Optimization of polysaccharides by acid extraction from rapeseed meal

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Abstract

The quadratic orthogonal rotation design with 5 factors was used to study the effects of acid concentration, liquid/solid ratio, time, temperature and extracting times on the yield of polysaccharide by acid extraction from rapeseed meal, the functional model of yield to 5 factors was established by RSREG software which is part of SAS8.0. The mathematical model for the simulation was presented and the regression equation is significant. The analysis on this model showed that the effect order of 5 factors on yield was: temperature > extracting times> time> liquid/solid ratio> acid concentration. The optimum technical condition was as follows: the rapeseed meal was marinated with 18-22 times 0.10-0.14mol/L HCl at 100°C for 1.4-1.8 hours, the extracting times were 4-5.The yield of soluble polysaccharide by acid extraction from rapeseed meal was over 3.1% under this condition.

Key words : polysaccharide from rapeseed meal, acid extraction, orthogonal rotation design, mathematical model, yield efficiency analysis

Introduction

Comprehensive utilization of rapeseed meal can increase economic efficiency of the rapeseed. Except for protein, polyphenols and phytic acid, polysaccharides is another major available composition of rapeseed meal. So far, there was almost no report on polysaccharides from rapeseed meal either at home or abroad, and the systemic research on it is just at initial stage. The anti-oxidative effect of polysaccharides from rapeseed meal has been approved by the early experiments (Yan Fengwei et al., 2004), which was a basis on the further research of polysaccharide of rapeseed meal.

Polysaccharides extracted from plants by acids have been testified to have apparent bio-functions which was not the same as correspondingly hot-water treatment (Dong Hongxin et al., 2004, Lin Yuman et al.,1998). Research and utilization on acidic polysaccharide of rapeseed meal was desirable to offer a valuable and abundance source of functional ingredient, and improve rapeseed comprehensive utilization efficiency. Presently there was no report on polysaccharides extracted by acid from rapeseed meal. This experiment take acid as extract solvent, quadratic rotation-orthogonal composite experimental design was employed with acid concentration, liquid/solid ratio, time, temperature and extracting times as variables to study the effects on the yield of polysaccharides. Optimization analysis was used to select the optimum conditions. This experiment aimed to improve the technical and yield of the polysaccharides extraction from rapeseed meal, and offer basic information to the next study.

Material and Methods

Experimental procedure: Dehulled double-low rapeseed cake and meal → extracted with 80% ethanol → plant residues → extracted with dilute hydrochloric acid → concentrated → deposited with 95% ethanol → dissolved deposition with water → determining yield of polysaccharides (Ruan Zheng et al., 2005, Zheng Xiaodong et al., 2000, Robert J et al., 2000)

Statistical manner and software : Quadratic rotation-orthogonal composite experimental design. RSREG software (part of SAS8.0) (Jiang Qiuyan et al., 2005, Yang Aimei et al., 1998)

Experimental arrangement: Quadratic rotation-orthogonal composite experimental design (1/2conduct) with 5 variables which is acid concentration (X_1), liquid/solid ratio (X_2), time (X_3), temperature (X_4) and extracting times (X_5). Response function is yield of polysaccharides. Every variable with 5 levels, the Factors and levels of the experiment show in Table.1. (Yang Aimei et al., 1998)

Table 1 Factors and levels of the experiment

Code	X_1(mol/L)	X_2 (g/ml)	X_3(hour)	X_4(°C)	X_5
-2	0.1	1:5	1	20	1
-1	0.2	1:10	1.5	40	2
0	0.3	1:15	2	60	3
1	0.4	1:20	2.5	80	4
2	0.5	1:25	3	100	5

Result

The effects of the five variables (acid concentration, liquid/solid ratio, time, temperature and extracting times) on the response functions (yield of polysaccharide) were shown in Table.2, The analysis of partial regression coefficient significance was shown in Table.3.

Table 2 Structured matrices and yield result

No.	X_1	X_2	X_3	X_4	X_5	Y(%)
1	1.000	1.000	1.000	1.000	1.000	1.64
2	1.000	1.000	1.000	-1.000	-1.000	0.69
3	1.000	1.000	-1.000	1.000	-1.000	1.89
4	1.000	1.000	-1.000	-1.000	1.000	0.51
5	1.000	-1.000	1.000	1.000	-1.000	1.64
6	1.000	-1.000	1.000	-1.000	1.000	0.51
7	1.000	-1.000	-1.000	1.000	1.000	2.58
8	1.000	-1.000	-1.000	-1.000	-1.000	0.46
9	-1.000	1.000	1.000	1.000	-1.000	2.14
10	-1.000	1.000	1.000	-1.000	1.000	0.63
11	-1.000	1.000	-1.000	1.000	1.000	2.17
12	-1.000	1.000	-1.000	-1.000	-1.000	0.35
13	-1.000	-1.000	1.000	1.000	1.000	2.11
14	-1.000	-1.000	1.000	-1.000	-1.000	0.51
15	-1.000	-1.000	-1.000	1.000	-1.000	1.54
16	-1.000	-1.000	-1.000	-1.000	1.000	0.45
17	2.000	0.000	0.000	0.000	0.000	1.57
18	-2.000	0.000	0.000	0.000	0.000	0.95
19	0.000	2.000	0.000	0.000	0.000	1.29
20	0.000	-2.000	0.000	0.000	0.000	0.95
21	0.000	0.000	2.000	0.000	0.000	1.10
22	0.000	0.000	-2.000	0.000	0.000	0.98
23	0.000	0.000	0.000	2.000	0.000	2.05
24	0.000	0.000	0.000	-2.000	0.000	1.09
25	0.000	0.000	0.000	0.000	2.000	1.23
26	0.000	0.000	0.000	0.000	-2.000	0.67
27	0.000	0.000	0.000	0.000	0.000	1.39
28	0.000	0.000	0.000	0.000	0.000	1.51
29	0.000	0.000	0.000	0.000	0.000	1.50
30	0.000	0.000	0.000	0.000	0.000	1.36
31	0.000	0.000	0.000	0.000	0.000	1.62
32	0.000	0.000	0.000	0.000	0.000	1.36
33	0.000	0.000	0.000	0.000	0.000	1.14
34	0.000	0.000	0.000	0.000	0.000	1.04
35	0.000	0.000	0.000	0.000	0.000	1.48
36	0.000	0.000	0.000	0.000	0.000	1.26

Regression equation for the response functions in the actual level of variables:

$$Y=1.331477+x_1*0.026250+x_2*0.063750+x_3*0.017083+x_4*0.552917+x_5*0.093750+(x_1*x_1)*(-0.007727)+x_1*x_2*(-0.055625)+x_1*x_3*(-0.075625)+x_1*x_4*(-0.066875)+x_1*x_5*(-0.055625)+(x_2*x_2)*(-0.042727)+x_2*x_3*(-0.011875)+x_2*x_4*0.021875+x_2*x_5*(-0.061875)+(x_3*x_3)*(-0.062727)+x_3*x_4*(-0.060625)+x_3*x_5*(-0.081875)+(x_4*x_4)*0.069773+x_4*x_5*0.059375+(x_5*x_5)*(-0.085227)$$

Analysis of variance: $R=0.9152$, $F=(R^2/20)/((1-R^2)/11)=2.84$, $P=0.0395$.Regression equation indicated significance, fitting is in condition.

The results indicated that among the variables, acid concentration (X_1) had a significant positive effect on the response function (yield of polysaccharides). Time (X_3), extracting times (X_5) had a positive effect on the response function. Among the various interactions, temperature (X_4) and liquid/solid ratio (X_2) had the positive effect followed by the positive effect of extracting times (X_5), all the others had the insignificant negative effects with each other. The quadratic effect of temperature had significant positive effect on the yield of polysaccharides, all the others had negative effect on the yield. It could be found that temperature take the most important role on the yield of polysaccharide apparently.

Some pretests have been done before the composite experimental design. Results of the pretest showed that when the acid concentration was 0.05~0.15mol/L, the yield of polysaccharides increased with the increase of acid concentration, nevertheless increased the acid concentration continuously, the yield of polysaccharides increased mildly and even decreased when beyond 0.3 mol/L; when the liquid/solid ratio was 1:5~1:30, yield of polysaccharides increased with the increase of liquid/solid ratio and increased mildly when it beyond 1:15; when time was 0.5~3.5 hours, yield of polysaccharides increased along with the increase of time and increased mildly when beyond 2 hours; when temperature was 20~100°C, yield of polysaccharides increased with the increase of temperature observably; when extracting times was 1~6, polysaccharides can be extracted completely from the material after 5 times extraction. The five levels of every variable selected based on above results, so despite the significance analysis indicated that except temperature, the other variables all had the insignificant effects on the response function, the optimum values of every factors had been included in its range. Similar conclusions could

be certified from the optimization analysis.

Table 3 analyze of partial regression coefficient significance

Variable Intercept	DF	Estimate	Error	T	Value
	1	1.26716	0.08382	15.12	<.0001
x1	1	0.02625	0.08025	0.33	0.7497
x2	1	0.06375	0.08025	0.79	0.4438
x3	1	0.01708	0.08025	0.21	0.8353
x4	1	0.55292	0.08025	6.89	<.0001
x5	1	0.09375	0.08025	1.17	0.2674
x11	1	-0.00773	0.07259	-0.11	0.9171
x12	1	-0.05563	0.09829	-0.57	0.5828
x13	1	-0.07563	0.09829	-0.77	0.4579
x14	1	-0.06688	0.09829	-0.68	0.5103
x15	1	-0.05562	0.09829	-0.57	0.5828
x22	1	-0.04273	0.07259	-0.59	0.5680
x23	1	-0.01187	0.09829	-0.12	0.9060
x24	1	0.02188	0.09829	0.22	0.8280
x25	1	-0.06188	0.09829	-0.63	0.5419
x33	1	-0.06273	0.07259	-0.86	0.4060
x34	1	-0.06063	0.09829	-0.62	0.5499
x35	1	-0.08187	0.09829	-0.83	0.4226
x44	1	0.06977	0.07259	0.96	0.3571
x45	1	0.05938	0.09829	0.60	0.5580
x55	1	-0.08523	0.07259	-1.17	0.2652

Discussion

Main factor effect analysis: Analysis of regression equation and regression coefficient indicated 5 variables effected on yield arranged with significance was: temperature > extracting times > time> liquid/solid ratio> acid concentration.

Individual factor effect analysis: Analysis of quadric regression model by reducing dimensionality, the effect equations of yield were:

$$Y_1 = 1.331477 + x_1 * 0.026250 + (x1 * x1) * (-0.007727)$$

$$Y_2 = 1.331477 + x_2 * 0.063750 + (x2 * x2) * (-0.042727)$$

$$Y_3 = 1.331477 + x_3 * 0.017083 + (x3 * x3) * (-0.062727)$$

$$Y_4 = 1.331477 + x_4 * 0.552917 + (x4 * x4) * 0.069773$$

$$Y_5 = 1.331477 + x_5 * 0.093750 + (x5 * x5) * (-0.085227)$$

Fixing the coded levels of the 5 variables at -2, -l, 0, 1, 2, according to 5 equations above, the estimated yield values and effect sizes of five factors and levels showed in Table 4. From Table 4 the same conclusions as above can be obtained.

Table 4 Estimated yield and effect sizes of five factors and levels

Function	-2	-1	0	1	2	Maximum value
Y_1	1.2481	1.2975	1.3315	1.3500	1.3531	MAX (X_1 = -1.5790) =1.2708
Y_2	1.0331	1.2250	1.3315	1.3525	1.2881	MAX (X_1 = 1.4203) =1.3358
Y_3	1.0464	1.2517	1.3315	1.2858	1.1147	MAX (X_1 = -0.4147) =1.2984
Y_4	0.5047	0.8483	1.3314	1.9542	2.7164	MAX (X_1 = 2) =2.7164
Y_5	0.8031	1.1525	1.3315	1.3400	1.1781	MAX (X_1 = -1.7114) =1.2423

Optimization analysis: 28 pieces of combination after optimization analysis using SAS (SAS Institute Inc., 1999) based on the regression equation were obtained, after frequency analysis based on the 28 pieces of combination, the yield expectation was over 3.1%. Results of the analysis showed that the optimum technical condition was as follows: temperature was 100°C, extracting times was 4~5, time cost in every extraction was 1.4~1.8 hours, liquid/solid ratio was 1:18~1:20, acid concentration was 0.10~0.14mol/L.

Conclusions

1) *5 variables effected on yield be arranged with significance :* temperature > extracting times> time> liquid/solid ratio> acid concentration.

2) *Optimum technical condition was as follows:* temperature was 100°C, extracting times was 4~5, time cost in every extraction was 1.4~1.8 hours, liquid/solid ratio was 1:18~1:20, acid concentration was 0.10~0.14mol/L. The yield of soluble

polysaccharide by acid extraction from rapeseed meal was over 3.1% under this condition.

3) *Validate tests:* validate tests were done by selecting 5 combinations accidental from the optimum extraction parameter, results showed in Table 5.

Table 5 Validate test results

C(mol/L)	R(g/ml)	t(hour)	T(°C)	N	yield (%)
1	0.10 1:20	1.8	100	4	3.24
2	0.10 1:20	1.5	100	5	3.32
3	0.12 1:18	1.8	100	5	3.20
4	0.14 1:18	1.5	100	5	3.12
5	0.14 1:20	1.5	100	4	3.17

C- acid concentration; R- liquid/solid ratio; t- time cost in every extraction; T- temperature; N- times

Result of validate tests indicated that the yield of soluble polysaccharide by acid extraction from rapeseed meal can actually be over 3.1% under this condition.

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