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Optimization of microwave assisted extraction of pectin from orange peel

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ABSTRACT

In this study, microwave-assisted extraction was applied for pectin extraction from the dried orange peel and Box–Behnken response surface design was used to study and optimize the effects of processing variables (microwave power, irradiation time, pH and solid–liquid ratio) on the yield of pectin. The amount of pectin extracted increased with increasing microwave power, while it reduces as the time, pH and solid–liquid ratio increased. From the results, second order polynomial model was developed and it adequately explained the data variation and significantly represented the actual relationship between independent variables and the response. An optimization study using Derringer's desired function methodology was performed and optimal conditions based on both individual and combinations of all independent variables (microwave power of 422 W, irradiation time of 169 s, pH of 1.4 and solid–liquid ratio of 1:16.9 g/ml) were determined with maximum pectin yield of 19.24%, which was confirmed through validation experiments.

1. Introduction

Citrus fruits are an important crop throughout the world and oranges account for approximately 65% of all the citrus fruits produced (Luengo, Álvarez, & Raso, 2013). Citrus fruits are mainly used as either fresh juice or packed drinks. Peel makes up roughly half of an orange's mass and is the main by-product from the industrial processing of this fruit. Wastes generated from citrus (Cerezal, Larrauri, & Pinera, 1995; Cho, Lee, & Kim, 2003; Contreras-Esquivel, Hours, Voget, & Mignone, 1999) processing industries are reported to be rich source of pectin. Pectin is a complex carbohydrate, which is a significant component of the primary cell wall of plants. Pectin is a polymer of α-galacturonic acid with a variable number of methyl ester groups. Pectin is a natural food additive used extensively in the food industry as a thickener, a texturiser, an emulsifier, stabilizer, gelling agent and other applications include fat replacers in spreads, salad dressings, ice cream and emulsified meat products (Liu, Shi, & Langrish, 2006). The world market demand for pectin is in excess of 30,000 tons annually and is growing by about 4–5% per annum (Yeoh, Shi, & Langrish, 2008). So, citrus-processing industries are commercially interested in recovering residual amounts of soluble solids after juice extraction. Therefore, it is necessary to establish a new method, by which the pectin could be extracted in a shorter time and in better quality.

Microwave assisted extraction (MAE) is an interesting alternative to conventional extraction methods and it has many advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost. Soxhlet method usually needs a few hours, even more than 20 h, while microwave-assisted extraction only needs a few minutes or seconds. Compared with supercritical fluid extraction, the apparatus of microwave-assisted extraction is simpler and cheaper (Hao, Han, Huang, Xue, & Deng, 2002). MAE increased the capillary-porous characteristics and the water absorption capacity of the plant material. These changes provide an opportunity for improving the extraction yield of target analytes from plant material (Kratchanova, Panchev, & Pavlova, 2004).

Pectin from orange peels has wide applications in the food industries. Since, the conversion of orange fruit peel into a valuable by product such as pectin offers great scope for utilization and also reduces the environmental pollution. Hence, the main objective of this study is to develop an MAE of pectin from orange peel and investigate the effect of process variables (microwave power, irradiation time, pH and solid–liquid ratio) and the response (pectin yield); and to obtain optimum conditions for maximum extraction yield of pectin from orange fruit peel. Thus, response surface

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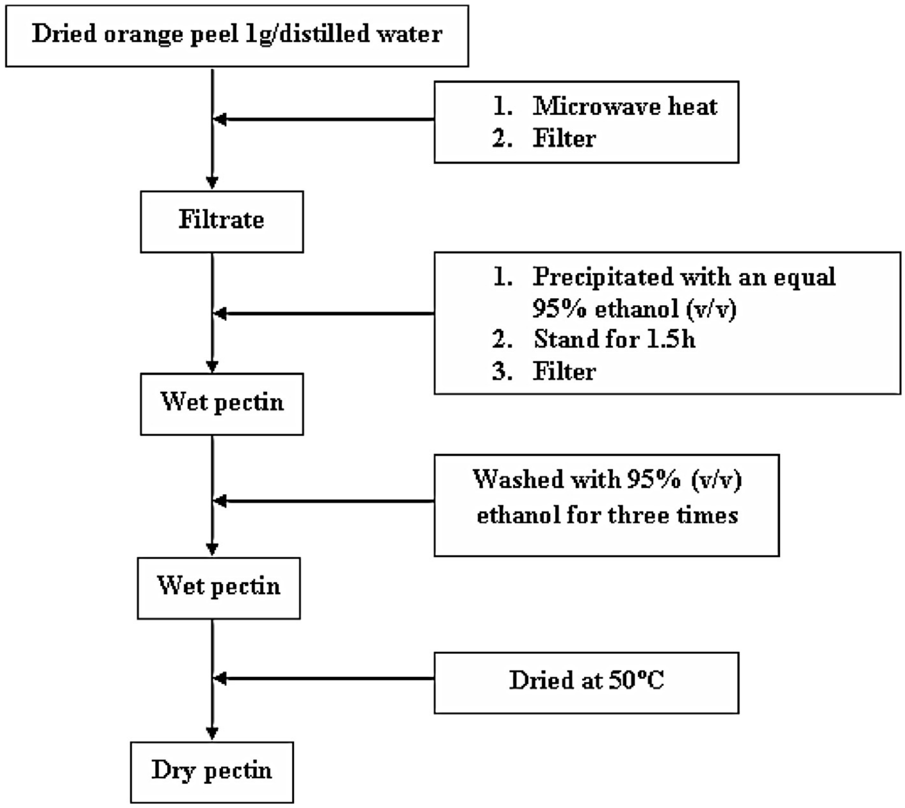

Fig. 1. Flow chart for pectin extraction from orange peel.

methodology (RSM) using Box–Behnken design was employed in this study. The advantage of this methodology is the simultaneous investigation of individual and interactive effects of process factors on the response from a small number of experiments (Prakash Maran, Sivakumar, Sridhar, & Thirgananasambandham, 2013).

2. Materials and methods

2.1. Raw materials and reagents

Orange (*Citrus reticulata*) fruit was obtained from local fruit orchard near Erode, Tamilnadu, India. The peels were removed, finely cutted and dried in a hot air oven (NSW, India) at 60 °C until it attains constant weight. Then the peels were pulverized and passed through a 40-mesh sieve to obtain powdered sample. The powder (moisture content of 12–14% on dry basis) was stored in bags and kept it dry environment prior to the experiments. All solvents and chemicals used in this study were analytical grade and purchased from Merk chemicals, Chennai, India.

2.2. Extraction of pectin from dried orange peel using MAE

MAE of pectin was performed according to the methods described by Wang et al. (2007) in an ordinary household microwave oven (LG, MH-3948 WB) at working frequency of 2450 MHz with adjustable microwave power and time. A flow chart for the extraction of pectin is depicted in Fig. 1. About 1 g of dried orange peel powder was weighed and placed into a 250 ml Pyrex beaker and different amount distilled water (10–20–30 ml) containing different pH values (1–1.5–2) was added. The pH of the solvent was adjusted with the help of sulfuric acid (0.05–0.016–0.005 mol/l). According to the experimental design (Table 1), the extraction was performed under different MAE conditions. The beaker was placed in the middle of the oven over a rotating dish and was exposed to microwave radiation at different powers (160–320–480 W) for the selected irradiation time (60–120–180 s). After microwave heating, the mixture in the Pyrex beaker was allowed to cool down to room temperature and filtered using filter paper (Whatman no-1). The filtered extract was centrifuged and the supernatant was precipitated with an equal volume of 95% (v/v) ethanol. The coagulated pectin mass was washed with 95% (v/v) ethanol for three times to remove the mono and disaccharides (Minkov, Minchev, & Paev, 1996).

2.3. Determination of pectin yield

After extraction, the wet pectin was dried at 50 °C in the hot air oven until its weight was constant and weighed. The pectin yield (PY) was calculated from the following equation proposed by Li, Jia, Wei, and Liu (2012).

$$PY = \left(\frac{m_0}{m}\right) \times 100 \qquad (1)$$

where m_0 (g) is the weight of dried pectin and m (g) is the weight of dried orange peel powder.

2.4. Experimental design and statistical analysis

Experimental datas were analyzed using Design-Expert 8.0.7.1 (State-Ease Inc., Minneapolis, MN, USA) statistical package including ANOVA to obtain the interaction between the process variables and the response. A single factor experimental method was used to study the effect of process variables (microwave power, extraction time, pH and solid–liquid ratio) on the pectin yield from orange peel. According to the results from single factor experiments, a three level four factors Box–Behnken response surface

Table 1
Box–Behnken experimental design matrix with observed and predicted values.

Run order	Microwave power (W)	Irradiation time (s)	pH	SL ratio (g/ml)	Pectin yield (%)	
					Experimental	Predicted
1	320	120	1.5	20	18.59	18.52
2	160	120	1.5	10	15.99	15.45
3	480	120	1.5	10	18.06	17.69
4	320	120	2	10	10.89	11.07
5	320	180	1	20	13.04	13.51
6	320	120	2	30	9.95	9.91
7	480	120	2	20	16.99	16.50
8	320	60	1	20	7.52	7.19
9	320	180	1.5	30	12.37	11.77
10	160	120	2	20	9.49	9.66
11	320	120	1.5	20	18.49	18.52
12	160	120	1.5	30	10.39	10.71
13	480	180	1.5	20	18.39	18.73
14	320	120	1	10	13.37	13.89
15	160	180	1.5	20	14.75	14.57
16	160	60	1.5	20	8.97	9.11
17	160	120	1	20	13.43	13.50
18	320	60	2	20	7.42	6.89
19	320	60	1.5	30	8.06	7.95
20	320	120	1	30	9.39	9.29
21	480	120	1.5	30	15.79	16.27
22	320	120	1.5	20	18.49	18.52
23	320	180	1.5	10	17.09	16.75
24	320	180	2	20	11.73	12.01
25	480	120	1	20	15.06	14.46
26	320	60	1.5	10	8.99	9.13
27	480	60	1.5	20	12.08	12.75

experimental design (BBD) was employed to investigate the individual and interactive effects of process variables on the pectin yield from orange peel. The range of independent variables and their levels are presented in Table 1 and it was coded (−1, 0 and +1) according to the method described by Prakash Maran et al. (2013) for statistical calculation. Twenty seven experiments were augmented with three replications at the center points in order to evaluate the pure error. The relationship between the response and four independent variables were approximated by the following second order polynomial equation and the generalized form of that equation was given in elsewhere (Prakash Maran, Sivakumar, Sridhar, & Thirgananasambandham, 2013). After analyzing the polynomial equation depicting the effect of independent variables on the responses, optimization process was carried out by Derringer's desired function methodology (Prakash Maran and Manikandan, 2012). After optimization, adequacy of the model equation for predicting the optimum response values was validated with experimental results.

3. Results and discussion

3.1. Experimental design and statistical analysis

The experimental results of PY from orange peel using four-factor-three level BBD was shown in Table 1. Pareto analysis of variance (ANOVA) was used to analyze the experimental data in order to examine the statistical significance of the model terms and the results are listed in Table 2. The higher model *F*-value (81.76) and the associated lower *p*-values ($p < 0.0001$) demonstrated that, the developed model was significant and indicates that most of the variation in the response can be explained by the regression equation. The lack of fit *F*-value (114.75) with low *p*-value ($p < 0.05$) indicated that the model could be used to predict the responses. The high value of R^2 (0.989), adj-R^2 (0.978) and pre-R^2 (0.940) clearly stated that, the form of the model chosen to represent the actual relationship between the response and independent variables is well correlated (Prakash Maran, Manikandan, Thirugnanasambandham, Nivetha, & Dinesh, 2013). Low values of coefficient of variance (4.30) clearly exhibiting a very high degree of precision and good reliability of the conducted experiments (Prakash Maran, Mekala, & Manikandan, 2013). In this study the signal to noise ratio is found to be >28, which indicates the best fitness of the developed models.

3.2. Model fitting

A second order polynomial equation was used to build a mathematical model to find the optimum conditions that maximize the extraction yield of pectin and study the combined relationships between the process variables and the response. The developed second-order models in term of coded variable are given below

$$\begin{aligned}\mathrm{PY} = {} & 18.52 + 1.95X_1 + 2.86X_2 - 0.45X_3 - 1.54X_4 + 0.13X_1X_2 \\ & + 1.47X_1X_3 + 0.83X_1X_4 - 0.3X_2X_3 - 0.95X_2X_4 + 0.76X_3X_4 \\ & - 0.55X_1^2 - 4.18X_2^2 - 4.44X_3^2 - 2.94X_4^2 \end{aligned} \quad (2)$$

3.3. Diagnostics of model adequacy

In addition to determination coefficient, the adequacy of the model was evaluated through various diagnostic plots such as predicted versus actual, normal % probability, and internally studentized residuals were construced and are shown in Fig. 2. The predicted values obtained from the developed model were quite close to the experimental values and lie reasonably close to the straight line and indicated the adequate agreement with real data (Fig. 2a). Fig. 2b shows the normal % probability plot of residuals for response was normally distributed, as they lie reasonably close to the straight line and shows no deviation of the variance. Internally studentized residuals plot was constructed to facilitate the satisfactory fit of the developed model and the plot (Fig. 2c) shows that, all the data points lie within the limits (±3).

Table 2
Analysis of variance for regression model of pectin yield.

Source	Coefficient estimate	Sum of squares	Degree of freedom	Standard error	Mean square	*F* value	*p*-Value
Model	18.52	365.49	14	0.33	26.11	81.76	<0.0001
X_1	1.95	45.44	1	0.16	45.44	142.29	<0.0001
X_2	2.86	98.21	1	0.16	98.21	307.57	<0.0001
X_3	−0.45	2.38	1	0.16	2.38	7.44	0.0183
X_4	−1.54	28.34	1	0.16	28.34	88.74	<0.0001
X_{12}	0.13	0.07	1	0.28	0.07	0.22	0.6475
X_{13}	1.47	8.61	1	0.28	8.61	26.98	0.0002
X_{14}	0.83	2.77	1	0.28	2.77	8.68	0.0122
X_{23}	−0.30	0.37	1	0.28	0.37	1.15	0.3054
X_{24}	−0.95	3.59	1	0.28	3.59	11.25	0.0057
X_{34}	0.76	2.31	1	0.28	2.31	7.24	0.0197
$X_1{}^2$	−0.56	1.64	1	0.24	1.64	5.14	0.0426
$X_2{}^2$	−4.18	93.07	1	0.24	93.07	291.48	<0.0001
$X_3{}^2$	−4.44	105.32	1	0.24	105.32	329.82	<0.0001
$X_4{}^2$	−2.94	45.98	1	0.24	45.98	144.00	<0.0001
Residual		3.83	12		0.32		
Lack of fit		3.83	10		0.38	114.75	0.0087
Pure error		6.667E−03	2		3.33E−03		
Cor total		369.32	26				
Std. dev.	0.57			R^2		0.989	
Mean	13.14			Adj-R^2		0.976	
C.V. %	4.30			Pred-R^2		0.940	
Press	22.05			Adequate Precision		28.09	

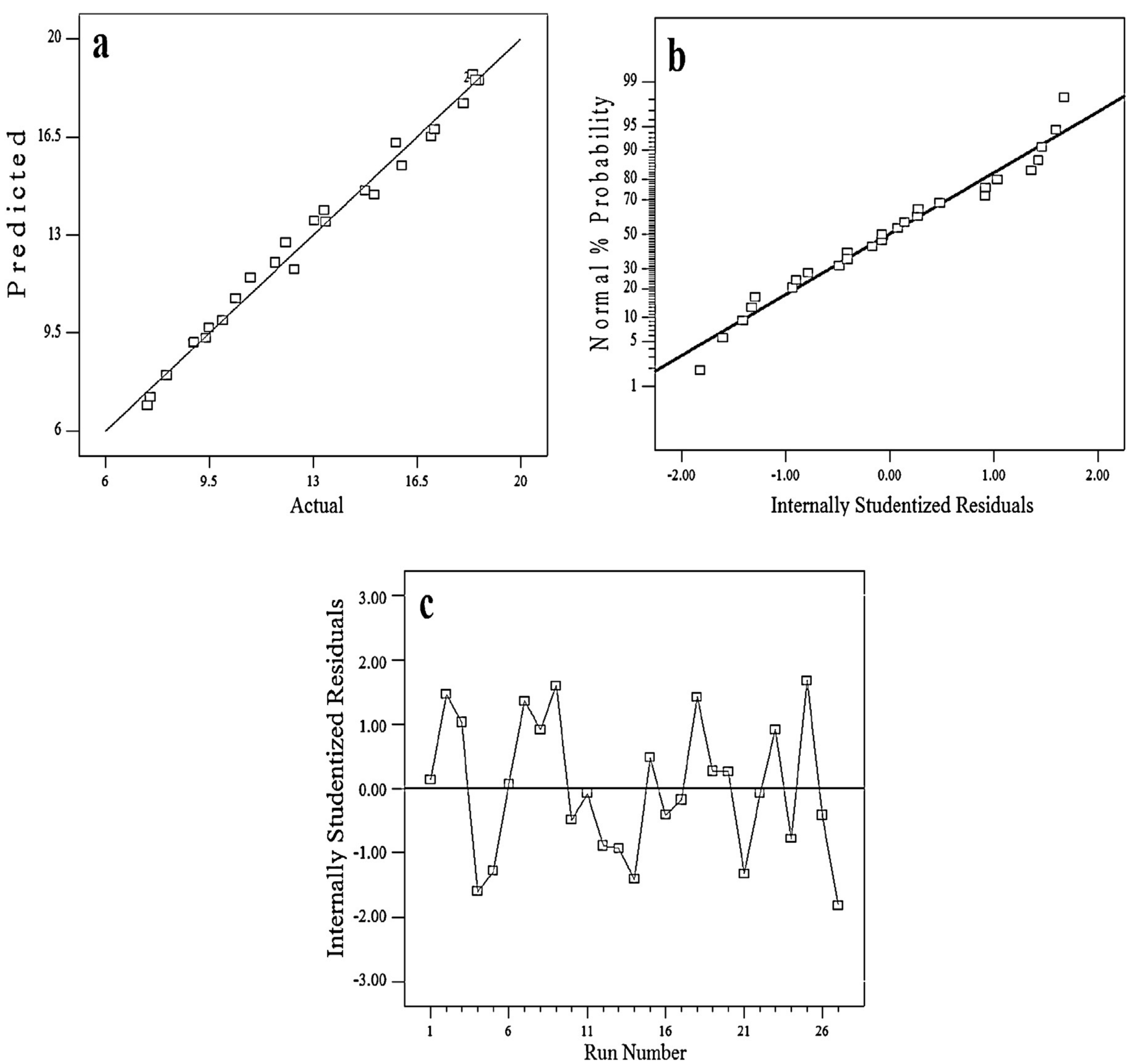

Fig. 2. Diagnostic plots for the model adequacy.

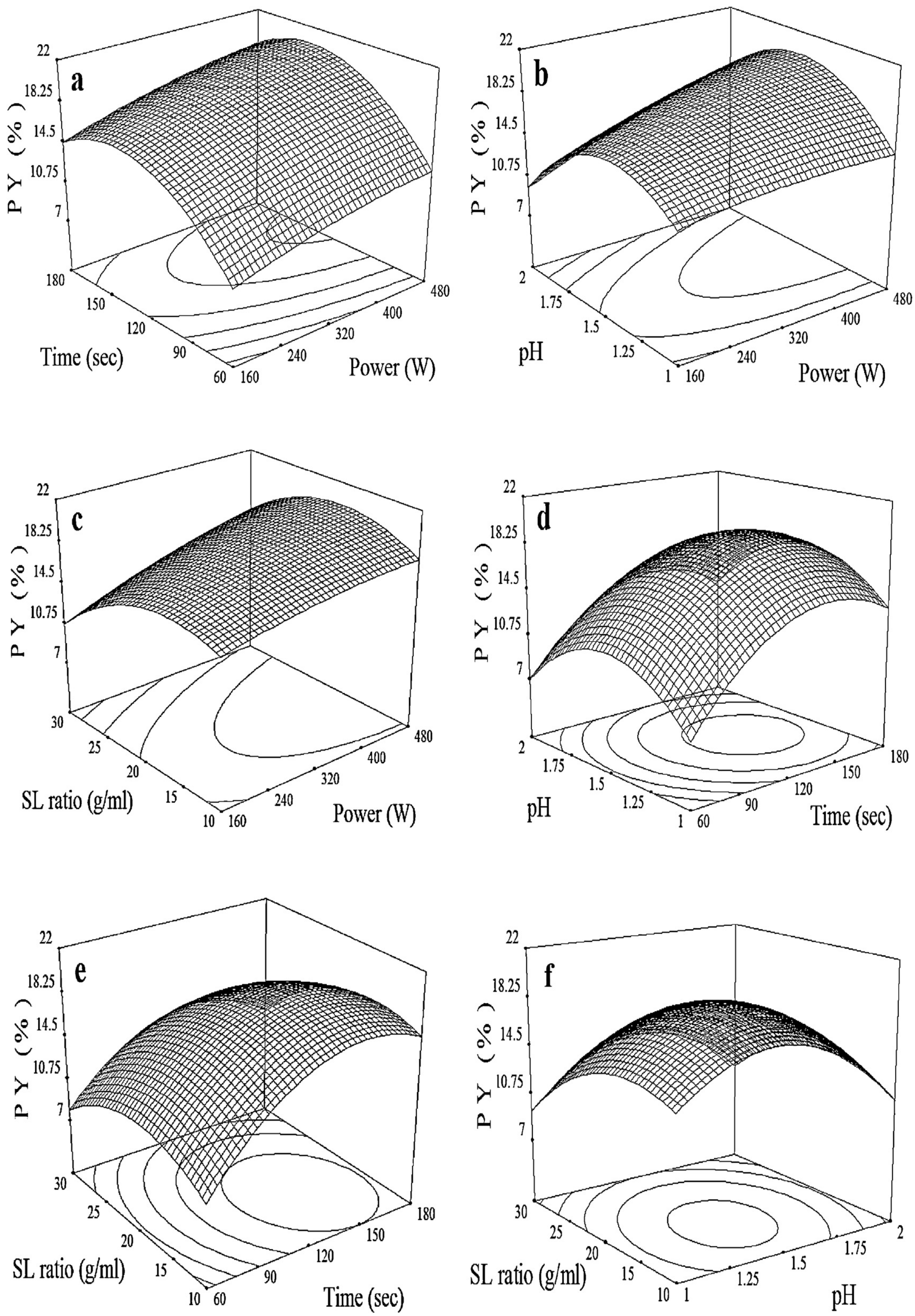

Fig. 3. Effect of process variables on the extraction yield of pectin.

3.4. Effect of process variables

3.4.1. Effect of microwave power

The extraction efficiency was improved by raising microwave power from 160–480 W (Fig. 3a–c). The accelerated extraction of pectin by increasing microwave power is related to the direct effects of microwave energy on the plant materials. Microwave radiation loosens the cell wall matrix and the skin tissues are rapidly and extensively opened up by the microwave (Kratchanova et al., 2004). This will lead to increased interaction between extracting agent and source material in extraction process. More electromagnetic energy was transferred on biomolecules by ionic conduction and dipole rotations, which result in power dissipated inside the solvent and plant material and then generate molecular movement and heating (Gfrerer & Lankmayr, 2005) on the extraction system quickly and improved the extraction efficiency.

3.4.2. Effect of irradiation time

Irradiation time is one of the key factors affecting the extraction yield of pectin and it is necessary to select a proper irradiation time to assure the maximum extraction of pectin from orange peel by MAE. From the results, it is observed that, the PY was increased steadily and reached the maximum at 125 s (Fig. 3a, d and e). This phenomenon could be explained that, the absorption of microwave energy in the extraction system promoted the thermal accumulation of the extraction solution leads to the dissolution of pectin into the solution until 125 s. However, the excessive time exposure in the microwave field may cause the degradation of pectin (Xianzhe, Fangping, Chenghai, & Xiangwen, 2011). So, the yield was decreased slowly, when the irradiation time extended beyond 125 s. Similar results were observed in the extraction of triterpenoid saponins from *Ganoderma atrum* (Chen, Xie, & Gong, 2007).

3.4.3. Effect of pH

From the results, it is found that, the PY was increasing evidently as the decreasing pH values (Fig. 3b, d and e). The lower acidic extraction solvent have the ability to contact with the insoluble pectin directly and favored the hydrolysis of the insoluble pectin constituents into soluble pectin, thus increasing the pectin recovery (El-Nawawi & Shehata, 1988) and reached maximum, when the pH value was 1.4. However, increasing pH value beyond 1.4, the PY was decreased. This might be due to the aggregation of pectin which retarded the pectin release.

3.4.4. Effect of solid–liquid ratio

The solvent quantity is also an important factor to affect the yield of pectin. Therefore, in this study, its influence on the extraction yield was evaluated. It can be seen that (Fig. 3c, e and f), the yields of pectin increased with increasing solid–liquid ratios up to 1:16 g/ml, which was probably due to the fact, larger volume of extraction solvent causing excessive swelling of the materials (Guo et al., 2001) and absorbs the microwaves directly by the materials. So, the cell walls were ruptured, which resulted in easy release of pectin into the surrounding medium. However, the increasing solid–liquid ratio was exceeded beyond 1:16 g/ml, the solution get saturated with the solute, which was negatively affected the mass transfer rate and barricaded the penetration of the pectin into the solution and decreases the extraction yield.

3.5. Optimization of extraction parameters and validation of optimized condition

The objective of optimization was to find out the MAE conditions which give the maximum extraction yield of pectin. The desirability function approach was applied in the optimization process. This numerical optimization technique evaluates a point that maximizes the desirability function (Prakash Maran, Manikandan, Vigna Nivetha, & Dinesh, 2013). The optimum extraction conditions and the maximum yield of pectin were obtained from desirability function approach was microwave power of 422 W, irradiation time of 169 s, pH of 1.4, solid–liquid ratio was 1:16.9 g/ml and the maximum yield of pectin was 19.24% with a desirability value of 0.968. The suitability of the optimized conditions for predicting the optimum response values was tested experimentally using the selected optimal conditions. Triplicate experiments were performed under the optimized conditions and the mean values (19.19 ± 0.07 for PY) obtained from real experiments, demonstrated the validation of the optimized conditions.

4. Conclusion

In this study, MAE was optimized for the extraction of pectin from orange peel. Four factors at three levels Box–Behnken response surface experimental design was successfully employed to optimize and study the individual and interactive effect of process variables such as microwave power, irradiation time, pH and solid–liquid ratio on the maximum extraction yield of pectin from orange peel. From the experimental results, a high correlated quadratic mathematical model was developed and employed to optimize the MAE process conditions for maximum recovery of pectin from orange peel. From response surface plots, all process variables significantly influenced the extraction efficiency of PY, independently and interactively. The optimal conditions determined by Derringer's desired function methodology were as follows: microwave power of 422 W, irradiation time of 169 s, pH of 1.4 and solid–liquid ratio of 1:16.9 g/ml with maximum pectin yield (19.24%). Under the optimal conditions, the experimental values (19.19 ± 0.07%) agreed with the predicted values (19.24%).

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