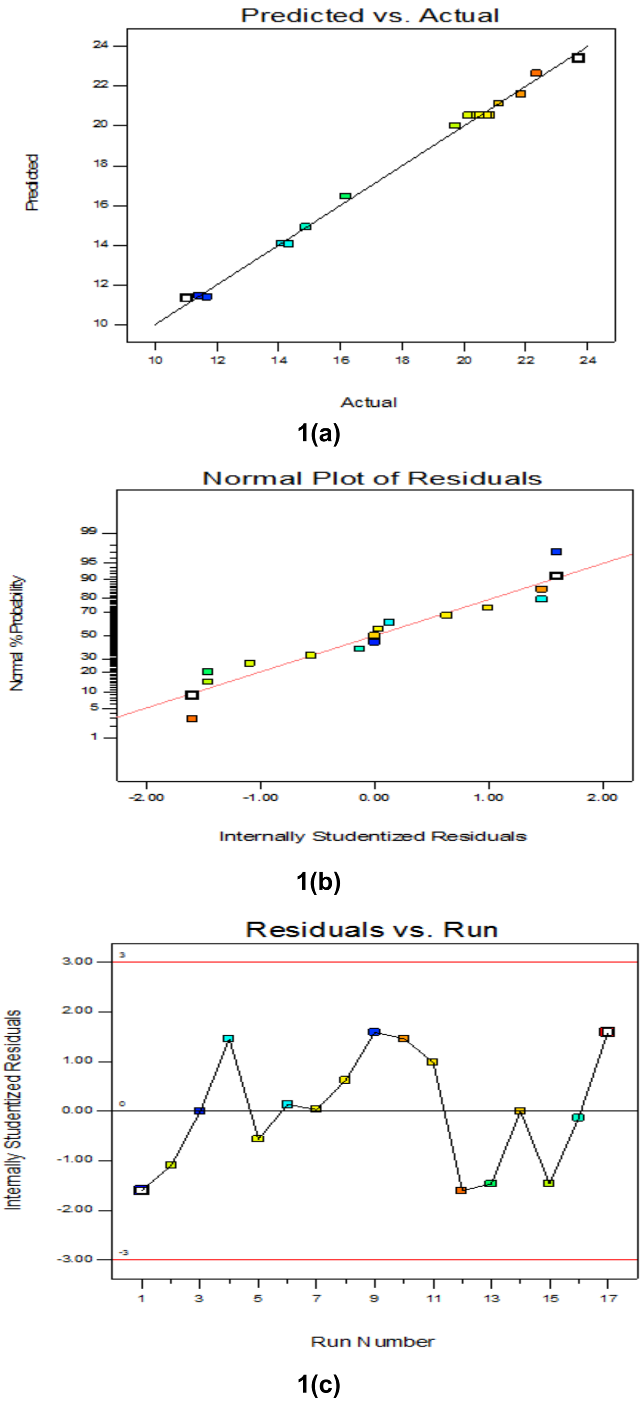
Microwave power affected the extraction efficacy significantly (Fig. 2a-c). It was seen that the peak pectin yield was 23.72% at the Microwave power of 480 W and the lower most pectin content was 11.03% at the microwave power of 160 W. The pectin yield was highest (23.72 %) at the maximum microwave power (480 W), thus microwave power had significant ($p<0.001$) effect at 0.1% level of significance on pectin yield. It can be clarified in detail that the microwave irradiation splits the wall of cells and disrupts the cell leading to quick decay due to electromagnetic energy [16]. Rise in the microwave power intensifies the temperature of the solution. At this point, the dielectric constant of water declines making pectin water soluble [17]. It is observed that rising the microwave power improves the infiltration of extraction solvent into the pectin source and through molecular interaction with the electromagnetic field, rapid transfer of energy between the extraction solvent and sample is noted, allowing the components from the dissolution to be extracted [18].

3.2.2 Effect of microwave time

Microwave time was also an important factor and presented a positive outcome on the yield of pectin and had significant ($p<0.001$) effect at 0.1% level of significance on pectin yield. (Fig. 2b-c). It has been recorded that a longer irradiation time favors the production of pectin [19]. This could perhaps be due the fact that with the more irradiation time, the better exposure of the orange peel powder to the solvent leads to the better dissolution of the orange peel powder and therefore, subsequent diffusion of pectin out from the raw solid materials [20]. In this study, it is observed that the pectin yield amplified with increase in time and reached at the peak pectin yield of 23.72% at the irradiation time of 2 minutes but decreases gradually with the further increase from 2 to 3 minutes. It is perceived that the elongated extraction process can destroy the pectin content owing to high temperature and excessive thermal accumulation in the extraction matrix. Besides, increased time of extraction results in pectic acid formation as by-product, hence, reducing the extraction yield of pectin [21].

3.2.3 Effect of pH

The pH was one of the significant factor that influenced the pectin yield and it is essential to choose an appropriate pH to guarantee maximum extraction of orange peel powder (Fig. 2b-c). From the current study it is verified that the yield amplified in reducing pH values. It was observed that the pH had significant ($p<0.001$) effect on pectin yield at 0.1% level of significance. The peak yield was 23.72% at pH 1 and the minimum yield was 11.03 % at pH 2. As the pH values decreases, the existence of H+ ions increase which enhances the hydrolysis of proto pectin. The high acid extraction solution had the capacity to merge with the insoluble pectin and supports the hydrolysis of the insoluble pectin soluble form, consequently increasing pectin extraction from plant materials [22].

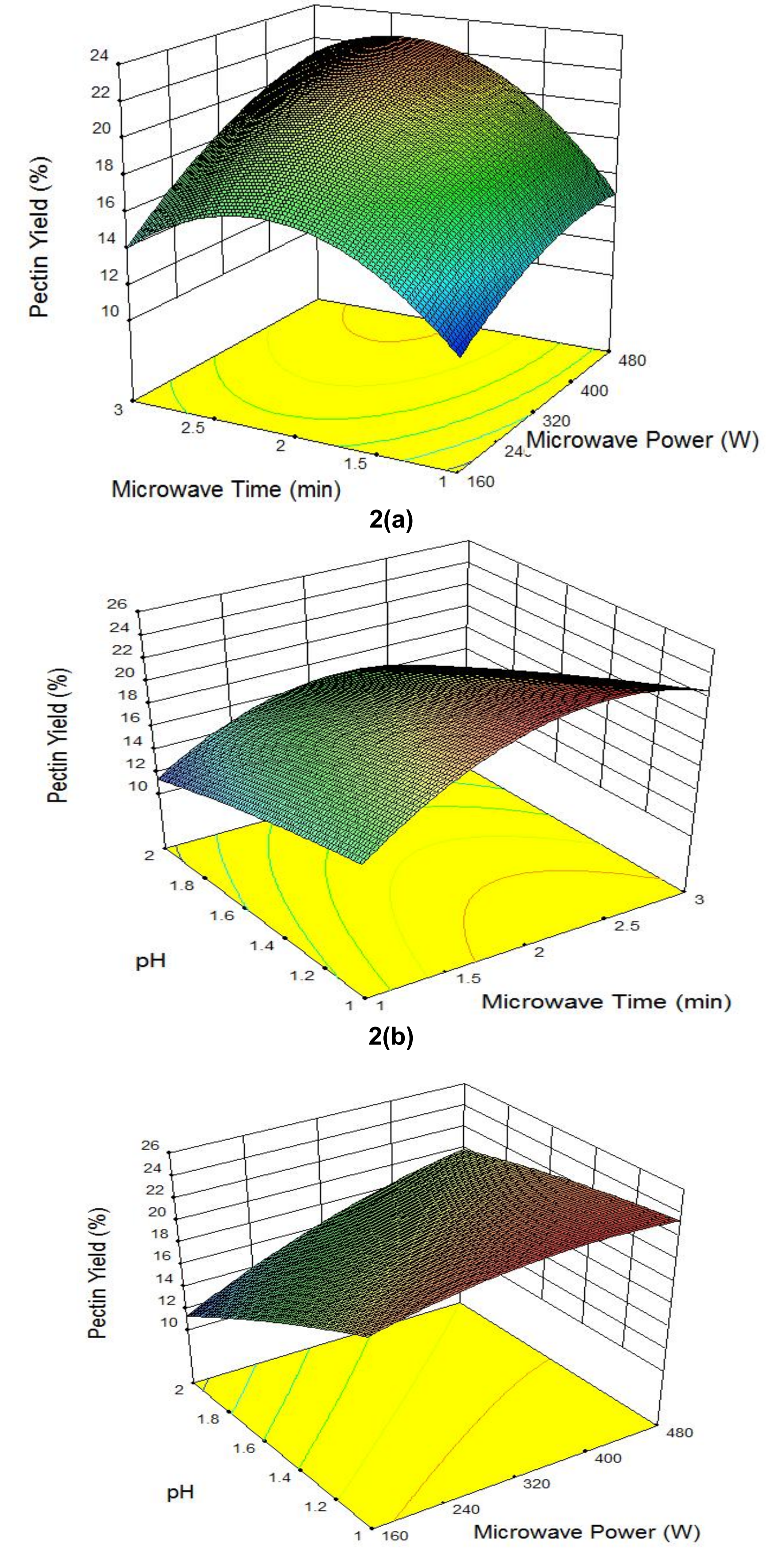


1(a)

1(b)

1(c)

Fig. 1. Diagnostic plots for the model adequacy



2(a)

2(b)

2(c)

Fig. 2. Effect of process parameters on the extraction yield of pectin

3.3 Optimization of Independent Parameters and Validation of Optimized Conditions

The optimization was done to achieve the microwave assisted extraction conditions which gave the highest yield of pectin. The technique of desirability function was employed in the optimization process. This approach estimates a point that amplifies the desirability function [23]. The optimal extraction conditions and the peak yield of pectin attained from desirability method was microwave power of 478 W, microwave time of 2.2 min and pH of 1.05 and the peak yield of pectin was 24.00% with a desirability value of 1. The appropriateness of the optimized conditions was confirmed by carrying out triplicate experiments. The mean values (23.74±0.26% for pectin yield) attained from the real experiments, established the validation of the optimized conditions.

4. CONCLUSION

Pectin is a food additive that is extensively utilized in the food and subsidiary industries. In the current analysis, extraction of pectin from orange peel using microwave was inspected under varied extraction conditions. The Box–Behnken design has verified to be appropriate to optimize the effect of microwave time, pH and microwave power on maximizing the pectin yield. The experimental results gave rise to a high correlated quadratic mathematical model which was implemented to optimize the microwave assisted extraction process conditions for peak retrieval of pectin from orange peel. From response surface plots, all process parameters remarkably affected the extraction efficacy of pectin yield, both interactively and independently. The optimal conditions determined by desired function methodology were as follows: microwave power of 478 W, microwave time of 2.2 min and pH of 1.05 with peak pectin yield (24.00%). Under the optimal conditions, the experimental values (23.74±0.26% for pectin yield) had a close confirmation with the predicted values (24.00%).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28180