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RESEARCH ARTICLE

Zhenliang ZHANG, Zide ZHANG, Xiaona ZHANG, Jing LI, Yan WANG, Congzhi ZHAO

An ultrasound-assisted extraction technology of almond dregs protein

Abstract Using quadratic regression orthogonal design rotation, the ultrasound-assisted alkaline extraction process of protein from almond dregs was optimized in the experiment. The results showed that the optimized parameters of the almond dregs protein extraction process were as follows: extracting temperature 37°C, pH value 10.1, and ratio of solid to liquid 1:29.5 ($g \cdot mL^{-1}$). The efficiency of protein extraction was 69.76% with twice extraction for each 30 min under the above conditions.

Keywords almond dregs, isolated protein, ultrasound

1 Introduction

Almonds are seeds of apricots, and they are rich in many nutrients, with great medical values (Li and Jia, 2007; Mandalari et al., 2008). The almond dreg is the by-product after pressing oil, usually used as feed or fertilizer with a more than 50% protein content, which has not been fully utilized. The isolated protein of almond is a plant protein, mainly used in the field of producing foodstuff, chemicals and cosmetics (Esteban et al., 1985). Simultaneously, it can be decomposed by enzymes and applied for processing functional foods to add to the value of the products (Alder, 1986; Kong et al., 2008; Sze and Sathe, 2009). The related studies have shown that almond is rich in proteins and trace elements and its proportion of amino acid is balanceable (Calixto et al., 1981). Therefore, almond dregs can be used as a good source of protein, giving great significance to the study of almond dregs.

At present, the method of alkaline extraction and acid precipitation has been widely used in the field of plant protein extraction (Rivas et al., 1991; Duncan and Geoffrey, 1997; Angelika et al., 2006; Shen et al., 2008; Zhou et al., 2009). However, there is no report about a method used in the extraction of protein from almond dregs. Also, the method of ultrasound-assisted extraction is convenient, rapid, simple, safe and easy to be applied in industrialized production (Wanasundara and Shahidi, 1996; Wang et al., 2008). In the present study, alkaline extraction and acid precipitation method with ultrasound-assisted extraction were used to extract almond dregs protein.

Response surface methodology (RSM) is a statistical method that uses quantitative data in the experimental design to optimize the processes or products (Giovanni, 1983; Myers and Montgomery, 2002). Thus, the optimization of maximum efficiency of protein extraction was investigated using RSM with three variables including extraction temperature, pH value and ratio of solid to liquid.

2 Materials and methods

2.1 Materials

The almond dregs were obtained from the local market in Baoding City, Hebei Province, China. It has a protein content of 53.85% and moisture content of 8.65%. All the chemicals and solvents used were of analytical grade.

2.2 Experimental methods

2.2.1 Ultrasound-assisted almond dregs protein extraction

Ultrasound-assisted extraction was performed in an ultrasonic cleaning bath (KQ5200B type, 40 kHz, 160 W,

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Zhenliang ZHANG, Zide ZHANG (✉), Xiaona ZHANG, Jing LI, Congzhi ZHAO
College of Food Science and Technology, Agricultural University of Hebei, Baoding 071001, China
E-mail: zhangzde@heinfo.net

Yan WANG
Hebei Jiaotong Vocational & Technical College, Shijiazhuang 050000, China

Kunshan Ultrasonic Instrument Co., Ltd., Jiangsu, China). The almond dregs were ground into powder with a cyclonic mill and passed through a 60-mesh sieve. Ten grams of the sample were placed into a 150-mL conical flask, and distilled water added according to a given ratio of solid to liquid (almond dregs $g \cdot mL^{-1}$). The pH value was adjusted with 2% NaOH. The conical flask was placed in an ultrasonic cleaning bath for extraction for 30 min. Then the sample was centrifuged ($4200\ r \cdot min^{-1}$) for 5 min and the supernatant was collected. The residue went through a secondary extraction (under the same conditions) and the supernatant was incorporated. Then the protein content in the supernatant was determined by the method of Coomassie Brilliant Blue.

2.2.2 Component determination

Association of Official Analytical Chemists [AOAC] (1996) descriptions were used to determine moisture content and protein content of almond dregs (Kjeldahl method, N×6.25). The protein content in the supernatant was determined by the method of Coomassie Brilliant Blue. With bovine serum albumin (BSA) as the standard, the standard curve was drawn and the regression equation was established: $Y = 0.0059X + 0.042$, $R^2 = 0.9951$. X represents the concentration of protein ($\mu g \cdot mL^{-1}$), Y represents absorbance.

2.2.3 Efficiency of protein extraction

The efficiency of protein extraction (%) = the protein content in supernatant/the protein content in the material × 100.

2.3 Experimental design

A three-variable, three-level Box-Behnken design (BBD) was applied for optimizing the extraction condition in order to obtain the high efficiency of protein extraction from the almond dregs. The three independent variables set were extraction temperature (X_1, °C), pH value (X_2), and ratio of solid to liquid (X_3, $mL \cdot g^{-1}$), and each variable was set at three levels (Table 1). A total of 15 experiments were designed (Table 2). Each experiment was performed twice and the average efficiency of protein extraction (%) was taken as the response, Y.

2.4 Statistical analyses

The efficiency of protein extraction obtained was taken as the dependent variable or response, Y. The model proposed for the response is given below:
$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$, where Y is the response variable, X_1, X_2 and X_3 are the coded independent

Table 1 Independent variable values of the process and their corresponding levels

level	variable		
	X_1 (extraction temperature)/°C	X_2 (pH value)	X_3 (ratio of solid to liquid)/($g \cdot mL^{-1}$)
−1	30	9	1: 20
0	35	10	1: 30
+1	40	11	1: 40

Table 2 Box-Behnken design and observed responses

number	X_1	X_2	X_3	response (Y, %)
1	0	1	1	56.04
2	−1	0	−1	50.07
3	0	−1	−1	58.48
4	1	−1	0	60.65
5	0	−1	1	53.81
6	−1	0	1	52.69
7	1	0	−1	62.10
8	1	1	0	63.19
9	0	1	−1	57.16
10	1	0	1	61.74
11	−1	1	0	60.52
12	−1	−1	0	45.60
13	0	0	0	69.71
14	0	0	0	69.01
15	0	0	0	67.54

variables, and β_0 is offset term, β_1, β_2 and β_3 are linear terms. β_{11}, β_{22} and β_{33} are squared effects, and β_{12}, β_{13} and β_{23} are interaction effects.

The quality of the fitted model was expressed by the coefficient of determination R^2. The fitted polynomial equation was expressed as a surface plot in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Floros and Chinnan, 1988; Rastogi and Rashmi, 1999). The software used in this study was Design-Expert 7.1.3 Trial, by State-Ease, Inc.

3 Results and discussion

3.1 Fitting the models

The analysis of variance for the experimental results of the Box-Behnken design is shown in Table 3. The model's P-value, 0.0198, implied that the model was significant ($P < 0.05$) (Box and Draper, 1987). The coefficient of determination (R^2) of the model was 0.9306, indicating that 93.06% of the variations could be explained by the fitted model. Thus, the model adequately represented the real relationship between the parameters chosen. The predicted second-order polynomial model was

$$\begin{aligned} Y =& 68.753 + 4.85X_1 + 2.30X_2 - 0.44X_3 \\ & - 3.10X_1X_2 - 0.75X_1X_3 + 0.89X_2X_3 \\ & - 5.49X_1^2 - 5.77X_2^2 - 6.61X_3^2. \end{aligned} \quad (1)$$

The results indicated that the effect of the extraction temperature was the major contributing factor to the efficiency of protein extraction; the pH value and the ratio of solid to liquid were insignificant.

3.2 Response surface plotting

To determine the optimal levels of the variables for the efficiency of protein extraction from the almond dreg powder, three-dimensional surface plots were constructed according to equation (1).

Figure 1(a) shows the effect of extraction temperature and pH value on the efficiency of protein extraction at a fixed ratio of solid to liquid of 1∶30. Figure 1(b) shows the effect of extraction temperature and ratio of solid to liquid on the efficiency of protein extraction at a fixed pH value of 10. Figure 1(c) shows the effect of pH value and ratio of solid to liquid on the efficiency of protein extraction at a fixed extraction temperature of 35°C.

A higher temperature was found to be beneficial to the extraction of the almond dreg proteins, and could accelerate the extraction. However, the costs rose with the increase of temperature. At the same time, the high temperature could lead to denaturation of proteins and affect the quality of the products. Therefore, the extraction temperature must be controlled strictly.

A significant increase of the protein extraction efficiency was observed at a pH value below 10. However, a higher pH value could increase the consumption of acid during acid precipitation. At the same time, stronger alkalinity might cause deaminization, decarboxylation, and the reaction of cystine and lysine.

With dietary fiber the almond dreg has the ability of water-swelling. In our study the materials were difficult to be stirred when at a small ratio of solid to liquid. Although the pH value and the ratio of solid to liquid displayed an insignificant effect on the efficiency of protein extraction, they should be chosen in an appropriate range.

3.3 Optimization of the process

If the partial derivative in equation (1) is zero, three equations can be constructed as follows:

Table 3 ANOVA for response surface mean mode

source	sum of squares	*df*	mean squares	*F* values	pH value
model	619.08	9	68.79	7.44	0.0198
X_1	188.18	1	188.18	20.36	0.0063
X_2	42.18	1	42.18	4.56	0.0857
X_3	1.56	1	1.56	0.17	0.6984
X_1^2	111.40	1	111.40	12.06	0.0178
X_2^2	122.95	1	122.95	13.31	0.0148
X_3^2	161.35	1	161.35	17.46	0.0087
X_1X_2	38.32	1	38.32	4.15	0.0937
X_2X_3	3.15	1	3.15	0.34	0.5846
X_1X_3	2.22	1	2.22	0.24	0.6448
residual	46.20	5	9.24	–	–
Cor total	665.29	14	–	–	–

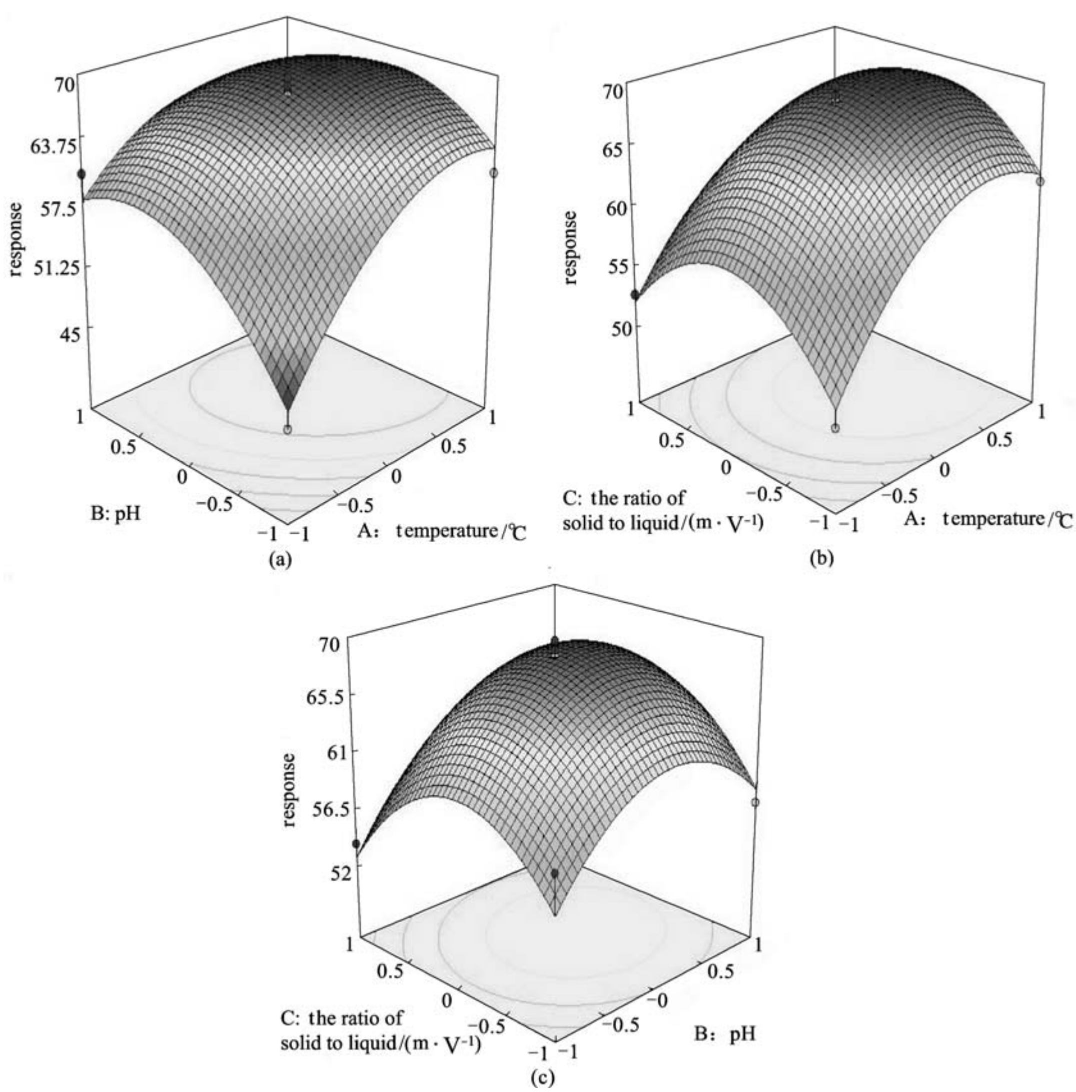

Fig. 1 Response surface plots of the efficiency of protein extraction affected by extraction temperature, pH value, and ratio of solid to liquid
Note: (a) is extraction temperature and pH value (ratio of solid to liquid, 1∶ 30); (b) is extraction temperature and ratio of solid to liquid (pH value, 10); (c) is ratio of solid to liquid and pH value (extraction temperature, 35°C).

$$10.98X_1 + 3.1X_2 + 0.75X_3 = 4.85, \quad (2)$$

$$3.1X_1 + 11.54X_2 - 0.89X_3 = 2.3, \quad (3)$$

$$0.75X_1 - 0.89X_2 + 13.22X_3 = -0.44. \quad (4)$$

Using equations (2)–(4) the following results were obtained: $X_1 = 0.40$, $X_2 = 0.08$, $X_3 = -0.05$.

The optimal conditions were as follows: extraction temperature, 37°C; pH value, 10.1; ratio of solid to liquid, 1∶29.5 ($g \cdot mL^{-1}$). The optimum efficiency of protein extraction was 69.76%.

3.4 Verification of results

The suitability of the model equation for predicting the optimum response values was tested using the recommended optimum conditions. The experimental values were found to be in agreement with the predicted ones (Table 4).

4 Conclusions

In the present paper, an ultrasound-assisted extraction of proteins from almond dregs was performed with a three-variable and three-level Box-Behnken design (BBD) based on RSM. Our experiment results showed that the extraction temperature was a major contributing factor to the extraction of proteins. The optimal efficiency of protein extraction was 69.76%. The adequacy of this model needs to be confirmed by further experiments.

Table 4 Optimum condition, predicted and experimental values of response at optimum conditions

optimum condition	coded level	actual level	
extraction temperature	0.4	37°C	
pH value	0.08	10.1	
ratio of solid to liquid	0.05	1∶29.5	
responses	predicted value	experimental value[a)]	
		mean	range
efficiency of protein extraction/%	69.76	69.60±1.01	68.59–70.61

Note: a) represents mean of three measurements.

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