

Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from *Lepidium perfoliatum* seeds

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ABSTRACT

Response surface methodology was used to determine the optimum processing conditions that give maximum extraction yield, viscosity, hue and emulsion stability, as well as, minimum protein content for the gum extracted from *Lepidium perfoliatum* seed. Temperature (45–75 °C), processing time (1.5–3.5h), pH (5–8) and water to seed ratio (30:1–60:1) were the factors investigated. Experiments were designed according to Central Composite Rotatable Design with these four factors, including central and axial points. For each response, a second-order polynomial model was developed using multiple linear regression analysis. Applying desirability function method, optimum operating conditions were found to be extraction temperature of 48.1 °C, pH of 8, water to seed ratio of 30:1 and process time of 1.5 h. At this optimum point, extraction yield, viscosity, protein content, hue and emulsion stability were found to be 17.36%, 463.07 mPa s, 2.84%, 60.47 and 88.96 %, respectively.

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1. Introduction

Hydrocolloids obtained from different sources (plant, epiphyte and animal extracts) are widely used in the food systems for various purposes, such as thickeners, stabilizers, gelling agents and texture modifiers. Although they are not true emulsifiers, hydrocolloids may be considered as stabilizers because they improve the long-term stability of food systems. Hydrocolloids from plants have also the advantage over those from animals due to more acceptability by consumer.

Plants seeds are a traditional and ancient source of gums. Most seeds contain starches as the principal reserve food stored for use by the embryonic plant, but many contain other polysaccharide polymers with certain functional properties as a useful source of commercial hydrocolloids. For instance, gums from seeds such as locust bean and guar (Glicksman, 1982), Flaxseed (Cui, Mazza, Oomah, & Billiaderis, 1994), white mustard (Balke & Diosady, 2000), Fenugreek (Brummer, Cui & Wang, 2003), *Prosopis flexuosa* (Ibanez & Ferrero, 2003), Mesquite (Estevez et al., 2004), Durian (Amin, Shamsuddin Ahmad, Yinyin, Yahya, & Ibrahim, 2007), *Lallemantia royleana* (Mohammad Amini, Haddad Khodaparast, & Farhoosh, 2007), *Salvia macrosiphon* (Bostan, Razavi, & Farhoosh, 2008) and *Gleditsia triacanthos* (Sciarini, Malsonado, Ribotta, Perez, & Leon,

2008) are important food additives and the characteristics of these hydrocolloids have been previously studied.

There are many other sources of hydrocolloids which have not been reported in the previous studies namely Qodume shahri. Two types of locally called Qodumes exist in Iran, Qodume shirazi (*Alyssum homolocarpum*) and Qodume shahri (*Lepidium perfoliatum*). The seeds of these plants have been used for hundreds of years in traditional Iranian medicinal prescriptions because of their pharmacological effects. Qodume shirazi (*A. homolocarpum*) seeds are known to contain a large amount of mucilaginous substance (Koocheki et al., 2008). Because of the shear thinning nature of its dispersions, *A. homolocarpum* seed gum could be suitable for application as a thickening and stabilizing agent (Koocheki, Mortazavi, Shahidi, Razavi, & Taherian, 2009; Koocheki, Kadkhodaei, Mortazavi, Shahidi, & Taherian, in press).

Any of 230 species of herbs constituting the genus *Lepidium*, of the Cruciferae family are distributed throughout the world and it is native to Egypt, Arabia, Iraq, Iran and Pakistan. Many, such as *L. perfoliatum*, are lawn and field weeds, but some are useful salad plants. Most species have long taproots, broad basal leaves differing from the narrow leaves on the flowering stalks, and spikelike arrangements of small, greenish or whitish, four-petalled flowers. Seeds are in a flat, round, dry fruit and each pod has 2 seeds. Seed is 2 mm long, ovate-oblong, reddish brown and narrowly winged all around. In traditional medicine, mucilage extracts from *L. perfoliatum* seeds are widely employed for the treatment of dry coughs, whooping cough, lung infections and demulcent (Amin, 2005).

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The most common method for the extraction of the seed mucilaginous material is aqueous extraction (Amin et al., 2007; Brummer, Cui, & Wang, 2003; Ibanez & Ferrero, 2003; Koocheki et al., 2008; Sepulveda, Sanz, Aliaga, & Aceituno, 2007). Conventional hot-water treatment has been used for extraction of polysaccharides which is time–temperature dependent. Different amounts of yield and rheological properties have been reported for various seeds extracted by varying conditions or cultivar of the seeds (Cui et al., 1994; Wu, Cui, Tang, & Gu, 2007). It is important, therefore, to optimize the extraction process in order to obtain the highest yield and quality polysaccharides. Our preliminary trials indicated that extraction time, temperature, pH, and water to seed ratio could have considerable influence on the properties of the crude polysaccharides.

In the extraction processes, where there are multiple independent variables affecting the responding factors, it is likely to use an optimization method that can determine all the factors. In addition, the possibility of interactions between the independent variables should be considered in order to determine the optimal experimental conditions (Cui et al., 1994).

Response surface methodology (RSM) has been reported to be an effective tool for optimization of a process when the independent variables have a combined effect on the desired response. RSM is a collection of statistical and mathematical system that has been successfully used for developing, improving and optimizing such processes (Bostan et al., 2008; Cui et al., 1994; Koocheki et al., 2008; Myers & Montgomery, 1995; Wu et al., 2007).

While the demands for new sources of hydrocolloids have been increased (Williams & Phillips, 2000a), the volume share of these ingredients depends on the security of their supply, quality and the price. Consequently, there could be a substantial market for *L. perfoliatum* seed gum to substitute a number of these hydrocolloids for a better price and suitable functionality.

Even though Qodume shahri seed produces a desirable amount of mucilaginous substances when it is wetted, but no study has been conducted on its extraction process. Therefore, the objectives of the present work are 1) to study the effect of extraction time, temperature, pH and water to seed ratio on the extraction yield, protein content, emulsion stabilization capacity, color and viscosity of mucilage from Qodume shahri seeds, and 2) to find out the optimum conditions for mucilage extraction from *L. perfoliatum* seeds using RSM. We anticipate that the results of this study could lead us to find the new source of hydrocolloid with novel functionalities.

2. Materials and methods

2.1. Materials

The Qodume shahri seeds were purchased from the local medical plant market, Mashhad, Iran. The seeds were manually cleaned to remove all foreign matter such as dust, dirt, stones, chaff, immature and broken seeds. All chemicals used were analytical grades unless otherwise specified.

2.2. Extraction procedure

Qodume shahri seed gum was prepared according to the revised method of our previous work (Koocheki et al., 2008). In brief, Qodume shahri seed gum was extracted from whole seeds using deionized water (Milli-Q, Millipore, Bedford, USA) at a water to seed ratio of 30:1–60:1 and pHs of 5–8. The pH was monitored continuously and adjusted by addition of 0.1 mol/L NaOH and/or HCl. The water bath temperature was adjusted for selected range of extraction from 45 ± 1.0 °C to 75 ± 1.0 °C. Water was preheated to the desired temperature before the seeds were added. The seed–water slurry was

stirred with an electric mixing paddle throughout the entire extraction period (1.5–3.5 h). The seeds were discarded and ultimately, the dispersion was dried in a conventional oven (overnight at 45 °C), milled and sieved using a mesh 18 sifter (Sciarini et al., 2008).

2.3. Analysis of samples

The yield of crude gum was computed as the percentage weight of powder gum over the total seeds weight. Crude protein content was measured using Kjeldahl method and considering 6.25 as the conversion rate of nitrogen to crude protein (Kjeldahl, 1883).

2.4. Viscosity measurement

The viscosity was measured using a rotational viscometer (Bohlin Model Visco 88, Bohlin Instruments, U.K.) equipped with a heating circulator (Julabo, Model F12-MC, Julabo Labortechnik, Germany). According to the viscosity of dispersions, appropriate measuring bob and cup (C30) was used for viscosity measurements. Hydrated samples (1% w/v) were loaded into the cup and allowed to equilibrate for 10 min at 25 °C. The samples were subjected to the specified shear at 46.16/s which was selected based on the perceived mouthfeel thickness of normal fluids (Baines & Morris, 1988).

2.5. Color measurement

Colors were measured using computer vision system. The system comprised of a digital camera (Canon A550, Kuala Lumpur, Malaysia), image-capturing box and image analysis software (Clemex Vision Professional, PE4, Longueuil, Canada). A sample holder was placed at the bottom of the box and the digital camera was fixed 25 cm above the sample. Lighting system consisted of two fluorescent lamps (Farhad lightening 10W, 0.09A, Mashhad, Iran) which were turned on for 10 min before image-capturing.

Identical volume of gum solutions (1% w/v) were poured into a small glassy plate placed on the sample holder which was covered with a white translucent background. Inspection was carried out with capturing and processing a single image of each solution. Threshold was set to distinguish gum solution from the background and plate which was selected from the gray transformation section of the software toolbox. The cut-off value was adaptively decided based on image scene. Using binary operation section of the software toolbox, chord size was employed to eliminate spots and unwanted objects from the picture.

Since the hue (H), saturation (S) and intensity (I) color space was less affected by illumination, the color features of the samples were extracted in HSI; the software offered possibility of extraction these values on object measurement section and only the hue value was then used to evaluate the effect of extraction conditions.

2.6. Stabilizing effect

For measuring the stabilizing effect of extracted mucilage, first a 0.25% dispersion was prepared using deionized water at 80 °C with continuous mixing for 30 min. The dispersion was then cooled to room temperature and stored overnight at 4 °C (to ensure the complete hydration) prior to use for emulsion preparation. Oil-in-water emulsions (O:W, 20:80 w/w) were prepared by adding 20 g corn oil (obtained from local market) into hydrated gum while mixing with the aid of a mechanical stirrer. After 3 min mixing, the crude emulsion was homogenized using Ultra-Turrax T-25 homogenizer (IKA Instruments, Germany) at 9500 revolution/min for 1 min at room temperature. Emulsions were then kept in a 80 °C water bath for 30 min following by centrifugation at 1200g for

10 min. Emulsion stability (ES) was evaluated based on following Equation (Sciarini et al., 2008):

$$ES = \left(\frac{f_{ev}}{i_{ev}} \right) \times 100 \quad (1)$$

where f_{ev} is the final emulsion volume and i_{ev} is initial emulsion volume.

2.7. Experimental design and statistical analysis

Response surface methodology (RSM) was used to estimate the effect of independent variables (extraction temperature, x_1 ; pH, x_2 ; time, x_3 and water to seed ratio, x_4) on the extraction yield (%), viscosity (mPas), protein content (%), color (hue) and emulsion stability (%). A Central Composite Rotatable Design was employed for designing the experimental data.

The RSM was applied to the experimental data using a commercial statistical package, Design-Expert version 6.01 (Statease Inc., Minneapolis, USA). Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design included star points, and six centre points to calculate the repeatability of the method (Montgomery, 2001). The response functions (Y) were extraction yield, viscosity, protein content, hue and emulsion stability. These values were related to the coded variables (x_i , $i = 1, 2, 3$ and 4) by a second order polynomial using below Equation:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 + \varepsilon \quad (2)$$

The coefficients of the polynomial model were represented by b_0 (constant term), b_1 , b_2 , b_3 and b_4 (linear effects), b_{11} , b_{22} , b_{33} and b_{44} (quadratic effects), and b_{12} , b_{13} , b_{14} , b_{23} , b_{24} and b_{34} (interaction effects). Statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of model was checked accounting for R^2 and adjusted- R^2 .

Numerical and graphical optimization technique of the Design-Expert software was used for simultaneous optimization of the multiple responses. The desired goals for each variables and response were chosen. All the independents variables were kept within range while the responses were either maximized or minimized.

3. Results and discussion

3.1. Model fitting

The second-order polynomial response surface model (Eq. (2)) was fitted to each of the response variables (Y). For the corresponding fitting of the explanatory models and the variation of the

extraction yield, viscosity, protein content, hue and emulsion stability, the sum of squares of the sequential model was analyzed. These analyses indicated that adding terms up to quadratic significantly improved the model (Table 1) and, therefore, could be the most appropriate model for the five response variables.

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 2. In addition, adj- R^2 and coefficient of variation (CV) were calculated to check the model adequacy.

The lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error (Montgomery, 2001). If there is a significant lack of fit which could be indicated by a low probability value, the response predictor is discarded. The lack of fit illustrated in Table 2, did not result in a significant p -value for selected variables, meaning that these models were sufficiently accurate for predicting the relevant responses.

Coefficient of determination, R^2 , is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for a good fitted model, R^2 should not be less than 80%. When R^2 approaches to the unity, signifies the suitability of fitting empirical model to the actual data. The lower value of R^2 shows the inappropriateness of the model to explain the relation between variables (Little & Hills, 1978; Mendenhall, 1975).

Our results showed that the R^2 values for these response variables were higher than 0.80, indicating the regression models were suitable to explain the behavior. The R^2 values for viscosity, protein, extraction yield, hue and emulsion stability were found to be 0.99, 0.94, 0.99, 0.98 and 0.96, respectively.

It should be noted that adding a variable to the model will always increase R^2 , regardless of whether the additional variable is statistically significant or not. Thus, a large value of R^2 does not always imply the adequacy of the model. For this reason, it is more appropriate to use an adj- R^2 of over 90% to evaluate the model adequacy. With the exception of protein content (adj- $R^2 = 0.88$), the adj- R^2 values were found to be higher than 0.93 for all the responses. Higher adj- R^2 indicated that non significant terms have not been included in the model.

Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. As a general rule, the coefficient of variation (CV) should not be greater than 10%. Daniel (1991) reported that a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model. Our results showed that the coefficients of variation were less than 10% for all the responses (Table 2), representing a better precision and reliability of the conducted experiments.

Fig. 1 shows that the polynomial regression model was in good agreement with the experimental results. In this figure, each of the observed values is compared to the predicted value calculated from

Table 1

Sequential model sum of squares or apparent viscosity, extraction yield, protein, hue and emulsion stability (ES).

Source	DF	Apparent viscosity		Yield		Protein		Hue		ES	
		Sum of squares	Pr > F	Sum of squares	Pr > F	Sum of squares	Pr > F	Sum of squares	Pr > F	Sum of squares	Pr > F
Mean	1	1,275,553.2		16,038.34		419.48		98,056.84		120,840.53	
Linear	4	551,625	<0.0001	418.28	<0.0001	7.00	<0.0001	95.83	0.0097	4588.83	<0.0001
Interaction	6	17,872.75	0.8374	5.63	0.995	0.24	0.6681	69.67	0.0297	207.88	0.8167
Quadratic	4	106,916.17	<0.0001	167.94	<0.0001	0.60	0.0161	67.48	<0.0001	1140.68	<0.0001
Cubic	8	125,306.38	0.5375	1.76	0.196	0.37	0.1583	2.48	0.6352	142.17	0.3724
Residual	7	531.5		0.79		0.15		2.78		95.92	
Total	20	1,971,382		16,632.75		427.85		98,295.08		127,016	

Table 2
ANOVA and regression coefficients of the second-order polynomial model for the response variables (actual values).

Source	DF	Apparent viscosity (mPa s)			Protein (%)			Extraction yield (%)			Hue			ES (%)		
		Coefficient	Sum of squares	p-Value	Coefficient	Sum of squares	p-Value	Coefficient	Sum of squares	p-Value	Coefficient	Sum of squares	p-Value	Coefficient	Sum of squares	p-Value
Model	14	2959.13	694,804.13	<0.0001	-4.89	7.84	<0.0001	-58.99	591.86	<0.0001	8.13	232.98	<0.0001	307.50	5937.38	<0.0001
<i>Linear</i>																
b_1	1	-45.20	461,482.67	<0.0001	0.13	4.18	<0.0001	1.41	341.79	<0.0001	0.98	51.63	<0.0001	-2.97	3528.38	<0.0001
b_2	1	-65.07	2480.67	0.0064	0.45	0.98	<0.0001	2.21	0.18	0.3265	3.57	3.35	0.0075	-10.77	70.04	0.053
b_3	1	-233.67	48,780.17	<0.0001	0.54	0.58	0.001	8.41	20.96	<0.0001	10.20	40.56	<0.0001	-27.26	477.04	<0.0001
b_4	1	-27.50	38,881.5	<0.0001	0.08	1.26	<0.0001	0.68	55.36	<0.0001	0.05	0.29	0.374	-2.78	513.38	<0.0001
<i>Quadratic</i>																
b_{11}	1	0.25	87,043.05	<0.0001	-0.001	0.37	0.0054	-0.01	127.32	<0.0001	-0.01	57.11	<0.0001	0.02	306.67	0.0005
b_{22}	1	5.65	4429.76	0.0276	-0.02	0.06	0.2062	-0.17	3.85	0.0003	0.07	0.74	0.1663	0.93	120.24	0.0148
b_{33}	1	7.96	1737.19	0.0004	-0.07	0.12	0.0845	-0.91	22.56	<0.0001	0.14	0.52	0.2403	0.97	25.74	0.222
b_{44}	1	0.20	54,825.19	0.001	-0.0004	0.26	0.0157	-0.01	54.68	<0.0001	0.001	1.66	0.0462	0.03	936.67	<0.0001
<i>Interaction</i>																
b_{12}	1	-0.08	49	0.4103	-0.004	0.15	0.0541	0.01	0.74	0.0552	-0.3	6.25	0.0007	-0.003	0.063	0.9508
b_{13}	1	1.68	10,100.25	<0.0001	-0.0002	0.0001	0.958	-0.02	1.13	0.0211	-0.09	26.47	<0.0001	0.2	150.06	0.0077
b_{14}	1	0.05	1980.25	<0.0001	-0.0003	0.076	0.1612	-0.001	0.25	0.2465	0.002	2.99	0.0106	-0.003	5.06	0.5806
b_{23}	1	-1.17	49	0.4103	-0.002	0.0001	0.958	-0.31	3.45	0.0004	-0.8	23.28	<0.0001	0.04	0.063	0.9508
b_{24}	1	0.13	144	0.1671	-0.001	0.011	0.582	0.002	0.039	0.639	-0.02	4.8	0.0022	-0.003	0.063	0.9508
b_{34}	1	1.24	5550.25	<0.0001	-0.001	0.002	0.8127	0.003	0.032	0.6731	-0.04	5.88	0.001	0.12	52.56	0.0888
Residual	15		1024.67			0.52			2.05			5.27			238.08	
Lack of fit	10		803.83	0.2639		0.47	0.062		2.05	0.2211		4.21	0.2311		144.75	0.6586
Pure error	5		220.83			0.055			0.5			1.06			93.33	
Total	29		695,828.8			8.37			594.41			238.24			6175	
R^2		0.989			0.938			0.996			0.978			0.961		
Adj- R^2		0.979			0.879			0.992			0.957			0.926		
CV		3.41			4.99			1.78			1.04			6.28		

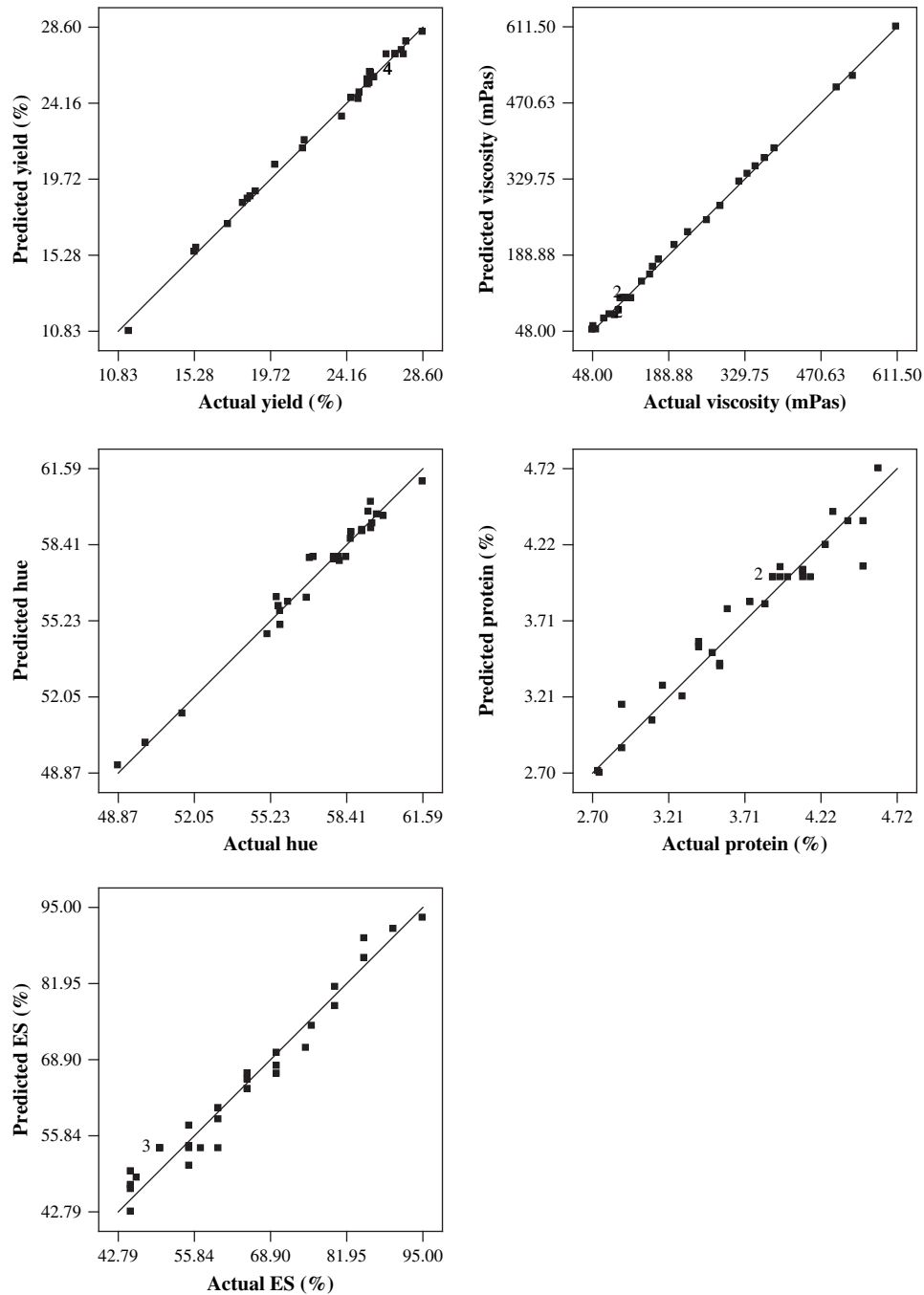


Fig. 1. Comparison between predicted and actual values of apparent viscosity, yield, protein, hue and emulsion stability of *L. perfoliatum* seed gum.

the model. The result suggests that the models used in this research were able to identify operating conditions for selective extraction of mucilage from *L. perfoliatum* seed.

3.2. Extraction yield

The p -values were used as a tool to check the significance of every coefficient. This value is necessary to understand the pattern of mutual interactions between the test variables. The smaller the magnitude of the p , the more significant is the corresponding coefficient. Values of p less than 0.05 indicate model terms are significant.

From the model of extraction yield, linear and quadratic effect of extraction temperature, time and water to seed ratio were

significant ($p < 0.0001$). For pH, although the linear effect of extraction pH was not significant but the coefficient of quadratic effects was significant (Table 2). Based on the sum of squares, the importance of the independent variables on yield could be ranked in the following order: extraction temperature > water to seed ratio > extraction time. It can be seen that among the interaction terms, extraction time with pH and extraction temperature with time were significant ($p < 0.001$, $p < 0.05$). The results also showed that variables with the largest effect were the linear and quadratic term of extraction temperature followed by linear and quadratic effect of water to seed ratio.

The relationship between independent and dependent variables is illustrated in three-dimensional representations of the response

surface generated by the model. The response surfaces were based on the coefficients presented in Table 2. The data were generated through keeping two variables at their respective zero level (centre value of the testing ranges) and varying the other two within the experimental range. The maximum predicted value is shown by the surface confined in smallest ellipse in the contour diagram. In general, exploration of the response surfaces indicated a complex interaction between the variables.

The variation of yield with extraction temperature and time at constant pH (6.5) and water to seed ratio (45:1) is presented in Fig. 2a. As it shows, the yield increased exponentially with temperature and time of treatment. At higher temperatures, the viscosity of mucilage, linked to the seeds, decreases and makes the seeds less sticky. As a result, the mucilage can be easily released and the extraction yield increases (Koocheki et al., 2008). The effect of time on yield was more pronounced at lower temperatures. At 75 °C after

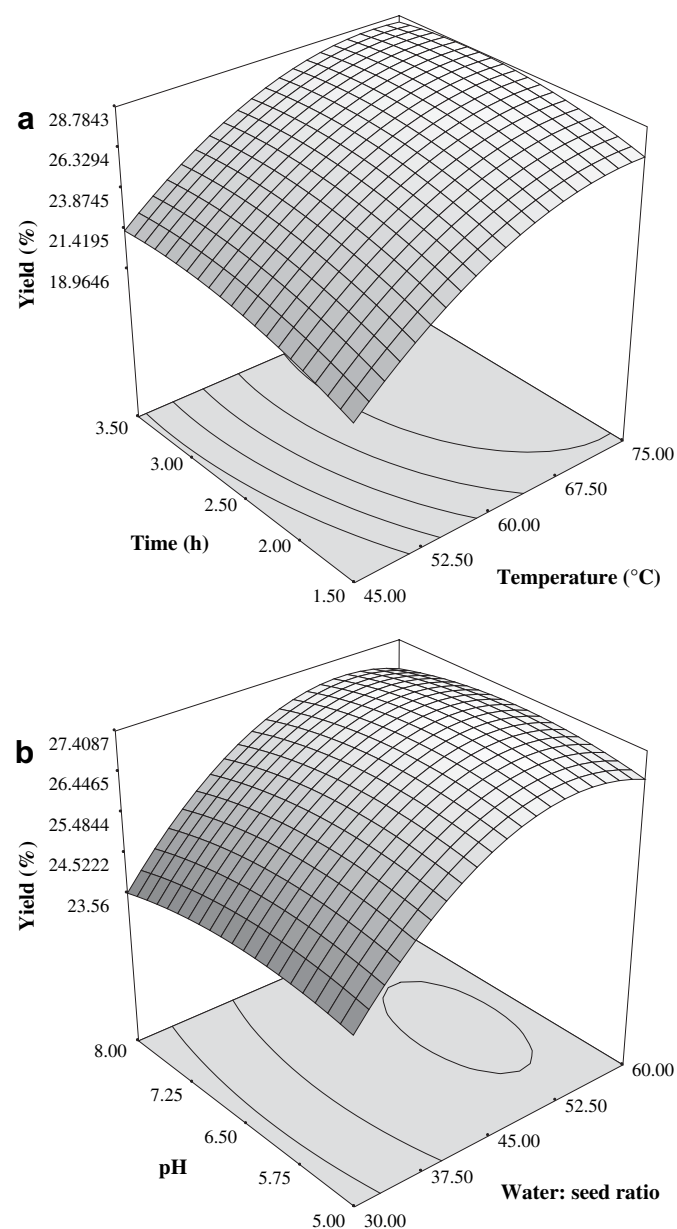


Fig. 2. Response surface for the effect of extraction temperature and time (a, water to seed ratio = 45:1 and pH = 6.5), and water to seed ratio and pH (b, temperature = 60 °C and time = 2.5 h) on the extraction yield of Qodume shahri seed gum.

3 h, yield reached nearly the equilibrium toward the time and extending the time did not have much effect on mucilage extraction.

Similar trend has been reported for flaxseed gum (Cui et al., 1994), Krueo Ma Noy pectin (Singthong, Ningsanond, Cui, & Goff, 2005), boat-fruited sterculia seeds polysaccharide (Wu et al., 2007), *Opuntia* spp. mucilage (Sepulveda et al., 2007), *A. homolocarpum* seed gum (Koocheki et al., 2008) and Yanang leaves gum extraction (Singthong, Ningsanond, & Cui, 2008).

It has also been cited that increase in polysaccharide yield is due to the strong effect of extraction time–temperature on the mass transfer rate of the water soluble polysaccharides in the cell wall (Shi, Chang, Schwarz, Wiesenborn, & Shih, 1996; Wu et al., 2007).

However, when extraction temperature and time were kept constant (60 °C and 2.5 h), increase in water to seed ratio was the reason for an exponential increase in the yield (Fig. 2b). This is due to the availability of more liquid which increases the driving force of mucilage out of the seeds.

Koocheki et al. (2008) and Sepulveda et al. (2007) also reported that when the volume ratio of water to seeds was increased, a greater mucilage yield obtained from *A. homolocarpum* and *Opuntia* spp. seeds. Conversely, Singthong et al. (2008) stated a higher extraction yield for Yanang leaves gum at a low ratio of solid to water.

Effect of pH on extraction yield was minor and in agreement with reported studies by Cui et al. (1994), Wu et al. (2007) and Koocheki et al. (2008) for flaxseed, boat-fruited sterculia seeds and Qodume Shirazi seeds gum. It is, however, contrary to the results found by Balke and Diosady (2000), Estevez et al. (2004) and Somboonpanyakul, Wang, Cui, Barbut, and Jantawat (2006) who reported that extraction in alkaline solution provided the highest yield. Based on our study, the optimal conditions for maximum extraction of 29.04% mucilage are given by software as: extraction temperature = 70.78 °C, pH = 5.8, time = 2.9 (h) and water to seed ratio = 49.9 to 1.

Comparing the extraction yield of Qodume shahri to the other gum indicated the higher value of extraction when using the optimal conditions. For instance, maximum extraction yield for different seeds gum which have been reported in previous studies are: Yanang gum 4.54% (Singthong et al., 2008), Flaxseed gum 7.9% (Cui et al., 1994), Malva nut gum 20% (Somboonpanyakul et al., 2006), *Durio zibethinus* seed gum 1.2% (Amin et al., 2007), *Opuntia* mucilage 19.4% (Sepulveda et al., 2007), Mesquite seed gum 24.9% (Estevez et al., 2004) and Fenugreek gum 22% (Brummer et al., 2003).

Therefore, from economical point of view, Qodume shahri seeds have the potential of becoming an important commercial gum as a novel source of food hydrocolloid.

3.3. Viscosity

The results in Table 2 indicated that all linear and quadratic parameters were highly significant ($p < 0.0001$, $p < 0.01$ or $p < 0.05$) for all extracted mucilage. The mutual interaction between temperature and time, temperature and water to seed ratio and time and water to seed ratio were also found to be significant ($p < 0.0001$). The variables with the largest effect were the linear and quadratic terms of temperature followed by the linear terms of time and water to seed ratio.

Fig. 3a shows the interaction between extraction temperature and time. From the plot it can be seen that when extraction temperature (°C) was increased from 45 °C to 75 °C the viscosity decreased in a parabolic manner. In addition, the extraction time also demonstrated a linear decrease for viscosity of the mucilage. This effect, according to Garcia-Ochoa and Casas (1992), is due to the interactions of the molecules in solution which become weaker at higher temperature. The reduction of gum viscosity with

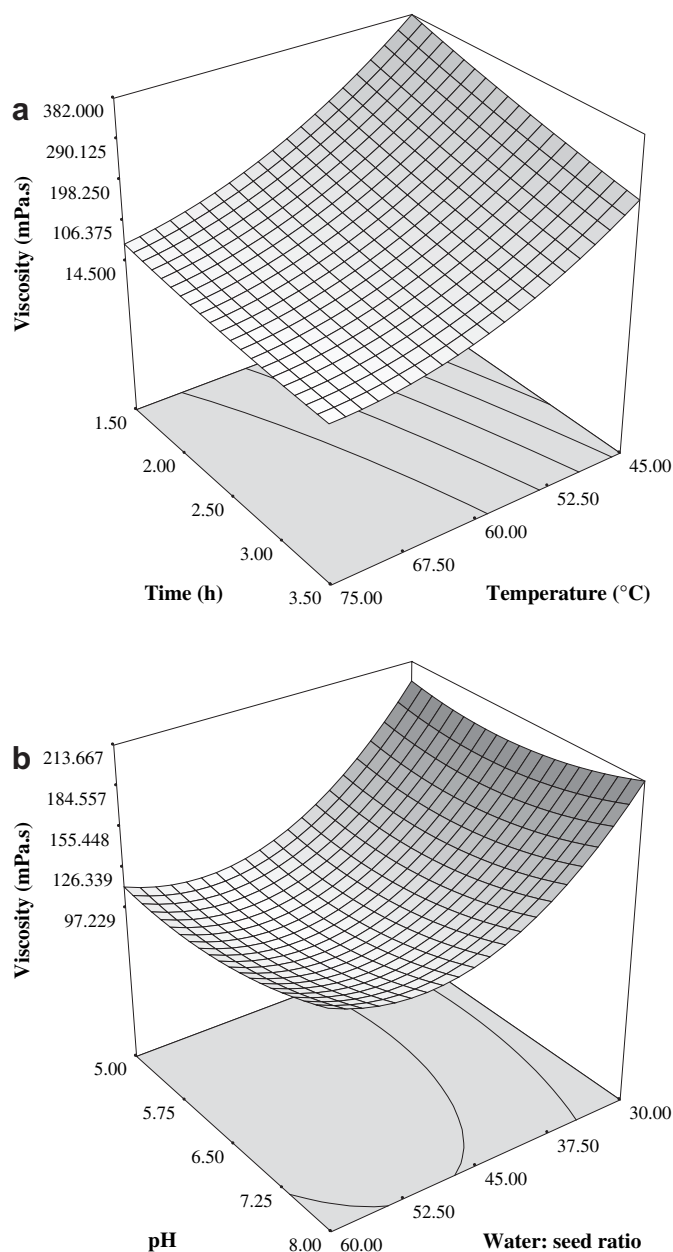


Fig. 3. Response surface for the effect of extraction temperature and time (a, water to seed ratio = 45:1 and pH = 6.5), and water to seed ratio and pH (b, temperature = 60 °C and time = 2.5 h) on the apparent viscosity of Qodume shahri seed gum (apparent viscosity was determined at 25 °C, pH: 7, gum concentration 1% and shear rate: 46.16/s).

temperature might be the result of molecules irreversible changes in their conformation (Estevez et al., 2004). These results were similar to those results obtained by Bostan et al. (2008) and Koocheki et al. (2008) for *S. macrocarpon* seed gum and *A. homolocarpum* seed gum, respectively. Nevertheless, Singthong et al. (2008) concluded that increase in extraction temperature and time resulted in increase in the viscosity of Yanang leaves gum.

Although increase in extraction time–temperature increased the extraction yield but high temperature might induce the change of gum molecule structure at the extended time. This might also be the reason for reduction in viscosity.

The viscosity of mucilage was water to seed ratio dependant and decreased with increase of water to seed ratio (Fig. 3b). At higher water to seed ratio, pH did not have much effect on the mucilage

viscosity while at lower water to seed ratio, increase in pH, slightly increased the viscosity. This small decrease in mucilage viscosity with decrease in pH at lower water to seed ratio might be due to the conformational changes in the molecules of the mucilage (Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000). Mohammad Amini et al. (2007) reported similar trend for Balangu seed gum. In contrast to our finding, Goycoola, Morris, and Gidley (1995), Ibanez and Ferrero (2003) and Koocheki et al. (2008) reported that due to a reduction in the weight of the molecules and the suppression of intermolecular association, alkaline conditions caused a reduction in viscosity.

The highest response for viscosity (519.8 mPa.s) in the ranges studied, observed when the gum was extracted at temperature = 45 °C, pH = 8, time = 1.5 (h) and water to seed ratio = 30 to 1. Comparatively, the viscosity of *L. perfoliatum* seed gum was somehow similar to xanthan gum and *L. royleana* seed gum (Riazi, Farhoosh, & Razavi, 2006; Mohammad Amini et al., 2007), however the viscosity was higher than *A. homolocarpum* seed gum (Koocheki et al., 2009), Salep, CMC (Riazi et al., 2006) and *S. macrocarpon* seed gum (Bostan et al., 2008). According to Yaseen, Herald, Aramouni, and Alavi (2005) the difference in viscosity occurs as a result of the different molecular weight, polymeric nature of the gums and the interactions between polymer chains when gums are dissolved or dispersed. Our result shows that *L. perfoliatum* seeds gum could be an essential viscosity builder and provider of the body and mouthfeel for industrial application.

3.4. Color (hue)

Fig. 4a illustrates the different bands of colors (Hue) which was used to evaluate the different colors such as yellow, green, red and etc.

It was suggested that the color developed by the extracted mucilage may be due to the passage of pigments or tannic substances from the tegument. The results given in Table 2 show that linear extraction temperature, time and pH had significant effects on hue ($p < 0.0001$, $p < 0.01$). Quadratic effects of extraction temperature and water to seed ratio were significant at less than 0.01% and 5% level, respectively. The results also illustrate that among the interactions, extraction time–temperature and time–pH had significant and similar effects on hue. The mutual interaction between extraction temperature and pH ($p < 0.001$), extraction temperature and water to seed ratio ($p < 0.01$), pH and water to seed ratio ($p < 0.01$) and extraction time and water to seed ratio ($p < 0.01$) were also found to be significant. From the results, it can be concluded that variables with the largest effects were the quadratic and linear terms of extraction temperature, the linear term of extraction time, followed by the interaction effect of extraction time–temperature and time–pH. In different extraction conditions hue varied between 48.87 and 61.59, meaning that the final gum color changed from brown to yellow (Fig. 4b).

The effect of changing extraction time–temperature on hue is given in Fig. 5a. At lower temperatures (°C), hue did not change with extraction time, whereas, at higher temperatures decreases along with increasing extraction time. Similar effect can be seen with increasing temperature alone. At lower extraction time (1.5 h), hue increased with increase in temperature up to 55 °C, but decreased afterwards. Meaning that at higher extraction time and temperature more color diffused into water and the final color of the gum changed into brown. Balke and Diosady (2000) also found that white mustard seed mucilage obtained at different temperatures were visibly different. They also concluded that those mucilage obtained at higher temperatures were highly colored.

Increase in pH did not change the color of the gum at lower water to seed ratio (no change in hue value) but at higher water to seed ratio decreased the hue value (Fig. 5b) and the color of the

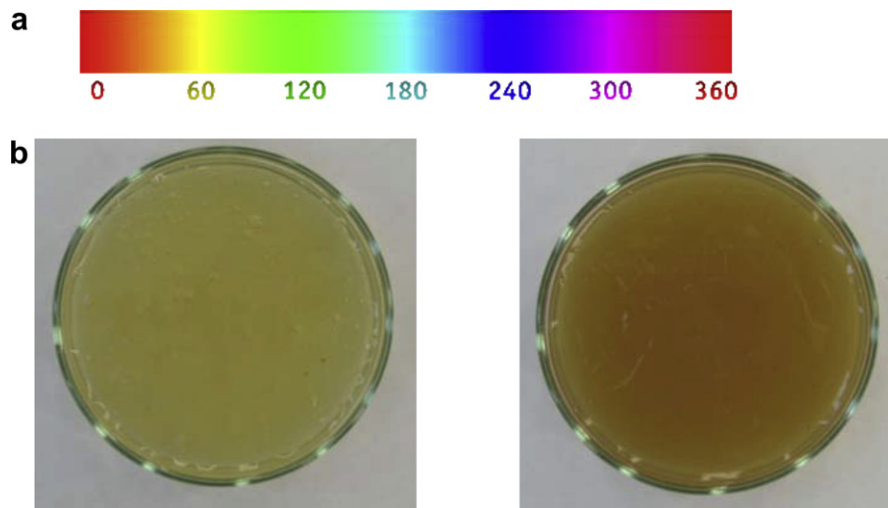


Fig. 4. Hue values for different colors (a); the lowest and highest hue for the gum extracted from *L. perfoliatum* seed (b).

gum turned into brown. Hue value did not change with water to seed ratio at low acidic conditions (high pH). However, at low pH, increase in water to seed ratio, augment hue value. From these results we can conclude that at acidic condition and higher water to seed ratio, the color of the gum was closer to yellow than at higher pH and lower water to seed ratio. This means either the pigments were more soluble in alkaline condition or the pigments gradated at lower pHs. The dissolved pigment in lower water to seed ratio, reached to the saturation and thus the content did not changed with pH. Ibanez and Ferrero (2003) also concluded that extraction of *P. flexuosa* seed gum in alkaline medium rendered a more colored product. The results reported by Estevez et al. (2004) indicated that the Mesquite seed gums presented a similar color, regardless of the pH of extraction. However, the acid-extracted gum was the whitish. According to Dakia, Blecker, Robert, Wathelet, and Paquot (2008), the yellowish color of the locust bean gum extracted with water may be due to the passage of pigment or tannic substances from the brown tegument or from the yellow germ to the endosperm. However, their results also showed that for gum whiteness, acid pre-treatment are preferred.

The gum extracted at temperature = 58 °C, pH = 8, time = 1.5 (h) and water to seed = 60:1 had the highest hue (60.76) among all the samples. The extracted gum had pale yellow color which was similar to the other commercial gums such as arabic gum (Williams & Phillips, 2000b, chap. 9) and guar gum (Glicksman, 1982).

3.5. Protein

The results tabulated in Table 2 showed that the linear effect of extraction temperature, pH and water to seed ratio were highly significant ($p < 0.0001$) while time were only significant at 0.1%. Quadratic effect of extraction temperature and water to seed ratio was significant at 1%. Among the interaction terms, only extraction temperature and pH was significant ($p < 0.05$) for the protein content. The importance of the independent variables on protein content could be ranked in the order of extraction temperature, water: seed ratio, pH and time.

There was a general trend showing an increase in protein content as the extraction time and temperature increased (Fig. 6a). At higher temperature the protein contamination was enhanced due to superior mass transfer rates. Higher water to seed ratio appeared to favor the incorporation of protein into the *L. perfoliatum* seed gum (Fig. 6b). At higher water to seed ratio, protein

content increased substantially with decrease in pH while in lower water to seed ratio, increase in protein content along with decrease in pH was much smaller. The increase in protein content with decrease in pH might be due to the easier ease of protein solubilization at lower pH region (Cui et al., 1994). Similar results were observed in the studies by Cui (2001) and Koocheki et al. (2008). In contrast with our results Estevez et al. (2004) reported that acid extraction resulted in lower protein content owing to molecular hydrolysis caused by the acid.

Lowest protein content was observed at temperature of 45.26 °C, pH of 7.96 and water to seed ratio of 30.16:1 in which the protein content reached a level of 2.74%. This value was lower than those reported by Somboonpanyakul et al. (2006) for Malva nut gum (8.4%), Sepulveda et al. (2007) for *Opuntia ficus indica* (7.3%), Ibanez and Ferrero (2003) for *P. flexuosa* seed gum (10.4%), but similar to the protein contents reported by Estevez et al. (2004), Singthong et al. (2005) and Brummer et al. (2003) for Mesquite seed gum (2.5%), Krueo Ma Noy pectin (3.29%) and Fenugreek gum (2.36%), respectively. According to Glicksman (1982) the protein content in commercial guar gum, locust bean gum and Tara gum was found to be 10%, 8% and 3.13%, respectively. Study by da Silva and Goncalves (1990) showed that the protein content of the crude gum reflects the natural presence of structural proteins and enzymes, but also a possible contamination with seed germ. The lower protein content is, therefore, related to the purity of hydrocolloid.

3.6. Emulsion stability (ES)

Extraction temperature, time and water to seed ratio had significant ($p < 0.0001$) and similar linear effects on the emulsion stability (Table 2). However, the linear effect of extraction pH was not significant. The results indicated that quadratic parameters of extraction temperature, pH and water to seed ratio had significant effect ($p < 0.01$, $p < 0.05$ and $p < 0.0001$) on stabilizing property of gum. Moreover, among the interaction effects, only the mutual interaction between extraction temperature and time was significant ($p < 0.01$). The importance of the independent variables on emulsion stability of mucilage could be ranked in the following order: extraction temperature > water to seed ratio extraction time (based on the sum of squares).

The stabilizing effect of mucilage decreased with increase in temperature and time (Fig. 7a). At higher temperatures, increase in extraction time had little effect on the stabilizing properties of

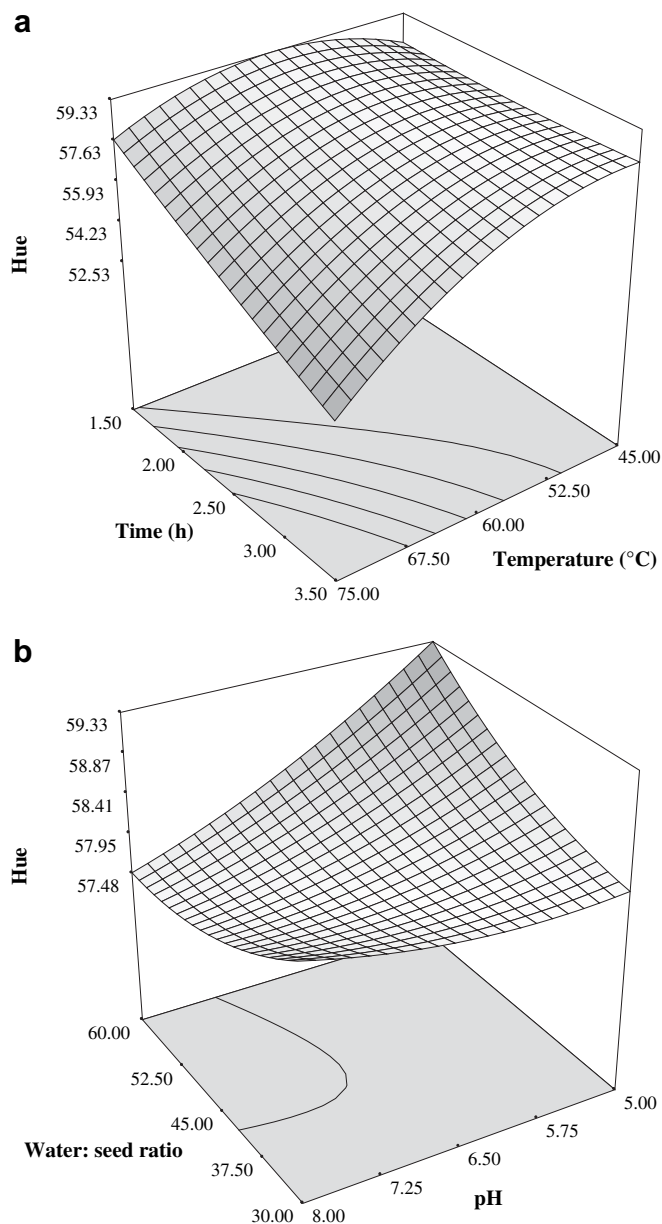


Fig. 5. Response surface for the effect of extraction temperature and time (a, water to seed ratio = 45:1 and pH = 6.5), and water to seed ratio and pH (b, temperature = 60 °C and time = 2.5 h) on the hue value of Qodume shahri seed gum.

mucilage extracted from *L. perfoliatum* seed. The stabilizing effect of mucilage depended on water to seed ratio and decreased exponentially with increase in water to seed ratio (Fig. 7b). At higher water to seed ratio, pH did not have significant effect on the stabilizing property of mucilage while at lower water to seed ratio, increase in pH, slightly increased this effect. The effects of these parameters on the stabilizing property of *L. perfoliatum* seed gum were similar to the effects observed for viscosity of this gum. Bostan et al. (2008) also reported that increase in temperature, decreased the stabilizing effect of hydrocolloid extracted from *S. macrosiphon* seed but pH had no effect on this factor.

Stoke's law states that the velocity at which a droplet moves is inverse to the viscosity, thus by increasing the viscosity of emulsion, the stability of an emulsion to gravitational separation can be increased (McClements, 2005; Taherian, Fustier, & Ramaswamy, 2007; Taherian, Fustier, Britten, & Ramaswamy, 2008). Polysaccharides are

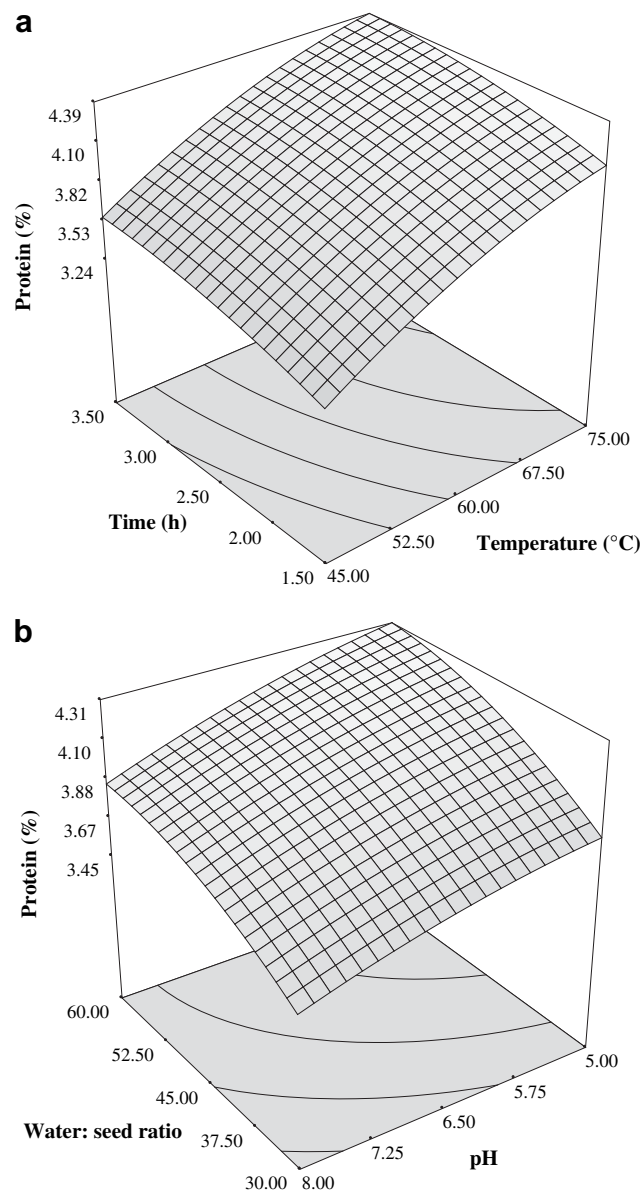


Fig. 6. Response surface for the effect of extraction temperature and time (a, water to seed ratio = 45:1 and pH = 6.5), and water to seed ratio and pH (b, temperature = 60 °C and time = 2.5 h) on the protein content of Qodume shahri seed gum.

good stabilizing agents because of their high molecular weight and gelation behavior which lead to the formation of a macromolecular barrier by increasing the viscosity of the aqueous phase and slowing coalescence between dispersed droplets (Papalamprou, Makri, Kiosseoglou, & Doxastakis, 2005). Therefore, change in the extraction conditions not only alters the viscosity but it will affect the stabilizing effect of extracted gum. Mucilage extracted at 45 °C, pH of 8, water to seed ratio of 30:1 after 1.5 (h) had the highest stabilizing effect (93.17%). Sciarini et al. (2008) used the same method for measuring the stability of emulsions prepared with xanthan gum, guar gum and *G. triacanthos* gum. Comparing our results with the results reported by Sciarini et al. (2008), at similar gum concentration, showed that the *L. perfoliatum* seed gum had similar emulsion efficiently to guar gum and *G. triacanthos* gum, nevertheless these values were lower than that reported for xanthan gum. In general, the thickening action of Qodume shahri seed gum in aqueous system could add body to formulations and stabilize the dispersions and emulsions.

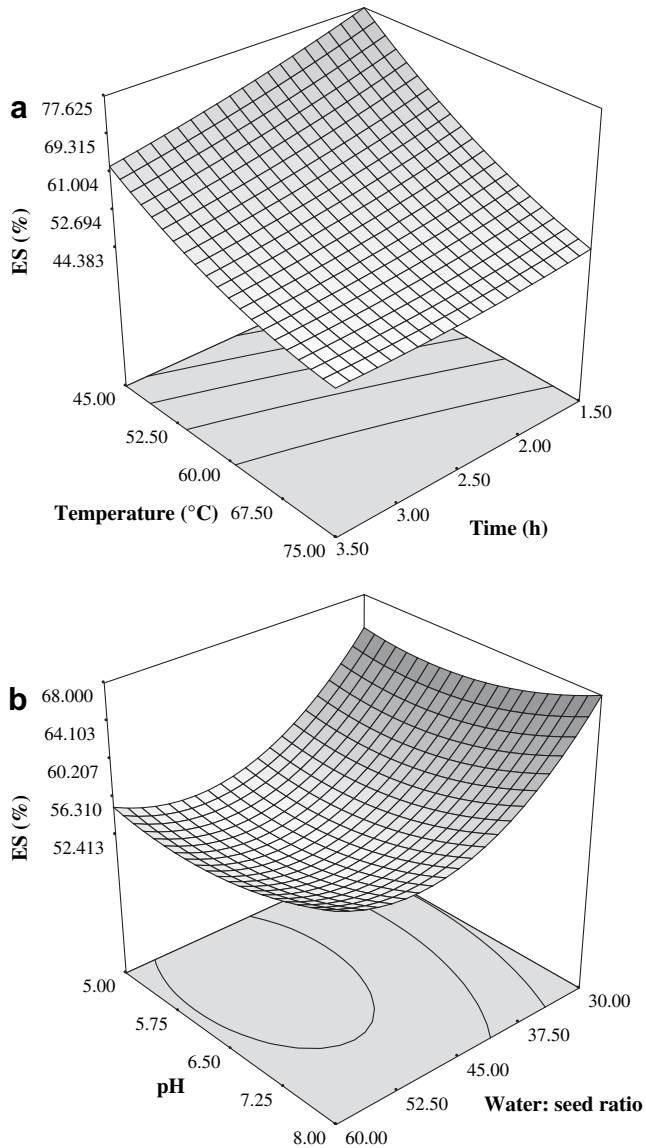


Fig. 7. Response surface for the effect of extraction temperature and time (a, water to seed ratio = 45:1 and pH = 6.5), and water to seed ratio and pH (b, temperature = 60 °C and time = 2.5 h) on the emulsion stability of Qodume shahri seed gum.

3.7. Optimization

Optimum condition for extraction of *L. perfoliatum* seed gum was determined to obtain maximum extraction yield, viscosity, hue and emulsion stability with minimum protein content in gum. This optimum condition is tabulated in Table 3 that provide the highest value of yield = 17.36 (%), viscosity = 463.07 (mPa s), hue = 60.47 and emulsion stability = 88.96 (%) with lowest protein content = 2.84 (%). This means that the optimum condition for extraction of *L. perfoliatum* seed gum were: extraction temperature of 48.1 °C, pH = 8, time = 1.5 h and water to seed ratio of 30:1 (Table 3).

The range of optimum extraction condition was determined by superimposing the contour plots of all the responses. Fig. 8 presents the overlaying contour plots for the five responses which were evaluated as a function of extraction time and temperature at constant water to seed ratio (45:1) and pH (6.5) as well as pH and water to seed ratio at constant temperature (45 °C) and time (1.7 h). These plots illustrate the determination of the best combination factors for extraction of *L. perfoliatum* seed gum. Fig. 8a represent

Table 3
Predicted optimum condition for extraction of Qodume shahri seed gum.

Factor	Low	High	Optimum
Temperature (°C)	45	75	48.1
pH	5	8	8
Time (h)	1.5	3.5	1.5
Water:seed ratio	30:1	60:1	30:1

the extraction temperature of 37.59–49.4 °C and time of 0.42–3.04 h at constant water to seed ratio of 45:1 and pH of 6.5 as the best combinations. The extraction pH of 2.5–8.69 and water to seed ratio of 31.64:1–50.13:1 at constant temperature of 45 °C and time of 1.7 h illustrated in Fig. 8b. A small shaded area, namely overlay area of the five responses is assigned as the optimum area of extraction that represents a higher amount of yield, viscosity, hue

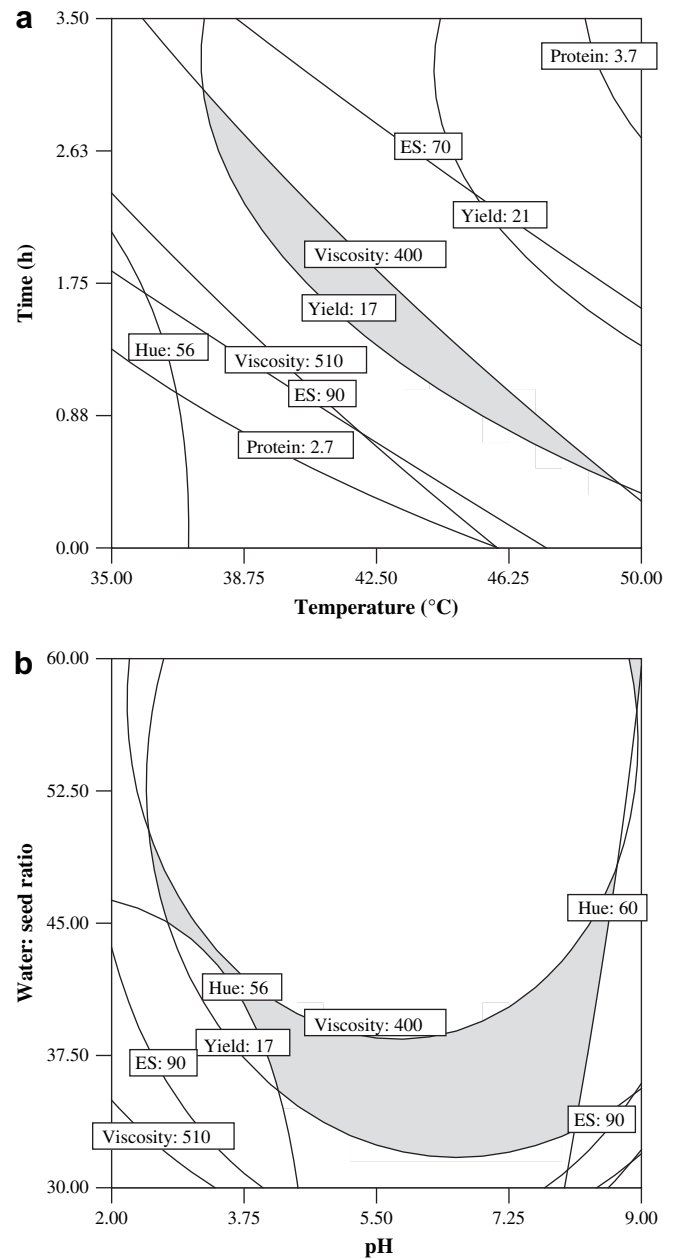


Fig. 8. The optimum region by overlaying contour plots of the five responses evaluated as a function of extraction temperature and time (a, at constant water to seed ratio = 45:1 and pH = 6.5) and pH and water to seed ratio (b, at constant temperature = 45 °C and time = 1.7 h).

and emulsion stability with low protein content. At these areas the extraction process is in its optimum condition.

4. Conclusion

Results showed that the effects of process variables including temperature, time, pH and water to seed ratio were statistically significant for extraction of mucilage. Second-order polynomial models were obtained for predicting extraction yield, viscosity, protein content, hue and emulsion stability. While increasing the extraction temperature increased the extraction yield and the protein content of the mucilage, it decreased the viscosity, hue and stabilizing effect of the gum. Lower pH at higher water to seed ratio seems to reduce the viscosity and emulsion stability of the hydrocolloid but it had no significant effect on yield extraction. However, at higher water to seed ratio, increase in pH, decreased the hue value. Extraction yield, hue and protein content of gum increased with increase in water to seed ratio from 30:1 to 60:1 whereas viscosity and emulsion stability decreased with increase in this variable. Optimum extraction condition for maximizing extraction yield, viscosity, hue and emulsion stability as well as obtaining minimum protein content comprised the condition in which the temperature, time, pH and water to seed ratio were set to 48.1 °C, 1.5 h, 8 and 30:1, respectively.

This investigation could help food industries to add a new source of hydrocolloid with certain functionality and for certain uses in their list of ingredients.

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