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Research Article

Fraction and Purification of Phosphatidylinositol from Soybean Phospholipid

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Abstract: Phosphatidylinositol (PI) was extracted from soybean phospholipid using alcohol-n-hexane. The optimum extraction conditions were 56% ethanol, the ethanol and n-hexane (1.7:1, by volume), 0.3 g/L Ca^{2+} and pH = 8.17 with ammonia solution in 56% ethanol. The purity of PI in soybean phospholipid was increased from 29.00 to 84.17% under the optimum condition.

Keywords: Fractionation degree, phosphatidylionsitol, purification, soybean phospholipid

INTRODUCTION

Phospholipid (PL) is a class of lipids including Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), and Phosphatidylinositol (PI). They are a major component of all cell membranes as they can form lipid bilayers. PI contains a phospholipid acid, an inositol, and phospholipid polar group's annular section which has a six-carbon inositol (Wang *et al*., 2002). 3, 4, 5-position hydroxyl group of the inositol ring of PI is phosphorylated with the respective kinase catalyzed to form a variety of these materials about PI derivatives that can be used as a precursor to produce a second messenger signal. PI involves in many cellular activities, such as transmembrane transport, ion transport, membrane vesicle transport, cell frame, and apoptosis (Berridge, 1984). Soybean phospholipids are abundant of phosphatidylinositol, so it is a good material to explore the method of seperation and refinement of PI. Using double solvent system to separate PI from soybean phospholipid can improve the extraction efficiency of PI in soybean phospholipid.

MATERIALS AND METHODS

Chemical reagents and instruments: Soybean phospholipid; PI standard; ethanol; n-hexane; ammonium hydroxide; acetic acid; isopropanol; distilled water. Hitachi HPLC with DAD detector.

HPLC analysis: Phenonmenex Luna Silia column, mobile phase: n-hexane: isopropanol: 2% acetic acid = 8:1:1, flow rate: 1.0 mL/min, column temperature: 30°C, the injection volume was 20 μL.

Preparation of standard solution: Weighed PI standard accurately, add quantitative mobile phase to prepare series of standard solution (1.0, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/mL, respectively).

Fractionation and purification method: Weighed soybean phospholipid and dissolved it into n-hexane, then added ammonia-solution-ethanol for separating PI. Put metal ions into ethanol. Stirred the ethanol with metal ions 15 min and placed it in 4°C for 12 h. Then, took it out and centrifuged at 5000 r/min, 10 min. Freeze-dried it to obtain high purity PI powder. Dissolved 1 mg powder with 1 mL mobile phase and detected it by HPLC.

The response surface experiment: To optimize the conditions, using Design-Expert 8.0 software design the response surface experiments. Based on single factor experimental results, used the concentration of ethanol, the volume ratio with ethanol-N-hexane and pH values as arguments, fractionation degree as response, to make the response surface optimization experiments in which each factor has three levels coding with -1, 0 and 1, respectively. Table 1 showed the response surface of level of factor.

Calculated the fractionation degree: Separating the components of phospholipids are based on their different solubility in solvent. Using ethanol-n-hexane system is to improve the content of PI in soybean phospholipid (Wu *et al*., 2006). Another Chen *et al*. (2005) studied the effect of the ethanol concentration about separating PI from soybean phospholipid, established the ethanol-n-hexane system concept of fractionation degree about soybean phospholipid.

(PI) and (PE) separately indicate the corresponding concentrations of PI and PE in ethanol and n-hexane, (g/mL). (PI) and (PE) separately indicate the corresponding percentage of PI and PE in extract of ethanol and n-hexane. Fractionation degree has a

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Table 1: Design of response surface methods

Level	Ethanol concentration (%)	n-hexane/ ethanol (V/V)	pH value
Code	X_1	X_2	X_3
-1	50%	1:1	7
0	55%	1:1.5	8
1	60%	1:2	9

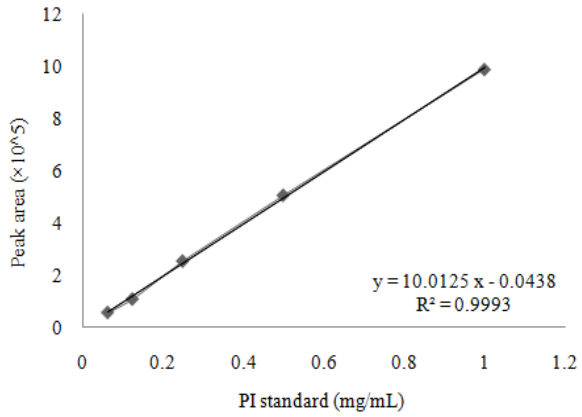


Fig. 1: Working curve of phosphatidylinositol

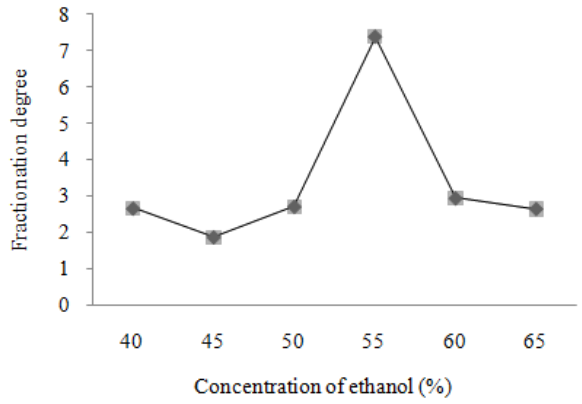


Fig. 2: Effect of concentration of ethanol on fractionation degree

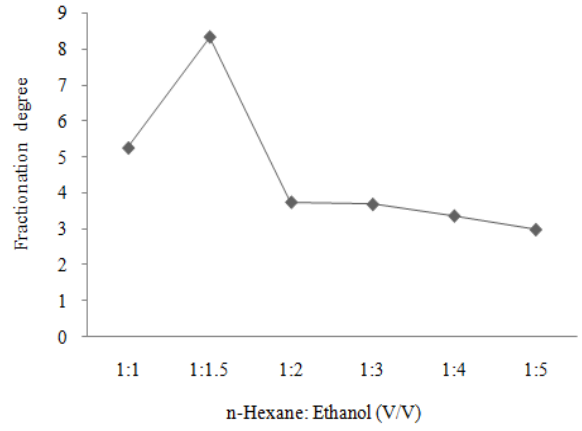


Fig. 3: Effect of the volume ratio with ethanol and n-hexane on fractionation degree

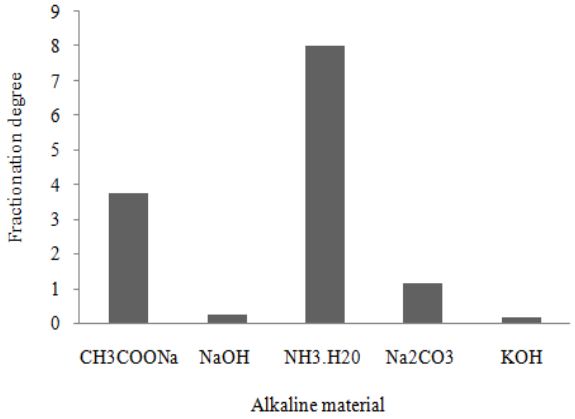


Fig. 4: Effect of different alkaline material on fractionation degree

relationship with the percentage of PI and PE in extract of ethanol and n-hexane:

$$Fractionat\ ion\quad Degree = \frac{[PI]_{ethanol}/[PI]_{hexane}}{[PE]_{ethanol}/[PE]_{hexane}} = \frac{((PI\%)_{ethanol}/(PE\%)_{ethanol}}{(PI\%)_{hexane}/(PE\%)_{hexane}} \quad (1)$$

RESULTS AND DISCUSSION

Determination of PI content: Drew standard curve with PI's peak area (Y) to the concentration of PI (X) that the concentration range was 0.3125 to 1.0 mg/mL. The regression equation is Y = 1001245x - 4380, R^2 = 0.9993. The result showed phosphatidylinositol had a good linear relationship in 0.3125 to 1.0 mg/mL, in Fig. 1.

The effect of the concentration of ethanol on fractionation degree: The fractionation degree initially increased gradually and then declined stabilized with the increase of the concentration of ethanol, which was shown in Fig. 2. Therefore, 55% ethanol was the optimum condition for fractioning PI and PE.

The effect of the volume ratio with ethanol and N-hexane on fractionation degree: The fractionation degree initially increased and then declined until stabilized with different volume ratio of ethanol and n-hexane, which was shown in Fig. 3. Therefore, the best volume ratio with ethanol and n-hexane was 1.5:1.

The effect of alkaline material and PH values on fractionation degree: As shown in Fig. 4 the fractionation degree is the highest after ammonia solution treatment among those other alkaline materials. PH value is relevant to the quantity of ammonia solution. When pH value was 7, the fractionation had no significant difference. When pH increased to 8, the fractionation degree increased to summit, which was

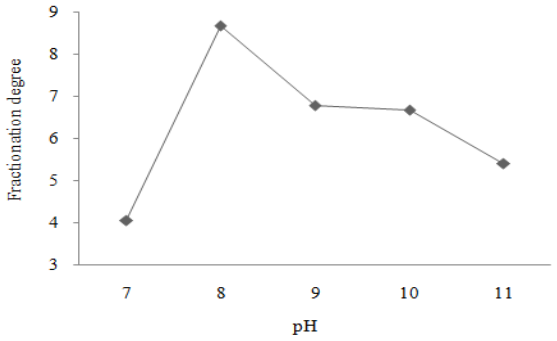


Fig. 5: Effect of pH on fractionation degree

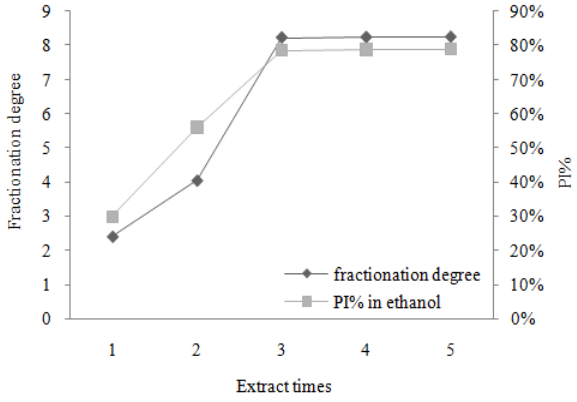


Fig. 6: Effect of extract times on fractionation degree

shown in Fig. 5. Therefore, pH8 was the best condition for PI extraction.

The effect of extract times on fractionation degree: As shown in Fig. 6, the fractionation degree rose gradually and then stabilized, with the increasing of the extract times. The content of PI in the ethanol had the same change. Three times extraction was best for the optimizing the fractionation degree without wasted laboratory reagents.

Analysis of the response surface experiment: The results of the response surface experiment were shown in Table 2, and the analysis was in Table 3.

Table 2: Design and results of response surface methods

Experiment	X_1	X_2	X_3	Fractionation degree
1	1	0	-1	6.22
2	0	0	0	8.63
3	-1	1	0	7.38
4	0	-1	-1	6.62
5	0	-1	1	6.78
6	0	0	0	8.51
7	1	0	1	7.01
8	1	-1	0	7.37
9	0	1	1	7.75
10	0	1	-1	6.53
11	-1	0	-1	5.92
12	-1	-1	0	6.58
13	0	0	0	8.70
14	0	0	0	8.47
15	1	1	0	7.90
16	-1	0	1	6.42
17	0	0	0	8.68

Analyzed the results of response surface and got the regression equation:

$$Y = 3.9791X_1 + 2.3755X_2 + 19.48525X_3 - 0.027X_1X_2 + 0.0145X_1X_3 + 0.53X_2X_3 - 0.03636X_1^2 - 1.526X_2^2 - 1.29650X_3^2 - 183.81175$$

Table 3, F value of model was 125.37, $p<0.0001$. It showed that the total regression was significant, which means the experimental data and secondary mathematical models were conformed and fitted well. F value of lake of Fit was 1.53, $p>0.05$. It showed that lack of fit was not significant, which means the model had the higher accuracy. The correlation coefficient R^2 was 99.83% and the correction coefficient of determination Adj.R^2 was 98.59%.

All of above, it showed that the model was fitted well, and the regression model was significant. The first term and the quadratic term had the higher significantly. It indicated that the impact of three factors on the response was not just a simple linear relationship. F value of lack of fit was small that mean each experimental point fitted well with the equation and the experimental errors were small. The regression equation, instead of the real experimental points, could

Table 3: ANOVA

Source	df	S.S.	M.S.	F value	Pr>F	Significant
Model	9	14.610	1.620	125.37	<0.0001	**
X_1	1	0.610	0.610	46.72	0.0002	*
X_2	1	0.610	0.610	47.14	0.0002	*
X_3	1	0.890	0.890	68.61	<0.0001	**
X_1X_2	1	0.018	0.018	1.41	0.2742	
X_1X_3	1	0.021	0.021	1.62	0.2433	
X_2X_3	1	0.280	0.280	21.69	0.0023	*
X_1^2	1	3.480	3.480	268.64	<0.0001	**
X_2^2	1	0.610	0.610	47.32	0.0002	*
X_3^2	1	7.080	7.080	546.50	<0.0001	**
Residual	7	0.091	0.013			
Lack of fit	3	0.048	0.016	1.53	0.3375	Not significant
Pure error	4	0.042	0.011			
Cor total	16	14.700				

*, **: Significance at 0.05 and 0.01 levels, respectively; S.S.: Sum of square; M.S.: Mean square

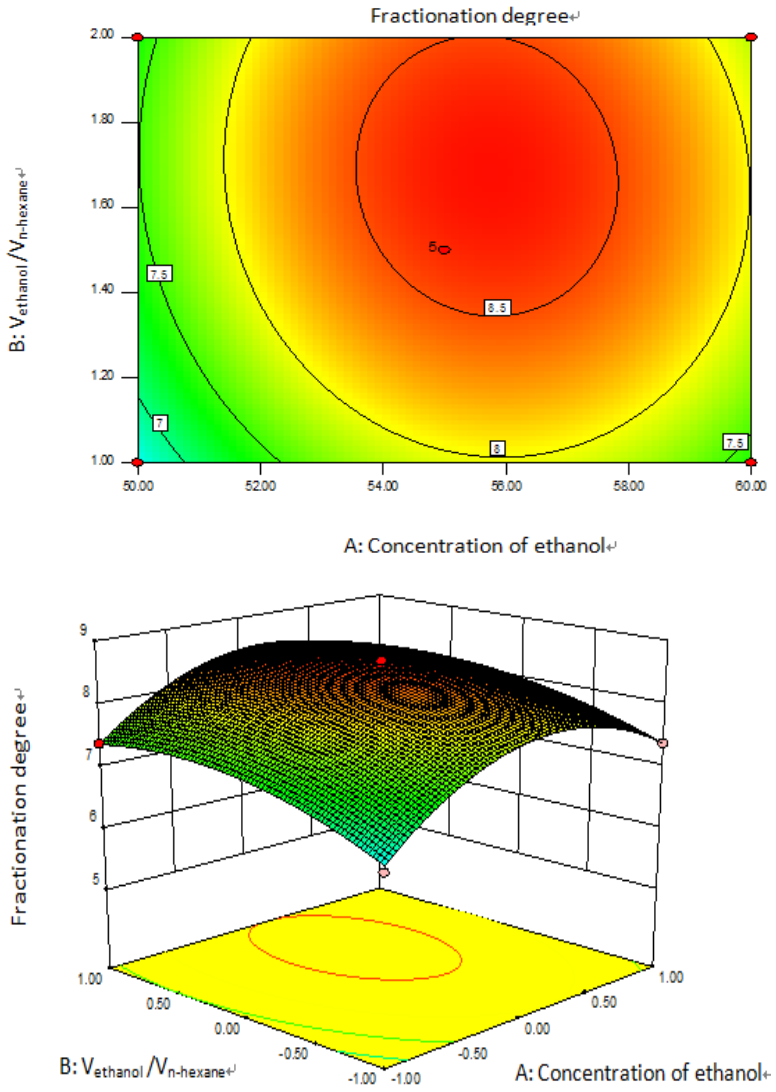
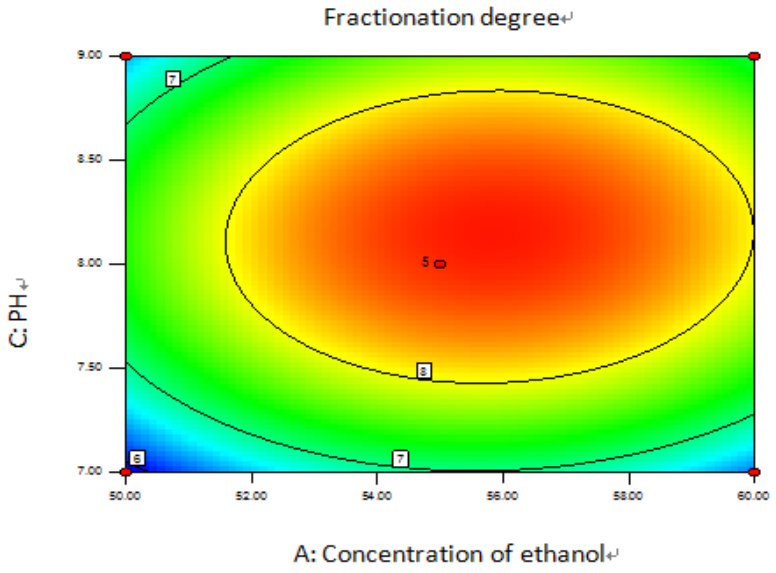


Fig. 7: Contour line and response surface graph of the concentration of ethanol and the volume ratio with n-hexane and ethanol



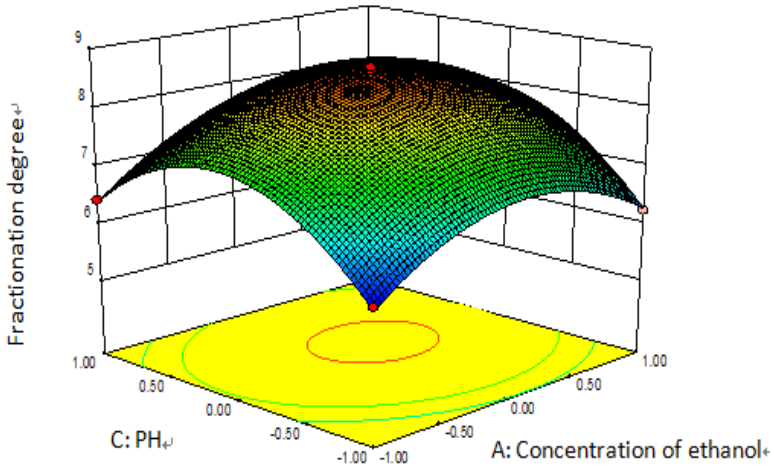


Fig. 8: Contour line and response surface graph of the concentration of ethanol and pH

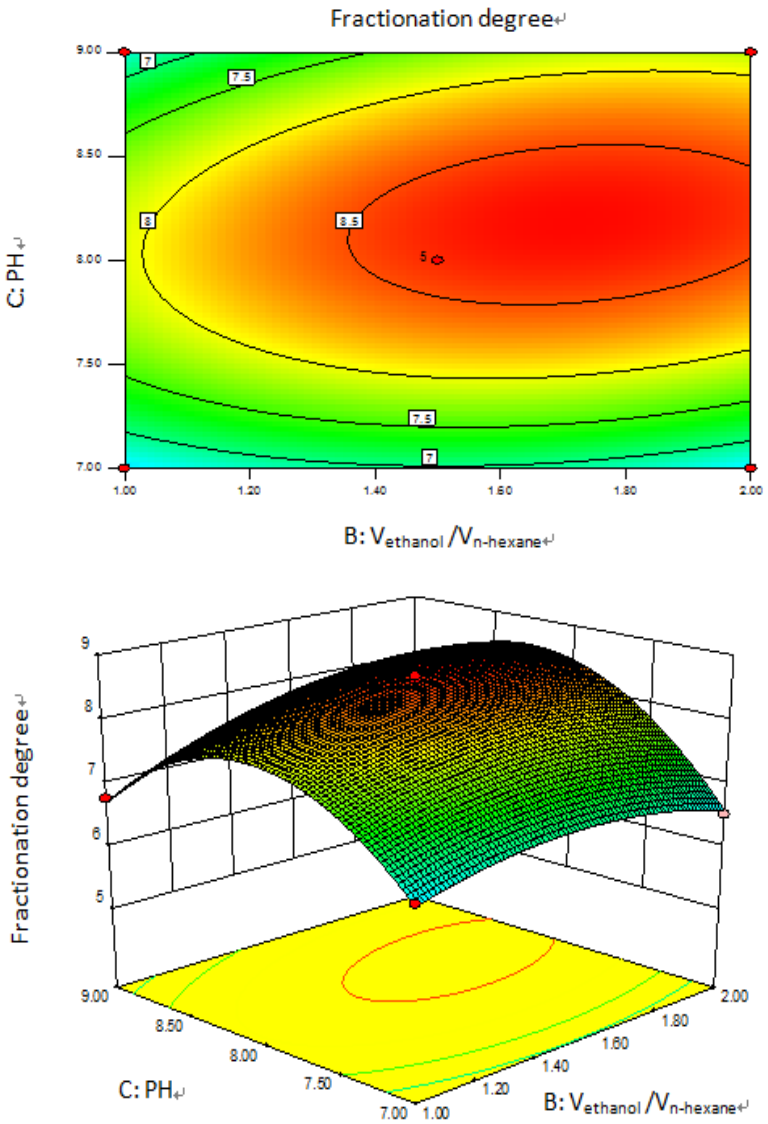


Fig. 9: Contour line and response surface graph of the volume ratio with n-hexane and ethanol and pH

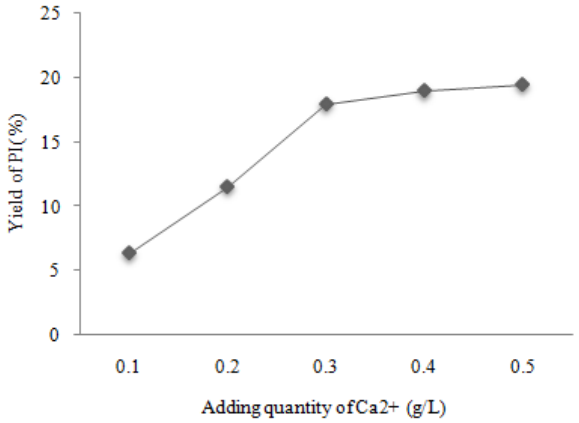


Fig. 10: Effect of adding quantity of Ca^{2+}

be used to analyze the experiment. The equation could be applied to the theoretical prediction of the reaction.

Figure 7 to 9, all the extreme values were in the center of contour line. And when the contour lines were more oval, the interactions of two factors were much greater. Then got the order of the impact with three factors to the fractionation degree (the volume ratio with ethanol and n-hexane>ethanol concentration>pH). According to the typical analysis, the stable point of this experiment was the maximum point. Therefore, the optimal combination was 55.72% ethanol, ethanol and n-hexane (1.7:1, by volume) and pH = 8.17 with ammonium hydroxide in 55.72% ethanol. The max theoretical value of the fractionation degree was 8.70.

According to the actual conditions, the optimum conditions were 56% ethanol, ethanol and n-hexane (1.7:1, by volume), and pH = 8.17 with ammonium hydroxide in 56% ethanol. Repeated the optimal combination to obtain resolution was 8.64, which closed with the theoretical value. And the purity of PI was 78.65%. Therefore, using the response surface methodology to optimize the fractionation of PI was accurate and reliable.

Determine the purification condition: The main purpose of this experiment was to separate the PC and PI by precipitated PI with metal ions.

With the increase of Ca^{2+}, the precipitation increased of PI gradually and then stabilized, which was shown in Fig. 10. So, 0.3 g/L Ca^{2+} is the optimal conditions.

In the repeat experiment, the yield of PI is 18.2%. The purity of sample is 84.17%.

CONCLUSION

The optimum conditions of the fractionation were 56% ethanol, the ethanol and n-hexane (1.7:1, by volume), 0.3 g/L Ca^{2+} and pH = 8.17 with ammonia solution in 56% ethanol. The purity of PI in soybean phospholipid was increased from 29.00 to 84.17% under the optimum conditions.

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