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Optimization of hydrolysis conditions for the production of glucomanno-oligosaccharides from konjac using β-mannanase by response surface methodology

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ABSTRACT

Glucomanno-oligosaccharides (GMO), usually produced from hydrolysis of konjac tubers with a high content of glucomannan, have a positive effect on *Bifidobacterium* as well as a variety of other physiological activities. Response surface methodology (RSM) was employed to optimize the hydrolysis time, hydrolysis temperature, pH and enzyme to substrate ratio (E/S) to obtain a high GMO yield from konjac tubers. From the signal-factor experiments, it was concluded that the change in the direct reducing sugar (DRS) is consistent with total reducing sugar (TRS) but contrary to the degree of polymerization (DP). DRS was used as an indicator of the content of GMO in the RSM study. The optimum RSM operating conditions were: reaction time of 3.4 h, reaction temperature of 41.0 °C, pH of 7.1 and E/S of 0.49. The results suggested that the enzymatic hydrolysis was enhanced by temperature, pH and incubation time. Model validation showed good agreement between experimental results and the predicted responses.

1. Introduction

Konjac glucomannan (KGM) is a neutral polysaccharide extracted from tubers of *Amorphophallus konjac* C. Koch (Nishinari & Williams, 1992), which composed of β-(1 → 4) linked D-mannose and D-glucose in the ratio of 1.6:1 (Katsuraya et al., 2003). There are some branching points at the C-3 position of both D-glucosyl and D-mannosyl residues (Maeda, Shimahara, & Sugiyama, 1980), and some at the C-6 positions of glucosyl units, with degree of branching of ca. 8% (Katsuraya et al., 2003). The chain also has a 5–10% acetyl group substitute (Takigami, 2000). The relatively high molecular weight (Mw) of KGM is 10^5–10^6 (Li & Xie, 2003).

When regarded as a food supplement, glucomanno-oligosaccharides (GMO) have shown better probiotic functions than glucomannan. Partially hydrolyzed glucomannan polysaccharide stimulates *Bifidobacterium* and *Lactobacillus acidophilus* growth better than its parent unhydrolyzed KGM (Chen, Fan, Chen, & Chan, 2005). Consuming 5% GMO in the diet for 4 weeks sufficiently promoted the growth of intestinal bifidobacteria and suppressed the growth of *Clostridium perfringens* compared with cellulose. The bifidogenic effects of GMO were greater than those of unhydrolyzed KGM as observed in the feces of Balb/c mice. The fermentation product of glucomannan and fiber likely caused the suppression of *C. perfringens* or *Escherichia coli* in the lower gut (Wang, Lai, Chen, & Chen, 2008). GMO with degree of polymerization (DP) of 5 exerted a greater prebiotic effect than a DP of 10. For that the growth of *Bifidobacterium adolescentis*, *Bifidobacterium breve* and *Bifidobacterium longum* increased in the order KGM < DP of 10 ≤ DP of 5. In addition, GMO (DP of 5) facilitated the growth of *B. adolescentis*, *B. breve*, *Bifidobacterium bifidum* and *B. longum* to the same extent as oligofructose, while KGM, GMO (DP of 5) and GMO (DP of 10) were better substrates than oligofructose for *L. acidophilus*. GMO produced antioxidative effects by increasing radical scavenging ability and eliminating lipid peroxide formation. Furthermore, fermentation of GMO with a DP of 5 produced great inhibitory effects on thiobarbituric acid-reactive substances formation (Wang et al., 2008). Because of their wide ranging applications as exemplified above and their potential applications for drug delivery (Alonso-sande, Teijeiro-Osorio, Remuñán-López, & Alonso, 2009), the number of publications dealing with uses of KGM and GMO has remarkably increased over the last 10 years. However, most of the review articles about KGM and GMO have focused on their chemical and physicochemical properties, such as chemical structure, molecular weight, gelation behavior, and ability to interact with other polymers, such as carrageenan or xanthan (Katsuraya et al., 2003). The research detailed herein is the first to report on the efficient production of GMO.

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A effective enzymatic hydrolysis of KGM with neutral β-mannanase was developed. Endo-β-1,4-D-mannanase catalyzes the random hydrolysis of β-1,4-mannosidic linkages in the main chain of mannan polymers thereby releasing linear and branched manno-oligosaccharides of various lengths (De Vries, 2003). The design of an efficient enzymatic hydrolysis of KGM was a multivariate process involving many factors that affect the efficiency of enzymatic hydrolysis. The classical method of determining optimum conditions by varying one parameter while keeping the other at a specified constant level is single-dimensional, laborious and time-consuming and often does not guarantee determination of optimal conditions (Wernimont, 1985). On the other hand, carrying out experiments with every possible factorial combination of the test variables is impractical because of the large number of experiments required (Haaland, 1989). In order to overcome these problems, optimization studies have been done using response surface methodology (RSM), a statistically designed experimental protocol in which several factors were simultaneously varied. This multivariate approach has advantages in terms of reductions in the number of experiments, improved statistical interpretation possibilities and reduced time requirements from overall analysis. RSM has been found to be successful and economical during optimization of various industrial processes (Deepak et al., 2008; Gan & Latiff, 2011; Wang, Cheng, Mao, Fan, & Wu, 2009; Ye & Jiang, 2011).

The objective of this research was to study and optimize enzymatic hydrolysis conditions of KGM using a commercial mannanase to provide a high yield of GMO. RSM was applied to optimize the hydrolysis conditions including enzyme to substrate ratio (E/S), hydrolysis pH, hydrolysis time and temperature.

2. Materials and methods

2.1. Materials

All chemicals, buffers and reagents were of analytical grade. Unless stated, all the chemicals were purchased from Sigma Chemical Co. (USA). Neutral β-mannanase was purchased from Bosar Biotechnology Company (Beijing, China). (No high molecular weights protein inside except mannanase as indicated by SDS-PAGE, data not shown.) Konjac was purchased from Guangshui City (Hubei, China).

2.2. Methods

2.2.1. Preparation of konjac powder

The konjac tuber was washed and peeled off and then was smashed with juice extractor. After deionized water was added at a ratio of 1:1000 (w/v), it was homogenated using a high isotropic mulser at 800 rpm for 10 min three times. Finally, the homogeneity was lyophilized (−60 °C, 13 Pa) and ground into powder.

2.2.2. Production of glucomanno-oligosaccharides

One gram of konjac powder was added to 50 ml 0.05 M Na_2HPO_4–citric acid buffer and then mixed with neutral β-mannanase (E/S ranging from 0.01 to 0.7, w/w) to start the reaction. The mixture was incubated at pH 2.0–10 for reaction times ranging from 0 to 7 h, while the temperature of the water bath was kept steady at a given temperature (reaction temperature ranged from 20 to 80 °C). The reaction was stopped by boiling the samples for 15 min and then the samples were centrifuged at 6000 rpm ($2535 \times g$). The supernatant was then tested for DRS and TRS and the DP was calculated.

2.2.3. Determination of the activity of neutral β-mannanase

1.00 g of β-mannanase was added into 700 ml 0.05 M pH 6.0 Na_2HPO_4–citric acid buffer and stirred for about 20 min at room temperature and then transferred into a 100 ml volumetric flask with 0.05 M pH 6.0 Na_2HPO_4–citric acid buffer and centrifuged at 4000 rpm ($1127 \times g$) for 10 min. The supernatant was used as the enzyme solution. Neutral β-mannanase activity was assayed according to the method of Bailey, Biely, and Poutanen (1992). A reaction mixture containing 2 ml of 1.0% (w/v) locust bean gum (LBG, Sigma, USA) and 2.0 ml of suitable diluted enzyme solution was incubated in 0.05 M pH 6.0 Na_2HPO_4–citric acid buffer at 40 °C for 20 min. The reaction was stopped by adding 5.0 ml of 1.0% (w/v) DNS. The reducing sugar content was determined by the DNS method using mannose as the standard (Miller, 1959). One unit of neutral β-mannanase activity was defined as the amount of enzyme that produces 1 μg of mannose equivalent per minute.

Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Level		
	−1	0	1
X_1: Time (h)	0.5	2.5	4.5
X_2: Temperature (°C)	20	50	80
X_3: pH	2	6	10
X_4: E/S (w/w)	0.1	0.4	0.7

2.2.4. Determination of the direct reducing sugar (DRS), total reducing sugar (TRS) and DP

As total sugar is defined as the total amount of reducing sugar after samples were degraded thoroughly, direct reducing sugar was named as reducing sugar before degraded corresponded to TRS. DRS, TRS and DP reflect the degree of degradation of polysaccharide. Thus, effect and termination of the enzymatic process can be monitored through DRS, TRS and DP. DRS was determined by the Somogyi method (Somogyi, 1945). TRS was detected by mixing 5 ml of diluted sample with 5 ml 8% H_2SO_4, boiling the sample for 2 h, cooling it to room temperature and adjusting the pH to 8–10 with 9% NaOH. Then the sample was diluted into 25 ml with deionized water and the DRS of the sample was tested with the Somogyi method. The DP was calculated by the following formula:

$$\mathrm{DP} = \frac{\mathrm{TRS}}{\mathrm{DRS}} \tag{1}$$

2.2.5. Experimental design

The hydrolysis parameters were optimized by RSM. A three level, four variable Box-Behnken factorial design (BBD) was adopted to determine the best combination for DRS. Temperature, pH, time and E/S ratio were chosen as independent variables. The range and central point values of four independent variables presented in Table 1 were based on the results of a single-factor experiment. All the experiments were done in triplicate and DRS was selected as the response (Y). Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The behavior of the system was explained by the following quadratic equation:

$$Y = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j \tag{2}$$

where Y is the dependent variable (DRS in real value); b_0, b_i, b_{ii} and b_{ij} are coefficients estimated by the model, X_i, X_j are levels of the independent variables. They represent the linear, quadratic and cross product effects of the X_1, X_2, X_3 and X_4 factors on the response, respectively.

SAS 9.0 software was used to estimate the response of the independent variables and also to plot the response surface graphs. The fitted polynomial equation is then expressed in the form of three dimensional surface plots in order to illustrate the

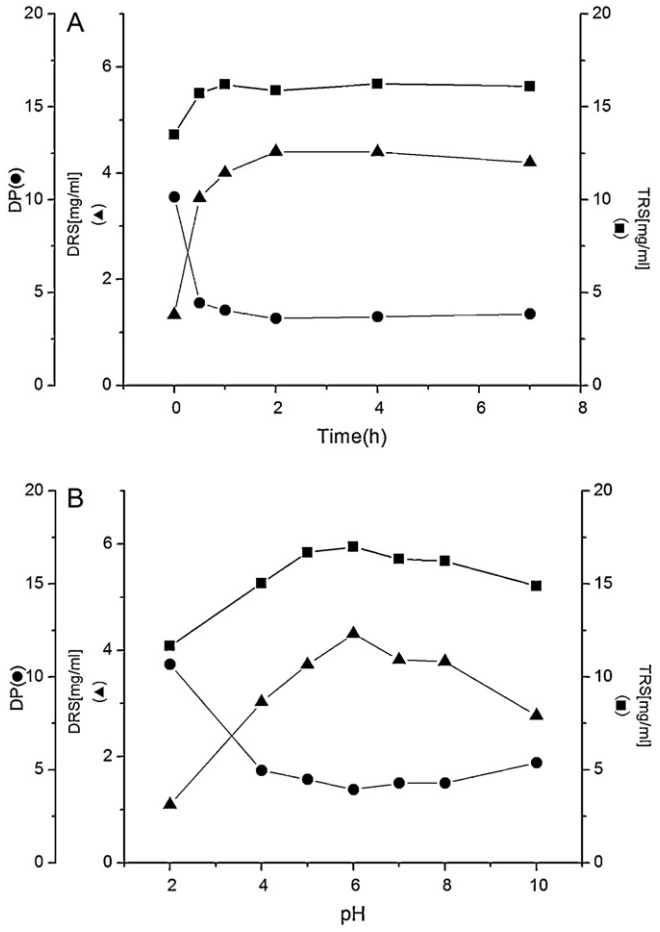

Fig. 1. Effect of hydrolysis time (a) and pH (b) on the DRS (▲), TRS (■) and DP (●).

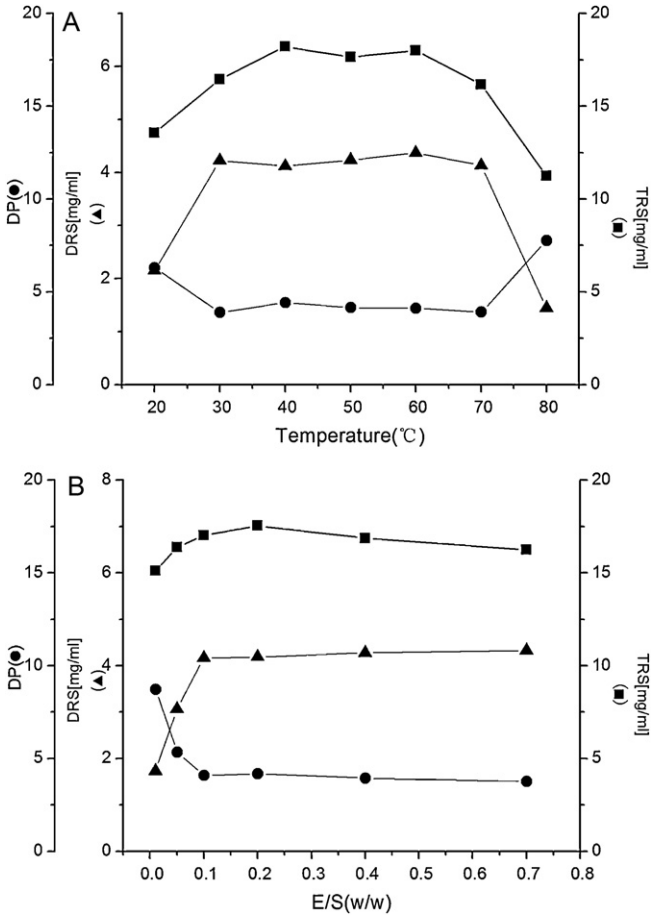

Fig. 2. Effect of hydrolysis temperature (a) and E/S (b) on the DRS (▲), TRS (■) and DP (●).

relationship between the responses and the experimental levels of each of the variables utilized in this study. The point optimization method is employed in order to optimize the level of each variable for maximum response. The combination of different optimized variables, which yield the maximum response, is determined in an attempt to verify the validity of the model. Subsequently, three additional confirmation experiments are conducted to verify the validity of the statistical experimental strategies.

3. Results and discussion

3.1. Effect of different times on the DRS, TRS and DP of glucomanno-oligosaccharides

The results obtained after enzymatic hydrolysis of konjac powder for different times is shown in Fig. 1a. The reaction time was set at 0 h, 0.5 h, 1.0 h, 2.0 h, 4.0 h, and 7.0 h, while the other parameters were set to temperature 40 °C, pH 6.0, and E/S 0.1. The DP of konjac glucomannan decreased within 1.0 h, and then plateaued until 7 h. DRS and TRS increased within the first 4 h, and then remained steady from 4 to 7 h. With an increase in hydrolysis time, the neutral β-mannanase activity showed a tendency to decrease. As a result, 4 h was determined to be the optimum hydrolysis time and 2.5 h was chosen as the center point with 1.5 h as the step change. Takahashi et al. (1984) found that the average DP of the hydrolysis products of KGM with β-mannanase showed a rapid rate of decrease at an early stage (1 h) and reached a DP of about 2 after 24 h of reaction time. These results are comparable to ours.

3.2. Effect of different pH on DRS, TRS and DP of glucomanno-oligosaccharides

The results obtained after enzymatic hydrolysis of konjac powder at different pH are shown in Fig. 1b. The hydrolysis process was carried out at pH of 2.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 10.0, while the other reaction conditions were temperature of 40 °C, time 1.0 and E/S 0.1. When the pH was in the range of 2.0–6.0, DRS and TRS both increased with increasing pH while the DP decreased. When the pH was in the range of 6.0–10.0, it was the opposite. Thus, the optimum pH for the hydrolysis of konjac powder was 6.0. The hydrolysate showed the maximum DRS and TRS and lowest DP at pH 5.0–7.0. These values agreed reasonably well with literature reports aiming to produce GMO at pH 6.8. Consequently, pH 6.0 was chosen as the center point with 4.0 as the step change.

3.3. Effect of different temperatures on DRS, TRS and DP of glucomanno-oligosaccharides

The results obtained after enzymatic hydrolysis of konjac powder at different temperatures is shown in Fig. 2a. The temperature was set at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, while other hydrolysis variables were set as follows: pH 6.0, time 1.0 h and E/S 0.1. From 20 °C to 30 °C, DRS and TRS increased while DP declined which was the contrary to the pattern at 70–80 °C. Between 30 and 70 °C, DRS, TRS and DP essentially did not vary. This mannanase has a broad optimal temperature range from 30 to 70 °C contrary to the mannanases reported previously with optimums in the range of 55–65 °C (Akino, Nakamura, & Horikoshi, 1988; Takahashi,

Kusakabe, Maekawa, Suzuki, & Murakami, 1983; Tsujisaka, Hiyama, Takenishi, & Fukumoto, 1972). In most cases, the rate of reaction increased with temperature while the stability of the enzymes declines (Foresti & Ferreira, 2007; Garcia, Sanchez, Martinez, & Aracil, 1999). An increase in enzymatic reaction rate with temperature is explained by transition state theory (Serri, Kamaruddin, & Long, 2006). Normally, use of a high temperature causes energy waste as well as the denaturation of enzyme. Thus the optimum temperature for hydrolysis of konjac powder by the enzyme was determined to be 40 °C. The hydrolysate showed maximum DRS and TRS at 40–60 °C. Thus, 50 °C was chosen as the center point with 30 °C as the step change.

3.4. Effect of different E/S ratio on DRS, and DP of glucomanno-oligosaccharides

The results from the enzymatic hydrolysis of konjac powder with different E/S are shown in Fig. 2b. E/S was set at 0.01, 0.05, 0.2, 0.4, 0.7, while other hydrolysis variables were set as follows: temperature 40 °C, pH 6.0, and time 1.0 h. The increase of E/S ratio resulted in an increase in DRS and TRS, but it was not sustainable. The results are likely due to greater hydrolysis of the konjac powder with increasing β-mannanase addition. With consideration of the cost, the center point for the E/S ratio was 0.4 with a step change of 0.3.

The initial soluble konjac mixture and the products produced by the hydrolysis were checked by TLC. It is indicated that there is no GMO at $t = 0$ and the tendency of DP is consistent with Figs. 1 and 2 (data not shown), which suggested that GMO were really produced from the hydrolysis. Based on the signal-factor experiments, it was concluded that the changing trend of DRS is consistent with TRS but contrary to that of DP. Hence, DRS can be measured as a signal to represent the volumes of glucomanno-oligosaccharides instead of testing all three parameters: DRS, TRS and DP.

3.5. Statistical analysis and the model fitting

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It solves multivariable data which is obtained from properly designed experiments to solve multivariable equations simultaneously. A 27-run BBD with four factors and three levels, including three replicates at the center point, was used to fit a second-order response surface in order to optimize the extraction conditions. Table 2 shows the process variables and experimental data. The results of the analysis of variance, goodness-of-fit and the adequacy of the models are summarized in Table 3. The DRS ranged from 0.137 to 3.619 (mg/ml). The maximum value was found at 4.5 h, temperature 50 °C, pH 6 and E/S 0.7. The parameters of the equation were obtained by multiple regression analysis of the experimental data. The following quadratic model explains the experimental data:

$$Y = 3.5033 + 0.2705X_1 - 0.4508X_2 + 0.7536X_3 + 0.1918X_4 - 0.2928X_1^2 - 0.9113X_2^2 - 1.5689X_3^2 - 0.3088\,X_4^2 - 0.2130X_1X_2 + 0.1155X_1X_3 + 0.0120X_1X_4 - 0.3020X_2X_3 + 0.0875X_2X_4 + 0.0818X_3X_4 \quad (3)$$

where Y is the predicted response in real value; X_1 the coded value of variable time; X_2 the coded value of variable temperature; X_3 the coded value of variable pH, X_4 the coded value of variable E/S.

The t-test and P values were used to identify the effect of each factor on DRS (Table 3). The P-values were used as a tool to check the significance of each coefficient. The smaller the P-value was, the more significant the corresponding coefficient was (Guo, Zou, & Sun, 2010). The time, temperature, pH, time * time, temperature * temperature, pH * pH had a significant effect ($P < 0.05$) on DRS. The most remarkable effects were the linear term of pH (X_3), the quadratic term of pH (X_3^2) and the quadratic term of temperature (X_2^2), followed by the linear term of temperature (X_2) and the linear term of time (X_1). These analytical data disagreed with most enzyme reactions where E/S or E/S * E/S has a significant effect (Dwevedi & Kayastha, 2009; Ferreira, Duarte, Ribeiro, Queiroz, & Domingues, 2009; Guo, Pan, & Masaru, 2009; Peričin, Radulović-Popović, Vaštag, Madarev-Popović, & Trivić, 2009). The interaction terms did not have significant influence ($P > 0.1$), which means that the interaction between the different factors did not influence the response. Results showed that among the independent variables, pH played a dominant role in the extent of hydrolysis. The fit of the model was checked by determination of the coefficient R^2, which was calculated to be 0.9338, indicating that 93.38% of the variability in the response of DRS can be explained by the model equation (3). This high R^2 value indicated that the models are well adapted to the responses. The statistical analysis of data revealed that linear, quadratic and interaction coefficients were significant. The ANOVA results also showed that there was a non-significant ($P > 0.05$) lack of fit which further validates the model. Eq. (3) shows the dependence of DRS on pH, temperature and time.

Table 2
Box-Behnken experimental design with the independent variables.

Run	Coded levels of variable				DRS (mg/ml)
	X_1	X_2	X_3	X_4	
1	−1	−1	0	0	2.235
2	−1	1	0	0	1.489
3	1	−1	0	0	3.173
4	1	1	0	0	1.575
5	0	0	−1	−1	0.828
6	0	0	−1	1	0.890
7	0	0	1	−1	2.041
8	0	0	1	1	2.430
9	−1	0	0	−1	2.190
10	−1	0	0	1	2.821
11	1	0	0	−1	2.940
12	1	0	0	1	3.619
13	0	−1	−1	0	0.283
14	0	−1	1	0	1.777
15	0	1	−1	0	0.649
16	0	1	−1	0	0.935
17	−1	0	−1	0	0.137
18	−1	0	1	0	2.161
19	1	0	−1	0	0.243
20	1	0	1	0	2.729
21	0	−1	0	−1	2.662
22	0	−1	0	1	2.757
23	0	1	0	−1	1.192
24	0	1	0	1	1.637
25	0	0	0	0	3.493
26	0	0	0	0	3.512
27	0	0	0	0	3.505

3.6. Optimization of hydrolysis conditions of polysaccharides

In order to determine the optimal levels of each variable for maximum DRS production, response surface plots were constructed by plotting the response (DRS production) against any two independent variables while other variables were fixed at their respective zero level. The results of DRS affected by time, temperature, pH and E/S are presented in Figs. 3 and 4.

As shown in Figs. 3 and 4, the DRS increased until pH, temperature, E/S, and time reached an optimum point and then decreased with further increases of pH, time, E/S, and temperature. This was probably because pH and temperature affected the enzyme hydrolysis activity. The 3-D response surface plot and the contour plots

Table 3
ANOVA analysis for the response variables.

Variables	DF	SS	MS	F	Pr > F
Model	14	28.5073	2.036236	12.09055	0.0001
X_1	1	0.878	0.878	5.214	0.0414
X_2	1	2.439	2.439	14.482	0.0025
X_3	1	6.815	6.815	40.463	0.0001
X_4	1	0.441	0.441	2.620	0.1315
X_1^2	1	0.835	0.835	4.959	0.0459
X_2^2	1	5.486	5.486	32.574	0.0001
X_3^2	1	14.907	14.907	88.512	0.0001
X_4^2	1	0.509	0.509	3.020	0.1078
$X_1 * X_2$	1	0.181	0.181	1.078	0.3197
$X_1 * X_3$	1	0.053	0.053	0.317	0.5839
$X_1 * X_4$	1	0.001	0.001	0.003	0.9543
$X_2 * X_3$	1	0.365	0.365	2.166	0.1668
$X_2 * X_4$	1	0.031	0.031	0.182	0.6773
$X_3 * X_4$	1	0.027	0.027	0.159	0.6973
Residual	12	2.020985	0.168415		
Lack of fit	10	2.0208	0.20208	2188.593	0.000457
Pure error	2	0.000185	0.000092		
Total	26	30.52829			
	R^2 = 93.38%	R^2_{Adj} = 85.66%	CV = 20.55615		

in Figs. 2a and 3a give the DRS as a function of hydrolysis time and temperature at a fixed pH (0 level) and E/S (0 level). A quadratic effect for both variables was observed, although temperature had greater influence on the response. DRS increased with time, but after 4 h of hydrolysis, a plateau of DRS was noticed. Also, higher values of DRS were observed when the temperature was between 30 °C and 50 °C. Figs. 3b and 4b show the effect of time and pH on DRS where DRS increased with increases in time. A further increase in pH resulted in reversal of this trend. Figs. 3c and 4c show the 3-D response surface plot and the contour plot at varying time and E/S at fixed temperature (0 level) and pH (0 level). DRS increased with increases in time, but decreased slightly with further increases in E/S. The effect of different temperatures and pH on DRS is shown in Figs. 2d and 3d when the other two variables (time and E/S) were fixed at 0 level. A quadratic effect for both variables was observed although pH had greater influence on the response. DRS increased as pH increased, but after a pH of 3, a plateau of DRS was noted. After pH 8, DRS started to decrease. In Figs. 3e and 4e, when the 3-D response surface plot and the contour plot were developed for DRS with varying temperature and E/S at fixed time (0 level) and pH (0 level), it indicated that DRS increased with increasing temperature. A further increase in E/S resulted in reversal of this trend. As shown in Figs. 3f and 4f, when the other two independent variables, temperature and time were kept at a zero level, DRS increased as E/S increased, although the influence of E/S was not obvious. Higher values of DRS could be noticed when E/S was between 0.3 and 0.5.

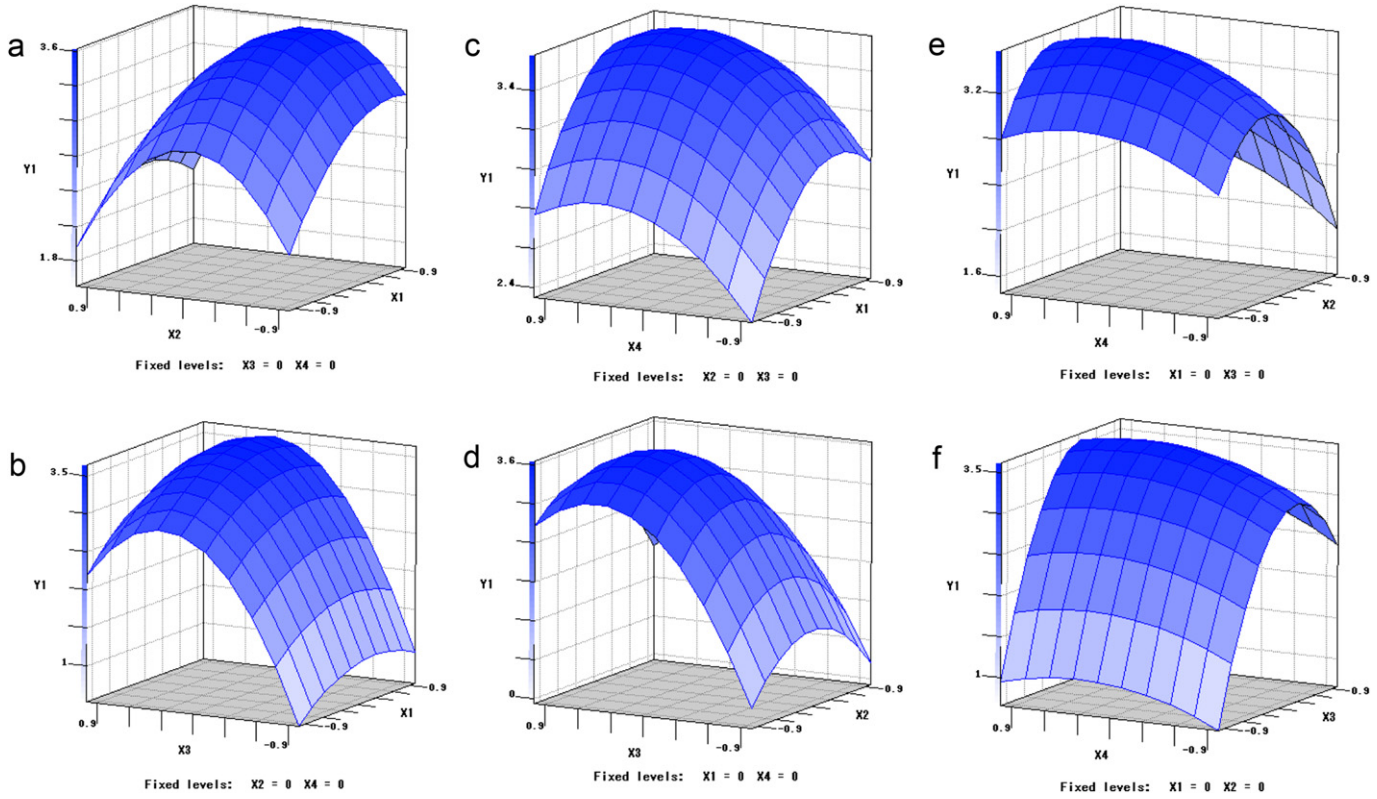

Fig. 3. Response surface (3-D) showing the effect of time, temperature, pH and E/S on the response *Y*.

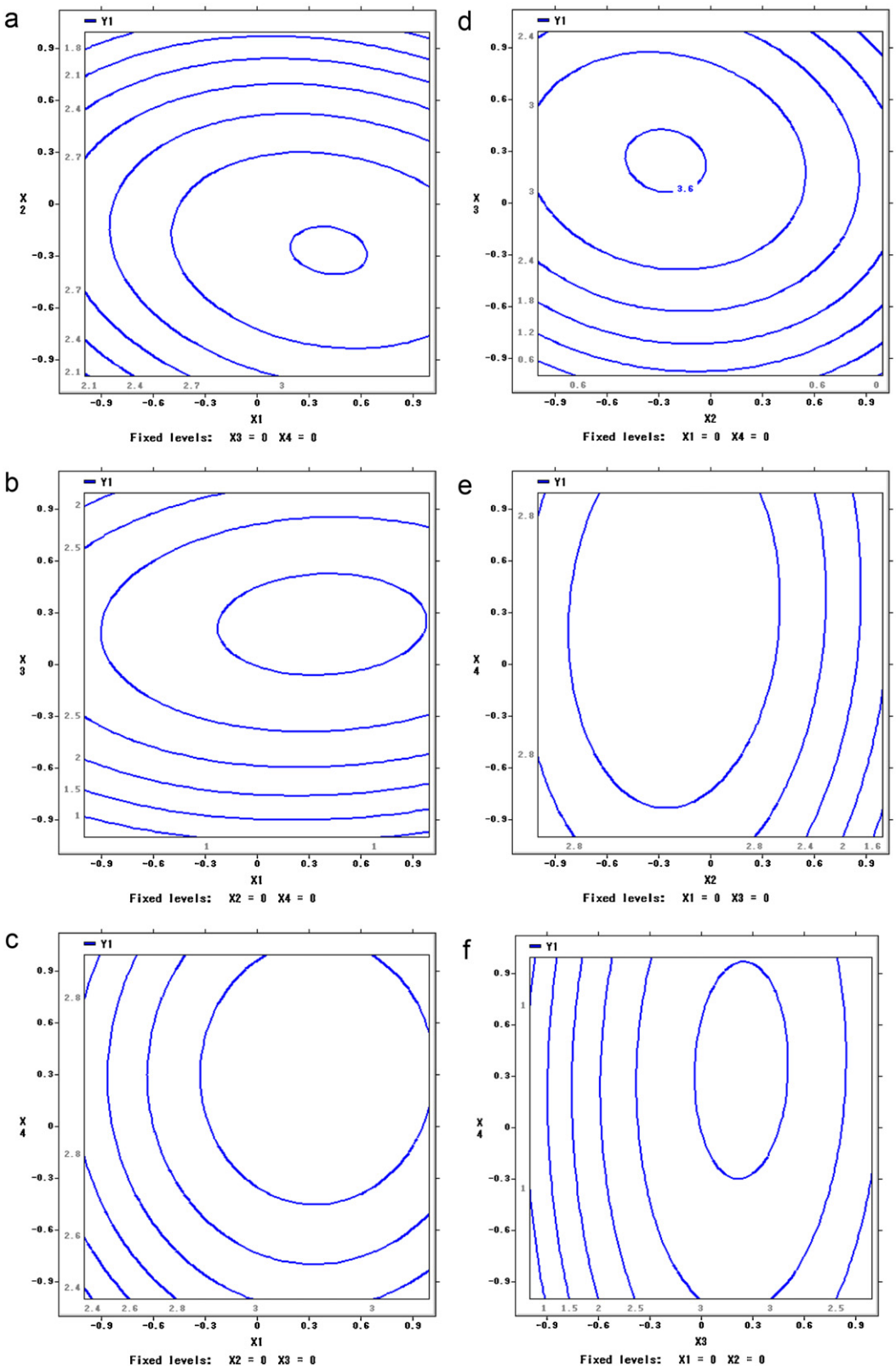

Fig. 4. Contour plots showing the effect of time, temperature, pH and E/S on the response *Y*.

Table 4
Results of validation experiment.

Optimum conditions for the hydrolysis		DRS	
		Predictive value	Observed value
Time	3.4 h	3.768 mg/ml	3.709 mg/ml
Temperature	41.0 °C		
pH	7.1		
E/S	0.49		

These results have confirmed that the response surface had a maximum point within the experimental range of the independent variables. The precise coordinates of the optimum and the levels for the three independent variables were obtained by analytical procedures. The stationary point (maximum) of the fitted model was found by deriving first derivatives of the function (3), as follows:

$$\begin{aligned} &0.2705 - 0.5856X_1 - 0.2130X_2 + 0.1155X_3 + 0.0120X_4 = 0 \\ &-0.4508 - 1.8226X_2 - 0.2130X_1 - 0.3020X_3 + 0.0875X_4 = 0 \\ &0.7536 - 3.1378X_3 + 0.1155X_1 - 0.3020X_2 + 0.0818X_4 = 0 \\ &0.1918 - 0.6176X_4 + 0.0120X_1 + 0.0875X_2 + 0.0818X_3 = 0 \end{aligned} \quad (4)$$

The system of linear equation (4) was solved and following results were obtained: $X_1 = 0.4673$, $X_2 = -0.2989$, $X_3 = 0.2767$ and $X_4 = 0.3137$. The calculated values (X_1, X_2, X_3 and X_4) correspond to the coded values of the independent factors for the maximum value of the response (DRS). The optimum conditions for the hydrolysis are presented in Table 4. It could be seen that under the optimal conditions, achieved DRS was 3.768 mg/ml.

3.7. Validation of the model

To confirm the validity of the suggested mathematical model, an additional experiment was conducted under the predicted optimal conditions. Two parallel probes were performed and the average DRS value (3.709 mg/ml) was measured at the sample standard deviation of 0.02. Using the *t*-distribution, for the one-side test, the critical value of the lower estimate, at a level of $a = 0.05$, was obtained (3.659 mg/ml). As the mean value (3.709 mg/ml) is higher than the critical value (3.659 mg/ml), there is no significant difference between the measured value and the calculated value for DRS at the optimal conditions. This indicates that the second-order polynomial model (Eq. (4)) can be used to predict DRS if different levels of time, temperature, pH and E/S are chosen as control factors for the hydrolysis of konjac powder with neutral β-mannanase, at a temperature of 41 °C, time of 3.4 h, pH 7.1 and E/S of 0.49 (Table 4). This result (especially time and E/S) exceeds experimental data allowed in single-factor experiment. But from the analysis of RSM, DRS increased when time or E/S increased, but increased little (as same as that in Figs. 1a and 2b). So it is acceptable. Furthermore, the balance between cost and GMO output will also be considered in practice.

Enzymatic hydrolysis has been shown to be an effective tool to produce glucomanno-oligosaccharides which have the potential to be used in different food products. Wang et al. (2008) defined DP as the ratio of total sugar to reducing sugar, and the total sugar was analyzed by the method of Dubois, Gilles, Hamilton, Robers, and Smith (1956), which is different from ours. So further investigation of functional, nutritive and pharmacological (antioxidant) properties of the obtained glucomanno-oligosaccharides should be undertaken in order to gather more information on the enzymatic processing of konjac powder.

4. Conclusions

From the results it can be concluded that the enzymatic hydrolysis of konjac powder was clearly enhanced by temperature, pH, and incubation time over the range tested. A concentration of DRS of 3.709 mg/ml was obtained under the experimental conditions of 41 °C, pH 7.1, incubation time 3.4 h and E/S 0.49. The model validation provided good agreement between the experimental results and the predicted response. The high yield of DRS under the optimal conditions indicated the potential of the method for the production of GMO at an industrial scale.

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