Combined Influence of Extraction Parameters on Degree of Esterification of Sugar Beet Pulp Pectin: A Preliminary Study

[1, 2] Deqiang Li, [3] Jianjiang Shang, [1] Xinfang Ma, [3] Xiaoyan Zhu and [1] Zhiyong Liu*

[1]*College of Chemistry and Chemical Engineering, Shihezi University, Shihezi, Xinjiang, 832003, China.*

[2]*College of Chemical Engineering, Xinjiang Agricultural University, Urumqi, Xinjiang, 830000, China.*

[3]*Department of Mechatronic Engineering, Yili Vocational and Technical College, Yili, Xinjiang, 835000, China.*

lzyongclin@sina.com*

(Received on 6th August 2012, accepted in revised form 10th December 2012)

Summary: A Box-Behnken design was employed to study the combined effects of variables of microwave-assisted extraction on pectin from sugar beet pulp. The independent variables were time and power of microwave-assisted extraction, pH of sulfuric acid and rate of solid to liquid (SLR). The combined effects of these variables on degree of esterification (DE) were studied. Results have shown that the generated regression model significantly explained the actual relationship between the independent variables and response. Besides that, pH was the most important variable which affected the DE. Higher acid concentration, extraction power and longer extraction time increased the DE of pectin due to Esterification. Through the response surface, the satisfactory conditions for extraction of high-ester sugar beet pulp pectin were obtained as follows: 1.02 of pH, 3.96 min of extraction time, 193.68W of power and 19.98 of SLR.

Keywords: Sugar beet pulp; Degree of esterification; Response surface methodology; Box-Behnken design.

Introduction

Sugar beet is one of the most important sugar crops all over the world. The process for the manufacture of sugar generates about 10 million tons fresh sugar beet which is an enormous undeveloped biological waste in China. The pulp which contains high percent of pectin is used to feed animals at little value. Hence, a viable way for enhancing added value and reducing environmental influence is necessary to make the sugar beet pulp into useful products.

Pectin is a carbohydrate which is composed of complex polysaccharides and present within the primary cell wall to think as "glue" that hold cellulose together of almost all higher plants. Pectin is mainly polysaccharides in which (1→4)-linked α-D galacturonates and the predomination is their methylester [1, 2]. Generally, pectin is used as gelling and emulsification agent for a long history in the food industry [3]. Due to the influence of forming sugar acid gel systems of different pectin which has different structures, few materials have been used as commercial pectin source. Sugar beet pulp pectin (SBPP) contains higher degree of acetylation and neutral sugar content and feruloyl group, and pool gelling property and emulsification as a result which significantly influence the usage in food process.

Along with the in-depth study, pectin has been used as the biosorbent for heavy metal removal. Schiewer and Patil [4] investigated the removal of cadmium by pectin-rich fruit wastes (derived from several citrus fruits, apples and grapes) and found that the citrus peel may be the most promising biosorbent. Moreover, Ho et al. [5] characterized and compared the pectin and PAM as flocculant and optimized the treatment conditions in Kaolin suspension. All these studies were in view of that the pectin could associate with divalent metal ion, and the structure of pectin significantly influenced the association. While, few literatures focused on the effects of pectin structure on the removal of heavy metals [6, 7].

Response surface methodology (RSM) makes the process faster and provides useful information with statistically valid results and has been testified to be an effective way for the above mentioned desired purpose [8-10]. RSM has been used to optimize the process of extraction of pectin and the property of pectins. The effects of conditions on the pectin were reported by Pinheiro *et al.* [11]. In their paper, the combined effect of variables (extraction time and citric acid concentration) and the relationship between variables and DE of pectin from passion fruit peel were investigated, but the conditions of extraction power and SLR which were also important to the properties were not studied.

The aim of this study was to determine the effect of operational conditions on the DE of SBPP for further studies of adsorption properties of heavy

*To whom all correspondence should be addressed.

metals. In our study, the DE of SBPP was determined, and the relationships between variables (time and power of microwave-assisted extraction, SLR and concentration of acid) and the response (DE of SBPP) were investigated by RSM.

Results and Discussion

Response Measurements

Experimental values obtained for the DE of pectin are shown in Table-1. The DE of SBPP ranged from 33.23 to 83.44%. Compared to the dates found in the literature, the DE of SBPP extracted using microwave was closed to Sun and Hughes [12]. The performance may be the result of abridgement of extraction time caused by the microwave which effectively decreased the degree of hydrolysis.

Table-1: Variable levels and responses of DE based on time and power of microwave-assisted extraction, pH of sulfuric and SLR.

Std	Variable levels				Response
	X_1(min)	X_2 (W)	X_3	X_4 (g/mL)	DE (%)
3	2.00	250	1.5	15	52.00
28	3.00	200	1.5	15	59.32
5	3.00	200	1	10	62.79
9	2.00	200	1.5	10	64.81
8	3.00	200	2	20	44.26
2	4.00	150	1.5	15	60.00
29	3.00	200	1.5	15	62.07
21	3.00	150	1.5	10	46.50
13	3.00	150	1	15	81.48
12	4.00	200	1.5	20	62.84
6	3.00	200	2	10	40.32
10	4.00	200	1.5	10	63.71
18	4.00	200	1	15	84.38
25	3.00	200	1.5	15	61.36
26	3.00	200	1.5	15	58.73
24	3.00	250	1.5	20	60.81
14	3.00	250	1	15	81.30
20	4.00	200	2	15	38.80
19	2.00	200	2	15	44.26
23	3.00	150	1.5	20	41.82
16	3.00	250	2	15	42.14
11	2.00	200	1.5	20	30.00
4	4.00	250	1.5	15	61.54
7	3.00	200	1	20	74.19
1	2.00	150	1.5	15	46.94
22	3.00	250	1.5	10	43.18
15	3.00	150	2	15	41.39
17	2.00	200	1	15	75.00
27	3.00	200	1.5	15	40.74

Model Prediction and Fitting

RSM was used to evaluate the effect of time and power of microwave-assisted extraction, pH of sulfuric acid and SLR on the DE of pectin extracted from sugar beet pulp, then to build a model that describes the behavior of DE. A second-order model in terms of coded variables was given by Eq. 1.

$$DE = 56.44 + 4.85X_1 + 1.90X_2 - 17.33X_3 - 0.62X_4 - 0.88X_1X_2 - 3.71X_1X_3 + 8.94X_1X_4 + 0.23X_2X_3 + 5.88X_2X_4 - 1.86X_3X_4 + 1.29X_1^2 - 1.85X_2^2 + 4.55X_3^2 - 4.88X_4^2 \quad (1)$$

The results of analysis of variance (ANOVA) are given in Table-2. As can be seen, the model *F*-value of 4.61 with a low probability *P*-value of 0.0036 indicted high significance of the model. The lack of fit for an *F*-value of 0.92 means that this term is not significantly relative to the pure error, the nonsignificant no significant value of lack fit (< 0.05) shows that the quadratic model is valid for this study.

Table-2: Results of analysis of variance (ANOVA) for DE.

Source	Sum of squares	DF	Mean square	*F*-value	*P*-value
Model	4812.38	14	343.73	4.61	0.0036
X_1	282.80	1	282.80	3.80	0.0717
X_2	43.47	1	43.47	0.58	0.4576
X_3	3604.12	1	3604.12	48.37	< 0.0001
X_4	4.55	1	4.55	0.061	0.8048
X_1X_2	3.10	1	3.10	0.042	0.8414
X_1X_3	55.02	1	55.02	0.74	0.4046
X_1X_4	289.94	1	289.94	3.87	0.0694
X_2X_3	0.22	1	0.22	2.902E-003	0.9578
X_2X_4	124,43	1	124,43	1.67	0.2172
X_3X_4	13.91	1	13.91	0.19	0.6722
X_1^2	10.85	1	10.85	0.15	0.7058
X_2^2	22.28	1	22.28	0.30	0.5931
X_3^2	134.12	1	134.12	1.80	0.2011
X_4^2	151.77	1	151.77	2.04	0.1754
Residual	1043.07	14	74.51		
Lack of fit	727.14	10	72.71	0.92	0.5860
Cor Total	5855.45	28			

From the results in Table-2, only the liner coefficient of the pH of acid variable was significance level of 0.05. The interaction of extraction and SLR has shown to be the second most important coefficient of this model.

Analysis of Response Surface

The mutual effects between the experimental variables and the response are illustrated in three dimensional representations of the response surfaces. The results from 3-level-4-factor experiments (Table-1) were used for constructing the plot. The main goal of response surface is to track efficiently for the optimum values of the variables, and the tendency of the contribution caused by all variables were obtained.

Fig. 1a shows the effects of extraction power from 150 to 250 W and extraction time from 2 to 4 min on DE of pectin, in the condition of the lowest

power and time, the DE of the pectin was obtained about 54.04%. With the increasing of the power and time, the DE of the pectin was augmenting. The mutual effects between extraction pH and SLR on DE of sugar beet pulp pectin were also investigated by the same strategy.

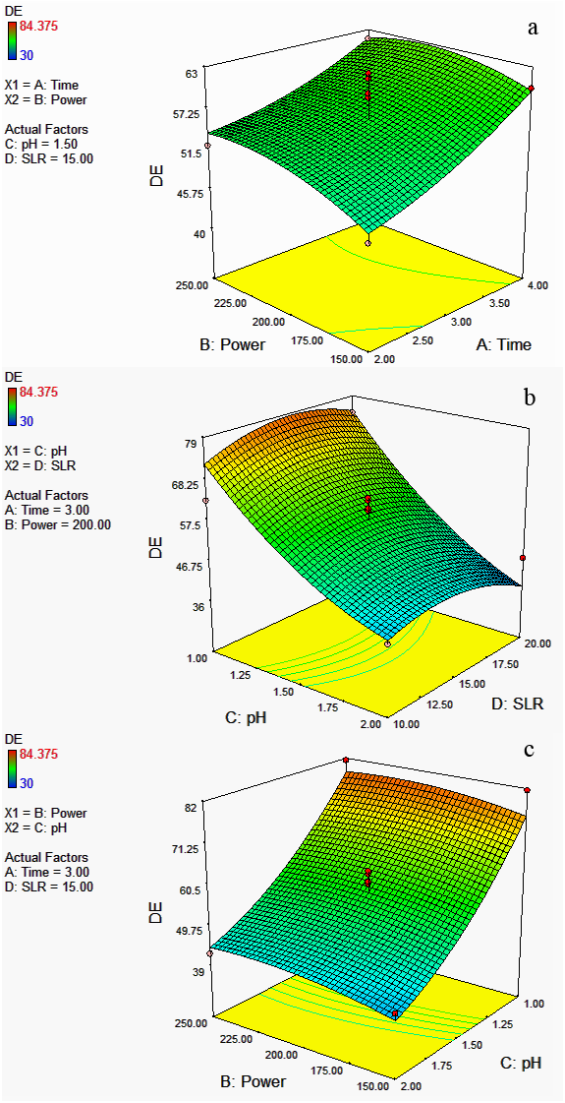

Fig. 1: Surface plots for pectin DE of sugar beet pulp floor: (a) Effect of extraction power and time on pectin DE with SLR 15, pH 1.5; (b) Effect of extraction pH and SLR on pectin DE with time 3.00 min, power 200.00W; (c) Effect of extraction power and pH on pectin DE with SLR 15, extraction time 3 min.

Fig. 1b shows the effects of pH from 1 to 2 and SLR from 1:10 to 1:20. It appears that the DE of SBPP was increasing with the decreasing of pH of sulfuric acid, the main causation caused increasing phenomenon may be that esterification reaction was occurred. In this process, an ester was made by the interaction of hydroxyl and carboxyl groups of pectin molecular (intramolecular and/or intermolecular) in the presence of a sulfuric acid catalyst. This outcome is opposed to the conclusion obtained by Pinheiro, *et al.* [11]. In their literature, citric acid instead of H_2SO_4 advanced the hydrolysis of pectin.

The effects of pH and power were clearly presented in Fig. 1c. Once again, the three-dimensional plots showed that the DE of SBPP was largening with the increasing of pH and power.

From the estimating of Box-Behnken design, a pH of 1.02, extraction time of 3.96 min, power of 193.68 and SLR of 19.98 were obtained for extraction of high-ester SBPP.

Experimental

Materials

Sugar beet pulp is provided by Xinjiang LüYuan Sugar Industry Co., LTD, (Hejing, Xinjiang Uygur Autonomous Region, China). All the chemical reagents purchased from Xian Chemical Reagent Co., Ltd. (Xi'an, Shanxi Province, China) and were of analytical grade and were used as such.

Pectin Extraction

The sugar beet pulp was bleached at 95°C for 5 min for decreasing the enzyme activities and dried in an air convection oven at 45°C. The pretreated sugar beet (5g) is marinated in distilled water with SLR 1:10, 1:15, 1:20. The pH of sulfuric acid was adjusted to three degrees, 1, 1.5 and 2.0. The mixture was heated with the different microwave power (150, 200, 250W) and microwave time (2, 3, 4 min) and then filtrated. The filtrate was cooled down to ordinary temperature and centrifuged. The supernatant was precipitated with twice volume of absolute alcohol and rested in order to float pectin sufficiency. The filtrate was separated by filtrating and rinsed three times with 95% ethanol. The pectin was dried to a constant weight at 45°C for 14 h.

Determination of Degree of Esterification

The DE of SBPP sample was determined by

FT-IR analysis. All samples were incorporated with KBr and pressed into flakes. The infrared spectra were recorded in the region of 4000-500 cm^{-1}. The DE was obtained from the ratio of the area upon the band at 1740 cm^{-1} corresponding to the number of esterified carboxylic acid groups to the total area of the bands at 1740 and 1630 cm^{-1} corresponding to the number of total carboxylic groups [13, 14].

The DE was calculated by using

$$\%DE = \left(\frac{A_{1740}}{A_{1740} + A_{1630}}\right) \bullet 100 \qquad (2)$$

where *DE* is the degree of esterfication of pectin, A_{1740} and A_{1630} are the area upon the band of 1740 and 1630 cm^{-1}, respectively.

Experimental Design

Optimization of conditions for pectin extraction from sugar beet pulp was carried out by using RSM. Four independent variables, time (X_1) and power (X_2) of microwave-assisted extraction, pH of acid (X_3) and SLR (X_4) were conducted followed the experimental design statistical analysis obtained by the full factorial Box-Behnken. All the levels were selected based on the preliminary experiments, and indicated in Table-3. All calculations and graphics were performed using the experimental design software design expert 7.0.

Table-3: 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	150	200	250
pH	X_3	1.0	1.5	2.0
SLR	X_4	10	15	20

In this work, the relationships between the DE and four selected variables were approximated by the following second order polynomial (Eq. 3.) function:

$$Y = \beta_0 + \sum_{i=1}^{a} \beta_i X_i + \sum_{i=1}^{a} \beta_{ii} X_i^2 + \sum_{i,j=1(i\neq 1)}^{a} \beta_{ij} X_i X_j \qquad (3)$$

where Y is the calculated response function, X_i the corresponding actual values of variables. 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; β_{ij} is interaction effects; and β_{ii} is quadratic effects.

Conclusion

Pectin was extracted from dried sugar beet pulp with 29 combinations of extraction power, SLR, pH and extraction time. The model obtained fits the study well, and only the pH showed significant effects on the DE from the ANOVA. By the analysis of the response surface, a divertive result that the DE of pectin was augmenting with the increasing of pH, time and power was opposed to the conclusion already published was obtained. The satisfactory condition was obtained in order to get a high-ester SBPP by using RSM.

Acknowledgements

This work was supported financially by funding from the National Scientific Personnel Service Enterprise Foundation of China (Contract grant number: 2009GJG41046).

References

1. B. L. Ridley, M. A. O'Neill and D. Mohnen. *Phytochemistry*, **57**, 929 (2001).
2. H. Abid, A. Hussain, S. Ali and J. Ali. *Journal of the Chemical Society of Pakistan*, **31**, 459 (2009).
3. E. Theuwissen, and R. P. Mensink. *Physiology and Behavior*, **94**, 285 (2008).
4. S. Schiewer, and S. B. Patil. *Bioresource Technology*, **99**, 1896 (2008).
5. Y. C. Ho, I. Norli, F. M. Abbas and A. N. *Morad. Bioresoure Technology*, **101**, 1166 (2010).
6. M. T. Kartel, L. A. Kupchik and B. K. Veisov. *Chemosphere*, 38, 2591 (1999).
7. F. T. Li, H. Yang, Y. Zhao and Ran Xu. *Chinese Chemical Letters*, **18**, 325 (2007).
8. M. U. Rahman, M. M. Yasinzai, R. B. Tareen, A. Iqbal, E. A. Odhano and S. Gul. *Journal of the Chemical Society of Pakistan*, **33**, 29 (2011).
9. G. Began, M. Goto, A. Kodama, T. Hirose. *Food Research International*, **33**, 341, (2000).
10. A. B. Shaibu and B. R. Cho. *International Journal of Advanced Manufacturing Technology*, **41**, 631 (2009).
11. E. R. Pinheiro, I. M. D. A. Silva, L. V. Gonzaga, E. R. Amante, R. F. Teófilo, M. M. C. Ferreira and R. D. M. C. Amboni. *Bioresource Technology*, **99**, 5561 (2008).
12. R. Sun, S. Hughes. *Polymer Journal*, **30**, 671 (1998).
13. A. K. Chatjigakis, C. Pappas, N. Proxenia, O. Kalantzi, P. Rodis and M. Polissiou. *Carbohydrate Polymers*, **37**, 395 (1998).
14. G. D. Manrique and F. M. Lajolo. *Postharvest Biology and Technology*, **25**, 99 (2002).