

Pistachio green hull pectin: Optimization of microwave-assisted extraction and evaluation of its physicochemical, structural and functional properties



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ABSTRACT

The optimal microwave-assisted extraction conditions of pistachio green hull (PGH) pectin (extraction yield of 18.13%) were in microwave power of 700 W, irradiation time of 165 s, and pH of 1.5. The physico-chemical, structural and functional properties of pectin was examined and the results showed that PGH pectin under optimum conditions was low methoxyl (about 12.1%) with molecular weight of 1.659 kg/mol, emulsifying activity of 58.3%, total phenolic content of 18.18 mg GalAE/g pectin and good surface activity (46.23 and 49.75 mN/m at 0.1 and 0.5% w/v). Also, high performance liquid chromatography analysis illustrated that the pectin is including galacturonic acid (66.0%), arabinose (26.2%), galactose (3.7%), rhamnose (2.7%) and xylose (1.0%). In addition, ¹H NMR and FTIR spectrums confirmed the presence of pectin in obtained supernatant. Surface morphology analysis and X-ray diffraction pattern showed that PGH pectin had a rough surface with crystalline structure.

1. Introduction

Pistachio (*Pistacia vera* L.), as one of the most important agricultural products in Iran (Goli, Barzegar, & Sahari, 2005), is including an edible seed in the center, a purple seed coat protected by a hard lignified shell and a green to yellow-red hull (Erşan, Güçlü Üstündağ, Carle, & Schweiggert, 2016). Pistachio green hull (PGH), as a large portion of pistachio (approximately one-third of dry matter weight), is a suitable source of phenolic acids, flavonoids, fatty acids, proteins, vitamins and polysaccharides such as pectin (Chaharbaghi, Khodaiyan & Hosseini, 2017; Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010) which is unfortunately discarded as an agricultural waste (Grace et al., 2016).

Thus, the use of it for the production of a value-added component can both increase the value of the pistachio production and decrease the environmental contaminant (Rajaei et al., 2010). So far, the several studies have been conducted on the antioxidant and phenolic compounds of PGH (Erşan et al., 2016; Rajaei et al., 2010; Grace et al., 2016; Rafiee, Barzegar, Sahari, & Maherani, 2017), but to the best of our knowledge, its polysaccharides (such as pectin) were less regarded (Chaharbaghi et al., 2017).

Pectin, as a heteropolysaccharide, is widely distributed in the primary cell wall and the middle lamella of plant tissues and played an important role in the viability of plant cell (Chaharbaghi et al., 2017). Its main structure is including poly- α -(1,4)-D-galacturonic acid residues

Abbreviations: PGH, pistachio green hull; DE, degree of esterification; LMP, low methoxyl pectin; HMP, high methoxyl pectin; GRAS, generally recognized as safe; LSR, liquid to solid ratio; RSM, response surface methodology; BBD, Box-Behnken design; TFA, trifluoroacetic acid; AA, ascorbic acid; GalA, galacturonic acid; Ara, arabinose; Gal, galactose; Rha, rhamnose; Xyl, xylose; Glu, glucose; Fru, fructose; GallAE, gallic acid equivalent; WHC, water holding capacity; OHC, oil holding capacity; EA, emulsion activity; ES, emulsion stability; ELV, the volume of emulsion layer; TVS, total volume of system; REL, the volume of remained emulsion layer; IEL, the volume of initial emulsion layer; RG-I, rhamnogalacturonan-I; RG-II, rhamnogalacturonan-II; HG, homogalacturonan; MR₁, the ratio of mol% of galacturonic acid to mol% of neutral sugars (the linearity of pectin structure); MR₂, the ratio of mol% of rhamnose to mol% galacturonic acid (the contribution of RG region in the pectin structure); MR₃, the ratio of mol% of arabinose and galactose to mol% of rhamnose (the length of branches attached to RG-I); Mw, average molecular weight; NMR, nuclear magnetic resonance; FT-IR, Fourier transform infrared; XRD, X-ray diffraction; HPLC, high performance liquid chromatography; GPC, gel permeation chromatography

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that neutral sugars connected to it as side chains. However, this carbohydrate is composed by several complex polysaccharides which homogalacturonan, rhamnogalacturonan I are the most important of it (Petkowicz, Vriesmann & Williams, 2017). The one of the most effective factors on the pectin application is degree of esterification (DE). Based on the factor, there are two different type of pectin with names of low methoxyl pectin (LMP; DE lower than 50%) and high methoxyl pectin (HMP; DE higher than 50%). Pectin, as a GRAS (generally recognized as safe) and natural additive, is used extensively in food (as a thickener, stabilizer, gelling agent and so on) and pharmaceutical (in drug encapsulation) industries (Xu et al., 2014). Also, this polysaccharide has health benefits on the blood fat, heart disease and so on (Liu, Fishman & Hicks, 2007). Due to its applications and functional properties, the universal demand for pectin increases 4–5% annually (Raji, Khodaiyan, Rezaei, Kiani & Hosseini, 2017).

Nowadays, the citrus peel and apple pomace are the most important sources for the commercial pectin production. However, due to high demand for this ingredient, many studies were done on other pectin sources such as banana peel (Swamy & Muthukumarappan, 2017), sour orange peel (Hosseini, Khodaiyan, & Yarmand, 2016a,b), papaya peel (Koubala, Christiaens, Kansci, Van Loey & Hendrickx, 2014), watermelon rind (Petkowicz et al., 2017), melon peel (Raji et al., 2017), carrot pomace (Jafari, Khodaiyan, Kiani & Hosseini, 2017) and so on.

Microwave-assisted extraction procedure involves irradiation of the immersed samples in a solvent. The energy of these waves leads to the molecules vibration and enhances their separation. This method has many advantages such as low operation time, consumed solvent and production cost and also high extraction yield (Hosseini, Khodaiyan, & Yarmand, 2016b). Therefore, it can cover disadvantages of traditional methods (such as aqueous method).

In microwave-assisted extraction method, the different factors such as irradiation power, irradiation time, pH and liquid/solid ratio (LSR) can be effective on the yield and quality of pectin (Lefsih et al., 2017). Therefore, the optimization of extraction conditions to achieve maximum production yield is necessary. Response surface methodology (RSM), as a group of statistical and mathematical techniques, has been extensively applied in processes optimization and investigation of the effect of different independent factors and their interactions on several responses (Pasandide, Khodaiyan, Mousavi & Hosseini, 2017).

In previous study, PGH was used to optimize pectin extraction using acidic method (Chaharbaghi et al., 2017). However, structural, physicochemical and functional properties of PGH pectin are still completely unclear. Therefore, the aim of this study was firstly, the optimization of microwave-assisted extraction conditions (microwave power, pH, extraction time and the liquid/solid ratio (LSR)) of PGH pectin using by RSM and secondly, the study of structural, physicochemical and functional properties of pectin obtained under optimum extraction condition.

2. Materials and methods

2.1. Materials and chemicals

Fresh raw pistachios (Akbari variety) obtained from a local store in Karaj, Alborz, Iran. After separating the soft green shell from pistachios, they were placed on a stainless steel tray and dried in an oven (45 °C) until a constant weight was achieved (about 16 h). The dried PGH were crushed in a blender and passed through a 40 mesh sieve. Then, the obtained PGH powder were packed in a dark bag and kept in dry and dark conditions for the next experiments. Citric acid (99%), hydrochloric acid (37%), sulfuric acid (95–97%), sodium hydroxide ($\geq 99\%$), TFA (trifluoroacetic acid) ($\geq 99\%$), Folin-Ciocalteu's phenol reagent, DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and Standard monosaccharides (galacturonic acid, arabinose, galactose, rhamnose, xylose, glucose, and fructose) were bought from Merck Chemical Co. (Darmstadt, Germany). Also, Standard dextrans (1, 5, 25, and 70 kDa) were purchased from

Pharmacosmos (Holbaek, Denmark).

2.2. Pectin extraction

First, the one factor at a time was used to reach to the best LSR (the liquid/solid ratio). After choosing the best LSR (15 v/w), the microwave-assist extraction of pectin from the dried PGH powder was carried out according to the method described by Li, Jia, Wei, and Liu (2012) with a little modifications. The extraction conditions are present in Table 1. After extraction, the obtained acidic aqueous solution was filtered and centrifuged at 10,000g for 15 min, respectively. Then, the ethanol (96%) was added and the solution was stored for 24 h in 7 °C. In the next step, the supernatant was separated by centrifuging (10,000g, 15 min), washed with ethanol (three times) and dried at 45 °C for 24 h, respectively. Finally, the PGH pectin yield was calculated as follows:

$$\text{Extraction yield (\%)} = \frac{\text{Dried pectin (g)}}{\text{Dried PGH powder (g)}} \times 100 \quad (1)$$

2.3. Experimental design

In order to reach the best LSR, the one factor at a time was applied. In this method, one of the factors (LSR) was variable (5, 10, 15, 20, 25, and 30 v/w) and the others were constant (power of 500 W, irradiation time of 120 s, pH of 2.25). After choosing the best LSR, this factor was considered constant in all experiments. On the other hand, a Box-Behnken design (BBD) with three variables in three levels (Table 1) was applied to evaluating the effect of the different factors (power, irradiation time and pH) on the extraction yield and DE of PGH pectin. It should also be noted that all computation and graphics were accomplished using the statistical software Design Expert 7.0 and Excel.

2.4. Degree of esterification (DE)

Due to the high importance of DE, this parameter was evaluated for all of the pectin samples by using titration method (Hosseini et al., 2016b). For this purpose, 100 mg pectin was mixed with 20 ml CO₂-free water and 2 ml ethanol. After complete dissolving of the samples and adding three drops of phenolphthalein reagent, the solution was titrated with NaOH (0.1 M) until the appearance of pink color (the first volume, V₁). Afterward, 10 ml NaOH (0.1 M) was added to the solution and stirred for 20 min. Then, 10 ml HCl (0.1 M) was added to the solution and agitated until the complete disappearance of the pink color. Finally, the remained HCl was titrated with NaOH (0.1 M) (the second volume, V₂). The DE of PGH pectin was calculated as follows:

$$\text{DE(\%)} = \frac{V_2}{V_2 + V_1} \times 100 \quad (2)$$

2.5. Chemical composition of PGH pectin

In this part, chemical parameters of PGH pectin extracted under optimal conditions were determined. For this purpose, moisture content of PGH pectin was estimated by losing weight after drying pectin at 105 °C for 24 h (Jiang et al., 2012). Ash content of pectin was measured after incinerating 1 g of PGH pectin in furnace at 550 °C for 6 h and cooling in desiccator. Protein content of pectin was determined by Kjeldahl method ($N \times 6.25$) (Yapo, Robert, Etienne, Wathélet, & Paquot, 2007).

2.6. Monosaccharides composition of PGH pectin

High performance liquid chromatography (HPLC) method was applied to determine the monosaccharide composition of PGH pectin (extracted under optimal conditions). For this purpose, pectin was first hydrolyzed by 2 M trifluoroacetic acid (TFA) solution at 85 °C for 3 h

and then immediately cooled in ice-water bath and centrifuged at 2500g for 15 min, respectively. On the next stage, TFA was removed from the hydrolysates by freeze-drying. The dried hydrolysates were dissolved in deionized water and then analysed by HPLC apparatus equipped with a Eurokat H column and a smartline RI detector 2300 (Knauer, Berlin, Germany). The mobile phase was 0.005 M sulfuric acid with a flow rate of 0.8 ml/min at 70 °C. Eventually, the monosaccharide composition was estimated according to the retention time of the standard monosaccharides: galacturonic acid (GalA), arabinose (Ara), galactose (Gal), rhamnose (Rha), xylose (Xyl), glucose (Glu), and fructose (Fru).

2.7. Determination of average molecular weight

The molecular weight of PGH pectin extracted under optimal conditions was estimated by gel permeation chromatography (GPC) as described by Wang, Chen and Lü, (2014). For this purpose, the Shimadzu LC-20A instrument equipped with Ultrahydrogel™ linear columns and RI detector was used. The flow rate of mobile phase (0.1 M NaNO₃ solution) was 1 ml.min⁻¹. Mw was determined according to the calibration curve of standard dextran (1, 5, 25, and 70 kDa).

2.8. Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC of PGH pectin extracted under optimal conditions was determined by method explained by Betancur-Ancona, Peraza-Mercado, Moguel-Ordonez and Fuertes-Blanco (2004). Briefly, 1 g pectin was added to 10 ml distilled water or sunflower oil (density of oil was 0.9 g/ml). Then, the mixture was vortexed for 1 min and centrifuged for 30 min at 3000g. The supernatant was removed and the residual was weighted. WHC and OHC were expressed as grams of water or oil held by 1 g pectin.

2.9. Total phenolic content

Total phenolic content of PGH pectin extracted under optimal conditions was determined by method of Sharma, Kamboj, Khurana, Singh and Rana (2015) with slight changes. Briefly, 0.5 ml of pectin solution (1% w/v) was prepared and 2.5 ml of Folin-Ciocalteu reagent (10% v/v) and 2 ml of sodium carbonate solution (7.5% w/v) were added to it, respectively. After 1 h incubation at room temperature, the absorbance of the mixture was measured at 750 nm. Gallic acid was used as standard (R² of the standard curve was 91.78%) and the total phenolic content of PGH pectin was expressed as milligram gallic acid equivalents/gram of PGH pectin (mg GallAE/g).

2.10. DPPH radical scavenging activity

Determination of antioxidant activity of PGH pectin extracted under optimal conditions was carried out by the method described by Chaouch, Hafsa, Rihouey, Le Cerf and Majdoub (2015) with a little modification. 1 ml sample in different concentrations (1–50 mg/ml) was added to 4 ml of methanolic DPPH solution (0.1 mM). The mixtures were vortexed and incubated in darkness for 30 min at room temperature. The absorbance of the mixtures was measured by a UV–Vis spectrophotometer (Spectrum SP-UV500DB) at 517 nm. Ascorbic acid (AA) in similar concentrations was used as standard. Lower absorbance of the samples shows higher antioxidant activity. The DPPH radical activity was estimated as follow:

$$\text{DPPH radical activity (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100 \quad (3)$$

2.11. Evaluation of emulsifying properties

In order to assess the emulsifying activity (EA) and emulsion stability (ES) of pectin extracted under optimal conditions, oil-in-water (O/W) emulsions were produced by adding 5 ml sunflower oil to 5 ml pectin aqueous solution (0.5% w/w) containing 0.02% sodium azide as a bactericide. Then, the pre-emulsions were mixed by a homogenizer at 10,000g for 4 min and centrifuged at 4000g for 5 min, respectively (Pasandide et al., 2017). Eventually, the EA was calculated by follows formula:

$$\text{EA(\%)} = \frac{\text{ELV}}{\text{TVS}} \times 100 \quad (4)$$

where ELV was the volume of emulsion layer and TVS was the total volume of system.

To determine the ES of pectin, a similar method was used for preparation of emulsion. Then, after 1 and 30 days at 4 °C and room temperature, the ES was calculated by follows formula:

$$\text{ES(\%)} = \frac{\text{REL}}{\text{IEL}} \times 100 \quad (5)$$

where REL was the remained emulsion layer volume and IEL was the initial emulsion layer volume.

2.12. Surface tension measurement

For evaluation of surface tension of PGH pectin (extracted under optimal conditions) solutions (0.1 and 0.5% w/v), a tensiometer (Nanometric-pazhoh Co. Tehran, Iran) with the Wilhelmy plate method by a mica plate was applied (Yapo, Wathelet, & Paquot, 2007).

2.13. Nuclear magnetic resonance (NMR) spectra

For this purpose, PGH pectin obtained under optimal conditions was dehydrated and dissolved in D₂O. Then, ¹H NMR analysis was performed by a Varian Unity Inova 500 MHz spectrometer in internal temperature of 20 °C. 32 scans were collected with a relaxation delay of 1 s and an acquisition time of 4.09 s.

2.14. Fourier transform-infrared (FT-IR) analysis

FT-IR spectroscopy of PGH pectin obtained under optimal conditions was done by a Bruker FT-IR Tensor 27 spectrometer (Billerica, Massachusetts, USA) with potassium bromide (KBr) method in the wavenumber range of 4000–600 cm⁻¹ and resolution of 4 cm⁻¹.

2.15. X-ray diffraction (XRD) analysis

The XRD pattern of PGH pectin extracted under optimal conditions was determined by using an X-ray diffractometer (PHILIPS, Amsterdam, Netherland). The sample was scanned with diffraction angle (2θ) between 10° and 80°. The step size and time per step were 0.05° (2θ) and 1 s/step, respectively.

2.16. Morphological analysis

For evaluation of the surface morphology of PGH pectin obtained under optimal conditions, a scanning electron microscope (Vega3, Tescan Co. Ltd., Brno, Czech Republic) was used. For this purpose, the pectin sample was fixed onto the stub and coated with gold by a gold sputter. Eventually, the sample was photographed with the accelerating voltage of 15 KV).

3. Results and discussion

3.1. Statistical analysis and optimization of pectin extraction

In order to optimize the extraction conditions, four factors (LSR, microwave power, irradiation time and pH) were selected as independent variables. So, the best LSR was selected by one factor at a time design and other factors were optimized by Box-Behnken design (BBD) in constant LSR.

One factor at a time design was ran in different LSRs (1:5, 1:10, 1:15, 1:20, 1:25, 1:30 v/w), while the other factors were fixed (microwave power: 500 W, irradiation time: 120 s and pH: 2.25). The data showed that, by increasing the LSR up to 1:15 v/w, the extraction yield was increased up to 4.8%, which can be due to increasing contact surface area between solvent and the plant matrix by increase in solvent ratio. However, by increasing the LSR beyond 1:15 v/w, the pectin penetration into the surrounding medium was decreased and the microwave irradiation energy were adsorbed by excessive solvent which had a negative effect on the pectin extraction yield (Hosseini et al., 2016b; Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2014). Hence the LSR of 1:15 v/w was used in all experiments of this study.

BBD as a collection of mathematical and statistical techniques which can examine the factors (microwave power, pH, and time) effect on responses (pectin extraction yield and DE of PGH pectin) were applied to process optimization. Experimental and predicted results for two responses were presented in Table 1. This table shows that the pectin extraction yield was ranged between 2.20 and 18% and the highest and lowest yield were due to run number six (microwave power of 700 W, time of 120 s, and pH of 1.5) and run number one (microwave power of 300, time of 60 s, and pH of 2.25), respectively. Also, by resolving the suggested model for the extraction yield (Eq. (6)), the highest extraction yield of PGH pectin was 18.13% in optimum extraction conditions (microwave power of 700 W, irradiation time of 165 s and pH of 1.5). In order to ensure the accuracy of the predicted model, three runs were done in mentioned conditions and the result ($18.13 \pm 0.53\%$) was very close to predicted value. Thus, this consequence showed that the proposed model is completely suitable for prediction of the extraction yield.

Table 1

Independent factors and their levels, experimental and predicted results (extraction yield and degree of esterification) of PGH (pistachio green hull) pectin.

Factors		Unit		Actual levels			
				−1	0	+1	
Microwave power (X_1)		W		300	500	700	
Irradiation time (X_2)		s		60	120	180	
pH (X_3)		–		1.5	2.25	3	
Run	X_1	X_2	X_3	EEY ^A	EDE ^A	PEY ^A	PDE ^A
1	300	60	2.25	2.2	39.47	2.04	42.40
2	700	60	2.25	3.6	48.72	3.51	47.50
3	300	180	2.25	3.8	53.34	3.89	54.56
4	700	180	2.25	4.2	37.14	4.36	34.21
5	300	120	1.50	12.8	14.28	14.55	11.57
6	700	120	1.50	18.0	11.96	17.37	13.40
7	300	120	3.00	4.8	82.76	4.82	81.32
8	700	120	3.00	4.0	61.54	3.95	64.24
9	500	60	1.50	14.0	10.71	14.11	10.49
10	500	180	1.50	16.4	16.94	16.26	18.42
11	500	60	3.00	3.2	80.76	3.34	79.28
12	500	180	3.00	4.0	70.00	3.89	70.22
13	500	120	2.25	4.2	55.56	4.07	53.41
14	500	120	2.25	4.2	53.50	4.07	53.41
15	500	120	2.25	3.8	51.16	4.07	53.41

^A EEY, EDE, PEY and PDE are experimental extraction yield, experimental DE (degree of esterification), predicted extraction yield and predicted DE, respectively.

Table 2

The results of ANOVA (analysis of variance) for extraction yield and DE (degree of esterification) of PGH (pistachio green hull) pectin.

Source	DF	Some of squares	Mean square	F-value	p-value
(A) Yield					
Model	9	406.407	45.156	179.67	0.000
Linear	3	263.830	87.943	349.91	0.000
Power	1	4.805	4.805	19.12	0.007
Time	1	3.645	3.645	14.50	0.013
pH	1	255.380	255.380	1016.10	0.000
Square	3	132.687	44.229	175.98	0.000
Power × Power	1	0.720	0.013	0.05	0.832
Time × Time	1	3.800	1.151	4.58	0.085
pH × pH	1	128.166	128.166	509.95	0.000
Interaction	3	9.890	3.297	13.12	0.008
Power × Time	1	0.250	0.250	0.99	0.364
Power × pH	1	9.000	9.000	35.81	0.002
Time × pH	1	0.640	0.640	2.55	0.171
Error	5	1.257	0.251		
Lack-of-Fit	3	1.150	0.383	7.19	0.125
Pure Error	2	0.107	0.053		
Total	14	407.664			
R^2			0.9969		
Adj R^2			0.9914		
Pred R^2			0.9543		
(B) DE					
Model	9	7935.07	881.67	83.02	0.000
Linear	3	7387.20	2462.40	231.87	0.000
Power	1	116.21	116.21	10.94	0.021
Time	1	0.63	0.63	0.06	0.818
pH	1	7270.37	7270.37	684.61	0.000
Square	3	224.47	74.82	7.05	0.030
Power × Power	1	83.33	105.81	9.96	0.025
Time × Time	1	32.74	42.33	3.99	0.102
pH × pH	1	108.40	108.40	10.21	0.024
Interaction	3	323.39	107.80	10.15	0.014
Power × Time	1	161.93	161.93	15.25	0.011
Power × pH	1	89.30	89.30	8.41	0.034
Time × pH	1	72.17	72.17	6.80	0.048
Error	5	53.10	10.62		
Lack-of-Fit	3	43.41	14.47	2.99	0.261
Pure Error	2	9.69	4.85		
Total	14	7988.17			
R^2	9		0.9934		
Adj R^2	3		0.9814		
Pred R^2	3		0.9103		

It should be stated that, in comparison to the other sources of pectin extraction yield (using microwave-assisted extraction) like banana peel (2.18%, Swamy & Muthukumarappan, 2017), dragon fruit peel (7.5%, Thirugnanasambandham, Sivakumar, & Maran, 2014), passion fruit peel (18.2%, Seixas et al., 2014) *Citrullus Lanatus* fruit rinds (25.79%, Maran et al., 2014), and orange peel pectin (19.24%, Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013), PGH pectin had a reasonable extraction yield (18.13%).

Analysis of variance (ANOVA) results were demonstrated in Table 2. As can be seen, the effect of the linear, square and the interaction models for both the extraction yield and DE were significant. The high F-value (179.67 and 83.02 for extraction yield and DE, respectively) and the low p-value ($p < 0.001$ for both extraction yield and DE) show that the proposed models were extremely significant and more than 99% of the responses can be explained by them, because the calculated determination coefficient for extraction yield and DE was 99.69 and 99.34%, respectively (Maran, Swathi, Jeevitha, Jayalakshmi & Ashvini, 2015). Also, lack-of-fit for two obtained responses was insignificant (0.125 and 0.261 for extraction yield and DE, respectively), which showed the experimental data was well fitted with the quadratic polynomial model and there is no need to the higher-order equations (Jafari et al., 2017; Li et al., 2012; Swamy & Muthukumarappan, 2017). The second-order equations of the extraction yield and DE were

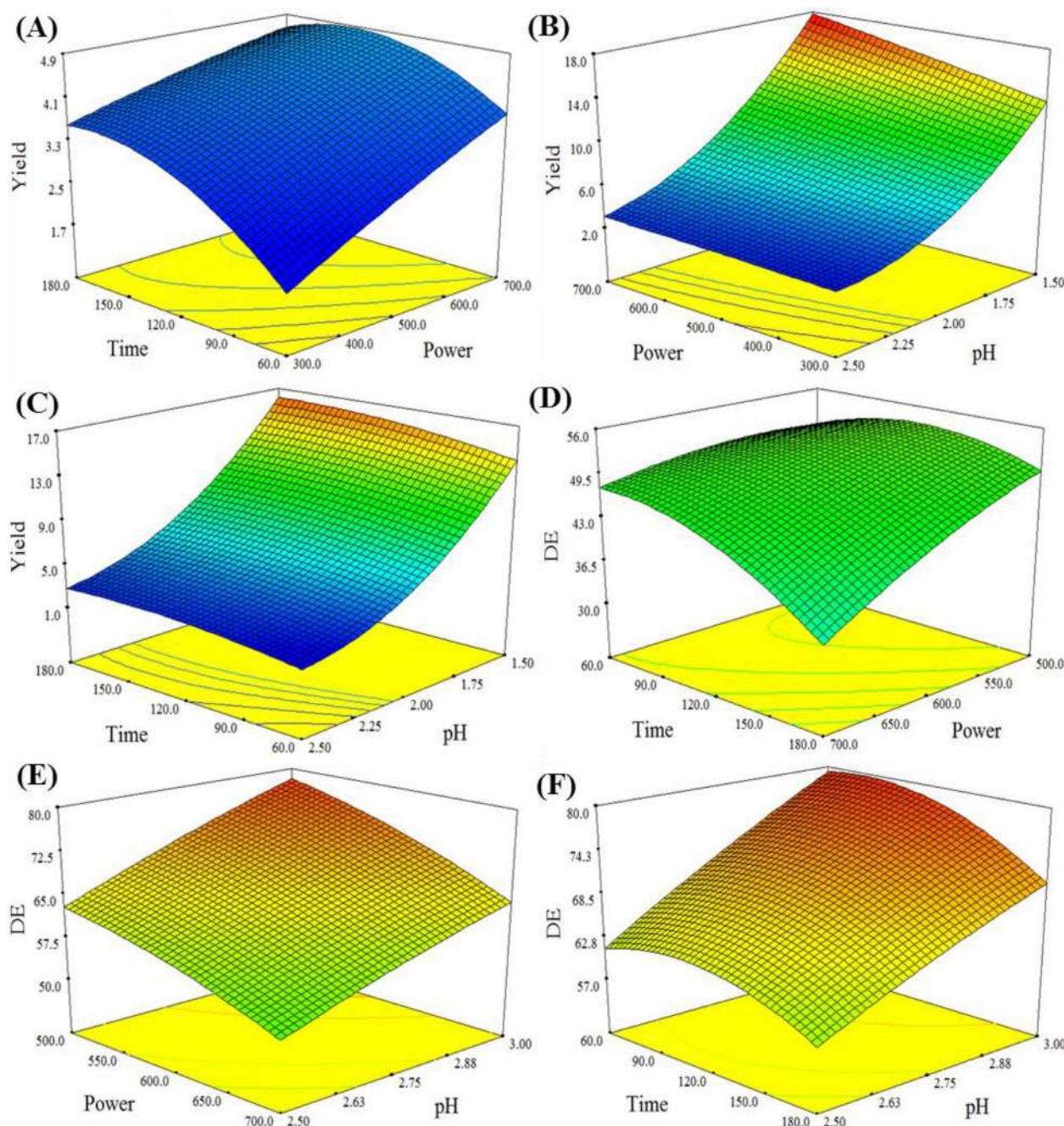


Fig. 1. Effect of independent factors (microwave power, irradiation time and pH) on the extraction yield (A, B and C) and degree of esterification (D, E and F) of PGH (pistachio green hull) pectin.

expressed in below:

$$\text{Yield}(\%) = 4.67 + 0.775X_1 + 0.675X_2 - 5.650X_3 - 0.058X_1^2 - 0.588X_2^2 + 5.892X_3^2 - 0.250X_1X_2 - 1.500X_1X_3 - 0.400X_2X_3 \quad (6)$$

$$\text{DE}(\%) = 53.41 - 3.81X_1 - 0.28X_2 + 30.15X_3 - 5.35X_1^2 - 3.39X_2^2 - 5.42X_3^2 - 6.36X_1X_2 - 4.73X_1X_3 - 4.25X_2X_3 \quad (7)$$

where X_1 is the coded independent factors (X_1 = microwave power, X_2 = irradiation time, X_3 = pH).

3.2. The role of extraction factors on PGH pectin yield

According to the extraction model and Table 2, all three factors (microwave power, time and pH) were significantly effective on

extraction yield, but pH was the most important. Therefore, it is very necessary to choose an appropriate pH. Fig. 1 shows the extraction yield was increased by lowering pH and the highest extraction yield was achieved in the lowest pH (1.5). This result was in line with several previous studies (Maran et al., 2013; Maran et al., 2015; Hosseini et al., 2016b). The effect of decrease in pH on extraction yield is probably because of improving infusion of pectin to extraction medium by plant matrix separation (Maran et al., 2013). The microwave power is another extraction factor which has an important role in both quantity and quality of extracted pectin. The results show that the extraction yield was increased by increasing the microwave power from 300 to 700 W. By increasing the microwave power, the energy adsorbed by plant matrix and the destruction of solid component was enhanced and thereby the release of pectin was facilitated (Bagherian, Ashtiani,

Fouladitajar & Mohtashamy, 2011). In this study, by increasing the irradiation time up to 165 s, the extraction yield was increased (Fig. 1A). This observation is probably attributed to increasing the exposure time of the PGH against microwave irradiation and consequently, increasing pectin extraction yield with a steep slope (Thirugnanasambandham et al., 2014). However, after this time, the extraction yield was decreased, which can be due to destruction of the pectin structure into its oligosaccharides (Bagherin et al., 2011).

3.3. The role of extraction condition on DE

Table 1 showed that DE of PGH pectin was ranged from 10.71 (run number six: microwave power = 700 W, time = 120 s, pH = 1.5) to 82.76% (run number seven: microwave power = 300 W, time = 120 s, pH = 3). Also, DE of pectin obtained from optimum extraction point was $12.1 \pm 2.72\%$. According to the results in Table 2 and predicted equation (Eq. (7)), pH is the most important factor affecting on DE. By decreasing pH, DE of extracted pectin was decreased (Fig. 1). Also, this figure showed that the long irradiation time and high microwave power lead to decrease in DE. The reduction of DE in rough conditions (low pH, high microwave power, and long irradiation time) is probably because of the deesterification of galacturonic acid (GalA) chains (Pasandide et al., 2017).

3.4. Chemical and monosaccharide composition of PGH pectin

Ash, moisture and protein content and also, the monosaccharide composition of PGH pectin extracted under optimum conditions (microwave power of 700 W, irradiation time of 165 s, pH of 1.5) were exhibited in Table 3. As can be observed, the ash, moisture and protein content of PGH pectin were about 5.16, 12.2 and 6.3%, respectively.

It is well known that pectin structure is including linear chains of D-galacturonic acid units that are occasionally interrupted by L-rhamnose and has side chains of D-galactose and L-arabinose units (Mohnen, 2008). As it can be seen in Table 3, GalA, as the backbone of smooth (homogalacturonan) and hairy (Rhamnogalacturonan) regions of pectin, is the most abundant monosaccharide in PGH pectin with a value of about 66% of total monosaccharides. On the other hand, arabinose, galactose and rhamnose were the main neutral monosaccharides (more than 95%) of PGH pectin, which are mainly related to the rhamnogalacturonan-I (RG-I). In addition, xylose and galactose were found with small percentages that are probably related to side chains in the rhamnogalacturonan-II (RG-II) region (Mohnen, 2008). It should be stated that the glucose may originate from the remnant of soluble sugars or non-pectic polysaccharides (Wang et al., 2016). Therefore, low amount of it (about 0.4% of total monosaccharides) illustrates that the extracted pectin in this study had very low amounts of other polysaccharides such as cellulose (Kpodo et al., 2017).

There are different molar ratios ($MR_1 - MR_3$) which can be estimated by monosaccharides composition to achieve to some primary information from the pectin structure (Kpodo et al., 2017; Wang et al., 2016). MR_1 is the molar ratio of GalA to the neutral monosaccharides and exhibits linearity of pectin. For a better understanding of the subject, we can compare PGH pectin with other by-product pectin. For instance, PGH pectin with a MR_1 value of 1.97 is less and more linear than the grapefruit peel pectin extracted using conventional and ultrasound-assisted method, respectively. MR_2 shows the contribution of RG region in the pectin structure and MR_3 exhibits the length of branches attached to RG-I (The value of MR_2 and MR_3 were 0.04 and 11.07, respectively) (Wang et al., 2016). The HG and RG-I molar ratio of PGH pectin was 63.7 and 35.3%, respectively. Thus, due to a higher percentage of HG molar ratios, it seems that “smooth” regions are more in PGH pectin structure. In addition, High value of MR_3 is indicating the long and extensive branches (mostly arabinan and arabinogalactan). Thus, this pectin can be chosen as a good emulsifier and stabilizer.

Table 3

Physicochemical and functional parameters of PGH (pistachio green hull) pectin obtained under optimal extraction conditions (microwave power of 700 W, irradiation time of 165 s, pH of 1.5).

Chemical composition (%)		Molecular weight ^C			
Ash	5.16 ± 0.19	Mw (kg/mol)	1.659		
Moisture	12.2 ± 0.3	Mn (kg/mol)	1.388		
Protein	6.3 ± 0.23	PDI (Mw/Mn)	1.19556		
Monosaccharide composition ^A (%)		Functional properties ^D			
GalA	66.0	DE (%)	12.1 ± 2.72		
Ara	26.2	WHC (g/g)	4.11 ± 0.36		
Gal	3.7	OHC (g/g)	3.02 ± 0.29		
Rha	2.7	EA (%)	24 °C	58.3 ± 5.4	
Xyl	1.0		4 °C	1 day	92.5 ± 3.1
Glu	0.4			30 day	89.8 ± 3.6
Fru	ND	ES (%)	24 °C	1 day	88.6 ± 4.2
Molar ratio ^B			30 day	87.9 ± 4.7	
HG	63.7%	Surface tension ^E (mN/m)			
RG-1	35.3%				
MR ₁	1.97		0.1 (%) w/v	46.23 ± 0.32	
MR ₂	0.04		0.5 (%) w/v	49.75 ± 0.48	
MR ₃	11.07				

^A GalA, Ara, Gal, Rha, Xyl, Glu, and Fru are galacturonic-acid, arabinose, galactose, rhamnose, xylose, glucose, and fructose, respectively. ND: not detected.

^B HG and RG-I are homogalacturonan and rhamnogalacturonan-I, respectively. HG = GalA - Rha; RG-I = 2Rha + Ara + Gal; MR_1 = GalA/(Rha + Ara + Gal + Xyl + Fru); MR_2 = Rha/GalA; MR_3 = (Ara + Gal)/Rha.

^C Mw: weight average molecular weight; Mn: number average molecular weight; PDI: poly-dispersity index.

^D DE, WHC, OHC, EA, and ES are degree of esterification, water holding capacity, oil holding capacity, emulsifying activity, and emulsion stability, respectively.

^E This parameter was determined in pectin concentrations of 0.1 and 0.5% w/v.

3.5. Average molecular weight (Mw) of PGH pectin

The pectin properties (like functional properties) significantly depended on its average Mw. As can be seen in Table 3, the average Mw of PGH pectin extracted under optimum conditions (microwave power of 700 W, irradiation time of 165 s, and pH of 1.5) was 1.659 kg/mol. This value was much lower than the data obtained from other sources (Jia, Zhang, Lan, Yang, & Sun, 2013; Wang et al., 2014; Bagherian et al., 2011), which can be due to the rough extraction conditions used in optimum point. The rough conditions (high microwave power, long extraction time and low pH) lead to degrade and break pectin network and thereby, significantly decrease in Mw (Bagherian et al., 2011). Thus, although the source of pectin and the type of extraction method are effective on the pectin Mw, the extraction condition is the most important factor.

3.6. WHC and OHC of PGH pectin

WHC and OHC, as two functional properties, display the capability of the sample to hold water and oil, respectively. In this study, WHC and OHC of PGH pectin obtained from optimum extraction point (microwave power of 700 W, irradiation time of 165 s, and pH of 1.5) were 4.11 ± 0.34 g/g and 2.02 ± 0.19 g/g, respectively. The WHC of PGH

pectin was extremely higher than the water-soluble polysaccharides of pistachio (1.46 g/g) and almond (1.95 g/g) by products (Sila et al., 2014). Various intrinsic and extrinsic factors such as the amount of free hydroxyl groups in chemical structure, porosity of pectin, type of extraction method and pH could affect the WHC (Bayar, Friji & Kammoun, 2018). The high WHC of PGH pectin shows that it could be effective to overcome the syneresis problems of some products like yoghurt. In the other hand, OHC of PGH pectin was higher than *Opuntia ficus indica* cladodes (1.23 ± 0.42 g/g) and commercial citrus pectin (0.93 ± 0.17 g/g) (Bayar et al., 2018). It seems that, the overall charge density and the hydrophilic nature of components are the most important factors affecting on the OHC of pectin (Bayar et al., 2017).

3.7. Total phenolic content

Total phenolic content of PGH pectin was determined by Folin-Ciocalteu method which is dependent on transfer of electrons from phenolic component to the Folin-Ciocalteu reagent in alkaline medium. This parameter is one of the most important factors in determination of antioxidant properties. The total phenolic content of PGH pectin extracted under optimal extraction condition (microwave power of 700 W, irradiation time of 165 s, and pH of 1.5) was 18.18 ± 1.09 mg gallic acid equivalents per g of PGH pectin (mg GalAE/g). The studies showed that very little research has been done on the pectin total phenolic content. However, in a research, total phenolic content of tamarind pectin was analysed and the results showed that this parameter in tamarind pectin is higher than PGH pectin. Therefore, it can be said that the antioxidant activity of PGH pectin is lower than tamarind pectin (Sharma et al., 2015).

3.8. Antiradical activity of PGH pectin

The DPPH radical scavenging, as one of the most practical tests for determination of the antioxidant activity, was applied to estimate the antiradical activity of PGH pectin obtained under optimal extraction condition (microwave power of 700 W, irradiation time of 165 s, pH of 1.5) and its results presented in Fig. 2A. According to these results, by increasing the concentration of PGH pectin, the antiradical activity was increased until (in pectin concentration of 50 mg/ml) approximately reached to the antiradical activity of ascorbic acid as standard sample. Also, the antiradical activity of PGH pectin at the concentration of 10 mg/ml was higher than the results obtained from *Opuntia ficus indica* cladodes pectin by Bayar et al. (2017). The suitable antiradical activity of PGH pectin, probably explained by its total phenolic and GalA content as well as low molecular weight (Chaouch et al., 2015; Bayar et al., 2017).

3.9. Emulsifying properties

In this part, the emulsifying activity (EA) and emulsion stability (ES) of PGH pectin in optimum extraction point (microwave power of 700 W, irradiation time of 165 s, and pH of 1.5) was estimated (Table 3). After centrifuging the prepared emulsion (0.5% w/w of pectin solution), three recognizable phases were observed (oil phase with low density in top, aqueous phase with high density in bottom, and emulsified layer in middle). The results showed that the EA of PGH pectin (58.3%) was higher than *Citrus medica* peel (46.5%) and sour orange peel pectin (40.7%) reported by Pasandide et al. (2017) and Hosseini et al. (2016b), respectively. Also, after one day at 4 and 24 °C, the stability of emulsions was 89.8 and 92.5%, respectively. However, after 30 days, the ES was decreased to 87.9 and 88.6% at 4 °C and 24 °C. These observations prove the results obtained from HPLC analysis and show that PGH pectin has a great potential to use as emulsifier and stabilizer in food products.

3.10. Surface properties of PGH pectin

In this stage, surface properties of pectin obtained under optimum extraction condition (microwave power of 700 W, irradiation time of 165 s, and pH of 1.5) were determined (Table 3). Surface tension of PGH pectin solutions was 46.23 ± 0.32 and 49.75 ± 0.48 mN/m at concentrations of 0.1 and 0.5% w/v, respectively. As it can be seen, with increase in pectin concentration, the surface tension was increased. This can be due to the higher viscosity of 0.5% pectin solution as previously suggested (Yapo, Wathelet, et al., 2007). Also, these values were lower and higher than the data obtained from sugar beet pulp (53.2 and 53.4 mN/m in concentrations of 0.1 and 0.5% w/v) and watermelon rind (40.17 mN/m in concentration of 4% w/v) pectin reported by Yapo, Wathelet, et al. (2007) and Petkowicz et al. (2017), respectively. It seems that, the surface activity of pectin has a direct relation with its Mw. However, the studies showed that the source and extraction conditions of pectin are also effective on this parameter.

3.11 ¹H NMR spectrum analysis of PGH pectin

¹H NMR spectrum of PGH pectin (in optimum extraction point: microwave power of 700 W, irradiation time of 165 s, pH of 1.5) is presented in Fig. 2B. In fact, the majority of chemical shifts were appeared within 3.2–5.1 ppm. A broad signal at 3.64 ppm was due to methoxyl groups connected to the carboxyl groups of galacturonic acid. The signals around 2 ppm is attributed to -CH₃ of the acetyl groups. The signals of 1.02, 1.10 and 1.18 ppm were probably derived from branched and unbranched methyl group links of rhamnose (Kpodo et al., 2017). The signals at 4.80 and 5 ppm were derived from H-5 proton near to the -COO⁻ and H-5 proton near to the -COOCH₃, respectively. Also, the signals around 5 ppm are due to H-1 protons of galacturonate and methyl galacturonate unites. The chemical shifts of H-4, H-3 and H-2 were appeared at 4.30, 3.97 and 3.57 ppm, respectively (Grassino et al., 2016). In result, ¹H NMR spectrum approved the presence of pectin polysaccharide structure in sample and was in line with the data published by Kpodo et al., (2017), Grassino et al., (2016) and Wang et al., (2016).

3.12. Fourier transform infrared (FT-IR) analysis

The IR spectrum of PGH pectin (in optimum extraction point: microwave power of 700 W, irradiation time of 165 s, pH of 1.5) was showed in Fig. 2C. The peaks between 1300 and 1000 cm⁻¹ are due to carbohydrates, so that the strong peaks at 1014.37 and 1068.37 cm⁻¹ are attributed to the high HG content (especially vibrations of C-O, C-C-H, C-C and OCH) which confirm the obtained results of HPLC analysis (Kpodo et al., 2017). The presence of pyranose and furanose were proved by three peaks in the range of 1010–1100 cm⁻¹. The bands at 1143.58 and 1226.50 cm⁻¹ were attributed to C-O-C vibrations of glycosides content (Hosseini et al., 2016a). The peaks at 1440.50 and 1365.35 cm⁻¹ were caused by C-H stretching vibrations. The peaks at 1628.50 and 1731.76 cm⁻¹ are specific to the free carboxylic groups (-COO⁻) and esterified carboxyl (-COOR), respectively (Grassino et al., 2016). The bands around 2900–2950 cm⁻¹ are attributed to C-H stretching including CH, CH₂ and CH₃ bending vibrations. A broad area between wavenumbers 3000 and 3500 cm⁻¹ was caused by the inter- and intra-molecular hydrogen links of O-H groups (Wang et al., 2016).

3.13. XRD analysis of pectin

X-ray diffraction is a technique used to obtain more information about molecules structure (crystalline or non-crystalline). The X-ray diffraction pattern of the PGH pectin (in optimum extraction point: microwave power of 700 W, irradiation time of 165 s, pH of 1.5) was depicted in Fig. 2D. The intensity of diffraction peaks at 17.41°, 22.81°, 29.31°, 32.41°, 32.66°, 38.76 and 72.31° (2θ) shows the crystallinity of

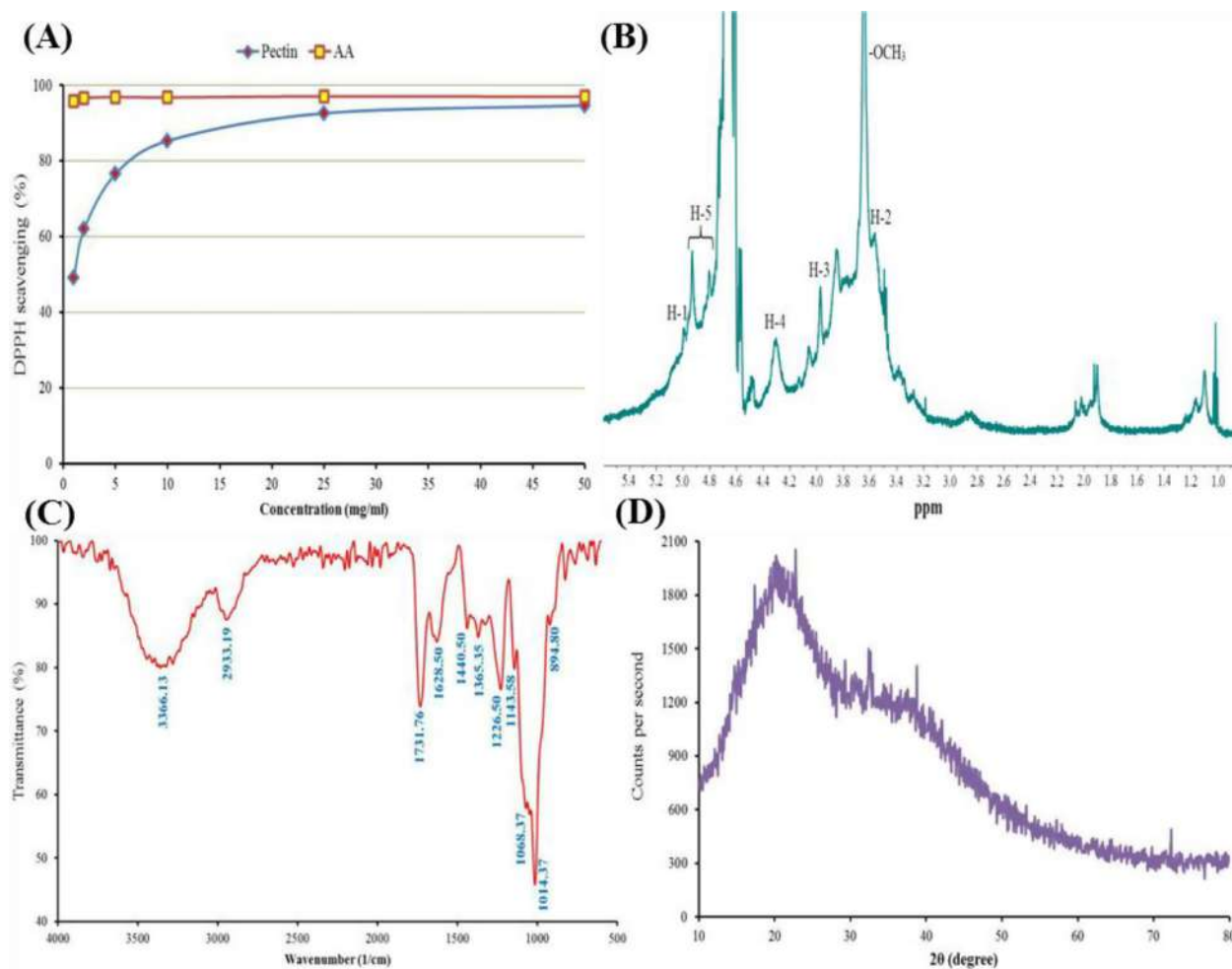


Fig. 2. (A) Antiradical activity, (B) Nuclear magnetic resonance (^1H NMR) spectrum, (C) Fourier Transform Infrared (FT-IR) spectrum and (D) X-ray diffraction (XRD) pattern of PGH (pistachio green hull) pectin obtained under optimal extraction conditions.

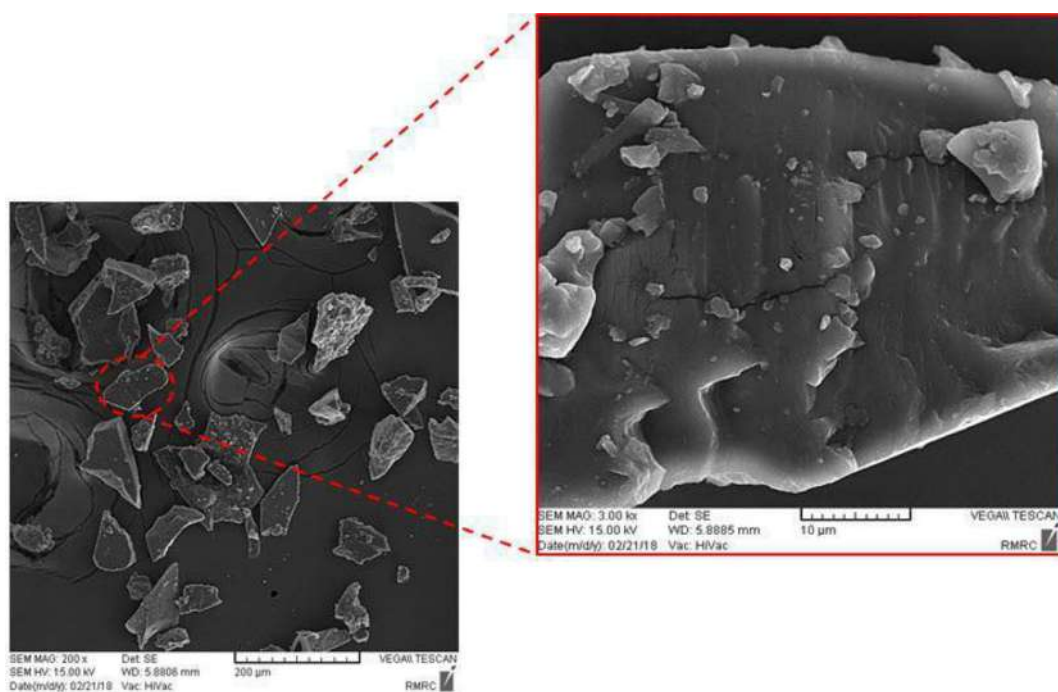


Fig. 3. Scanning electron micrograph of PGH (pistachio green hull) pectin obtained under optimal extraction conditions.

PGH pectin. This result was similar to other studies on different by-products pectin (Sharma et al., 2015; Jiang et al., 2012). However, some other researchers illustrated that pectin is non-crystalline (Kumar & Chauhan, 2010). According to obtained results, it can be noted that the crystallinity or non-crystallinity of pectin is probably due to nature, source and the type of extraction method (; Jiang et al., 2012).

3.14. PGH pectin surface morphology

SEM is a type of surface imaging method which provides information about surface topography and structure. The obtained images from this method are caused by interaction between sample atoms and electron beam emitted by electron gun of device (Vernon-Parry, 2000). Therefore, this method can be used to study the effect of extraction conditions on the pectin surface structure. The surface morphological properties of pectin obtained under optimum extraction conditions (microwave power of 700 W, irradiation time of 165 s, pH of 1.5) indicated that PGH pectin has a rough, relatively ruptured and slightly wrinkled surface (Liew, Ngoh, Yusoff & Teoh, 2016) (Fig. 3), which is probably attribute to sudden increasing temperature and the internal pressure during extraction process. In a similar study, Liew et al. (2016) also reported that creating a coarse surface in pectin extracted by microwave is due to quick rise in temperature.

4. Conclusion

In this study, extraction optimization of pectin from PGH, as a by-product of pistachio processing factories, was performed using Box-Behnken design. The maximum extraction yield of PGH pectin was $18.13 \pm 0.53\%$ in optimum conditions (microwave power of 700 W, irradiation time of 165 s, and pH of 1.5). In this conditions, PGH pectin had low DE ($12.1 \pm 2.72\%$) and Mw (1.659 kg/mol), and also, high GalA (about 66.0%) and total phenolic content (18.18 ± 1.09 mg GallAE/g pectin). HPLC analysis showed high percentage of HG (about 63.7% of PGH pectin structure) which could be concluded that the “smooth” regions were more in pectin structure. Also, Due to extensive and long branches of arabinan and arabinogalactan, PGH pectin could be suggested as a good emulsifier and stabilizer (emulsifying activity of $58.3 \pm 5.4\%$). High GalA and total phenolic content and also, low Mw were caused high antioxidant activity of PGH pectin. The ^1H NMR and FTIR spectrums confirmed the presence of esterified pectin in obtained samples. SEM and XRD pattern showed that PGH pectin had a rough surface and crystalline structure, respectively.

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