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Box–Behnken experimental design for investigation of microwave-assisted extracted sugar beet pulp pectin

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ABSTRACT

Optimization of conditions for sugar beet pulp pectin (SBPP) extraction was investigated using response surface methodology (RSM). The average molecular weight ($\overline{M_v}$) of twenty-nine SBPP samples was determined by viscometry. The relationships between four variables and two responses were studied using Box–Behnken experimental design. The constructed models were adequate to explain the relationships between independent variables and responses. All studied factors had great influence on the yield. The satisfactory conditions for SBPP extraction were obtained as follows: 152.63 W of power, 3.53 min of time, 1.57 of pH of sulfuric and 18.92 of SLR. Among the studied factors, pH of sulfuric acid had the greatest influence on $\overline{M_v}$.

1. Introduction

Pectin is a family of complex polysaccharides present in cell walls of all land plants (Axelos, Thibault, & Lefebvre, 1989). The main components of pectin are polysaccharides in which (1 → 4)-linked α-D-galacturonates and methyl esters predominate (Joye & Luzio, 2000; Westereng, Michaelsen, Samuelsen, & Knutsen, 2008).

Pectin has a long safe history of use as gelling agent, thickener and stabilizer in food industry. Unlike commercial pectin extracted from citrus peel and apple pomace, sugar beet pulp pectin has a higher degree of acetylation, a higher neutral sugar content, feruloyl groups and a lower molecular weight (Pippen, McCready, & Owens, 1950; Rombouts & Thibault, 1986). Existence of feruloyl group and lower molecular weight have been attributed to poor gelling property in the condition of high concentrations of sugar when acid is present (Pippen et al., 1950; Roboz & Van Hook, 1946). However, there has been considerable interest in the potential use of SBPP as a replacement for gum Arabic in the stabilization of oil-in-water emulsions for the beverage industry. Otherwise, pectin has been attempted to develop as drug delivery system (Liu, Fishman, Kost, & Hicks, 2003; Liu, Fishman, Hicks, & Kende, 2005; Liu, Kende, Ruthel, Fishman, & Hicks, 2006), flocculant (Ho, Norli, Abbas, & Morad, 2010) and biosorbent (Schiewer & Patil, 2008).

Commercial pectin is extracted from apple pomace and citrus peel at high temperature in the present of acid. Recent years, microwave-assisted method which can distinctly reduce extraction time and energy has been employed for natural products extraction (Fishman, Chau, Cooke, & Hotchkiss, 2008; Fishman, Chau, Hoaglad, & Hotchkiss, 2006; Kratchanova, Pavlova, & Panchev, 2004). In the aspect of pectin extraction, microwave-assisted extraction can prominently increase yield and the $\overline{M_v}$, heighten the degree of esterification and acetylation.

Recently, extraction and characterization of SBPP and the relationships between factors and yield were studied. In fact, all the investigations using full factorial design are tedious and RSM, which is a collection of statistical and mathematical techniques, has been proved to be an effective way for the desired purpose (Kaur, Sarkar, Sharma, & Singh, 2009; Masmoudi et al., 2008; Pinho, Melo, Mansilha, & Ferreira, 2011). Most of these studies were concerned with the extraction of pectin using conventional heating, from which effect of variables on yield, properties and structure had been obtained. Few if any of these studies were directed toward understanding of combined effects of processing variables on the yield and $\overline{M_v}$ of microwave-assisted extracted SBPP.

In this research, the $\overline{M_v}$ was obtained by viscometry. We have studied the relationships between the variables (pH of sulfuric acid, SLR, time and power of extraction) and the responses (yield and $\overline{M_v}$) and obtained satisfactory conditions for extraction using Box–Behnken experimental design.

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Table 1
Experimental domain of the Box–Behnken design.

Factors	Coded symbols	Levels		
		−1	0	1
Extraction time (min)	X_1	2	3	4
Extraction power (W)	X_2	100	1	10
pH of Sulfuric acid	X_3	150	1.5	15
SLR	X_4	200	2	20

2. Material and methods

2.1. Materials

Sugar beet pulp was purchased from the Xinjiang LüYuan Sugar Industry Co., Ltd. (Hejing, Xinjiang Uygur Autonomous Region, China). All the chemical reagents purchased from Tianjin Fuguang Chemical Reagent Co., Ltd. (Tianjin, China) were of analytical grade.

2.2. Pectin extraction

Firstly, sugar beet pulp powder was bleached at 95 °C for 5 min and then dried in an air convection oven at 45 °C. The pretreated powder was marinated in distilled water with SLR 1:10, 1:15, 1:20. The concentration of sulfuric acid was adjusted to be 0.05, 0.016 and 0.005 mol/L (pH = 1, 1.5, 2). The mixture was heated with three powers of 150, 200, 250 W and three times, 2, 3, 4 min. The filtrate (containing pectin) was cooled down and centrifuged. The supernatant was precipitate with absolute alcohol and left to rest in order to float pectin sufficiency. The pectin was dried to a constant weight at 45 °C for 14 h.

Table 2
Variable levels and responses of pectin yield and molecular weight based on power and time of extraction, sulfuric concentration and SLR.

Std.	Variable levels				Response	
	X_1 (min)	X_2 (W)	X_3	X_4 (g/mL)	Yield (%)	$\overline{M_v}$ (10^3)
3	2.00	250.00	1.50	15.00	15.8	7.8
9	2.00	200.00	1.50	10.00	12.4	85.4
19	2.00	200.00	2.00	15.00	5.2	191.1
11	2.00	200.00	1.50	20.00	7.6	27.1
1	2.00	150.00	1.50	15.00	7.4	19.3
17	2.00	200.00	1.00	15.00	22.8	38.5
28	3.00	200.00	1.50	15.00	13.6	142
5	3.00	200.00	1.00	10.00	19.0	47.8
8	3.00	200.00	2.00	20.00	8.4	315.3
29	3.00	200.00	1.50	15.00	14.6	85.4
21	3.00	150.00	1.50	10.00	8.0	90.8
13	3.00	150.00	1.00	15.00	19.0	34.6
6	3.00	200.00	2.00	10.00	7.2	179.5
25	3.00	200.00	1.50	15.00	12.4	144
26	3.00	200.00	1.50	15.00	15.6	147
24	3.00	250.00	1.50	20.00	21.8	65
14	3.00	250.00	1.00	15.00	27.4	18.8
23	3.00	150.00	1.50	20.00	6.8	53.3
16	3.00	250.00	2.00	15.00	16.2	168.3
7	3.00	200.00	1.00	20.00	30	49.8
22	3.00	250.00	1.50	10.00	10.2	164.1
15	3.00	150.00	2.00	15.00	5.0	222.6
27	3.00	200.00	1.50	15.00	10.6	144
2	4.00	150.00	1.50	15.00	22.8	65.2
12	4.00	200.00	1.50	20.00	25.6	40.51
10	4.00	200.00	1.50	10.00	13.4	150.4
18	4.00	200.00	1.00	15.00	32.4	27.4
20	4.00	200.00	2.00	15.00	6.6	160
4	4.00	250.00	1.50	15.00	24.2	26.9

Table 3
Results of analysis of variance (ANOVA) for yield.

Source	Sum of squares	DF	Mean square	*F*-value	*P*-value
X_1	241.20	1	241.20	27.77	0.0001
X_2	180.96	1	180.96	20.84	0.0004
X_3	867.00	1	867.00	99.83	<0.0001
X_4	75.00	1	75.00	8.64	0.0108
X_1X_2	12.25	1	12.25	1.41	0.2547
X_1X_3	16.81	1	16.81	1.94	0.1859
X_1X_4	72.25	1	72.25	8.32	0.2547
X_2X_3	1.96	1	1.96	0.23	0.6421
X_2X_4	40.96	1	40.96	4.72	0.0476
X_3X_4	24.01	1	24.01	2.76	0.1186
X_1^2	31.73	1	31.73	3.65	0.0766
X_2^2	3.76	1	3.76	0.43	0.5211
X_3^2	43.40	1	43.40	5.00	0.0422
X_4^2	6.66	1	6.66	0.77	0.3959
Model	1617.96	14	115.57	13.31	<0.0001
Residual	121.59	14	8.68		
Lack of fit	106.44	10	10.64	2.81	0.1657
Pure error	15.15	4	3.79		
Cor total	1739.55	28			

2.3. Pectin extraction rate

Yield of pectin was calculated as follows (Eq. (1)):

$$\text{Yield of pectin } (\%) = \left(\frac{m_0}{m}\right) \times 100\% \quad (1)$$

where m_0 (g) is the weight of dried product; m (g) is the weight of dried powder.

2.4. Average molecular weight determination

The $\overline{M_v}$ of pectin samples were determined by viscometry. Intrinsic viscosities of the sample solutions were determined using the method developed by Kar and Arslan (1999).

The relative viscosity (η_r) was calculated by the following equation:

$$\eta_r = \frac{\eta_p}{\eta_s} = \frac{t_1 d_1}{t_2 d_2} \quad (2)$$

where the η_s and η_p are the viscosities of solvent and solution, respectively; t_1 and t_2 are the times of solution and solvent flowing in the viscometer, respectively; d_1 is the density of solution and d_2 is the density of solvent.

Table 4
Results of ANOVA for $\overline{M_v}$.

Source	Sum of squares	DF	Mean square	*F*-value	*P*-value
X_1	853.62	1	853.62	0.55	0.4720
X_2	101.50	1	101.50	0.065	0.8025
X_3	866,800	1	866,800	55.49	0.0001
X_4	2323.81	1	2323.81	1.49	0.2427
X_1X_2	179.56	1	179.56	0.11	0.7396
X_1X_3	100.00	1	100.00	0.064	0.8039
X_1X_4	665.38	1	665.38	0.43	0.5246
X_2X_3	370.56	1	370.56	0.24	0.6638
X_2X_4	948.64	1	948.64	0.61	0.4488
X_3X_4	4475.61	1	4475.61	2.87	0.1126
X_1^2	239,600	1	239,600	15.34	0.0016
X_2^2	153,500	1	153,500	9.82	0.0073
X_3^2	1657.00	1	1657.00	1.06	0.3205
X_4^2	327.29	1	327.29	0.21	0.6542
Model	139,700	14	9847.82	6.30	0.0007
Residual	218,700	14	1562.12		
Pure error	14	4	3.50		
Cor total	159,700	28			

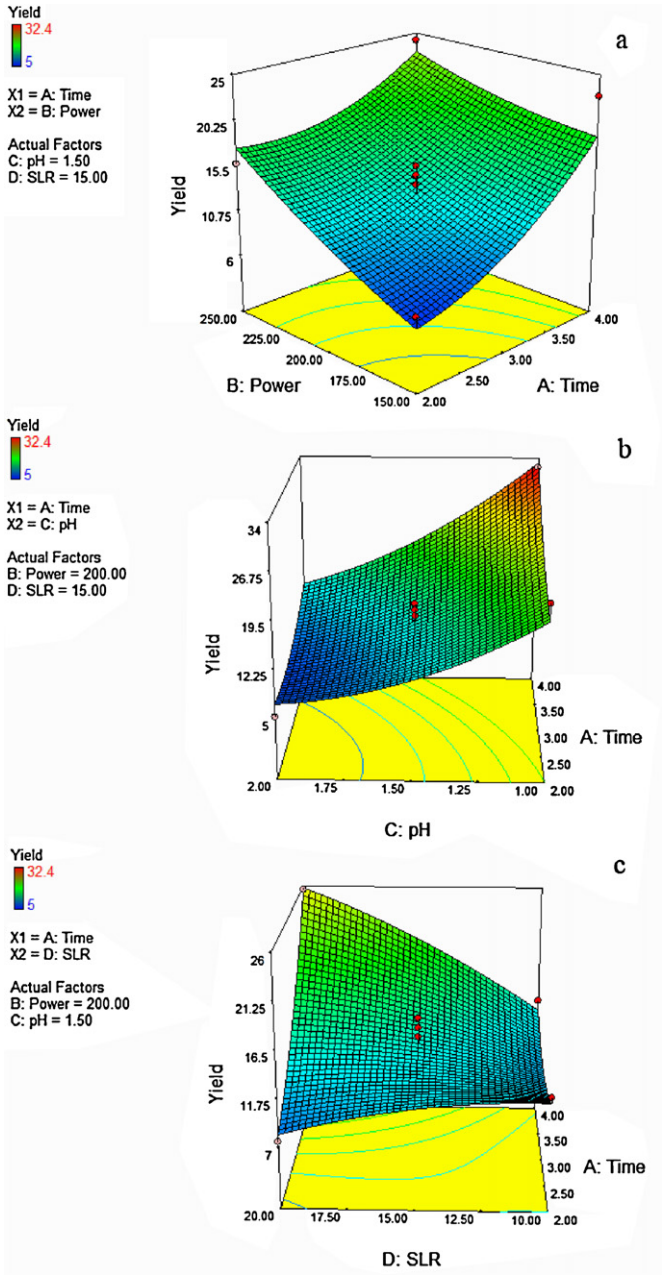

Fig. 1. Surface plots for pectin yield of sugar beet pulp floor: (a) effect of power and time on pectin yield with SLR 15, concentration 1.5; (b) effect of concentration and time on pectin yield with SLR 15, power 200 W; (c) effect of SLR and time on pectin yield with concentration 1.5, power 200 W.

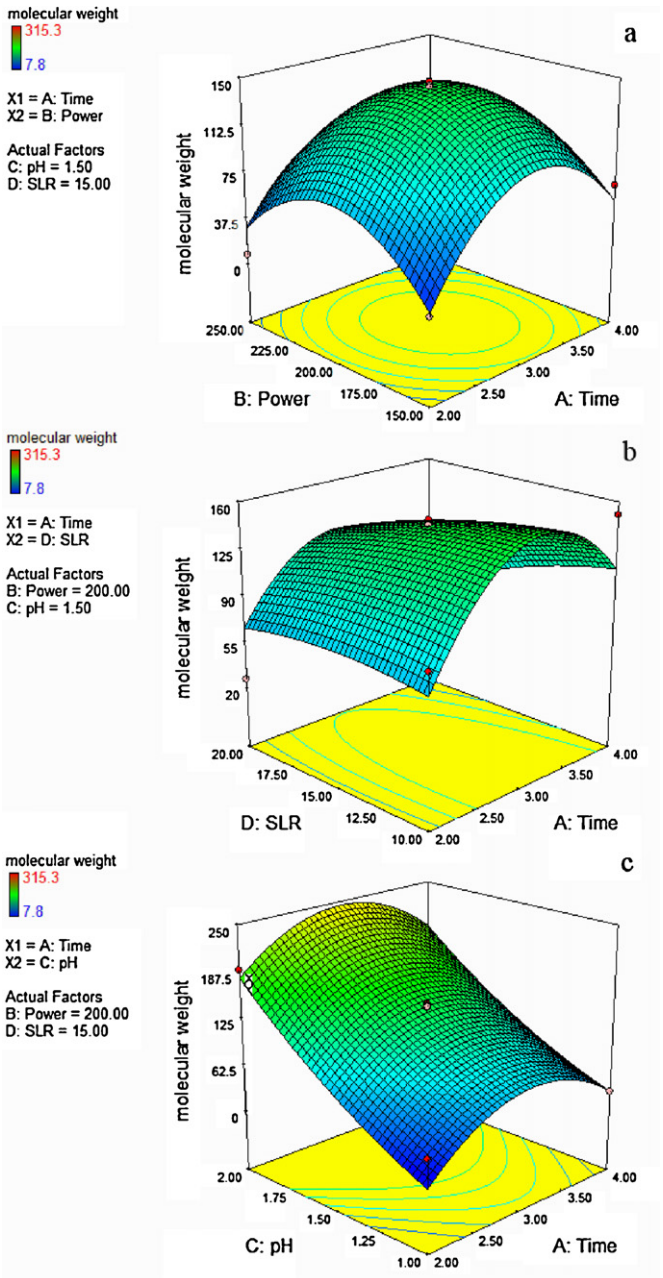

Fig. 2. Surface plots for pectin $\overline{M_v}$ of sugar beet pulp floor: (a) effect of power and time on pectin molecular weight with SLR 15, concentration 1.5; (b) effect of SLR and time on pectin molecular weight with concentration 1.5, power 200 W; (c) effect of concentration and time on pectin molecular weight with SLR 15, power 200 W.

Relative viscosity was translated to specific viscosity (η_{sp}) by the equation:

$$\eta_{sp} = \frac{\eta_p - \eta_s}{\eta_s} = \eta_i - 1 \tag{3}$$

The molecular weight was calculated by the Mark Houwink equation:

$$\eta_i = k\overline{M_v}^a \tag{4}$$

where $\overline{M_v}$ is the viscosity–average molecular weight, k and a are constant values of 0.3 mL/g and 0.613 at 25 °C (Chen & Joslyn, 1967), respectively.

2.5. Experimental methodology

RSM is a statistical method that used quantitative date from appropriate experimental design to determine optimal conditions. Therefore, RSM with Box–Behnken was employed to determine the optimum conditions for SBPP extraction. The experimental factors were ascertained on the basis of the results of preliminary experiments.

In this work, the relationships between the responses and the four selected variables were approximated by the following second order polynomial (Eq. (5)) function:

$$Y = \beta_0 + \sum_{i=1}^{a}\beta_i X_i + \sum_{i=1}^{a}\beta_{ii}X_i^2 + \sum_{i,j=1(i\neq 1)}^{a}\beta_{ij}X_iX_j \tag{5}$$

where Y is the calculated response function and X_i is the corresponding actual value of variable. β_0 is the estimated regression coefficient of the fitted response at the center point of the design; β_i is the regression coefficient for liner effect terms; βij is interaction effects; and β_{ii} is quadratic effects.

2.6. Box–Behnken design

A Box–Behnken design was used to estimate the model coefficients. The levels of the four retained variables are indicated in Table 1. All calculations and graphics were performed by using the experimental design software Design Expert 7.0.

3. Results and discussion

3.1. Response measurements

Experimental values obtained for the yield and $\overline{M_v}$ of the pectin are shown in Table 2. The microwave-assisted acid-extracted pectin yield ranged from 5.2 to 32.4% of dry weight and the $\overline{M_v}$ situated between 7.8×10^3 and 315.3×10^3.

3.2. Estimated model

RSM was used to evaluate the effects of variables on the yield and $\overline{M_v}$ of SBPP, then build a model to find the best setting of the variables that maximize the extraction of yield and study the combined relationships between the conditions and the $\overline{M_v}$. The second-order models in term of coded variable are given by Eqs. (6) and (7).

$$\begin{aligned}\text{Yield} = {} & 13.36 + 4.48X_1 + 3.88X_2 - 8.5X_3 + 2.50X_4 - 1.75X_1X_2 \\ & - 2.05X_1X_3 + 4.25X_1X_4 + 0.70X_2X_3 + 3.20X_2X_4 \\ & - 2.45X_3X_4 + 2.21X_1^2 + 0.76X_2^2 + 2.59X_2^3 - 1.01X_4^2 \end{aligned} \quad (6)$$

$$\begin{aligned}\overline{M_v} = {} & 144.00 + 8.43X_1 - 2.91X_2 + 84.99X_3 - 13.92X_4 \\ & - 6.70X_1X_2 - 5.00X_1X_3 - 12.90X_1X_4 - 9.62X_2X_3 \\ & - 15.40X_2X_4 + 33.45X_3X_4 - 60.78X_1^2 - 48.64X_2^2 \\ & + 15.98X_3^2 - 7.10X_4^2 \end{aligned} \quad (7)$$

The results of analysis of variance (ANOVA) for yield are given in Table 3. As can be seen, the model F-value of 13.31 with a low probability P-value of less than 0.0001 indicted high significance of the model. The lack of fit for an F-value of 0.1657 meant that this term was not significantly relative to the pure error, the nonsignificant value of lack fit (<0.05) showed that the quadratic model was valid for this study. From the results in Table 3, the liner coefficient, the interaction of extraction power and SLR and the quadratic coefficient of pH were significant by t-test at a level of 0.05. The pH had shown to be the most important variable and extraction time the second important factor of this model.

The results of ANOVA for $\overline{M_v}$ are given in Table 4. From Table 4, the model F-value of 6.30 and low probability P-value of 0.0007 revealed that the second-order model fitted the experimental data well. The linear effect of pH exhibited a significant effect on $\overline{M_v}$. Whilst the quadratic terms of time and power were significant.

The relative effect of each variable to the purpose was directly measured by the regression coefficient in Eqs. (6) and (7). A positive sign for the regression coefficient in the fitted model indicates the ability of the factor to increase the responses, whilst a negative sign decrease the intention.

3.3. Analysis of response surface

The relationships between the experimental variables and the responses are illustrated in three dimensional representations of the response surfaces. These plots are presented in Figs. 1 and 2, respectively. The main goal of response surface is to track efficiently for the optimum values of the variables such that the response is maximized. By analyzing the plots, the best response range can be calculated.

From Fig. 1 it is clearly evident that all the variables exerted quadratic effect on pectin extraction. The yield of SBPP was increasing evidently as the decreasing of pH of sulfuric acid, extending of time and increasing of extraction power. One explanation of the trend is that proper acid and power hydrolyze the insoluble pectin to soluble. Besides, the extended extraction time increases the hydrolysis of insoluble pectin. The possible explanation for the decrease of yield when the SLR increased to a proper quantity was that the increased solution enhanced dissolving capacity, but too much solution led to additional burden of further treatment.

Fig. 2 shows the three-dimensional response surface plot for $\overline{M_v}$ of pectin. It can be seen from Fig. 2a that the $\overline{M_v}$ increases firstly, and decreases afterwards with the increase of extraction time and power. As shown in Fig. 2b, the $\overline{M_v}$ of samples change within limits as the increase of SLR. Fig. 2c shows that it is possible to obtain higher $\overline{M_v}$ as the increase of pH. The possible explanation for this trend was that pectins underwent more hydrolysis as extraction process went on. Part of water-soluble pectin of lower $\overline{M_v}$ was extracted in the beginning of the process. As the progress going on, the insoluble pectin hydrolyzed and dissolved in the solution, but the hydrolysis of water-soluble pectin was taken up at the last step of process.

4. Conclusions

RSM was used for optimization of pectin extraction. Pectin was extracted from dried sugar beet pulp with 29 combinations of power and time of microwave-assisted extraction, SLR and pH of sulfuric acid. The linear effect of pH, extraction power and time greatly impacted the yield, and only the pH exhibited a significant effect on $\overline{M_v}$, and the relationship between $\overline{M_v}$ and extraction time was consistent with previous publication (Fishman et al., 2006). The yield and $\overline{M_v}$ of pectin were varied from 5.2 to 32.4% and 7.8×10^3 to 315.3×10^3, respectively. The optimal pectin yield was achieved at 152.63 W of power, 3.53 min of time, 1.57 of pH and 18.92 of SLR.

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