ORIGINAL ARTICLE

Optimization of enzymatic hydrolysis of guar gum using response surface methodology

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Abstract Guar gum is a polysaccharide obtained from guar seed endosperm portion. Enzymatically hydrolyzed guar gum is low in viscosity and has several health benefits as dietary fiber. In this study, response surface methodology was used to determine the optimum conditions for hydrolysis that give minimum viscosity of guar gum. Central composite was employed to investigate the effects of pH (3–7), temperature (20–60 °C), reaction time (1–5 h) and cellulase concentration (0.25–1.25 mg/g) on viscosity during enzymatic hydrolysis of guar (*Cyamopsis tetragonolobus*) gum. A second order polynomial model was developed for viscosity using regression analysis. Results revealed statistical significance of model as evidenced from high value of coefficient of determination ($R^2 = 0.9472$) and $P < 0.05$. Viscosity was primarily affected by cellulase concentration, pH and hydrolysis time. Maximum viscosity reduction was obtained when pH, temperature, hydrolysis time and cellulase concentration were 6, 50 °C, 4 h and 1.00 mg/g, respectively. The study is important in optimizing the enzymatic process for hydrolysis of guar gum as potential source of soluble dietary fiber for human health benefits.

Keywords Guar gum · Viscosity · Process optimization · Response surface methodology · Central composite design

Introduction

Guar gum is a highly viscous water soluble heteropolysaccharide composed of linear backbone of β-1, 4-linked mannose units with α-1, 6-linked galactose units randomly attached as side chains (Dea and Morrison 1975). It is derived from the seeds of plant *Cyamopsis tetragonolobus* (Cluster bean), an annual plant indigenous to Indian sub continent growing in semi arid conditions. Guar gum finds extensive application as an additive in various food products, viz. sauces, soups, desserts and baked food products due to its stabilizing and thickening properties. In last few decades, guar gum has been studied extensively for its health benefits (Krotkiewski 1984; Ellis et al. 1996; Butt et al. 2007; Mudgil et al. 2011). Partially hydrolyzed guar gum (PHGG) has attracted attention as a water-soluble dietary fiber as it shows physiological effects such as increasing defecating frequency and lowering the pH of feces of both healthy men and constipated women and reducing serum cholesterol, free fatty acid, and glucose concentrations in humans (Greenberg and Sellman 1998; Heini et al. 1998; Trinidad et al. 2004; Minekus et al. 2005; Stewart and Slavin 2006; Yoon et al. 2006). PHGG can be produced by enzymatic hydrolysis, acid hydrolysis, irradiation, microwave and ultrasonication techniques (Tayal and Khan 2000; Cheng et al. 2002; Singh and Tiwari 2009; Gupta et al. 2009a). For food applications, PHGG production is preferred using enzymes like endo-β-D-mannanase (Yoon et al. 2008) and pectinase (Shobha et al. 2005). During enzymatic hydrolysis, various parameters such as pH, temperature, hydrolysis time and enzyme concentration control viscosity reduction (Wientjes et al. 2001; Kurakake et al. 2006; Mahammad et al. 2006; Mudgil et al. 2012).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques used to develop,

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improve, and optimize processes. Its use leads to the rapid and efficient development of new and improved products and processes with the least cost. The basic principle of RSM is to relate product properties to regression equations that describe inter-relations between input parameters and product properties (Montgomery 1984). It defines the effect of the independent variables, alone or in combination, on the processes. In addition to analyzing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the processes (Myers and Montgomery 1995). In the present study, RSM was employed to standardize the process parameters like pH, temperature, hydrolysis time and cellulase concentration for maximum viscosity reduction during hydrolysis process.

Materials and methods

Raw material and sample preparation

Guar gum sample was of commercial food grade obtained from Hindustan Gums & Chemicals Ltd., Haryana, India. Before analysis, sample was passed through 200 mesh sieve to obtain a fine powder with uniform particle size. The sample was stored at refrigerated conditions.

Physicochemical analysis

Moisture, fat, protein, crude fiber and ash content were determined according to the methods of AOAC (1990). Gum content or galactomannan content was determined by subtracting the values of above parameters from hundred. Viscosity was analyzed using viscometer (Brookfield, USA) with spindle No. 2 at 20 rpm and 25 °C temperatures.

Experimental design

Central Composite Design (CCD) was used to study the effect of four independent variables at five levels on response pattern and to determine the optimum combination of variables. The independent variables optimized were X_1(cellulase concentration), X_2 (pH), X_3 (hydrolysis temperature), X_4 (hydrolysis time) for dependent response Y (viscosity).

The model proposed for response Y was

$$Y = b_0 + \sum_{n=1}^{4} b_n X_n + \sum_{n=1}^{4} b_{nn} X_n^2 + \sum_{n(m}^{4} b_{nm} X_n X_m \quad (1)$$

where b_0 is the value for the fixed response at the central point of the experiment; and b_n, b_m and b_{nm} are the linear, quadratic and cross-product coefficients, respectively.

Enzymatic hydrolysis

Guar gum (4 g in 396 g distilled water) was enzymatically hydrolyzed with selected 30 combinations of independent variables like cellulase concentration (0.25–1.25 mg/g), pH (3–7), temperature (20–60 °C) and time (1–5 h) as per central composite design. In central composite design the experiments were designed such that the effect of two independent variables on dependent response value was studied, while the other independent variables were taken at zero coded levels. Aqueous guar gum solutions were prepared with selected pH and cellulase concentration. Guar gum hydrolysis process was carried out with cellulase from *Aspergilus niger* enzyme at selected pH, temperature and reaction time in BOD shaking Incubator (NSW, India) at 100 rpm agitation. After selected process time for hydrolysis; viscosity of guar gum solutions was analyzed using viscometer (Brookfield, USA) with spindle No. 2 at 20 rpm and 25 °C temperatures.

Statistical analysis

The data was analyzed for analysis of variance (ANOVA) and regression models using Stat-Ease software (Design-Expert 8.0.4.1). A second-order polynomial was fitted to the data to obtain regression equations. The terms in the regression equations was examined for statistical significance (n=3).

Results and discussion

Physicochemical analysis

Physicochemical analysis of guar gum is shown in Table 1. Guar gum contained 83.64 % of galactomannan. Galactomannan is the reserve food material in endosperm of guar seeds. High galactomannan content is responsible for the viscosity property of guar gum. Present study showed that physicochemical parameters of guar gum like moisture, ash, crude fiber, protein, fat, galactomannan content and viscosity were consistent with the values reported for guar gum by other researchers (Chudzikowski 1971; Blake et al. 1997; Parvathy et al. 2007; Gupta et al. 2009b).

Enzymatic hydrolysis

Enzymatic hydrolysis of guar gum was carried out with 30 combinations of four independent variables, viz. cellulase concentration, pH, temperature and reaction time as per experimental designs presented in Table 2. Results showed that experimental viscosity value varied from 4 to 35 cps (1 cp=0.001 Pa.s). Analysis of independent and dependent

Table 1 Physicochemical quality characteristics of guar gum

Physicochemical Parameters	
Moisture (%)	8.9±0.17
Ash (%)	0.31±0.02
Crude fiber (%)	2.3±0.02
Protein (%)	4.4±0.05
Fat (%)	0.27±0.06
Galactomannan content (%)	83.64
pH	7.5±0.01
Viscosity (cps)	5900±100

[a] n=3

Table 2 Experimental design for enzymatic hydrolysis of guar gum with respective coded variables and response value (Y)

Experiments	Coded Variables[a]				Response[b] 'Y'	
	X_1	X_2	X_3	X_4	Actual	Predicted
1	1.0	1.0	−1.0	1.0	11.0	10.4
2	0.0	0.0	0.0	2.0	8.0	8.4
3	0.0	0.0	0.0	0.0	9.0	10.1
4	1.0	−1.0	−1.0	1.0	10.0	9.6
5	−1.0	1.0	−1.0	1.0	17.0	18.8
6	1.0	1.0	−1.0	−1.0	15.0	18.6
7	1.0	1.0	1.0	−1.0	11.0	10.2
8	1.0	1.0	1.0	1.0	5.0	4.5
9	−1.0	−1.0	1.0	−1.0	13.0	13.4
10	−1.0	−1.0	−1.0	−1.0	21.0	23.8
11	1.0	−1.0	−1.0	−1.0	14.0	14.4
12	−1.0	1.0	1.0	1.0	9.0	8.4
13	0.0	0.0	0.0	0.0	12.0	10.1
14	0.0	0.0	0.0	−2.0	27.0	24.4
15	0.0	0.0	0.0	0.0	11.0	10.1
16	0.0	0.0	0.0	0.0	11.0	10.1
17	1.0	−1.0	1.0	−1.0	8.0	8.5
18	−2.0	0.0	0.0	0.0	21.0	20.0
19	0.0	0.0	−2.0	0.0	25.0	23.0
20	0.0	−2.0	0.0	0.0	8.0	6.4
21	−1.0	1.0	−1.0	−1.0	35.0	32.5
22	2.0	0.0	0.0	0.0	8.0	6.7
23	0.0	2.0	0.0	0.0	14.0	13.4
24	0.0	0.0	0.0	0.0	10.0	10.1
25	−1.0	1.0	1.0	−1.0	17.0	19.6
26	0.0	0.0	2.0	0.0	7.0	6.7
27	0.0	0.0	0.0	0.0	8.0	10.1
28	1.0	−1.0	1.0	1.0	4.0	6.2
29	−1.0	−1.0	−1.0	1.0	13.0	13.5
30	−1.0	−1.0	1.0	1.0	7.0	5.6

[a] where X_1: enzyme concentration (mg/g), X_2: pH, X_3: Temperature (°C) and X_4: Hydrolysis time (h)

[b] where Y: viscosity (cps)

variables was done to obtain regression equation that could predict the response under the specified range. The regression equation obtained for viscosity Y was as follows:

$$Y = 10.17 − 3.33\,X_1 + 1.75\,X_2 − 4.08\,X_3 − 4.00\,X_4$$
$$+ 0.81\,X_1^2 − 0.063\,X_2^2 + 1.19\,X_3^2 + 1.56\,X_4^2 − 1.12\,X_1\,X_2$$
$$+ 1.13\,X_1\,X_3 + 1.38\,X_1\,X_4 − 0.63\,X_2\,X_3 − 0.88\,X_2\,X_4$$
$$+ 0.63\,X_3\,X_4$$

where X_1=Cellulase concentration, X_2=pH, X_3=Temperature and X_4=Hydrolysis time.

Regression model was used to calculate the predicted viscosity values and compared with experimental viscosity values. High value of coefficient of determination (R^2= 0.9472) shows the adequacy of the applied model. The statistical analysis data showed that linear and quadratic terms were significant while the interaction coefficients were non-significant (Table 3). The ANOVA also revealed that there was a non-significant (probability P>0.05) lack of fit that further validates the model. The conditions for maximum hydrolysis were optimized by fixing the two variables at coded zero level (Table 2) while varying the remaining two variables and predicting the response variable (viscosity value). Figure 1a shows relationship of viscosity with cellulase concentration and pH when hydrolysis time and temperature were fixed at coded zero level or 3 h and 40 °C, respectively. Changes in cellulase concentration and pH revealed that maximum viscosity reduction was obtained when cellulase concentration was at higher limit and pH was at lower limit. Decrease in pH and increase in cellulase concentration increased the viscosity reduction in guar gum. The effect of pH on viscosity was more pronounced when the cellulase concentration was at minimum level while decrease in pH did not show any significant effect on viscosity reduction at maximum level of cellulase concentration. The effect of enzyme treatment on viscosity reduction was noticed more pronounced at higher pH level than at lower pH level.

Increase in temperature and cellulase concentration showed slight decrease in viscosity as evidenced in

Table 3 Analysis of variance (ANOVA) of independent variables for optimiztion of enzymatic hydrolysis of guar gum

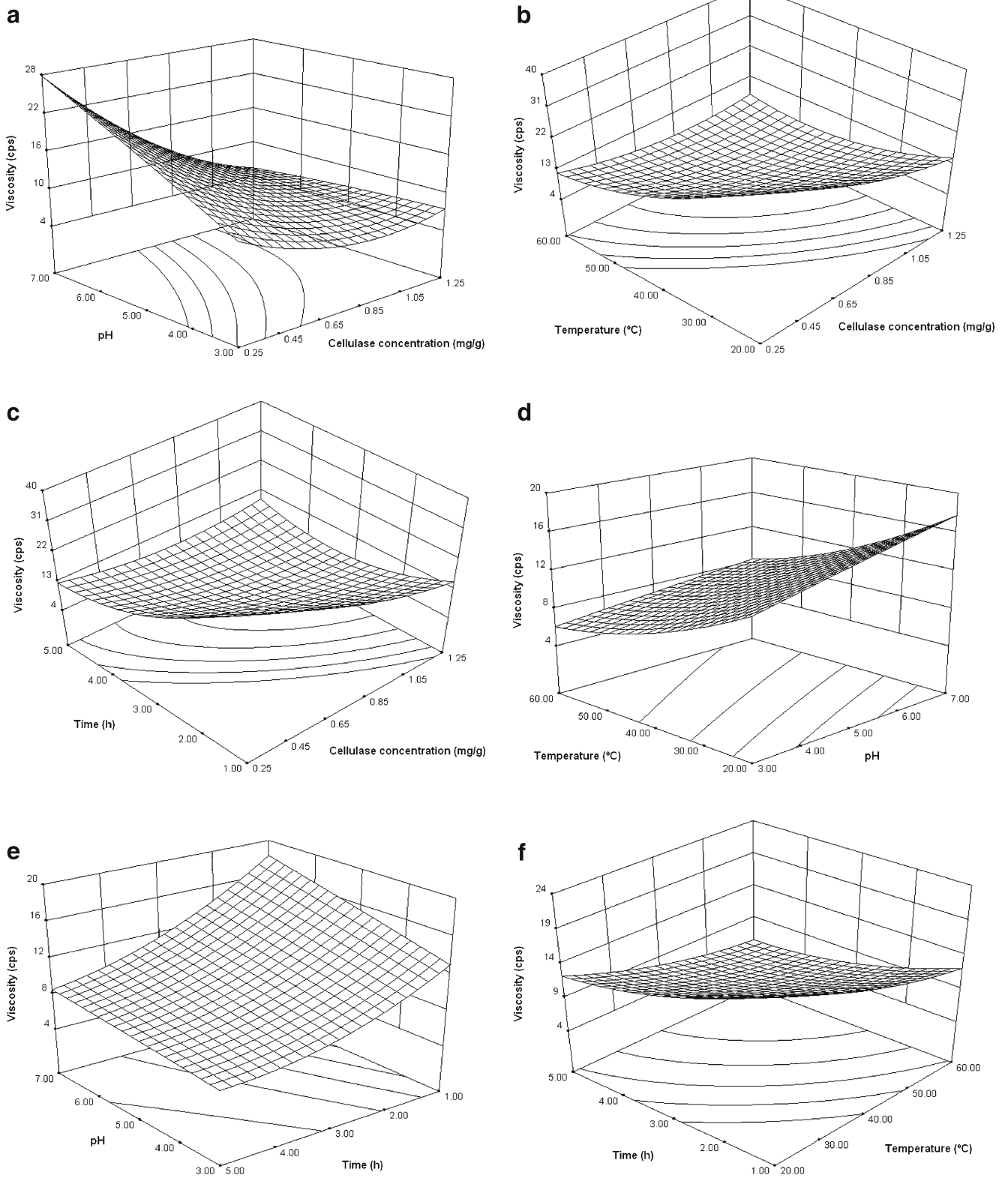| Source	Degree of freedom	F-value	Probability
Regression	14	19.2	0.000
Linear	4	57.1	0.000
Square	4	5.35	0.007
Interaction	6	3.23	0.139
Lack of fit	10	2.9	0.125
Pure error	5		
Total error	29		

Fig. 1 Effect of pH, cellulase concentration, hydrolysis time and temperature on viscosity of guar gum. (a) Hydrolysis time 3 h and hydrolysis temperature 40 °C, (b) pH 5 and hydrolysis time 3 h, (c) pH 5 and hydrolysis temperature 40 °C, (d) Cellulase concentration 0.75 mg/g and hydrolysis time 3 h, (e) Cellulase concentration of 0.75 mg/g and hydrolysis temperature 40 °C and (f) Cellulase concentration 0.75 mg/g and pH 5

Fig. 1b. Effect of temperature on viscosity reduction was more pronounced at lower cellulase level (i.e. 0.50 mg/g) than at higher cellulase concentration level (i.e. 1.0 mg/g). At lower temperature (30 °C), effect of cellulase concentration on viscosity reduction was more than at the higher temperature (50 °C). Maximum viscosity reduction was observed somewhere in the middle range of cellulase concentration and the hydrolysis time, when the pH and temperature were kept at 5 and 40 °C (Fig. 1c). Hydrolysis time was comparatively effective in viscosity reduction in guar gum whereas cellulase concentration had little effect. Effect of time on viscosity reduction was more profound at lower cellulase concentration of 0.50 mg/g.

Variation in hydrolysis temperature and pH revealed that maximum viscosity reduction was obtained when temperature was at maximum level and the pH was at minimum level while hydrolysis time and cellulase concentration were kept at 3 h and 0.75 mg/g, respectively (Fig. 1d). Effect of temperature was more pronounced in viscosity reduction of guar gum at maximum pH, while pH showed more viscosity reduction at minimum level of temperature. Maximum viscosity reduction was observed at higher hydrolysis time on lower pH value when the cellulase concentration and hydrolysis temperature were kept at 0.75 mg/g and 40 °C, respectively (Fig. 1e). The reduction in viscosity value was more sensitive in the region of pH 5–6 and hydrolysis time of 2–3 h. Changes in hydrolysis time and temperature revealed that maximum viscosity reduction was achieved when hydrolysis time and temperature values were maintained at moderate levels, while the pH and cellulase concentration were kept at 5 and 0.75 mg/g, respectively (Fig. 1f). Hydrolysis time showed greater impact on viscosity reduction at hydrolysis temperature of 30 °C, while hydrolysis temperature showed similar effect on viscosity reduction with respect to hydrolysis time of 2 h.

Studies on optimization of enzymatic hydrolysis of guar gum with cellulase using response surface methodology could not be traced; however, studies without optimization of process variables have been reported. Shobha et al. (2005) reported significant reduction in viscosity (280 cps) of guar gum on pectinase treatment. Yoon et al. (2008) reported that enzymatic treatment of guar gum with β-mannanase caused substantial reduction in viscosity (10 cps). Pullulanase treatment of guar gum also showed reduction in viscosity of guar gum but to a lesser extent (Shobha and Tharanathan 2009). All these studies are in agreement with the present study suggesting that enzymatic hydrolysis of guar gum substantially reduces the viscosity. In the present study, dramatic reduction in viscosity (4 cps) of guar gum by cellulase was noticed which is significant from the point of view of commercial utilization of guar gum as a source of soluble dietary fiber.

Confirmative Test

Confirmative test was carried out using optimum levels of independent variables (cellulase concentration 1.00 mg/g, pH 6, temperature 50 °C and hydrolysis time 4 h). The predicted value was slightly lower than the experimental value. From regression equation, 4.5 cps viscosity was predicted at optimum values of independent variables whereas experimental value of viscosity was 5 cps. Thus, the confirmative test validated the experimental results as well as regression model.

Conclusion

Enzymatic hydrolysis of guar gum was carried out using 30 selected combinations of cellulase concentration, pH, hydrolysis temperature and time (independent variables). The experimental value of viscosity (response variable) varied from 4 to 35 cps. The second-order model developed for viscosity exhibited a non-significant (probability $P>0.05$) value for lack of fit and high value for the coefficient of determination (0.9472). The surface graphs indicated that maximum viscosity reduction was obtained by treatment of guar gum with cellulase at concentration of 1.00 mg/g, pH of 6, temperature of 50 °C and hydrolysis time of 4 h. It is concluded that cellulase concentration and pH were more effective than temperature and hydrolysis time. The predicted and experimental values were in agreement with each other. The optimum conditions can be used for the preparation of enzymatically hydrolyzed guar gum.

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