Chemical Modification of Insoluble Fraction of Persian Gum (Mountain Almond tree gum)

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Abstract: Persian gum, mountain almond tree (*AmygdalusScoparia*) exudate, is a transparent edible gum which can be found in different forests of Iran (Fars and Azarbayjan Provinces). This gum has and could have many pharmaceutical, food and industrial applications. However, it is not used in a commercial practice in food production. This gum consists of soluble (% 25–30) and insoluble fractions (% 70–75). Therefore, in the present study, with a view to utilize the gum for broader applications, modification of insoluble fraction was carried out using acrylamide in the presence of sodium hydroxide under different reaction conditions. The effect of insoluble fraction of Persian gum (0.5–1.5g) and acrylamide concentration (0.08–0.112 mol), temperature (30–60 °C) and time (1–3 h) on solubility increasement, was investigated using response surface methodology (RSM). Maximum solubility (>65%) was obtained in 1.5 % on dry basis of soluble fraction of Persian gum, 0.08 mol acrylamide at 60°C for 3 h. Intrinsic viscosity and molecular weight determination proved modification reaction led to reduction in molecular weight. In addition, effect of pH and electrolytes on rheological properties showed soluble fraction of optimum modified gum is an anionic hydrocolloids.

***Keywords*: Persian gum; Gum modification; Solubility; Intrinsic viscosity; Rheological properties.**

1. Introduction

Persian gum, mountain almond tree (*Amygdalus scoparia*) exudate, is a transparent edible gum which can be found in different forests of Iran (Fars and Azarbayjan Provinces). This gum has and could have many pharmaceutical, food and industrial applications. However, it is not used in a commercial practice in food production. The structure of the back bone of gum, β– (1→3) linked D–galactopyranose with random distribution of β– (1→6) linked D–galactopyranose and α– (1→ 3) linked L–arabinofuranose units as side chain (G/A:2.1), was stablished by Smith hydrolyse and NMR studies [11]. This gum consists of soluble (% 25–30) and insoluble fractions (% 70–75) [1]. Therefore, the present study aims to chemically modify the insoluble fraction of Persian Gum to improve the solubility and make it more useful/ industrially exploitable. Chemical modification of insoluble fraction was carried out using acrylamide in the presence of sodium hydroxide under different reaction conditions.

Response surface methodology (RSM) is a collection of mathematical and empirical techniques useful for establishing models, and for optimizing processes even in the presence of complex interactions [8,9]. This procedure not only determines the interaction between parameters, but also reduces the number of experimental trials, development time and overall cost [10]. In the current study, a central composite design (CCD) was applied to assay the effect of four independent variables–Insoluble fraction of persian gum (0.5–1.5 %), Acrylamide content (0.084–0.112 mol), Temperature (30–60 °C) and Time (1–3 h) –on the response variable (solubility increasement of insoluble fraction). Therefore, the objective of this work was to find an optimum quantity of independent variables for accessing the maximum solubility increasement.

2. Materials and Methods

2.1. Materials

Persian Gum was obtained from Azarbayjan province forests. Sodium hydroxide, acrylamide and acetic acid were purchased from Merck Chemicals Co (Darmstadt, Germany). Ethanol was provided by Ghadir chemical company (Ghom, Iran) and methanol was purchased from Dr. Mojallali chemical industries (Tehran, Iran).

2.2. Methods

2.2.1. Chemical Modification

In this investigation, 21 mixtures were prepared. The modification reaction was performed as follows: insoluble fraction of Persian gum (0.5–1.5 g on dry basis) was dispersed in 25 ml distilled water and stirred, then 25 ml NaOH aqueous solution was added, the end pH was 14 (optimum pH for reaction). After 10 minutes, acrylamide (AA) (0.084–0.112 mol) was added with stirring. At this end, the reaction was allowed to proceed for the desired temperature (30–60 °C) and time (60–180 min). These components were prepared for the optimization procedure based on a four–factor central composite design (CCD) (Table 1). The reaction mixture was cooled and neutralized with glycial acetic acid and precipitated by pouring the reaction contents in the ethanol with stirring. The precipitated product was separated, and washed twice with aqueous methanol (80:20) followed by pure methanol. The products were initially dried at room temperature followed by in electric oven at 60±2 °C for 2h [6].

Table 1. Matrix of the central composite design (CCD) and experimental data obtained for the response variables.

Run	Block	Independent variables				Response variables	
		Insoluble fraction of Persian gum (x_1, g)	Acrylamide (x_2, mol)	Temperature (x_3, °C)	Time (x_4, h)	Solubility (%)	N (%)
1	1	0.5	0.08	30	1	20	0.572
2	1	1	0.10	45	2	30.2	0.657
3	1	1.5	0.08	60	3	64	0.85
4	1	1	0.10	45	3.68	27	0.67
5	1	0.16	0.10	45	2	18	0.52
6	1	0.5	0.08	60	1	54	0.32
7	1	1	0.1	45	2	29.2	0.65
8	1	1.5	0.11	60	1	24.2	0.9
9	1	0.5	0.11	30	3	34	0.56
10	1	1	0.10	19.77	2	24	0.71
11	1	1.5	0.11	30	1	22	0.91
12	1	1	0.07	45	2	36.4	0.9
13	1	1	0.10	70.23	2	28	0.87
14	1	0.5	0.11	60	3	35	0.324
15	1	1	0.10	45	0.32	36	1.3
16	1	1.5	0.08	30	3	24	0.7
17	1	1	0.12	45	2	27.4	0.678
18	1	1	0.10	45	2	30	0.685
19	1	1	0.10	45	2	30.4	0.7
20	1	1.84	0.10	45	2	37	0.9
21	1	1	0.10	45	2	29.6	0.86

2.2.2. Analysis and measurement

- Nitrogen content

The nitrogen content of the derivative product was determined by Kjeldahl method.

- Solubility

In this experiment, the modified gums were grinned and modified gums powder were disolved in distilled water (2%). They were kept for 24 h at 10 °C and centrifuged (14000 rpm for 15 min). The solubility increasement was calculated as follows:

$$\text{solubility} = \frac{\text{dry material content in supernatant (g)}}{\text{initial drymaterial content (2 g)}} \times 100$$

- Intrinsic viscosity

Intrinsic viscosity of soluble fraction of Persian gum and soluble fraction of modified gum at optimum conditions were determined by a Canon-Fenske glass capillary viscometer. Tests were performed at 25 ± 0.1 °C using a circulating water bath.

Reduced viscosity was calculated as follows:

$$\eta_{red} = (\eta - \eta_0)/\eta_0 c$$

Where η is the sample viscosity, $\eta_0$is the viscosity of the solvent (distilled water) and c is the concentration of gum dispertion (g/ml). Intrinsic viscosity [η] was calculated by linearly extrapolating the reduced viscosity to zero concentration from concentrations of 1.87×10^{-4} – 1.87×10^{-2}%.

- Molecular weight determination

The molecular weights of soluble and insoluble fraction of Persian gum and soluble fraction of modified gum at the optimum conditions were determined by means of a Zetasizer (Nano ZS, Malvern Instruments, UK) with the following specifications: standard medium, Toluene; refractive index, 1.5; scattering angle, 90°; temperature, 25°C.

- Rheological properties

The pH values of dispersions were adjusted at 3 and 11 by 0.1 M HCl and 0.1 M NaOH solutions respectively (natural pH was 5). Moreover, the different electrolyte concentrations at similar ionic strength were adjusted using 3 M NaCl and 1 M $CaCl_2$ solutions. Then, their rheological properties were measured using Brookfield rheometer (LV DV3 Ultra).

- FT-IR spectroscopy

The chemical structure changes were examined by FT-IR (Fourier transform infrared) 'PerkinElmer Spectrum 10.03.06' analysis.

- Acrylamide determination

Residual acrylamide in soluble fraction of modified gum was determined using a HPLC- system (AGILLENT 6410 QQQ, America) coupled with a mass spectrometer. Analytical separation was achieved using a zorbax c_{18} 5 µm (250×4 mm) column. The elution mode was isocratic, using a mixture of acetonitrile and water (3: 97, v/v) as LC

eluent. The flow rate was 0.5 ml/min and the injection volume was 30 µl.

- Experimental design and statistical analysis

RSM was used to determine the effect of four most significant reaction mixtures namely insoluble fraction of Persian gum (0.5–1.5 g, x_1), AA (0.084–0.112 mol, x_2), temperature (30–60°C, x_3) and time (1–3 h, x_4) on solubility increasement of insoluble fraction (Y_1). Twenty one treatments were conducted based on the central composite design (CCD), each at three coded levels -1, 0 and 1 (Table 1). Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The responses functions (y) were related to the coded variables (x_i, i=1, 2, 3, 4) by a second-order polynomial using equation below:

$$Y= b_0+ b_1 X_1 \square b_2 X_2+ b_3 X_3 \square b_4 X_4 \square b_1b_2 X_1X_2+b_1b_3 X_1X_3+ b_1b_4 X_1X_4 \square b_2b_3 X_2X_3+ b_2b_4 X_2X_4 + b_3b_4 X_3X_4 \square b_{11} X_1^2+ b_{22} X_2^2 \square b_{33} X_3^2+ b_{44} X_4^2$$

The coefficients of the polynomial 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).

The correlation between the response and independent variables can be readily seen in the response surface and contour plots. These plots show the simultaneous interaction of two factors on the responses and find the location of optimum experimental variables [3].

3. Results and discussion

3.1. Preliminary investigation

The aims of preliminary tests were to (1) determine the effect of pH on modification reaction and then (2) to find the best pH leading to more solubility of insoluble fraction of Persian gum. The findings showed that the most solubility was resulted in pH=14 (data not shown).

3.2. Model fitting and statistical analysis

The negative or positive effects of two independent variables– Insoluble fraction of Persian gum (x_1), acrylamide (x_2), temperature (x_3) and reaction time (x_4)–on dependent variables (Y) were considered using RSM, and their interactive relationship was studied. Analysis of variance (ANOVA) was performed to investigate the adequacy of the suggested models and identify the significant factors. The results showed that the solubility increasement was directly related to the linear effect of gum concentration (p=0.003) and temperature (p<0.0001), the mutual interaction of gum and acrylamide concentration, gum concentration and temperature, acrylamide concentration and temperature and reaction temperature and time were also significant. The independent and dependent variables were fitted by the second-order polynomial equation to the experimental data as shown below:

$$Y= 29.42+ 4.75 A \square 2.25 B+ 4.25 C \square 2.25 D \square 9.38 AB+ 4.12 AC+ 0.63 AD \square 5.35 BC+ 2.10 BD + 3.85 CD \square 0.16 A^2+ 0.94 B^2 \square 0.54 C^2+ 0.84 D^2$$

Fig.1 shows the surface plot for solubility increasement as a function of significant mutual interaction of independent variables.

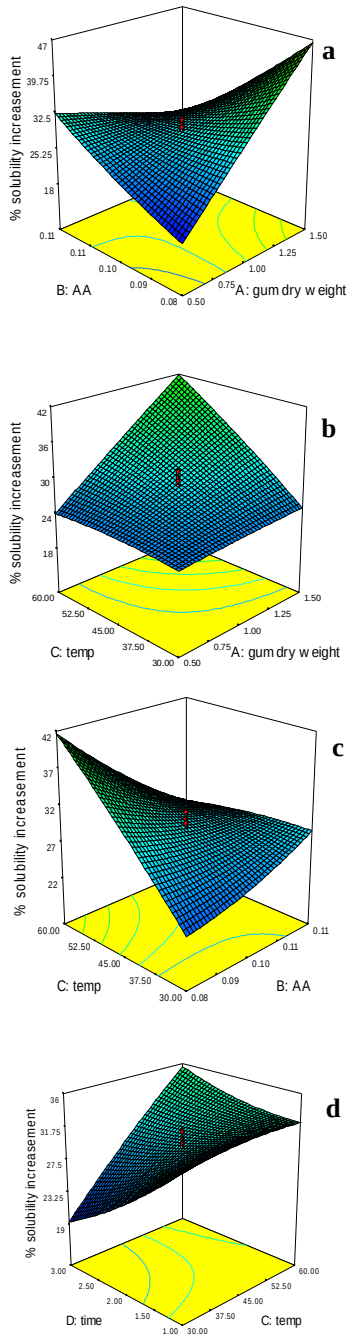

Fig. 1. 3D plots showing the effect of significant mutual interaction of independent variables on solubility increasement.

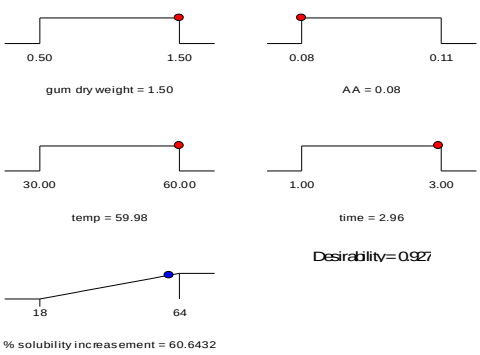

Fig. 2. Schematic representation of the optimum values of factors, response, and the corresponding levels.

3.3. Optimization

Numerical procedures were carried out for predicting the optimum level of independent variables to obtain maximum solubility increasement. The RSM package response optimizer determined the overall optimum region to be at 1.5 g on dry basis insoluble fraction of Persian gum, 3 mol acrylamide, 59.98 °C for 2.96 h. Fig. 2 shows the results of the optimization. According to this figure, the corresponding predicted response values under the optimum conditions for solubility increasement was %60.6432.

3.4. FTIR spectroscopy

From the FTIR spectra (Fig. 3) , it is evident that soluble fraction of modified gum shows a broad band at 3373 cm-1 for OH stretching vibrations, and symmetrical and asymmetrical N–H stretching vibrations, which are merged with OH group stretching vibrations. Another band appears at 1575 cm^{-1} due to the amide I band of carbonyl stretching and N–H bending. The peak at 1058 cm-1 is also observed due to C–O–C stretching vibration of the ether group. On reacting gum with acrylamide in the presence of sodium hydroxide using the wet process the following reaction are expected to occur [5].

Gum □ OH + CH_2= CH □ $CONH_2$ → Gum □ O □ CH_2 □ CH2 □ $CONH_2$ (1)

Gum □ CH_2 □ CH_2CONH_2 + H_2O → Gum □ CH_2 □ CH_2 □ COONa + NH_3 (2)

CH_2= CH □ $CONH_2$ + H_2O → CH_2 = COONa + NH_3 (3)

CH_2= CH □ $CONH_2$ + H_2O → CH_2= CH □ COONa + NH_3(4)

CH_2 = CH □ $CONH_2$ + H_2O → HO □ CH_2 □ CH_2 □ $CONH_2$ + H_2O (5)

CH_2 = CH □ $CONH_2$+ HO → CH_2 □ CH_2 □ $CONH_2CONH_2$ □ CH_2 □ CH_2 □ O □ CH_2CH_2 □ $CONH_2$ (6)

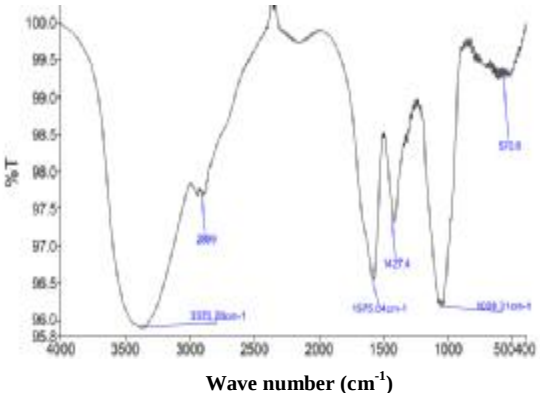

Fig.3. FT-IR spectrum of modified gum

3.5. Intrinsic viscosity

Intrinsic viscosity of soluble fraction of Persian gum and soluble fraction of modified gum at the optimum conditions at C 0, as determined by linear extrapolation of η_{red}, are shown in Fig. 4. Chemical modification of Persian gum resulted in decrease in intrinsic viscosity from 876.185 to 441.225 ml/g. The modification treatment led to reduction in molecular weight of Persian gum (from 134 to 42.4 KDa). That could be a reason for lower intrinsic viscosity and solubility increasement.

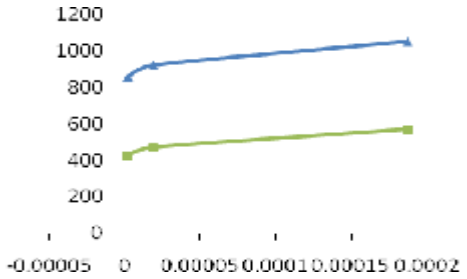

Fig. 4. Reduced viscosities of soluble fractions of Persian gum ( ) and optimum modified gum ( ) in the concentration range 1.87×10^{-4}–1.87×10^{-2} %.

3.6. Rheological properties

3.6.1. Influence of pH

Fig. 5 displays the influences of pH on the steady flow viscosity of soluble fraction of modified gum at the optimum conditions. As can be seen, the viscosity increases at pH 3–5, and almost remains constant at pH 5–11. The effect of pH on the viscosity of polysaccharide dispersions has been widely reported [2,4,7,12,14]. But for polysaccharide dispersions such as flaxseed gum [4] and konjac gum [12], the initial increase of the viscosity with pH increasing was attributed to the ionization of the carboxyl groups in the gum molecules leading to the extension of molecules (There is 10% uronic acids units in Persian

gum and expected reactions may cause carboxyl group formation).

3.6.2. Influence of electrolytes

Fig. 6 shows the effects of electrolyte (NaCl and $CaCl_2$) on the steady flow viscosity. It can be seen that the addition of electrolytes causes decrease of viscosity. The decrease of viscosity arises from the screening effect of the electrolyte on the intermolecular H-bonding and the intramolecular electrostatic repulsion among negatively charged groups in anionic gum molecules[4]. The screening effect of the electrolyte on the intermolecular H-bonding can cause the decrease of the three-dimensional network strength while that on the intramolecular electrostatic repulsion may cause the curling (weak due to the steric hindrance) of gum coils, resulting in the decrease of the viscosity of the gum solution [7]. Therefore, soluble fraction of Persian gum may be an anionic hydrocolloid and can be used in acidic milk beverages like mixture of milk–orange juice.

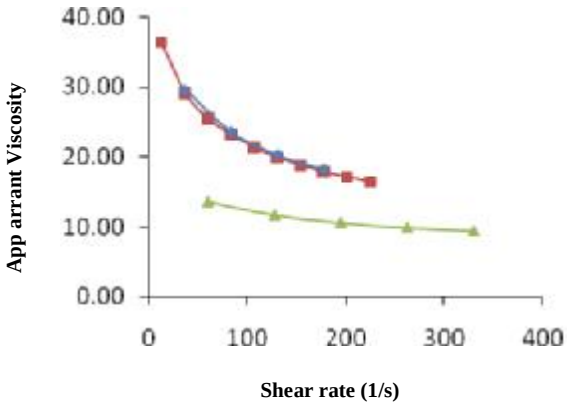

Fig. 5. Influence of pH on steady flow viscosity of soluble fraction of modified gum at the optimum conditions (soluble fraction of optimum modified gum, ■ pH=11▲ pH=3)

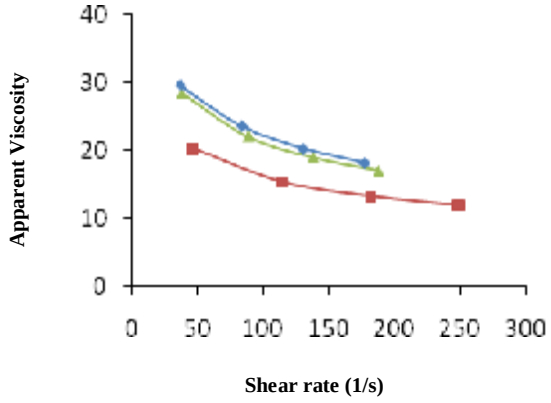

Fig. 6. Influence of electrolytes on steady flow viscosity of soluble fraction of optimum modified gum (soluble fraction of optimum modified gum, Influence of NaCl, Influence of $Cacl_2$).

3.7. Acrylamide determination

Results showed that there are 99 ng acrylamide per milliliter of soluble fraction of modified gum. According to recent exposure assessments, the daily dietary intake of AA with diet should be 1 μg/kg bw [13]. Therefore, we can use permissible amounts of this modified gum in food products.

4. Conclusions

This study showed that central composite design and response surface methodology could be successfully used in optimizing reaction variables for gum modification. RSM predicted that a set level of 1.5 (g on dry basis) insoluble fraction of Persian gum, 0.08 mol acrylamide, temperature 59.98 °C for 2.96 h would provide the optimum condition for solubility increasement. The modified gum under optimized conditions showed 60.6432% solubility increasement. Results showed that molecular weight reduction could be a reason for solubility increasement.

5. References

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