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Ultrasound-assisted extraction of pectin from sisal waste

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ABSTRACT

In this study, an efficient ultrasound-assisted extraction (UAE) of pectin from sisal waste was investigated and optimized. Response surface methodology (RSM) based on a three-level four-factor Box–Behnken response surface design (BBD) was employed to optimize the extraction conditions (ultrasonic power, extraction temperature, extraction time and solid–liquid ratio). Analysis of variance showed that the contribution of a quadratic model was significant for the pectin extraction yield. The experimental yield (29.32%) was obtained under the optimal condition (ultrasonic power of 61 W, temperature of 50 °C, time of 26 min and SL ratio of 1:28 g/ml) was well agreement with predicted values. Therefore, ultrasound-assisted extraction could be used as an alternative method to extract pectin from sisal waste with the advantages of lower extraction temperatures, shorter extraction time and reduced energy consumption.

1. Introduction

Sisal (*Agave sisalana*) is a monocotyledonous plant of great economic interest and grows well all round the year in hot climate and arid regions. Sisal products are widely used in marine industries and agriculture because of their strength, durability, stretchability, affinity for certain dyes, and resilience in saltwater. Some potential innovations include the use of the material as an organic fertilizer, a supplement in ruminant feed and a raw material in the production of medicine (Debnath, Pandey, Sharma, Thakur, & Lal, 2010). However, only 4% of the sisal leaves are used to create fibre. By-products from sisal extraction can be used for making biogas (Mshandete, Björnsson, Kivaisi, Rubindamayugi, & Mattiason, 2006), pharmaceutical ingredients (Santos & Branco, 2014) and polymer composites (Wu, 2011). The biomass left after fibres have been removed is now flushed away as waste. This sisal waste of material is composed of water (85%), parenchymal tissue, short fibres, primary and secondary metabolites and inorganic compounds (Silva & Beltrão, 1999). Currently, this waste is mainly land filled and dumped in nearby rivers, where microorganisms degrade it. As a result, the untreated waste causes consumption of oxygen in the recipient watercourses which leads to oxygen-deficient water zones with a negative effect on fish and other living organisms. Thus, recycling of sisal waste is crucial because it reduces environmental pollution and may also provide other highly useful and commercializable materials (Botura et al., 2013). This has led to focus on developing technologies aimed at determining applications for the remaining plant materials, especially the residue.

Recently, industrially, pectin is extracted from apple pomace, sugar beet pulp, and citrus peels using water acidified with a strong mineral acid, notably, nitric, hydrochloric or sulphuric acid (the so-called conventional acid extraction) with elevated temperature (Koubala et al., 2008). Acidic wastewater and environmental concerns make alternative extraction method to extract pectin from plant materials. In connection with the emerging concept of 'Green Chemistry', various novel methods including ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE) and accelerated solvent extraction (ASE) have been developed, as more environmentally friendly and energy efficient technologies, to enhance the recovery of valuable compounds from plant tissues (González-Centeno et al., 2014). Among them, UAE technology has received considerable attention due to its beneficial properties, involving shorten the extraction time, reduce the organic solvent waste, increase the extraction yield and enhance the quality of extracts when compared to conventional extraction (Tao, Wu, Zhang, & Sun, 2014). In fact, ultrasound has been recognized as an alternative approach to traditional extraction methods (Ebringerová & Hromádková, 2010; Rastogi, 2011; Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Galanakis, 2013).

Some studies have indicated the presence of pectin in waste sisal leaves (Aspinall & Cañas-Rodriguez, 1958; Silva & Beltrão, 1999; Santos, Espeleta, Branco, & de Assis, 2013). However, the feasibility

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of using ultrasound for the extraction of pectin from sisal waste has not yet been explored in the literature. Hence, the objective of this present work is to investigate and optimize the effect of ultrasonic power, temperature, time and solid–liquid ratio on the extraction of pectin from sisal waste using four factors three level Box–Behnken response surface design. 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 & Manikandan, 2012; Maran, Sivakumar, Thirgananasambandham, & Sridhar, 2014a). The optimized controlled conditions determined in this study should offer important reference values for any subsequent studies.

2. Materials and methods

2.1. Materials

Sisal waste utilized in this study was obtained from a sisal fibre processing unit near Chennai, Tamil Nadu, India. The sisal waste was dried in a hot air oven at 50 °C until it attains constant weight. The dried residuals were crushed into powder using a mill, stored in an air tight container and kept in dry environment prior to experiments. Analytical grade ethanol (99% purity) was purchased from Merck Chemicals, Mumbai.

2.2. UAE of pectin

Experiments were performed using an ultrasonic device (VCX 400, Sonics and Materials, USA and 0–400 W) with a 2.00 cm flat tip probe with provisions to set required output power, temperature and time. The extraction was performed by continuous ultrasound waves at a frequency of 20 kHz and ultrasonic generator probe was directly submerged into the suspension (10 g of dried power with appropriate volume of distilled water). The experiments were carried out in triplicates according to Table 1 and the average results were reported. During the extraction period, an amplitude controller was used to set the desired level of ultrasonic power and temperature was controlled at a desired level within ±1 °C. After extraction, the suspension was 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 in order to remove the mono and disaccharides (Maran, Sivakumar, Thirgananasambandham, & Sridhar, 2013). 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 (dry basis) from the following method described by Maran, Sivakumar, Thirgananasambandham, and Sridhar (2014b).

$$\mathrm{PY}\,(\%) = \left(\frac{m_0}{m}\right) \times 100 \tag{1}$$

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

2.3. Experimental design

In this study, four factors with three levels Box–Behnken response surface experimental design (BBD) was chosen to study and optimize the influence of process variables such as ultrasonic power (50–70 W), extraction temperature (40–60 °C), extraction time (10–30 min) and SL ratio (20–40 g/ml), on the maximum extraction yield of pectin from sisal waste. A total number of 29 experiments with five replicates (used to estimate experimental error) at the centre point were established and total number of experiments (N) was calculated from the following equation (Maran, Sivakumar, Sridhar, & Thirgananasambandham, 2013).

$$N = 2K(K-1) + C_0 \tag{2}$$

where K is the number of factors and C_0 is the number of central point.

Experimental data were fitted to a second-order polynomial mathematical equation in order to express the relationship between independent variables and responses. The generalized form of second-order polynomial equation was given as follows:

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j X_j + \sum_{j=1}^{k}\beta_{jj}X_j^2 + \sum_{i}\sum_{i<j=2}^{k}\beta_{ij}X_iX_j \tag{3}$$

where Y is the response; X_i and X_j are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters ($k = 4$ in this study) (Maran, Manikandan, Vigna Nivetha, & Dinesh, 2013). Statistical analysis of the experimental data was performed using the Stat Ease Design Expert 8.0.7.1 statistical software (Stat-Ease Inc., Minneapolis, USA).

2.4. Optimization and validation of optimized condition

Numerical optimization technique was adapted in this study to optimize the process conditions. For optimization of process variables, the regression model developed in this study was used to determine the optimal condition which could provide maximum pectin yield. The nature of the optimal condition (point of maximum or minimum or a saddle point response) was also evaluated by transforming the developed regression model into conical form and the Eigen values were computed using MATLAB software.

To determine the validity of optimized condition, additional triplicate experiments were performed under optimal conditions and average values of the experiments were compared with the predicted values of the optimized conditions in order to find out the accuracy and suitability of the optimized conditions.

3. Results and discussion

3.1. Experimental data analysis

The experimental data was fitted to various models (linear, interactive (2FI), quadratic and cubic) and the results were shown in Table 2. The results demonstrated that, linear and interactive (2FI) models exhibited lower R^2, adjusted R^2, predicted R^2 and also high p values, when compared with quadratic model. Cubic model was found to be aliased. Therefore the quadratic model incorporating linear, interactive and quadratic terms was chosen to describe and study the effect of process variables on the pectin yield from sisal waste.

3.2. Model fitting and statistical analysis

By applying multiple regression analysis on the experimental data, Design-Expert software generated the second-order polynomial equation which can express the relationship between process variables and the responses. The final equation obtained in terms of coded factors is given below.

$$\begin{aligned}\text{Pectin yield}\,(\%) = {} & 30.55 + 1.93X_1 - 2.15X_2 + 2.69X_3 - 0.24X_4 \\ & - 0.59X_1X_2 - 0.052X_1X_3 - 1.52X_1X_4 + 2.92X_2X_3 + 1.36X_2X_4 \\ & + 0.41X_3X_4 - 2.58X_1^2 - 5.81X_2^2 - 3.42X_3^2 - 2.43X_4^2\end{aligned} \tag{4}$$

Table 1
Box–Behnken design matrix and observed results.

Std order	Power (W)	Temperature (°C)	Time (min)	SL ratio (g/ml)	Pectin yield (%)		Residual error
					Observed	Predicted	
1	50	40	20	30	21.87	21.79	0.08
2	70	40	20	30	26.95	26.83	0.12
3	50	60	20	30	18.43	18.67	−0.24
4	70	60	20	30	21.14	21.34	−0.20
5	60	50	10	20	22.91	22.65	0.27
6	60	50	30	20	27.82	27.22	0.60
7	60	50	10	40	20.64	21.36	−0.72
8	60	50	30	40	27.17	27.56	−0.39
9	50	50	20	20	21.85	22.32	−0.47
10	70	50	20	20	28.62	29.22	−0.60
11	50	50	20	40	25.57	24.89	0.68
12	70	50	20	40	26.26	25.71	0.55
13	60	40	10	30	23.06	23.69	−0.63
14	60	60	10	30	14.02	13.56	0.46
15	60	40	30	30	22.87	23.25	−0.37
16	60	60	30	30	25.49	24.77	0.72
17	50	50	10	30	20.15	19.88	0.27
18	70	50	10	30	24.19	23.83	0.36
19	50	50	30	30	25.04	25.36	−0.32
20	70	50	30	30	28.87	29.11	−0.24
21	60	40	20	20	26.54	26.06	0.48
22	60	60	20	20	18.74	19.03	−0.28
23	60	40	20	40	23.18	22.86	0.32
24	60	60	20	40	20.84	21.28	−0.45
25	60	50	20	30	29.87	30.55	0.68
26	60	50	20	30	30.56	30.55	−0.01
27	60	50	20	30	30.14	30.55	0.41
28	60	50	20	30	30.26	30.55	0.28
29	60	50	20	30	30.52	30.55	0.03

Pareto analysis of variance (ANOVA) was used to analyze the experimental data and the results are listed in Table 3. The higher model *F*-value (105.41) and the associated lower *p* values ($p < 0.0001$) demonstrated the significance of developed models and also indicate the best fit of the developed model (Maran, Sivakumar, Thirgananasambandham, & Kandasamy, 2013). The high R^2 (0.991), adj-R^2 (0.981) and pre-R^2 (0.946) values clearly demonstrate the model precision in exhibiting the relationship between the response and independent variables (Maran, Mekala, & Manikandan, 2013). Low values of coefficient of variance (2.38) displayed the high degree of precision and good reliability of the conducted experiments (Maran, Manikandan, & Mekala, 2013). The predicted residual sum of square (PRESS) value of 27.8 shows the fit of this model with each point in the design. In this study, the adequate precision (signal to noise ratio) was found to be >40 for response, which indicates the best fit of the developed model (Maran, Manikandan, Thirugnanasambandham, Vigna Nivetha, & Dinesh, 2013).

3.3. Diagnostics of model adequacy

In addition to determination coefficient, influential plots (normal % probability, predicted versus actual, internally Studentized residual and Cook's distance plot) were constructed (Fig. 1) from the observed and predicted experimental data. From the results, it was observed that, the data lie close to the straight line (Fig. 1a and b) and exhibit adequate agreement between real and the data obtained from the developed model (Maran, Priya, & Manikandan, 2013). The residual values are in the determined range (Fig. 1c and d) and there is no strong evidence of influential outliers in the observed data (Maran, Manikandan, Priya, & Gurumoorthi, 2013). Hence, trends observed in Fig. 1 revealed that, no obvious patterns were found and residuals appeared to be randomly scattered.

3.4. Effect of process variables

In this study, the pectin recovery was varied markedly from 14.02% to 30.55% with different levels of independent factors. To understand and study the individual and interactive effect of independent variables on the response, three dimensional (3D) response surface plots were constructed by maintaining two factors at its constant level (in turn its central level), whereas the other two factors were varied in their range (Maran, Manikandan, Priya, et al., 2013) and the results are depicted in Fig. 2.

3.4.1. Effect of different ultrasonic power

The effect of different ultrasonic power (50–70 W) on the extraction yield of pectin was studied and the results were shown in Fig. 2. Increase in the yield with the increase of ultrasonic power (up to 65 W) was observed and then decreased. As ultrasonic wave passed through the liquid medium, more bubble were created and collapsed (Quan, Sun, & Qu, 2009), which leads to microjet formation and acoustic streaming (Vinatoru, 2001). The violent shock

Table 2
Adequacy of the model tested.

Source	Sequential sum of squares *p* value	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
Linear	0.0230	0.365	0.260	0.173	424.87	
2FI	0.7422	0.468	0.173	−0.058	516.59	
Quadratic	<0.0001	0.991	0.981	0.946	27.80	Suggested
Cubic	0.0296	0.985	0.976	0.855	36.51	Aliased

Table 3
ANOVA and significance of regression coefficients.

Source	Coefficient estimate	Sum of squares	Standard error	95% CI low	95% CI high	Mean square	*F* value	*p* value
Model	30.55	508.76	0.26	29.98	31.11	36.34	105.41	<0.0001
X_1	1.93	44.54	0.17	1.56	2.29	44.54	129.19	<0.0001
X_2	−2.15	55.56	0.17	−2.52	−1.79	55.56	161.17	<0.0001
X_3	2.69	86.88	0.17	2.33	3.05	86.88	252.01	<0.0001
X_4	−0.24	0.67	0.17	−0.60	0.13	0.67	1.94	0.1855
X_{12}	−0.59	1.40	0.29	−1.22	0.04	1.40	4.05	0.0637
X_{13}	−0.05	0.01	0.29	−0.68	0.58	0.01	0.03	0.8619
X_{14}	−1.52	9.24	0.29	−2.15	−0.89	9.24	26.80	0.0001
X_{23}	2.92	34.00	0.29	2.29	3.55	34.00	98.62	<0.0001
X_{24}	1.36	7.43	0.29	0.73	1.99	7.43	21.55	0.0004
X_{34}	0.41	0.66	0.29	−0.22	1.04	0.66	1.91	0.1886
X_1^2	−2.58	43.27	0.23	−3.08	−2.09	43.27	125.51	<0.0001
X_2^2	−5.81	218.91	0.23	−6.30	−5.31	218.91	634.98	<0.0001
X_3^2	−3.42	75.91	0.23	−3.92	−2.93	75.91	220.20	<0.0001
X_4^2	−2.43	38.35	0.23	−2.93	−1.94	38.35	111.23	<0.0001
Residual		4.83				0.34		
Std. Dev.	0.59							
Mean	24.65							
C.V. %	2.38							
Ade. Pre.	27.80							

wave and high-speed jet generated by ultrasound provokes the swelling of the materials by mixing of molecules with solvents and enlargement of the pores in the materials (Luque-García & Luque de Castro, 2003), which allows higher diffusivity across the plant materials and enhanced the extraction yield of pectin. However, beyond ultrasonic power of 65 W can cause an increase in bubble numbers in solvent during cavitation, which might reduce the efficiency of the ultrasound energy transmitted into the medium (Filgueiras, Capelo, Lavilla, & Bendicho, 2000) and decreased the pectin yield.

3.4.2. Effect of different temperature

The temperature in polysaccharide extraction has to be chosen very carefully, as it may affect the process in a non-desirable way. Hence, experiments were carried out to study the effect of temperature over the extraction yield of pectin from sisal waste. From the results, it was observed that, the yield of pectin was increased linearly with increasing temperature from 40 to 50 °C and then decreased (Fig. 2). Increase in temperature can accelerate solubility and diffusivity of solvent in to the plant materials and increases the extraction yield (Yang & Zhai, 2010). On the other hand, temperature has a greater influence on the cavitation threshold, which is responsible for acoustic cavitation and also results in the formation of cavitational nucleus. Cavitation effect works by imploding cavitation bubbles or cavities through relative greater force ruptured the cavitational nucleus and disrupted the structure of the material during extraction, which enhanced desorption and solubility of pectin in the surrounding medium and improved the yield. Beyond the temperature of 50 °C, the surface tension and viscosity of the solvent were reduced, which can change the characteristics of ultrasonic cavitation and intensity of mass transfer enhancement and decrease the extraction yield.

3.4.3. Effect of different extraction time

Effect of time on the extraction of pectin from sisal waste was investigated and the results were shown in Fig. 2. There was a steady increase in the yield of pectin up to 25 min and then it decreases on further increase in time. This phenomenon could be explained that, swelling and hydration of plant material could be accelerated by the cavitation effect of the ultrasonic waves during the earlier period of extraction (Wang, Wu, Dai, Chen, & Shao, 2012). The asymmetric collapse of micro-bubbles near surfaces was also associated with micro-jets that could cause the disruption and good penetration of solvent (Vilkhu, Mawson, Simons, & Bates, 2008) into the matrix (Sun & Tomkinson, 2002) through diffusion improves the washing out of pectin content from plant material to surrounding solvents and enhanced the extraction performance. However, heating effect and exposure of ultrasound treatment for longer extraction time cause the structural destruction and decomposition of pectin, which reduced the extraction yield.

3.4.4. Effect of solid–liquid ratio

Solid–liquid (SL) ratio is the critical variable that may affect the extraction yield. Thus, the extraction yield was measured at different SL ratios ranging from 1:20 to 1:40 g/ml and the results were depicted in Fig. 2. Increase in SL ratio up to 1:30 g/ml enhanced the contact area between material and solvent; lower the concentration and viscosity of the extraction solvent which could lead to dissolve the pectin molecules in the solvent and augmented the yield. But the intensity of cavitation falls down with an increase in the viscosity of the medium, since the formation of cavitation requires the negative pressure in the rarefaction region of wave function overcome the natural cohesive forces (Xu et al., 2014). Therefore, after 1:30 g/ml, further increase in SL ratio would not increase the extraction yield.

3.5. Optimum extraction condition for maximizing the pectin yield

Regression model (Eq. (4)) developed on this study was used to determine the optimum extraction condition for maximum extraction yield of pectin from sisal waste. In order to obtain the optimum values, first and second-order derivatives of the regression equation (Eq. (4)) were derived with respect to X_1, X_2, X_3 and X_4 respectively.

The first order derivative of Eq. (3) gives the following equations:

$$\frac{\partial Y}{\partial X_1} = 1.93 - 5.16X_1 - 0.59X_2 - 0.052X_3 - 1.52X_4 \quad (5)$$

$$\frac{\partial Y}{\partial X_2} = -2.15 - 0.59X_1 - 11.62X_2 + 2.92X_3 + 1.36X_4 \quad (6)$$

$$\frac{\partial Y}{\partial X_3} = 2.69 - 0.052X_1 + 2.92X_2 - 6.84X_3 + 0.41X_4 \quad (7)$$

$$\frac{\partial Y}{\partial X_4} = -0.24 - 1.52X_1 + 1.36X_2 + 0.41X_3 - 4.86X_4 \quad (8)$$

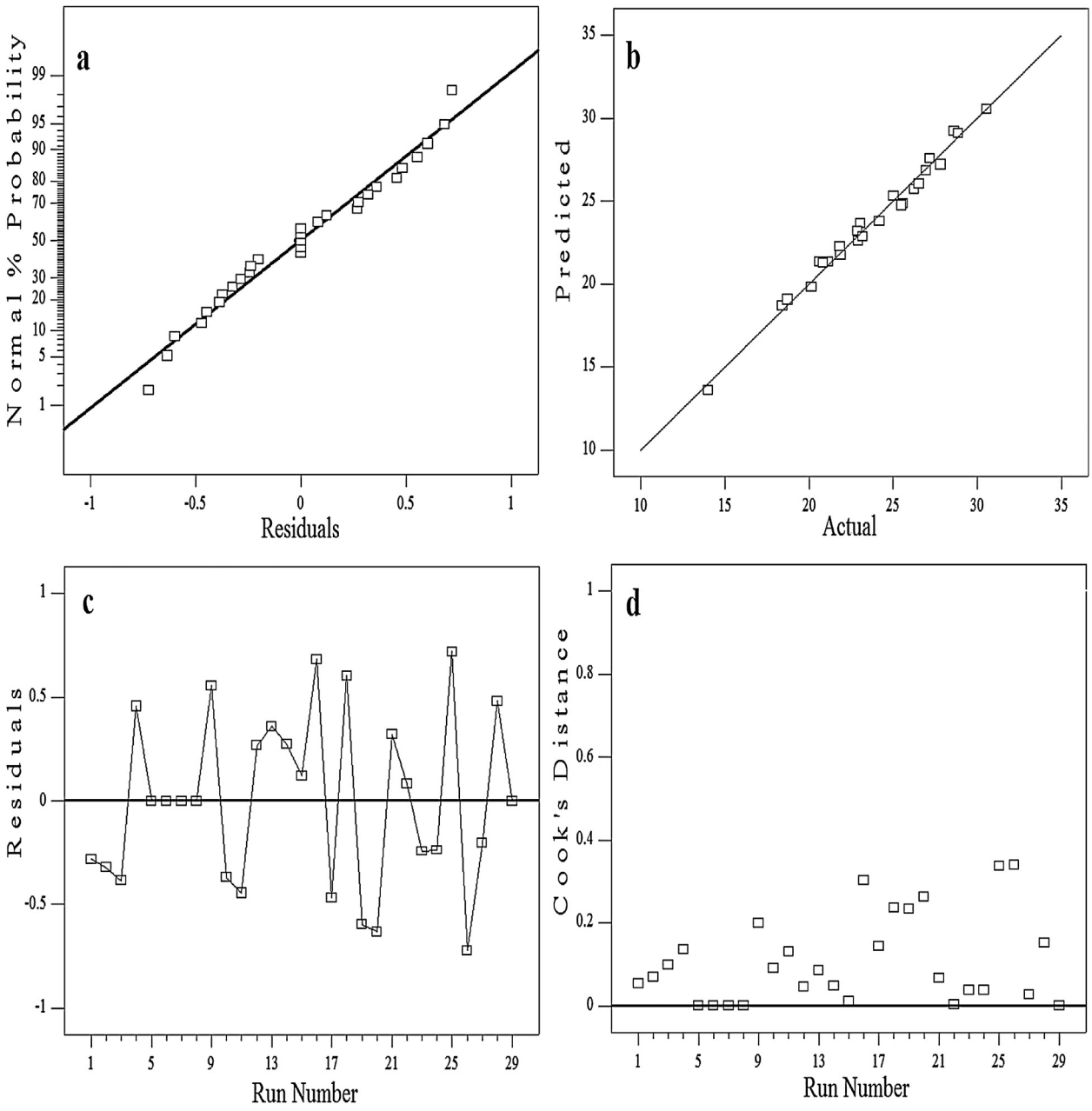

Fig. 1. Model adequacy plots for response.

Second-order derivatives of Eqs. (5)–(8) yield the following equations:

$$\frac{\partial^2 Y}{\partial X_1^2} = -5.16 \quad (9)$$

$$\frac{\partial^2 Y}{\partial X_2^2} = -11.62 \quad (10)$$

$$\frac{\partial^2 Y}{\partial X_3^2} = -6.84 \quad (11)$$

$$\frac{\partial^2 Y}{\partial X_4^2} = -4.86 \quad (12)$$

Since all the second-order derivatives showed negative values, it signifies the applicability of maximization. Thus, Eqs. (5)–(8) were equated with zero and solved for X_1, X_2, X_3 and X_4 to get the maximum values:

$$1.93 - 5.16X_1 - 0.59X_2 - 0.052X_3 - 1.52X_4 = 0 \quad (13)$$

$$-2.15 - 0.59X_1 - 11.62X_2 + 2.92X_3 + 1.36X_4 = 0 \quad (14)$$

$$2.69 - 0.052X_1 + 2.92X_2 - 6.84X_3 + 0.41X_4 = 0 \quad (15)$$

$$-0.24 - 1.52X_1 + 1.36X_2 + 0.41X_3 - 4.86X_4 = 0 \quad (16)$$

Algebraic solution to the above four equations given in coded form of optimal condition was found to be: $X_1 = 0.127$ W, $X_2 = -0.007\,^{\circ}$C, $X_3 = 0.548$ min and $X_4 = -0.196$ g/ml. The corresponding experimental parametric values were: 61.27 W (ultrasonic power), 49.93 °C (temperature), 25.48 min (time) and 1:28.04 g/ml (SL ratio) respectively. Under the optimal conditions, the predicted yield was 31.15%.

The optimal point as obtained above can be a point of maximum or minimum or a saddle point response. In order to determine

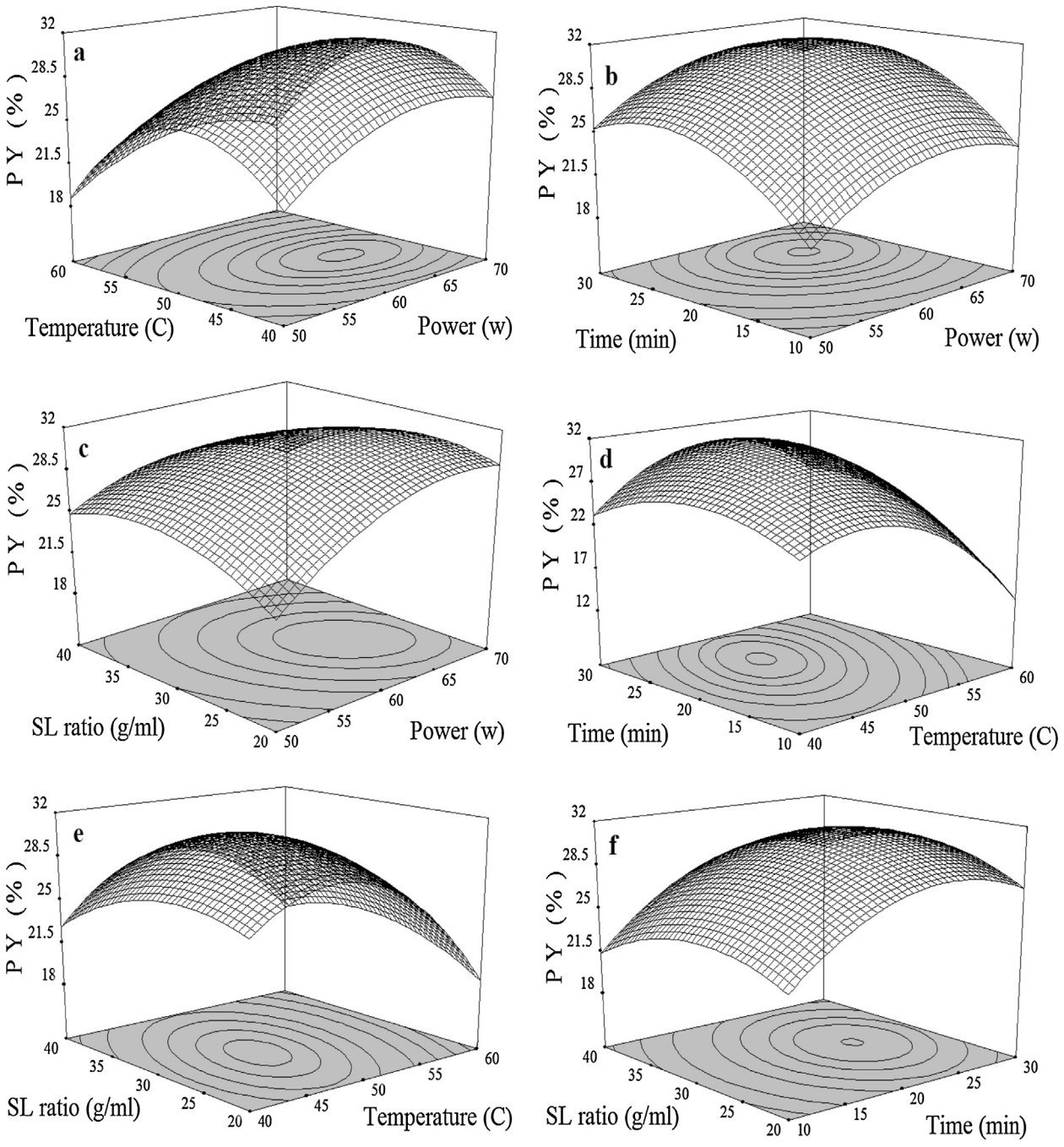

Fig. 2. Response surface plots showing the effect of process variable on pectin yield.

the nature of the optimal point, the regression equation was transformed to the canonical form and the Eigen values were computed using MATLAB 7.1 software. The Eigen values obtained were negative ($X_1 = -6.46$, $X_2 = -1.53$, $X_3 = -3.36$ and $X_4 = -2.90$) and it indicates that, the determined optimal condition was a maximum point.

3.6. Validation of optimized condition

However, considering the operability in actual production, the optimal conditions can be modified as follows: 61 W of ultrasonic power, 50 °C of temperature, 26 min of time and 1:28 g/ml of SL ratio respectively. To compare the predicted results with experimental value, the experiments (triplicates) were carried out at the modified optimal extraction conditions. Under the modified conditions, the experimental yield of pectin was $29.32 \pm 0.16\%$ and was well matched with predicted values (29.43%). As a result, Box–Behnken design was considered to be an accurate and decisive tool for predicting the maximum extraction yield of pectin from sisal waste using UAE technique.

4. Conclusions

In this study, an efficient UAE process was successfully developed to extract pectin from sisal waste. BBD was applied to study and determine the optimal conditions of ultrasonic power, temperature, extraction time and SL ratio for the UAE of pectin from sisal waste. The maximum yield of pectin (29.43%) was obtained under

the following optimal condition: ultrasonic power of 61 W, temperature of 50 °C, time of 26 min and SL ratio of 1:28 g/ml. Under this condition, the actual yield of 29.32 ± 0.16% pectin was acquired. UAE has a strong potential as a method for the extraction of pectin from sisal plant waste material.

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