Optimize the Extraction Conditions of Pectin Extracted from Saveh Pomegranate Peels

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ABSTRACT: *In recent years, numerous studies have been done on the beneficial use of food waste. Pomegranate is widely used in food industries, and the skin of this fruit containing the valuable substance, pectin, is discarded as waste. The general aim of this study was to investigate the effect of temperature (35, 65, and 95 ºC), time (40, 120, and 200 min), and pH (1, 2, 3) on the galacturonic acid percentage, Degree of Esterification (DE), and yield rate of pectin extracted from pomegranate peels and optimize extraction conditions. Therefore, 15 treatments were designed using the response surface methodology Box-Behnken and the obtained results were analyzed and optimized by using the response surface method at a confidence level of 95% in Minitab 16 software. The results of the analysis of variance (ANOVA) showed that the linear effect of temperature, time and pH had significantly ($p \leq 0.05$) affected the amount of pectin extracted from pomegranate peels. Results showed that hard extraction conditions (lower pH, higher temperature, and greater time) have increased the extraction yield and the amount of galacturonic acid of pectin, while these conditions have reduced the degree of esterification of pectin. According to the results, the yield of pectin extracted from pomegranate peels, the percentage of galacturonic acid, and the degree of esterification varied from 6.96% to 8.65%, 60.31% to 84.64%, and 52.30% to 65.21%, respectively. The multiple optimum extraction conditions to achieve maximum yield of pectin extraction (8.65%) and galacturonic acid (83.49%) with 97.60% desirability were obtained at 94.39 °C, time 200 min, and pH = 1.24. The results of this study proved that pectin can be extracted from Saveh pomegranate peel with desirable quality properties and used in food formulations*

KEYWORDS: *Degree of esterification; Galacturonic acid; Pectin extraction; Pomegranate peels; Yield.*

INTRODUCTION

Pectin is a polysaccharide that occurs naturally in most plants although it is commercially extracted mainly from citrus peels and apple pulp [1]. In the food industry, pectin is used as a gelling agent, especially for the production

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of gellies and jams. Pectin is a sugar-acidic derivative polymer derived from the gelatinous structures of fruits and vegetables [2]. Maximum amount of pectin is found in unripe fruits and following this stage, the quantity and the quality of the extracted pectin is diminished Galacturonic acid is the most predominant chemical building block of pectin in fruits and vegetables [3]. The acids in the polymer may be methylated or free acids such as protopectin, gelatinous acid, pectinic acid, and pectin [4]. Pectin is characterized by good taste release, desirable processing properties, and stability at low pH value. Today pectin is used as a thickening and stabilizing in addition to a gelling agent [5]. Pectin is used in different products such as fruit-based and bakery products, dairy products, milk-fermented drinks, and confectionary products as well as in the pharmaceutical industry [6]. Pectin is found in almost all fruits and vegetables thus researchers have conducted many studies on its extraction from fruit wastes [7]. Pectin extraction methods include acid extraction, Microwave-assisted Extraction, and Ultrasound-assisted extraction. The microstructure and rheology of pectin gels are exaggerated by numerous factors, such as pH, temperature, sucrose content, and Ca^{2+} ion concentration. The gelling capability of pectin depends on its viscosity and solubility, which are a measure of its molecular weight [8]. *Nikbakht et al.*, (2019) showed that pectin can be extracted from apple pulp for use in some food products as a gel, thickener, tissue, emulsifier, and stabilizer. Apple pectin was extracted by microwave method. Pectin's obtained from each sample was analyzed by FT-IR. The highest extraction efficiency was obtained under microwave power of 720 watts, pH 1.5, and exposure time of 7 minutes equal to 15.11% [9].

Kashani et al., (2021) extracted pectin from potato peel by acidic method and reported that temperature, pH, and extraction time are the most important factors affecting the extraction yield and quality of produced pectin [8]. In another research, *Kashani et al*., (2020) extracted pectin from potato peel and used it in jelly formulation. The results showed that there was no significant difference between the physicochemical, texture, and organoleptic properties of jelly prepared with pectin extracted from potato peel and jellies prepared with commercial pectin from citrus and apple [7].

Moorthy et al., (2015) extracted pectin from pomegranate peel using ultrasound assist by using Box-Behnken method.

They reported that Box-Behnken is suitable method to optimize the effect of process variables. Their result showed that the highest yield (23.87%) of pectin extracted from pomegranate peel was observed in solid:liquid ratio 1:17.52 g/mL, pH=1.27, extraction time= 28.31 min and extraction temperature= 61.90 °C [10]. The Pomegranate from *punicase* family with scientific name of *Punica granatum* is native to tropical regions of Iran. The amount of pomegranate production in Iran was 1086629 tons in (2015). Pomegranate production in year 2018 in Iran was about 700000 tons that we can predict that it reaches to 915000 tons this year. Pomegranate that used in jam making, sauce, concentrate of fruit juice and jelly and eating. Pomegranate peels (pomegranate peels nearly composes 30 to 40% of whole weight of fruit. Considering that pomegranate peel makes up about 30 to 40% of the total weight of the fruit, therefore, the optimal use of pomegranate peel waste seems necessary. Pomegranate peels has valuable mixes that is anti-microbe, anti-oxidant, and colorful and pectin. By regarding low amount of this valuable thing one can decrease waste and using it for producing valuable thing [2]. By regarding low amount of this valuable thing one can decrease waste and using it for producing valuable thing [11]. *Girma et al.,* (2016) was extracted by ultrasound. The optimum temperature, time and pH for the extraction of pectin for banana and mango peels were determined to be 82°C, 105min and 2 respectively. The yields of pectin under these optimum conditions were found to be 11.31% and 18.5% for both peels respectively [12]. *Chakrabandhu et al.,* (2019) extracted the banana peels by using ultrasound method and and found yeild of pectin 7.88% [13].

Aklilu (2021), studied about the extraction of pectin from banana peel using microwave-assisted extraction methods. The optimum conditions were initiated to be temperature of 60 °C, extraction time of 102 min, liquid–solid ratio of 40% (v/w) and pH of 2.7. The yield of pectin and degree of esterification under these optimum conditions were 14.34% and 63.58, separately. Temperature, time, liquid–solid ratio and pH revealed a significant ($p < 0.05$) effect on the pectin yield and degree of esterification [14].

By expanding this amount of waste to the total waste of the country's conversion industries, we can realize the high economic value of these industries, which in addition to their high profitability, also prevent environmental pollution.

Extraction method, extraction conditions and variety of products can have a significant effect on the characteristics of pectin extracted from waste. To the authors' knowledge, there is no comprehensive study on optimization and extraction condition pectin from pomegranate Saveh peels. By optimizing the extraction conditions and presenting models to readers, researcher and manufacturers can extract higher pectin from pomegranate peel waste with desired quality. The overall objective of this study was to optimize the extraction conditions of pectin extracted from Saveh pomegranate peels.

EXPERIMENTAL SECTION

Materials

Pomegranate was purchased from a garden in Saveh, Iran. Sulfuric acid, citric acid, hydrochloric acid, sodium hydroxide, sodium tetraborate, sodium azide, meta-hydroxy reagent in phenyl, and phenolphthalein reagent was purchased from Merck, Germany. Di galacturonic (Sigma, USA) and sunflower oil (Oila, Iran) were purchased.

Determination of appropriate acid for pectin extraction from pomegranate peels

Pomegranate peels were separated, thoroughly washed with water, and cut into small pieces. Next, it was dried in an oven at 65 ℃ for 24 hours to reach a constant weight, powdered by a mill, and sieved with mesh No. 60 [15]. The moisture of dried pomegranate peel powder was 8%. The prepared powders are stored in a polyethylene container in a dry place for later use. In the first stage, as an initial study and determination of the type of acid appropriate for extraction of pectin from pomegranate peels, the effect of citric acid, nitric acid, phosphoric acid, and sulfuric acid on the yield of extracted pectin at 90 °C for 90 min in acidified water at pH 2.5 was examined.

Extraction of pectin from pomegranate peels and yield

To extract pectin from pomegranate peels in different conditions performed according to the method of *Moorthy et al.*, (2015) [10] with some modifications. The experiment range for each factor has been choosing based on the results of first experiments. First 3 g of dried powder of pomegranate peels poured in erlenmeyer with 90 ml acidified distilled water with citric acid (appropriate acid) in defined pH= (1, 2, 3) they were mixed and in warm water with temperatures (35, 65, 95 °C) in known time (40, 120, 200 min) by magnetic agitator was stirred and warmed (Table 1).

After needed time for extraction, samples exit from warm water bath and solution was smoothed twice with cross cloth and for splitting of remained particles, gained solution was centrifuge for 20 minutes in 4000 rpm. For splitting of solid from liquid after centrifuge, it was exit from filter paper number 41. Extract of pectin put in refrigerator and after reaching to 4 °C, 96% ethanol with ration of 1 to 2 (extract to alcohol) added to pectin and one night it was in refrigerator until pectin deposited and balanced again. Then pectin deposit separated from liquid by centrifuge in 4000 rpm about 15 minutes. Liquid was separated and remained deposit for deleting of impurities washed with 70% ethanol and then 96% ethanol. After each washing with alcohol, by centrifuge in 4000 rpm, in 10 minutes, remained deposit separated from liquid. At last gained pectin dries with freezing method. Dried pectin after weighting (for measuring extraction yield) changed to powder and it was packing in polyethylene bags and put in refrigerator. The following equation was used to obtain pectin production yield [10]:

$$Y_{pec}\ (\%) = \frac{P}{B_i} \times 1000 \qquad (1)$$

Galacturonic acid

The amount of galacturonic acid was measured by using the method of *Monsoor et al.*, (2001) [16]. The galacturonic acid content of pectin extracted from pomegranate peel was measured by colorimetric method using metahydroxy di phenyl reagent by visible ultraviolet spectrophotometer 2502 CE, UK). 0.05 g of the extracted pectin were mixed with a certain amount of deionized water in a 250 mL flask. It was stirred with a magnetic stirrer until thoroughly dissolved. 1 mL of this diluted solution containing pectin was transferred to 3 test tubes immersed in an ice/water mixture one of which for measuring the absorbance of control sample and two tubes for measuring the absorbance of pectin sample. Next, 6 mL of sodium tetraborate solution were added to each test tube and the tubes were vortexed to mix the content thoroughly.

Table 1: Temperature, time and pH for extraction of pectin from pomegranate.

Row	pH	Time (Min)	Temperature (ºC)
1	3	120	35
2	1	40	65
3	1	120	35
4	2	40	35
5	1	200	65
6	2	200	35
7	2	200	95
8	2	120	65
9	2	120	65
10	2	40	95
11	2	120	65
12	1	120	95
13	3	40	65
14	3	200	65
15	3	120	95

They were heated in a hot water bath at 100°C for 6 minutes and then immediately cooled to the room temperature. 0.1 mL of metahydroxy diphenyl reagent was added to the two test tubes containing pectin sample and 0.1 mL of 0.5% sodium hydroxide solution was added to the test tube containing control sample and their contents were thoroughly mixed. The tubes were kept at room temperature for 15 minutes and then the absorbance of the contents of each tube was measured at 250 nm. The calibration curve was drawn by measuring the absorbance of standard galacturonic acid solutions at standard concentrations of 20, 40, 60, 80 and 100 µg/mL at 520 nm and the concentration of galacturonic acid in each pectin sample was measured by using linear regression equation of calibration curve based on dry weight of pectin.

Determination of Degree of Esterification (DE)

The Degree of Esterification (DE) of pectin was measured by the titration method with some variations. To do this, 0.2 g of 2 mL of 98% ethanol and 20 mL of deionized distilled water were stirred by a magnetic stirrer at 40 °C and after complete dissolution, 2 drops of phenolphthalein were added and then titrated with normal 0.1 sodium hydroxide until a pale pink color appeared (the consumed volume of sodium hydroxide was recorded as V_1). 10 mL of 0.1 M sodium hydroxide were added to the neutralized solution and stirred for 2 hours by a magnetic stirrer for saponification of pectin. 10 mL of normal 0.1 hydrochloric acid was added and stirred until the pink color disappeared. Excess acid was titrated with normal 0.1 sodium hydroxide to the same end point. The consumed volume of sodium hydroxide was recorded as V_2 [17]. The DE was calculated by Equation 2:

$$\mathrm{DE}(\%) = \frac{V_2}{(V_1 + V_2)} \times 1000 \qquad (2)$$

Yield percentage by regarding Equation (2) calculated (*Ansari et al.* 2017) [17].

Emulsifier Activity (EA) and Emulsion Stability (ES)

To estimate the emulsifying activity and emulsion stability of the extracted pectin under optimal conditions, the method of *Dalev* and *Simeonova* (1995) [18] with some variations was used on days 1 and 30 at 4 and 23℃. To prepare the emulsions, 5 ml of sunflower oil was added to 5 mL volumetric 5% pectin solution (containing 0.02% sodium azide as antibacterial) for emulsification. The emulsions then were centrifuged at 4000 rpm for 5 min. at room temperature. Emulsifier activity by regarding equation 3, and emulsion stability according Equation (4) calculated as follows:

$$\text{Emulsifier activity (\%)} = \frac{\text{Emulsified layer volume}}{\text{Total sample volume}} \times 100 \qquad (3)$$

$$\text{Emulsion stability (\%)} = \frac{\text{Volume of remaining emulsion layer}}{\text{volume of initial emulsion layer}} \times 100 \qquad (4)$$

FT-IR spectrum

The FT-IR spectrum was plotted with an accuracy of 4 cm by FT-IR spectrometer (PERKIN Elmer Co., MA, USA) using KBr plate method in the range of 4000 to cm^{-1} [19].

Molecular weight of pectin

First, in order to measure the intrinsic viscosity of pectin ([η]), the concentrations of 0.1, 0.2, 0.3, and 0.4 of pectin in aqueous solution containing 5 Molar sodium acetate and 0.1 Molar chloride sodium and 0.04 0% sodium azide was prepared. The pectin solution was filtered through a 0.45-micron membrane filter and their flow time was measured by capillary tube viscometer No. 518.10. The solution temperature was kept constant by immersing the viscometer in a water bath at a controlled temperature of 25 °C. The intrinsic viscosity of pectin was estimated by plotting the pectin

concentration in the horizontal axis against the natural logarithm of relative viscosity divided by concentration (Keramer equation) and specific viscosity by concentration (Huggins equation) in the vertical axis [20, 21].

$$(\text{Keramer equation}) \quad (\ln \eta_{rel})/C = [\eta] + K''[\eta]^2 \qquad (5)$$

$$(\text{Hggins equation}) \quad (\eta_{sp})/C = [\eta] + K'[\eta]^2\, C \qquad (6)$$

The intersection point mean of these two graphs with vertical axis was considered as the intrinsic viscosity of pectin according to DL/ G.

Finally, the average molecular weight of pectin is from the Mark Houwink Sakurada equation, where K and N are constant numbers that depend both on the temperature and the soluble and solvent properties [21]. Both K and α depend on temperature, solute and solvent characteristics respectively. A large number of models have been used to deduce [η]-Mw relationships. In this work the following values were assumed K = 1.410−6 and α = 1

$$\text{Equation mark houwink sakurada} \quad [\eta] = KM_v^a \qquad (7)$$

Analysis

In the current study the effect of three independent variables including temperature (35, 65 and 95 °C), time (40, 120 and 200) and pH (1, 2 and 3) on the yield, galacturonic acid percentage and esterification degree of pectin extracted from the potato peels were examined. It should be noted that the range of independent variables was determined by pretreatment. The Response surface method is used to optimize the conditions of extraction. The designing of treatments was done by response surface methodology (Box- Behnken). Therefore 15 treatments were designed. Box Behnken method was used to analyze the yield data, degree of esterification, and galacturonic acid, and analyze their optimum conditions. Analyzing the data of the emulsion stability section and determination of appropriate acid was performed using one-way ANOVA (Duncan) at 5% probability level using Minitab16 software.

RESULTS AND DISCUSSION

Determination of appropriate acid for pectin extraction

The results of determining the suitable acid for the extraction of pectin from pomegranate peel are presented in Fig. 1. The highest (8.28%) and the lowest (8.12%) yield of pectin extraction were observed when citric acid and phosphoric acid were used, respectively. The results of statistical analysis revealed a significant ($p \leq 0.05$) difference between the amounts of pectin extracted with different acids. As can be seen in Table 2, citric acid was recognized as the most effective for pectin extraction from pomegranate peel, because of chelator characteristics and existence of mixes which absorb humidity in comparison with other acids worked more effectively in pectin extraction [18]. Therefore, citric acid was used in the optimization method of pectin extraction yield. Citric acid is a natural and safe food component and is more attractive than other more generally used mineral acids. Therefore, citric acid has been the preferred acid for extraction in terms of economic and environmental attention [19]. *Jamsazzadeh Kermani et al.*, (2014), reported that citric acid has ability of extracting much pectin than hydrochloric acid and sulfuric acid due to chelating characteristic of citric acid [22]. Citric acid can ban much amount of solved pectin’s, then extract them, so the yield of pectin extraction with citric acid than two other acids that hadn’t chelate characteristics goes higher. Based on Fig. 1. the least amount of pectin (8.12%) was for phosphoric acid. According to the results of some researchers [18,19,22], mineral acids such as hydrochloride acid, and phosphoric acid, sulfuric acid, nitric acid, and organic acids such as lactic acid and acetic acid citric acid, tartaric acid, have all been used for pectin extraction tests. This result is in agreement with the results of other studies that have determined citric acid as the best for extracting pectin [20, 21,22]. Bagherian showed that the pectin yield was 19.16% using a conservative method of extraction on grapefruit peels [23]. The increase in pectin yield is seen while residual peels were used can be clarified by the thermal treatment during the hydro-distillation which damaged the structure of the peels thus increasing interaction between acidic solution and raw material through the extraction, thus leading to an effective increase of pectin yield [24].

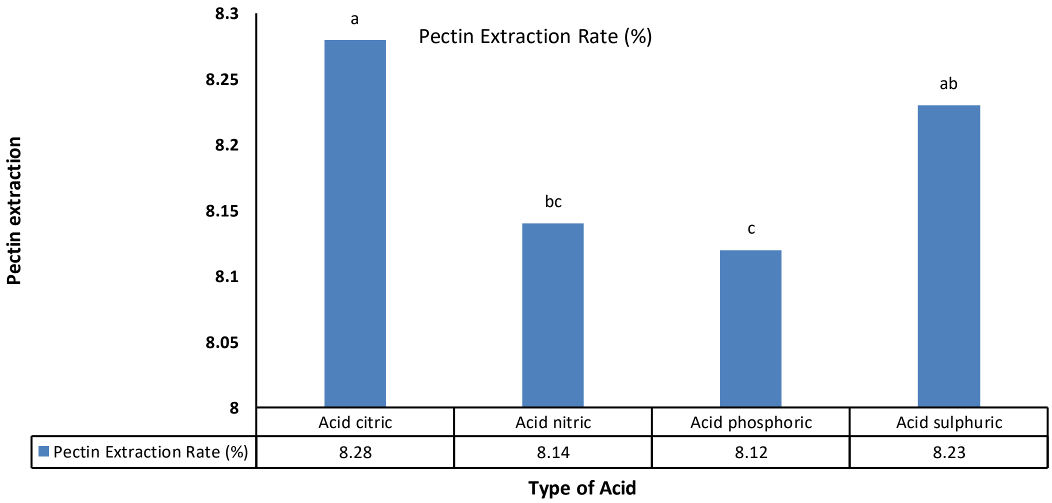

Fig.1: The effect of citric acid, nitric acid, phosphoric acid and sulfuric acid on the amount of pectin extracted from pomegranate peels.

Yield, galacturonic acid and degree of esterification of pectin extracted from pomegranate peels in different conditions

Table 2 shows the results of the yield of pectin, galacturonic acid amount and degree of esterification extracted from pomegranate peels in different conditions (pH, temperature and time). By regarding results different condition of extraction has much effect on pectin extraction in a way that production yield was different from 6.96% to 8.65%. The highest amount of extracted pectin from pomegranate peels was 8.65% with temperature=95 °C, time=120 minutes and pH=1. The lowest yield 6.96% was achieved at temperature 35 °C, time=40 min, and pH = 3.

The maximum amount of galacturonic acid from pomegranate peels was 84.64% at temperature=65 ºC, time=200 minutes, and pH=1. The lowest percentage of galacturonic acid for extracted pectin was 60.31% at 65 °C, 40 minutes, and pH=3.

The pomegranate (*Punica granatum*) is an individual of the fruits contains the main bioactive phenolic elements belonging to the Punicaceae family and has been generally used as botanical parts in herbal medicines and dietary supplements. Pomegranate is gradually consumed as numerous processed products, such as juices, wines, jams, jellies, and extracts. In pomegranate juice processing, 1 ton of fresh fruit makes 669 kg of by-product pomegranate marc containing 78% peel and 22% seeds [15]. *Akbari-Adergani* evaluated pectin from pomegranate peel by microwave method and reported that pectin extraction efficiency under optimal conditions (power 700 watts, time 120 seconds and pH equal to 1.5) was 20.42% [19]. *Ebrahim Zadeh* and *Azad Bakht* (2006) showed that he highest percentage of pectin extraction in Italian, *Shahsavari* and *Sangin* oranges was obtained with 27, 25, and 20%, respectively [21].

The highest amount of esterification degree for extracted pectin from pomegranate peels was 65.21 in 35 ºC, time=120 minutes, pH=3. The lowest amount of esterification degree for pectin was 52.30% in 95 ºC, time=120 minutes, and pH=1. Akbari-Adergani evaluated pectin from pomegranate peel by microwave method and reported the degree of pectin esterification is equal to 33.24% [19].

The reason for increased yield with increasing extraction time could be the increase in time required for the complete release of pectin into acids solution [19, 20]. Increased yield with increasing time could be due to further increase in solubility of polysaccharides and pectin in the extraction solvent and in mass transfer from the substance to the solution which resulted in increased rate and yield of pectin extraction [22] also as the temperature increased, the diffusion of efficient and therefore the diffusion rate increased [22]. It should be noted that increased yield with decreasing pH was related to the effect of acid on the cell wall of the primary product as well as the release of pectin in the extraction solution, so the stronger the acid, the higher the decomposition of the cell wall, pectin release and pectin production [24-27]. *Khan et al*., (2015) optimized the pectin extraction condition

Table 2: Comparison between yield, galacturonic acid and degree of esterification of pomegranate peels extracted under different conditions.

Treatment	Temperature (ºC)	Time (Min)	pH	Yield of pectin (%)	Predicted Yield of pectin (%)	Galacturonic acid (%)	Predicted Galacturonic acid (%)	Degree of Esterification (%)	Predicted Degree of Esterification (%)
1	35	120	3	6.96	6.965	62.43	61.480	65.21	64.265
2	65	40	1	7.65	7.644	66.30	66.714	59.00	58.126
3	35	120	1	7.25	7.232	62.51	65.427	57.27	57.837
4	35	40	2	7.11	7.124	68.33	64.999	61.00	61.306
5	65	200	1	7.75	7.779	84.64	80.359	54.95	54.311
6	35	200	2	7.15	7.139	70.57	71.934	58.60	5.671
7	95	200	2	8.45	8.426	76.56	79.981	55.50	55.194
8	65	120	2	7.58	7.580	77.31	77.030	59.50	59.303
9	65	120	2	7.56	7.580	76.54	77.030	59.11	59.303
10	95	40	2	8.15	8.161	73.31	71.946	57.07	56.999
11	65	120	2	7.60	7.580	77.24	77.030	59.30	59.303
12	95	120	1	8.65	8.645	76.31	77.260	52.30	53.265
13	65	40	3	7.15	7.121	60.31	54.591	63.00	63.639
14	65	200	3	7.25	7.256	66.33	65.916	62.14	63.014
15	95	120	3	7.85	7.867	67.56	64.642	61.62	61.052

from citrus peel and reported that pectin yields in the pH range of 1-2.5 were higher than those at pH 3-4 [28]. Other researchers extracted pectin from citrus peel [28], orange (citrus sinensis)) peels [29], banana peels [27], and Mango peels [30] their amount of yield under optimal conditions were 10.1, 30.28, 29.28, and 22.35% respectively.

The results showed that time and pH have a high effect on the galacturonic amount of extracted acid of pectin becomes more and it can be because of dividing non-pectin mixes because other polysaccharides of cell wall like cellulose, hemicellulose, arabinan, galactose are extracted too. Another reason for the increase in galacturonic acid extracted from pectin by time increase is an increase in hydrolysis of the neutral sugar side chain for the pectin structure itself. These side chains that are a part of pectin and connected to linear change with covalent bonds in *Rhamnogalacturonan*-II region are composed of arabinan and galacton and in *Rhamnogalacturonan*-II, there are 4 lateral chains with 11 different sugars like the opposite. With time increase in extraction, these lateral chains separate more [22]. *Garna et al*., (2007) and *Abid et al*., (2017) showed that the galacturonic acid amount isn't affected by process temperature that is matched this research results [26, 31]. *Emaga et al*., (2008) extracted pectin form banana peels and reported that the pH was the most effective factor in the percentage of galacturonic acid, also they reported that galacturonic acid percentage isn't affect by extraction temperature [32]. *Yapo* (2007) extracted pectin from sugar beet in different situations and understood that the pH was the most effective factor on galacturonic acid percent and temperature had a medium effect on galacturonic acid percent that matches with this research results. In interaction of a carboxylic acid (groups of -COOH) factor with an alcohol (groups of -OH) factor that gained ester and water, is called yield [33].

Esterification degree is one of the most important parameters for recognition of its usage that based on the definition shows a number of methanol moles near 100 moles of galacturonic acid. The importance of metoxyl group is for their near relation and other characteristics of pectin like a solvent, power of jelly making, kind, and properties of produced jelly, sensitively to salt, etc. Metoxyl amount shows pectin kind and usage [5]. In reality by pH decreased, temperature and time

Table 3: Results of analysis of variance (ANOVA) for second order regression model for yield, galacturonic acid level and degree esterification (DE) of extracted pectin from pomegranate peels.

Source	Pectin yield		Galacturonic Acid		Degree Esterification	
	F-value	P-value	F-value	P-value	F-value	P-value
Regression	426.39	0.000*	4.07	0.068	16.59	0.003*
Liner	1188.37	0.000*	7.34	0.028*	46.63	0.000*
Temperature (A)	2928.54	0.000*	6.85	0.047*	30.02	0.003*
Time (B)	39.84	0.000*	6.82	0.048*	9.77	0.026*
pH (C)	596.73	0.000*	8.36	0.034*	100.1	0.000*
Square	60.95	0.000*	3.71	0.096	2.1	0.219
Temperature×Temperature (A^2)	132.57	0.000*	2.76	0.158	3.4	0.124
Time ×Time (B^2)	8.63	0.032*	0.39	0.561	0.32	0.595
pH × pH (C^2)	28.3	0.003*	8.99	0.030*	2.15	0.203
Interactions	29.85	0.001*	1.16	0.412	1.05	0.447
Temperature × Time (A×B)	18.47	0.008*	0.02	0.897	0.17	0.697
Temperature × pH (A×C)	71.07	0.000*	1.14	0.334	0.46	0.529
Time × pH (B×C)	0	1.00	2.31	0.189	2.52	0.173
Lack of fit	3.15	0.250	150.29	0.007	43.57	0.023
R^2 = R-Squared	99.84%		87.99%		96.76%	
R-Sq(adj)	99.64%		86.37%		90.93%	
CV.%	6.21		6.75		6.12	

of extraction, the yield was increased but the esterification degree of pectin decreased much more because of the breakdown of pectin ester bonds in a hard situation of acid hydrolysis. Other researchers reported that more difficult extraction condition from the point of acidic pH and high temperature decreases poly galacturonic chain [23]. It is thought that a high esterification degree shows pectin with less damage because the ester bond towards glycosides bonds of alpha 1 and 4 between galacturonic acids has less resistance to acid hydrolysis [29]. In pectin extraction from banana peels, *Emaga et al*., (2008) [32] reported that aside from pH and temperature effects, an increase of extraction time, decreased metoxyl pectin.

Analysis of variance and regression model of yield, Galacturonic Acid (GA) And Degree of Esterification (DE) of pectin extracted from pomegranate peels

Results of analysis of variance of response surface model and predicted regression model for yield, galacturonic acid level, and degree of esterification of extracted pectin from pomegranate peels are shown in Table 3 and Table 4.

The results of Table 3 showed that the linear and square effects of temperature, time, and pH, and interaction effects of temperature × time and temperature × pH were significant ($P\leq 0.05$) on the yield of extracted pectin from pomegranate peels. The value of the of R-Sq in this model was = 99.84 and its modified coefficient of explanation were R-Sq (adj) =99.64 and coefficient of variance = 6.21% which indicated the good fit of the model to the experimental data.

The linear effect of temperature, time, and pH, and the square effects of pH were significant ($P\leq 0.05$) on galacturonic changes. Amount of R-Sq=87.99% and R-Sq (adj) =86.37% and coefficient of variance = 6.75% of galacturonic acid showed good fitness of the model to experimental data.

The linear effect of temperature, time and pH, were significant ($P\leq 0.05$) on degree esterification changes.

Table 4: Predicted regression model for yield, galacturonic acid level and degree of esterification of extracted pectin from pomegranate peels.

Source	Model
Yield (%)	$14.773 + 6.10A + 1.180B - 0.990C + 1.447AB - 0.639AC - 0.0533BC + 3.278A^2 - 0.319B^2 + 0.053C^2$
Galacturonic acid (%)	$76.9426 - 2.8661A - 0.0756B + 1.7217C - 0.0350AB + 1.4775AC + 0.4842BC - 7.6861A^2 - 1.4178B^2 - 7.9328C^2$
Esterification Degree (%)	$53.9463 - 17.8617A - 1.3106B + 1.0042AB - 2.8433AC - 0.2000BC + 4.2728A^2 + 0.2128B^2 - 2.1222C^2$

Amount of R-Sq=96.76% and R-Sq (adj) = 90.93% and coefficient of variance = 6.12% that showed good fitness of the model.

According to the results of Table 3, The high R^2, R^2-adj and low values of coefficient of variance of all response variable clearly demonstrated a good correlation between experimental and predicted data, therefore the regression model of all response variables (Table 4) have the ability to predict the experimental data.

Results of Temperature × Time, pH ×Time, pH × Temperature on yield of pectin extracted from pomegranate peels

As can be seen, Fig. 2 (a) shows the yield of pectin extraction from pomegranate peels at constant pH=2 and temperature × time variations. The amount of yield significantly increased with increasing time and temperature. The yield 8.4% and higher than what was observed at temperatures >90 °C for 160 to 200 minutes.

Fig. 2 (b) shows the yield of extraction of pectin from pomegranate peels under constant time conditions of 120 min. with changes in pH and temperature. The amount of yield significantly increased with increasing temperature and decreasing pH. The yield of 8.50% and above it was observed at temperatures > 95 °C and pH 1-1.5.

Fig. 2 (c) shows the yield of extraction of pectin from pomegranate peels at a constant temperature of >65 °C, and the variation of pH and time. By increasing the time and decreasing the pH the rate of yield significantly increased. The yield of 7.7% and higher than was observed at time 74 to 200 min and pH 1-1.35.

Ansari et al. (2017) investigated the efficiency and physicochemical properties of pectin extracted from eggplant skin lesions. The researchers stated that for the private physicochemical study of the resulting pectin, the emulsion stability test was performed at different days and temperatures, and the flow behavior at different concentrations was investigated. According to the results, the highest extraction efficiency of eggplant skin was 30.28% in severe conditions of extraction at 90 ° C, 150 min time, and pH 1.5 [17]. *Geng et al.*, (2014) examined the pectin extraction conditions in Aloe peel and the results showed that the highest pectin extraction conditions were 90 ° C, pH 1.5 and 120 min [34].

Single optimization condition of yield of extracted pectin from pomegranate peels

Fig. 3 shows the optimal conditions of pectin extraction from pomegranate peel. As can be seen in the Figure, it was predicted that the maximum yield or the amount of pectin extracted from pomegranate peel would be 8.7312% with 100% desirability at 95℃, for 200 min. and at pH 1.

Interaction effect of Temperature × Time, pH ×Time, pH × Temperature on galacturonic acid of extracted pectin from pomegranate peels

Based on, Fig. 4 (a) shows the galacturonic acid of pectin extraction from pomegranate peels at constant pH=2 and temperature × time variations. The amount of galacturonic acid significantly increased with increasing time and temperature. The galacturonic acid 80% and higher than it was observed at temperatures 70 to 95 ℃ for 175 to 200 minutes.

Fig. 4 (b) shows the galacturonic acid of extraction of pectin from pomegranate peels under constant time conditions of 120 minutes with changes in pH and temperature. The amount of galacturonic acid significantly increased with increasing temperature and decreasing pH. The galacturonic acid of 78% and above it was observed at temperatures 68 to 95 °C and pH 1.1-1.8.

Fig. 4 (c) shows the galacturonic acid of extraction of pectin from pomegranate peels at constant temperature of 65°C, and variation of pH and time. By increasing the time

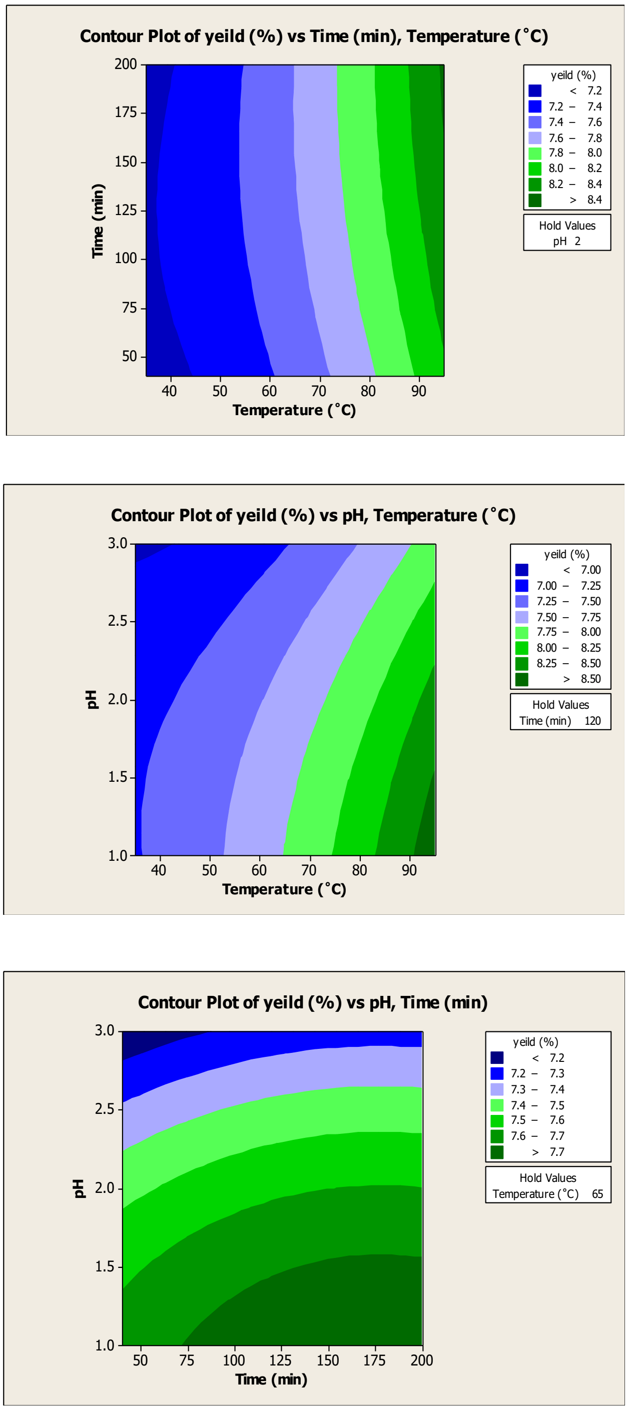

Fig. 2: Interactions effects on pectin extraction yield from pomegranate peels a) Temperature × Time b) Temperature × pH, c) Time × pH.

and decreasing the pH the rate of galacturonic acid significantly increased. The galacturonic acid of 80% and higher than it was observed at time 165 to 200 min and pH 1-1.8.

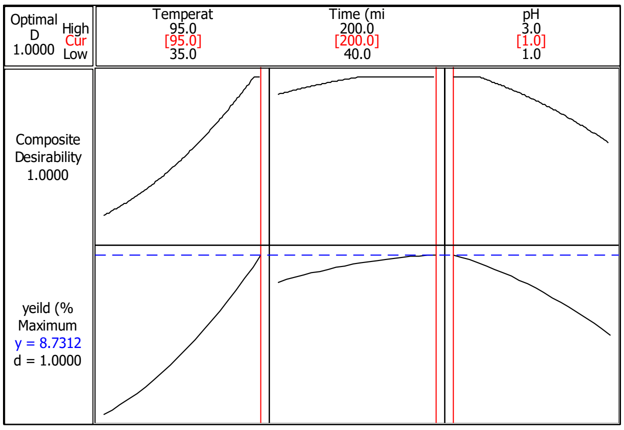

Fig. 3: Optimum condition of yield of extracted pectin from pomegranate peels.

Single optimization condition of galacturonic acid of extracted pectin from pomegranate peels

Fig. 5, shows the optimal conditions for galacturonic acid of pectin extracted from pomegranate peel. As shown in Fig. 4, the maximum galacturonic acid of pectin extracted from pomegranate peel was 83.607% with 95.75% desirability at 88.93 °C for 200 minutes and at pH 1.28. The predicted results were confirmed by confirmatory tests in duplicate and no significant difference was observed between the predicted and actual values.

Interaction effect of Temperature × Time, pH ×Time, pH × Temperature of degree of esterification extracted pectin from pomegranate peels

Based on, Fig. 6 (a) shown the degree of esterification of pectin extraction from pomegranate peels at constant pH=2 and temperature × time variations. The amount of degree of esterification significantly increased with decreasing time and temperature. The degree of esterification 61% and higher than it was observed at temperatures 35 to 48 °C for 40 to 70 minutes.

Fig. 6 (b) shows the degree of esterification of extraction of pectin from pomegranate peels under constant time conditions of 120 minutes with changes in pH and temperature. The amount of degree of esterification significantly increased with decreasing temperature and increasing pH. The degree of esterification of 64% and above it was observed at temperatures 35 to 55 °C and pH 2.9-3.

Fig. 6 (c) shows the degree of esterification of extraction of pectin from pomegranate peels at constant temperature of 65°C, and variation of pH and time.

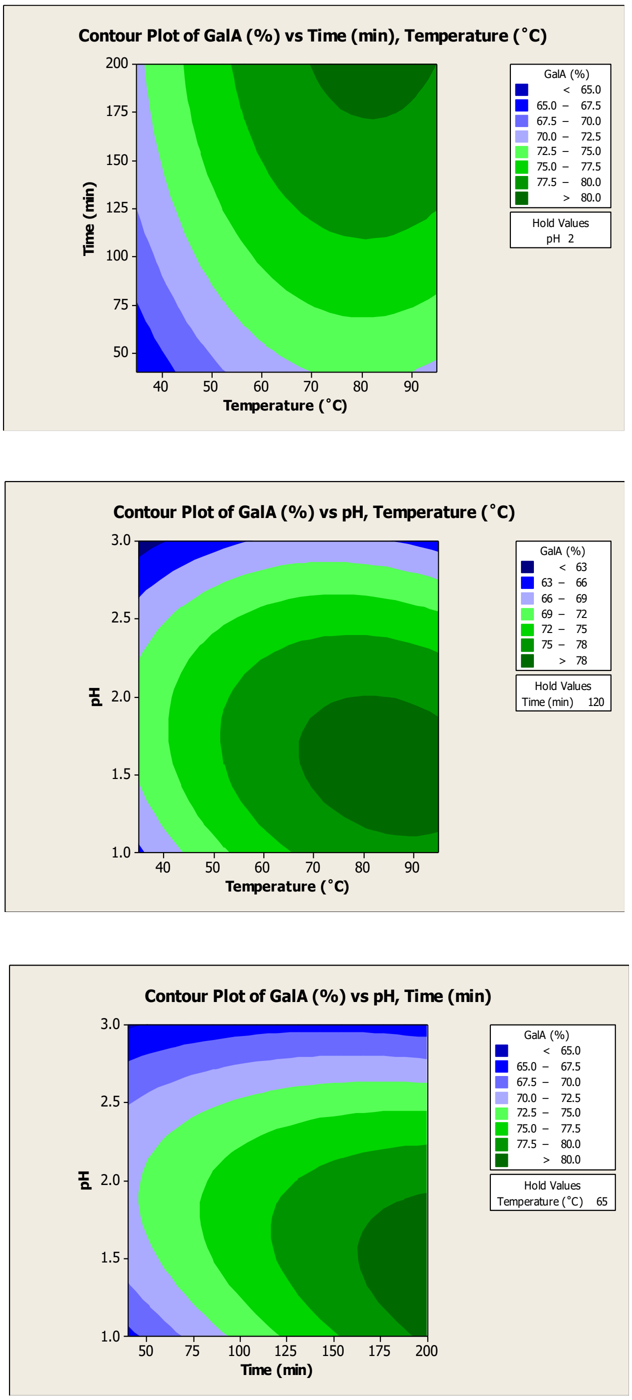

***Fig. 4:** Interactions effects on galacturonic acid of pectin extracted from pomegranate peels a) Temperature × Time b) Temperature × pH, c) Time × pH.*

By increasing the pH the rate of degree of esterification significantly increased. The degree of esterification of 62.5% and higher than it was observed at time 40 to 200 min and pH 2.7-3.

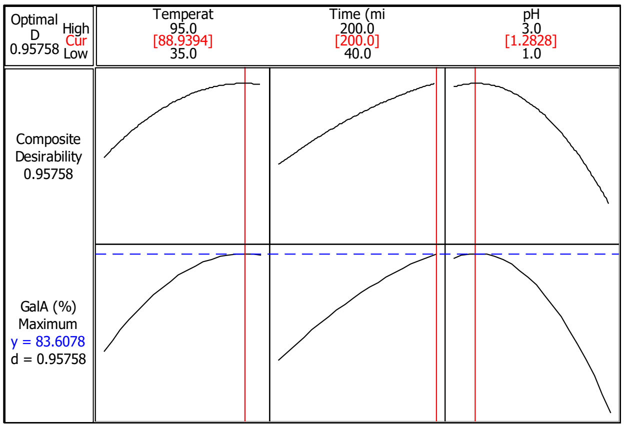

***Fig. 5:** Optimal conditions of galacturonic acid pectin extracted from pomegranate peels.*

Ziari and *Zakai Ashtiani* (2008) investigated the effect of various factors on the yield and purity of pectin extracted from wheat bran using the acid hydrolysis method and optimal conditions were obtained. It was observed that the pre-modification operation improves the quality of the final product. Also, the effect of temperature on the amount of efficiency was greater than the effect of pH and the effect of pH was greater than the effect of time. The best results in terms of yield and purity (amount of galacturonic acid in the extracted pectin) were obtained using alcohol-modified wheat bran (EIM) at an extraction pH of 1.5 and a temperature of 90 °C for 90 minutes [35].

Single optimization condition of the Degree of Esterification (DE) of extracted pectin from pomegranate peels

Fig. 7 shows the optimal conditions for DE of pectin extracted from pomegranate peel. As shown in Fig 7, it was predicted that the maximum DE of pectin extracted from pomegranate peel would be 64.498% with 94.47% desirability at 37.42 ℃ for 52.92 minutes at pH 3. The predicted results were confirmed by confirmatory tests in duplicate and no significant difference was observed between the predicted and actual values.

Multiple optimizations of yield and percentage of galacturonic acid extracted from pomegranate peels

The diagram of multiple optimization of yield and galacturonic acid of pectin extracted from pomegranate peel is shown in Fig. 8. Because the yield and purity of pectin (galacturonic acid) are two more important factors in pectin extraction, they were optimized simultaneously. The optimal conditions for obtaining maximum yield and

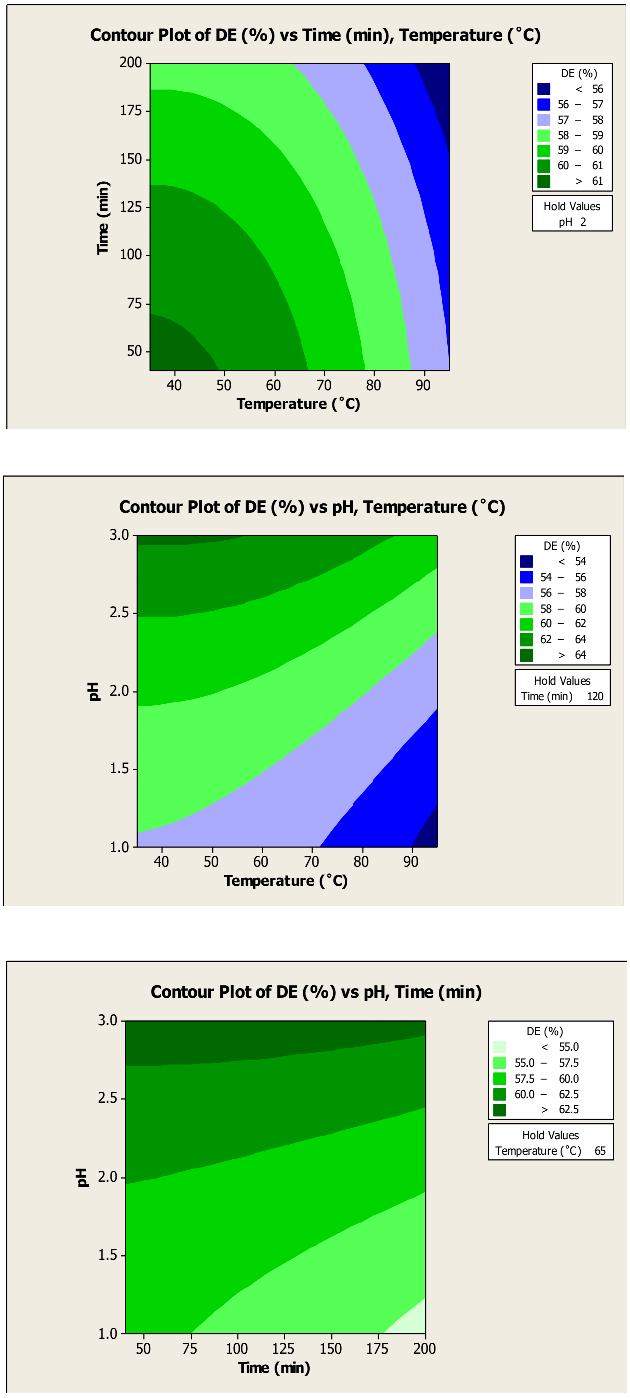

Fig. 6: Interactions effects on degree of esterification of pectin extracted from pomegranate peels a) Temperature × Time b) Temperature × pH, c) Time × pH.

galacturonic acid with 97.60% desirability was predicted to have a temperature of 94.39 °C, time of 200 minutes, and pH value of 1.24 where the yield of 8.65 and 83.49% galacturonic acid were obtained. The predicted results

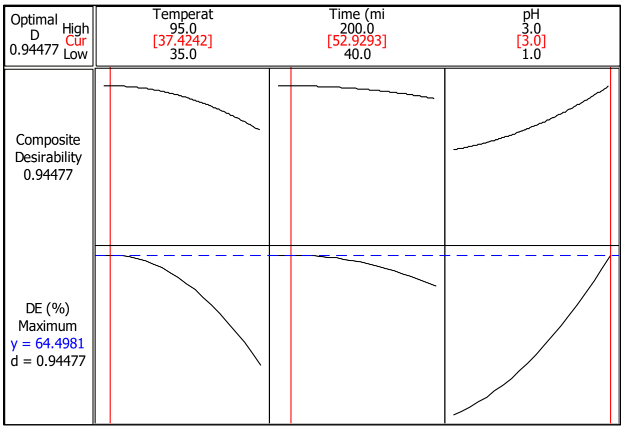

Fig. 7: Optimal conditions for degree of esterification of pectin extracted from pomegranate peels.

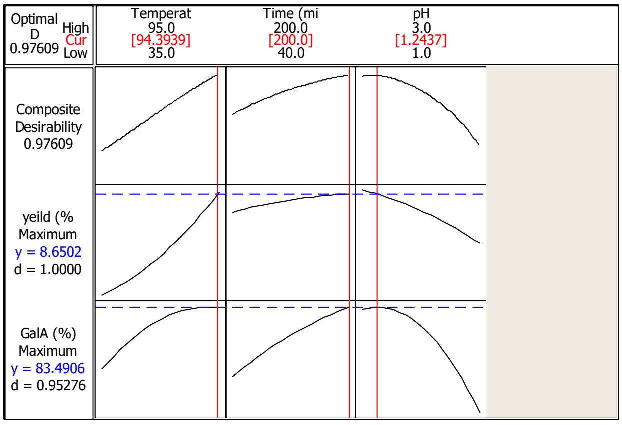

Fig. 8: Multiple optimizations of yield and percentage of galacturonic acid extracted from pomegranate peels.

were confirmed by confirmatory tests in duplicate and no significant difference was observed between the predicted and actual values.

Measuring of emulsifier activity and stability

The stability of pectin emulsion extracted from pomegranate peel under optimal conditions was investigated by using 0.5% W/W water solution pectin (Table 5). After centrifugation of the emulsions, three detectable phases were observed: oil phase at the top due to lower density, emulsion phase in the middle due to medium density and aqueous phase at the bottom because of higher density. As the results showed, the stability of pectin emulsion was much higher at 4℃ than 23℃ and decreased over time. The emulsion stability one day after production and storage at 4℃ and 23 °C was 90% and 78%, respectively and after 30 days decreased to 81% and 70%.

The results showed that the storage temperature had a significant effect ($P \leq 0.05$) on changes in the strength of emulsions extracted from pectin. The results showed that the resistance of the emulsion prepared from pectin extracted from pomegranate peel at 4 °C significantly higher than 23 °C.

Ebrahim Zadeh and *Zadbakht*, (2006) pectin emulsifier properties from some skin of citrus surveyed and they gained similar results [21]. These researchers said that the stability range of the emulsion of pectin by different acids in 4 ºC after 1 day was between 74.3-79.4 and in 23ºC after 1 day was between 62.1-69.3%. Also this factor after 30 days in 4ºC was between 74.2-79.3 and in 23ºC was between 62.1-69.2%. As a result, we can say that emulsion stability in different temperatures was different and in 4ºC was more than 23ºC.

FT-IR Spectrum

Fig. 9 shows the FT-IR spectroscopy of pectin extracted from pomegranate peel under optimal conditions. As shown in the Figure, the strong absorbance region, 3200-3500 cm^{-1} for pectin extracted from pomegranate peel indicates the stretching absorbance of OH due to the vibrations of intra- and extra molecular hydrogen bonds. Stretching bonds of OH occur in a range of frequencies and represent various components including stretching bonds generated by free carboxyl groups in the vapor phase and bending bonds in carboxylic acid groups. In the pectin sample, the mentioned area also is related to the intra- and extracellular vibrations of hydrogen bonds in the galacturonic acid polymer. OH tensile bonds happen in a range of frequency and shows different parts like tensile bonds from the free carboxyl group in the steam phase and also bending bonds in a carboxylic acid. In a sample of pectin, this region is about shaking inside and outside of cells of hydrogen bonds in the galacturonic acid polymer. The existing peak in 2800-3000 cm^{-1} is a sample of CH shaking and is composed of tensile and bending shakes of CH_3, CH_2, and CH [36]. The molecule weight of extracted pectin from pomegranate peels in the best situation was 146 k Dalton. Regarding the molecule weight of business pectin that was between 50 to 150 k Dalton, extracted pectin in this research is in the range of business pectin. Pectin molecule weight based on the first material and extraction method is very different. If the molecule weight of extracted pectin is more, it is composed of a powerful and coherent jelly [37].

Table 5. Emulsifier stability of pectin extracted from pomegranate peels under optimum conditions.

	Day 1		Day 30	
Temperature	4 ºC	23 ºC	4 ºC	23 ºC
Emulsifier stability	90	78	81	70

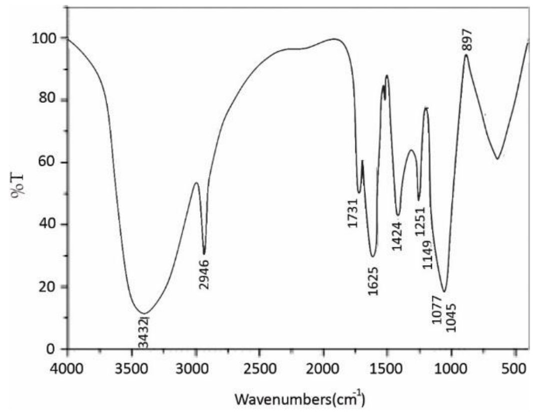

Fig. 9: FT-IR spectrum of pectin extracted from pomegranate peels.

Molecule weight of extracted pectin from pomegranate peels

The molecular weight of pectin extracted from pomegranate peel under optimal conditions was 146 k Dalton. Regarding the molecule weight of business pectin that was between 50 to 150 k Da, extracted pectin in this research is in the range of business pectin. Pectin molecule weight based on first material and extraction method is very different. If molecule weight of extracted pectin is more, it composed a powerful and coherent jelly [37]. *Mosayebi et al*., (2017) [25] studied the optimized conditions of the pectin extraction using ultrasonic waves from the black mulberry pomace. The results of their study showed that the molecular weight of the extracted pectin was 50.03 KDa. *Bagherian et al*. [23] reported the molecular weight of pectin extracted from grapefruit ranged from 56.4 to 84.4 KDa.

CONCLUSIONS

In this research extracted pectin from pomegranate peels in acidic method based on temperature, time and pH had been done. Based on results, pectin yield from pomegranate peels was form 6.96-8.65%, galacturonic acid from 60.31-84.64% and degree of esterification from 52.30-65.21% was variable. The multiple optimization condition of extraction to gain the highest amount of yield and galacturonic acid was in temperature= 94.39ºC, time=200 minutes, and pH=1.24 with 97.60% desirability. Based on the results of analysis variance the temperature, time, and pH had a significant effect on pectin extracted from pomegranate peels. Based on chemical and food laws and regulations the minimum permitted galacturonic acid content of commercial pectin is 65%. Since the amount of galacturonic acid of pectin extracted from pomegranate peel (84.64%) is higher than other sources, therefore pectin extracted from pomegranate peel in this study can be used as a novel and cheap resource for use in food products. Readers can use the optimal model of pectin extraction from pomegranate peel in this study and predict other extraction conditions.

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