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Investigation of combined effects of independent variables on extraction of pectin from banana peel using response surface methodology

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ABSTRACT

Combined effects of key factors (pH value, extraction temperature, extraction time, salting out time) on extraction of pectin from banana peel was investigated by response surface methodology (RSM). When enriched in pectin, this research can be useful for preparation of banana products. Results showed that extraction temperature and time had the most significant combined effect on the improvement of extraction rate of pectin from banana peel, followed by the combined effects of extraction time and salting-out temperature. Besides, the extraction rate of pectin from banana peel also obviously affected by the interaction effects of pH and salting-out temperature. The fitted mathematical model allowed us to plot response surfaces and to determine optimal extraction conditions. Results showed that the optimum conditions were established for extraction of banana peel pectin: pH value 1.5, extraction temperature 85.5 °C, extraction time 2 h, and salting-out temperature 70.0 °C.

1. Introduction

Banana (*Musa supientum*) is an important food crop in both tropical and subtropical regions (Emaga, Robert, Ronkart, Wathelet, & Paquot, 2008). In 2004, the production of banana reached about 72 million tons (Su, Du, & Wang, 2001). With the development of the processing industries of banana and the increase in the production of processed fruit products, a large quantity of banana peel was wasted or cheaply consumed as animal feed, which was obviously un-economy and unfriendly to the environment. Therefore, banana-processing industries have been searching for applications of these by-products which proved to be a source of important natural compounds, such as pectin (Cordenunsi, Shiga, & Lajolo, 2008; Willats, McCartney, Mackie, & Knox, 2001).

Pectin is a structural component of non-starch polysaccharides substances and the main adhesive components in plant cell wall, widely existing in the sap of green plants (Schols & Voragen, 1996; Willats et al., 2001). It is well accepted that pectin is a high value functional food ingredient because of its excellent emulsifying properties and stability which can be used as gelling agent and stabilizer (May, 1990).

Commercial pectin is extracted at high temperatures by hydrolyzing protopectin using acids such as sulfuric, phosphoric, nitric, and hydrochloric or citric acid (May, 1990; Minkov, Minchev, & Paev, 1996). Microwave-assisted extraction (Wang et al., 2007) and enzymatic method (Shkodina, Zeltser, Selivanov, & Ignatov, 1998) also have been employed in recently years. Lots of researches showed that pH, temperature, extraction time, agitation and solid to liquid ratio had effects on the yield and the quality of pectin (Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008; Levigne, Ralet, & Thibault, 2002; May, 1990).

Many researchers focused on the extraction of pectin with the conventional materials, including pumpkin (Shkodina et al., 1998), peach promace (Pagán, Ibarz, Llorca, Pagán, & Barbosa-Cánovas, 2001), sugar beet (Levigne et al., 2002), apple pomace (Wang et al., 2007), citrus peel (Kratchanva, Pavlova, & Panchev, 2004; Ros, Schols, & Voragen, 1996), etc. Tapping into the trend for alternative sources of pectin, Emaga, Robert, et al. (2008) reported that pectin extracted from banana skin could find application as a gelling agent. In their research, emphasis was especially laid on the characteristics of the banana peel pectin. Till now, little work has been done on investigation of the combined effects of the processing variables on the extraction of pectin from banana peel.

In fact, when many factors and interactions affected desired response, both the optimization of the process and investigation of their combined effects were lengthy and tedious. Response surface methodology (RSM) was proved to be an effective way for the above-mentioned purpose, which was a collection of statistical and mathematical techniques (Atkinson & Donev, 1992; Box & Behnken, 1960; Chandrika & Fereidoon, 2005; Fabio & Antonio, 1996; Wang et al., 2007). It had been successfully used for developing, improving, and optimizing processes, the main advantage of which is the reduced number of experimental trials needed to eval-

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uate multiple parameters and their interactions. So, it was less time-consuming than other conventional methods (Wang et al., 2007).

In this paper, RSM was firstly employed for the extraction of banana peel pectin. The aim of this research was to develop an approach that would bring a better understanding of the combined effects of the key processing variables (pH value, extraction temperature, extraction time, salting-out temperature) on the desired response (extraction rate of pectin) , as well as to look for optimum conditions of the pectin extraction from banana peel.

2. Materials and methods

2.1. Materials and apparatus

Local wild banana was commercially purchased from Guangzhou, China. Thermostable α-amylase and neutrase are purchased from Yuli Biological Technology Co., Ltd. (Guangzhou, China). All solvents and chemicals used were purchased from Tianjin Chemicals Co., Ltd. (Tianjin, China), and were certified analytical grade. HH-2-type constant-temperature-bath equipment was produced by Fuhua Electrical Appliance Co., Ltd. (Jintan, China). AEL-200 Electronic Analytical Balance and PHS-25 type pH instrument were produced by Yulung Instrument Co., Ltd. (Shanghai, China). 101A-1-type electric heater thermostat oven was blast produced by Shanghai Experimental Instrument Factory (Shanghai, China). SHZ-D-type vacuum pump was produced by Yingyu-Yuhua Instrument Factory (Gongyi, China) DZG-6050SA-type vacuum drying oven produced by Senxin experimental equipment Co., Ltd. (Shanghai, China).

2.2. General pectin extraction procedure

General extraction was carried out in a glass flask immersed in a water bath as follows: Fresh banana peel was cut into small pieces (about 4 mm × 4 mm), soaked in hot water at 85 °C for 10 min, and then washed with cold water. The washed banana peel pieces were enzymatically treated with 16 U/g thermostable α-amylase at 60 °C for 10 min, followed by further enzymatic treatment with 16 AU/g neutrase at 45 °C for 30 min. Pectin was extracted from the pretreated banana peel by 0.3% of sodium hexametaphosphate solution at 85 °C for 1.5 h. The extracts were filtrated to remove solid particles and vacuum condensed. Into 100 ml of the condensed mixture, 7 ml of saturated aluminum sulfate solution was added (pH 2) at 70 °C for 45 min. After precipitation by an equal volume of 96% (v/v) ethanol, the obtained mixture was statically cooling down. Then, the obtained pectin was centrifuged, washed three times with 70% ethanol and centrifuged again.

2.3. Experimental design

RSM with central composite design was used to determine the optimum condition for pectin extraction from banana peel. A central composite design was chosen to look for the best experimental conditions of four independent factors affecting the extraction process which were: X_1: pH of the mixture; X_2: extraction temperature (°C); X_3: extraction time (h); and X_4: salting-out temperature (°C). For each factor, the experimental range was chosen on the basis of results of preliminary experiments. Experiments in the centre of the design were performed in order to make the estimation of pure error possible. All the experiments were carried out at random in order to minimize the effect of unexplained variability in the observed responses due to systematic errors. All calculations and graphics were performed using electronic worksheets from Design Expert 7.0 software.

2.4. Pectin extraction rate

Yield of pectin, subject of this study, was calculated as follows:

$$\text{Yield of pectin } (\%) = (m_0/m) \times 100$$

m_0 (g) is the dried product weight; m (g) is the dried raw material weight.

3. Results and discussion

3.1. Regression analysis of relationship between desired response and independent variables

Thirty experiments were carried out according to the conditions indicated in Table 1. Response values (pectin extraction rate) were reported in the last column of this table. Regression analysis (in Table 2) was made to the experimental data aiming at an optimal region for the responses studied. The significance of each coefficient was determined using the F-test and p-value in Table 2. The corresponding variables would be more significant if the absolute F-value becomes greater while p-value becomes smaller (Atkinson & Donev, 1992). It showed that the variables with the largest effect were the interaction effects of extraction temperature and time (X_2X_3), the quadratic term of extraction time (X_3^2), the interaction effects of extraction time and salting-out temperature (X_3X_4), and the linear terms of pH (X_1) and salting-out temperature (X_4), followed by the interaction effects of pH and salting-out temperature

Table 1
Central composite design with the experimental responses values for yield of pectin.[a]

No.	X_1[b]	X_2[c]	X_3[d]	X_4[e]	Yield (%)
1	1	−1	−1	−1	0.986
2	0	0	1	0	0.680
3	0	0	0	0	1.06
4	0	0	−1	0	0.908
5	1	1	1	1	0.816
6	0	0	0	−1	0.969
7	−1	0	0	0	1.06
8	1	1	−1	−1	0.934
9	−1	−1	1	−1	0.758
10	0	0	−1	0	1.06
11	1	0	1	−1	0.799
12	1	0	−1	1	0.561
13	−1	1	−1	1	0.758
14	0	0	0	0	1.06
15	0	1	0	0	0.945
16	−1	1	−1	−1	0.750
17	0	−1	0	0	0.768
18	1	−1	1	1	0.823
19	1	1	−1	1	0.360
20	−1	−1	1	1	0.723
21	0	0	0	0	1.06
22	−1	−1	−1	1	0.929
23	−1	1	1	−1	1.14
24	1	0	0	0	1.07
25	−1	1	1	1	1.05
26	0	0	0	0	1.06
27	0	0	0	1	0.926
28	0	0	0	0	1.06
29	−1	−1	0	−1	0.877
30	1	1	1	−1	0.702

[a] The extraction conditions were described in Section 2.2.
[b] The three levels (−1, 0, and 1) of factor X_1(pH) represented 1.5, 2.0, and 2.5, respectively.
[c] The three levels (−1, 0, and 1) of X_2 (extraction temperature) represented 80, 85, and 90 °C, respectively.
[d] The three levels (−1, 0, and 1) of X_3 (extraction time) represented 1.0, 1.5, and 2.0 h, respectively.
[e] The three levels (−1, 0, and 1) of X_3 (salting-out temperature) represented 65, 70, and 75 °C, respectively.

Table 2
Estimated regression model of relationship between response variables (pectin extraction) and independent variables (X_1, X_2, X_3, X_4) and variance analysis of items of regression equation of pectin yield.

Item	Quadratic sum	DF	Mean square error	*F*-value	*p*-value
X_1	0.055	1	0.055	3.20	0.0940
X_2	0.0030	1	0.0030	0.18	0.6806
X_3	0.010	1	0.010	0.59	0.4534
X_4	0.052	1	0.052	3.04	0.1019
X_1X_2	0.037	1	0.037	2.13	0.1649
X_1X_3	0.0002	1	0.0002	0.013	0.9101
X_1X_4	0.039	1	0.039	2.28	0.1522
X_2X_3[a]	0.084	1	0.084	4.83	0.0441
X_2X_4	0.0016	1	0.0016	0.093	0.7649
X_3X_4	0.056	1	0.056	3.24	0.0922
X_1^2	0.024	1	0.024	1.40	0.2555
X_2^2	0.032	1	0.032	1.87	0.1917
X_3^2	0.078	1	0.078	4.53	0.0502
X_4^2	0.0011	1	0.0011	0.063	0.8052
Model[a]	0.67	14	0.048	2.78	0.0296
Residual	0.26	15	0.017		
Lack of fit	0.26	10	0.026	2730.05	0.0001
Net error	0.00005	5	0.00001		
Correction term	0.93	29			

[a] Significance level of .05.

(X_1X_4). Analysis of variance (ANOVA) for the model was also carried out to validate the model (in Table 2). Results showed that the regression was significant (p-value <0.05).

3.2. Analysis of response surface

The relationship between the responses and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Figs. 1–6).

3.2.1. Interaction effects of extraction temperature and pH on pectin extraction rate

Fig. 1 showed a tortuose response surface of the extraction temperature and pH value on the extraction yield of pectin from banana peel, indicating that the extraction rate of pectin increased rapidly at first and then decreases by increasing extraction temperature and/or decreasing pH value. These effects were markedly shown for extraction temperatures of 65–85 °C and low pH levels. One possible explanation for the increase of the extraction rate was the improvement of solubility of the extracted pectin by increasing temperature in a certain range. Besides, the diffusion coefficient also normally increased and so that improved the rate (Coulson & Richardson, 1978). Moreover, proper acidic conditions favored the hydrolysis of the insoluble pectin constituents into soluble pectin, thus increasing the pectin recovery (El-Nawawi & Shehata, 1988).

3.2.2. Interaction effects of extraction time and pH value on pectin extraction rate

The response surface in Fig. 2 showed the combined effects of extraction time and pH, indicating that longer extraction time firstly led to higher extraction rate of pectin. Further prolonging the extraction time, however, decreased the extraction rate. And a properly low pH value gave higher yields of banana pectin. The

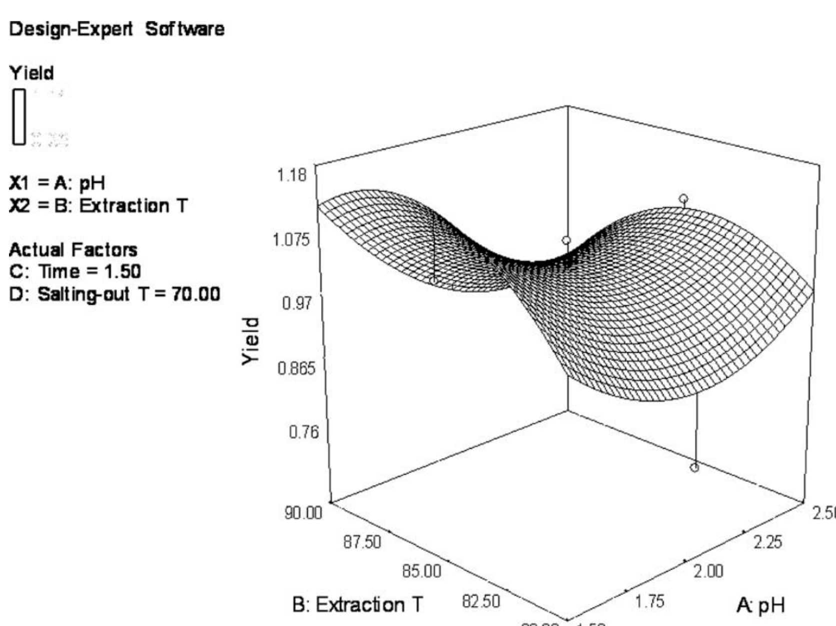

Fig. 1. Combined effects of extraction pH and temperature on extraction rate of pectin.

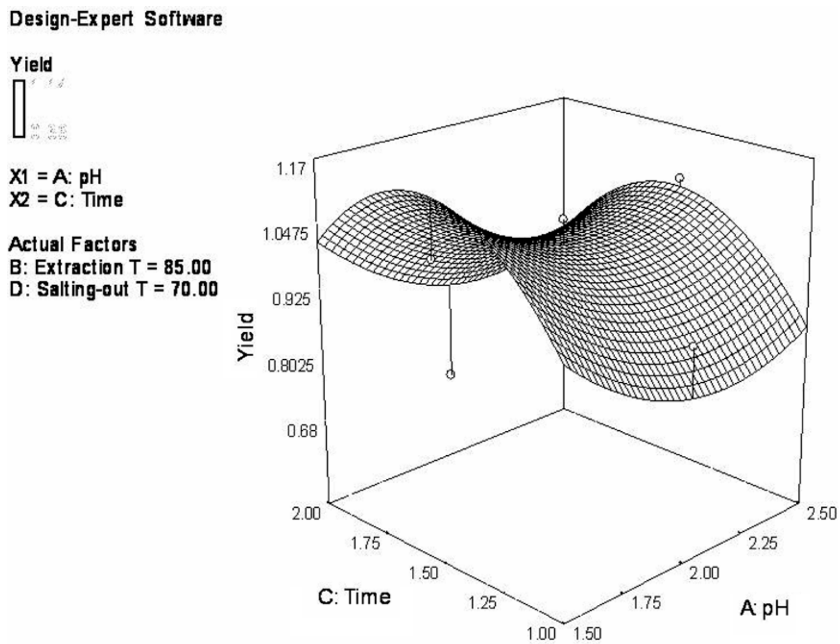

Fig. 2. Combined effects of extraction pH and extraction time on extraction rate of pectin.

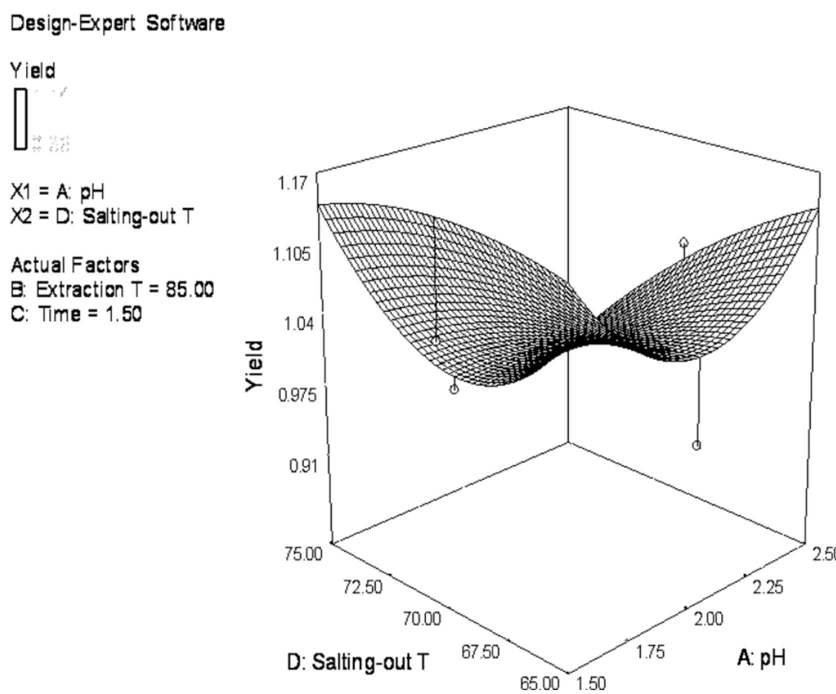

Fig. 3. Combined effects of extraction pH and salting-out temperature on extraction rate of pectin.

maximum extraction rate was reached at the optimum conditions (pH of 1.5 and extraction time of 2 h).

3.2.3. Interaction effects of pH and salting-out temperature on pectin extraction rate

In Fig. 3, the examination of three-dimensional plots showed that the extraction rate of pectin increased when increasing salting-out temperature and/or decreasing the pH. These effects were markedly shown for salting-out temperatures of 65–70 °C and low pH levels. When salting-out temperature and pH value were regulated to 70 °C and 1.5, respectively, the extraction rate of pectin from banana peel reached the maximum value.

3.2.4. Interaction effects of extraction time and temperature on pectin extraction rate

Both extraction time and temperature displayed significantly quadratic effects on the yield of pectin in the response surface and contour plots in Fig. 4. The extraction rate of pectin first increased rapidly with the increase of both extraction time and temperature, but then decreased steadily. It was inferred that the greater extraction rate of pectin could be obtained at the following

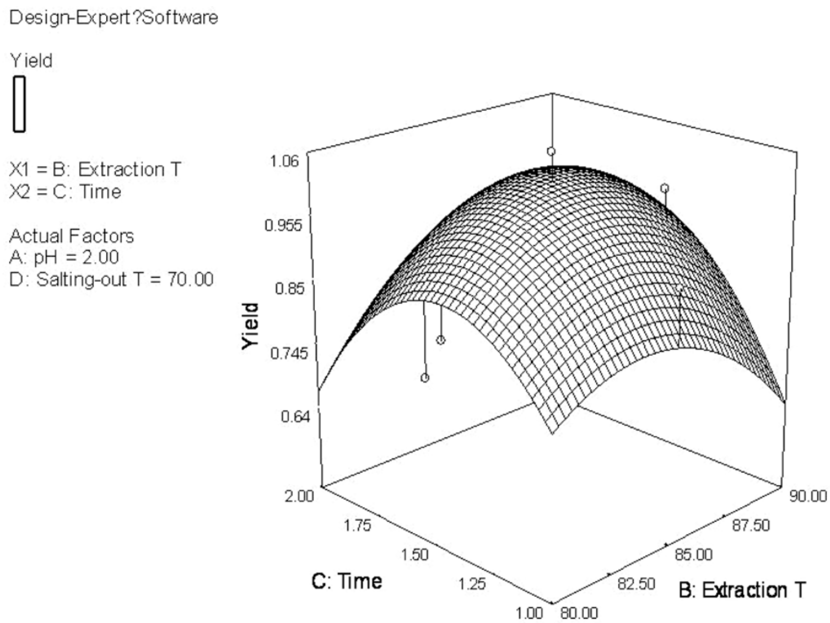

Fig. 4. Combined effects of extraction temperature and time on extraction rate of pectin.

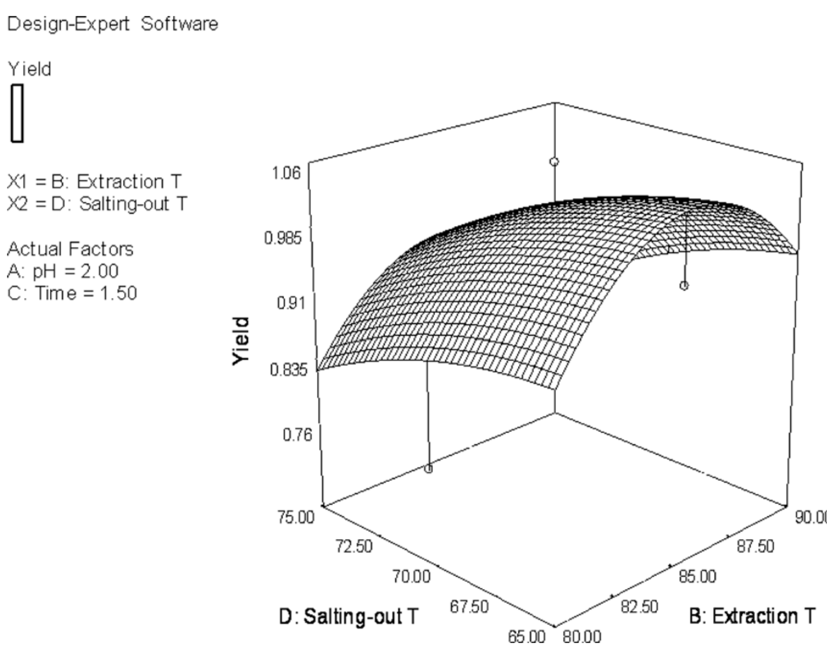

Fig. 5. Combined effects of extraction and salting-out and salting-out temperature on extraction rate of pectin.

conditions: about 85.5 °C of extraction temperature and 1.5 h of extraction time. The reason was that the pectin was not so easy to be dissolved out from the plant cell wall if the extraction temperature was too low and extraction time was too short. Besides, much high extraction temperature and too long extraction time may lead to degradation of pectin chain molecules, thus affecting pectin extraction rate.

3.2.5. Interaction effects of extraction and salting-out temperatures on pectin extraction rate

The correlative effects of salting-out temperature and extraction temperature on pectin extraction rate are demonstrated in Fig. 5, indicating parabolic relationships between extraction/salting-out temperatures and pectin extraction rate. A higher salting-out temperature may led to a slow increase of extraction rate of pectin, while increase of the extraction temperature may rapidly improve extraction rate of pectin and then decrease it. The highest extraction rate of pectin can be achieved by changing the extraction temperature and salting-out temperature to 85.5 and 70 °C, respectively.

3.2.6. Interaction effects of extraction time and salting-out temperature on pectin extraction rate

Fig. 6 depicted response surface of the interaction effects of the last investigated two variables, namely extraction time and salting-out temperature on the yield of pectin. It was found that extraction rate of pectin first increased rapidly and then slowly decreased along with the increase of extraction time. And it was

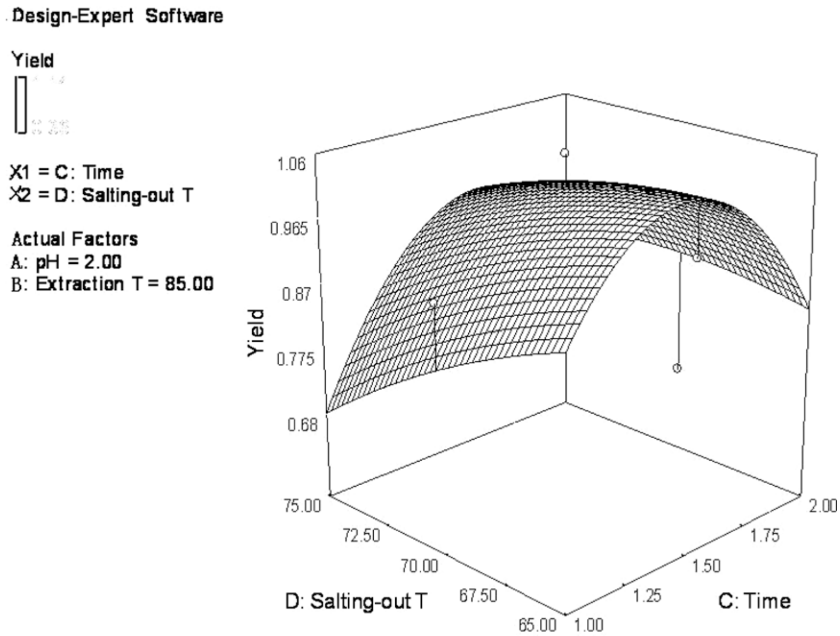

Fig. 6. Correlative effects of extraction time and temperature on extraction rate of pectin.

slowly decreased by increasing the salting-out temperature. It was inferred that much higher salting-out temperature resulted in the degradation of the chain of pectin molecules, thus affecting the extraction yield of pectin.

4. Conclusion

This research revealed that RSM was an effective tool for estimating the interaction effect of key independent variables in the extraction of pectin from banana by-product. Extraction temperature and time showed more significant combined effect on the response value, i.e. extraction rate of pectin from banana peel, than the quadratic term of extraction time and the interaction effects of extraction time and salting-out temperature. Besides, the linear terms of pH and salting-out temperature and the interaction effects of pH and salting-out temperature also obviously influenced the extraction rate of pectin from banana peel. By using the response surface, the optimum condition of these operating variables was also graphically obtained to reach a high extraction rate of pectin from banana peel. These results demonstrated the successful extraction of pectin from banana peel, providing potential benefits to industrial extraction of pectin, from both an economic and environmental points of view.

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