



Ultrasonic and heating extraction of pistachio by-product pectin: physicochemical, structural characterization and functional measurement

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Received: 10 July 2019 / Accepted: 28 October 2019 / Published online: 19 November 2019
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Abstract

In this study, Box–Behnken design was applied to optimize the ultrasound-assisted extraction (UAE) and heating extraction (HE) conditions for pectin production from pistachio green hull (PGH). The pectin extraction yield at optimal condition for UAE (ultrasound power of 150 W, pH of 1.5 and time of 24 min) and HE (temperature of 70 °C, time of 90 min and liquid to solid ratio of 40 v/w) methods was about 12.0 and 10.3%, respectively. In these conditions, the galacturonic acid content of UAE-pectin and HE-pectin was about 59.33 and 75.11%, respectively. Both pectin samples showed good emulsifying activity, water holding capacity and oil holding capacity. In addition, total phenol content, antiradical and surface activities of HE-pectin were higher than UAE-pectin. The degree of esterification of pectin produced by the HE method was higher than the UAE method, which was confirmed by FT-IR and ¹H-NMR analysis. The results of XRD patterns showed an amorphous structure with several crystalline portions for PGH pectin. The rheological properties of pectin solutions illustrated that in high frequencies, both pectin samples had a similar behavior (elastic), while in low frequencies, HE-pectin showed a viscous behavior. In the final, it should be stated that the UAE method significantly increased pectin yield using a lower processing time and consumed solvent, however, HE-pectin presented a better quality in comparison to UAE.

Graphic abstract



Keywords Pectin extraction optimization · Pistachio green hull · Characterization · Rheological properties

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Introduction

Pectin, as a complex polysaccharide (including mostly homogalacturonan (HG) and rhamnogalacturonan I (RG I)), is rich in galacturonic acid. HG is an unbranched homopolymer composed of α -(1 $\rightarrow$ 4) D-galacturonic acid units and RG I is a branched part with α -(1 $\rightarrow$ 4) D-galacturonic acid and α -(1 $\rightarrow$ 2) L-rhamnose units. Also in minor amounts, the other branched parts such as rhamnogalacturonan II (RG II) and xylogalacturonan (XG) are attached to the galacturonic acid units. The portion of the carboxyl groups of galacturonic acid residues are esterified (mostly with methoxyl groups) [1] and based on it, pectin is divided into two groups: low methoxyl (LM, DE < 50%) and high methoxyl (HM, DE > 50%). These two types of pectin samples have different properties and applications. HM pectin gels at low pH (pH < 3.5) and in presence of high sucrose concentrations (60–65%), while LM pectin forms a gel in presence of calcium ions with or without the addition of sugar [2, 3].

This macromolecule is widely used as a gelling, stabilizing and thickening agent in the food industry [4]. Also, this polymer has many pharmaceutical effects such as healing wounds [5], anti-metastasis activity, anti-ulcer activity, lowering cholesterol and many more [6]. Thus, because of these nutritional benefits and technological applications, the worldwide consumption and demand for pectin in global markets is growing by 5% annually [7].

Conventionally, pectin is produced from apple pomace and citrus peels by the hydrothermal method. However, this method has disadvantages including long processing time, high consumed energy, and a large amount of generated wastewater [8–10]. Thus, novel efficient technologies such as ultrasound-assisted extraction (UAE) were developed as an alternative.

UAE technique is the application of sound waves in the frequency range of 20 kHz–1 MHz, to interact with the materials. The cavitations in this technique produced high energies around the solvent, caused cell disruption for better penetration of solvent into the cell and increased the mass transfer rate [11]. UAE, as a green-chemistry extraction technique, increases the efficiency of the pectin extraction process and provides a clean, fast, and environmentally friendly process [12, 13]. In addition, ultrasound can break pectin into its smaller fragments, and thereby lead to the modification of its physicochemical and functional properties [14]. Therefore, due to its great advantages, this method was used to study pectin extraction from different sources such as grapefruit peel [14], sunflower head [15], sisal waste [16], pomegranate peel [17], eggplant peel [18], sour orange peel [19], and grape pomace [20].

Pistachio (*Pistacia vera* L.) is a valuable agricultural product with high nutritional and functional value [21].

Iran is the largest pistachio producer and exporter in the world in 2017 [22]. Green hull is the most (about 60%) portion of agro-industrial by-products of the fresh pistachio processing which includes a large number of proteins, essential oils, antioxidants, phenolic compounds, and carbohydrates (such as pectin) [23]. This by-product is mostly discarded as waste and leads to environmental problems [24], while with some investments, it can be used as a good source of pectin.

Previously we studied pistachio green hull (PGH) pectin extracted by microwave-assisted extraction (MAE) method [25]. However, the studies showed that the pectin properties are influenced by the extraction method and pectin obtained from different methods especially the conventional and new methods has various properties. To the best of our knowledge, there is not any study on the effect of the extraction process on the yield and properties of PGH pectin. Therefore, in the current study, we intended to optimize the variables of heating extraction (HE) method (independent variables: temperature, extraction time, and liquid to solid ratio (LSR) at three levels) and UAE method (independent variables: ultrasound power, pH, and extraction time at three levels), evaluate and compare the yields, and various properties of both pectin samples and the previous study [25].

Materials and methods

Raw material and chemicals

Fresh pistachios (Akbari variety) were bought from a grocery market in Karaj, Iran. The green hulls were separated, cut into small pieces using a lab knife and dried at 45 °C for 48 h. The dried PGH were pulverized with a grinder and passed through a 40 mesh sieve. The powdered PGH were packed and placed in a dark and dry condition until next uses.

Citric acid, hydrochloric acid, sulfuric acid, sodium tetraborate and sodium hydroxide were purchased from Merck Chemical Co. (Darmstadt, Germany). Also, the other applied chemicals and solvents were analytical grade.

Pectin extraction

UAE process was performed on an ultrasonic device (Ultrasonic Co., Iran) with a cylindrical titanium probe (10 mm diameter) [18], with three factors of ultrasound power, irradiation time and pH each in three levels (Table 1) with a constant liquid/solid ratio (LSR) (see Sect. 2.3). HE method with three factors of temperature, heating time and LSR at three levels were performed at a constant pH of approximately 6.5, according to Table 1. After extraction, the mixture was centrifuged (10,000×g for 15 min), coagulated by

Table 1 Box–Behnken design matrix, experimental and predicted data for PGH pectin extracted by UAE and HE methods

Heating extraction (HE)					Ultrasound-assisted extraction (UAE)					
Factors	Unit	Actual levels			Factors	Unit	Actual levels			
		− 1	0	+ 1			− 1	0	+ 1	
Temperature (X ₁)	°C	70	80	90	Power (X ₁)	W	90	120	150	
Time (X ₂)	min	30	60	90	pH (X ₂)	–	1.5	2.25	3	
LSR (X ₃)	v/w	20	30	40	Time (X ₃)	min	10	20	30	
Run	Heating extraction (HE)					Ultrasound-assisted extraction (UAE)				
	X ₁	X ₂	X ₃	EY ^a	PY ^a	X ₁	X ₂	X ₃	EY ^a	PY ^a
1	70	30	30	3.7	3.63	90	1.50	20	10.8	10.81
2	90	30	30	8.0	8.06	150	1.50	20	11.8	11.91
3	70	90	30	8.6	8.53	90	3.00	20	7.0	6.88
4	90	90	30	3.0	3.06	150	3.00	20	8.2	8.18
5	70	60	20	6.6	6.75	90	2.25	10	6.6	6.56
6	90	60	20	6.8	6.82	150	2.25	10	7.4	7.26
7	70	60	40	9.0	8.97	90	2.25	30	7.2	7.33
8	90	60	40	8.0	7.85	150	2.25	30	9.0	9.03
9	80	30	20	6.0	5.91	120	1.50	10	10.0	10.02
10	80	90	20	5.2	5.11	120	3.00	10	5.4	5.55
11	80	30	40	6.7	6.78	120	1.50	30	10.8	10.65
12	80	90	40	7.4	7.48	120	3.00	30	7.5	7.47
13	80	60	30	7.4	7.40	120	2.25	20	8.0	7.86
14	80	60	30	7.6	7.40	120	2.25	20	7.8	7.86
15	80	60	30	7.2	7.40	120	2.25	20	7.8	7.86

^aEY and PY are experimental extraction yield and predicted extraction yield, respectively

96% ethanol and placed in 4 °C for 24 h, respectively. The coagulates were separated by centrifuging at 10,000×g for 20 min and washed three times with 96% ethanol. In the final step, the precipitates were dried at 45 °C for 16 h. The pectin yield was calculated by the following equation:

$$\text{Yield (\%)} = \frac{\text{dried weight of extracted pectin}}{\text{dried weight of PGH powder}} \times 100 \quad (1)$$

Experimental design

In the UAE method, the best LSR point was appointed using one factor at a time technique. The LSR factor was considered as a variable in different levels of 15, 20, 25, 30, 35, and 40 v/w, while the other factors of UAE were kept at a constant level (center point). The best LSR was considered as a constant factor in all experiments. Then, a three-factorial three-leveled Box–Behnken design (BBD) was applied to optimize and explore the effect of the three factors of UAE method including ultrasound power (90–150 W), pH (1.5–3),

and sonication time (10–30 min); and HE method including temperature (70–90 °C), heating time (30–90 min), and LSR (20–40 v/w) on the pectin yield. For each method, the BBD consisted of 15 experiments with three replicates at the center point for evaluating the experimental error. The design and independent variables of each method were listed in Table 1. The optimization process for both methods was confirmed by the analysis of variance (ANOVA) as listed in Table 2. The optimal point of extraction process (UAE or HE) was predicted by fitting the obtained data into the following second-order polynomial model (Eq. 1):

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_{i=1}^k \sum_{j=2}^k \beta_{ij} X_i X_j \quad (2)$$

where Y was the response function; β_0 , β_j , β_{jj} and β_{ij} were the constant, linear, quadratic and interactive coefficients, respectively; k was the number of independent variables ($k=4$); X_i and X_j were the coded independent variables.

Statistical analysis of experimental data was accomplished by Stat-Ease Design Expert (v 7.0) software (Stat-Ease Inc., USA).

Table 2 The results of the analysis of variance (ANOVA) for the regression model of PGH pectin extracted by UAE and HE methods

Source	DF ^a	Some of squares	Mean square	F-value	p value
<i>Yield of UAE</i>					
Model	9	44.20	4.91	176.44	0.000
Linear	3	35.39	11.79	423.86	0.000
X ₁	1	2.88	2.88	103.47	0.000
X ₂	1	29.26	29.26	1051.30	0.000
X ₃	1	3.25	3.25	116.81	0.000
Square	3	8.12	2.70	97.28	0.000
X ₁ ²	1	0.36	0.46	16.64	0.010
X ₂ ²	1	6.09	5.57	200.43	0.000
X ₃ ²	1	1.66	1.66	59.70	0.001
2-Way interaction	3	0.68	0.22	8.17	0.023
X ₁₂	1	0.01	0.01	0.36	0.575
X ₁₃	1	0.25	0.25	8.98	0.030
X ₂₃	1	0.42	0.42	15.18	0.011
Error	5	0.13	0.02		
Lack of fit	3	0.11	0.03	2.81	0.273
Pure error	2	0.03	0.01		
Total	14	44.33			
R ²			0.9969		
Adj. R ²			0.9912		
Pred. R ²			0.9580		
<i>Yield of HE</i>					
Model	9	39.56	4.40	127.42	0.000
Linear	3	5.83	1.94	56.40	0.000
X ₁	1	0.55	0.55	15.98	0.010
X ₂	1	0.01	0.01	0.14	0.719
X ₃	1	5.28	5.28	153.08	0.000
Square	3	8.30	2.76	80.22	0.000
X ₁ ²	1	0.08	0.08	2.41	0.181
X ₂ ²	1	7.49	7.49	217.32	0.000
X ₃ ²	1	0.45	0.45	13.11	0.015
2-Way interaction	3	25.42	8.47	245.65	0.000
X ₁₂	1	24.50	24.50	710.22	0.000
X ₁₃	1	0.36	0.36	10.43	0.023
X ₂₃	1	0.56	0.56	16.30	0.010
Error	5	0.17	0.03		
Lack of fit	3	0.09	0.03	0.77	0.607
Pure error	2	0.08	0.04		
Total	14	39.73			
R ²	9		0.9957		
Adj. R ²	3		0.9878		
Pred. R ²	3		0.9582		

^aDegree of freedom

Physicochemical characterization of PGH pectin

The moisture content of extracted pectin in optimum conditions of HE and UAE methods was determined using the lost weight of dried pectin in an air-circulating oven (105 °C for 24 h). Also, the ash content of PGH pectin was

determined by calcination of pectin in a furnace for 6 h at 550 °C. Protein content was estimated for both HE and UAE pectin using the Kjeldahl procedure and conversion factor of 6.25. The galacturonic acid (GalA) content was estimated by the meta-hydroxydiphenyl method as previously described by Blumenkrantz and Asboe-Hansen [26]. Briefly, 200 µg

of pectin sample was dissolved in 1 ml distilled water and then, 6 ml of concentrated sulfuric acid containing 0.0125 M sodium tetraborate (borax) was added to it. The mixture was vortexed and heated in a boiling bath for 5 min and then, cooled immediately in an ice-water bath. In the next step, 20 μ L of 0.15% meta-dihydroxydiphenyl solution (dissolved in 0.5% sulfuric acid) was added to it. The mixture was vortexed and after 5 min, the pink color appeared in it. The absorbance of the mixture was recorded at 520 nm by a UV–Vis spectrophotometer (SP-UV500DB, Spectrum Instruments, Australia). A standard curve of GalA (with an R^2 value of 0.9924) was used to estimate the GalA content of the pectin sample. The degree of esterification of pectin samples was determined using the titrimetric method [27]. The total phenolic content of pectin samples was determined using the Folin Ciocalteu method [25]. The surface tension measurement was performed on a Nanometric-pazhoh tensiometer (Tehran, Iran) using the Wilhelmy plate method with a mica plate [28].

Functional measurement of PGH pectin

Water holding capacity (WHC) and oil holding capacity (OHC) were determined as previously described by Bayar et al. [29]. WHC or OHC was stated as gram of water or oil held per gram of pectin. Emulsifying activity (EA) and emulsion stability (ES) of PGH pectin determined using the method described by Jafari et al. [30]. ES was determined after 1 and 30 days storing at 4 °C and room temperature. Foam capacity (FC) and foam stability (FS) of PGH pectin were determined as described by Bayar et al. [29]. Briefly, 10 ml of pectin aqueous solutions (2 and 4% w/v) were prepared and vortexed for 3 min at room temperature. FC and FS were calculated as follow:

$$FC(\%) = \frac{V_0 - V_T}{V_T} \times 100 \quad (2)$$

$$FS(\%) = \frac{V_{30} - V_T}{V_T} \times 100 \quad (3)$$

where V_0 : total volume after vortexing ($t=0$), V_{30} : total volume after 30 min and V_T : the initial volume of solution.

The antiradical activity of PGH pectin was estimated using DPPH radical scavenging method as previously described by Bayar et al. [31]. Ascorbic acid (AA) in similar concentrations was used as a standard antioxidant sample. The DPPH radical scavenging activity of pectin samples was measured as follow:

$$\text{DPPH radical scavenging activity (\%)} : \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100 \quad (4)$$

Structural analysis of PGH pectin

Fourier transform infrared (FTIR) spectroscopy of PGH pectin was done on a Thermo Avatar System 370 FTIR spectrometer (Thermo Electron Corporation, USA) using KBr pellets over wavenumber of 4000–500 cm^{-1} . H-NMR spectra analysis was accomplished on a Varian Unity-Inova (500 MHz) NMR spectrometer (Palo Alto, United States). The XRD patterns of PGH pectin samples were performed on a PHILIPS X-ray diffractometer (Amsterdam, Netherlands) over the diffraction angle (2θ) of 10°–80° and step size of 0.05° (2θ).

Surface morphology analysis

The surface morphology of PGH pectin was studied using a Vega3 scanning electron microscope (Tescan Co. Ltd., Brno, Czech Republic). For this purpose, the powder of pectin samples were coated with gold and photographed at an accelerating voltage of 15 kV.

Rheological properties of PGH pectin

In this part, PGH pectin solutions were first prepared (2% w/v) and then, the viscoelastic properties of them were studied employing Anton Paar MCR 302 rheometer (Graz, Austria). The storage (G') and loss modulus (G'') were determined through oscillation frequency sweep with frequencies ranging from 0.1 to 10 Hz at room temperature. It should be noted that the experiments were performed in a constant strain of 0.01% (linear viscoelastic region of PGH pectin) [32].

Results and discussion

Model fitting and optimization process

In order to optimize the UAE conditions, the best LSR was selected (20 v/w) and then, the chosen LSR was fixed in all experiments. Afterward, Box–Behnken design (BBD), as a great tool to examine different factors effects on responses, was used to optimize the other factors of UAE method (ultrasound power, pH, and time) and also the factors related to HE (temperature, time, and LSR) method. The experimental and predicted results for responses of HE and UAE were shown in Table 1. According to these results, PGH pectin extraction yield was ranged from 5.4 (run number 10) to 11.8% (run number 2) for UAE and 3.7 (run number 1) to 9% (run number 7) for HE. Also, at the optimum point of UAE (ultrasound power of 150 W, pH of 1.5 and time of 24 min) and HE (temperature of 70 °C, time of 90 min and LSR of 40 v/w) methods, the extraction yields were 12.0 and 10.3%,

respectively. To ensure the correctness of predicted models for UAE (Eq. 5) and HE (Eq. 6), three experiments were repeated for each method at optimum points and the extraction yields were 12.0 ± 0.53 and $10.3 \pm 0.75\%$, respectively. These results show the high accuracy of the proposed model for both HE and UAE methods. In the previous study, the yield of PGH pectin extracted by MAE (18.13%) was higher than these two methods [25]. Also, both yields were lower than the yield of some other commercial and non-commercial sources (in optimum conditions of different extraction methods) such as sour orange peel (17.95% in temperature of 95 °C, time of 90 min and LSR of 25 v/w), grapefruit peel (27.34% in power intensity of 12.56 W/cm², extraction temperature of 66.71 °C, and sonication time of 27.95 min), apple pomace (15.75% in extraction time of 20.8 min, pH of 1.01, solid:liquid ratio of 0.069 and microwave power of 499.4 W), orange peel (19.24% in microwave power of 422 W, irradiation time of 169 s, pH of 1.4 and solid–liquid ratio of 1:16.9 g/ml), and higher than durian rind (8.80% in extraction temperature of 85 °C, heating time of 4 h and pH of 2.30) and dragon fruit peel (7.50% in microwave power of 400 W, temperature of 45 °C, extracting time of 20 min and solid–liquid ratio of 24 g/ml) [3, 14, 25, 33–36].

Analysis of variance (ANOVA) results (Table 2) showed that the models were significant in terms of linear, square and interaction for both UAE and HE methods. Higher F-value (176.44 and 127.42 for UAE and HE, respectively) and lower p value (p value < 0.001 for both UAE and HE) of proposed models represent that the suggested models are significant and only less than 1% of the obtained data could not be explained by these models (R^2 was 0.9969 for UAE and 0.9957 for HE) [37]. In addition, the calculated lack-of-fit for both models (Eqs. 5, 6) was insignificant (the obtained lack-of-fit for UAE and HE was 0.273 and 0.607, respectively) which shows the proposed square polynomial models are extremely well fitted for experimental data of UAE and HE. Thus, it could be concluded that both UAE and HE models are perfectly suitable and sufficient for explaining the effect of variables of responses [38].

$$\text{Yield of UAE (\%)} = 7.87 + 0.60X_1 - 1.91X_2 + 0.64X_3 + 0.35X_1^2 + 1.23X_2^2 - 0.67X_3^2 + 0.05X_1X_2 + 0.25X_1X_3 + 0.32X_2X_3 \quad (5)$$

where X_1 , X_2 , and X_3 were ultrasound power, pH, and time, respectively.

$$\text{Yield of TE (\%)} = 7.40 - 0.26X_1 - 0.03X_2 + 0.81X_3 - 0.15X_1^2 - 1.42X_2^2 + 0.35X_3^2 - 2.48X_1X_2 - 0.30X_1X_3 + 0.38X_2X_3 \quad (6)$$

where X_1 , X_2 , and X_3 were temperature, time, and LSR, respectively.

Effect of extraction factors on the pectin yield

Figure 1a, c show that by decreasing pH, the pectin extraction yield was increased. It is well known that a medium with low pH could be extremely effective on plant cell destruction and pectin leakage as the other researchers reported [38]. Ultrasound power (Fig. 1a, b) and irradiation time (Fig. 1a, c) were the other effective factors on the pectin extraction yield. An increase in pectin recovery with increasing ultrasound power can be due to cavitation production by ultrasound waves. This cavitation could improve medium penetration into the plant matrix and consequently increase pectin leakage [10, 17]. Also, Fig. 1b shows that pectin recovery was enhanced by increasing irradiation time and then dropped. This observation is may be related to the increase of exposing time of plant matrix against the cavitation effect of ultrasound waves and in consequence, dramatically increase of pectin yield. However, with an excessive rise of irradiation time, the yield is decreased, which is probably due to fragmentation and destruction of pectin structure [8].

In the HE method, Fig. 1e, f indicate that pectin yield was increased with an increase in LSR, which may be attributed to increasing the contact area between medium and plant material which leads to growing liquid–solid concentration gradient and improving pectin extraction yield [3]. In this study, temperature had a negative effect on pectin yield. This observation may is related to the thermal degradation of the PGH pectin structure [8]. Also, Fig. 1d, f illustrate that the longer time of extraction leads to higher extraction yield, which this result is in agreement with many other studies [3, 8, 37].

Physicochemical analysis

The physicochemical parameters of PGH pectin samples extracted at the optimum point of UAE (ultrasound power of 150 W, pH of 1.5, and time of 24 min) and HE (temperature of 70 °C, time of 90 min, and LSR of 40 v/w)

were evaluated and the results were presented at Table 3. The moisture content of UAE and HE pectin was about

9.86 and 10.24%, respectively. The ash content of both pectin samples was almost similar (approximately 6.44 and 6.08% for UAE and HE methods, respectively) and slightly higher than the results of PGH pectin extracted by MAE (5.16%) [25]. The protein content of pectin obtained from

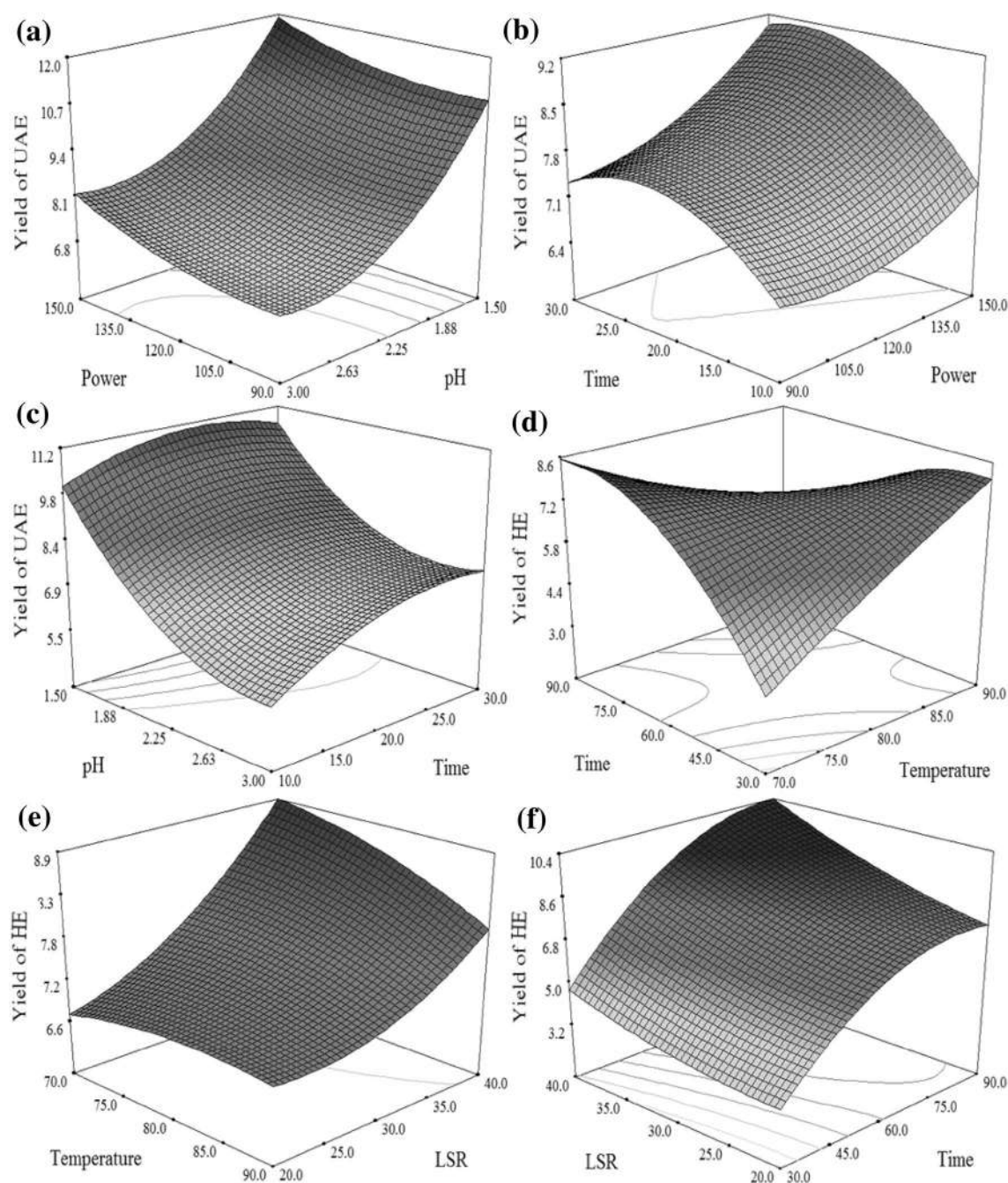


Fig. 1 Effect of independent variables for UAE and HE methods on the PGH pectin extraction yield

HE method (about 7.08%) was higher than UAE (about 5.41%) and MAE method (6.3%) while these values for PGH pectin were higher than watermelon rinds pectin (3.9%) [1, 25]. Table 3 shows that the difference between the GalA content of PGH pectin extracted by UAE and HE methods was significant (about 59.33 and 75.11%, respectively). However, the obtained value for this parameter in the previous study (66.0%) was higher than the UAE and lower than HE [25]. Obviously, this difference in GalA

content is because of the different extraction methods. In the UAE method, the acidic medium in UAE and MAE may causes degradation in GalA units of pectin structure but in HE method, we had a mild extraction and thereby, a higher GalA content. These results were also in line with data reported by other researchers [32, 39]. DE of extracted pectin under optimal conditions of UAE and HE was determined titrimetrically and results showed that DE of pectin extracted by UAE can be categorized

Table 3 Extraction yield and physicochemical parameters of PGH pectin extracted by UAE and HE methods

Chemical parameter	Pectin sample	
	UAE	HE
Yield (%)	12.0 ± 0.53 ^a	10.3 ± 0.75 ^b
Moisture (%)	9.86 ± 0.78 ^a	10.24 ± 0.31 ^a
Ash (%)	6.44 ± 0.12 ^a	6.08 ± 0.39 ^a
Protein (%)	5.41 ± 0.57 ^b	7.08 ± 0.19 ^a
GalA* content (%)	59.33 ± 2.32 ^b	75.11 ± 2.41 ^a
DE* (%)	41.34 ± 2.12 ^b	59.54 ± 0.46 ^a
TPC* (mg gallic acid equivalent/g pectin)	22.32 ± 0.59 ^a	41.60 ± 0.60 ^b
Surface tension (mN/m) at 0.1 (% w/v)	45.34 ± 0.49 ^a	40.73 ± 0.21 ^b
Surface tension (mN/m) at 0.5 (% w/v)	42.88 ± 1.09 ^a	37.25 ± 0.66 ^b

Different letters in the same row indicate significant differences ($p < 0.05$)

*GalA, DE and TPC are galacturonic acid, degree of esterification and total phenolic content, respectively

as LM-pectin (low methoxyl pectin) because it was lower than 50% ($41.34 \pm 2.12\%$). But the pectin obtained from the HE method had a DE value of $59.54 \pm 0.46\%$ and recognized as HM-pectin (high methoxyl pectin). This result was in accordance with previously published data by Wang et al. for grapefruit peel pectin [32]. It seems that the lower DE in the pectin extracted by the UAE method is due to the extraction of pectin fractions with low DE in an acidic condition such as chelate-soluble pectin, which leads to decrease in average DE of the whole extracted pectin. TPC of extracted pectin samples of UAE and HE methods was evaluated using the Folin-Ciocalteu procedure which is basically relied on the electrons transfer from phenolic constituents to the Folin-Ciocalteu reagent. In the UAE method, this parameter had a value of 22.32 ± 0.59 mg GAE/g pectin, which was lower than the data obtained from HE method (41.60 ± 0.60 mg GAE/g pectin). However, both of them (UAE and HE pectin) had a higher value than the MAE method due to the harsh extraction condition and sudden temperature increase in the MAE method [25]. The values were lower than the TPC of tamarind pectin [40], which can be due to the type of used source. The surface tension of pectin for UAE and HE methods in a concentration of 0.1% was 45.34 ± 0.49 and 40.73 ± 0.21 mN/m, respectively. Also, this factor for pectin extracted by these two methods in concentration of 0.5% w/v was 42.88 ± 1.09 and 37.25 ± 0.66 mN/m, respectively. This result shows that pectin obtained from the UAE method had more surface tension value than HE method which was related to the higher protein content and TPC of pectin of HE method. Also, it could be concluded that by increasing

Table 4 Comparison of functional properties of PGH pectin extracted by UAE and HE

Parameter	Pectin sample	
	UAE*	HE*
Water holding capacity (g/g)	4.21 ± 0.25 ^a	4.32 ± 0.32 ^a
Oil holding capacity (g/g)	1.52 ± 0.16 ^a	1.06 ± 0.21 ^b
Emulsifying activity (%)		
23 °C	53.83 ± 1.85 ^a	49.00 ± 1.52 ^b
Emulsion stability (%)		
After 1 day		
4 °C	95.01 ± 0.62 ^b	97.28 ± 0.85 ^a
23 °C	73.00 ± 1.53 ^b	93.13 ± 0.69 ^a
After 30 days		
4 °C	80.55 ± 2.43 ^b	94.12 ± 2.77 ^a
23 °C	71.45 ± 1.17 ^b	74.36 ± 1.11 ^a
Foaming capacity (%)		
2% (w/v)	6.14 ± 1.45 ^b	10.68 ± 1.63 ^a
4% (w/v)	9.21 ± 2.27 ^b	21.54 ± 2.68 ^a
Foam stability (%)		
2% (w/v)	2.25 ± 1.31 ^b	4.84 ± 1.01 ^a
4% (w/v)	5.32 ± 2.52 ^b	14.38 ± 1.96 ^a

Different letters in the same row indicate significant differences ($p < 0.05$)

*Pectin extracted by UAE and HE methods (under optimal extraction conditions)

pectin concentration to 0.5% w/v, the surface tension of pectin solutions was decreased which probably is because of the higher TPC and protein content at a concentration of 0.5% w/v. The decrease of surface tension with an increase of TPC is probably attributed to the accumulation of phenolic compounds in the air–water interface, an increase in the surface pressure and thereby decrease in surface tension [1, 41]

Functional measurements

Functional properties of pectin samples extracted at the optimum point of UAE (ultrasound power of 150 W, pH of 1.5, and time of 24 min) and HE (temperature of 70 °C, time of 90 min, and LSR of 40 v/w) were evaluated and the results were listed at Table 4. WHC and OHC is an index representing the ability of pectin sample to retain water and oil, respectively. WHC of PGH pectin samples obtained by UAE and HE methods were 4.21 ± 0.25 and 4.32 ± 0.32 g water/g pectin, respectively. The values were higher than the value of PGH pectin extracted by MAE (4.11 g/g) [25]. Also, these results were higher than the commercial apple pectin (2.00 g/g) [42]. It is noteworthy that, in addition to the extraction method and nature of plant source, many other factors could have an effect on WHC (such as porosity and

particle size of the sample). On the other hand, OHC pectin obtained from UAE and HE methods were 1.52 ± 0.16 and 1.06 ± 0.21 g oil/g pectin, respectively, while the value for pectin of MAE was 2.02 g/g [25]. The OHC values were higher than the results (1.01 g oil/g) obtained by Bayar et al. [31] and lower than the commercial apple pectin (2.22 g/g) [42]. It should also be noted that OHC, as an important property in food processing industries, is depended on many factors like total charge density and the hydrophobic nature of components [25].

EA of pectin produced from UAE and HE was 53.83 ± 1.85 and $49.00 \pm 1.52\%$, respectively. These observations were higher than the data obtained for *Citrus medica* peel pectin (46.2%) [43], and sugar beet pulp pectin (43–47%) [44]. In fact, pectin with high emulsifying properties could be used as both thickening and emulsifying agents in the formulation of various food systems (like mayonnaise) [45]. It can be seen that the ES of pectin extracted from the UAE method was lower than the HE method in all conditions of the store. As it is illustrated in Table 4, the emulsions were more stable at low temperatures, which was similar to obtained data from sour orange peel pectin [38]. Thus, it can be said that EA and ES are depended on different factors that the plant source and extraction method are the most effective in this regard.

Foaming is due to trapping the gas bubbles in the sample and creating a homogeneous dispersion of gas–liquid,

gas–semi-solid or gas–solid. This is a suitable property in food processing industries due to obtaining the final products with better stability and sensory quality [1]. Indeed, pectin with high foaming properties can enhance sensory features in aerated food products [45]. Thus, FC and FS of both pectin samples were determined. As expected, FC (in both concentrations of 2 and 4% w/v) of pectin extracted from UAE (6.14 ± 1.45 and $9.21 \pm 2.27\%$, respectively) was much lower than HE method (10.68 ± 1.63 and $21.54 \pm 2.68\%$, respectively) which is maybe due to higher protein content and TPC in pectin of HE method. However, both of the FC values for extracted pectin from PGH were lower than *Opuntia ficus indica* cladodes pectin (25%) [29]. In addition, by increasing pectin concentrations from 2 to 4%, the foaming stability of samples was increased in both UAE and HE methods which these results were in agreement with the results of surface tension and the data obtained by Bayar et al. [31]. On the other hand, pectin resulting from HE method (particularly in concentration of 4% w/v) had foam with good stability (in 30 min) in comparison with UAE method (2.25 ± 1.31 and $4.84 \pm 1.014\%$ for UAE, and 5.32 ± 2.52 and $14.38 \pm 1.96\%$ for HE in pectin concentrations of 2 and 4% w/v, respectively). However, the highest FS of PGH pectin was lower than the highest FS value of *Opuntia ficus indica* cladodes pectin (20%) [29].

DPPH radical scavenging activity of PGH pectin samples was evaluated (Fig. 2). With an increase in the concentration

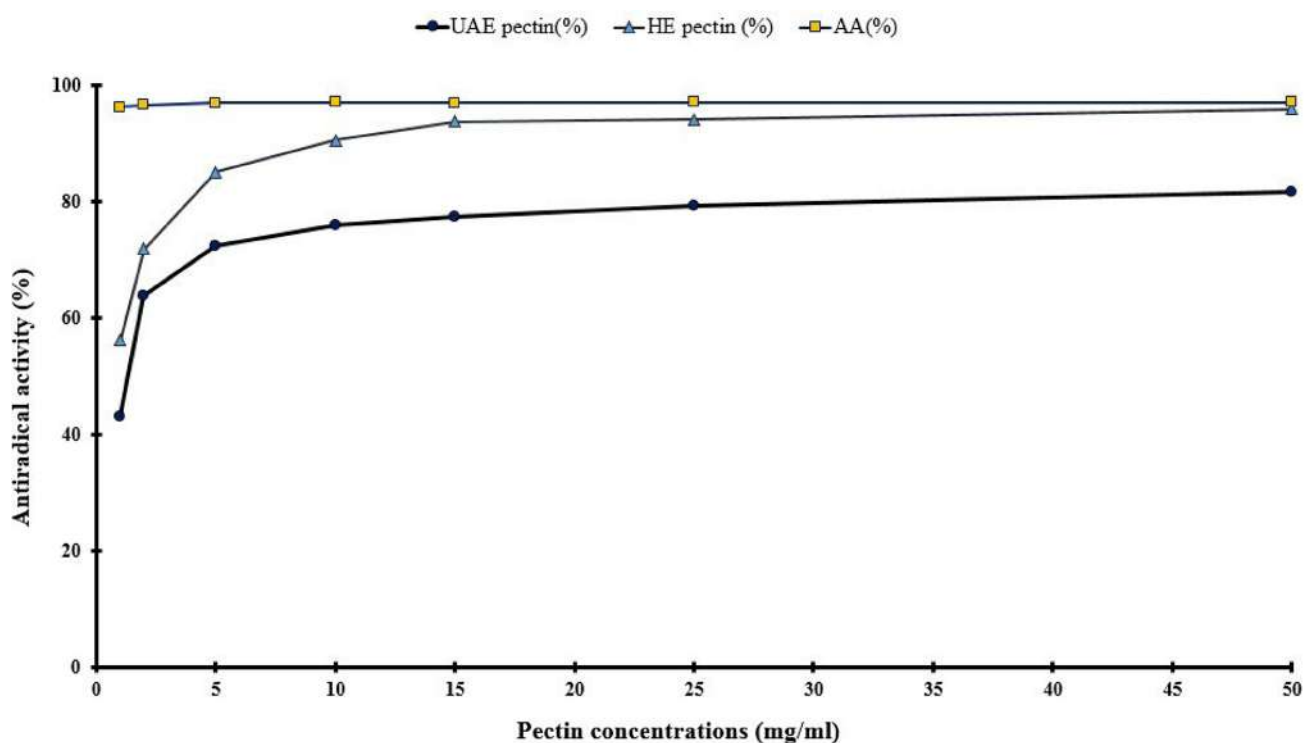
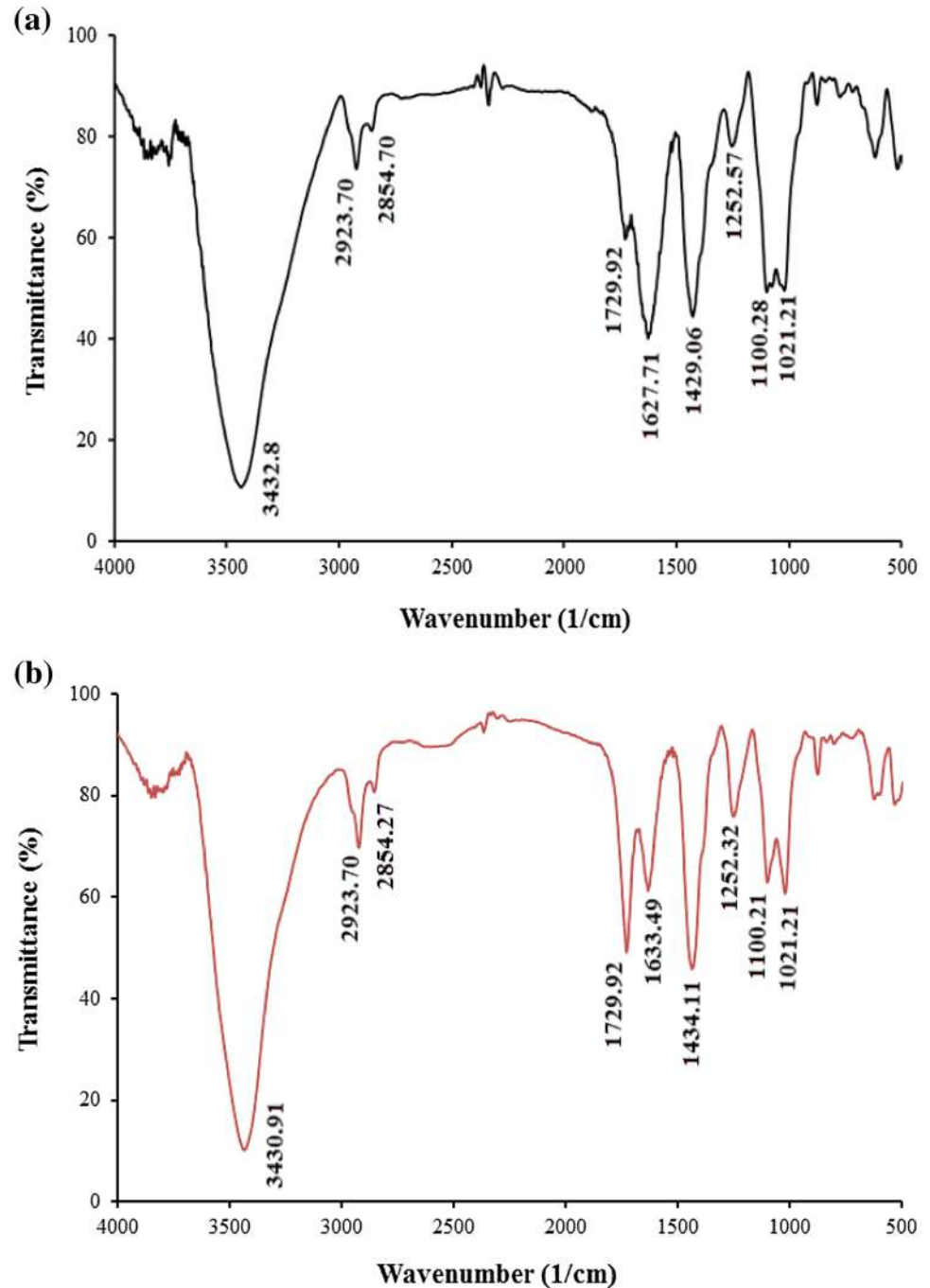


Fig. 2 DPPH antiradical activity of PGH pectin (0 to 50 mg/ml) extracted by UAE and HE methods and ascorbic acid (AA) as standard

of pectin sample radical scavenging activity was increased. Also, it can be seen that by increasing pectin content, anti-radical activity was increased until approximately fixed after a concentration of 15 mg/ml (for UAE about 80% and for HE about 95%). This dose-dependent manner was previously observed for several pectin samples extracted from different sources [25, 29, 40]. In the high concentrations of pectin, the radical scavenging activity of the HE pectin sample was very close to the activity of ascorbic acid (as a strong antioxidant sample), while in the UAE method, DPPH scavenging

activity of pectin was very lower than this value. In addition, at a concentration of 10 mg/ml, the radical scavenging activity of UAE and HE pectin samples were higher than the obtained results for pectin obtained by the MAE method in the previous study [25]. Thus it could be concluded that pectin obtained from the HE method was more antioxidant than UAE and MAE method which is due to its higher TPC and GalA content.

Fig. 3 FT-IR spectra of PGH pectin obtained from UAE (a) and HE (b) methods



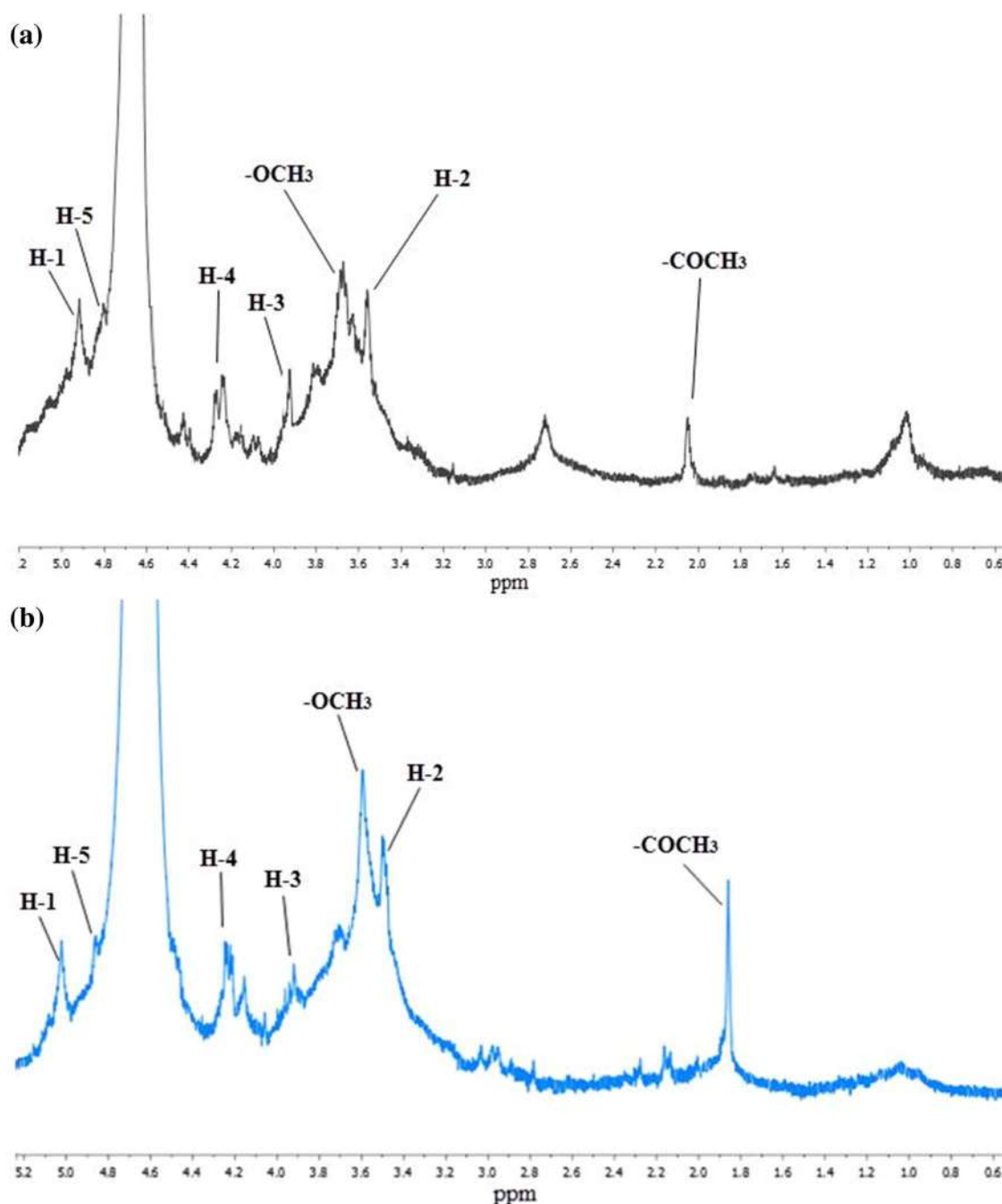


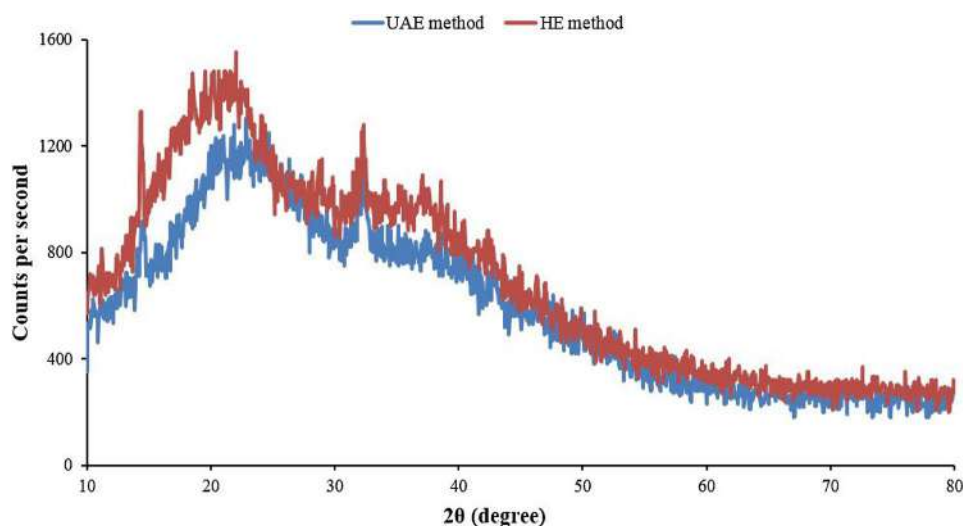
Fig. 4 ^1H -NMR spectra of PGH pectin extracted by UAE (a) and HE (b) methods

Structural analysis

The FT-IR spectra of PGH pectin samples are shown in Fig. 3. Both pectin samples had approximately similar absorption peaks at 500–4000 cm^{-1} . The peaks of 1021, 1100, and 1252 cm^{-1} in the obtained pectin spectra of UAE and HE pectin samples were due to the glycoside bonds

(C–O–C) and showed the backbone of pectin structure [38]. The peaks around 1400 cm^{-1} (1429 and 1434 cm^{-1} for UAE and HE, respectively) were caused by stretching vibrations of C–H. Two intense peaks around 1600–1650 cm^{-1} and 1700–1750 cm^{-1} were caused by free carboxyl groups ($-\text{COO}^-$) and esterified carboxyl groups ($-\text{COOR}$), respectively [46]. The signals of 2856 and 2923 cm^{-1} were referred

Fig. 5 XRD (X-ray diffraction) patterns of PGH pectin extracted by UAE and HE methods



to C-H bonding vibrations of CH, CH₂ and CH₃. The broad peaks ranged from 3000 to 3600 cm⁻¹ were due to intra- and inter-molecular hydrogen links of O-H groups [25, 27]. Comparison between FT-IR spectra of pectin obtained from UAE (Fig. 3a) and HE (Fig. 3b) methods exhibited that the peaks around 1729 cm⁻¹ (esterified carboxyl groups) for these two methods had different intensity (the peak intensity for pectin of HE method was more than UAE method). Also, the peak due to free carboxyl groups (1627 cm⁻¹ for UAE and 1633 cm⁻¹ for HE) in pectin resulting from UAE method was more intense than HE method. Therefore, it could be concluded that pectin obtained from UAE method has a lower DE than HE method and is an LM pectin, while pectin extracted from HE method is an HM pectin [32].

The H-NMR spectra of pectin samples are presented in Fig. 4. The broad signal around 3.6 ppm signifies the presence of methyl groups in D-galacturonic acid units. Also, the peaks around 2 ppm refer to the presence of acetyl groups connected to carboxyl groups of D-galacturonic acid units [47]. These two peaks are specific for pectin and the intensity of them illustrates DE of pectin (higher intensity = higher DE). According to Fig. 4a, b, DE of pectin obtained from the UAE method is lower than the HE method, which is in line with the obtained result from DE measurement and FT-IR spectroscopy analysis. The region ranged from 3.4 to 5.1 ppm is due to proton peaks of D-galacturonic acid units. For pectin extracted from UAE method (Fig. 4a), the chemical shifts around 4.9, 4.8, 4.3, 3.9 and 3.5 ppm refers to H-1, H-5, H-4, H-3 and H-2 protons of D-galacturonic acid units, respectively. For pectin obtained from HE method (Fig. 4b), these peaks appeared around 5, 4.9, 4.3, 3.9 and 3.5 ppm, respectively [39]. With respect to the presented spectra, it can be concluded that all the chemical shifts approve the presence of polygalacturonic acid units in the PGH pectin extracted by UAE and HE methods. It should be noted that

all the presented chemical shifts were in accordance with previous studies [1, 32, 39].

XRD pattern of PGH pectin extracted under optimum conditions of UAE and HE was analyzed to achieve more information about structure (crystalline and amorphous) of pectin. Figure 5 shows the obtained XRD patterns of pectin extracted from UAE and HE methods. According to the appeared characteristic peaks for pectin resulting from HE method in 11.1°, 14.3°, 22.0°, 28.2°, 32.3°, 38.1°, and 42.6° (2θ), an amorphous structure with several crystalline portions is suggested for the pectin [40]. Similar results were reported for PGH pectin extracted by the MAE method [25]. For pectin obtained from UAE method, similar signals with lower intensity and also, the disappearance of several sharp peaks were observed, which was in agreement with the results reported for grapefruit peel pectin by Wang et al. [32].

Morphological properties of PGH pectin

The surface morphology of PGH pectin was studied using SEM to achieve the visual information about surface topography of pectin extracted under the optimum conditions of UAE and HE. Figure 6 shows SEM images of pectin particles with different magnifications. These images illustrate that pectin obtained from HE method is intact and extremely rough (Fig. 6d), while the ultrasound waves lead to a decrease in roughness and create a relatively smooth surface in pectin (Fig. 6b). In a similar study, Wang et al. reported that UAE leads to disruption in crosslinks between molecules and reorganization of the pectin matrix and thereby, the creation of a smooth surface in it [14]. In another study, Liew et al. also achieved a similar result for pomelo peel pectin [48].

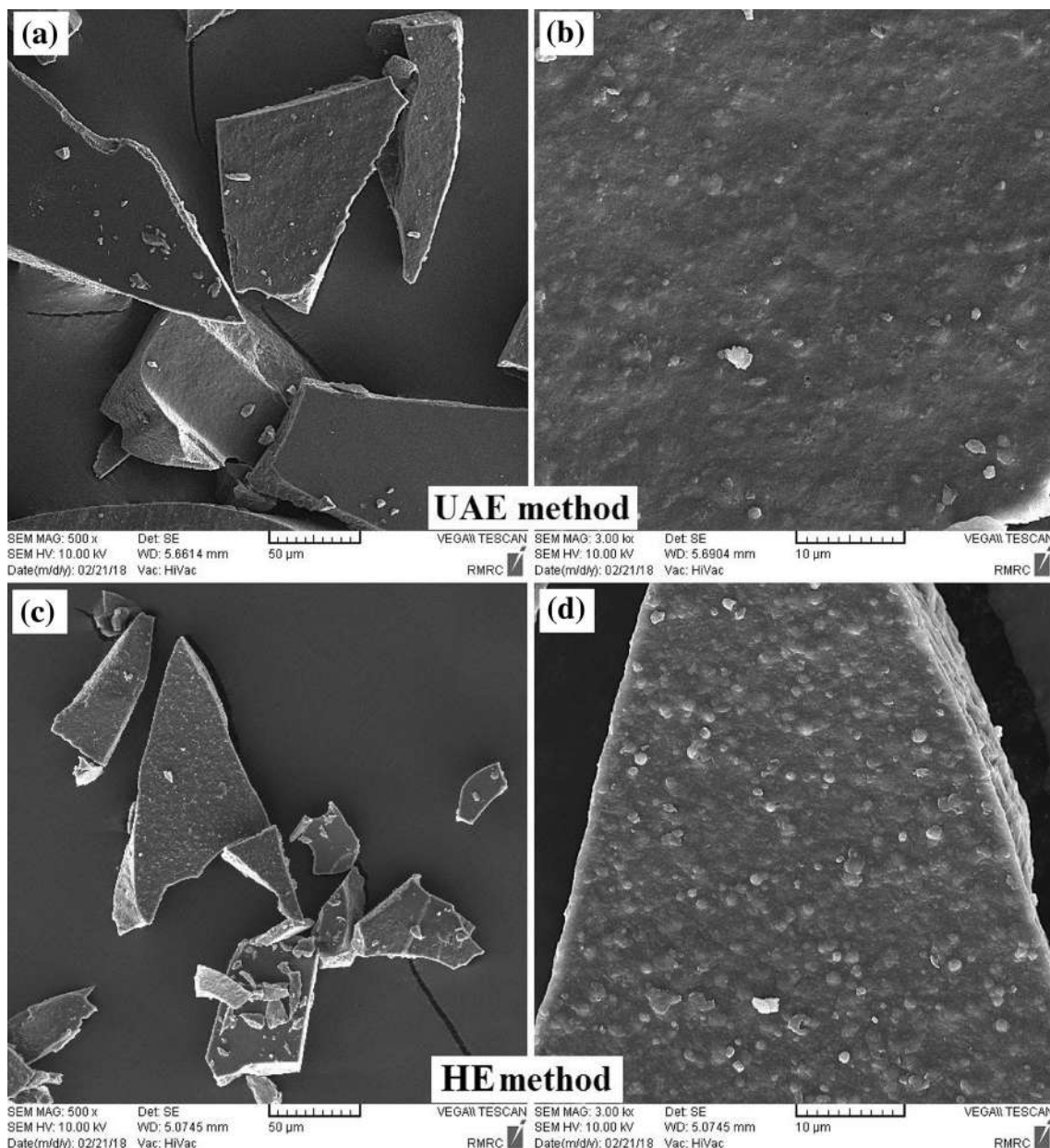


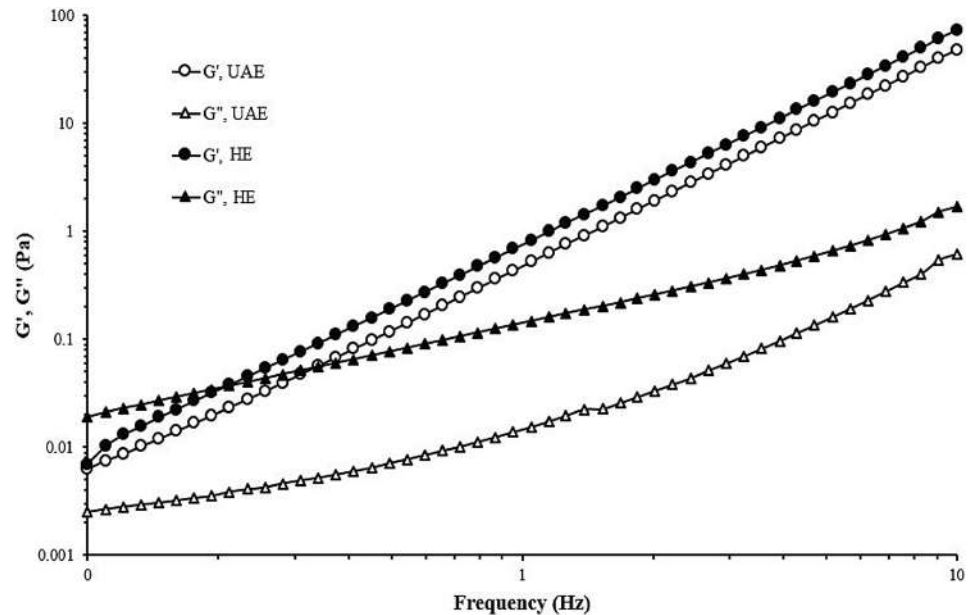
Fig. 6 SEM images of PGH pectin extracted by UAE (a, b) and HE (c, d)

Rheological properties of PGH pectin

To study viscoelastic properties of extracted pectin at optimum conditions of UAE and HE methods, frequency sweep was performed (in a pectin concentration of 2% w/v) on a range of 0.1 to 10 Hz at a constant strain of 0.01% (linear viscoelastic region of PGH pectin). In brief, the storage modulus (G') and the loss modulus (G'') are due to elastic and viscous behaviors of pectin solutions, respectively [7]. For pectin obtained from HE method, G'' values were higher than G' values at low frequencies and vice versa at higher frequencies, while for UAE method, G' values were

higher than G'' values at all frequencies (Fig. 7). Zhang et al. obtained similar results for ultrasound treated and untreated apple pomace pectin [49] and suggested that the crossover between viscous and elastic behavior in untreated pectin solution indicates viscoelastic behavior of pectin and also, the start of elastic behavior in it. According to Fig. 7, it can be said that pectin obtained from HE and UAE methods showed a viscous ($G'' > G'$, liquid-like) and elastic ($G' > G''$, gel-like) behaviors at low frequencies, while in higher frequencies the pectin solutions had a similar (elastic) behavior and both of them were gel-like. On the other hand, G' and G'' of pectin extracted from HE method were much higher

Fig. 7 Frequency sweep of PGH pectin extracted by UAE and HE methods at 0.01% strain



than the similar modulus for UAE method which this difference was probably due to electrostatic interaction between positive charges of protein content and negative charges of galacturonic acid content in pectin solutions [1]. The same results were reported for the conventional and ultrasonic extraction of grapefruit peel pectin [32].

Conclusion

In this research, the UAE and HE processes were used for the extraction of pectin from PGH. The variables of both methods were optimized using BBD considering pectin yield as a response. The ultrasound power of 150 W, pH of 1.5 and extraction time of 24 min for UAE method and the temperature of 70 °C, extraction time of 90 min and LSR of 40 v/w for HE method were determined as optimal conditions with the extraction yields of 12.0 ± 0.53 and $10.3 \pm 0.75\%$, respectively. In these conditions, a comprehensive comparison between UAE-pectin and HE-pectin showed that the UAE method provided higher pectin yield during a considerably lower time while, HE-pectin method had higher galacturonic acid content, TPC and antiradical activity (DPPH radical scavenging) value than pectin from UAE method. Both the obtained pectin showed good emulsifying properties, WHC and OHC. In addition, HE method pectin showed lower surface tension value than UAE which was in agreement with the results obtained from foaming properties. It should be noted that DE of pectin extracted from the HE method was higher than the UAE method, which was in accordance with FT-IR and H-NMR analysis. Additionally, pectin extracted from both methods had a crystalline structure based on XRD patterns. The surface morphology showed that the surface

roughness of pectin obtained from the HE method was more than the UAE method. The study of rheological properties of PSG pectin indicated that G' and G'' of pectin extracted from the HE method in the concentration of 2% w/v were much higher than the UAE method. In the final, it should be stated that the UAE method significantly increased the yield with a lower time and consumed solvent, however, HE-pectin presented a better quality in comparison to UAE. Therefore, there are some limitations and disadvantages for the UAE process and still need to study on UAE to be industrially usable for pectin extraction.

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