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OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF POLYSACCHARIDES FROM QUINCE BRANCH BY RESPONSE SURFACE METHODOLOGY (RSM)

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Abstract: *The objective of this study is to investigate the impact of ultrasonic waves on extraction yield of Quince branch crude polysaccharides (QBCP). To optimize the effects of the ultrasonic-assisted extraction (UAE) processing parameters on the extraction yield of QBCP, a response surface methodology (RSM) was employed. Four independent variables were investigated: extraction temperature of 50◦C to 90◦C, extraction time of 20 to 60 minutes, pH of 3 to 90, and solid to volume ratio of 1 to 13%. The obtained experimental data was fitted to a second-order polynomial equation using multiple regression analysis and also analyzed by appropriate statistical methods (ANOVA). The response surface methodology (RSM) showed that the significant quadratic regression equation with high R^2 (=0.9872) was successfully fitted for extraction yield of QBCP as function of independent variables. The optimum conditions for the extraction procedure of crude polysaccharides from quince branch were identified as follows: extraction temperature of 80◦C, extraction time of 30 minutes, pH equal to 4.51 and solid to volume ratio of 4.3%. Under these conditions, the experimental yield was (9.03 ± 0.98%), which is in close agreement with the value predicted by the model. In general, extracted polysaccharide has various usages in pharmacy and food industries.*

Key words: *Polysaccharide, Ultrasonic waves, RSM, Quince branch.*

1. Introduction

The history of the usage of plants and their biologically active compounds dates back to thousands of years ago. Herbal medicine has been using herbs and their extracts to treat human and animal diseases for many years. The low prevalence of non-infectious diseases and the existence of claims about the effects of some nutritional compounds on the prevention and treatment of certain

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diseases have attracted the attention of researchers to the extraction of bioactive compounds from plant sources [40]. These types of properties in plant compositions refer to vitamins, lipids, phenolic acids, proteins, and polysaccharides. Polysaccharides, due to Structural and combinational variations, can carry biological information and physiologic functions compared to other plant compounds, such as proteins and nucleic acids. Polysaccharides extracted from plant sources have a number of effects including an antiviral effect, boosting immunity system, anticancer activity, antioxidant activity, liver protection, anti-clotting effect, anti-inflammatory activity, hematopoietic activity and probiotic effect [14], [47, 48]. Polysaccharides are high molecular weight macromolecules that can be easily dissolved and dispersed in water under appropriate conditions [21]. They can modulate rheological properties of food, and are generally used as food thickeners, texture modifiers, stabilizers and emulsifiers for various usages [19], [34]. The properties of water and fat absorption of polysaccharides are physiologically very important because they can be effective in controlling obesity or blood lipid status and improving the health status [8], [10]. Thus, the extraction of bioactive polysaccharides from new sources is one of the latest subjects available to many researchers. The quince is a scientific name of (*Cydonia vulgaris* Pers., *Cydonia oblonga* Miller, *Cydonia maliformis* Miller and *Pyrus cydonia*). It is native to the east and center of Asia, and is distributed in the northern forests of Iran from Astara to Katul in Gorgan [34]. The plant belongs to the *Rosaceae* family and along with apples and pears, it is located in the *Pomoideae* family [3].

Various chemicals such as polyphenols, terpenes, organic acids, amino acids and glycosides have been isolated and identified from different parts of the plant such as leaf, fruit, stem, root, seed and skin [12, 13]. A brief review of the medical explanation showed that various parts of the quince tree were used as traditional remedies for coughing, bronchitis, nausea, fever, diarrhea, bladder inflammation, constipation, hemorrhoids, diabetes, and hypertension [18]. During the recent years, the possibility of commercial use of Quince has been studied. Powder extracted from quince branches reduces digestive problems such as diarrhea, constipation, indigestion and etc. Quince branches and leaves also strengthen the stomach and liver as well. Studies have shown that the usage of quince branch and leaf extraction affects the biochemical profiles associated with coronary heart disease. It is also beneficial for the cardiovascular system. In addition, its branch is helpful in lowering blood cholesterol, blood pressure and preventing blood clots [1].

In 1781, Francis Galton discovered the existence of ultrasound waves for the first time [9]. Ultrasonic is a producer device of sonic and sinusoidal waves, by creating a vacuum under the pressure of liquid vapor and subsequently formation of bubble within the liquid that leads to the cavitation phenomenon in the liquid. In this method, ultrasound waves with a frequency higher than 20 kHz penetrate into the material that causes elongation and expanding, resulting in the formation of cavities within the plant material [23]. Ultrasound-assisted extraction technology is the common method that is used to extract plant polysaccharides [41].

The current study is aimed at optimizing the process for ultrasonic-assisted extraction of quince branch crude polysaccharides (QBCP), using response surface methodology (RSM) and studying the effects of extraction time, extraction temperature, number of extraction and Solid to Volume ratio on the extraction yield of QBCP.

2. Materials and Methods

2.1. Materials

The quince branches were collected from the margin of forest areas in Behshahr (Abbasabad Forest), Mazandaran province, at the end of autumn. The quince branches were cleaned after collecting and were washed with distilled water, freeze-dried, ground and impurities had been separated. After drying, it was ground in a mixer and turned into a soft powder with a laboratory mill. All other chemicals and solvents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of Quince Branch Crude Polysaccharides (QBCP)

The extraction of (QBCP) was performed by using a method described by Pan et al. [30]. The quince branches (2000 g) were ground in a blender to obtain a fine powder and then were extracted for three times with 80% EtOH at 60◦C and 2h each time to defat and remove some colored materials, oligosaccharides, and some small molecule materials under reflux in the Soxhlet’s machine. The pretreated samples were separated from the organic solvent by centrifugation (3000 × g for 10 min). Each dried pretreated sample (20g) was extracted by water in a designed extraction temperature (A: 50, 60, 70, 80, 90◦C), extraction time (B: 20, 30, 40, 50, 60 min), Solid to Volume (C: 1, 4, 7, 10, 13 g/mL), and pH (D: 3, 4.5, 6, 7.5, 9). The water extraction solutions were separated from insoluble residue through the nylon cloth (pore diameter: 38m), concentrated and then precipitated by the addition of ethanol to a final concentration of 80% (v/v) to obtain QBCP. The precipitate was air-dried at the 50°C until its weight was constant, and then was weighted with a scales [30]. The percentage of polysaccharides extraction yield (%) is calculated as follows (Equation 1):

$$\text{Yield of QBCP\% (W/W)} = \frac{\text{Weight of dried QBCP polysaccharides}}{\text{Weight of Quince branches power}} \times 100 \quad (1)$$

2.2.2. Experimental Design and Statistical Analysis

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for improving and optimizing processes, and it is used to examine the relationship between one or more response variables and a set of quantitative experimental variables or factors [38], [44]. According to the results of single-factor tests, the optimal conditions for UAE of QBCP were determined by RSM with the standard four variables and five levels chosen [13]. The effects of independent variables (extraction temperature, A; extraction time, B; solid to Volume ratio, C; pH, D) on QBCP extraction yield was estimated.

The range of independent variables and their levels are represented in Table 1. In the present study, the design consisted of

30 experimental points (16 factorial points, 8 axial points, and 6 center points). All the 30 experiments were performed to study the effect of independent variables on the response and the extraction conditions were optimized. The experiments were randomized to reduce errors caused by external factors during the experimental process. The response values in each trial were analyzed using Design-Expert and fitted to a second-order polynomial regression model containing the coefficient of linear, quadratic and interaction terms and expressing a mathematical relationship between independent variables (X_1, X_2, X_3 and X_4) and response (Y):

The behavior of the system was explained by the following quadratic (Equation 2):

$$Y(\%) = \beta_i + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i\langle j=2}^{4} \beta_{ij} X_i X_j \quad (2)$$

where: Y is the measured response associated with each factor lever combination; β, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linearity, square and interaction, respectively; X_i and X_j are the coded independent variables.

An analysis of variance (ANOVA) with 95% confidence level was then carried out for each response variable in order to test the model significance and suitability.

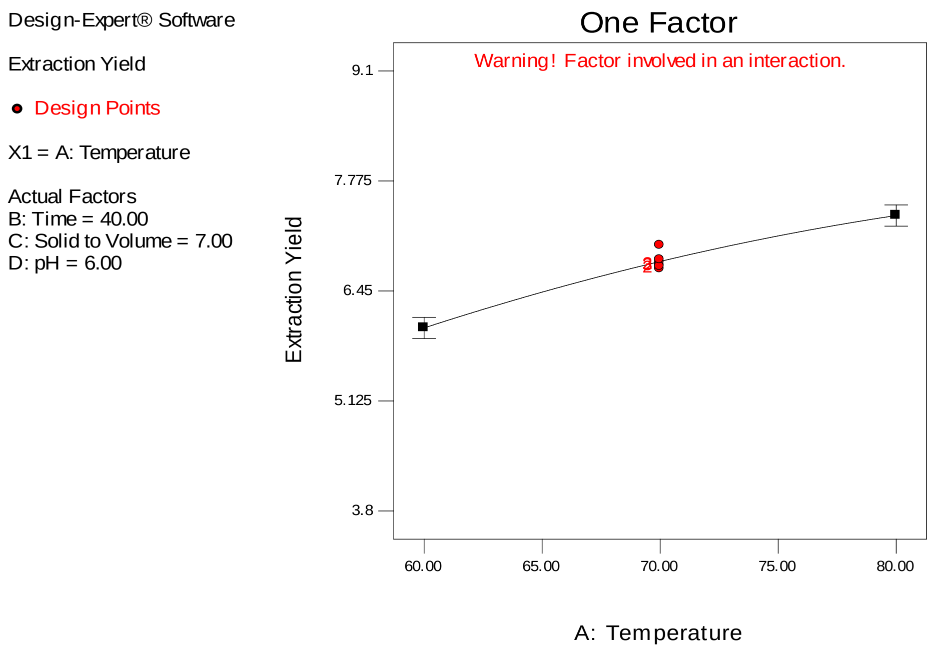
3. Results and Discussion

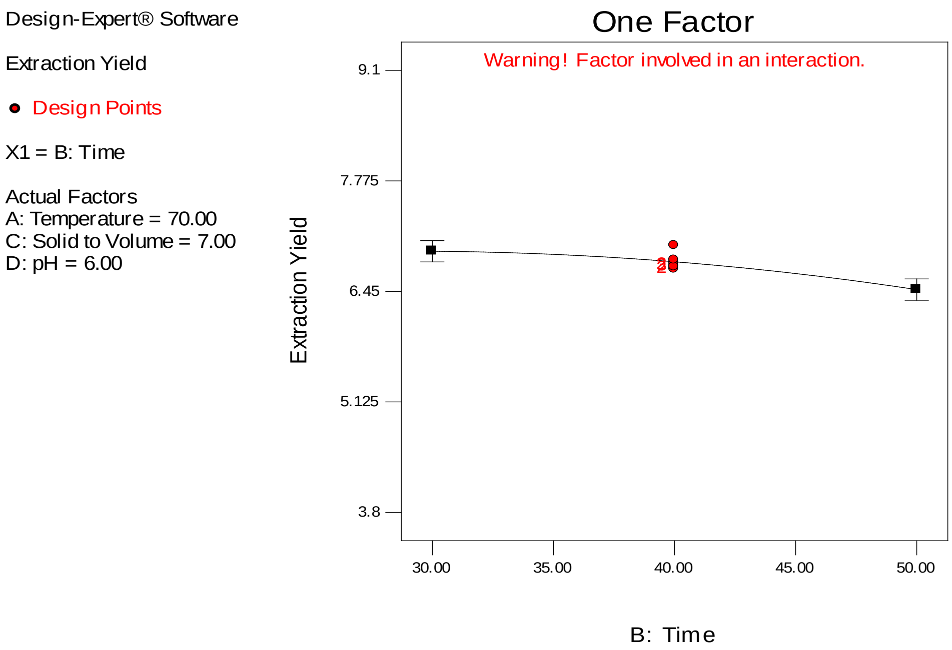
3.1. Effects of Independent Variables on Extraction Yield

3.1.1. Effect of the Extraction Temperature on QBCP Yield

In order to study the effect of different temperatures on the extraction yield of quince branch, extraction process was carried out under different temperatures (50, 60, 70, 80 and 90◦C). In this case, other factors were maintained at the central point. Three variables of extraction time, solid to Volume ratio and pH at the central point were fixed at 40 minutes, 70 ◦C and pH equal to 6, respectively. As shown in Figure 1A, the efficiency of polysaccharide extraction increases linearly as the temperature rises. A significant increase in QBCP yield was observed by increasing the extraction temperature from 60◦C to 80◦C because higher extraction temperatures can enhance enzyme catalyzing activities in favor of releasing the polysaccharides. The reason for the increase in efficiency when the temperature reaches 60◦C to 80◦C is due to increased solubility of hydrocolloids [7]. As shown in Figure 1A, the maximum extraction yield of QBCP was observed when the extraction temperature was 80◦C. The rate of interaction between raw materials and water molecules increases with extraction temperature, thereby improving the performance of polysaccharides [43].

It has also been proved that increases in temperature lead to some limitations and the cost of extraction increases [2]. In some sources, the increase in temperature has a direct effect on yield, since high temperatures reduce viscosity, accelerate molecular movement and increase the solvent conduction coefficient to the solid; consequently, it increases solubility and diffusion of material into the solution and increases the extraction yield [35]. The extraction coefficient increases with rising the extraction temperature due to the increase of the polysaccharides solubility [7]. High temperatures decrease the number of cavitation bubbles and weaken the impact of cavity collapse on homogenized samples, thus the enzyme activity decreases [29].





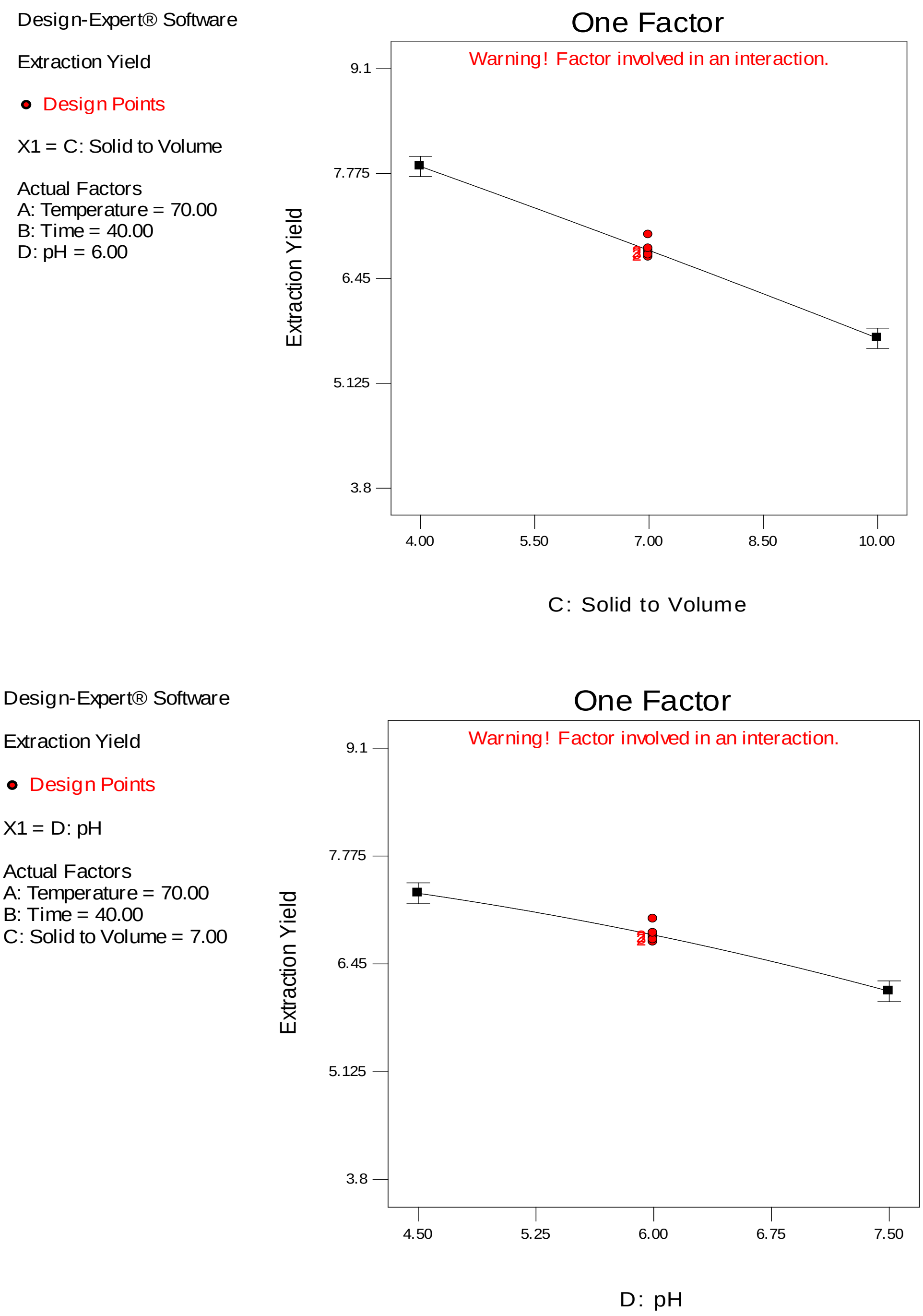


Fig. 1. *Effects of different times, temperatures, solid to volume, and pH on the extraction yield of QBCP*

3.1.2. Effect of the Extraction Time on QBCP Yield

The effect of different times on the extraction yield of QBCP is shown in Fig. 1b. Extraction was carried out under different time conditions while other extraction variables were set as follows: extraction temperature of 70°C, pH of 6, and ratio of solid to Volume of 7. As shown in Figure 1B, with increasing time from 30 to 50 minutes, the extraction yields decrease. It has been reported that longer extraction time makes a positive effect on the yield of polysaccharides and the production of hydrocolloid [22], [46]. This might be due to the time requirement of the exposure of the polysaccharides to the release medium where the liquid penetrated into the dried raw material, dissolved the polysaccharides and subsequently diffused out from the raw material. In addition, ultrasonic facilitates the diffusion of intracellular polysaccharides into the outer solvent. Long extraction time causes polysaccharides destruction [26].

3.1.3. Effect of the Extraction Solid to Volume Ratio on QBCP Yield

The effect of different ratio of solid to Volume (1, 4, 7, 10, and 13 mL/g) on the extraction yield of QBCP is shown in Figure 1C, when the other three factors (extraction temperature, extraction time, and pH) were fixed in the central points. The extraction yields of the polysaccharides significantly decreased from 7.83% to 5.77% as the ratio of solid to Volume increased from 4 to 10, due to the decrease in the driving force for the mass transfer of the polysaccharides [6]. A possible explanation is that increasing the ratio of solid to Volume may increase diffusivity of the solvent within cells and enhancing desorption of the polysaccharides from the cells [37]. According to (Figure 1C), increasing the solid to Volume ratio leads to a reduction of the extraction yield of the polysaccharide of about 5.8%. The less solubility of polysaccharides in the solvent is the reason of the enhancement of yield.

3.1.4. Effect of the Extraction pH on the QBCP Yield

Figure 1D shows the effect of pH on the QBCP extraction yield while fixing the other extraction conditions as follows: extraction time, 40; extraction temperature, 70; and solid to Volume ratio, 7. As shown in Figure 1D, by increasing pH from 4.50 to 7.50, the extraction yield decreases from 7.35 to 2.6 percent approximately. The hydrolysis of polysaccharides at lower pH condition will be further. Accordingly, by increasing polysaccharides dissolution, the extraction efficiency will be higher. Also, pH affects the activity of various enzymes [46].

3.2. Statistical Analysis and Model Fitting

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. Based on the results of single-factor tests, the independent variables and levels used in the response surface design of QBCP extracted by UAE are given in Table 1.

Table 1

Independent variables and their levels used in the response surface design

Independent variables	Factor level				
	-2	-1	0	1	2
Extraction Temperature (◦C, A)	50	60	70	80	90
Extraction Time (min, B)	20	30	40	50	60
Solid to Volume (g/ml, C)	1	4	7	10	13
pH (D)	3	4.5	6	7.5	9

Table 2

Experimental results of the response surface methodology

Run[a]	Variable levels[b]				Extraction yield (%)		Residual
	A	B	C	D	Actual value	Predicted value	
1	70	40	13	6	7.49	7.44	0.049
2	80	50	10	7.5	8.88	9.03	-0.15
3	70	40	7	3	7.34	7.31	0.035
4	70	40	7	9	8.37	8.38	-0.02
5	70	40	7	6	5.87	6.06	-0.19
6	60	50	4	4.5	7.69	7.23	0.46
7	70	40	7	6	5.26	5.2	0.061
8	60	30	4	4.5	5.67	5.85	-0.18
9	70	20	7	6	6.01	5.93	0.08
10	60	50	4	7.5	7.96	7.98	-0.016
11	80	30	10	7.5	5.97	6.39	-0.41
12	80	50	10	4.5	8.02	7.92	0.091
13	80	30	4	7.5	4.18	4.12	0.065
14	90	40	7	6	5.61	5.75	-0.14
15	50	40	7	6	3.89	3.84	0.049
16	60	30	10	4.5	4.96	4.96	-4.03E-03
17	70	40	7	6	5.06	4.96	0.1
18	60	30	10	7.5	7.62	7.67	-0.048
19	70	40	1	6	6.74	6.85	-0.11
20	70	40	7	6	6.09	5.93	0.16
21	70	40	7	6	9.02	8.88	0.14
22	60	50	10	7.5	4.45	4.53	-0.088
23	80	30	10	4.5	7.59	7.65	-0.058
24	60	30	4	7.5	5.36	5.24	0.11
25	80	30	4	4.5	6.76	6.8	-0.048
26	60	50	10	4.5	6.79	6.8	-0.012
27	70	60	7	6	6.71	6.8	-0.091
28	80	50	4	7.5	6.74	6.8	-0.062
29	70	40	7	6	7	6.8	0.19
30	80	50	4	4.5	6.82	6.8	0.02

[a] Experiments were conducted in a random order.

[b] A: Temperature (◦C), B: Time (min), C: Solid to Volume (ml/g), D: pH

All 30 random sequential experiments under various extraction conditions are performed to study the reciprocal influence of independent variables (extraction temperature, extraction time, pH and solid to Volume ratio) on the QBCP extraction yield and to optimize the operating parameters. The actual and predicted values according to the factorial design are presented in Table 2. Correlation analysis between predicted values and actual values can evaluate the suitability of the response surface model [24].

Applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial (Equation 3):

$$\begin{aligned} Y(\%) = 6.80 + 0.68A - 0.23B - 1.09C - 0.60D - 0.13AB + 0.10AC + 0.12AD - \\ - 0.18BC + 0.15BD - 0.11CD - 0.12A^2 - 0.10B^2 - 0.025C^2 - 0.090D^2 \end{aligned} \tag{3}$$

where A, B, C and D are extraction temperature, extraction time, solid to Volume ratio and pH, respectively.

The fit statistics of extraction yield for the selected quadratic predictive model is shown in Table 3. The Analysis of variance (ANOVA) showed that the lack of fit relative to pure error at the 95% confidence level was not significant, thus it indicates that the model can be used to predict the polysaccharides extracted from quince branch [24]. ANOVA was performed to investigate the adequacy of the suggested model (Eq. (3)) and identify the significant factors. The line of the best fit with the slope of 0.9872 and R2=0.9872 were obtained, indicating 98.72% of the variations could be explained by the fitted model. R2adj is necessary for considering the influence of a number of independent variables [39]. For a robust statistical model, Adj-R2 should be close to R2 [42]; here, the value Adj-R2 was 0.9753, which indicates that 97.53% of the total variation in the yield was attributed to the experimental variables studied. The Adj-R^2 is the correlation measure for testing the goodness-of-fit of the regression equation [17]. In addition, Adj-R2, Pre-R2 and the coefficient of variation (C.V.) are calculated to check the model adequacy. Pre-R2 is 0.9308, which is in a reasonable agreement with the Adj-R2 of 0.9753 (Adj-R^2, Pre-R^2<0.2), and indicates a high degree of correlation between the observed and predicted data from the regression model [11]. The coefficient of variation (C.V.) was 3.23%, indicating the reliability of the experimental values (<5.00%) [36]. The F-test showed the model had a very high F-value (82.88) and a very low P-value, indicating this model was highly significant [32]. The F-value (5.95) and the P-value (0.0314) of the lack-of-fit test showed there was no lack-of-fit factor in the model (Table 3). The low PRESS 3.63 value suggests for the adequacy of the fitted quadratic models for predictive applications. Adequate precision measures the signal-to-noise ratio where a ratio greater than 4 is desirable [27]. Therefore, the ratio of 34.761 indicates an adequate signal. This model can be used to navigate the design space [15].

As shown in Tables 4 and 5, the independent variables (A, B, C, D), interaction effects (AB, AC, AD, BC, BD, CD) and quadratic effects (A^2, B^2, C^2 and D^2) were significant, with very small P-values (P<0.05). The other term coefficients were not significant (P>0.05). Therefore, the regression equation can be used to describe the real relationship between the variables and the yield of QBCP.

Table 3

The significance of each response variable effect showed by using F ratio and P-value in the nonlinear second order model

	Variables	DF[a]	SS[b]	MS[c]	F-value	p-value
Linear effects	A	1	11.00	11.00	246.89	< 0.0001
	B	1	1.27	1.27	28.47	< 0.0001
	C	1	28.28	28.28	634.58	< 0.0001
	D	1	8.69	8.69	195.12	< 0.0001
Interaction effects	AB	1	0.26	0.26	5.89	0.0283
	AC	1	0.17	0.17	3.92	0.0663
	AD	1	0.22	0.22	4.84	0.0440
	BC	1	0.54	0.54	12.02	0.0034
	BD	1	0.35	0.35	7.85	0.0134
	CD	1	0.19	0.19	4.17	0.0592
Quadratic effects	A^2	1	0.42	0.42	9.38	0.0079
	B^2	1	0.30	0.30	6.63	0.0211
	C^2	1	0.017	0.017	0.38	0.5448 ns
	D^2	1	0.22	0.22	4.97	0.0415

[a] Degrees of Freedom; [b] Mean Sum of squares; [c] Mean sum of squares.

Table 4

Testing the significance of the regression coefficients associated with different experimental factors

Factor[a]	**Coefficient estimate**	**DF**	**Standard error**	**95% CI low**	**95% CI high**	**F-value**	**p-value[b]**
Intercept	6.80	1	0.086	6.62	6.99	-	-
A	0.68	1	0.043	0.59	0.77	246.89	< 0.0001 **
B	−0.23	1	0.043	−0.32	−0.14	28.47	< 0.0001 **
C	−1.09	1	0.043	−1.18	−0.99	634.58	< 0.0001 **
D	−0.60	1	0.043	−0.69	−0.51	195.12	< 0.0001 **
AB	−0.13	1	0.053	−0.24	−0.016	5.89	0.0283 *
AC	−0.10	1	0.053	−0.22	7.983E-003	3.92	0.0663 *
AD	0.12	1	0.053	3.580E-003	0.23	4.84	0.0440 *
BC	-0.18	1	0.053	-0.30	-0.071	12.02	0.0034 *
BD	0.15	1	0.053	0.035	0.26	7.85	0.0134 *
CD	-0.11	1	0.053	-0.22	4.733E-003	4.17	0.0592 *
A^2	-0.12	1	0.040	-0.21	-0.038	9.38	0.0079 **
B^2	−0.10	1	0.040	−0.19	−0.018	6.63	0.0211 *
C^2	−0.025	1	0.040	−0.11	0.061	0.38	0.5448 ns
D^2	−0.090	1	0.040	−0.18	−3.941E-033	4.97	0.0415 *

* 0.01 ≤ p < 0.05; ** p < 0.01

[a] A: extraction temperature (°C), B: extraction time (min), C: Solid to Volume (ml/g), D: pH;

[b] p<0.05 indicates statistical significance. ns = not significant at p≤0.05.

Table 5

Analysis of variance (ANOVA) for the fitted models

	DF[a]	Coefficient	Sum of Squares	Mean Square	F-value	p-value
Model	14		51.70	3.69	82.88	< 0.0001
Residual	15		0/083	0.012		
Lack of Fit	10		0.62	0.062	5.95	0.0314 ns
Pure Error	5		0.052	0.010		
Cor Total	29		52.37			
R^2		0.9872				
Adj- R^2		0.9753				
C.V		3.23				
PRESS		3.63				
Standard deviation		0.21				
Adeq Precision		34.761				

[a] Degrees of Freedom

3.3. Effect of Parameter Interactions on the QBCP Extraction Yield

Response surface plots showing the effects of the variables were presented in Figure 2, according to the regression analysis. To visualize the combined effects of two operational parameters on the extraction yield, the response was generated as a function of two independent variables: triaxial response surfaces and planar contour plots, and was determined by using the Design Expert software. Two variables were kept constant at their respective central test range values and the other two variables varied within their experimental ranges in order to understand their main and interactive effects on the dependent variables. We could evaluate the effects of independent variables and their interactions with regard to the QBCP extraction yield based on the above graphical representation [44]. Moreover, we could also find the extreme value of extraction conditions from the highest peak of response surface plots and the center point of contour plots.

As Figure 2A shows, the QBCP extraction yield was influenced by the ultrasonic temperature and the ultrasonic time. At the central point, the other two variables (solid to Volume ratio and pH) were fixed at 7 and 6 respectively. Accordingly, ultrasonic temperature had a residual effect on efficiency. As the temperature increases up to 80°C, the yield increases linearly in the range of X variations. The reason for increasing the yield with temperature enhancement was probably because of mass transition improvement due to increasing polysaccharide solubility and reducing viscosity of solvent [4, 5]. Also, by increasing ultrasonic time from 30 to 50 minutes, there was no significant effect on polysaccharide extraction yield. According to the given significant linear and quadratic effects of ultrasonic temperature ($P \leq 0.01$), the presence of curvature in the form of the procedure and contour is expected.

Figure 2B shows the effects of ultrasonic temperature (A) and pH (D) on the QBCP yield and their interactions while the other two variables (ultrasonic time and solid to Volume ratio) were fixed at 40°C and 7 respectively. Accordingly, by increasing the ultrasonic temperature from 60◦C to 80◦C, the extraction yield increases. Increasing the extraction temperature accelerates the solvent evaporation, increasing energy costs and enhancement of extraction impurities [16]. Also, as pH increases from 4.5 to 7.5, the extraction yield is significantly reduced. Additionally, the viscosity decreases as the temperature and pH decrease. Therefore, it can be concluded that by increasing the temperature along with the pH reduction, the polysaccharide extraction yield increases.

As shown in Figure 2C, when the ultrasonic time and pH were fixed, the ultrasonic temperature (A) and ratio of solid to Volume (C) had an analogical effect on the QBCP yields. Therefore, by increasing ultrasonic temperature from 60◦C to 80◦C, as well by as increasing the ratio of solid to Volume from 4 to 10, the extraction yield was reduced. The high temperature leads to increasing surface tension and vapor pressure in the small bubble that causes the ultrasonic waves to fluctuate.

Figure 2D shows the interaction effect of ultrasonic time and pH on the QBCP yields while the other two factors were fixed as follow: ultrasonic temperature 70◦C, ratio of solid to Volume 7. Accordingly, with the increase of the ultrasonic time from 30 to 50 minutes, the extraction yield was reduced to a low level and reached 6.5 approximately. Also, as pH increases from 4.5 to 7.5, the extraction yield decreased significantly and reached about 6. Increasing the extraction time resulted in reduced yield, since long periods of time causes the destruction of the molecule. Therefore, shorter periods can be considered more appropriate. It can be concluded that by decreasing the temperature along with the decrease of pH, the yield of the extraction of polysaccharides increases.

In Figure 2E, when the 3-D response surface plot and the contour plot were developed for the extraction yield of QBCP with varying extraction time and ratio of solid to Volume at fixed ultrasonic temperature 70◦C and the pH 6, it can be seen that the yield of QBCP decreased and approximately reached 6 with the increase of the solid to Volume ratio from 4 to 10. Accordingly, by increasing ultrasonic time from 30 to 50 minutes, no significant effect on polysaccharide extraction yield was observed. By increasing the ratio of solid to Volume at the time to reach the balance, concentration increases ($P<0.05$) as well as the resulting polysaccharide further extraction. In general, it can be said that by reducing the solid to Volume ratio, the polysaccharide extraction yield increases.

Figure 2F illustrate the 3D response surface plot and the contour plot at varying pH and solid to Volume ratio at fixed ultrasonic temperature (70°C), ultrasonic time (40 minute). It indicated that the maximum extraction yield of QBCP can be achieved when pH and the ratio of solid to Volume were 4/5 and 4, respectively. As the solid to Volume ratio increases, the extraction yield decreases. One possible explanation is that increasing the ratio of solid to Volume may increase the penetration of the solvent into the cell and improve the removal of the polysaccharides from the cell. However, the pH had no significant synergistic effect on polysaccharide yield. A desirability ramp was developed from optimal points via the numerical optimization technique.

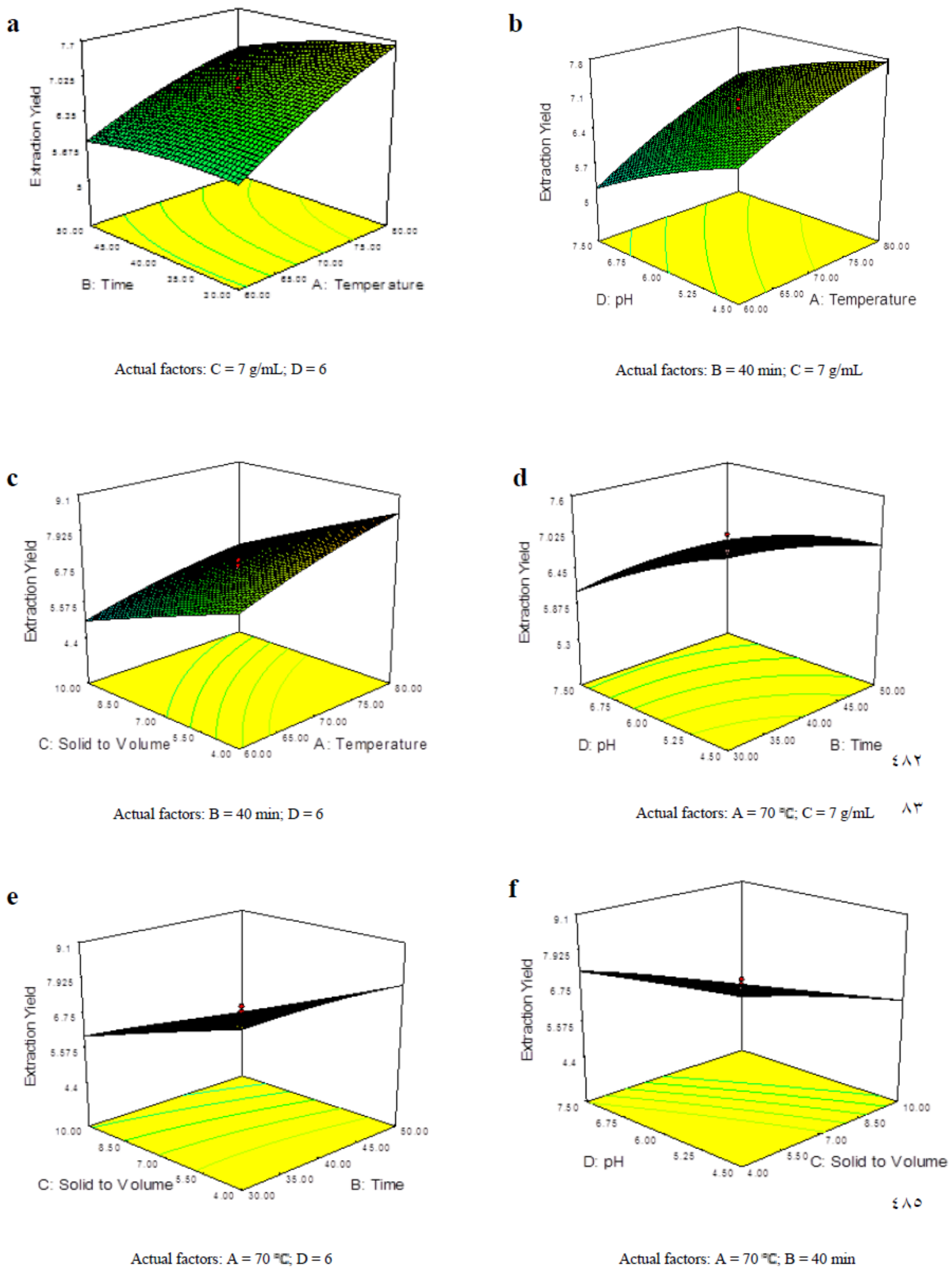


Fig. 2. *Response surface plots (3D) showing the interactive effects of extraction temperature (A), extraction time (B), solid to volume ratio (C), and pH (D) on the QBCP extraction yield*

A. Model adequacy checking

Generally, it is necessary to check that the fitted quadratic polynomial model gives a sufficient approximation to the actual values. Unless the model shows an adequate fit, proceeding with an investigation and optimization of the fitted response surface likely gives poor or misleading results [48]. In addition, to the determination of the coefficient, the adequacy of the models was also evaluated by the residuals [25]. The residuals from the least squares fit play an important role in judging model adequacy [27]. As shown in Figure 3, the normal probability plot is a suitable graphical method for judging residuals normality. The normality assumption was satisfied as the residual plot approximated along a straight line. Figure 4 shows that the residuals scatter randomly on the display, suggesting the variance of the original.

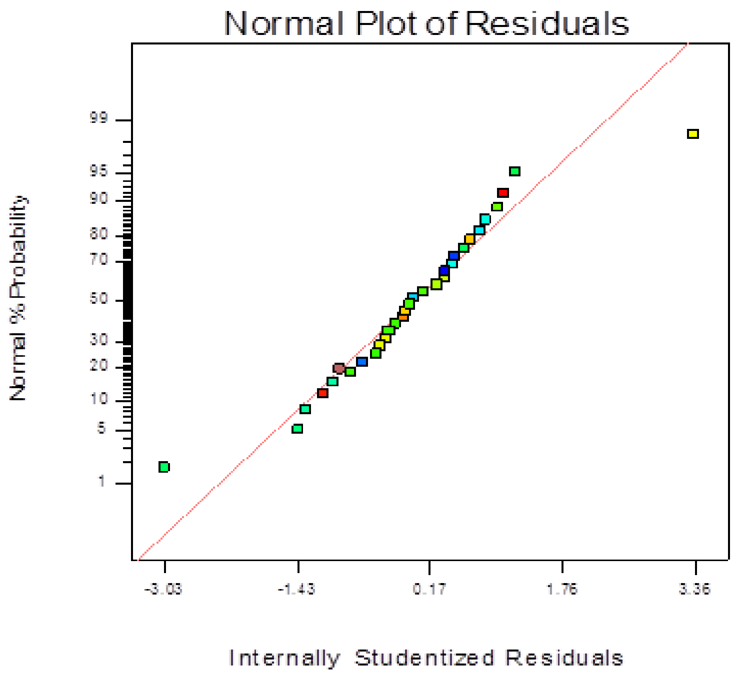


Fig. 3. *Normal probability of internally studentized residuals*

The point that the residuals scatter randomly on the display suggests that the variance of the original observation is constant for all values of Y. Both of the plots (Figures 3 and 4) are satisfactory, so we conclude that the empirical model is adequate to describe the QBCP extraction yield by response surface.

B. Verification of predictive model

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. Good agreement must exist between the values predicted using the model equations and the experimental values at the points of interest. To ensure that the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition [45]. The optimal values of the selected variables were obtained by solving the regression equation using the Design-Expert software. The suitability of the model equation for predicting optimum response value was investigated under the following optimal conditions: extraction temperature of 80°C, extraction time of 30.03 min, pH of 4.51 and ratio of solid to Volume of 4.3 mL/g (Table 6).

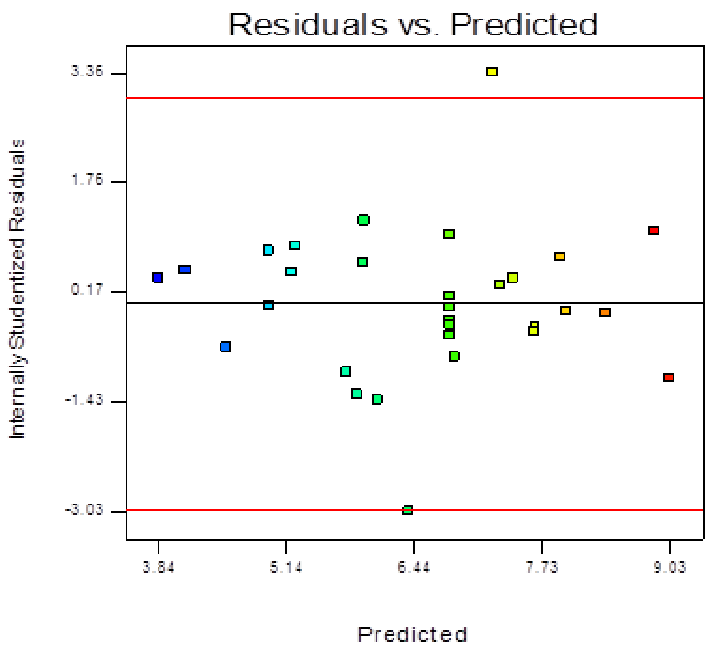


Fig. 4. *Plot of internally studentized residuals vs. predicted response*

Table 6

Predicted and experimental values of the responses at optimum conditions

Optimum Condition				Extraction yield (%)	
Extraction Time	Extraction Temperature	pH	Solid to Volume	Experimental	Predicted
30.03	80	4.51	4.0	9.03± 0.098[a]	9.02004

[a] Mean ± standard deviation (N=3).

The conditions were determined to be optimum by the RSM optimization process and were also used to predict the values of the response. Under these conditions, the experimental extraction yield of QBCP was (9.03 ± 0.98%), which agreed with the predicted value 9.02004%. No significant difference (*P*>0.05) was found between the experimental and the predicted values. Therefore, the results indicated the suitability of the model employed and the success of RSM in optimizing the extraction conditions. The results indicated that the experimental values were in accordance with the predicted ones, and also suggested that the regression model was accurate and adequate for the extraction of QBCP.

4. Conclusion

Extraction of biologically active polysaccharides of new sources is one of the most important issues for many researchers. Currently, extraction with ultrasonic waves is a dynamic development topic in applied research and industry. The advantages of this method are the cheapness, simplicity and efficiency, effective degradation of plant tissue, penetration of solvent into it, increasing extraction yield and increasing reaction speed. In this study, the effect of ultrasonic waves on extraction temperatures (50◦C to 90◦C), extraction times (20 to 60 minutes), different pH (3 to 9) and various ratios of solid to Volume (1 to 13 ml/g) on the field of polysaccharide extraction were studied using the RSM method. The effect of independent variables of ultrasonic time, pH, and solid to Volume ratio in the evaluated surfaces on the efficiency of the polysaccharide extraction has a decreasing effect, but the effect of ultrasonic temperature on incremental extraction yield has an increasing effect. The maximum yield is achieved in the ratio of solvent to low dry matter and high temperature. Based on the single factor experiments, RSM was used to determine the optimal process parameters that gave a high extraction yield. The results of the research indicate the usefulness of the methodology of response procedure in optimizing the polysaccharide extraction process from quince branch. ANOVA showed that the effects of extraction temperature (◦C), extraction time (min), pH and the ratio of solid to Volume (g/ml) were significant and quadratic models were obtained for predicting the response. The optimum conditions for polysaccharide extraction yield were investigated using the numerical optimization technique with Design Expert 8 software. The interaction of two factors on polysaccharide extraction yield was studied, while the other two variables were fixed at the central point. The optimum conditions for the extraction procedure of crude polysaccharides from quince branch were identified as follows: extraction temperature of 80◦C, extraction time of 30 minutes, pH of 4.51 and solid to Volume ratio of 4.3%. Under these conditions, the experimental yield of polysaccharides was (9.03 ± 0.98%), which was close to the predicted yield value (9.02004%). The results of this study indicated that extracted polysaccharides from quince branch can be considered a potential source of natural antioxidants.

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