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Short communication

Microwave assisted extraction of pectin from waste *Citrullus lanatus* fruit rinds

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ABSTRACT

In this present study, microwave-assisted extraction (MAE) was applied to extraction of pectin from waste *Citrullus Lanatus* fruit rinds. Extraction parameters which are employed in this study are microwave power (160–480 W), irradiation time (60–180 s), pH (1–2) and solid–liquid ratio (1:10–1: 30 g/ml) and they were optimized using a four factor three levels Box–Behnken response surface design (BBD) coupled with desirability function methodology. The results showed that, all the process variables have significant effect on the extraction yield of pectin. Optimum MAE conditions for the highest pectin yield from waste *C. Lanatus* fruit rinds (25.79%) were obtained with microwave power of 477 W, irradiation time of 128 s, pH of 1.52, solid–liquid ratio of 1:20.3 g/ml respectively. Validation experiment results were well agreed with predicted value.

1. Introduction

Citrullus lanatus (Watermelon) is a fruit of great economic importance, with worldwide production estimated at approximately 93,700 million tons (Tarazona-Díaz, Viegas, Moldao-Martins, & Aguayoa, 2011). Watermelon is enjoyed by many people across the world as a fresh fruit, partly owing to it being low in calories, highly nutritious and thirst-quenching. Watermelon biomass can be categorized as three main components which are the flesh, seed, and rind. Flesh contributes approximately 68% of the total weight, the rind approximately 30%, and the seeds approximately 2% (Kumar, 1985). Rind is one of the major solid wastes generated by several restaurants, cottage fruit juice producers and food industries in India. It is discarded indiscriminately into the environment and thereby constituting environmental challenges. This waste rind is not presently being utilized for any value added processes due to limited research activities focusing on the possible conversion of the waste to other valuable products thereby making it available for dumping as solid waste. Therefore, the development of new, environmentally friendly method to manage that problem is great concern. Since, the conversions of waste *C. lanatus* fruit rind (WCLFR) into valuable by product such as pectin offers great scope for utilization and also creates more income for farmers, food processors and more importantly reduce environmental impacts of the waste. Pectin is defined as a complex mixtures of polysaccharides that make up approximately one third of the cell-wall of most of the plants. Pectin is primarily made up of D-galacturonic acid joined by α-(1–4) glycosidic linkages (Wang et al., 2007). Pectins are now used as thickeners, water binders, and stabilizers and texturizing fat replacer to mimic the mouth-feel of lipids in low-calorie foods (May, 1990). Among other uses, it has been considered in the class of dietary fibers known to have a positive affect on digestive processes and to help lower cholesterol (Braddock, 1999).

Over the past decade, various novel extraction techniques have been introduced (microwave-assisted extractions (MAE), supercritical fluid extraction (SFE), and pressurized solvent extraction (PSE)) and investigated to recover valuable crude extracts from various waste biomass. Among the various extraction techniques, MAE has been accepted as a potential and powerful alternative to conventional extraction techniques, owing to its special heating mechanism, moderate capital cost and its good performance under atmospheric conditions (Eskilsson & Björklund, 2000), shorter time, less solvent, higher extraction rate and better products with lower cost (Maran, Sivakumar, Thirgananasambandham, & Sridhar, 2013a). Hence, the objectives of this present study is to develop easy, rapid MAE method to extract pectin from waste *C. lanatus* fruit rinds and also to investigate and optimize the process variables such as microwave power, irradiation time, pH and solid–liquid

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ratio on the extraction yield of pectin using a four factors three level Box–Behnken response surface design coupled with desired function methodology. Box–Behnken design has proved to be an extremely valuable tool, it not only helps in determining the accurate optimum values of experimental parameters but also provides the possibility to evaluate the interaction between variables with a reduced number of experiments (Maran et al., 2013a; Prakash Maran, Sivakumar, Sridhar, & Prince Immanuel, 2013). The optimized controlled conditions determined in this study should offer important reference values for any subsequent studies.

2. Materials and methods

2.1. Raw materials and reagents

WCLFR were obtained from local fruit juice centre near Perundurai, Tamilnadu, India. The rinds were finely cutted and washed in tap water and blanched for 5 min. Blanched material was loaded on stainless trays and dried in a hot air oven at 60 °C until it attains constant weight. The dried rinds were pulverized and passed through a 40-mesh sieve in order to obtain powdered sample. The powdered samples were packed in dark bags and stored it in dry environment prior to the experimental analysis. All solvents and chemicals used in this study were analytical grade and purchased from Merk chemicals, Chennai, India.

2.2. Microwave assisted extraction of pectin

Extraction of pectin was performed according to the method described by Wang et al. (2007) with the help of an microwave equipment (LG, MH-3948 WB) at working frequency of 2450 MHz with adjustable microwave power and irradiation time under different MAE conditions (Table 1). A 250 ml Pyrex beaker containing a mixture of 1 g of dried rind powder with different amount distilled water (10–30 ml) containing different pH values (1–2) were placed in the middle of the microwave equipment over a rotating dish and exposed to different microwave power (160–480 W) and irradiation time (60–180 s). The pH of the solvent was adjusted with the help of hydrochloric acid. At selected time, extraction process was carried out and after that, the mixture in Pyrex beaker was allowed to cool down to room temperature, filtered using filter paper (Whatman No. 1), centrifuged (5500 rpm for 15 min) 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). After extraction, the wet pectin was subjected to drying at 50 °C in the hot air oven until it attains a constant weight. The pectin yield (PY) was calculated from the following equation proposed by Li, Jia, Wei, and Liu, (2012).

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

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

2.3. Experimental design

A Box–Behnken response surface experimental design (BBD) with four factors (microwave power (X_1), irradiation time (X_2), pH (X_3) and solid–liquid ratio (X_4)) at three levels (Table 1) was performed to optimize and investigate the individual and interactive effects of process variables on the pectin yield (response) from WCLFR. For each factor, an experimental range was based on the results of preliminary single-factor experiments. The experiments were conducted in a randomized order and the data were analyzed by multiple regression analysis in order to develop an empirical second order regression polynomial mathematical model, which exhibits the relationship between response and independent variables. The generalized form of second order polynomial mathematical model was given in elsewhere (Maran, Sivakumar, Sridhar, & Thirgananasambandham, 2013). The construction and analysis of the experimental design, analysis of variance (ANOVA) to obtain

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

Run order	Microwave power (X_1, (W))	Irradiation time (X_2 (s))	pH (X_3)	SL ratio (X_4 (g/ml))	Pectin yield (%)		
					Experimental	Predicted	Residual
1	160	60	1.5	20	14.56	14.81	−0.25
2	480	60	1.5	20	17.25	18.15	−0.90
3	160	180	1.5	20	20.34	20.13	0.21
4	480	180	1.5	20	23.98	24.42	−0.44
5	320	120	1	10	18.96	19.57	−0.61
6	320	120	2	10	15.68	16.63	−0.95
7	320	120	1	30	14.98	14.71	0.27
8	320	120	2	30	15.54	15.61	−0.07
9	160	120	1.5	10	21.58	20.96	0.62
10	480	120	1.5	10	23.65	23.12	0.53
11	160	120	1.5	30	15.98	16.36	−0.38
12	480	120	1.5	30	21.38	21.85	−0.47
13	320	60	1	20	13.09	12.74	0.35
14	320	180	1	20	18.63	19.15	−0.52
15	320	60	2	20	13.01	12.34	0.67
16	320	180	2	20	17.32	17.52	−0.20
17	160	120	1	20	19.02	19.13	−0.11
18	480	120	1	20	20.65	20.02	0.63
19	160	120	2	20	15.08	15.17	−0.09
20	480	120	2	20	22.58	21.93	0.65
21	320	60	1.5	10	14.58	14.57	0.01
22	320	180	1.5	10	22.68	22.26	0.42
23	320	60	1.5	30	13.65	13.53	0.12
24	320	180	1.5	30	17.96	17.43	0.53
25	320	120	1.5	20	24.18	24.11	0.07
26	320	120	1.5	20	24.08	24.11	−0.03
27	320	120	1.5	20	24.08	24.11	−0.03

Table 2
Analysis of variance (ANOVA) for the fitted quadratic polynomial model.

Source	Coefficient estimate	Sum of squares	Degree of freedom	Standard error	95% CI low	95% CI high	Mean square	*F* value	*p*-value
Model	24.11	369.09	14	0.40	23.25	24.98	26.36	55.43	<0.0001
X_1	1.91	43.82	1	0.20	1.48	2.34	43.82	92.13	<0.0001
X_2	2.90	100.75	1	0.20	2.46	3.33	100.75	211.83	<0.0001
X_3	−0.51	3.12	1	0.20	−0.94	−0.08	3.12	6.56	0.0249
X_4	−1.47	25.93	1	0.20	−1.90	−1.04	25.93	54.52	<0.0001
X_{12}	0.24	0.23	1	0.34	−0.51	0.99	0.23	0.47	0.5041
X_{13}	1.47	8.61	1	0.34	0.72	2.22	8.61	18.11	0.0011
X_{14}	0.83	2.77	1	0.34	0.08	1.58	2.77	5.83	0.0327
X_{23}	−0.31	0.38	1	0.34	−1.06	0.44	0.38	0.80	0.3900
X_{24}	−0.95	3.59	1	0.34	−1.70	−0.20	3.59	7.55	0.0177
X_{34}	0.96	3.69	1	0.34	0.21	1.71	3.69	7.75	0.0165
X_1^2	−0.56	1.65	1	0.30	−1.21	0.09	1.65	3.46	0.0874
X_2^2	−4.18	93.22	1	0.30	−4.83	−3.53	93.22	196.01	<0.0001
X_3^2	−4.49	107.74	1	0.30	−5.15	−3.84	107.74	226.54	<0.0001
X_4^2	−2.98	47.51	1	0.30	−3.64	−2.33	47.51	99.89	<0.0001
Residual		5.71	12				0.48		
Lack of fit	5.70	10				0.57	171.01	0.0058	
Pure error	6.667E-03	2				3.33E-03			
Cor total		374.80	26						
Std. Dev.	0.69			R^2				0.985	
Mean	18.68			Adj R^2				0.967	
C.V. %	3.69			Pred R^2				0.912	
Press	32.85			Adeq. precision			23.51		

the interaction between the process variables and the response, quality of fit of the polynomial model (coefficient of determination (R^2), adjusted coefficient of determination (adj-R^2) and predicted coefficient of determination (pre-R^2)) and optimization of process conditions were obtained using Design-Expert 8.0.7.1 (State-Ease Inc., Minneapolis, MN, USA) statistical package. After optimization, triplicate verification experiments were performed under the optimal conditions and the average value of the experiments was compared with the predicted values of the developed model equation.

3. Results and discussion

3.1. Statistical analysis and model building

The experimental data and the process variables for the pectin yield at different extracted conditions are presented in Table 1. The results of the analysis of variance, adequacy and fitness of the models are summarized in Table 2. The results showed that, *p* values of developed model were below 0.0001, which in turn indicate the fitness of model was highly significant ($p < 0.0001$) (Prakash Maran, Mekala, & Manikandan, 2013). The results also demostrated that, the proposed regression model for pectin yield was satisfactory with high R^2 (0.985), adj-R^2 (0.967) and pre-R^2 (0.912) values respectively, which exhibited a close agreement between the experimental and the theoretical values predicted by polynomial model (Prakash Maran, Sivakumar, Thirgananasambandham, & Sridhar, 2013). Meanwhile, the very low value of coefficient of variation (CV) (3.69) clearly indicated a very high degree of precision and a great deal of reliability of the experimental values (Prakash Maran, Manikandan, Vigna Nivetha, & Dinesh, 2013). By applying multiple regression analysis on the experimental data, the dependent variable and independent variable are related by the following second-order polynomial equation:

$$Y(\%) = 24.11 + 1.91X_1 + 2.9X_2 - 0.51X_3 - 1.47X_4 + 0.24X_1X_2 + 1.47X_1X_3 + 0.83X_1X_4 - 0.31X_2X_3 - 0.95X_2X_4 + 0.96X_3X_4 - 0.56X_1^2 - 4.18X_2^2 - 4.49X_3^2 - 2.98X_4^2 \quad (2)$$

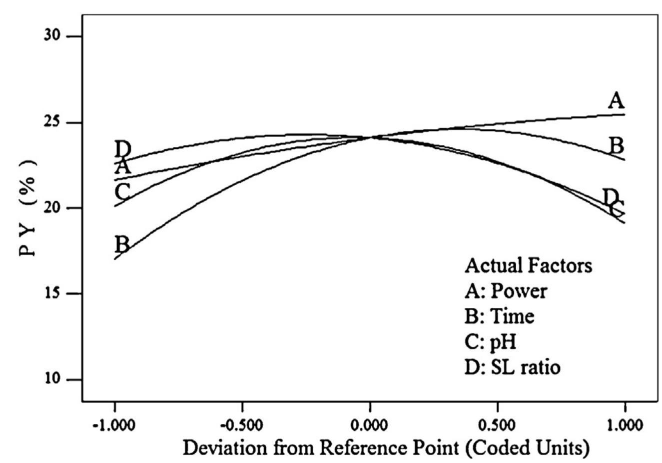

Fig. 1. Perturbation plot showing the effect of process variables.

3.2. Influence of process variables on pectin yield

3.2.1. Perturbation plot

In generating the perturbation plots, all the factors were plotted on the same response graph. This graph could be used to find factors that most affect the response. A steep slope or curvature in a factor shows that the response is sensitive to that factor. A relatively flat line shows insensitivity to change in that particular factor (Gupta & Ako, 2005). The response (PY) was plotted against the deviation from the reference point by changing only one factor over its entire range while holding all other factors constant as shown in Fig. 1. From the Fig. 1, it was observed that, pH and solid–liquid ratio have great influence on the extraction yield compared with microwave power and irradiation time.

3.2.2. Effect of microwave power

The extraction of pectin was increased by raising microwave power from 160 - 480 W (Fig. 2a–c). The increasing microwave irradiation energy can enhance the penetration of solvent into the plant matrix and deliver efficiently to materials through

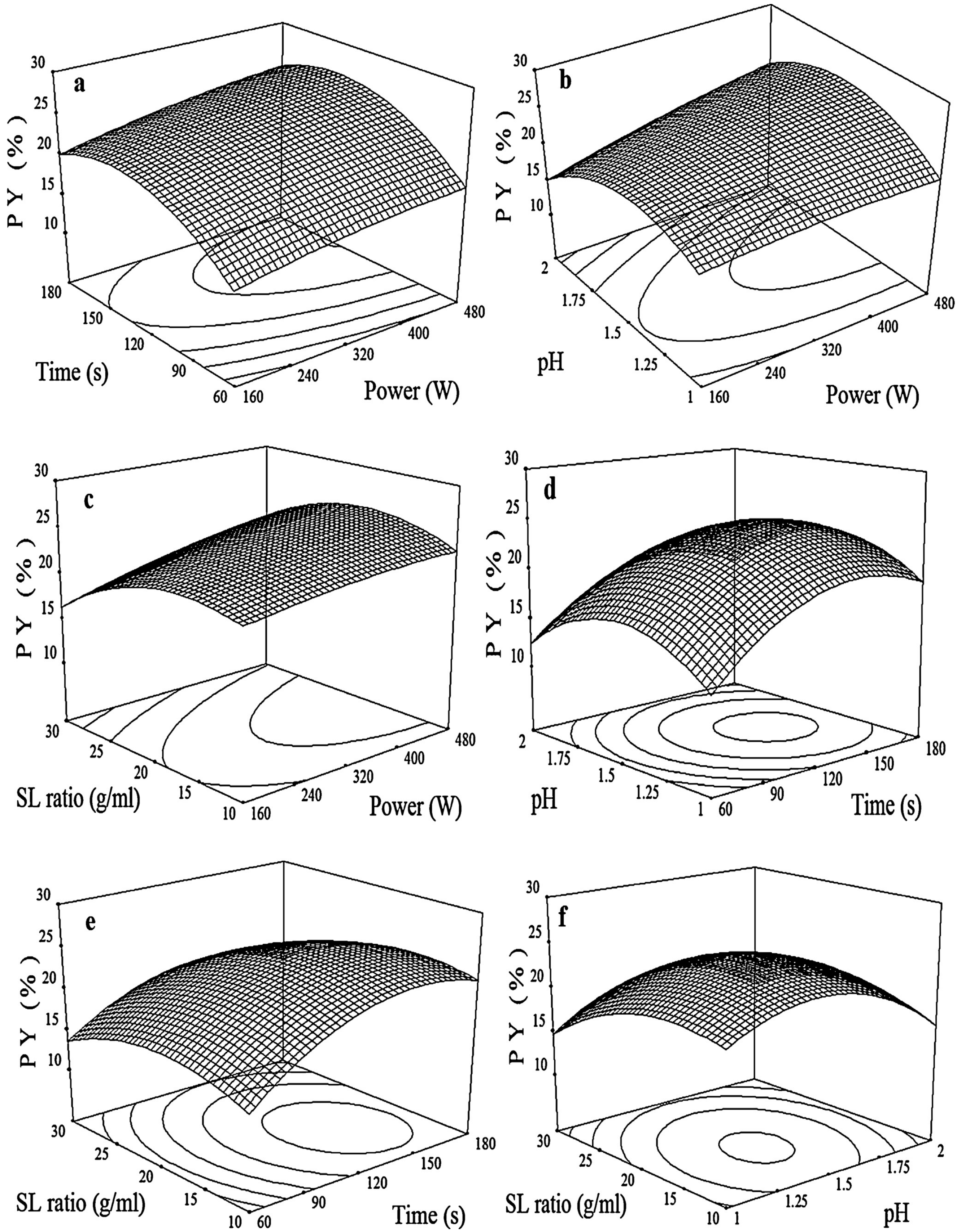

Fig. 2. Effect of process variables on the pectin yield.

molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted (Yan et al., 2010). As a polar solvent, water can efficiently absorb microwave energy and leads to efficient heating. Moreover, the microwave irradiation accelerates cell rupture by sudden temperature rise and internal pressure increase inside the cells of plant sample, which promotes the destruction of sample surface and in turns the exudation of pectin within the plant cells into the surrounding solvents (Zhang, Yang, & Liu, 2008) and increased the extraction yield.

3.2.3. Effect of irradiation time

Studies were carried out at different irradiation times (60–180 s) in order to select a proper irradiation time to enhance the extraction yield of pectin. From the results, it was observed that, the extraction yield of pectin was increased rapidly and reached maximum at 128 s and decreased slowly (Fig. 2a, d–e). This phenomenon could explain the fact that, the thermal accumulation within extraction solution due to the absorption of microwave energy promoted the dissolution process of pectin into solution until 128 s and decreased the yield gradually. Whereas, too long irradiation time may lead to degradation of pectin chain molecules, thus affecting pectin extraction rate (Maran, Sivakumar, Thirgananasambandham, & Sridhar, 2013b).

3.2.4. Effect of pH

In order to evaluate the pH effects on pectin extraction yield, it was extracted at different pH (1–2) levels and the results were shown in Fig. 2b, d and f. From the figures, it was found that, the extraction yield of pectin was increased at pH values from 1 to 1.52. The low pH reduced the molecular weight of pectin and it can be partially solubilized from plant tissues without any degradation and hence the pectin molecules proneness to precipitation. Both these effects contrarily influence the extraction yield of pectin (Faravash & Ashtiani, 2007). Beyond the pH value 1.52, aggregation of pectin could occur, which retarded the pectin release and decreased the pectin yield.

3.2.5. Effect of solid–liquid ratio

To determine the most appropriate solid to solvent ratio to extract pectin, rind to water ratios of 1:10–1:30 were used in this study and the results were in Fig. 2c, e–f. As shown in figures, the pectin extraction was significantly increased up to the solid-to-liquid ratio of 1:20.3. The pectin content was decreased with the further increase in solid-to-liquid ratio. The solvent (water) can efficiently absorb microwave energy and leads to enhance swelling of plant material, which is favorable to increase the contact surface area between the plant matrix and the solvent, resulting in increases the extraction yield of pectin up to solid–liquid ratio of 1:20.3. But, higher solvent would decrease the microwave adsorption of material, because more energy was absorbed by the solvent. Therefore, the break of material cell wall and mass transmission might be negatively influenced (Li et al., 2010) and decreased the pectin yield.

3.3. Optimization and validation of optimized condition

In this study, desired function method (Prakash Maran, Manikandan, & Mekala, 2013) was used to find out the optimum MAE conditions which gave maximum extraction yield of pectin from dried WCLFR. The optimum extraction conditions was found to be: Microwave power of 477 W, irradiation time of 128 s, pH of 1.52, solid–liquid ratio of 1:20.3 g/ml and maximum pectin yield of 25.79% respectively with desirability value of 0.948. In accordance with the optimization results obtained from desirability function, verification experiments (triplicate) were carried out at the optimized condition. The average experimental yield of pectin was $25.53 \pm 0.27\%$. The experimental yield was very close to the predicted values. Thus, it can be seen that the second-order model was adequate to describe the influence of the selected MAE operating variables on the extraction yields of pectin.

4. Conclusions

In this present study, the extraction conditions for enhanced extraction of pectin from WCLFR by MAE process were optimized with a four factors three level Box–Behnken response surface design coupled with desirability function methodology. The results showed that, all the process variables had significant effect on the extraction yield of pectin and a high correlated quadratic polynomial mathematical model was developed. The optimal conditions were determined to be: microwave power of 477 W, irradiation time of 128 s, pH of 1.52, solid–liquid ratio of 1:20.3 g/ml and pectin yield of 25.79% respectively.

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