

Valorization of walnut processing waste as a novel resource: Production and characterization of pectin

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Abstract

Microwave-assisted extraction of pectin from walnut green husk (WGH) was optimized using Box-Behnken design. The effects of microwave power, pH, irradiation time, and liquid/solid ratio on the extraction yield were investigated. The characteristics of WGH-pectin extracted at the optimal condition were evaluated through physicochemical, structural, and functional analysis. Under optimal condition (microwave power of 500 W, pH of 1.5, irradiation time of 3 min, and LS ratio of 20 ml/g), WGH-pectin had an extraction yield of 18.0 g/100 g, galacturonic acid content of 68.45 g/100 g, and a molecular weight of 260.289 kDa. It was also high-methoxyl pectin (54.07%) which was confirmed by FTIR and NMR spectroscopy. Furthermore, it showed significant antioxidant activity, good value for water and oil-holding capacity, and emulsifying properties. The XRD pattern of WGH-pectin showed an amorphous nature with several crystalline portions.

Practical applications

Walnut green husk is a potential byproduct of walnut processing sectors and a great resource of valuable compounds such as pectin, which is generally discarded into the landfill in the form of waste. However, it can be used as an inexpensive high-potential resource of pectin. For this purpose, microwave-assisted extraction was optimized to achieve the highest production rate. Then, the isolated pectin was characterized to study the physicochemical, structural, and functional properties in order to show its capability as a natural food ingredient.

1 | INTRODUCTION

The agro-industry sectors annually generate billions of tons of waste including non-edible portions of fruits and vegetables such as peel, husk, and shell. These residues could cause pollution, as well as severe managerial and economic problems (Lin et al., 2013). Walnut (*Juglans regia* L.) is a popular and largely consumed nut with numerous nutritional, health, and sensory attributes (Wang et al., 2016). The green husk of walnut (WGH) is produced in a large quantity, generally considered as a waste and discarded at landfills which could cause serious problems for the surrounding plants due to the presence of

juglone in its composition (Fernández-Agulló et al., 2013). However, this by-product is a rich source of valuable compounds such as phenolic compounds (Akbari et al., 2012; Oliveira et al., 2008), polysaccharides, and other active components and using it, as a source of value-added compounds, not only increases the value of the walnut production but also offers economic and environmental benefits. Different works have been conducted to use WGH as a source of phenolic compounds (Akbari et al., 2012; Espín et al., 2000; Fernández-Agulló et al., 2013), but its polysaccharides are less considered.

Pectin, as one of the most important polysaccharides, is a soluble dietary fiber present in the cell wall of all land-growing plants

and generally acts as the cementing material for the cellulosic network. It is composed of a main linear chain of α -(1,4)-D-galacturonic acid (GalA) units interrupted occasionally by α -(1,2)-L-rhamnose residues. The GalA residues are partially esterified and based on it, pectin is categorized in two different classes: pectin with a degree of esterification (DE) lower than 50% (LMP) and with a DE value higher than 50% (HMP) (Kazemi, Khodaiyan, Hosseini, et al., 2019c). Technological properties and its application as thickener, gel former, stabilizer, and fat replacer in the food industry (Wang et al., 2016) and also several beneficial health and nutritional effects as a dietary fiber and prebiotic including the reduction of postprandial glycaemic responses, the maintenance of normal blood cholesterol concentrations and reduction of energy intake in pharmaceutical sciences have led to growing demand in the global markets for this polysaccharide (Encalada et al., 2019). Thus, it is necessary for finding and investigating new and inexpensive sources to meet the pectin market demand (Hosseini et al., 2019).

As a green chemistry alternative to the conventional extraction process by mineral acid solutions at high temperature and long time, microwave-assisted extraction technique is a clean and economically viable method that can degrade cell wall and release pectin at a considerably short time with lower consuming energy and solvent (Lefsih et al., 2017). This environmentally friendly method has been widely used for the extraction of pectin from different sources such as eggplant peel (Kazemi et al., 2019a), lime peel (Rodsamran & Sothornvit, 2019), potato pulp (Yang et al., 2019), jackfruit peel (Xu et al., 2018) and *Opuntia ficus indica* (Lefsih et al., 2017). However, to the best of our knowledge, no research was available on the extraction of pectin from walnut green husk (WGH). The current work is aimed to optimize the microwave-assisted extraction factors including microwave power, exposure time, pH, and liquid/solid (LS) ratio toward achieving the highest pectin yield. Furthermore, the extracted pectin from WGH at the optimal extraction point was characterized through physicochemical, structural, and functional properties.

2 | MATERIALS AND METHODS

2.1 | Plant material and chemicals

Fresh green walnuts were acquired from a local fruit store in Karaj city (Alborz, Iran). The green husks were separated and dried in an oven at 50°C for 36 hr. The dried husks were cut into small pieces, mechanically ground using a lab mill, sieved using a 40-mesh screen, and stored in a dark dry place. The obtained fine powder of WGH (particle size: <420 μ m) was used in the pectin extraction process.

Concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), citric acid, sodium hydroxide (NaOH), sodium tetraborate, and sodium carbonate were bought from Merck Co. (Darmstadt,

Germany). Ethanol 96% was purchased from Ghadir Co. (Tehran, Iran). All other chemicals and reagents were of analytical grade.

2.2 | Microwave-assisted extraction process

The extraction process was carried out using a microwave oven (Butane Industry Co., Tehran, Iran) equipped with a digital control system for irradiation time and microwave power (adjustable from 200 to 1,000 W). Pectin was extracted from 5 g of WGH fine powder (< 400 μ m) according to the method as previously described (Kazemi et al., 2019d). The extraction yield was calculated from the following equation (Equation 1):

$$\text{Pectin yield (g/100 g)} = \frac{\text{Dried pectin (g)}}{\text{WGH fine powder (g)}} \times 100 \quad (1)$$

2.3 | Experimental design

Box-Behnken design (BBD), as a collection of mathematical and statistical techniques, investigates the individual and interactive effects of variables on the response simultaneously with a lower number of experiments in comparison to other empirical designs. Therefore, BBD was used to optimize the microwave-assisted extraction of pectin from WGH. For this purpose, based on the preliminary experiments (data not shown) and previous studies, four independent variables in a specific range were chosen as follow: microwave power (300–700 W), pH (1.5–3), irradiation time (1–3 min), and LS ratio (15–25 ml/g). The experimental design with 27 experiments including three central points was used to evaluate the experimental error (Table 1). The analysis of variance (ANOVA) was employed to validate the obtained data for the optimization process (Table 2). The optimal condition was predicted using the quadratic polynomial equation (Equation 2). The generalized form of the equation is given as follow:

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

where Y is the predicted response; X_i and X_j are the independent parameters; β_0 , β_j , β_{jj} and β_{ij} are the intercept, linear, quadratic, and interaction coefficients, respectively; k is the number of independent parameters (in this work: k = 4). Statistical analysis of all experimental data was carried out using the Stat-Ease Design Expert (version 7.0) statistical software (Stat-Ease Inc., Minneapolis, USA).

2.4 | Characterization of extracted pectin

In this part, the physicochemical, structural, and functional features of the extracted pectin from WGH at the optimal extraction conditions were determined.

TABLE 1 Microwave-assisted extraction variables and their levels with experimental and predicted responses

Factors	Unit	Actual levels				
		-1	0	+1		
Microwave power (X_1)	W	300	500	700		
pH (X_2)	-	1.5	2.25	3		
Irradiation time (X_3)	min	1	2	3		
LS ratio (X_4)	ml/g	15	20	25		
Run No.	X_1	X_2	X_3	X_4	EY ^a (g/100 g)	PY ^a (g/100 g)
1	300	1.50	2	20	12.80	12.14
2	700	1.50	2	20	10.80	11.10
3	300	3.00	2	20	1.20	0.74
4	700	3.00	2	20	3.40	3.91
5	500	2.25	1	15	1.80	1.54
6	500	2.25	3	15	8.00	7.64
7	500	2.25	1	25	1.60	1.81
8	500	2.25	3	25	4.60	4.71
9	300	2.25	2	15	3.20	3.88
10	700	2.25	2	15	4.20	4.35
11	300	2.25	2	25	1.60	1.95
12	700	2.25	2	25	3.80	3.62
13	500	1.50	1	20	6.60	6.65
14	500	3.00	1	20	3.20	3.75
15	500	1.50	3	20	17.40	17.55
16	500	3.00	3	20	1.40	1.85
17	300	2.25	1	20	2.00	1.94
18	700	2.25	1	20	2.40	1.91
19	300	2.25	3	20	5.20	5.34
20	700	2.25	3	20	7.80	7.51
21	500	1.50	2	15	10.60	10.84
22	500	3.00	2	15	4.40	3.94
23	500	1.50	2	25	11.80	11.91
24	500	3.00	2	25	0.80	0.21
25	500	2.25	2	20	6.80	6.60
26	500	2.25	2	20	6.40	6.60
27	500	2.25	2	20	6.60	6.60

^aEY and PY were experimental and predicted yield, respectively.

2.4.1 | Physicochemical analysis

The moisture content was determined by drying the pectin sample in an oven at 105°C for 24 hr (Kazemi et al., 2019b). The ash content was determined by a combustion of 1.0 g of dry pectin sample at 550°C for 6 hr (Hosseini et al., 2019). The protein content was estimated using nitrogen quantification by the Kjeldahl method and with a conversion factor of 6.25 (Yapo et al., 2007). The GalA content of the pectin sample was estimated using the colorimetric method (Blumenkrantz & Asboe-Hansen, 1973). The DE of GalA residues was measured using the titrimetric method (Pasandide et al., 2018). Total phenolic content was estimated using the Folin-Ciocalteu method (Hosseini et al., 2019) and expressed as mg of gallic acid

equivalent per g of pectin sample (mg GAE/g pectin). The average molecular weight (M_w) of the pectin sample was determined using gel permeation chromatography (GPC) with a Shimadzu LC-20A system, equipped with a Waters Ultrahydrogel column and refractive index detector. The mobile phase was NaNO₃ solution (0.1 M) with a flow rate and temperature of 1 ml/min and 35°C, respectively.

2.4.2 | Structural analysis

The dried pectin sample was analyzed by Fourier transform-infrared (FT-IR) and hydrogen-1 nuclear magnetic resonance (H-NMR) spectroscopy to confirm the presence of pectin structure in the extracted

TABLE 2 Analyze of variance for the fitted model of pectin yield

Source	DF	Some of squares	Mean square	F value	p value
Pectin yield					
Model	14	449.451	32.104	105.16	<.0001
Linear	4	326.900	81.725	267.71	<.0001
X_1	1	3.203	3.203	10.49	.007
X_2	1	257.613	257.613	843.87	<.0001
X_3	1	60.750	60.750	199.00	<.0001
X_4	1	5.333	5.333	17.47	.001
Square	4	67.701	16.925	55.44	<.0001
X_1^2	1	10.957	10.957	35.89	<.0001
X_2^2	1	18.089	18.089	59.26	<.0001
X_3^2	1	5.157	5.157	16.89	.001
X_4^2	1	15.565	15.565	50.99	<.0001
2-Way interaction	6	54.850	9.142	29.95	<.0001
X_1X_2	1	4.000	4.000	13.10	.004
X_1X_3	1	1.210	1.210	3.96	.070
X_1X_4	1	0.360	0.360	1.18	.299
X_2X_3	1	40.960	40.960	134.17	<.0001
X_2X_4	1	5.760	5.760	18.87	.001
X_3X_4	1	2.560	2.560	8.39	.013
Error	12	3.663	0.305		
Lack-of-Fit	10	3.583	0.358	8.96	.105
Pure Error	2	0.080	0.040		
Total	26	453.114			
R^2			0.9919		
Adj R^2			0.9825		
Pred R^2			0.9541		

sample. Furthermore, X-ray diffraction (XRD) spectroscopy was employed to achieve further information about its structure.

FTIR spectrum was recorded using the KBr method on a Bruker Tensor-27 spectrometer (Billerica, MA, US) in a wavelength range of 4000–600 cm^{-1} . A total of 10 scans were measured with a resolution of 4 cm^{-1} .

H-NMR spectrum was taken on Varian Unity INOVA -500 (500 MHz) spectrometer (Varian, Inc.). A total of 32 scans were recorded at an internal temperature of 30°C and an acquisition time of 4.0 s.

XRD measurement was performed on a PHILIPS diffractometer (Amsterdam, Netherland) with the diffraction angle range of 10–80° (2 θ), a step size of 0.05° (2 θ), and time/step of 1 s.

2.4.3 | Functional analysis

Water and oil-holding capacities were determined using the method described by Kazemi et al. (2019b). Also, the emulsion capacity (EC) and stability (ES) were evaluated according to the method as

described by Yapo et al. (2007). The ES of the prepared emulsions was measured after 1 and 30 days storing at 4 and 24°C.

The antioxidant activity of the pectin sample was measured using DPPH free radical scavenging assay by a UV/Vis spectrophotometer (Spectrum SP-UV500DB). The dried pectin sample was dissolved in double-distilled water and prepared in different concentrations (0.5, 1, 2.5, 5, 10, 15, and 25 mg/ml). Four milliliters of DPPH solution (ethanol, 0.1 mM) were added to 1 ml of sample and vortexed (1 min). The mixture was incubated in a dark place for 30 min at room temperature. The absorbance of the mixture was measured at 517 nm. In addition, ascorbic acid (AA) as a standard antioxidant, in a similar concentration was used for comparison. DPPH radical scavenging activity was estimated as follow (Equation 3):

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

where A_{sample} is the absorbance of the sample (1 ml of the sample with 4 ml of DPPH solution); A_{control} is the absorbance of the control (1 ml of distilled water with 4 ml of DPPH solution).

3 | RESULTS AND DISCUSSION

3.1 | The optimal extraction conditions of pectin from walnut green husk

3.1.1 | Experimental data and statistical analysis

BBD was employed to optimize the microwave-assisted extraction of pectin from WGH and determine the effect of microwave power, pH, irradiation time, and LS ratio on the mentioned parameter. The results including experimental and predicted results are listed in Table 1. The actual pectin yields were ranged from 0.8 g/100 g (run number 24) to 17.4 g/100 g (run number 15). The obtained data were modeled by a second-order polynomial mathematical equation (Equation 4) as follows:

$$\begin{aligned} \text{Pectin yield (g/100g)} = & 6.60 + 0.52X_1 - 4.63X_2 + 2.25X_3 - 0.67X_4 - 1.43X_1^2 \\ & + 1.84X_2^2 - 0.98X_3^2 - 1.71X_4^2 + 1.0X_1X_2 - 0.55X_1X_3 \\ & + 0.30X_1X_4 - 0.23X_2X_3 - 1.20X_2X_4 - 0.80X_3X_4 \end{aligned} \quad (4)$$

where, X_1 , Microwave power (W); X_2 , pH; X_3 , Irradiation time (min); X_4 , LS ratio ml/g.

The analysis of variance (ANOVA) for the fitted second-order polynomial model of pectin yield is listed in Table 2. The high F value (105.16) and a low p value (<.0001) of the developed model demonstrated the model was significant. Also, the calculated determination coefficient (R^2 : 0.9919) indicated only less than 1% of all variations could not be explained by the developed model. In addition, the obtained p value for lack-of-fit was .105, which indicated the suggested model was adequate for predicting the pectin yield under any combination of the

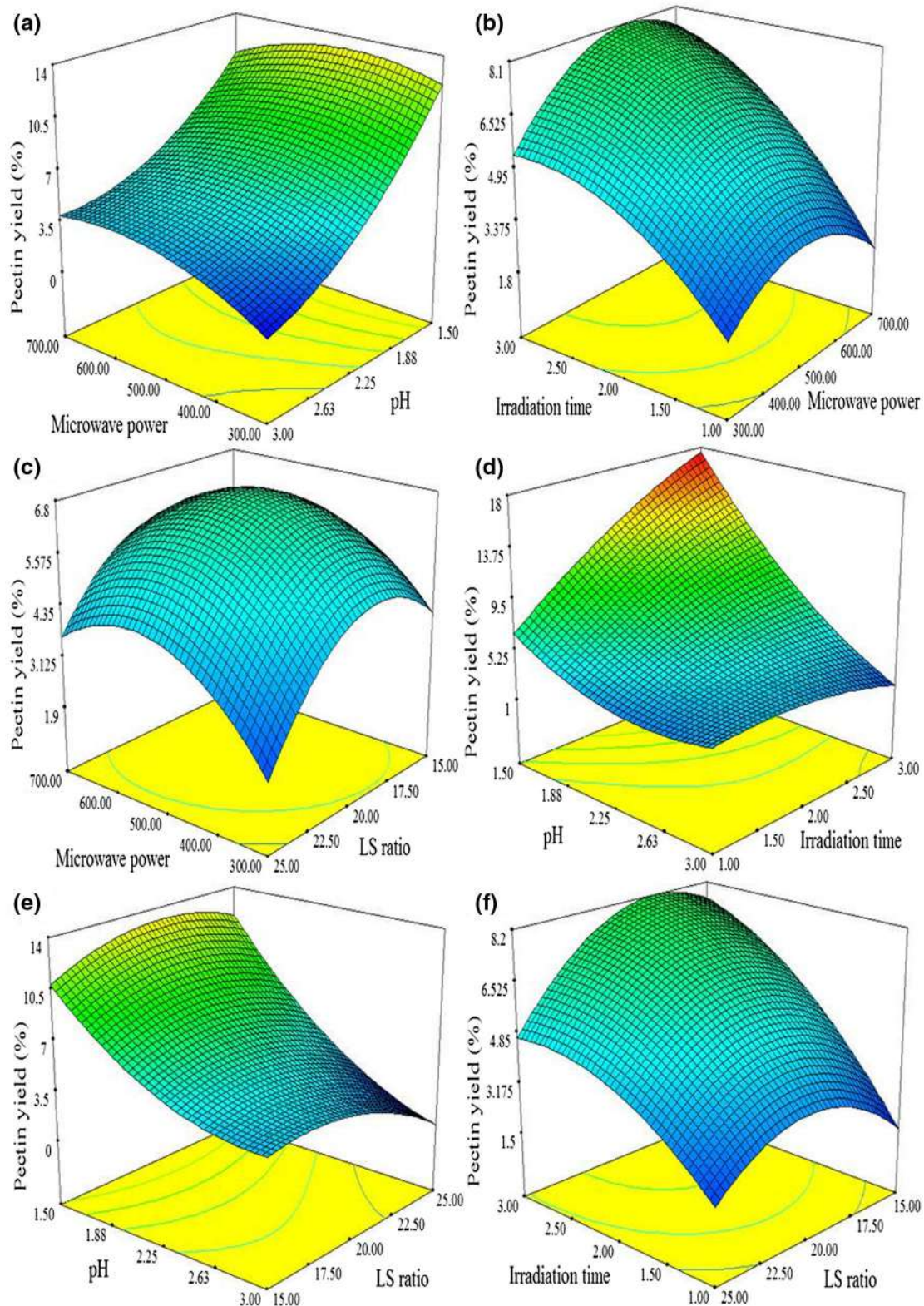


FIGURE 1 3D plots showing the effects of microwave-assisted extraction variables on pectin yield

values of the independent variables (Xu et al., 2018). Thus, the experimental results were well-fitted with the predicted values and the obtained responses were significantly explained by the suggested model.

The data in Table 2 showed that all of the variables significantly affected the pectin yield. According to Equation (4), the factor of pH

in linear (X_2) and quadratic (X_2^2) terms; and interaction between pH and LS ratio (X_2X_4) in interaction term, were the most effective variables on pectin yield. For better comprehension three dimensional (3D) plots, presenting the interaction effects of variables on pectin yield, were developed in Figure 1a–f.

3.1.2 | Effect of microwave power on pectin yield

According to Figure 1a–c, the pectin yield was dramatically increased by an increase in microwave power from 300 to 500 W, which was probably due to the increase in the absorbed energy by surrounding medium and plant matrix and so increase in the liberation of pectin from WGH (Hosseini et al., 2016). However, the pectin yield was decreased beyond 500 W, probably due to the thermal degradation of the pectin (Maran & Prakash, 2015).

3.1.3 | Effect of pH on pectin yield

The selection of the best pH point is extremely necessary to achieve the highest yield in the pectin extraction process (Hosseini et al., 2016; Kazemi et al., 2019a). So, pH is one of the most effective factors. Figure 1a,d,e clearly shows this fact and clarifies that a decrease in pH level from 3 to 1.5 causes a considerable increase in pectin yield. The low pH improves the infusion of the extraction solvent into the plant tissue and increases the hydrolysis of insoluble pectin to soluble form (Maran & Prakash, 2015).

3.1.4 | Effect of irradiation time on pectin yield

The irradiation time was one of the key variables for pectin extraction from WGH. Figure 1b,d,f show that the pectin yield is steadily increased with increasing the irradiation time from 1 to 3 min. This might be due to the increase of the reaction time which provides more opportunity for pectin to release from the plant matrix into the medium (Lefsih et al., 2017).

3.1.5 | Effect of LS ratio on pectin yield

A remarkable increase in the pectin yield was observed with an increase in LS ratio up to 20 ml/g Figure 1c,f, which was maybe due to the increase of the surface area between solvent and plant material. But beyond this LS ratio, the electromagnetic energy was directly absorbed by the excessive solvent, which led to a decrease in the energy absorption of the plant material and so decrease in the mass transfer rate of pectin (Hosseini et al., 2016; Maran & Prakash, 2015).

3.1.6 | Validation of model

The optimal condition of pectin extraction from WGH was selected considering the maximum production yield of pectin, which was at a microwave power of 500 W, pH of 1.5, irradiation time of 3 min, and LS ratio of 20 ml/g with a predicted pectin yield of 17.6 g/100 g. To validate the model, three experiments were carried out at the mentioned conditions. A mean value of 18.0 g/100 g was obtained which was not significantly different than the predicted data.

3.2 | Physicochemical analysis of pectin sample

The physicochemical properties of the extracted pectin are shown in Table 3. The observations indicated that the ash content of the pectin sample (5.78 g/100 g) was found to be lower than eggplant peel pectin extracted by microwave-assisted extraction (9.03 g/100 g; Kazemi et al., 2019b) and higher than the potato pulp pectin (1.42 g/100 g; Yang et al., 2019), apple pomace pectin (1.89 g/100 g) and citrus peel pectin (1.96–2.38 g/100 g; Kravtchenko et al., 1992). The protein content of the WGH-pectin sample with a value of 2.01 g/100 g was higher than the protein content of apple pomace pectin with a value of 1.6 g/100 g (Kravtchenko et al., 1992), and also was lower than the protein content of citrus peel pectin with a value of 3.0–3.3 g/100 g (Kravtchenko et al., 1992). Based on FAO, the protein content of pectin should not exceed 15.6 g/100 g (Hosseini et al., 2016). It should, however, be noted that higher content of protein can have a positive impact on the emulsifying properties. According to Table 3, the GalA content value of the isolated pectin sample (68.45 g/100 g) was higher than the recommended value (65 g/100 g) for using in food products and pharmaceutical purposes (Wikiera et al., 2016). The DE value of the GalA residues of the isolated pectin was found to be 54.07%, which showed the obtained pectin should be recognized as HMP. DE has a profound effect on the functional properties and HM pectin is generally used as a thickening and gelling agent in the formulation of fruit jams, jellies, and preserves; as a stabilizer in order to prevent hardening in the cakes, or improve the quality of cheese (Lu et al., 2019). It is noteworthy that, the obtained DE value was in accordance with FTIR and NMR spectroscopy analysis (see Section 3.4). The TPC value of WGH-pectin was 35.37 mg GAE/g pectin, which was higher than pectin from grapefruit peel (4.21–7.06 mg GAE/g pectin; Wang et al., 2015), and *Averrhoa bilimbi* (13.9 mg GAE/g pectin; Shafie et al., 2019). This difference in the phenolic compounds can be related to the applied source as well as the method and conditions of extraction. The Mw of WGH-pectin extracted by microwave-assisted extraction was estimated using GPC. The result revealed that WGH-pectin had an Mw value of 260.289 kDa, which was higher than jackfruit peel pectin (51.5–52.3 kDa; Xu et al., 2018), potato pulp pectin (153.7 kDa; Yang et al., 2019), and sugar beet pulp pectin (49.73–67.12 kDa; Huang et al., 2018). The Mw/Mn value was higher than 1.0, suggesting that WGH-pectin had a wide range of molecular weight distribution and it could be considered as a heterogeneous natural polysaccharide.

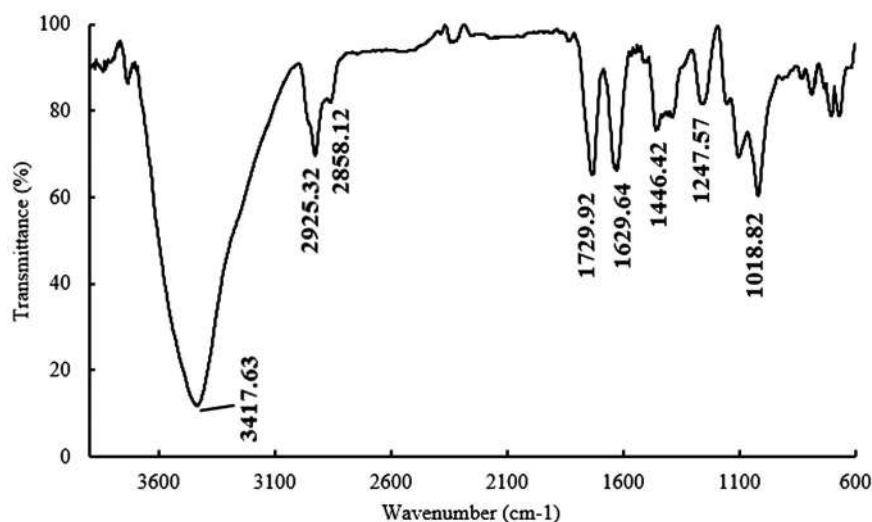
3.3 | Structural analysis of pectin sample

The FTIR spectrum of WGH-pectin is shown in Figure 2. The strong signal at about $3,417\text{ cm}^{-1}$ was attributed to the hydroxyl groups, which were originated from the inter- and intra-molecular hydrogen linkage of the GalA residues. The stretching and bending vibrations of C–H groups (CH , CH_2 , and CH_3) were observed at around $2,925\text{ cm}^{-1}$. The characteristic signals at $1,729\text{ cm}^{-1}$ and $1,629\text{ cm}^{-1}$ were assigned to the esterified and free carboxyl groups of GalA residues. The DE value of the pectin sample is related to these two peaks and

TABLE 3 Physicochemical and Functional properties of walnut green husk pectin

Physicochemical parameters			
Moisture (g/100 g)			3.98 ± 0.74
Ash (g/100 g)			5.78 ± 0.16
Protein (g/100 g)			2.01 ± 0.38
GalA content (g/100 g)			68.45 ± 2.28
DE (%)			54.07 ± 2.41
TPC (mg GAE ^a /g pectin)			35.37 ± 1.68
Mw (kDa)			260.289
Mn (kDa)			136.270
Mw/Mn			1.91010
Functional parameters			
Water-holding capacity (g water/g pectin)			5.47 ± 0.64
Oil-holding capacity (g oil/g pectin)			1.03 ± 0.41
Emulsion capacity (%)	–	24°C	58.90 ± 2.27
Emulsion stability (%)	After 1 day	4°C	96.26 ± 1.67
		24°C	94.00 ± 2.24
	After 30 days	4°C	89.06 ± 2.86
		24°C	83.53 ± 3.15
IC ₅₀ value of DPPH scavenging activity (mg/ml)			6.42

^aGAE was gallic acid equivalent.

FIGURE 2 FTIR spectrum of walnut green husk pectin

can be measured by the ratio of the area of 1,729 cm^{-1} over the sum of the areas of 1,729 cm^{-1} and 1,629 cm^{-1} (Yang et al., 2019). As can be seen the signal at 1,729 cm^{-1} is slightly stronger than 1,629 cm^{-1} , meaning the DE value of WGH-pectin is higher than 50%. This result was in line with the titrimetric measurement of DE value. In addition, the absorption signal at 1,247 cm^{-1} was originated from the stretching vibration of the C=O bond, suggesting the presence of GalA residue. The signals in the range of 1,200–1,000 cm^{-1} were attributed to the C–O–C vibrations of glycosides (Lu et al., 2019). These results were in accordance with the characteristic absorption signals of esterified GalA-rich pectin (Hosseini et al., 2016; Kpodo et al., 2017; Wang et al., 2016; Yang et al., 2019).

In order to achieve further structural characteristics of WGH-pectin, the H-NMR spectrum of WGH-pectin was determined (Figure 3). The intense signal at 3.65 ppm was related to the methyl groups of the GalA residues. In addition, the weak signals at around 1.95 ppm and 2.10 ppm were attributed to the acetyl groups of the GalA residues (Yang et al., 2019), suggesting that the major DE value was due to the high methyl groups in WGH-pectin structure. The signals at 3.55, 3.90, 4.20, 4.90, and 5.00 were originated from the protons linked to C-2, C-3, C-4, C-5, and C-1 of galacturonate and methyl galacturonate residues. The results confirmed the predominant presence of esterified GalA-rich residues and were in accordance with other studies (Kpodo et al., 2017; Wang et al., 2016).

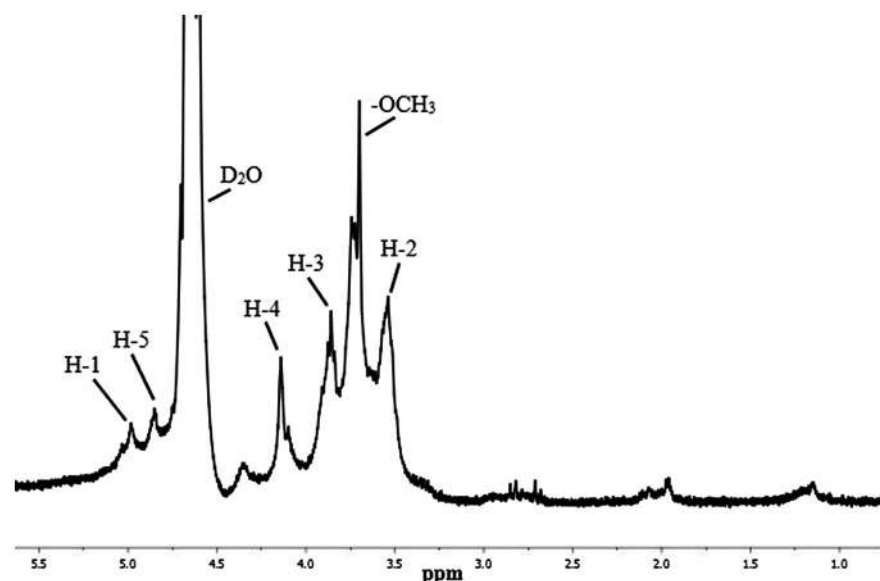


FIGURE 3 ^1H -NMR spectrum of walnut green husk pectin

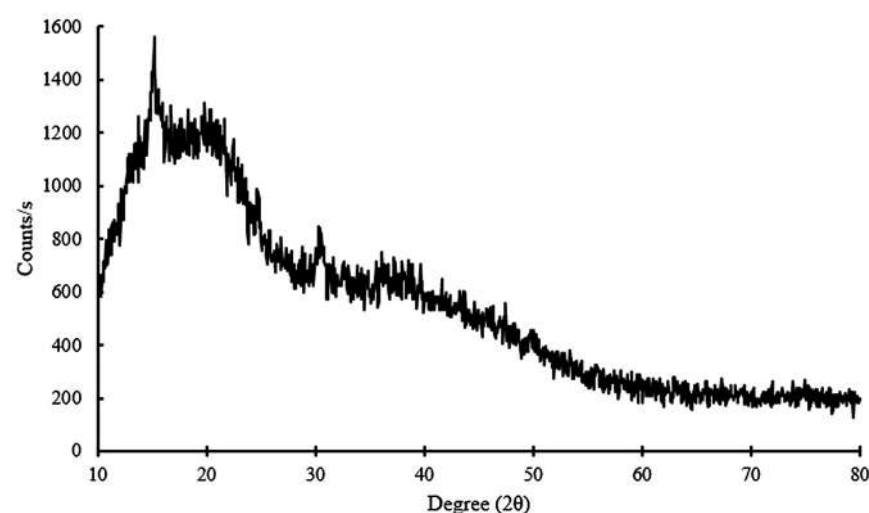


FIGURE 4 XRD pattern of walnut green husk pectin

XRD pattern in Figure 4 indicated an amorphous structure for the pectin sample, which was in line with previous studies (Hosseini et al., 2019; Wang et al., 2016). However, the relatively strong signals at 13.71° , 15.62° , 16.71° , 18.26° , 19.81° , 20.31° , 21.61° , 24.76° , 30.56° , and 36.11° (2θ) reveal that there are some crystalline portions in the structure of pectin. It is obvious that the crystallinity or non-crystallinity of pectin is related to its chemical composition. These crystalline portions were probably due to the presence of small amounts of non-pectin polysaccharides such as cellulose (Kazemi et al., 2019d).

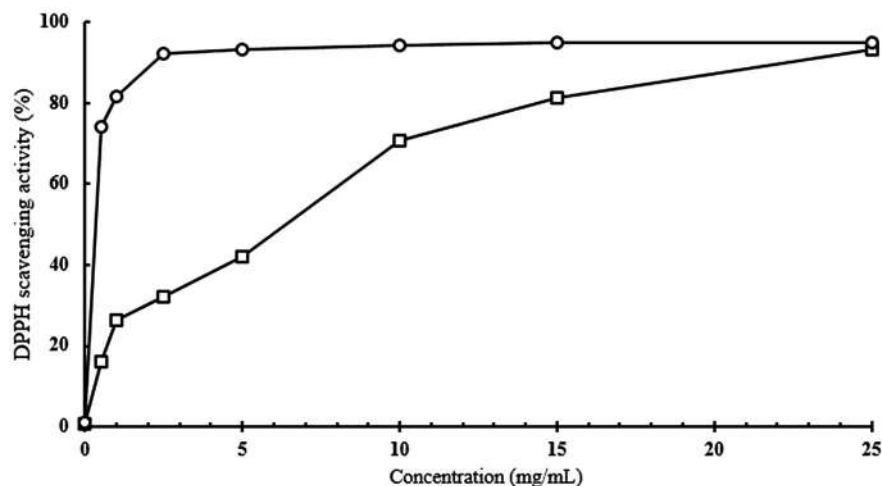
3.4 | Functional features

From Table 3, the water-holding capacity of WGH-pectin was found to be 5.47 g water/g pectin, which was higher than *Averrhoa bilimbi* pectin (3.70 g/g; Shafie et al., 2019). The higher water-holding capacity shows higher interaction between pectin and water. The chemical structure of extracted pectin and other factors such as particle size, porosity, pH, and ionic strength could be effective on this parameter

(Elleuch et al., 2011). Also, Rubio-Senent et al. (2015) reported that the water-holding capacity of pectin could be affected by the C-OH groups of GalA residues and higher GalA residues represent higher water-holding capacity. Oil-holding capacity of WGH-pectin was 1.03 g/g, meaning each gram of WGH-pectin could hold about one gram of sunflower oil. Pectin with a high value of oil-holding capacity can provide good textural and sensory properties for high-fat food systems. However, various factors have an effect on this parameter such as overall charge density and the hydrophilic nature of the pectin constituents (Elleuch et al., 2011).

Polysaccharides are generally recognized as strong hydrophilic and non-surface active materials. Conversely, proteins are widely applied in food products as an emulsifier agent due to their hydrophobic and hydrophilic side chains. Thus, the polysaccharides containing a small fraction of surface-active proteins could act as both stabilizing and emulsifying agent (Ghribi et al., 2015). Therefore, the emulsifying activity (EA) and emulsion stability (ES) of WGH-pectin were investigated in order to evaluate the functionality of extracted pectin. The EA of WGH-pectin was found to be 58.90%, which was similar to

FIGURE 5 DPPH radical scavenging activity walnut green husk pectin (□) and ascorbic acid (○)



pistachio green hull pectin (58.3%; Kazemi et al., 2019d) and slightly higher than sour orange pectin (53.42%; Hosseini et al., 2019). The result suggested that WGH-pectin can be considered as a good emulsifier, as its EA was higher than 50% (Abdul-Hamid & Luan, 2000). In addition, the stability of prepared oil-in-water emulsions was investigated by storing for 1 and 30 days at 4°C and room temperature (Table 3). The result suggested that the emulsions were more stable at low temperatures. Similar results were reported by Hosseini et al. (2019), Pasandide et al. (2018), and Yapo et al. (2007).

In order to evaluate the antioxidant activity of WGH-pectin, DPPH free radical scavenging activity of pectin extracted at the optimal extraction point was measured and compared with ascorbic acid as shown in Figure 5. A dose-dependent manner was observed for the scavenging activity of pectin, which was in accordance with previous studies (Bayar et al., 2018; Hosseini et al., 2019; Xu et al., 2018). The obtained results showed that although WGH-pectin showed a significant DPPH scavenging activity, ascorbic acid (as a strong antioxidant) had a higher scavenging activity in all concentrations. In addition, the estimated IC_{50} value for pectin (6.42 mg/ml) was found to be lower than *Averrhoa bilimbi* pectin (>10 mg/ml; Shafie et al., 2019) and *Opuntia ficus indica* cladodes pectin (8 mg/ml; Bayar et al., 2017). The lower IC_{50} value of WGH-pectin could be explained by its higher GalA content and higher TPC value (Bayar et al., 2018).

4 | CONCLUSION

Pectin was obtained for the first time from WGH powder using a microwave-assisted extraction method. BBD was employed to achieve the highest extraction yield. The results showed that pectin extraction yield in the optimal conditions (microwave power: 500 W; pH: 1.5; irradiation time: 3 min; and LS ratio: 20 ml/g) was 18.0 g/100 g. Different analyses revealed that the extracted WGH-pectin was rich in GalA residues and total phenolic compounds. In addition, the FTIR and NMR spectroscopy suggested the dominant presence of a high methylated GalA-rich structure in the extracted sample. The XRD pattern showed an amorphous structure with several crystalline regions in the structure of pectin. Furthermore, the functional analysis showed a considerable value for water and oil-holding capacity,

emulsifying activity, emulsion stability, and antioxidant (DPPH free radical scavenging) activity. The obtained results in the current study were in favor of the use of WGH-pectin as a potential ingredient in the formulation of various food products.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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