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Optimization of pectin extraction from steam distillated orange peels through an experimental factorial design

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ABSTRACT: This study examined variables that influence the acid-extraction of pectin from Steam Distillated Orange Peels using two different acids; an organic acid (citric acid) and a mineral one (sulfuric acid). The effects of these acids on extraction of pectin were investigated by response surface methodology. Extraction parameters which are employed in this study are temperature of extraction (X1: 50°C-80°C), acid concentration (X2: 0.05M-0.1M), acid hydrolysis time (X3: 30min-60min) and. In the case of citric acid, results showed that extraction temperature, time and acid concentration had the most significant combined effect on the extraction rate of pectin from Steam Distillated Orange Peels, followed by the combined effects of extraction temperature and acid concentration. While results showed, in the case of the sulfuric acid, that all the combinations have a significant effect on pectin yield, but the combined effect of the three factor is the most significant followed by the combined effect of time and temperature extraction, then the combined effect of the acid concentration and time extraction and finely the acid concentration and temperature extraction as the less significant combined effect. Pectin yields varied from 11.32% to 28.23% using citric acid and from 11.6% to 30.30% using sulfuric acid.

KEYWORDS: Pectin, Response Surface Methodology, Citrus Solid Waste, Optimization, Combined Effect.

1 INTRODUCTION

Pectins are polysaccharides from plant cell walls, widely used in the food industry as gelling and stabilizing agents. In addition, May and Mohnen reports that pectin has several positive effects on human health, including reduced serum cholesterol, reduced serum glucose and reduced cancer incidence amongst other effects [1], [2].

The extraction of pectin from citrus wastes is mainly accomplished, pectin content of citrus peel is usually high, 25 - 30% of the dried peel mass and this yield may change according to the acid used in the extraction and different factors such as acid concentration, time and temperature of extraction. Many works are available on the extraction of pectins with mineral acids, such as sulfuric acid [3], [4], hydrochloric acid [5], [6], [7], [8], nitric acid [9], [10], tartaric acid [11] and with organic acids such as citric acid [12], [11], [13], [14]. In this study we choose to use the sulfuric acid for the good pectin yield that it provide and the citric acid that could be better than the other extractors from an economic as from an environmental point of view.

Few works has been done on the combined effects of variables factors on pectin extraction from steam distillated orange peels. In fact, both the optimization of the process and investigation of their combined effects are time consuming.

Experimental factorial design is the most effective way for the above-mentioned purpose, which was a collection of statistical and mathematical techniques [15], [16], [17], [18], [19], [20].

In this paper, experimental factorial design was employed for the extraction of steam distillated orange peel pectin. The aim of this research is to develop an approach that allow a better understanding of the combined effects of three different processing variables (acid concentration, time and extraction temperature) on the desired response (pectin yield) , as well as to look for optimum conditions of the pectin extraction from steam distillated orange peels (SDOP).

2 MATERIALS AND METHODS

2.1 RAW MATERIALS

Orange peels were collected from local restaurant as solid waste after jus extraction. The peels were washed dried and steam distillated for essential oil extraction. Once the steam distillation completed, the steam distillated orange peels were collected, dried and crushed for pectin extraction.

2.2 PECTIN EXTRACTION

Pectin was extracted from SDOP with aqueous sulfuric acid and citric acid (1:30, w/v) under reflux, using a mechanical blender in the extraction conditions established by preliminary tests. After centrifugation (3000g for 10 min), each extract obtained was filtered, treated with ethanol 96% (v/2v; extract/ethanol) and stirred slowly and stored in a refrigerator overnight in order to fully achieve pectin precipitation [21], [22]. Afterward, the polysaccharides were removed by centrifugation, washed three times with ethanol and dried under vacuum. The weight was monitored until stabilization.

2.3 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Response surface methodology was used to determine the optimum condition for pectin extraction from steam distillated orange peels in each case (sulfuric and citric acid). The variables were monitored using a 3^2 factorial design and each experiment was realized in triplicate.

Experimental factorial design was used to determine the optimum condition for pectin extraction from SDOP in each case (sulfuric and citric acid). The variables were monitored using a 3^2 factorial design and each experiment was realized in triplicate.

The variables used were: extraction temperature (X1), acid concentration (X2) and extraction time (X3). The minimum, maximum levels for citric acid concentration were set at 0.05M and 0.1M, respectively, and for the effect of extraction time they were set to 30 and 60 min, respectively. While the minimum, maximum levels for the extraction temperature were 50°C and 80°C. The levels were selected based on previous studies (unpublished results). The complete design consisted of 8 experiments and each one of theme was realized in triplicates. 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. The response function (y) measured was the pectin yield.

2.4 PECTIN EXTRACTION RATE

Pectin yield was calculated as follows:

$$\text{Pectin yield (\%)} = (m_0/m)*100$$

m0 (g) is the dried pectin weight;
m (g) is the dried raw material weight

3 RESULTS AND DISCUSSION

3.1 REGRESSION ANALYSIS OF RELATIONSHIP BETWEEN DESIRED RESPONSE AND VARIABLES

Twenty four experiments were carried out according to the conditions indicated in Table 1. Response values (pectin extraction yield under each acid condition) were reported in the last column of this table. For both acids, regression analysis (Table 2 for citric acid and table 3 for the sulfuric acid) was made to the experimental data aiming at an optimal region for the responses studied (pectin yield). F-test and p-value (Table 2 & 3) were used to the determination of the significance of each coefficient; it would be more significant if the absolute F-value becomes greater while p-value becomes smaller [15].

Table 1. Design with the experimental responses values for pectin yield using citric acid and sulfuric acid

Experiments	Temperature (X1)	Concentration (X2)	Time (X3)	Yield (%) citric acid	Yield (%) sulfuric acid
1	1	-1	-1	12.81	15.45
2	-1	-1	1	13.43	17.41
3	1	-1	-1	11.32	14.62
4	1	1	-1	28.12	19.24
5	-1	-1	-1	21.58	11.6
6	1	1	-1	28.23	20.44
7	1	-1	1	19.10	21.39
8	-1	-1	1	13.05	18.24
9	1	1	-1	26.36	20.86
10	1	-1	1	20.16	22.92
11	1	1	1	21.61	24.66
12	1	-1	-1	12.35	15.06
13	-1	-1	1	13.59	18.68
14	-1	1	-1	17.73	13.85
15	1	-1	1	19.69	21.76
16	-1	1	1	23.87	29.35
17	-1	-1	-1	19.86	11.96
18	-1	1	-1	16.89	14.57
19	-1	-1	-1	18.25	12.09
20	-1	1	1	24.00	30.3
21	1	1	1	23.58	24.48
22	1	1	1	22.11	24.29
23	-1	1	-1	16.30	13.69
24	-1	1	1	24.38	29.24

3.1.1 CITRIC ACID CASE

Table (2) and Figure (1) showed that the variables with the most significant effect were the interaction effects of acid concentration, extraction temperature and time (X1*X2*X3), followed by the combined effect of the acid concentration and temperature extraction (X1*X2) and the combined effect of extraction temperature and time (X1*X3) was less significant than the previous combined effects, while acid concentration and time (X2*X3) was the least significant combined effect. The linear terms of the effect of concentration (X2) was highly significant, followed by the term of temperature extraction (X1), while the term of time extraction (X3) was the least significant. The graphical representation is below (3.1.2 SULFURIC ACID CASE).

Table 2. Estimated regression model of relationship between response variables (pectin extraction) and independent variables (X1, X2 and X3) and variance analysis of items of regression under citric acid conditions

Effects	Sum of Squares	Degree of Freedom	Mean square error	F Ratio	Prob> F
X1	21.113	1	21.113	26.183	0.0001
X2	253.435	1	253.435	314.301	<0.0001
X3	3.205	1	3.205	3.974	0.06353
X1*X2	40.482	1	40.482	50.204	<0.0001
X1*X3	1.193	1	1.193	1.479	0.2416
X2*X3	0.393	1	0.393	0.487	0.4953
X1*X2*X3	258.924	1	258.924	321.108	<0.0001
Model	578.743	7	82.678	102.534	<0.0001
Error	12.902	16	0.806	<0.0001	
C. Total	591.645	23	25,724		

3.1.2 SULFURIC ACID CASE

Regression analysis (in Table 3) was made to the experimental data aiming at an optimal region using sulfuric acid for the pectin extraction. Table 3 showed that the variables with the most significant effect were the interaction effects of acid concentration, extraction temperature and time (X1*X2*X3), followed by the combined effect of time and temperature extraction (X1*X3) and the combined effect of the acid concentration and time (X2*X3), finely the combined effect of acid concentration and temperature extraction (X1*X2) was less significant between all combined effects. The linear terms of time extraction (X3) was highly significant, followed by the term of acid concentration (X2), while the temperature extraction (X1) has the least significant effect. These effects were illustrated graphically in Figure 2.

Table 3. Estimated regression model of relationship between response variables (pectin extraction) and independent variables (X1, X2 and X3) and variance analysis of items of regression under sulfuric acid conditions

Source	Sum of Squares	Degree of Freedom	Mean square error	F Ratio	Prob> F
X1	24.382	1	24.382	75.2961	< 0.0001
X2	169.549	1	169.549	523.608	< 0.0001
X3	410.771	1	410.771	1268.562	< 0.0001
X1*X2	13.878	1	13.878	42.858	< 0.0001
X1*X3	41.686	1	41.686	128.736	< 0.0001
X2*X3	16.750	1	16.750	51.729	< 0.0001
X1*X2*X3	54.451	1	54.451	168.158	< 0.0001
Model	731.465	7	104.495	322.707	<0.0001
Error	5.181	16	0.3234	<0.0001	
C. Total	736.646	23	32.028		

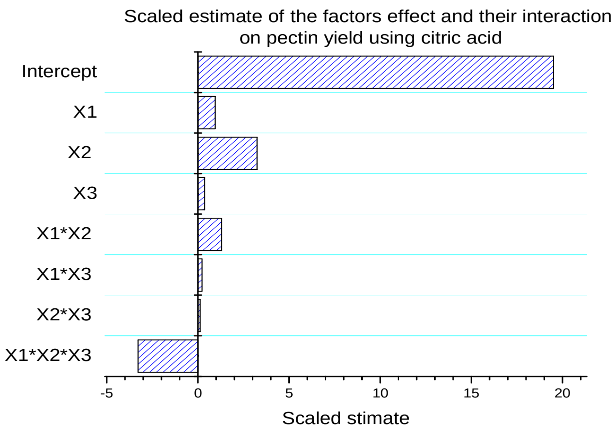

Fig. 1. Scaled estimate of the factors effect and their interaction on pectin yield using citric acid

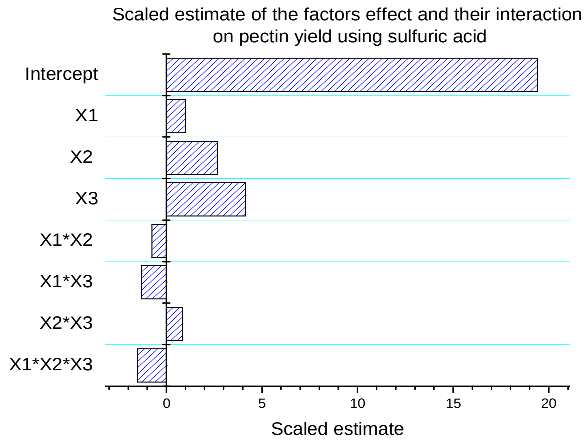

Fig. 2. Scaled estimate of the factors effect and their interaction on pectin yield using sulfuric acid

Analysis of variance (ANOVA) for the model was carried out to validate the model (in Tables 2and 3). Results showed that the regression was significant (p-value<0.05).

3.2 Analysis of response surface of the pectin extracted with citric acid and sulfuric acid

The relationship between the responses and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Figs. 3–5).

3.2.1 Interaction effects of temperature (X1) and acid concentration (X2) on extraction yield.

Fig. 3 represents a response surface of the extraction temperature and citric acid concentration value on the extraction yield of pectin from SDOP. It showing that the extraction rate of pectin increased by increasing both extraction temperature and acid concentration value. These effects were markedly shown for extraction temperatures at 80°C and at high concentration level. The possible explanation for the increase of the extraction rate is what Coulson and Richardson has shown that the improvement of solubility of the extracted pectin by increasing temperature in a certain domain of influence. Besides, the diffusion coefficient also normally increased and so that improved the pectin yield [23]. Also, the acidic conditions allow the hydrolysis of the insoluble pectin constituents into soluble pectin, which increases the pectin yield [24].

Fig. 4 represent a response surface of the extraction temperature and sulfuric acid concentration value on the extraction yield of pectin from SDOP, showing that the extraction rate of pectin increased by increasing extraction temperature and/or decreasing acid concentration value. These effects were markedly shown for extraction temperatures at 80°C and at a low concentration level. As reported above, the solubility of the extract pectin is improved by increasing the temperature which improves the pectin yield.

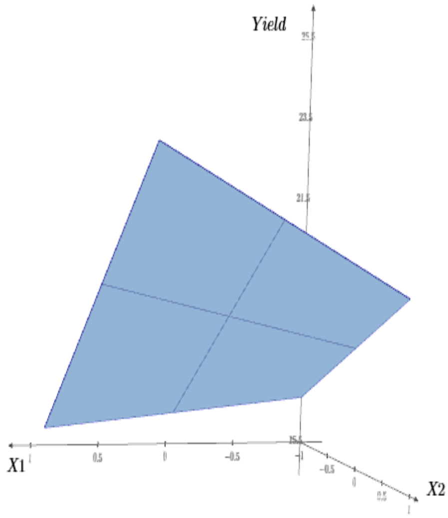

Fig. 3. (citric acid case)

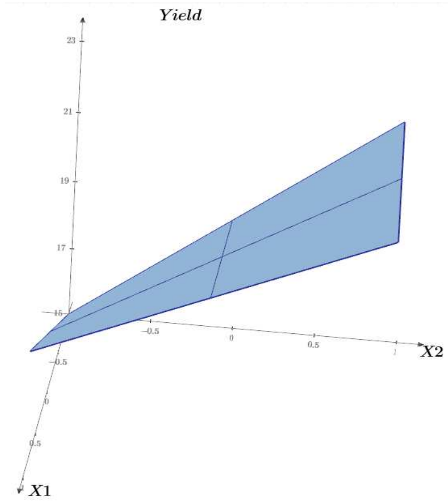

Fig. 4. (sulfuric acid case)

Interaction effects of extraction temperature and acid concentration.

3.2.2 INTERACTION EFFECTS OF EXTRACTION TIME AND ACID CONCENTRATION VALUE ON PECTIN EXTRACTION YIELD.

The response surface in Fig. 5 showed the combined effects of extraction time and citric acid concentration show that longer extraction time and a high acid concentration provides a high pectin yield. Further prolonging the extraction time at low acid concentration increased the extraction rate. The maximum extraction rate was reached at the optimum conditions (concentration of 0.1 M and extraction time of 60min).

The response surface in Fig. 6 showed the combined effects of extraction time and acid concentration, indicating that longer extraction time firstly led to higher extraction rate of pectin. Prolonging the extraction time, even at low acid concentration, increase the extraction rate. The highest pectin yield was reached at the optimum conditions (concentration of 0.1 M and extraction time of 60min.

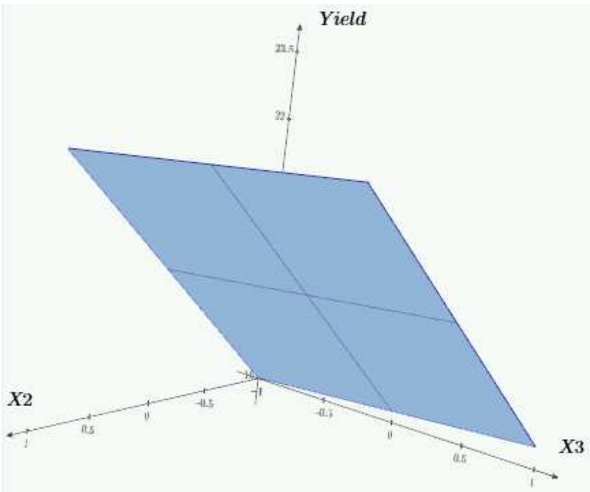

Fig. 5. (citric acid case)

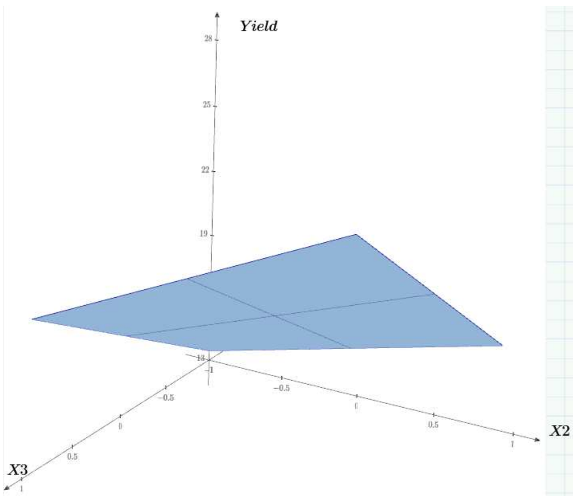

Fig. 6. (sulfuric acid case)

Interaction effects of extraction time and acid concentration.

3.2.3 INTERACTION EFFECTS OF TIME AND TEMPERATURE ON PECTIN EXTRACTION YIELD

In Fig. 7, the examination of three-dimensional plots showed that the extraction rate of pectin increased when increasing both time and temperature. These effects were markedly shown for temperatures 80°C and long duration 60 min. 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. Furthermore, a too long extraction time and a very high temperature can lead to some degradations of pectin chain, thus lowering the pectin yield.

Both extraction time and temperature (Fig. 8) displayed significantly effects on the yield of pectin. The extraction rate of pectin first increased rapidly with the decrease of both extraction time and temperature. It was inferred that the greater extraction rate of pectin could be obtained at the following conditions: about 50°C of extraction temperature and 30min of extraction time. In the citric acid case, long time extraction and a high temperature were necessary to get a high yield, but in the sulfuric acid case short time extraction and a low temperature were good enough to get a high pectin yield. These results can be very interesting knowing that pectin chain may be deteriorated by a hot temperature and longtime extraction.

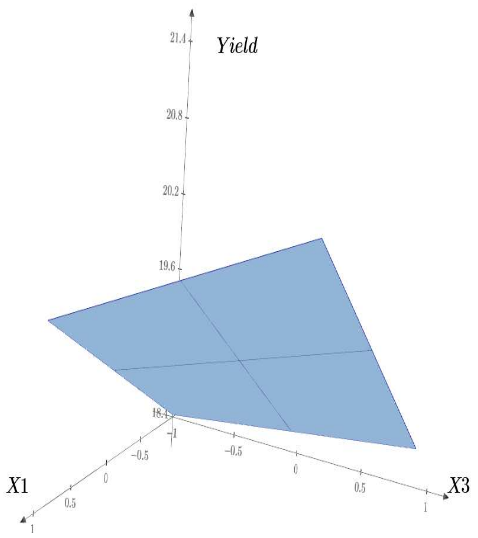

Fig. 7. (citric acid case)

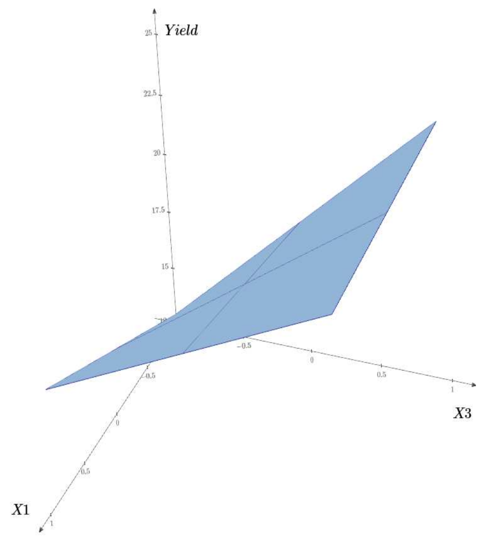

Fig. 8. (sulfuric acid case)

Interaction effects of extraction time and temperature.

4 CONCLUSION

Experimental factorial design was used as tool to estimate the interaction effect of independent variables in the extraction of pectin from steam distillated orange peels. The combined effect of the three factors was significant in both case of pectin extraction (citric and sulfuric acids).

Extraction temperature and acid concentration showed more significant combined effect on the response value obtained using citric acid to the pectin extraction, than the quadratic term of the other combined effects. Besides, the linear terms of acid concentration and temperature and their interaction also obviously influenced the extraction rate of pectin from steam distillated orange peels. The optimum condition of these operating variables was also graphically obtained to reach a high extraction rate of pectin. While in the sulfuric acid case, the linear terms of extraction time and concentration were more significant than all the combined effects. The interaction of temperature extraction and time has a significant effect on the pectin yield, followed by the interaction of temperature extraction and acid concentration.

These results demonstrated the successful extraction of pectin from steam distillated orange peels, providing potential benefits to industrial extraction of pectin, from both an economic and environmental points of view.

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