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Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology

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Abstract

The study investigated enzymatic pretreatment of oat bran, using Viscozyme L to enhance protein extraction. Response surface methodology (RSM) was used to study the effects of pretreatment variables of Viscozyme L concentration (6–30 FBG), pH (3.0–5.0), incubation time (0.5–2.5 h) and temperature (35–55 °C) on protein extraction from oat bran. The results indicated that the generated regression model represented the relationship between the independent variables and the responses. Protein extraction from oat bran was mainly affected by pH and incubation temperature. From the RSM-generated model, the optimum conditions of enzymatic pretreatment were identified as Viscozyme L concentration 30 FBG/10 g of oat bran, pH 4.6, incubation time 2.8 h and temperature 44 °C. Under the optimum conditions, the predicted protein extracted from oat bran was 55.7%, whereas, the experimental extracted protein was 56.2%. The RSM-predicted and experimental extracted proteins were not significantly different from each other. The enzymatic pretreatment method under the optimum conditions extracted significantly more protein (56.2%) than did the alkaline (pH 9.5) method (14.76%). Viscozyme L pretreatment of oat bran improved protein extraction.

Keywords: Oat bran; Viscozyme L; Enzymatic pretreatment; Protein extraction; Response surface methodology

1. Introduction

Oats are potential sources of low-cost proteins with good nutritional value (Hischke, Potter, & Graham, 1968; Ma, 1984). Preparations of oat protein concentrates (OPC) and isolated oat protein (IOP) have been extensively reported (Cluskey, Wu, Wall, & Inglett, 1973; Ma, 1983a; Ma, 1983b; Ma, 1985). In these studies, oat flour and ground oat groats were the major starting raw materials for oat protein products. Youngs (1972) reported that oat bran protein was higher than oat flour, but gave little information about optimization of oat bran protein extraction and characterization. Oat bran was reported to be a good source of β-glucan or soluble fibre (Nnanna & Gupta, 1996; Wood, 1986; Wood, Weisz, & Fedec, 1991). The β-glucan or soluble fibre from oat bran was linked to prevention of cardiovascular diseases and lowering of cholesterol. Protein extraction from oat bran will increase the concentration of β-glucan or soluble fibre and in turn the value of oat by-product. Nnanna and Gupta (1996) reported that oat bran is increasingly used in formulation of low-calorie or low glycemic index food products because of the health benefits of β-glucan or oat fibre.

The alkali method is reported to be the most commonly used procedure for protein extraction from oat flour (Cluskey et al., 1973; Ma, 1983a; Ma, 1983b; Ma, 1985). Although the protein content of oat bran is higher than that of oat flour, protein extraction from oat bran is more difficult than from oat flour because of the high fibre. Also, a good protein yield from oat bran is obtained only at more alkaline conditions. It was reported that high alkaline conditions may, however, reduce the nutritive value of protein

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by encouraging the formation of lysinoalanine (Wang, Hettiarachchy, Qi, Burks, & Siebenmorgen, 1999). The enzymatic pretreatment method, utilizing carbohydrases, was reported to improve the extraction of plant proteins at neutral and slightly basic pH levels (Ansharullah et al., 1997; Ghose & Haldar, 1969; Grossman, Rao, & Da Silva, 1980; Wang et al., 1999). In previous studies, carbohydrases were thought to be involved in disintegration of cell wall matrix, and facilitating of protein extraction. Viscozyme L is a multi-component carbohydrase (Anon, 1991), and can effectively hydrolyze plant cell wall polysaccharide. This may have advantage in cleaving the linkages within the polysaccharide matrix and hence liberate more intercellular constituents, such as protein. However, investigation of the extraction of protein from oat bran using Viscozyme L has not been previously reported.

In the enzymatic pretreatment method, effective enzymatic pretreatment is critical for carbohydrate hydrolysis and protein extraction. Several factors, such as enzyme concentration, incubation time, temperature and pH, may affect the efficiency of enzymatic pretreatment, and their effects may be either independent or interactive. When many factors affect the desired responses, response surface methodology (RSM) becomes an effective tool for optimizing the process (Triveni, Shamala, & Rastogi, 2001). The advantages of using RSM are reported to be reduction in the number of experimental trials needed to evaluate multiple parameters, and the ability of the statistical tool to identify interactions (Chen, Chen, & Lin, 2004; Lee, Ye, Landen, & Eitenmiller, 2000). In addition to analyzing the effects of the independent variables, the experimental methodology also generates a mathematical model that accurately describes the overall process. RSM was successfully utilized for optimization of enzymatic reactions (Ansharullah et al., 1997; Shieh, Akoh, & Koehler, 1995).

The objectives of the study were: (1) to develop an enzymatic pretreatment method, using Viscozyme L, to extract protein from oat bran; (2) to optimize enzymatic pretreatment conditions utilizing RSM; (3) to compare the enzymatic pretreatment method with the alkali (pH 9.5) method used for protein extraction from oat bran.

2. Materials and methods

2.1. Materials

Oat bran was supplied by Rongkang Cereal and Oil Co. Ltd. (Shanxi, China). Viscozyme L was obtained from Novozymes Inc. (Copenhagen, Denmark). The activity of Viscozyme L was 120 Fungal Beta-Glucanase Units (FBG) ml^{-1}, in which 1 FBG is the amount of enzyme required under the standard conditions (30 °C, pH 5.0 and 30 min reaction time) that can hydrolyze barley β-glucan to reducing carbohydrates, with a reducing power corresponding to 1 μmol glucose min^{-1}. All the other reagents were of analytical grade.

2.2. Sample preparation

Oat bran was ground using an electric grinder and sieved through 40 mm mesh. The ground oat bran was defatted by shaking with hexane at 1:3 (w/v) bran-to-solvent ratio for 1 h at room temperature. The suspension was filtered utilizing a Buchner funnel; the solids were washed with hexane, and air-dried to obtain defatted oat bran product (DOB) with 10% moisture.

2.3. Enzymatic pretreatment

Ten grammes of DOB sample were mixed with 100 ml of deionized water at 1:10 (w/v) ratios and blended to obtain a homogeneous slurry. After the pH (3.0–5.0) of the slurries was adjusted, Viscozyme L (6–30 FBG) was added. The slurries containing enzymes were incubated in a water bath, fitted with a thermostatic vibrator (HZS-H Model, Donglian Electronic and Technology Development Co., China) at 200 rpm for a selected period of time (0.5–2.5 h) at different temperatures (35–55 °C). The enzymatic pretreatment variables are presented in Table 1.

2.4. Protein extraction

Subsequent to the enzymatic pretreatment, the slurries were adjusted to pH 9.5 with 2 M NaOH solution immediately, and further incubated for 30 min at 50 °C under shaking conditions. The resultant suspensions were centrifuged at 4000*g* for 30 min, and the supernatants were used for protein determination.

2.5. Protein determination

The protein contents of defatted oat bran, enzyme, supernatants and by-product were determined using the Kjeldahl method (AOAC, 1990) and multiplying the nitrogen content with protein conversion factor of 6.25. The extracted oat protein was expressed as:

$$\text{extracted protein (\%)} = \frac{\text{total protein in supernatant} - \text{protein in enzyme}}{\text{total protein in oat bran}} \times 100\%$$

The proximate composition (protein, moisture, fat, ash and carbohydrate) of oat bran, enzyme, extracted protein and by-product of protein extraction were determined, and the results presented in Table 2.

2.6. Experimental design

A statistical tool utilizing five levels, four variables and central composite rotatable design (CCRD) (Cochran & Cox, 1992), with 31 individual points, was employed to study the effects of enzymatic pretreatments on protein extraction from oat bran. The independent variables and their levels were selected, based on the preliminary experiments in our laboratory (data not shown). The independent

Table 1
Independent variables and their levels used for the central composite rotatable design (CCRD) and optimization of enzymatic pretreatment conditions

Independent variable	Symbol		Levels				
	Uncodified	Codified	−2	−1	0	+1	+2
Amount of enzyme (FBG)	X_1	x_1	6	12	18	24	30
Time (h)	X_2	x_2	0.5	1.0	1.5	2.0	2.5
pH	X_3	x_3	3.0	3.5	4.0	4.5	5.0
Temperature (°C)	X_4	x_4	35	40	45	50	55

Table 2
Proximate composition (%) of Viscozyme L, defatted oat bran, oat bran protein and oat by-product[a]

component	Viscozyme L	Defatted oat bran	Extracted protein	By-product
Protein	9.74 ± 0.42	17.6 ± 0.41	81.7 ± 0.89	6.09 ± 0.36
Moisture	–	10.04 ± 0.29	6.84 ± 0.17	13.62 ± 0.70
Fat	–	0.79 ± 0.04	0.83 ± 0.12	0.30 ± 0.06
Ash	–	4.20 ± 0.22	3.42 ± 0.10	6.58 ± 0.35
Carbohydrate[b]	–	67.4	7.21	73.4

[a] Values represent the means of three determinations ± standard deviation.
[b] Calculated by difference.

variables X_i were coded as x_i, which are defined as dimensionless, according to the Eq. (1):

$$x_i = (X_i - X_0)/\Delta X_i \quad (1)$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the centre point, and ΔX_i is the step change value. The independent variables and their levels are presented in Table 3. The 31 runs were performed in a totally random order to minimize bias. Each experiment had two replications and the average extracted protein was taken as the response, Y. The responses generated from the experiment are presented in Table 4.

2.7. Statistical analysis

The response surface regression (RSREG) procedure of the Statistical Analysis System (SAS Institute, Inc., 1990) was used to fit the experimental data to the second-order polynomial equation to obtain coefficients of the Eq. (2).

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \quad (2)$$

where Y is the response variable, x_i and x_j are the coded independent variables, and β_0, β_i, β_{ii} and β_{ij} are the regression coefficients of variables for intercept, linear, quadratic and interaction regression terms, respectively. The analysis of variance (ANOVA) tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of each coefficient in the polynomial was tested using the Student t-test. The regression coefficients were used for statistical calculations to generate response surfaces and contour plots.

2.8. Verification of model

The optimum conditions of enzyme pretreatment depended on enzyme concentration, pH, incubation time and temperature, and were obtained using RSM. For verification of the model, the oat bran protein was extracted under optimal conditions and the extracted protein was determined. The experimental and predicted values were compared in order to determine the validity of the model.

Table 3
Central composite rotatable design (CCRD) and responses[a]

Run	Independent variables[b]				Response (Y)[c]
	x_1	x_2	x_3	x_4	
1	−1	−1	−1	−1	30.17
2	−1	−1	−1	+1	23.09
3	−1	−1	+1	−1	51.66
4	−1	−1	+1	+1	41.51
5	−1	+1	−1	−1	30.99
6	−1	+1	−1	+1	20.59
7	−1	+1	+1	−1	52.41
8	−1	+1	+1	+1	44.01
9	+1	−1	−1	−1	36.94
10	+1	−1	−1	+1	25.98
11	+1	−1	+1	−1	51.03
12	+1	−1	+1	+1	47.27
13	+1	+1	−1	−1	37.69
14	+1	+1	−1	+1	28.54
15	+1	+1	+1	−1	55.98
16	+1	+1	+1	+1	51.16
17	−2	0	0	0	33.99
18	+2	0	0	0	44.01
19	0	−2	0	0	32.93
20	0	+2	0	0	45.79
21	0	0	−2	0	17.58
22	0	0	+2	0	37.96
23	0	0	0	−2	30.86
24	0	0	0	+2	31.09
25	0	0	0	0	44.68
26	0	0	0	0	46.52
27	0	0	0	0	47.37
28	0	0	0	0	46.11
29	0	0	0	0	45.86
30	0	0	0	0	47.49
31	0	0	0	0	42.17

[a] Non-randomized.
[b] Coded symbols and levels of independent variables refer to Table 1.
[c] Averages of duplicated determination from different experiments.

Table 4
Significance of regression coefficients of the fitted second-order polynomial model for response (Y)

Term	Regression coefficient	Standard error	t Value	P Value
β_0	45.742857	1.939613	23.58	<0.0001
Linear				
β_1	2.508333	1.047511	2.39	0.0292
β_2	1.643333	1.047511	1.57	0.1363
β_3	8.408333	1.047511	8.03	<0.0001
β_4	−2.677500	1.047511	−2.56	0.0211
Quadratic				
β_{11}	−0.846131	0.959651	−0.88	0.3910
β_{22}	−0.756131	0.959651	−0.79	0.4423
β_{33}	−3.653631	0.959651	−3.81	0.0015
β_{44}	−2.852381	0.959651	−2.97	0.0090
Cross product				
β_{12}	0.661250	1.282933	0.52	0.6133
β_{13}	−0.528750	1.282933	−0.41	0.6857
β_{14}	0.458750	1.282933	0.36	0.7253
β_{23}	0.653750	1.282933	0.51	0.6173
β_{24}	−0.051250	1.282933	−0.04	0.9686
β_{34}	0.653750	1.282933	0.51	0.6173

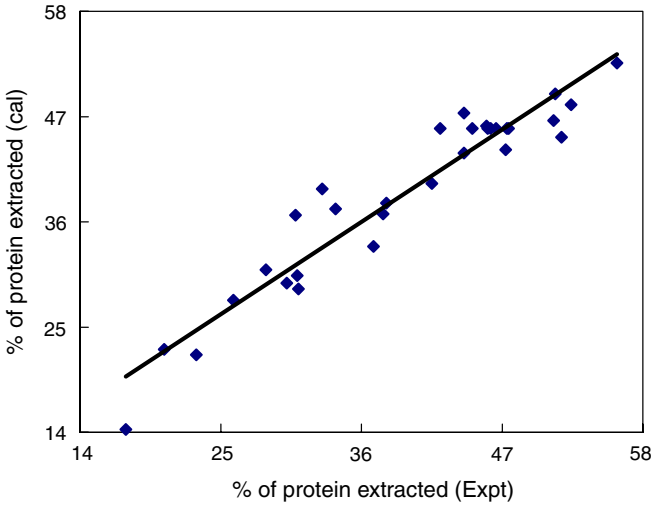

Fig. 1. Correlation of calculated protein with experimental extracted protein (%).

2.9. Alkaline extraction method

The DOB slurries in water (1:10, w/v) were adjusted to pH 9.5 with 2 M NaOH solution. The suspension was incubated in the water bath at 50 °C for 30 min with constant shaking.

3. Results and discussion

3.1. Fitting the models

The study utilized RSM to develop a prediction model for optimizing the Viscozyme L pretreatment conditions of protein extraction from oat bran. The experimental conditions and the corresponding response values from the experimental design are presented in Table 3. The independent and dependent variables were analyzed to obtain a regression equation that could predict the response within the given range. The values of the coefficients in the equation are presented in Table 4. The regression equation for protein extraction (Y) is as follows:

$$\begin{aligned} Y = {} & 45.7429 + 2.5083x_1 + 1.6433x_2 + 8.4083x_3 \\ & - 2.6775x_4 - 0.8461x_1^2 + 0.6613x_1x_2 - 0.5288x_1x_3 \\ & + 0.4588x_1x_4 - 0.7561x_2^2 + 0.6538x_2x_3 - 0.0513x_2x_4 \\ & - 3.6536x_3^2 + 0.6538x_3x_4 - 2.8524x_4^2 \end{aligned} \quad (3)$$

The plot of experimental values of extracted protein (%) versus those calculated from Eq. (3) indicated a good fit, as presented in Fig. 1. The results of analysis of variance (ANOVA) for the CCRD are shown in Table 5. For the model fitted, the coefficient of determination (R^2), which is a measure of degree of fit (Haber & Runyon, 1977), was 0.864. This implies that 86.4% of the variations could be explained by the fitted model. Joglekar and May (1987) suggested that, for a good fit of a model, R^2 should be at least 0.80. The probability (P) value of the regression model significance was less than 0.001. Therefore, the developed model could adequately represent the real relationship among the parameters chosen.

Table 5
Analysis of variance (ANOVA) of the regression parameters for the response surface model

Regression	Degree of freedom	Sum of squares	R^2	F Value	P Value
Linear	4	2084.672550	0.674	19.79	<0.0001
Quadratic	4	559.066367	0.181	5.31	0.0065
Cross product	6	28.554950	0.009	0.18	0.9781
Total model	14	2672.293867	0.864	7.25	0.0002

3.2. Effects of independent variables on responses

The effects of Viscozyme L pretreatment conditions of oat bran on protein extraction by the regression coefficients of fitted second-order polynomial are presented in Table 4. It was evident that the linear terms except for time, and two quadratic terms (pH and temperature) were significant ($P < 0.01$ or $P < 0.05$), whereas all the cross-product terms were not insignificant ($P > 0.5$). The results indicated that the effects of pH and temperature were the major contributing factors to protein extraction from oat bran. Within the experimental range, however, incubation time had no significant effects ($P > 0.05$) on the oat protein extraction.

To aid visualization, the response surfaces and contour plots of enzymatic pretreatment conditions are shown in Figs. 2–4. The incubation time had no significant effect on oat bran protein extraction and was maintained at 1.5 h (coded zero level). The response surface and contour

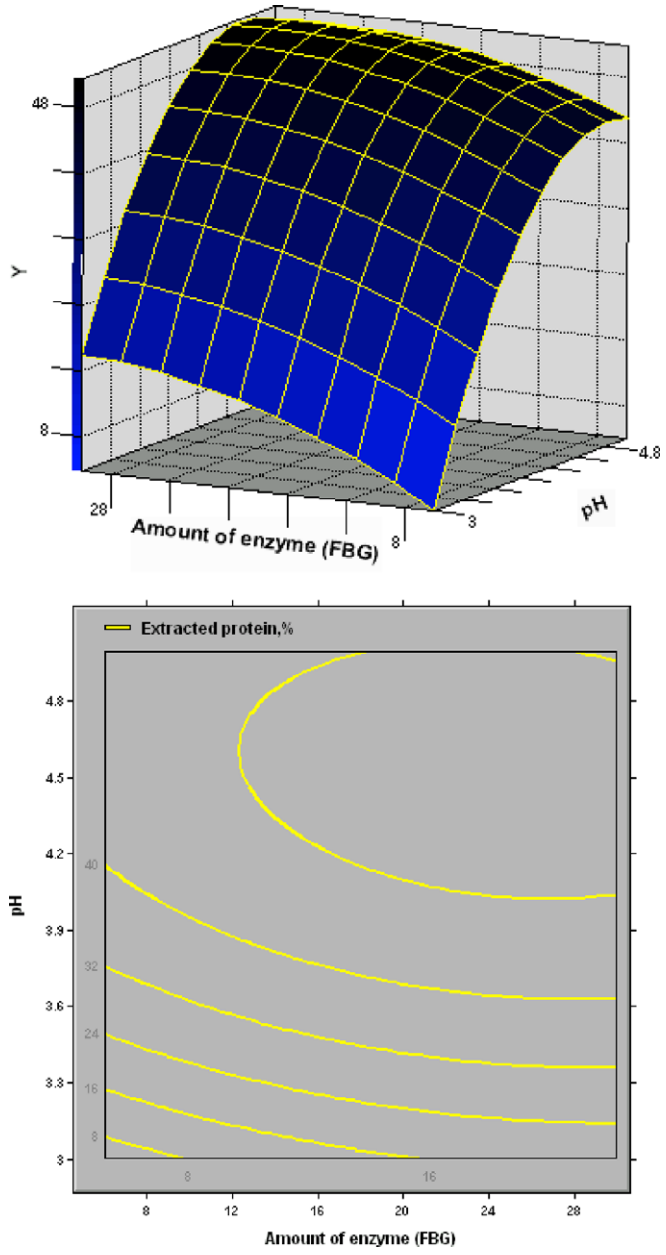

Fig. 2. Response surface and contour plots for the effects of enzyme concentration and pH on protein extraction from oat bran at 45 °C temperature and incubation time of 1.5 h.

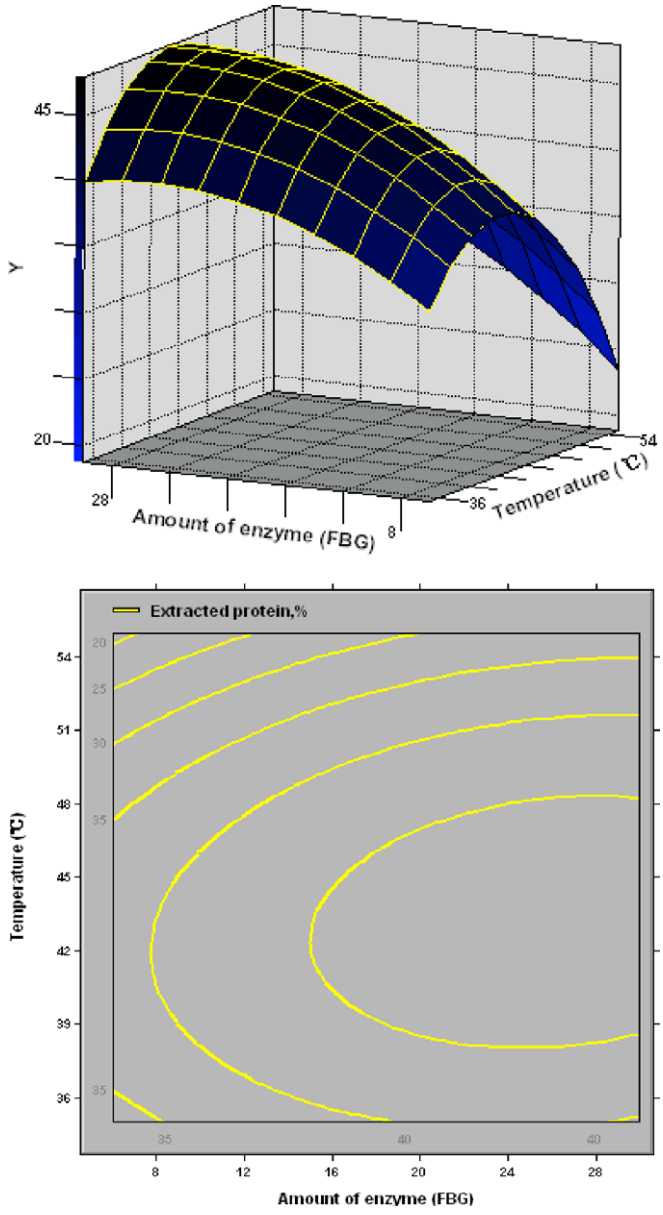

Fig. 3. Response surface and contour plots for the effects of enzyme concentration and temperature on protein extraction from oat bran at pH 4.0 and incubation time of 1.5 h.

plots of the effects of enzyme concentration and pH on oat bran protein extraction at 45 °C temperature are presented in Fig. 2. The results indicated that enzyme concentration displayed a linear effect on the response, and the extracted protein increased with an increase of enzyme concentration. However, pH demonstrated a quadratic effect on the response; hence extracted protein increased up to about pH 4.6, followed by a decline with its further increase. The effects of enzyme concentration and temperature on oat bran protein extraction at pH 4.0 are shown in Fig. 3. Temperature exerted a quadratic effect on the response, yielding maximum protein extraction at 44 °C temperature, whereas, the effect of enzyme concentration was linear, regardless of the incubation temperature used in the study. Both temperature and pH exerted quadratic effects on protein extraction, and the maximum protein extraction was obtained at 44 °C temperature and pH 4.6 (Fig. 4).

3.3. Optimum conditions and model verification

From the model, optimum conditions for enzymatic pretreatment of oat bran protein extraction were obtained as presented in Table 6. Under the optimum conditions of enzyme concentration 30 FBG, pH 4.6, incubation time 2.8 h and temperature 44 °C, a maximum response of 55.7% protein was predicted. The suitability of the model equation for predicting the optimum response value was

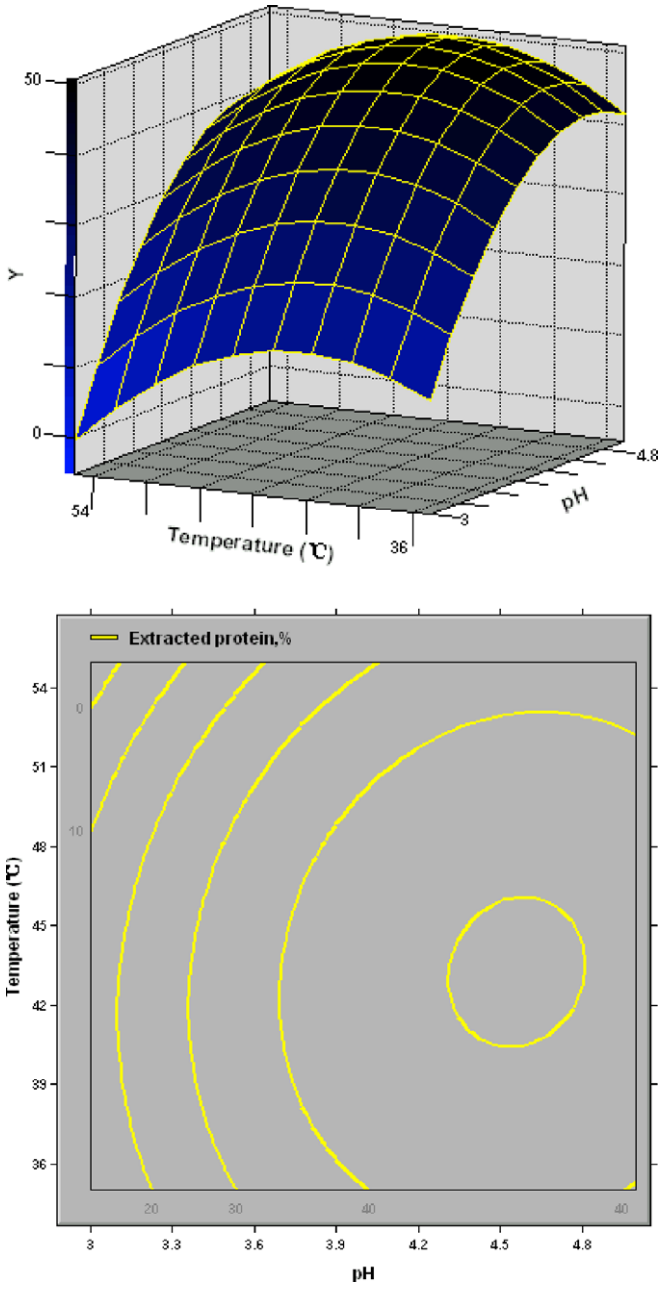

Fig. 4. Response surface and contour plots for the effects of pH and temperature on protein extraction from oat bran with enzyme concentration of 18 FBG and incubation time of 1.5 h.

tested by additional independent experiments using the recommended optimum conditions (Table 6). The results indicated that the experimental protein value (56.2%) was not significantly different from the predicted protein value (55.7%).

Comparing the experimental extracted protein (56.2%) with the alkaline extracted protein (14.7%), the enzymatic pretreatment method extracted significantly more protein under the optimum conditions than did the alkaline (pH 9.5) method (Table 7). The results indicated that enzymatic pretreatment of oat bran enhanced protein extraction greatly. Our findings were consistent with previous studies

Table 6
Optimum conditions of enzymatic pretreatment, predicted and experimental protein values from RSM

Amount of enzyme (FBG)	Optimum condition			Extracted protein (%)	
	Time (h)	pH	Temperature (°C)	Predicted value	Experimental value[a]
30	2.8	4.6	44	55.7	56.2 ± 1.03

[a] Means ± standard deviation of triplicate determinations from different experiments.

Table 7
The effects of Viscozyme L pretreatment on oat bran protein extraction

Treatments	Extracted protein (%)[a]
Enzymatic pretreatment method	56.2 ± 1.03
Alkaline method[b]	14.8 ± 3.22

[a] Means ± standard deviation of triplicate determinations from different experiments.
[b] With alkaline solution at pH 9.5.

on the role of enzymes in enhancing protein extraction from buckwheat bran and rice ran (Ansharullah et al., 1997; Grossman et al., 1980; Wang et al., 1999). Grossman et al. (1980) reported that pectinase and hemicellulase enhanced protein extraction from buckwheat bran. Ansharullah et al. (1997) worked on protein extraction from rice bran using Viscozyme L and Celluclast 1.5 L, and extracted more than 50% protein under optimum conditions enzyme concentration, pH, incubation temperature and time, Wang et al. (1999) utilized phytase and xylanase for protein extraction from rice bran, and concluded that the use of carbohydrases was beneficial in enhancing the yield. In these studies, carbohydrases were thought to be involved in disintegration of cell wall tissue, facilitating protein extraction. The decrease in viscosity caused by degradation of β-glucan in the oat bran may have been helpful in solubilizing and extracting oat protein in this study.

4. Conclusions

The enzymatic pretreatment conditions of enzyme concentration, pH, incubation time and temperature were optimized using RSM to improve protein extraction from oat bran. From the RSM results, the optimum conditions of Viscozyme L concentration 30 FBG/10 g of oat bran, pH 4.6, incubation time 2.8 h and temperature 44 °C were obtained with the highest protein predicted value of 55.7%. The predicted protein value was subsequently confirmed by verification experiments. Under the optimum conditions, the experimental protein of 56.2% was obtained, which was not significantly different from the RSM predicted protein (55.7%). The enzymatic pretreatment method extracted significantly more protein (56.2%) from oat bran than did the alkaline (pH 9.5) method (14.8%). The enzymatic pretreatment method was more efficient in the oat bran protein extraction than was the

alkaline method. The results indicated that Viscozyme L pretreatment of oat bran enhanced protein extraction.

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