Optimization of pectin extraction from pistachio green hull as a new source

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\textbf{A B S T R A C T}

In this paper, the influence of pH (0.5–2.5), temperature (50–90 °C), extraction time (30–150 min) and liquid/solid ratio (10–50 v/w) on the acidic extraction yield and degree of esterification (DE) of pistachio green hull pectin were studied by using central composite design. The results indicated that the yield and DE of pectin ranged from 7.31 to 19.02% and 26.00 to 53.01%, respectively. The optimization of pectin extraction condition showed that the optimal condition was pH of 0.5, temperature of 90 °C, time of 30 min and liquid/solid ratio of 50 v/w. In this condition, the experimental yield (22.1 ± 0.5%) was fine accord among predicted yield (23.42%). Also, under the optimal extraction condition, the galacturonic acid content of pectin was about 65%. The FTIR spectrum showed that the pectin extracted under optimal condition is rich from polygalacturonic acid.

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\section{1. Introduction}

Pectin is a complex mixture of polysaccharides that exists in the primary cell wall and the middle lamella of plant tissue (Hosseini, Khodaiyan, & Yarmand, 2016b). The main structure of pectin is a backbone of α(1 → 4) linked D-galacturonic acid with different degree of methyl esterification (Thakur, Singh, Handa, & Rao, 1997). Based on degree of esterification (DE), the pectin is divided into two groups: Low methoxyl and High methoxyl pectin. In the low methoxyl pectin, the esterification of acidic groups is less than 50% while it is more than 50% in high methoxylated pectin (Liew, Chin, & Yusof, 2014). Pectin is used as a thickener, a texturiser, an emulsifier, stabilizer and gelling agent in food industry (Kratchanova, Pavlova, & Panchev, 2004). Pectin is also used in the pharmaceutical and cosmetic industry. In this case, pectin is usually used to decrease blood cholesterol, soothe pain, and decrease heart disease and gallstones (Hosseini, Khodaiyan, & Yarmand, 2016a). Nowadays, the commercial pectin is usually obtained from citrus peel, apple pomace and sugar beet pulp (Mesbah, Jamal, & Farahnaky, 2005). However, due to the high demand of pectin in all over world, the many studies have been done on other sources of this polysaccharide, such as, cocoa husks (Chan & Choo, 2013), sisal waste (Santos, Espeleta, Branco, & de Assis, 2013), peach pomace (Pagan, Ibarz, Llorca, & Coll, 1999), banana peel (Qiu et al., 2010), Citrullus lanatus fruit rinds (Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2014), dragon fruit peel (Thirugnanasambandham, Sivakumar, & Maran, 2014), mulberry branch bark (Liu, Cao, Huang, Cai, & Yao, 2010), papaya peel (Maran & Prakash, 2015), pomegranate peel (Moorthy, Maran, Muneeswari, Naganyashree, & Shivamathi, 2015), carrot pomace (Jafari, Khodaiyan, Kiani, & Hosseini, 2017) and melon peel (Raji, Khodaiyan, Rezaei, Kiani, & Hosseini, 2017).

Pistachio (Pistacia vera L., Anacardiaceae) is an important agricultural product of Iran. Iran is the largest producer of pistachio nuts in the world and production of pistachio in this country is about 478,600 tons per year (On the basis of an FAO report in 2013) (Rafiee, Barzegar, Sahari, & Maherani, 2017). The pistachio is widely consumed globally related to its sensory and nutritional properties and health-related benefits (Grace et al., 2016). A large portion of the pistachio is its green hull (∼35–45%). This green hull, which is a good source of fatty acids (such as myristic, palmitic, margaric, stearic, elaïdïc, oleic and linoleic acid), phytoestrogens (such as campesterol, stigmasterol, beta-sitosterol), carotenoids (such as alpha-carotene, beta-carotene, trans- carotene, lutein and zeaxanthin), chlorophylls (such as chlorophyll a and b), tocopherols (such as alpha-tocopherol, gamma-tocopherol and delta-tocopherols), proteins, mineral salts, vitamins and polysaccharides (such as pectin) (Grace et al., 2016; Mohammadi Moghaddam, Razavi, Malekzadegan, & Shaker Ardekani, 2009), is usually discarded as waste of this product. Therefore, the use it for production of pectin...
as a value-added product can be valuable both in terms of nutritional and environmentally friendly.

To the best of our knowledge, there is no specific study on the extraction of pectin from pistachio green hull (PGH). Therefore, the aim of this study was the optimization of acidic (citric acid) process conditions (pH, temperature, extraction time and solid/liquid ratio (LSR)) for extraction of pectin from this new source (PGH) and also, the study of the physicochemical properties of pectin obtained under optimal conditions. For this purpose, a central composite design (CCD), which is a collection of mathematical and statistical techniques for empirical model building (Bitaraf, Khodaiyan, Mohammadiar, & Mousavi, 2012), was used to optimize the pectin extraction conditions.

2. Materials and methods

2.1. Samples preparation

Pistachio utilized in this work (Akbari variety) was purchased from local market in Tehran, Iran and the green hulls of pistachios were removed using a steel knife. In the next step, these hulls were dried in the oven at 40°C for 24 h, were ground in blender and passed through a 40 mesh sieve, respectively. The obtained powder was packed in dark bags and kept it in dry environment prior to the experimental analysis.

Meta-hydroxydiphenyl reagent was provided from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals and reagents were analytical grade and supplied by Merck Chemical Co. (Darmstadt, Germany).

2.2. Pectin production and recovery

The acidic (citric acid) extraction of pectin from PGH was performed according to Jafari et al. (2017) with some modifications. The used conditions for pectin extraction were presented in Table 1. After pectin extraction under these conditions, to separate the insoluble material, the grouts were filtered through two layers of gauze and were centrifuged at 4000 × g for 15 min, respectively. Then, pectin was precipitated with an equal volume of 98% (v/v) ethyl alcohol. After 16 h at refrigerator temperature (about 7°C), the coagulum obtained washed twice with ethyl alcohol and dried in oven at 50°C. The yield of pectin extraction was calculated as follows (Jafari et al., 2017; Thirugnanasambandham et al., 2014):

\[
\text{Pectin yield (g/l) = \frac{\text{Dried pectin (g)}}{\text{Initial dry powder of PCH (g)}} \times 100}
\]

2.3. Degree of esterification (DE)

The DE of pectin samples were determined by using the titration method (Wai, Alkarkhi, & Easa, 2010) with slight modifications. First, dried powder of pectin (100 mg) was wetted with 2 mL ethanol and dissolved in 20 mL deionized water at 40°C. After dissolving the samples, five drops of reagent (phenolphthalein) were added and the solution titrated with NaOH (0.1 M). The result obtained in end-point was recorded as the first titer or V1. Subsequently, 10 mL of NaOH (0.1 M) were added and for hydrolysis, the solution was stirred for 30 min. Then, 10 mL HCl (0.1 M) were added and the solution was stirred vigorously the pink color completely disappeared. After adding phenolphthalein reagent (five drops), the excess HCl was titrated with NaOH (0.1 M) until a pale pink color was obtained. This used volume of NaOH was recorded as the second titer or V2. The DE of PGH pectin was calculated as follows (Hosseini et al., 2016a):

\[
\text{DE} = \frac{V_2 (\text{mL})}{V_1 (\text{mL}) + V_2 (\text{mL})} \times 100
\]

The galacturonic acid content was measured with colorimetric (3,5-dimethylphenyl reagent) method according to Blumenkrantz and Asboe-Hansen (1973) with minor modifications. First, 6 mL of sulfuric acid/tetraborate was added to tubes containing 1 mL of the sample (200 µg/mL) and the tubes were cooled in an ice-water bath. Then, the tubes were shaken in a vortex mixer, were heated in a boiling water bath (6 min), and were cooled in an ice-water bath, respectively. After adding reagent and shaking for 5 min, the absorbance of samples was determined at 520 nm. The galacturonic acid content was calculated by comparison with a standard curve. For this purpose, the standard curve was obtained from standard galacturonic acid solutions in concentration of 0–250 µg/mL.

2.4. Total galacturonic acid content

The galacturonic acid content was measured with colorimetric (3,5-dimethylphenyl reagent) method according to Blumenkrantz and Asboe-Hansen (1973) with minor modifications. First, 6 mL of sulfuric acid/tetraborate was added to tubes containing 1 mL of the sample (200 µg/mL) and the tubes were cooled in an ice-water bath. Then, the tubes were shaken in a vortex mixer, were heated in a boiling water bath (6 min), and were cooled in an ice-water bath, respectively. After adding reagent and shaking for 5 min, the absorbance of samples was determined at 520 nm. The galacturonic acid content was calculated by comparison with a standard curve. For this purpose, the standard curve was obtained from standard galacturonic acid solutions in concentration of 0–250 µg/mL.

2.5. Evaluation of intrinsic viscosity and molecular weight

The intrinsic viscosity of pectin solution was obtained by a capillary viscometer (LAUDA, New Jersey, USA) at 25°C. 0.1 g of dried pectin was dispersed in 100 mL of an aqueous solution containing 0.1 mol/L sodium chloride (to reduce the electro-viscous effects), and the density of the solutions was determined by a pycnometer. The relative viscosity of the pectin solution was calculated as follows (Chen & Joslyn, 1967):
where \( \eta_f \) is the relative viscosity, \( \eta \) is the pectin solution viscosity (mPa s), \( \eta_i \) is the solvent viscosity (mPa s), \( t_i \) is the time taken by pectin solution to flow in the viscometer (s), \( d_i \) is the pectin solution density (g/cm\(^3\)), and \( d_s \) is the solvent density (distilled water with density of 0.997 g/cm\(^3\)). The intrinsic viscosity was calculated as follows (Tangler & Rao, 1987):

\[
\eta = 1 + [\eta] C
\]

(4)

where \([\eta] \) is the intrinsic viscosity (L g\(^{-1}\)) and C is the pectin concentration (g L\(^{-1}\)). Finally, the molecular weight (MW) of pectin (g mol\(^{-1}\)) was estimated by the Mark Houwink–Sakurada equation as follows (Kar & Arslan, 1999):

\[
[\eta] = k[MW]^a
\]

(5)

where \( k \) and \( a \) are constants. These two constants for pectin dissolved in a sodium chloride solution (0.1 mol/L) are 4.36 \times 10^{-5} \text{ L g}^{-1}\text{ and 0.78, respectively} (Venzon et al., 2015).

2.6. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of PGH pectin was recorded by means of a Perkin Elmer FTIR spectrometer (Perkin Elmer Co., MA, USA) using the KBr disk method. It should also be noted that the range and resolution of this spectra were 3500–600 and 4 cm\(^{-1}\), respectively.

2.7. Experimental design

In this study, a central composite response surface experimental design with four factors in five levels and seven replications at the center point (to approximate the experimental error) was applied to optimize and explore the effect of process variables (pH \( (X_1) \), temperature \( (X_2) \), time \( (X_3) \) and LSR \( (X_4) \)) on the responses (yield and DE of PGH pectin). These factors and their levels were shown in Table 1. Also, data obtained in laboratory were fitted to the empirical second order polynomial equation in order to express the relationship between process variables and responses. The generalized form of this equation was expressed as follows (Ghasemlou, Khodaiyan, & Gharibzahedi, 2012; Moodhyti et al., 2015):

\[
\text{Yield or DE(%) = } \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + e_i
\]

(6)

where \( \beta_0 \) is a constant coefficient; \( \beta_i \), \( \beta_{ii} \) and \( \beta_{ij} \) are the coefficients of the linear, quadratic and interactive terms, respectively; \( k \) is the number of independent variables (\( k = 4 \) in this research); \( X_i \) and \( X_j \) are coded variables; and \( e_i \) is the error. It should also be noted that all the computation and graphics in this study were conducted using the statistical software Design Expert 7.0.

3. Results and discussion

3.1. Accuracy and variance analysis of the regression model

In this study, CCD was applied to investigate the effect of independent variables on extraction yield and DE of PGH pectin. According to used design, 31 runs for 16 factorial points, 8 axial points and 7 center points were carried out. The design matrix and also the experimental and predicted responses are given in Table 1.

Generally, the analysis of variance (ANOVA) shows whether model variations are significant compared to the variability of the results obtained in laboratory or not. The results of ANOVA are presented in Table 2.

The adequacy of the obtained model was determined by the coefficient of determination (R\(^2\)) and adjusted determination coefficient (adjusted R\(^2\)). The determination coefficient of yield and DE (0.9655 and 0.9875, respectively) of PGH pectin showed that 96.55 and 98.75% of the total variation could be explained by the model (Khodaiyan, Razavi, & Mousavi, 2008). Also, the adjusted determination coefficient for both yield and DE (0.9353 and 0.9765, respectively) was high and this showed that most variation of extraction yield and DE of PGH pectin could be predicted by the obtained model. On the other hand, the p-value of the model was lower than 0.0001 (significant) for yield and DE and lack of fit was insignificant for these two parameters (0.097 and 0.634 for yield and DE, respectively). These results demonstrated that the models were well adapted to the responses (Hosseini et al., 2016a). The relationship between the responses (yield and DE) and independent variables (pH, temperature, time, and LSR) were given below:

\[
\text{Yield(%) = +13.49 - 3.14X}_1 + 0.75X_2 + 0.23X_3 + 0.15X_4 - 0.08X_1^2 + 0.31X_2^2 + 0.09X_3^2 + 0.08X_4^2 + 0.10X_1X_2 + 0.07X_1X_3 - 0.18X_1X_4 - 0.04X_2X_3 - 0.07X_2X_4 - 0.06X_3X_4
\]

(7)

\[
\text{DE(%) = +41.51 + 7.31X}_1 - 0.26X_2 + 1.98X_3 - 0.20X_4 - 0.63X_1^2 + 0.90X_2^2 + 0.35X_3^2 + 0.55X_4^2 + 0.47X_1X_2 - 0.92X_1X_3 + 0.13X_1X_4 - 0.08X_2X_3 - 0.17X_2X_4 - 0.12X_3X_4
\]

(8)

where \( X_1 \) is a coded independent factor (\( X_1 = \text{pH} \), \( X_2 = \text{temperature} \), \( X_3 = \text{extraction time} \), and \( X_4 = \text{LSR} \)).

3.2. Effect of independent variables on the yield of PGH pectin

Perturbation plot for extraction yield of PGH pectin is presented in Fig. 1(a). This graph could be applied to find variables that have greatest effect on the response. A steep slope or a relatively flat line for a factor shows that the response is sensitive or insensitive to it factor, respectively (Maran et al., 2014). In this part, the perturbation graph showed that the pH of extraction solution (in comparison with the other three factors) had greatest role in production of pectin from PGH.
Fig. 1. Perturbation plot for extraction yield (a) and DE (b) of PGH pectin (A = pH; B = Temperature; C = Extraction time and D = LSR).

Fig. 2, as a 3-D response surface plot for yield of PGH pectin, shows that the yield of pectin was increased with decreasing pH value. Ma et al. (2013) studied the extraction of pectin from sugar beet pulp, and reported that this increase in yield with decrease in pH value is probably due to disruption of the cell walls in strong acidic conditions and thus, the increase in release and dissolve of pectin in acidic solution. This result also is in line with the study of Raji et al. (2017), who studied the extraction optimization of pectin from melon peel.

The results obtained from Fig. 2 indicated that the yield of pectin was increased with an increase in extraction temperature. Samavati (2013), who studied the influence of extraction conditions on the polysaccharide yield from Abelmoschus esculentus, reported that the increase in extraction yield when temperature is increased could be due to an increase in penetration of the solvent into the solid matrix and so, this better penetration of solvent caused the increase of the polysaccharides mass going out from the solid particles into the solution. These observations also is similar to results obtained from sour orange peel and waste durian rinds pectin by Hosseini et al. (2016a) and Maran (2015), respectively.

The extraction time is another important factor in pectin extraction. The results showed that with increase in extraction time, the extraction yield also was increased (Fig. 2). This result agreed with Jafari et al. (2017)' work who obtained pectin from carrot pomace. These researchers stated that this increase in pectin yield with increasing extraction period is probably due to supplying more reaction time and thus increasing mass transfer of pectin from the solid particle into the liquid medium. Furthermore, Samavati and Manoochehrizade (2013), who studied extraction conditions on polysaccharide yield from Malva sylvestris, also obtained a similar result with the current study.

In many researches, LSR was used as independent variables for pectin extraction. In this study, the results showed that the LSR has a positive effect on the pectin yield, so that with increase in LSR, the pectin yield also is increased (Fig. 2). This increase in yield is probably due to an increase in driving force for the pectin mass transfer (Bendahou, Dufresne, Kaddami, & Habibi, 2007). In other study, Santos et al. (2013), who investigated the influence of extraction conditions on the pectin yield from sisal waste, also achieved similar results.

3.3. Determination of optimum conditions

In this research, desired function method was applied to optimize the pectin extraction conditions from PGH (Maran, Swathi, Jeevitha, Jayalakshmi, & Ashvini, 2015). The optimum extraction conditions for achieve to maximum extraction yield were as follows: pH 0.5, temperature of 90 °C, extraction time of 30 min, LSR of 50 v/w, and also the maximum yield of PGH pectin was 23.42%. Under these conditions, triplicate experiments were performed and the mean values for these real experiments were 22.1 ± 0.5%. As can be seen, the experimental yield was close to the predicted yield and so, the validation of optimized conditions (the yield of 23.42% for PGH pectin) was demonstrated.

3.4. Effect of independent variables on the DE of PGH pectin

The results obtained from used acidic conditions for extraction of pectin (DE was between 26.00 and 53.01%) showed that the PGH pectin can be considered as LMP. This type of pectin makes gel with or without the presence of sugar. Therefore, LMP can be used as a food additive for the gel formation in low calorie products (Chan & Choo, 2013; Wai et al., 2010).

Fig. 3 indicated that DE of PGH pectin with an increase in pH value (as most influential factor on DE (Fig. 1(b))), extraction time, LSR, and a decrease in temperature was increased. These observations are consistent with the results obtained by most researchers (Hosseini et al., 2016a; Wai et al., 2010). For example, Jafari et al. (2017) reported that DE of carrot pomace pectin was decreased with a large increase and decrease in temperature and pH value, respectively. This decrease in DE can be due to increase in de-esterification of polygalacturonic chains (Mort, Qiu, & Maness, 1993).

3.5. Evaluation of galacturonic acid content, intrinsic viscosity and molecular weight

The pectin known as a polysaccharide rich from galacturonic acid and based on FAO and EU regulations, the galacturonic acid content of pectin should not be less than 65% (Bagherian, Ash-tiani, Foualditajar, & Mohtashamy, 2011). Also, the galacturonic acid content is a good index of pectin purity, so that a direct relationship between this parameter and purity of pectin was existed (Liang et al., 2012). In current study, the galacturonic acid content of pectin extracted under optimal extraction conditions (pH of 0.5, temperature of 90 °C, extraction time of 30 min, LSR of 50 v/w) was evaluated and the results showed that the amount of this parameter was about 65%. Therefore, it can be stated that PGH pectin has a good potential for production of pectin with a suitable purity. Also, this result is approximately similar to data obtained from passion fruit peel (66.6%) by De Oliveira et al. (2016).
In this experiment, the relative viscosity, the intrinsic viscosity and the molecular weight of the PGH pectin obtained under optimal extraction conditions (pH of 0.5, temperature of 90°C, extraction time of 30 min, LSR of 50 v/w) were calculated. The results showed that these three parameters were about 1.07, 0.07 Lg⁻¹ and 12875.3 g mol⁻¹ (or ~12.875 KDa), respectively. It should also be noted that the intrinsic viscosity and molecular weight obtained from PGH pectin were lower than the data of orange pomace pectin and melon peel pectin reported by Venzon et al. (2015) and Raji et al. (2017), respectively.

3.6. FTIR spectrum of PGH pectin

Fourier transform infrared spectroscopy is a structure spectroscopic technique that uses to characterize the bonding structure of atoms. This process is due to interaction of infrared radiation with matter (Tucureanu, Matei, & Avram, 2016). In this part, the FTIR spectrum of PGH pectin obtained under optimal extraction condition (pH of 0.5, temperature of 90°C, extraction time of 30 min, LSR of 50 v/w) and also, the comparison between wavenumbers of FTIR spectra related to PGH and commercial pectin are shown in Fig. 4 and Table 3, respectively. The peak at around 3282 cm⁻¹ was related to the stretching vibrations of OH groups of pectin and also, the peak of 2929 cm⁻¹ was due to CH (CH, CH₂ and CH₃) vibrations (Tian, Zhao, Guo, & Yang, 2011). The CO groups of pectin usually have three peaks: the peak of 1757 cm⁻¹, which is attributed to CO of the carboxylic acid methyl ester, and the peaks at around 1698 and 1401 cm⁻¹, which are related to CO of free carboxyl groups (Jafari et al., 2017). In the end, the bonds between 800 and 1200 cm⁻¹ (as the finger print region) are unique to a compound and its inter-
**Fig. 3.** Effect of independent variables (pH, temperature (°C), time (min), and LSR (v/w)) on the DE of the PGH pectin.

<table>
<thead>
<tr>
<th>FTIR wavenumber (cm⁻¹)</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGH</td>
<td>Commercial pectin</td>
</tr>
<tr>
<td>3282</td>
<td>3329</td>
</tr>
<tr>
<td>2929</td>
<td>2940</td>
</tr>
<tr>
<td>1757</td>
<td>1753</td>
</tr>
<tr>
<td>1698 and 1401</td>
<td>1630 and 1441</td>
</tr>
<tr>
<td>800–1200</td>
<td>800–1200</td>
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<tr>
<td></td>
<td>O−H</td>
</tr>
<tr>
<td></td>
<td>C−H</td>
</tr>
<tr>
<td></td>
<td>C=O from methylesterified carboxyl groups</td>
</tr>
<tr>
<td></td>
<td>C=O from free carboxyl groups</td>
</tr>
<tr>
<td></td>
<td>Finger print</td>
</tr>
</tbody>
</table>

Table 3
Assignments of FTIR wavenumbers in the range 3500–600 cm⁻¹ of PGH and commercial pectin.

According to the results obtained in this section, it can be stated that the produced precipitate is a polysaccharide-rich polygalacturonic acid (Santos et al., 2013).

**4. Conclusion**

In this study, the central composite design was used to study the effect of extraction conditions (pH, temperature, time and liquid/solid ratio) on the yield and DE of pistachio green hull pectin. The results showed that the yield and DE of pectin ranged from
7.31 to 19.02% and 26.00 to 53.01%, respectively. Also, the maximum yield of pistachio green hull pectin obtained at the optimal conditions (pH of 0.5, temperature of 90°C, time of 30 min and liquid/solid ratio of 50 v/w) was 22.1±0.5%, which was close to the predicted yield (23.42%). Under this condition, the galacturonic acid content, intrinsic viscosity and molecular weight of pectin were about 65%, 0.071 g L⁻¹ and 12875.3 g mol⁻¹, respectively. Also, the results obtained from FTIR spectrum showed that the pectin produced in optimum condition is rich from polygalacturonic acid.

**References**


