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Optimization of pectin extraction from dried peel of citrus grandis

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Summary

The influence of acid concentration, temperature, and extraction time on pectin extracted from dried peel of Citrus grandis have been investigated. The dependence of pectin yield and of the relative viscosity of pectin solution on experimental conditions has been described by mathematical models.
To obtain suitable pectin for immobilizing biocatalysts, the extraction should be carried out at low acid concentration, low temperature, and for long periods of time.

Introduction

The pectin content of citrus peel is usually high, 25 - 30 % of the dried peel mass [1]. In Vietnam a large quantity of 2 - 3 cm thick Citrus grandis peel is wasted. It is, therefore, worthwile to get pectin from this seemingly waste material to different uses in food industry [1], entrapping microbial cells [2] etc.
Until now pectin is mostly used in food industry. For this purpose a relatively wide range of molecular weights of pectin is allowed. However, in order to form stable polymer beads in fixing biocatalysts, pectin should be in the first place long enough. To this end, this attempt has been made to get the pectin extraction under control by means of mathematical models.
Changes of pectin yield and solution viscosity as functions of extraction conditions have been established. The knowledge of how these factors effect molecular weight of pectin, can make it possible to produce polysaccharide carrier for immobilized biocatalysts.

Materials and Methods

Extraction

100 g of Citrus grandi dried peel was pretreated with 1 l of hot water. 500 ml of dilute hydrochloric acid were added to the 600 g wet material. After mixing, the mixture was put into a water bath for a fixed period of time.
The liquid phase was then separated. When an equal volume of ethanol was poured into it under stirring, pectin was precipitated. This gelatinous precipitate was filtered and dried. The yield of raw pectin was determined gravimetrically.

Determination of Solution Viscosity

1 g of dried pectin was dissolved in 100 ml of 0.9 % NaCl solution under slightly alkaline condition. The relative viscosity of pectin solution was determined at 25^{o} C by means of an Ubbelohde viscosimeter No. 3.

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Choice of Main Influences and Experimental Matrix

The extraction can be affected by many factors such as the ratio of liquid and solid phase, particle sizes atc. There is evidence of the fact that the three variables - acid concentration, temperature and reaction time - play a decisive role.

To avoid disturbing reactions of pectin in acid solution like decarboxylation and ring forming reactions, the extraction was done at a temperature much lower than applied in usual pectin extraction from citrus peel [1].

A 3-factor statistical design was developed according to [3], The levels corresponding to the factors in this plan were;

acid concentration	$0.72 < c < 2.16$	$x_1 = (c - 1.44) / 0{,}72$
temperature (^{o} C)	$50 < t < 70$	$x_2 = (T - 60) / 2$
reaction time (h)	$5 < t < 9$	$x_3 = (t - 7) / 2$

The measured responses were: y_1 : pectin yield (%) y_2 : relative viscosity of pectin solution with the concentration of 1g/ml in comparison with 0,9 % NaCl solution

Results and Discussion

15 experiments were carried out under conditions according to the matrix in Table 1. The model which corresponds to the response value of the statistical design mentioned above has the following quadratic expression:

$$Y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_33 \\ + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_{33}x_3^2 \tag{1}$$

A program for quadratic regression has been run on a Personal Computer Apple II to

- calculate coefficients bi
- verify the significance of each coefficient by the t-test at 95 % confidence level
- formulate mathematical model whereby insignificant coefficients are eliminated
- calculate response value according to the model and prove whether the model can be accepted
- predict the optimum value vy using simplex method.

For the significance test variances are needed. They were determined by a four-time repetitive measurement under the conditions:

$x_1 = x_2 = x_3 = 0$	$c = 1.44$ %	$T = 70^o$ C	$t = 7$ h
for pectin yield	$s_1^2 = 0.216$	$f = 3$	
relative viscosity	$s_2^2 = 0.0369$	$f = 3$	

After calculating all the coefficients of (1) we have obtained the models for pectin yield and relative viscosity in the following form:

$$Y_1 = 9.37 - 0.07x_1 + 0.63x_2 + 0.16x_3 - 0.17x_1x_2 - 0.18x_1x_3 - 0.14x_2x_3 \\ + 0.05x_1^2 + 0.03x_2^2 + 0.31x_3^2 \tag{2}$$

$$Y_2 = 0.75 - 0.53x_1 + 0.04x_2 + 0.07x_3 - 0.06x_1x_2 - 0.07x_1x_3 - 0.26x_2x_3 \\ + 0.34x_1^2 + 0.005x_2^2 + 0.15x_3^2 \tag{3}$$

Table 1: Results of pectin extraction under different experimental conditions

Run	acid c (%)	T (^oC)	t (h)	coded variables x_1	x_2	x_3	pectin yield y_1	Y_1	rel. viscosity y_2	Y_2
1	0.72	50	5	-1	-1	-1	8.63	8.74	1.37	1.35
2	2.16	50	5	1	-1	-1	8.33	8.74	0.50	0.30
3	0.72	70	5	-1	1	-1	10.09	9.99	1.85	1.88
4	2.16	70	5	1	1	-1	10.22	9.99	0.89	0.82
5	0.72	50	9	-1	-1	1	8.74	8.74	1.93	1.88
6	2.16	50	9	1	-1	1	8.81	8.74	0.43	0.82
7	0.72	70	9	-1	1	1	10.75	9.99	1.01	1.35
8	2.16	70	9	1	1	1	9.07	9.99	0.13	0.30
9	2.31	60	7	1.215	0	0	9.97	9.37	0.79	0.60
10	0.56	60	7	-1.215	0	0	9.14	9.37	2.09	1.89
11	1.44	72.2	7	0	1.215	0	10.03	10.13	0.90	0.75
12	1.44	47.8	7	0	-1.215	0	9.02	8.61	1.00	0.75
13	1.44	60	9.34	0	0	1.215	9.68	9.37	0.85	0.75
14	1.44	60	4.57	0	0	-1.215	8.35	9.37	0.58	0.57
15	1.44	60	7	0	0	0	9.71	9.37	0.60	0.75

y : experimental response value   Y : response value calculated by equ. (4) and (5)

By eliminating insignificant coefficients the models are simplified:

$Y_1 = 9.37 + 0.63x_2$ (4) $Y_2 = 0.75 - 0.53x_1 + 0.34x_1^2 - 0.26x_2x_3$ (5)

The response values are calculated according to the simplified models (Y_1 , Y_2 in Table 1). The Fisher test indicates that the models are accepted.

Equ.(4) shows that under the employed experimental conditions pectin yield depends strongly on temperature only. Greater amount of pectin is available at higher temperature, perhaps due to the acceleration of transfer processes in this solid-liquid reaction.

The great values of b_1 and b_{11}in equation (5) indicate that the relative viscosity of pectin in solution depends greatly on acid concentration, x_1. The effect of acid concentration on the viscosity is illustrated in Fig. 1. In the investigated variable range of the acid concentration, 0.72 - 2.16 %, lower acid concentration garantees the production of higher viscosity pectin.

A study of the negative coefficient $b_{23} = - 0.26$ in equation (5) shows that temperature and extraction time have contradictory effects on the viscosity of pectin solution.

In an extraction for short period of time - Fig. 1. a: $x_3 = -1$; $t = 5$ h - the greatest viscosity is reached at higest temperature - $x_2 = 1$; $t = 70^o$ C. This means that the long pectin macromolecules can not quickly be extracted at low temperature. If the temperature increases, the transfer process of great macromolecules into the liquid phase becomes better, so that the pectin solution viscosity increases with increasing temperature.

In the 9 hour extraction - Fig. 1. b: $x_3 = 1$; $t = 9$ h -, however, the viscosity decreases with increasing temperature. The glucosidic linkage of pectin molecules can be disrupted more easyly in the acid liquid phase than in the solid phase. Thus, in this case the viscosity is determined primarily by the depolymerization process. So, the lowest temperature - $x_2 = - 1$; $T = 50^o$ C - yields pectin of the highest viscosity.

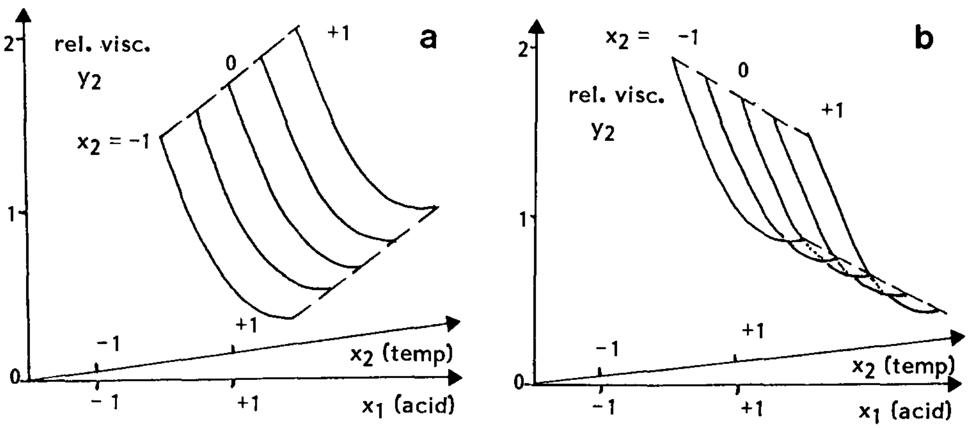

Figure 1: Changes of the relative viscosity of pectin solution at the different extraction conditions according to equ. (5). a. $x_3 = -1$; $t = 5$ h b. $x_3 = 1$; $t = 9$ h

Many kinds of reactions of polysaccharide in acid medium can result in pectin molecules of very different character or with differing functional groups. Therfore, to produce polymer for fixing biocatalysts we have chosen from the two optimum conditions the one, by which the temperature is kept low: $x_1 = -1$ $x_2 = -1$ $x_3 = 1$. After decodification, we obtain the following optimum values:

$$c = 0.72\ \% \quad T = 50^{o}\ C \quad t = 9\ h$$

Pectin extraction as performed at these optimum conditions provides us with pectin of high molecular weight. From its solution it is able to form large, thin and stable films for IR analysis [4]. Membranes of pectin extracted under other conditions are not stable and can be obtained only with the help of another polymer like polyvinylalcohol.

We also have used high viscosity pectin for entrapment of yeast cells. Mechanically stable immobilized cells have been built. It is proved by these results that pectin from dried peel of Citrus grandis may become a good carrier for microbial cells.

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