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THE OPTIMAL MANUFACTURE OF POLYSACCHARIDES FROM SOYBEAN AS STABILIZATION OF OIL-IN-WATER EMULSION

Ngo Thi Lan Huong[1], Dao Anh Hoang[1], Nguyen Van Khanh[2], Nguyen Thi Ly[1], Nguyen Thi Le[1], Hoang Le Son[1,*]

[1]National Institute of Medicinal Materials, Hanoi, Vietnam;

[2]University of medicine and pharmacy, Vietnam National University

*Corresponding author: hoangson.med@gmail.com

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Summary

The Optimal Manufacture of Polysaccharides from Soybean as Stabilization of Oil-in-Water Emulsion

The Resolution IV Factorial design (RIVF) and response surface methodology (RSM) combination with desirability function (DF) techniques were integrated to estimate optimal manufacture of the soybean polysaccharide that lead to maximizing the yields (Y_1) and content of polysaccharide (Y_2) and minimizing the value of emulsifying activity index -EAI (Y_3). The RIVF was used to determine the variable factors (X_1-X_3) as well as the fixed factor (X_4-X_6). The three mathematical models were developed by using RSM with experimental results. The DF was used to optimize the mathematical model and predict the optimal value of factors. The optimal parameters of manufacture were the extraction two time in 2.5h (X_2) with ratio of solid to water pH 6 (X_4) as 1:10 (X_3) at temperature 90°C (X_5) and the purification with the ratio water to ethanol 87% (X_1) as 1:5 (X_6). The manufacture also was confirmed through experiments. This polysaccharide could be the good candidates of excipients to stabilize oil-in-water emulsion.

Keywords: *Soybean, polysaccharides, Emulsifying activity index, EAI, Desirability function.*

1. Introduction

In 2019 years, FDA US was added the new polysaccharide excipient that originated from soybean in catalogue of inactive ingredients for approved drug products. The application only was in tablet and delayed release tablet formulation. Although, the soybean polysaccharide (SP) as an acidic polysaccharide was shown potential emulsifier for pharmaceutical applications in several literature due to a negatively charged amphipathic macromolecule, a high water solubility, a stability in acidic PH, and low bulk viscosity [1]. The chemical structure of soybean polysaccharide was an interesting glucomannan-type in the excipient industry due to a main rhamno-galacturonan backbone and a branchy by β-1,4-galactan and α-1,3- or α-1,5-arabian chains [2],[3]. The manufacture condition for polysaccharides as emulsifier from soybean have yet identified. Therefore, the purpose of this study was determined that the value factor effected to the responses including the manufacturing yields (Y_1), the content of polysaccharide (Y_2), and the emulsifying activity index (EAI-Y_3) according to the Box-Behnken factorial design and desirability functions. Particularly, EAI was the emulsifying property and measured by the amount of oil that could be emulsified per unit polysaccharide [4].

2. Material and methods

Materials and chemicals

The soybean seed (*Glycine max*) were purchased from the Hanoi traditional market. The soybean seed were divided into the hull and soybean cotyledons meal by hand. The soybean cotyledons meals were ground up and stored at room temperature. The D-glucose was purchased from Shyuanye (China). All other reagents used were of analytical grade. Distiller water was used in the preparation of all solutions.

Defatted and decolor of soybean meal powdered and purification of soybean oils

Soybean meal powdered (~500g) defatted at 40°C with five volumes of *n*-hexane for 8h in Soxhlet extractor. The defatted powders were left in a fume hood overnight to let the residual solvent fully evaporate [5]. After the vacuum evaporation *n*-hexane extraction, the residue which was the raw soybean oils could be remove phospholipids and mono-, di-acylglycerols contents by acetone precipitation and Florisil methods, respectively. The acetone solvent was added into the residue (80:1, v/v), stirred the mixture for 15 min, and kept the suspension in deep freezer at -20°C for 2 h. The inattention phospholipids and the supernatants could separate by centrifuging for 30 min at 3000 rpm. The acetone was removed under reduced pressure on a rotary evaporator [6]. This residue subsequently was stirred with Florisil (4%, w/w) and after 18h centrifuged for 30 min at 3000 rpm.

The purified oil was stored in a shade bottle at room temperature to use for further use [5]. The defatted soy power was extracted two times with ethanol 96% (solid/liquid, 1:10) at 67°C in 1 h (the unpublished optimization data). The suspension was filtered, and the filter cake could be dry in fume hood overnight to use the extracted polysaccharides [7].

Extraction and purification of the polysaccharides

The extraction and purification of PS were performed by M. Garcia-Vaquero *et al.* (2017) and modified [8]. Brief, the defatted and decolor power (10g) were extracted two time with the range of an extracted temperature from 60 to 90°C (X_5), extracted time from 1 to 3 h (X_2), a sample-to-distilled water ratio from 1:10 to 1: 20 (X_3). The pH (X_4) of solvents from 2.0 to 6.0 was adjusted by acid HCL. The residue was obtained as precipitate in ethanol (concentration of ethanol (X_1) from 50 to 90% and a ratio of water/liquid (X_6) from 1: 5 to 1:10) after centrifugation (5000 g for 30 min). In this residue, the remaining protein could be subtracted to achieve the purified polysaccharide by acetic acid at pH 4.3 in the water solutions.

The emulsifying activity index (EAI)

The emulsifying activity index (EAI) was determined by turbidimetric methods with UV spectrophotometer [7]. The stock emulsion was included 0.1 g PS powder, 10ml a 0.1M phosphate buffer at pH 6.8, and 1.0 ml the soybean oil and pre-homogenized using a magnetic stirrer at 500 rpm for 30 min. This stock emulsion was diluted with 0.1% (w/v) sodium dodecyl sulphate (SDS) in a vortex. The absorbance of diluted emulsion was measured using a UV spectrophotometer at wavelength 500 nm. The turbidity of the emulsion was calculated using given below.

$$T=2.303 \times \frac{A}{L} \times D$$

Where, T is the turbidity of the emulsion (m^{-1}), A is the absorbance, D is the dilution factor and L is the light path length (m).

The emulsion activity index (EAI, m^2/g) was calculated as:

$$EAI = \frac{2 \times To}{\emptyset \times C \times 1000}$$

Where, T_0 is the turbidity of fresh emulsion, $\emptyset$ is the oil volume fraction and C is the concentration of PS present in the PS dispersion (mg/ml).

Determination of the content and the yield

The content of PS was determined by phenol-H_2SO_4 spectrophotometric method with D-glucose as standard [9]. The calibration curve was applied as $y = 0.058x - 0.0071$ (R^2=0.996) at 490 nm by UV spectrophotomerter (UV-1800, Shimadzu). And the yield of PS was expressed as g PS/g soybean meal x 100%.

Screening factor design (Resolution IV Factorial design)

The Resolution IV Factorial design was used to determine the important factor in processing of polysaccharide due to the minimization of number experiment [10]. The six factors, three responses and the detail of design was showed in table 1. Each factor was considered at two levels (-1 and +1). The responses were the manufacturing yields (Y_1) and the content of polysaccharide (Y_2) and the value of EAI (Y_3).

Table 1. The factor level for Resolution IV Factorial design

Manufacture	Independent Variables		Code	Level	
				-1	1
Extraction	Temperature	(°C)	X_5	60	90
	Time	(h)	X_2	1	3
	Solid/liquid	(w/v)	X_3	0.05	0.1
	pH		X_4	2	6
Purification	Ethanol	%	X_1	50	90
	Water/Ethanol	(v/v)	X_6	0.1	0.2

Response surface methodology (The Box-Behnken factorial design)

Based on screening factor experiments, the Box-Behnken factorial test (BBD) with three level - three main factors was designed to propose a model for response [11]. All experiments were carried out in a randomized order to minimize any effect of extraneous factors. The polynomial model proposed for response (Y) was the equation:

$$Y= Ao + \sum_{i=1}^{3} AiXi + \sum_{i=1}^{3} AiiXi^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3} AijXij$$

where Y is the predicted response, A0 is constant, and Ai, Aii, and Aij are coefficients estimated by the model. X_i and X_j are levels of the factors. They represent the linear, quadratic, and cross-product effects of the X_1, X_2, and X3 factors on the response, respectively. The model evaluated the effect of each factor to a response. Design-Expert software (version 11.0.6.1, Stat-Ease, Inc., Minneapolis, USA) was used for the ANOVA analysis of the obtained experimental data. [12]

Desirability functions

Desirability functions have been used extensively to simultaneously optimize several responses [13]. The procedure calls for introducing for each response $Y_j(x)$,j = 1,2,…,m, a function dj(Yj(x)) with a range of values between 0.0 and 1.0 that measures how desirable it is that response Yj(x) takes on a particular value. Here x denotes the vector of factors or independent variables x' = (x_1, x_2,…,x_k). Once this function is defined for each of the m responses of interest, an overall objective function (the total desirability) is defined as the geometric mean of the individual desirability:

$$D(x) = [d1(Y1(x))d2(Y2(x))\dots dm(Ym(x))]^{\frac{1}{m}}$$

Derringer and Suich (1980) proposed the alternative functions:

$$dj(Yj(x)) = \begin{cases} 0 & if\ Yj(x) \le Yminj \\ \left[\frac{Yj - Yminj}{Ymaxj - Yj}\right]^r & if\ Yminj \le Yj(x) \le Ymaxj \\ 1 & if\ Yj(x) \ge Ymaxj \end{cases}$$

In equations, r,s, and t are user-specified weights that allow the experimenter to specify tighter or wider desirability functions around a target value (T_j) for a response j. The quantities Y_{minj} and Y_{maxj} denote the desired bounds for response j.

3. Results and Discussion

Determination of three important factors

In processing polysaccharides from defatted and decolor soybean powder, six factors were investigated that could be effect to three responses, including the manufacturing yields (Y_1) and the content of polysaccharide (Y_2) and the value of EAI (Y_3). The run experiments by a Resolution IV Factorial design along with the measurement value for the responses were display in Table 1. The important factors could be revealed by analyzing the Pareto chart (Fig. 1) [14]. The ethanol concentration in purification manufacture - coding X_1 factor were certainly important effected the yield response due to the above Bonferroni limit line. Among the possibly important effect with the above the *t*-value limit line, X_2, X_3, X_4 and X_5 factor were individual effected as well as interacted with other factor such as in X_2X_5 in the Y_1 response, X_2X_5, X_3X_5, and X_2X_3 in the Y_2 response and X_1X_3, X_4X_5 in the Y_3 response. Specially, the X_4 factor (pH) were possibly the negative effect in the EAI value. The acidic extraction was drag on increasing the EAI value in opposite the requirement for EAI response. Further, the increase value of temperature extraction always obtained higher than the extraction yield. Of course, this factor was absent effect to the most important for response as EAI. In consequence, the X_4, X_5, X_6 factors were fixed at high level, respectively, 90°C, pH = 6.0, ratio of ethanol 1:5. Three factors X_1, X_2 and X_3 were the variable value in BBD design.

Fitting the models

The ranges for three variables factor, namely, the ethanol concentration (X_1) for purification parameters and extraction time (X_2), and solid to liquid ration (X_3) for extraction parameters at three level (+1, 0, −1) in BBD were selected based on the observation of a Resolution IV Factorial design.

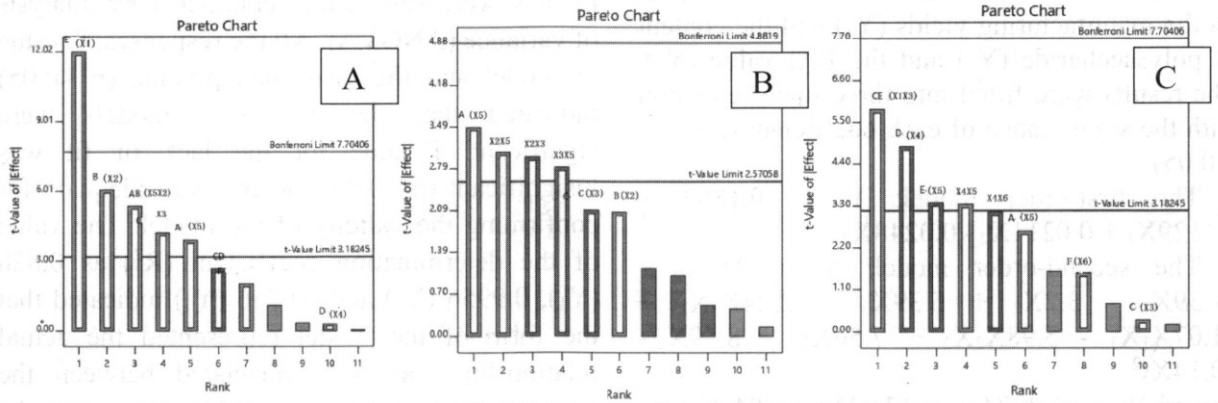


Fig. 1. Pareto chart for response analysis were included the manufacturing yields (A) and the content of polysaccharide (B) and the EAI value (C)

Table 2. Experimental values of responses for screening design of experiments

Run	X_1	X_2	X_3	X_4	X_5	X_6	Y_1	Y_2	Y_3
	%	h	w/v		°C	v/v	g	%	m^2/g
1	90	1	0.1	2	90	0.1	0.65	5.01	20678.2
2	50	3	0.1	2	60	0.1	0,.3	2.23	16051.0
3	50	3	0.05	6	60	0.2	0.28	7.01	17729.6
4	90	3	0.05	6	60	0.1	0.54	4.51	9056.1
5	90	1	0.1	6	60	0.2	0.66	12.6	10650.5
6	90	3	0.05	2	60	0.2	0.51	6.09	14540.8
7	90	3	0.1	6	90	0.2	1.04	10.04	23678.5
8	90	1	0.05	6	90	0.2	0.55	8.42	7152.8
9	50	1	0.1	6	90	0.1	0.14	12.65	13132.6
10	50	3	0.05	2	90	0.1	0.39	84.83	26217.7
11	50	1	0.05	2	60	0.1	0.14	4.31	25933.6
12	50	1	0.1	2	90	0.2	0.28	4.06	26530.6

Note: X_1: % ethanol; X_2: time; X_3: solid/liquid; X_4: pH; X_5: temperature; X_6: water/liquid. Y_1: the manufacturing yields, Y_2: the content of polysaccharide, Y_3: the emulsifying activity index.

Table 3. The Box-Behnken experimental design and response value

Run	Factors			Response		
	X_1 (%)	X_2 (h)	X_3 (w/v)	Y_1 (g)	Y_2 (%)	Y_3 (m^2/g)
1	70	1	0.05	0.73	10.3692	5450.34
2	70	3	0.05	0.186	17.004	5342.28
3	90	1	0.075	0.294	16.1306	7109.34
4	90	2	0.05	0.385	11.7268	7127.01
5	90	3	0.075	0.377	13.144	6871.28
6	70	2	0.075	0.118	20.011	5395.82
7	70	2	0.075	0.156	17.2412	4899.3
8	50	2	0.05	0.126	11.0916	5114.57
9	70	2	0.075	0.151	14.3188	3522.56
10	70	3	0.1	0.201	15.826	1915.17
11	50	1	0.075	0.084	24.3368	1670.82
12	50	3	0.075	0.085	12.378	3926.29
13	70	2	0.075	0.209	18.7919	4138.12
14	70	1	0.1	0.129	33.1228	10715.3
15	90	2	0.1	0.242	81.5072	11752.6
16	50	2	0.1	0.1	36.5795	9212.34
17	70	2	0.075	0.184	17.2897	9363.17

Statistical analysis and the model fitting

In table 3, the results of 17 experimental combinations of three variables factors were recorded in terms of responses. These experimental combinations of different extraction variables were carried out to know their impact on the manufacturing yields (Y_1) and the content of polysaccharide (Y_2) and the EAI value (Y_3). The results were fitted into three under equation with the significance of each coefficient (*p*-value < 0.05).

The first-order model: $Y_1 = 0.1882 + 0.1129X_1 + 0.0211X_2 - 0.0248X_3$

The second-order model: $Y_2 = 15.53 + 11.39X_1 - 3.2X_2 + 5.39X_3 + 2.24X_1X_2 + 11.07X_1X_3 - 5.98X_2X_3 + 7.56X_1^2 - 8.59X_2^2 - 10.14X_3^2$

and $Y_3 = 5210.74 + 1617.03X_1 + 504.35X_2 + 1320.17X_3 - 2173.03X_2X_3 + 1867.97X_3^2 - 2731.41X_2X_3^2$

Where X_1, X_2 and X_3 were the coded parameters for the ethanol concentration in purification, extraction time, and ratio of raw material to water in extraction, respectively. The models were statistically conducted by analysis of variance (ANOVA). All the responses, F-value of model and the associated p-value ($p < 0.05$) indicated that the regression models were significant. F-value for the lack of fit was insignificant ($p > 0.05$) thereby was adequate for confirming the validity of the model. The value of the determination coefficient (R^2) as 0.828 (Y_1), 0.9956 (Y_2) and 0.6746 (Y_3) indicated that the form of the model represented the actual relationship was well correlated between the response and variables ($p<0.05$). These results indicated that the model equations were adequate

for predicting the manufacturing yield (Y_1), the contents (Y_2) of PS, and the EAI value (Y_3) under the conditions of any combination of the variables values.

The relationship between the responses and experimental variables could be illustrated graphically to investigate interactions of the variables. Each plot shows a pair of factors by keeping the other factor constant at its middle level. In fig. 2A were the first-order model that indicated the individual effect on the manufacturing yield of PS (Y1). When the X1, X2 and X3 were increased in experimental domain, the value of Y1 also increased. The absence of factor interaction could affect the Y1. Due to the second-order model, the stationary point of Y_2 (the content of polysaccharide) and Y_3 (the value of EAI) were existed in fig. 2B, 2C that could represent a point of maximum response and a point of minimum response, or a saddle point. Fig. 2B, 2C also were revealed that the present of interaction between factor, details, X_1X_2, X_1X_3 and X_2X_3 in Y2 and only X_2X_3 in Y_3. The Y2 was found to increase rapidly in range of extraction time from 1.5h to 2.5h. The time extraction was over point-break at 2.5h then the decreased value of Y2 were trend (Fig 2B). The EAI value decreased with the increasing of three factors from 2 h to 2.5 h, from 70% to 80% and ratio of raw to water from 0.07 to 0.09 (Fig 2C).

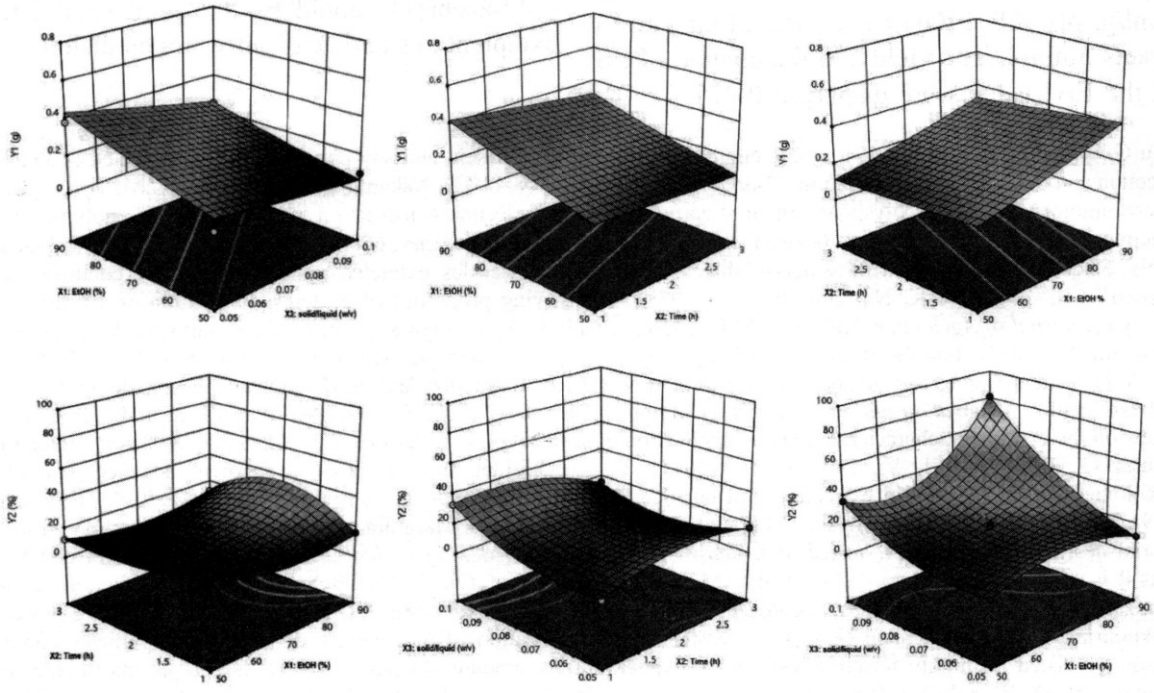
Optimization of the extraction parameters by desirability function

The optimal condition is established by using "desirability function - D" converts three objective functions into a single overall desired function by means of a mathematical transformation. The objective functions for responses with the maximum values of manufacturing yields, contents of PS and minimum value of EAI were indicated as follows:

$$D1=\begin{cases}0 & Y1 \le 0.084\\ \frac{Y1-0.084}{0.385-Y1} & 0.084 < Y1 < 0.385\\ 1 & Y1 \ge 0.385\end{cases}$$

$$D2=\begin{cases}0 & Y2 \le 10.3692\\ \frac{Y2-10.3692}{81.5072-Y2} & 10.3692 < Y2 < 81.5072\\ 1 & Y2 \ge 81.5072\end{cases}$$

$$D3=\begin{cases}0 & Y3 \le 1670.82\\ \frac{Y3-1670.82}{11752.6-Y3} & 1670.82 < Y3 < 11572.6\\ 1 & Y3 \ge 11572.6\end{cases}$$



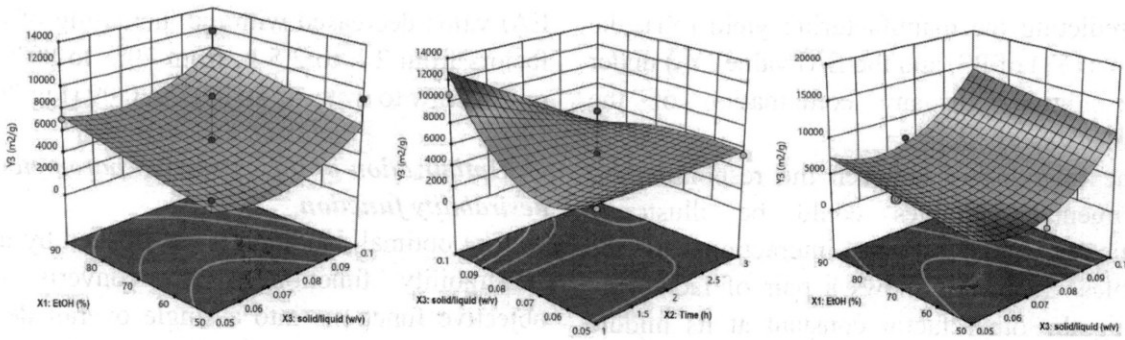


Fig. 2. The relationship between the responses and experimental variables could be illustrated by the response surface plot including the manufacturing yields (**A**) and the content of polysaccharide (**B**) and the EAI value (**C**). Each plot shows a pair of factors by keeping the other factor constant at its middle level.

The desirability function was calculated according to the formula $D=\sqrt[3]{D1.D2.D3}$. The optimal values of the variables were performed under the following conditions: extraction temperature of 90°C, time of 2.5h, solid/liquid of 1:10 (g/ml), pH 6.0, Ethanol 87% and water/liquid ratio of 1:5. The yield and the content of PS and the EAI value were predicted respectively 0.254, 52.92 and 7162.48.

Verification of the predictive model

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. Additional experiments by using the optimum conditions for PS process were carried out including extraction time of 2.5h, extraction temperature 90°C, ratio of water to raw material 10 ml/g, pH=7.0, EtOH 87%, ratio of ethanol to extracts 5ml/ml. The yield and the content of PS and the EAI value were displayed 0.275 ± 0.02, 55.86 ± 3.10 and 7353.63 ± 195.61, respectively.

4. Conclusions

This work reported on the application of the resolution IV factorial design, response surface method (RSM), and desirability function (DF) techniques in order to determine the optimal manufacture conditions of polysaccharide from soybean that lead to maximizing the yields and content of polysaccharide and minimizing the value of emulsifying activity index (EAI). The value of the optimal extraction was determined at temperature 90°C and pH 6.0 in 2.5 h with water to soybean ratio at 10:1. The optimal precipitation conditions were that ethanol concentration and ratio of ethanol to extracts was adjusted to 87% and 5:1, respectively. This polysaccharide could be the good candidate of excipient to stabilize oil-in-water emulsion.

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