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Optimization of mucilage extraction conditions from *Plantago major L.* seed using response surface methodology

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Abstract

Identification of a new source of hydrocolloids is of interest due to their important effects on the textural attributes of food products. The objective of this study was to investigate the extraction conditions of *Plantago major L.* seed mucilage using a central composite rotatable design of response surface methodology. Temperature (25–85°C), pH (3–9) and water to seed ratio (50:1-50:4) were the factors investigated. Results showed that temperature was major factor in the extraction yield, whereas water to seed ratio and pH had minor effects on the yield. The maximum and minimum yields were 18.95% (conditions: temperature= 85 °C, water to seed ratio = 31.3 and pH= 6) and 6.35% (conditions: temperature = 25 °C, water to seed ratio= 31.3 and pH= 6), respectively. The optimal conditions were obtained at the temperature of 60 °C, water to seed ratio of 48.9 and pH of 3 in which predicted value for the extraction yield was 11.84%. The rheological properties of the mucilage, extracted at the optimal conditions, were investigated as a function of concentration at three levels of 3, 4 and 5% w/v, and shear rate ranged from 14 to $300 s^{-1}$. Mucilage dispersions showed non-Newtonian shear-thinning behavior at all studied concentrations. The Power law model well described the rheological behavior of the mucilage solutions with high determination coefficients (R^2>0.99). The flow behavior index (n) varied in the range of 0.30 to 0.36. The consistency coefficient (k) was in the range 6.13-17.81 $Pa.s^n$. Overall, *Plantago major L.* seed mucilage could be attended as a new beneficial source for use as a food thickening agent.

Keywords: *Plantago major L.*, Response surface methodology, Mucilage.

Introduction

Hydrocolloids are a wide range of polysaccharides and proteins that are widely used in food processing to provide thickening and gelling aqueous solutions, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation, the controlled release of flavors, etc. They can perform a significant influence on the textural and organoleptic properties of food products at concentrations of less than 1% (Phillips and Williams, 2009). Humans have traditionally used the gum and mucilage obtained from different plants for food preparation (Koocheki *et al.*, 2009b). Starch and its derivatives, galactomannans, carrageenans, pectin, agars, alginates, gum arabic and cellulose are mostly used as hydrocolloid in food systems (Karazhiyan *et al.*, 2009). Beside these commercial hydrocolloids, new sources of gum and mucilage from different seeds such as flaxseed, white mustard, fenugreek, prosopis flexuosa, mesquite, durian, *Lallemantia royleana*, *Salvia macrosiphon* and *Gleditsia triacanthos* have been introduced by researchers in the last decades (Koocheki *et al.,* 2009b). Each of them has individual composition which possibly confers particular functional properties.

Plantago major L. (PM) is a perennial plant that belongs to the *Plantaginaceae* family. Some of its common names are Soldier's Herb, Broad-leaved Plantain, Hen Plant, Lambs Foot, Road weed and White Man's Foot. PM produces large amounts of seeds (up to 20000 per plant). The seeds are quite small with an ovate shape and a slightly bitter taste. The seed endosperm has highly thickened cellulosic walls with the cell lumen filled with oil and

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protein. It forms the major part of the seeds and surrounds the embryo completely. The seeds are located in capsules (8–16 per capsule) and become sticky in humid weather due to the swelling of the polysaccharides present mainly in the seed coat. The seeds contain up to 30% mucilage including the monosaccharides glucose, fructose, xylose and rhamnose as well as the disaccharide sucrose and the trisaccharide planteose. The outer seed coat contains polysaccharides that swell in contact with water and forms mucilage with high viscosity (Samuelsen, 2000). The leaves and the seeds possess anti-bacterial, haemostatic, anti-complementary, anti-inflammatory, anti-septic, laxative, anti-nociceptive, anti-leukaemia, anti-carcinoma, anti-viral, ophthalmic and diuretic properties, and are also used as a remedy for dysentery and diarrhea, and treatment of parasitic worms (Samuelsen *et al.*, 1999; Türel *et al.*, 2009).

Aqueous extraction is the common method for the extraction of mucilaginous components from different seeds. Previous studies have been showed that different factors may influence the extraction parameters such as yield, protein content and rheological properties of the extracted gum (Cui *et al.*, 1994; Wu *et al.*, 2007; Koocheki *et al.*, 2009b; Razavi *et al.*, 2009; Bostan *et al.*, 2010; Karazhiyan *et al.*, 2011). The most important of these factors are the ratio of water to seed, pH and temperature, which could be different for various seeds and must be determined in a laboratory. Although the effects of some extraction conditions may be predictable, most of them are not known to researchers; for example an increase in water to seed ratio might result in increase of the extraction efficiency, but the influence of some extraction factors such as pH might be complex and sometimes minor (Cui *et al.*, 1994; Wu *et al.*, 2007; Koocheki *et al.*, 2009b; Bostan *et al.*, 2010), or sometimes noteworthy (Razavi *et al.*, 2009; Karazhiyan *et al.*, 2011). For this reasons, it is necessary to evaluate different extraction conditions for each of the gum or mucilage resources and select the best conditions from the aspects of the extraction yield, impurity, energy consumption, rheological characteristics and so on.

Response surface methodology (RSM) is a useful technique for the investigation of several input variables which influence the performance and quality characteristics of the product or process under investigation. The technique provides mathematical and statistical procedures to study relationships between one or more responses (dependent variables) and a number of factors (independent variables) (Karazhiyan *et al.*, 2011). RSM has been used to study the effect of the different extraction conditions on the hydrocolloids obtained from different sources and optimize the extraction process (Cui *et al.*, 1994; Wu *et al.*, 2007; Koocheki *et al.*, 2009b & 2010; Razavi *et al.*, 2009; Bostan *et al.*, 2010; Karazhiyan *et al.*, 2011). There is no published data for optimizing the mucilage extraction from PM seeds. Therefore, the objectives of this research were 1) to investigate the effect of the extraction temperature, pH and water to seed ratio on the extraction yield of mucilage from PM seeds, 2) to find out the optimum conditions for mucilage extraction from PM seeds using RSM. Furthermore, rheological parameters of the mucilage, extracted under optimum conditions, were measured.

Materials and methods

Materials

PM seeds were prepared from the local medical plant market, Mashhad, Iran. The seeds were manually cleaned to remove all foreign matter such as dust, capsules, stones and chaffs. All chemicals used in the assay were purchased from Dr Mojallali chemical laboratories (Iran) unless otherwise noted.

Extraction procedure

PM seeds mucilage was extracted from the whole seeds using of the distilled water (25 to 85°C) at pH 3-9. The seeds dispersed in the water and the slurry was mixed over 10 minutes and extraction was carried out using a centrifugal basket extractor after 2.5 hours hydration. The extract was vacuum filtered, dried at 70^{o}C, milled and then screened to achieve the fine powder. The extraction yield was calculated as percentage of hydrocolloid powder to the seed weight.

Preparation of mucilage dispersions

The mucilage powder, extracted under optimum conditions, was slowly added to the distilled water for approximately 15 min under constant stirring rate at room temperature. Then, it was stored at the room temperature for 24 h to complete hydration prior to rheological assessment.

Rheological measurement

Apparent viscosity of mucilage dispersions were measured at constant temperature of 25^{o}C and different shear rates ranged from 14 to 300 s^{-1} at three concentration levels (3, 4, and 5% w/v) using a rotational viscometer (Visco 88, Bohlin instruments, UK) equipped with C30 measuring spindles (based on viscosity of dispersion) and a heating circulator (Julabo, Model F12- MC, Julabo Labortechnik, Germany). For each test, about 25 ml sample was transferred to sample compartment (bob and cup) following by 9, 10, 12 min pre-shearing at 50 s^{-1} to obtain uniform solution and time independent conditions for concentrations 3, 4 and 5% w/v, respectively. The shear rate was increased linearly from 14 to 300 s^{-1} in 4 min. The flow behavior index (n) and consistency index (k) values were computed by fitting the power law model (Eq. 1) using Slide Write software version 2.0.

$$\tau = k\dot{\gamma}^{n} \qquad (1)$$

Where, τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency coefficient ($Pa.s^n$) and n is the flow behavior index (dimensionless). The measurements were performed at least two replications.

Experiment design

The optimization experiments were carried out according to a central composite rotatable design (CCRD) with three variables including extraction temperature (25-85^{o}C), pH (3-9) and water to seed ratio (50:1-50:4). As presented in Table 1, the coded values of the independent variable are arranged as -1.68 (lowest level), -1, 0 (medium level), +1 and +1.68 (highest level) which have the same predictive power in all directions from the centre point with the best precision. The complete design consisted of 20 experimental points including 6 replications of the centre point and the experiment was carried out in the random order. Parameter in mucilage extraction that was measured as dependent variable was the extraction yield. The data were analyzed using the Design Expert software (version 6.0.2) to fit the following quadratic polynomial equation:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^{\,2} + \sum_{i=1}^{3} \beta_{ij} x_i x_j \qquad (2)$$

Where, Y is the dependent variable (extraction yield) and β_0 is a constant, β_i, β_{ii} and β_{ij} are regression coefficients of the model, while x_i and x_j are the code of the independent variables.

Results and discussion

Statistical analysis and modeling

The experimental data for extraction yield under different conditions are presented in Table 1. The maximum yield (18.95%) was obtained at the condition of temperature 85°C; water to seed ratio 31.3 and pH 6. The minimum yield was 6.35% that acquired at the temperature 25°C, water to seed ratio 31.3 and pH 6. The average yield of six middle points was 7.95% and standard deviation of ±0.245 that represent high reproducibility among those points. The second-order polynomial response surface model (Eq. 3) was fitted to the response variable i.e. yield (Y). For the corresponding fitting of the explanatory models and the variation of the extraction yield, the sum of squares of the sequential model was analyzed (Table 2). The value of lack-of-fit for regression equation of quadratic model was small in comparison with other model indicating that this model has a good fitness.

Regression analysis and ANOVA were used for fitting the model and to examine the statistical significance of the terms. The estimated regression coefficients of the quadratic polynomial models for the response variables, along with the corresponding coefficients of determination (R^2) are given in Table 3. After elimination of non-significant terms, final equation (Eq. 3) for response variable fitted to empirical data:

$$Y = 7.93+3.65X_1 + 0.26X_3 +1.8X_1^2 + 0.55X_2^2 +0.25X_3^2 \quad (3)$$

Where, X_1 , X_2 and X_3 are temperature, pH and water to seed ratio, respectively.

Table 1. Process variables and experimental data for the three factors at five levels of response surface design of *Plantago major L.* seed mucilage.

Run order	Actual levels			Code levels			Yield (%)
	T (°C)	pH	Water to seed ratio				
1	55	9.0	31.3	0	1.68	0	9.1
2	37	4.2	20.1	-1	-1	-1	6.73
3	73	7.8	42.4	1	1	1	14.4
4	55	6.0	31.3	0	0	0	8.021
5	73	7.8	20.1	1	1	-1	13.82
6	37	4.2	42.4	-1	-1	1	7.2
7	55	6.0	31.3	0	0	0	7.604
8	37	7.8	20.1	-1	1	-1	7.133
9	73	4.2	20.1	1	-1	-1	14.33
10	55	3.0	31.3	0	-1.68	0	10.19
11	55	6.0	31.3	0	0	0	8.229
12	55	6.0	31.3	0	0	0	8.125
13	55	6.0	50.0	0	0	1.68	8.666
14	55	6.0	31.3	0	0	0	8.021
15	85	6.0	31.3	1.68	0	0	18.95
16	55	6.0	31.3	0	0	0	7.708
17	73	4.2	42.4	1	-1	1	14.97
18	25	6.0	31.3	-1.68	0	0	6.79
19	55	6.0	12.5	0	0	-1.68	7.9
20	37	7.8	42.4	-1	1	1	7.76

Table 2. Analyze of sequential model sum of squares for the extraction yield.

Source	Sum of squares	DF	Prob > F	Lack of fit tests Prob > F
Mean	1885.35	1		
Linear	183.19	3	< 0.0001	< 0.0001
2FI	0.52	3	0.98	< 0.0001
Quadratic	48.63	3	< 0.0001	0.054
Cubic	0.10	4	0.98	0.005
Residual	1.63	6		
Total	2119.43	20		

Extraction yield

From the model of extraction yield, linear effect of extraction temperature and water to seed ratio and quadratic effect of all independent factors were significant ($P<0.05$), whereas no interaction terms were significant (Table 3). The results also showed that variables with the largest effect were the linear and quadratic terms of extraction temperature. This is because of the water temperature had great effects on the mass transfer rate of the water- soluble polysaccharides in the cell wall (Shi *et al.,* 1996). Based on the sum of squares, the importance of the independent variables on the yield could be ranked in the following order: extraction temperature > water to seed ratio > pH.

The relationship between independent and dependent variables is illustrated in three-dimensional representations of the response surface and contour plots generated by the model (Figs 1a, b; 2a, b; 3a, b).

Table 3. ANOVA and regression coefficients of the second-order polynomial model for the extraction yield.

Source	DF	Coefficient	Sum of squares	P-Value
Model	9	7.930	232.35	< 0.0001
Linear				
β_1	1	3.650	182.24	< 0.0001
β_2	1	-0.017	3.806E-003	0.88
β_3	1	0.260	0.95	0.04
Quadratic				
β_{11}	1	1.800	46.56	< 0.0001
β_{22}	1	0.550	4.42	0.0005
β_{33}	1	0.25	0.93	0.04
Interaction				
β_{12}	1	-0.260	0.52	0.11
β_{13}	1	0.015	1.891E-003	0.92
β_{23}	1	0.012	1.176E-003	0.93
Residual	10		0.17	
Pure error	5		0.059	
Total	19			
R^2		0.993		
Adj- R^2	0.985			
C.V.		4.282		

The variation of yield with extraction temperature and water to seed ratio at constant pH 6 is presented in Figs. 1a and b. As it shows, the yield increased exponentially with temperature. It can be explained by the fact that the viscosity of the seeds mucilage reduces with increasing temperature and adhesiveness of seeds might be less than low temperatures. As a result, the mucilage can be easily released from the seeds and the extraction yield raises (Koocheki *et al.,* 2009b). Similar plots were drawn for the temperature and pH at water to seed ratio of 31.3 (Figs. 2a and b). In the plots demonstrated for pH and water to seed ratio (Figs. 3a and b), extraction yield at initial and terminal pH was maximum and as the water to seed ratio increased, it enhanced exponentially. This is because more water would dissolve more mucilaginous substances of the seeds.

Effect of temperature on the extraction yield

Temperature was the major factor affecting the extraction yield of PM seed mucilage. As the temperature increased from 25°C to 85°C, the yield increased from 6.79 to 18.95%, irrespective of the changes in pH or water to seed ratio. This may be due to the fact that high temperature led to solubility increase of mucilaginous components and weakening of adhesive force between this components and seed hull. Other researchers have reported the same results for flaxseed gum, boat-fruited sterculia, wild sage, basil and *Lepidium perfoliatum* seeds (Cui *et al.*, 1994; Wu *et al.,* 2007; Koocheki *et al.,* 2009b; Razavi *et al.,* 2009; Bostan *et al.,* 2010; Karazhiyan *et al.,* 2011). Influence of temperature on the yield has not always been noteworthy at high degree. For instance, research of Sepulveda *et al.* (2007) showed that the temperature had no remarkable influence on the mucilage yield of *Opuntia ficus indica*. Also in other study, the highest yield of polysaccharides was obtained at 80 °C from *Opuntia milpa alta* seed, while using high temperature decreased the yield because of increasing the hydrolysis of polysaccharides (Cai *et al.,* 2008).

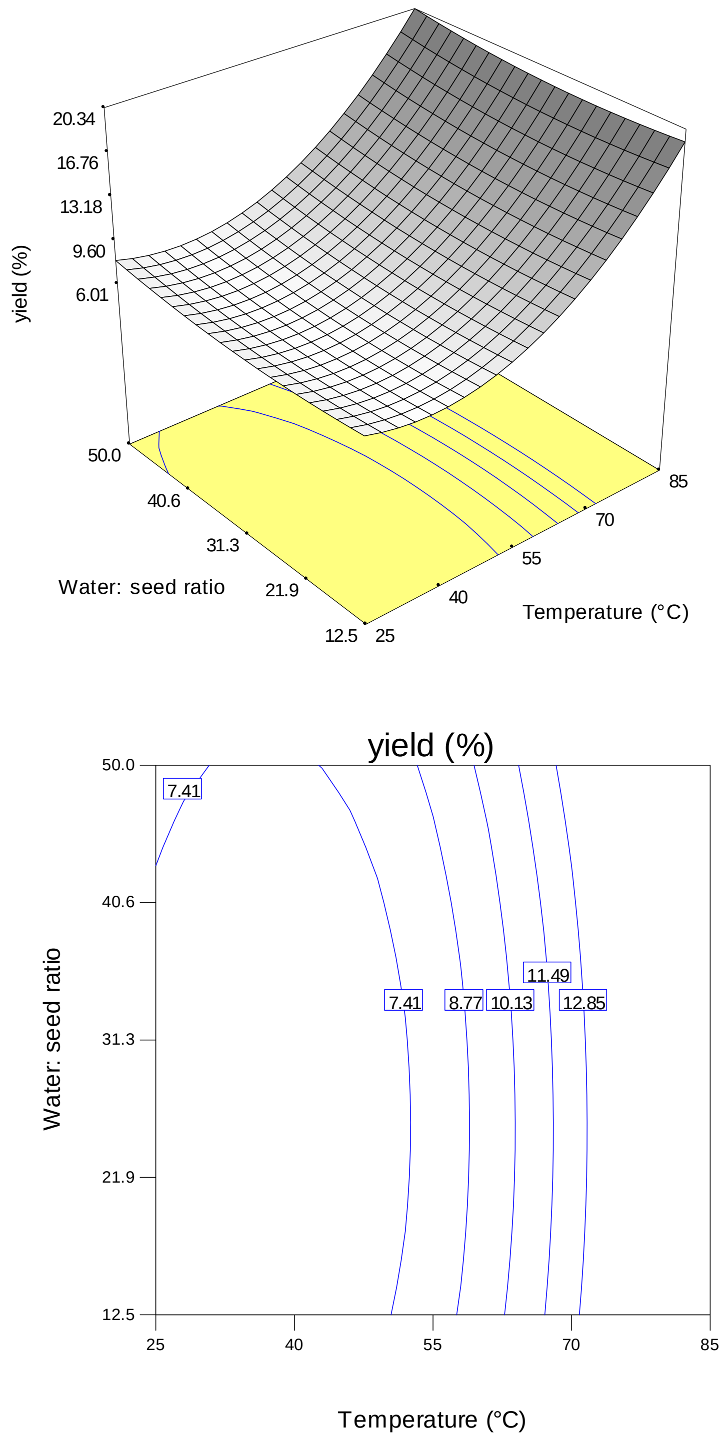

Figure 1. Response surface (a) and contour (b) plots for the effect of temperature and water to seed ratio on the yield extraction.

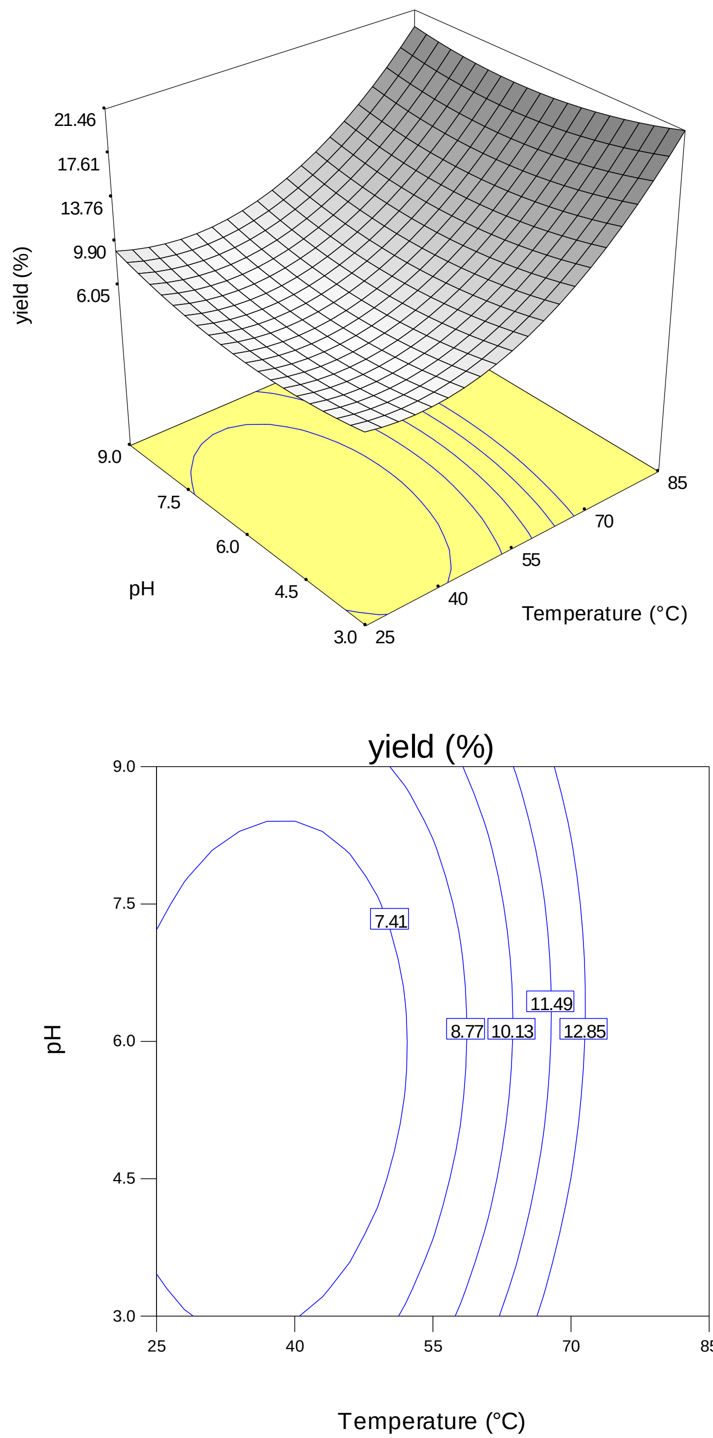

Figure 2. Response surface (a) and contour (b) plots for the effect of pH and temperature on the yield extraction.

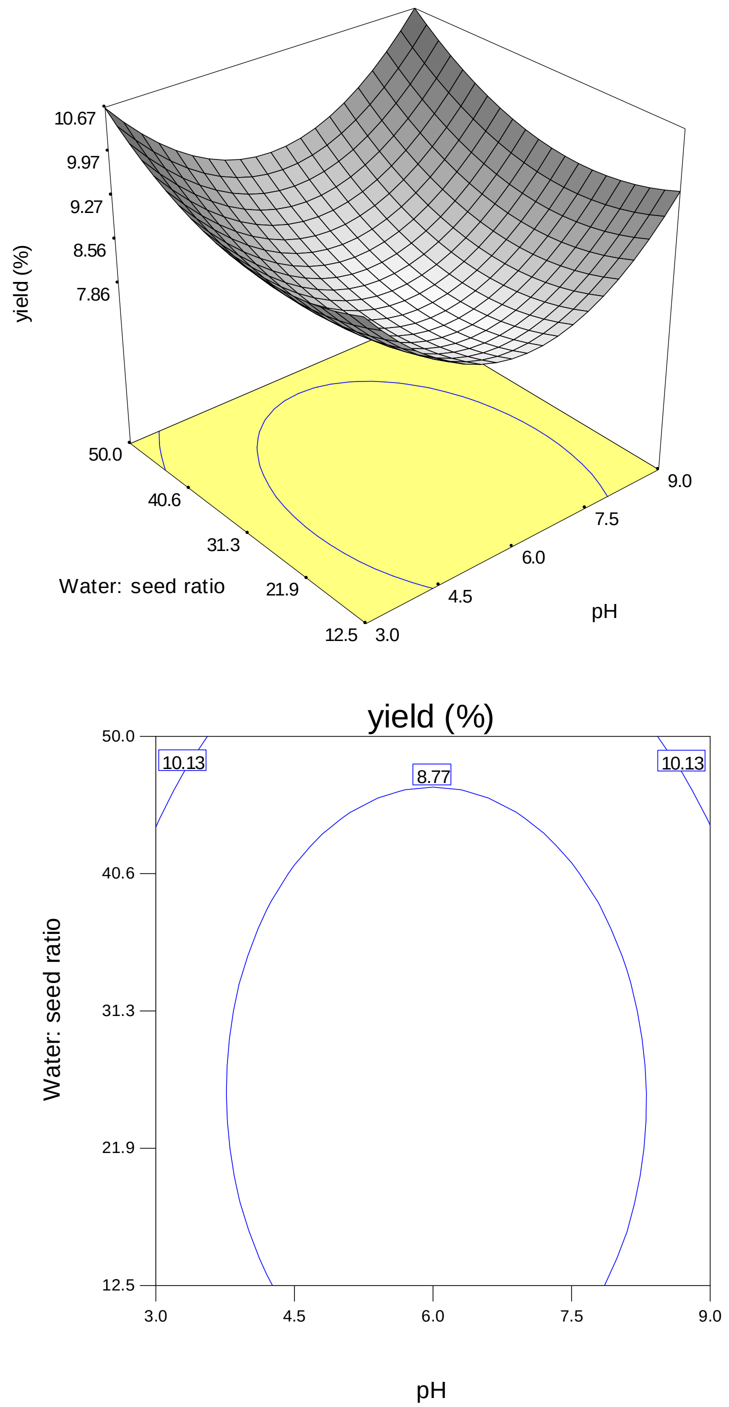

Figure 3. Response surface (a) and contour (b) plots for the effect of pH and water to seed ratio on yield extraction.

Effect of water to seed ratio on the extraction yield

Water to seed ratio had significant effect on the extraction yield of PM seed mucilage. As water to seed ratio increased, the extraction yield continuously increased too. This is due to the availability of more liquid which increases the driving force of mucilage out of the seeds

(Koocheki *et al.,* 2009b). Similar effects were reported for boat-fruited sterculia, wild sage and *Lepidium perfoliatum* seeds (Wu *et al.,* 2007; Koocheki *et al.,* 2009b; Bostan *et al.,* 2010). However, water to seed ratio showed non-significant effect on the extraction yield of flaxseed gum (Cui *et al.,* 1994). Also, Sepulveda *et al.* (2007) reported that mucilage yield of *Opuntia ficus indica* indicated a small tendency to increase when the amount of water used for the extraction was increased. Cai *et al.* (2008) observed that the yield of the extracted polysaccharides from *Opuntia milpa alta* seeds initially increased as the ratio of water to seed increased to 3-4 folds, but more increasing of this ratio caused to the yield reduction.

Effect of pH on the extraction yield

The yield of mucilage extraction from PM seed was not influenced by the pH. This result is in agreement with findings of Koocheki *et al.* (2009b) and Bostan *et al.* (2010) for *Lepidium perfoliatum* and wild sage seeds, respectively. Cui et al. (1994) reported the impact of pH on the mucilage separation is minor and only very acidic pH was relatively suitable for the extraction. The pH had significant effect on the yield of polysaccharide of boat-fruited sterculia seeds and the neutral pH provided the maximum extraction yield (Wu *et al.,* 2007).

Optimization

The optimum conditions for extraction of PM seed mucilage were determined to achieve the extract with appropriate quality and yield. For this purpose, the temperature was set in 60 °C, pH and water to seed ratio was selected in range of 3-9 and 12.5-50, respectively and the extraction yield was set on maximum. After optimization, 10 points was suggested that prime point (temperature= 60°C, pH= 3 and water to seed ratio= 48.9) with desirability of 0.66 was selected (Table 4). The predicted extraction yield under these conditions was 11.84%.

Table 4. Ten optimized conditions to achieve to the maximum extraction yield.

Number	Temperature (°C)	pH	water to seed ratio	yield (%)	Desirability
1	60	3	48.9	11.84	0.660
2	60	9	50	11.71	0.652
3	60	8.8	50	11.56	0.643
4	60	3	43.8	11.41	0.634
5	60	9	43.9	11.15	0.616
6	60	9	12.5	10.75	0.591
7	60	9	13.1	10.72	0.589
8	60	9	28.6	10.47	0.571
9	60	8.6	12.5	10.39	0.566
10	60	3.8	12.5	10.36	0.564

Rheological properties

The PM seed mucilage dispersions showed a non-Newtonian behavior and shear-thinning (Pseudoplastic) flow. When the flow behavior index is less than 1, it means that the dispersion macromolecules have oriented in network and they have aligned in the direction of the shearing force (Cancela *et al.,* 2005). The power law was suitable model to describe the flow behavior of the PM seed mucilage dispersions with high determination coefficients (R^2>0.99) for each concentration level. As the concentration of the mucilage solution increased, the consistency coefficient (k) increased, while flow behavior index values (n) decreased (Table 5). Similar results have been observed for some other hydrocolloids such as *Alyssum homolocarpum* mucilage (Koocheki *et al.,* 2009a), salep (Farhoosh and Riazi, 2007) and carboxy methylcellulose (Cancela *et al.,* 2005).

Table 5. The power law equation parameters for *Plantago major L.* seed mucilage dispersions at different concentrations*.

Parameter	3%	4%	5%
n (dimensionless)	0.36± 0.02	0.32± 0.03	0.30± 0.007
k (Pa.s^n)	6.13± 0.33	10.36± 0.54	17.81± 0.22
R^2	0.998	0.998	0.996

*The values are means of at least two replications.

Values of flow behavior index of PM seed mucilage were lower than those of the salep, cashew, starch and pectin dispersions (Mothe and Rao, 1999; Marcotte *et al.*, 2001) indicating great tendency of this mucilage to shear-thinning behavior. Whatever the mucilage solution possess high pseudoplasticity, the mouth feel characteristic will be favorite. The effect of shear rate on the apparent viscosity of the PM seed mucilage is shown in Fig. 4. It can be seen that the apparent viscosity of mucilage dispersions were shear rate dependent at all concentrations and it was maximum in low shear rates, but the apparent viscosity decreased sharply at shear rate around 50 s^{-1}. The apparent viscosity was governed of the mucilage concentration, and as the concentration increased, the apparent viscosity enhanced. This can be explained by the fact the higher solid contents generally lead to an increase in the viscosity resulting from mainly molecular movements and interfacial film formation (Maskan and Gogus, 2000).

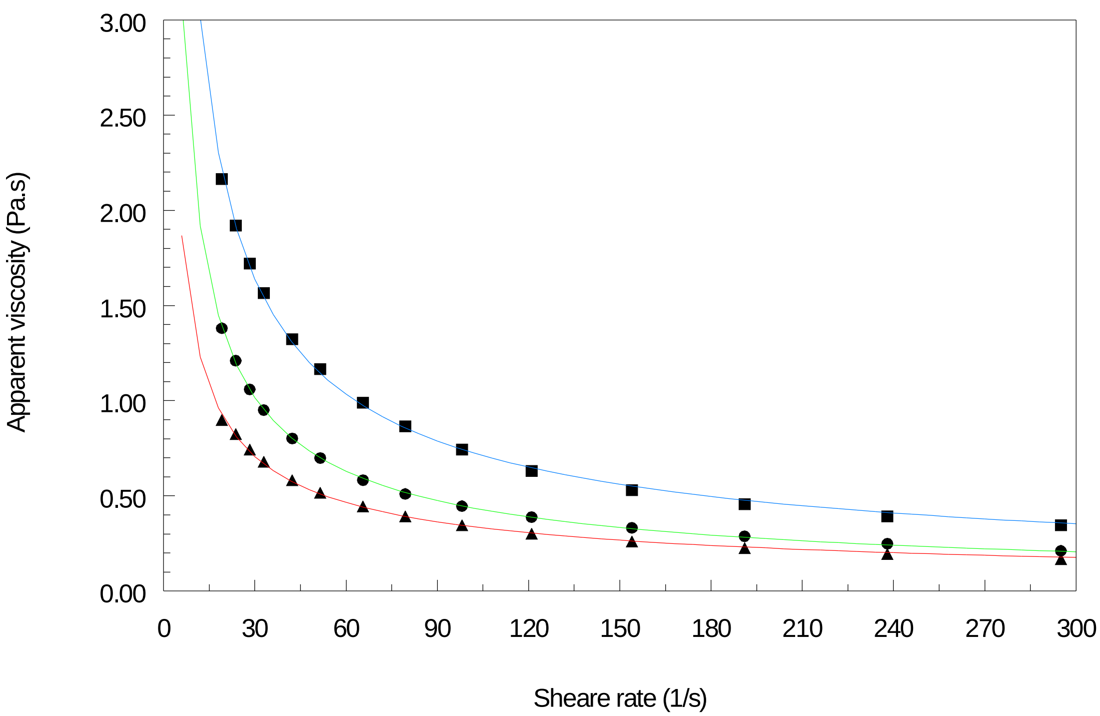

Figure 4. Apparent viscosity as a function of shear rate of *Plantago major L.* seed mucilage dispersions (▲3%, ● 4% and ■ 5%) at temperature 25°C.

Conclusion

This study investigated of the optimal conditions for the mucilage extraction from PM seeds. Results indicated the temperature was most important factor in the extraction process and as the temperature increased, the extraction yield increased. A pseudoplastic behavior observed for the mucilage dispersions at all concentrations. The power law model well described the rheological behavior of the mucilage dispersions with high determination coefficients. Increasing the solution concentration increased consistency coefficient, while the flow behavior index decreased.

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بهینه‌یابی شرایط استخراج موسیلاژ از دانه بارهنگ توسط روش سطح پاسخ

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چکیده

به دلیل نقش و جایگاه هیدروکلوئیدها در ویژگی‌های بافتی فراورده‌های غذایی، شناسایی منابع جدید آنها حائز اهمیت و مطلوب است. هدف از این پژوهش بررسی شرایط استخراج موسیلاژ از دانه بارهنگ توسط طرح مرکب مرکزی چرخش‌پذیر روش سطح پاسخ بود. درجه حرارت (25-85 °C)، pH (9- 3) و نسبت آب به دانه (50 به 1 الی 50 به 4) فاکتورهای مورد بررسی بودند. عامل اصلی تاثیرگذار بر میزان بازدهی استخراج، درجه حرارت بود، در حالی‌که نسبت آب به دانه و pH تاثیر بسیار کمی داشتند. بیشترین میزان بازدهی 95/18% در شرایط دمایی 85°C، نسبت آب به دانه 3/31 و pH برابر6 و کمترین راندمان 35/6% در دمای 25°C، نسبت آب به دانه 3/31 و pH برابر 6 به‌دست آمد. شرایط بهینه به‌دست آمده دمای 60°C، نسبت آب به دانه 9/48 و pH برابر 3 بود. در شرایط بهینه مقدار پیش‌بینی شده برای بازدهی 84/11% بود. رفتار رئولوژیکی موسیلاژ استحصال شده در شرایط بهینه در سه غلظت 3، 4 و 5 درصد وزنی- حجمی و سرعت برش بین 14 تا 300 بر ثانیه مورد بررسی قرار گرفت. محلول‌های موسیلاژی رفتار شل شوندگی با برش (غیرنیوتنی) را در تمامی غلظت‌ها از خود نشان دادند. مدل قانون توان توانست به خوبی رفتار رئولوژیکی محلول‌های موسیلاژی بارهنگ را با ضریب تبیین بالا (R^2>0/99) توصیف نماید. شاخص رفتار جریان (n) در محدوده 0/3 تا 0/36 تغییر یافت. ضریب قوام (k) نیز در محدوده $Pa.s^n$ 13/6 تا 81/17 متغیر بود. نتایج نشان داد موسیلاژ به‌دست آمده از دانه بارهنگ می‌تواند به‌عنوان قوام‌دهنده در غذاها مورد استفاده قرار گیرد.

واژه‌های کلیدی: بارهنگ، روش سطح پاسخ، موسیلاژ.

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