

Optimization of Ultrasound-assisted Extraction of Quince Seed Gum through Response Surface Methodology

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ABSTRACT

Ultrasound-assisted extraction was used to determine the optimum processing conditions and to achieve the maximum extraction yield and viscosity of the gum extracted from quince seed. Using Response Surface Methodology (RSM) to design the experiments and adopting the Central Composite Rotatable Design (CCRD), the effect of extraction temperature (25-55°C), time (3-10 minutes), and pH (6-8) were evaluated on efficiency of gum extraction yield (%) and viscosities (mPas). The Chemical composition of the extracted gum was determined using AOAC standard methods. Apparent viscosity of the gum was measured by rotational viscometer. Using multiple linear regression analysis, a second-order polynomial model was developed for each response. The quince seed gum showed pseudoplastic behavior. Optimum operating conditions based on the highest yield and viscosity was predicted by RSM as an extraction temperature of 38.03°C, pH of 6.35 and the extraction (ultra-sonication) time of 7.68 minutes. At this optimum point, extraction yield and viscosity were 14.09% and 52.4 mPas, respectively.

Keywords: Central composite rotatable design, Extraction Yield, Pseudoplastic behavior, Viscosity.

INTRODUCTION

The use of ultrasound techniques in food technology has attracted plentiful attention (Jambrak *et al.*, 2009). Ultra-sonication with low-frequency (high intensity) has many potential applications in food processing as well as extraction, emulsification, homogenization, freezing, crystallization, and filtration (Chemat and Khan, 2011), due to their mechanical, chemical and biochemical effect through the cavitation phenomenon (Soria and Villamiel, 2010).

During this phenomenon, the waves pass over an elastic medium and subsequently cycles of contraction and expansion are produced, bubbles are formed rapidly, growth and, finally, because of pressure,

fluctuations of acoustic waves, collapse violently (Iida *et al.*, 2008) which produce hot spot, macroturbulence and a lot of energy (McClements, 1995). Ultrasound waves improve extraction by disrupting biological cell walls and creating pores through them. These lead to high penetration of solvents into the cellular matrix which, in turn, facilitate and accelerate mass transfer (Vinatoru, 2001). Increasing extraction yield in a given time is one of the advantages of using sono-chemistry in food processing (Toma *et al.*, 2001; Rodrigues *et al.*, 2008).

Water-soluble gums are highly hydrophilic polysaccharide biopolymers. Some of them can form gels under certain conditions while others can act only as thickeners (Glicksman, 1982). These gum are from various parts of plants, e.g. seeds, endosperm, tree exudates, seeds wall and

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tuber or roots (Phillips and Williams, 2009). They often have some functional properties in food systems and play different roles as emulsifiers, foaming agents, stabilizers, texture modifiers, thickeners, dietary fiber, coating agents, and packaging films (Phillips and Williams, 2009 ; Dickinson, 2003). These functional properties are sensitive to preparation methods (Jaya and Durance, 2009).

It has been demonstrated that different methods and variables, such as extraction temperature, Water to Seed ratio (W/S), pH, salts concentration, nature of the solvent, extraction time, as well as the cultivars from which gum are extracted have an influence on hydrocolloids yield and rheological properties (Cui *et al.*, 1994; Wu *et al.*, 2007). According to these studies, extraction yield was increased with increasing extraction temperature and W/S ratio. Optimizing the extraction procedure resulted in the higher extraction yield, purity, and viscosity of the extracted gum. The extraction condition of various plant-based gums have been optimized by previous researchers (Cui *et al.*, 1994; Wu *et al.*, 2007). Rheological properties are considered to be directly related to the structural features (Amin *et al.*, 2007) and strongly depend on the source of gum, concentration, chemical composition, extraction method and any further processing conditions. For this reason, the rheology of many hydrocolloids dispersions has been extensively studied (Phillips and Williams, 2009).

Quince is a small deciduous tree that belongs to *Cydonia vulgaris* family (*Cydonia oblonga* Miller). It has a bright golden yellow pome fruit. In folk medicine, it is believed that some parts of this plant such as leaves, fruits, and seeds have an excellent medical effect and have been used for a variety of diseases such as cardiovascular diseases, hemorrhoids, bronchial asthma, cough (Jouki *et al.*, 2014), gastric tonic, anti-diarrheal, anti-inflammatory and ulcer healing agent especially within the gut, suitable for uterine

and hemorrhoid bleeding, antiemetic and astringent (Hemmati *et al.*, 2012; Minaiyan *et al.*, 2012). This plant is native to central Asia and found abundantly in the north of Iran. Iran supplies about 75% of the world quince production (Bergman *et al.*, 1996; Minaiyan *et al.*, 2012). This fruit has about 10% seed in its center. The seeds are extensively used as an account of the mucilage that exists in seeds coat (Fekri *et al.*, 2008) and once it is soaked in water, the seeds form mucilage around the outer surface. Several analytical methods were carried out to determine some constituents of quince seed gum (Ferrerres *et al.*, 2003; Silva *et al.*, 2005).

Nowadays, a number of studies have been devoted to use different methods of extraction and investigated the effect of these methods on functional properties in a broad range of hydrocolloids (Dogan *et al.*, 2007; Mu *et al.*, 2010). In order to decline the extraction time and organic solvent usage, there is an increasing interest for novel extraction methods such as Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) (Rajaei *et al.*, 2010).

There is no published report available on the use of ultrasound in the extraction of quince seed gum, so, the main purpose of the present study was to extract gum from quince seed under different operating conditions of pH, temperature, and extraction time using ultra sound bath and to evaluate the effects of these parameters on extraction yield and its viscosity.

MATERIALS AND METHODS

Quince seeds were purchased from the local market in Gilan, a northern province in Iran. Impurities of seeds were separated by hand, then, the clean seeds were stored in a cool and dry place. Chemicals with analytical grade were purchased from the Merck Company (Merck KgaA, Darmstadt, Germany).

Quince Seed Gum Extraction

Clean seeds were mixed with distilled water with water to seed ratio of 25:1 (v/w). According to CCRD, the Temperature were adjusted from 14.77 to 65.23°C and pH from 5.32 to 8.68 by 0.1 (Mol l⁻¹) NaOH and HCl, respectively. All these limits were chosen by primary experiments. Samples were placed in an ultrasonic water bath (Starsonic 35 Digit. Liarre, Italy; ultrasonic frequency 50-60 kHz, power 100W) equipped with automatic temperature controller to prevent temperature changes during a definite time of sonication (i.e. 0.61-12.39 minutes). In order to separate seeds from resultant gum, the slurry was centrifuged (4,000 RPM, 10 minutes) (Z 36 HK Refrigerated High Speed Centrifuge, Hermel/Labnet, USA). Supernatant were oven dried at 60°C for 16 hours.

The extraction value of quince seed gum was calculated in each treatment as the dry weight of extracted gum relative to initial dry seeds (Koocheki *et al.*, 2010). Extraction yield was calculated by Equation (1):

$$Y = 100 \times (M_1/M_2) \quad (1)$$

Where, Y is extraction yield (%), M_1 and M_2 are gram masses of extracting gum and quince seed, respectively.

The dried gum was ground, packed and stored in 4°C refrigerator for further experiments.

Proximate Analysis of Hydrocolloids

Moisture and ash content were measured by AOAC method (Official Methods of Analysis, 1984). Fat content was estimated by AOAC standards using Soxhlet extraction with hexane and total nitrogen content was determined by the semi-automatic Kjeldahl method. Protein estimation (%) was done as $N \times 6.25$ (Anderson and Weiping, 1991) and the carbohydrate content was obtained by calculation.

Rheological Behavior

In order to determine rheological properties, aqueous solution of each sample (0.5% dry basis) was prepared by dispersing the gum in distilled water containing 0.01% (w/w) sodium azide, then, stirred at 1,000 rpm at an ambient temperature for 1 h. The samples were left overnight for complete hydration of biopolymers (Ibañez and Ferrero, 2003).

Rheological behaviors of quince seed gum were measured 24 h after preparation by viscometer (Brookfield LV-DV II Pro; Brookfield Engineering Laboratories, Inc., Middleboro, USA) equipped with UL adapter. The sample chamber was placed in a water jacket connected to a bath with constant temperature to determine viscosities at constant temperature of 25°C. Measurements were done and duplicated at 50 RPM and responses with torque smaller than 10% were discarded.

Experimental Design and Statistical Analysis

The maximum extraction yield and rheological properties of extracted gum were targeted as responses and the optimization of this method was considered as well.

Experiments were designed using Response Surface Methodology (RSM) based on Central Composite Rotatable Design (CCRD) including central and axial points. Ultra-sonication time, temperature and pH were independent variables and yield and viscosity were dependent responses. To evaluate the influence of three independent variables (pH, extraction temperature, and ultra-sonication time) on responses of quince seeds extraction yield (%) and viscosity (mPas), the statistical technique of RSM was applied using Design Expert software version 6.0.4 (Stat-Ease Inc., Minneapolis, USA). RSM provided mathematical and statistical procedures to study the relationship between responses and a number of factors (Diniz and Martin,



1996). By using model analysis, lack of fit and coefficient of determination (R^2), the model adequacies were determined (Joglekar and May, 1987). By determining the range of each variable and responses, the appropriate point with suggested levels of parameters and response were predicted and procedure optimization was done. RSM has been used by several researchers to evaluate the effect of the different extraction conditions (Somboonpanyakul *et al.*, 2006; Milani *et al.*, 2011) and to optimize the procedure (Cui *et al.*, 1994; Koocheki *et al.*, 2010).

Experimental design and the effects of various variables on the responses (extraction yield and apparent viscosity) are shown in Table 1. Twenty experimental points were established according to three independent variables with actual levels. The generalized polynomial model for relating the response to independent variables is given by Equation (2):

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

Where, Y is the predicted response or dependent variable, β_0 is the offset term, β_i is the regression coefficients in linear terms of factors, β_{ii} is a regression coefficient for quadratic effects and β_{ij} is the interaction coefficient. On the other hand, x_i is the independent variable. In reduced model, only the statistically significant variables ($P < 0.05$) were included.

RESULTS AND DISCUSSION

Proximate Analysis

The proximate composition of quince seed gum extracted by ultra-sonication was determined (randomly 3 treatments of number 5, 11 and 18 were chosen with neutral pH) and average results were about 9.84% moisture, 90.16% dry weight,

Table 1. Experimental design in the composite rotatable center mode of response surface methodology and the effects of independent variables on the yield and apparent viscosity of quince seed gum.

Run No.	Independent variables			Dependent variables	
	pH	Ultra-sonication time (Min)	Temperature (°C)	Yield (%)	Apparent viscosity (mPas) ^a
1	8.00	10.00	25.00	11.71	55.70
2	8.00	3.00	25.00	9.50	53.80
3	6.00	3.00	55.00	13.87	40.90
4	8.00	10.00	55.00	16.29	42.10
5	7.00	6.50	40.00	12.71	51.20
6	8.68	6.50	40.00	15.08	53.90
7	7.00	6.50	40.00	13.43	54.30
8	7.00	6.50	40.00	13.85	51.20
9	8.00	3.00	55.00	13.58	46.30
10	6.00	10.00	55.00	15.13	43.40
11	7.00	6.50	14.77	12.91	49.20
12	7.00	6.50	40.00	14.15	50.70
13	7.00	12.39	40.00	13.97	45.50
14	7.00	6.50	40.00	14.27	55.10
15	7.00	6.50	65.23	15.65	33.60
16	6.00	3.00	25.00	8.40	54.30
17	7.00	6.50	40.00	13.43	52.10
18	7.00	0.61	40.00	7.02	58.10
19	5.32	6.50	40.00	15.00	50.20
20	6.00	10.00	25.00	11.22	53.40

^a Apparent viscosity at 50 RPM.

12.59% ash, 71.6% carbohydrate, 3.16% fat and 2.81% protein, all on dry basis. In a recent report, Fekri *et al.* (2008) showed 4.38% moisture, 95.62% dry weight, 8.24% ash and 20.9% protein in quince seed gum. Different values of chemical ingredients may arise because of the difference between the type of seeds and extraction procedure (Fekri *et al.*, 2008). Extraction yield in our study was higher than those reported for flax seed, basil seed, and dragon head (Fekri *et al.*, 2008).

Model Fitting

Regression analysis and ANOVA were studied to relate the dependent variables to the independent ones and the model fitting was conducted by RSM software. In order to predict quadratic polynomial model for responses, multiple regression coefficients was conducted by means of least square and, according to parameters values, the second order polynomial response surface model [Equation (2)] was fitted to these two response variables (yield and apparent viscosity). ANOVA analysis indicated that

the second order polynomial model was adequate for responses with indicated parameters. Experimental models for yield and apparent viscosity derived from meaningful parameters were shown by Equations (3) and (4), respectively.

$$\text{Yield (\%)} = 2.19 + 1.83 B + 0.11 C - 0.11 B^2 \tag{3}$$

$$\text{Apparent Viscosity (mPas)} = 42.29 - 0.46 B - 0.99 C - 0.02 C^2 \tag{4}$$

Where, *A*, *B* and *C* represent pH, ultrasonication time (min) and temperature (°C), respectively. ANOVA analysis of quadratic polynomial model of yield and apparent viscosity are shown in Table 2. The percentage of *R*² and *adj-R*² values of quadratic equation regression models were 85.03 and 82.23 for extraction yield, and 87.01 and 84.58 for apparent viscosity. The high value of *R*² indicated high percentage of response variability that was explained by these models. It must be noticed that the value of *R*² does not always clearly mean the good regression model, so, it is better to evaluate the adequacy of the model by using *adj-R*² (Myers *et al.*, 2009). Higher values of *adj-R*² implied that all the terms in the model were significant.

Table 2. ANOVA analysis of quadratic polynomial model of yield and apparent viscosity (P< 0.05).

Reference	Yield				Apparent viscosity			
	Degree of freedom	Coefficient estimate	F Value	Prob> F ^a	Degree of freedom	Coefficient estimate	F Value	Prob> F
Model	9	13.67*	7.54	0.002	9	52.42*	9.05	0.001
A	1	0.19	0.35	0.5693	1	0.89	1.40	0.2635
B	1	1.51	22.02	0.0009	1	-1.60	4.58	0.0581
C	1	1.66	26.39	0.0004	1	-5.18	47.78	<0.0001
A2	1	.33	1.09	0.3207	1	-0.016	5.061E-004	0.9825
B2	1	-1.28	16.55	0.0023	1	-0.10	0.021	0.8886
C2	1	0.06	0.036	0.8532	1	-3.78	26.88	0.0004
AB	1	0.10	0.062	0.8085	1	-0.49	0.25	0.6293
AC	1	-0.09	0.046	0.8353	1	0.29	0.086	0.7750
BC	1	-0.13	0.099	0.7599	1	-0.34	0.12	0.7374
Lack of fit	5		7.63	0.0218	5		3.58	0.0941

^a Values of "Prob> F" less than 0.0500 indicate model terms are significant. * Intercept.



Extraction Yield of Gum

The effect of temperature, ultra-sonication time and pH on yield value of gum extracted from quince seed are presented in Figure 1 (a, b, and c). Yield value varied from 7.02 to 16.29% due to extraction conditions. This response was significantly influenced by ultra-sonication time and temperature and was increased by increasing these variables, while pH had no significant effect on this value ($P < 0.05$).

Unlike the usual method of extraction, UAE caused destruction of plant cell wall in a short time. In fact, several factors affect the operation of ultrasound waves including moisture content, particle size, type of solvent, frequency, pressure, time, and temperature (Wang and Weller, 2006). The present study showed a broad range of extraction yield (7.02 to 16.29%) depending on extraction condition. The upper range of extraction was 16.29% that was higher than what is (13.7%) reported by Abbastabar (2013), who achieved the optimum condition of quince seed mucilage extraction with water to seed ratio of 96.2 (w/w) at 60.77°C and pH of 6.6 for 3 hours. Jouki *et al.* (2014) extracted about 11.58% of quince seed gum during 5 minutes at 65°C temperature and water to seed ratio of 25.1:1 (v/w) by conventional method while Hemmati *et al.* (2012) had reached 9.86% yield at water to seed ratio of 20:1 (v/w) during 30 minutes at 40°C. The differences might be attributed to different methods and conditions of extraction, origin of seeds, environmental condition and the seed genotype (Fekri *et al.*, 2008; Qian *et al.*, 2012). One of the advantages of UAE is to increase the extraction yield in a shorter period of time by vacuolation (Vinatoru, 2001). The treatment with minimum ultra-sonication time at 40°C showed almost the lowest yield value of 7.02%. At constant time of ultra-sonication (e.g 3 or 6.5 minutes), extraction yield was increased with increasing the temperature and the effect of this parameter on yield value was greater than the others. These results clearly

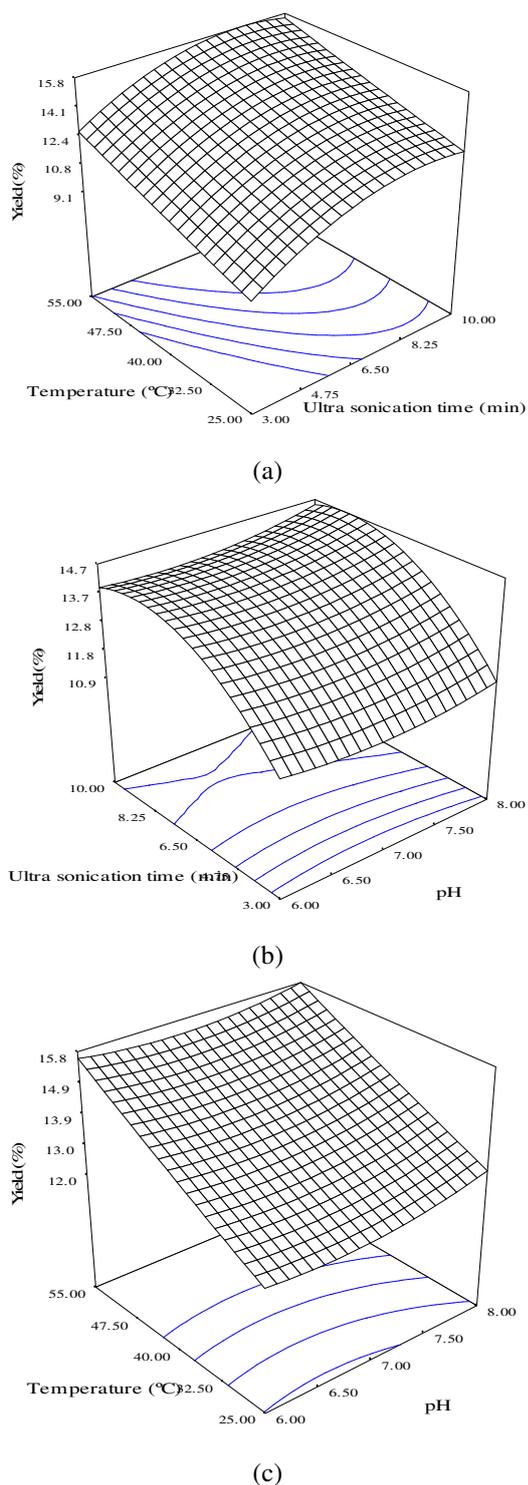


Figure 1. Response surface plots for extraction yield of quince seed gum as a function of: (a) Ultra-sonication time and temperature (pH: 7); (b) Ultra-sonication time and pH (Temperature: 40°C), and (c) Temperature and pH (Ultra-sonication time: 6.50).

show the effect of temperature on extraction and they are in agreement with the report of Cai *et al.* (2008) who showed the enhancing effect of increasing the temperature and time of extraction on yield value of cotton seed gum. However, excessive extraction time can cause some changes on the polysaccharide structure (Cai *et al.*, 2008).

The extraction at elevated temperature resulted in faster and easier mass transfer of water soluble polysaccharide from the cell wall (Wu *et al.*, 2007). High temperature decreased the viscosity of seed slurry, so that the gum around seeds became less sticky, and facilitating the extraction procedure (Koocheki *et al.*, 2009).

In the present study, the effect of pH on extraction yield was not significant, which could be due to the resistance of structural linkage of gum in dilute acid or alkali medium at ambient temperature (Kirchner and Tollens, 1874). However, there are different results about the effect of pH on extraction yield. Our finding was in agreement with the Cui *et al.* (1994), Wu *et al.* (2007) and Koocheki *et al.* (2009) who reported minor effect of pH on gum extracted from seeds. Some authors reported the positive effect of alkaline condition on extraction yield. This may be because of hydrolyzing insoluble constituents into soluble ones in alkaline medium which increases the extraction yield (Balke and Diosady, 2000; Estévez *et al.*, 2004;

Somboonpanyakul *et al.*, 2006).

Viscosity

Quince seed gum showed a strong shear thinning behavior like *L. sativum* gum (Karazhiyan *et al.*, 2009). Figure 2 implied pseudoplastic behavior of 3 different samples (treatment 4, 10 and 14) that were chosen randomly. Results indicated that ultra-sonication time and temperature had significant effects on the apparent viscosity ($P < 0.05$) while pH did not have any impact on this response which was similar to extraction yield. The effect of these variables (temperature, time of ultra-sonication, and pH) on gum apparent viscosity are presented in Figure 3 (a, b and c). Apparent viscosity of the gum was reduced by increasing the extraction temperature. It might be due to irreversible effect of temperature on molecular conformation (Estévez *et al.*, 2004).

In this study, increasing pH increased the viscosity, but this effect was not significant. This finding was in agreement with Karazhiyan *et al.* (2011). However, some authors (Goycoolea *et al.*, 1995; Ibañez and Ferrero, 2003; Koocheki *et al.*, 2010) have reported a reduction in viscosity in alkaline

conditions due to reduction in molecular weight and the suppression of intermolecular

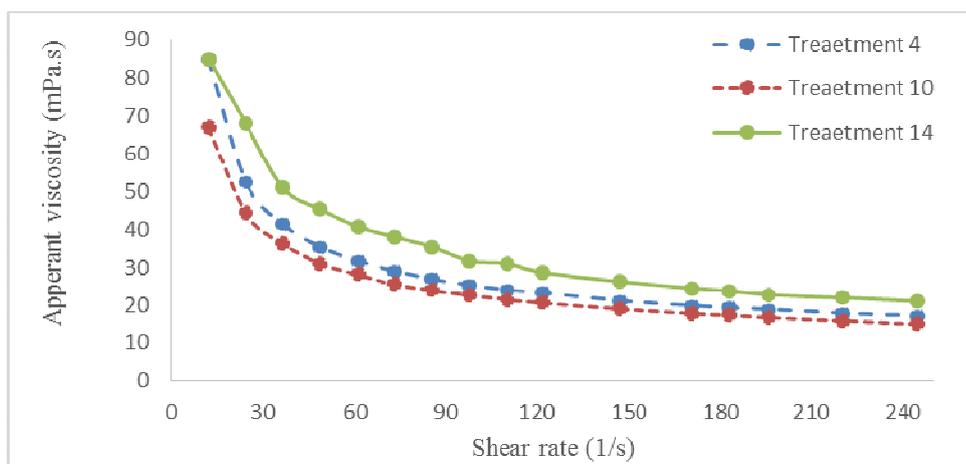
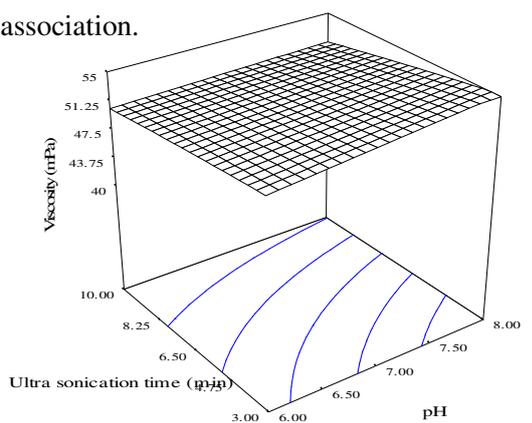


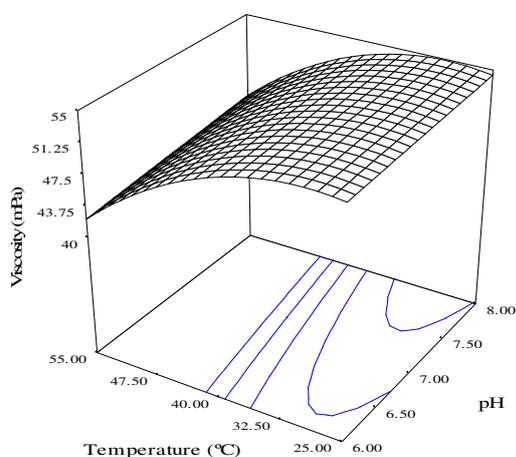
Figure 2. Pseudoplastic behavior of 3 randomly selected extracted quince seed gum.



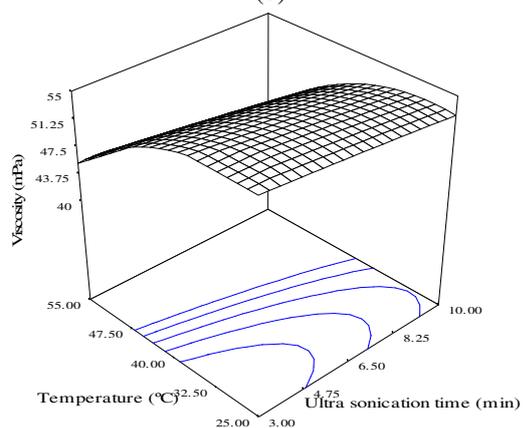
association.



(a)



(b)



(c)

Figure 1. Response surface plots for extraction yield of quince seed gum as a function of: (a) Ultra-sonication time and temperature (pH: 7); (b) Ultra-sonication time and pH (Temperature: 40°C), and (c) Temperature and pH (Ultra-sonication time: 6.50).

Optimization

In order to obtain the maximum extraction yield and apparent viscosity of Quince seed gum, the optimization of the experiment was done using RSM. Extraction parameters such as temperature, pH, and ultra-sonication time were selected in the experimental range of 25-55°C, 6-8, and 3-10 minutes, respectively, and high amount of the extracted gum and its viscosity were set as the main objectives. The optimum condition for extraction that could lead to maximum amount of responses of about 14.09% yield and 52.4 (mPas) apparent viscosity (at a shear rate of 48.9 s⁻¹) was obtained at 38.03°C, 7.68 minutes extraction (ultrasonication) time, and pH of 6.35. To validate the results, the optimized condition given by the model was checked empirically and the results were compared to the predicted responses of the model. This comparison showed the adequacy of the models (about 81% for yield and 86% for apparent viscosity).

CONCLUSIONS

The main objective of this study was to improve extraction of gum from quince seed by ultrasound assisted method and optimizing the extraction condition that would result in maximum yield and apparent viscosity of the extracted hydrocolloid. The most important variables in the extraction procedure which affected both yield and viscosity were ultra-sonication time and temperature, whereas pH was not significantly effective. Increasing time and temperature increased extraction yield, but decreased viscosity of the extracted gum.

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بهینه سازی شرایط استخراج صمغ دانه "به" با امواج فراصوت و از طریق روش سطح پاسخ

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چکیده

جهت تعیین شرایط بهینه استخراج صمغ دانه به استخراج با کمک امواج فراصوت مورد استفاده قرار گرفت و دستیابی به حداکثر بازدهی و ویسکوزیته آن بررسی شد. روش سطح پاسخ و طرح مرکب مرکزی چرخش پذیر برای طراحی آزمایشات استفاده شد و اثر درجه حرارت ($25-55^{\circ}\text{C}$)، مدت زمان استخراج (۳-۱۰ دقیقه) و pH (۶-۸) بر میزان بازدهی استخراج (%) و ویسکوزیته (میلی پاسکال) صمغ استخراج شده از دانه "به" بررسی شد. ترکیب شیمیایی صمغ استخراج شده با استفاده از روش های استاندارد AOAC تعیین گردید. ویسکوزیته ظاهری صمغ توسط ویسکومتر چرخان اندازه گیری شد. با استفاده از تجزیه و تحلیل آماری خطی چندگانه، برای هر پاسخ مدل چندجمله ای درجه دوم به دست آمد. صمغ دانه "به"، رفتار سیال غیر نیوتنی و رقیق شونده با برش نشان داد. شرایط بهینه جهت دستیابی به حداکثر راندمان استخراج و ویسکوزیته ظاهری توسط روش سطح پاسخ پیش بینی شد و این شرایط عبارت بود از درجه حرارت $38/03^{\circ}\text{C}$ ، مدت زمان استخراج با کمک امواج فراصوت $7/68$ دقیقه و pH برابر با $6/35$ که در آن میزان بازدهی و ویسکوزیته به ترتیب $14/09\%$ و $52/4$ میلی پاسکال ثانیه بود.