

High-methylated pectin from walnut processing wastes as a potential resource: Ultrasound assisted extraction and physicochemical, structural and functional analysis

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ABSTRACT

The ultrasound-assisted extraction of pectin from walnut processing waste was optimized by Box-Behnken design. The highest extraction yield ($12.78 \pm 0.83\%$) was obtained at ultrasound power of 200 W, sonication time of 10 min, pH of 1.5 and LS ratio of 15 v/w. The resulting pectin in these conditions was rich in galacturonic acid (69.44%) and was high in degree of esterification (59.21%) which was confirmed by NMR and FTIR spectra. In addition, the molecular weight distribution analysis showed that the obtained pectin had a heterogeneous natural with low molecular weight (6.30–158.48 kDa). The XRD spectrum of the walnut pectin showed an amorphous structure with few crystalline portions. Furthermore, walnut green husk pectin had good emulsifying properties, water and oil holding capacities, and radical-scavenging activity. Given that the obtained result, the extraction of pectin from this by-product in addition to being able to reduce environmental problems, it can also provide financial benefits for the walnut production sector.

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1. Introduction

Walnut (*Juglans regia* L.) is one of the most important and valuable agricultural products in the world with a production quantity of about 3.8 million tons per year, based on the Food and Agriculture Organization (FAO) report [1]. Iran is the second-largest walnut producer in Asia and the third in the world. Walnut green husk as the major by-product (about 50–60% w/w) of walnut processing sectors is usually unused and discarded as agricultural waste [2], while it is a suitable resource for the valuable components such as polyphenols, flavones, terpenoids, carbohydrates, and other bioactive compounds [3]. Therefore, the utilization of it as a source of the valuable compounds, not only promotes the value of the walnut production but also will decrease the disposal problems [4]. In addition, the resulting valuable components could be used as a food ingredient in the formulation of various food products [5]. Most of the previous studies were focused on extraction and characterization of phenolic compounds from this waste, for instance, the extraction optimization of juglone using different methods [2–4,6,7], the extraction and characterization of walnut green husk extract using different types of solvents [2,7,8] and also, the evaluation of antioxidant, antimicrobial, and antitumor activity of phenolic

constituents from walnut green husk [2,3,8]. However, to the best of our knowledge, no single study has reported the extraction of pectin from walnut green husk.

Pectin is a natural anionic hydrocolloid occurred in the middle lamella, the primary and secondary cell wall of terrestrial plants. D-galacturonic acid (GalA) units are the basic constituents of pectin, which present in three main polymeric forms: homogalacturonan (a linear chain of GalA units), rhamnogalacturonan-I (a repeating disaccharide of rhamnose and GalA), and rhamnogalacturonan-II (a homogalacturonan backbone with side chains of neutral monosaccharides) [9]. Depending on the esterification degree (DE) of GalA units, pectin is categorized into two classes: high methoxyl (HM) pectin with DE value higher than 50% and low methoxyl (LM) pectin with DE value lower than 50%. The different forms of pectin present different applications in the food industry. For instance, HM pectin can gel at acidic pH (2–3.5) and in the presence of sugars (55–75%), while LM pectin can gel in the wide range of pH (2–6) and in the presence of divalent cations such as Ca^{2+} [10]. Pectin has diverse technological properties which make it a suitable ingredient to use as stabilizing, gelling, thickening, and emulsifying agents in the food industry. It also has numerous applications in various areas such as cosmetics and personal care products, edible packaging films, and pharmaceutical industries [11]. In addition to its technological features, pectin is suggested to have various health-promoting benefits such as wound healing, lipase inhibiting

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and cholesterol decreasing effects [12]. Due to its applications and benefits, over the last years, pectin was a fast-growing functional ingredient in the global hydrocolloids market [9].

In the industry, pectin is produced using a conventional method with mineral acids, high temperature and long extraction time. But, some new innovative extraction techniques have been developed and employed to improve the extraction process and pectin quality. One of these techniques is the ultrasound-assisted extraction method. This green chemistry method can recover pectin in an environmentally-friendly manner, with low temperature, energy and solvent requirement, considerably short extraction time, and high efficiency [13,14]. This technique was previously used to extract pectin from various commercial and non-commercial sources such as sour orange peel [10], *Musa balbisiana* waste [13], eggplant peel [15], grapefruit peel [12], mango peel [12], and potato pulp [17]. The main sources for commercial pectin production are citrus peels (85.5%), apple pomace (14.0%) and sugar beet pulp (0.5%) [11]. According to the rising demand for pectin in the global markets (more than 5% per year), it is necessary finding additional inexpensive novel sources such as walnut green husk [15].

In the current study, walnut processing waste (walnut green husk) was used as a new inexpensive source for pectin production using the ultrasound-assisted extraction method. Box-Behnken design (BBD) was employed to optimize the extraction process factors and to evaluate the relationship between independent variables and response (pectin yield). Furthermore, the physicochemical, structural and functional properties of the walnut green husk pectin (WP) were investigated.

2. Materials and methods

2.1. Chemicals and raw material

Fresh walnuts were bought from a grocery store located in Karaj, Iran. The green husk of walnuts was removed using a lab knife, cut into small pieces, placed in a steel tray and dried at 45 °C for 18 h, respectively. The dried green husk pieces were ground using an IKA M 20 grinding mill (IKA Co., Germany) and passed through a 40-mesh sieve to get uniform size range. The resulting powder was kept in a dark dry place for next uses. The chemical composition of the obtained walnut green husk powder was evaluated as about 92–94% total solids (70 °C, 24 h), 13.5–15% w/w ash content (550 °C, 6 h), 5.3–6.2% w/w protein content (Kjeldahl method, 6.25), 1.5–3% w/w total fat content (Gerber method) and 66–70% w/w total carbohydrate content (phenol–sulfuric acid colorimetric method, 490 nm).

Hydrochloric acid (37%), sulfuric acid (98–95%), citric acid (99%), sodium hydroxide (99%), sodium tetraborate (Borax) and sodium carbonate were purchased from Merck Chemical Co. (Darmstadt, Germany). Ethanol (96%) was bought from Ghadir Co. (Tehran, Iran). All other chemicals and reagents were of analytical grade.

2.2. Pectin recovery

The ultrasound-assisted extraction of pectin from walnut green husk was performed on an ultrasonic device (Ultrasonic Co., Iran) with a cylindrical titanium probe (10 mm diameter), constant frequency of 20 kHz and a digital control system for adjusting sonication time and ultrasound power. The extraction process was carried out following the procedure previously described [10]. Briefly, 5 g of walnut green husk powder was mixed with acidified distilled water (using citric acid). Then, the ultrasound probe was immersed into the mixture and the power and time were set up using a control system of the ultrasonic device. After the extraction process, the obtained mixture was filtered and centrifuged at 10,000g for 20 min to eliminate impurities. The clarified mixture was precipitated by an equal volume of ethanol (96%) and was cooled at 4 °C for 18 h. Afterwards, the precipitated pectin was isolated using centrifugation (10,000g, 20 min), was washed twice with ethanol and was dried at 45–50 °C for 16 h, respectively. The pectin

yield (%) was expressed as gram of dry collected pectin per 100 g of walnut green husk powder.

2.3. Optimization process and experimental data analysis

In this study, BBD was applied to optimize and explore the effect of ultrasound power (100–200 W), sonication time (10–30 min), pH (1.5–3), and liquid-to-solid (LS) ratio (15–25 v/w) on the pectin yield. The design and levels of independent variables were listed in Table 1. The optimum point of ultrasound-assisted extraction was estimated by fitting the obtained data into a second-order polynomial model, as given in the following equation (Eq. (1)):

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

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).

2.4. Characterization of WP

The isolated WP at the optimal conditions was characterized through various properties. Each experiment was repeated three times and the results were reported based on the mean value $\pm$ SD.

Table 1
Ultrasound-assisted extraction variables and their levels with experimental and predicted responses.

Factors		Unit	Actual levels			
			−1	0	+1	
Ultrasound power (X_1)		W	100	150	200	
Sonication time (X_2)		min	10	20	30	
pH (X_3)		–	1.5	2.25	3	
LS ratio (X_4)		v/w	15	20	25	
Run No.	X_1	X_2	X_3	X_4	EY* (%)	PY* (%)
1	100	10	2.25	20	2.40	2.66
2	200	10	2.25	20	4.20	4.53
3	100	30	2.25	20	2.20	2.09
4	200	30	2.25	20	3.40	3.36
5	150	20	1.50	15	9.40	9.52
6	150	20	3.00	15	2.60	2.76
7	150	20	1.50	25	8.20	8.26
8	150	20	3.00	25	2.40	2.49
9	100	20	2.25	15	3.20	3.03
10	200	20	2.25	15	4.00	4.10
11	100	20	2.25	25	1.80	1.77
12	200	20	2.25	25	3.60	3.83
13	150	10	1.50	20	10.60	10.50
14	150	30	1.50	20	7.00	7.23
15	150	10	3.00	20	2.00	1.83
16	150	30	3.00	20	3.20	3.37
17	100	20	1.50	20	8.00	8.01
18	200	20	1.50	20	10.40	10.08
19	100	20	3.00	20	2.20	2.24
20	200	20	3.00	20	3.60	3.31
21	150	10	2.25	15	4.00	3.88
22	150	30	2.25	15	3.00	2.91
23	150	10	2.25	25	3.20	3.01
24	150	30	2.25	25	2.40	2.24
25	150	20	2.25	20	2.20	2.23
26	150	20	2.25	20	2.10	2.23
27	150	20	2.25	20	2.40	2.23

* EY and PY were experimental and predicted yields, respectively.

2.4.1. Moisture, ash and protein content

The moisture content was measured gravimetrically by drying in an oven (105 °C, 24 h). The ash content was estimated by completely combusting the WP sample in a furnace (550 °C, 6 h). The protein content was determined using the Kjeldahl method with a factor of 6.25.

2.4.2. GalA content and DE measurement

The GalA content of WP was estimated using the colorimetric method according to Blumenkrantz and Asboe-Hansen [18]. The DE value of pectin was determined by titration. Briefly, 200 mg of WP was wetted with 2 mL of ethanol and was completely dissolved in 20 mL distilled water. Then, the prepared mixture was titrated with sodium hydroxide solution (0.1 M, V_1) in the presence of phenolphthalein as an indicator. Afterward, the solution was mixed with 10 mL of 0.1 M sodium hydroxide. The prepared mixture was agitated for 1 h at room temperature. Then, 10 mL of hydrochloric acid solution (0.1 M) was added and the mixture was stirred until the pink color disappeared. The solution was titrated again with 0.1 M sodium hydroxide solution (V_2). The DE value of WP was calculated according to the following equation (Eq. (2)):

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

2.4.3. Total phenolic content (TPC) measurement

TPC of WP was estimated according to the Folin-Ciocalteu method using a UV/Vis spectrophotometer (Spectrum SP-UV500DB). Briefly, 0.5 mL of WP aqueous solution (1% w/v) was prepared into a 10 mL sample tube. Then, 2.5 mL of Folin-Ciocalteu reagent (10% v/v) and 2 mL of sodium carbonate solution (7.5% w/v) were added to the sample tube. The obtained mixture was vortexed for 1 min and was incubated for 1 h at room temperature. The absorbance of the sample was recorded at 750 nm. The standard curve of gallic acid ($R^2 = 99.37\%$) was used for calculation of the TPC value of WP and the result was expressed as mg gallic acid equivalents per g of WP (mg GAE/g WP) [15].

2.4.4. Gel permeation chromatography

The average molecular weight (Mw) of WP was determined using a Shimadzu LC-20A instrument (Kyoto, Japan), equipped with a Waters Ultrahydrogel™ column and refractive index detector (mobile phase: 0.1 M NaNO₃; flow rate: 1 mL/min; temperature: 35 °C; injection volume: 20–50 µL). The average molecular weight of WP sample was calculated using the calibration curve of standard dextrans (1–3000 kDa).

2.4.5. Fourier transform-infrared (FTIR)

FTIR spectroscopy was performed on a Bruker Tensor-27 spectrometer (Billerica, MA, US) using KBr pellets. The spectrum of WP powder was recorded by 10 scans over 4000–600 cm⁻¹ at a resolution of 4 cm⁻¹.

2.4.6. Hydrogen-1 nuclear magnetic resonance (H-NMR)

H-NMR spectroscopy was carried out on a Varian Unity-Inova (500 MHz) spectrometer (Varian, United States). The spectrum of WP was recorded at an internal temperature of 30 °C and an acquisition time of 4.0 s.

2.4.7. X-ray diffraction (XRD)

The XRD pattern of WP was acquired by a Philips X'pert pro PW1730 X-ray diffractometer (Amsterdam, Netherlands) with radiation of Cu-Kα, $\lambda = 1.54056 \text{ \AA}$ in the range of 10–80° (2Theta) and step size of 0.05° (2Theta).

2.4.8. Water-holding capacity and oil-holding capacity

Water-holding capacity (WHC) and oil-holding capacity (OHC) were measured using the method described by Bayar et al. [5] with

some modifications. In brief, 1 g of WP powder was placed into a 15 mL centrifuge tube and mixed with 10 mL of distilled water or sunflower oil (with a density of 0.9 g/mL). The WP mixture was vortexed for 1 min at room temperature (~25 °C) and subsequently, the mixture was centrifuged at 3000g for 20 min. The upper phase was removed and the centrifuge tube was tilted to a 45° angle and drained for 30 min on a filter paper. The WHC and OHC of WP were expressed as a gram of water or oil bound per gram of WP (g water or oil/g WP).

2.4.9. Emulsion properties

The emulsifying activity (EA) and emulsion stability (ES) of WP were investigated according to the method of Yapo et al. [19] with some modifications. In brief, 50 mg of the WP sample was suspended in 10 mL distilled water and then mixed with 10 mL of sunflower oil. The water/oil mixture was homogenized using an ultrasound device for 10 min and the emulsified mixture was centrifuged at 3000 × g for 5 min. The EA was calculated as the following equation (Eq. (3)):

$$\text{Emulsifying activity (\%)} = \frac{\text{Volume of emulsion layer}}{\text{Total volume of fluid}} \times 100 \quad (3)$$

After storing the prepared emulsion at 4 °C and room temperature for 1 and 30 days, the ES was measured using the following equation (Eq. (4)):

$$\text{Emulsion stability (\%)} = \frac{\text{Remained volume of emulsion layer}}{\text{Initial volume of emulsion layer}} \times 100 \quad (4)$$

2.4.10. Radical-scavenging activity

The antiradical activity of WP was evaluated using the DPPH assay. For this purpose, WP solution sample was prepared in the different concentrations (0.5, 1, 2, 5, 10, 25 mg/mL). 4 mL of DPPH ethanol solution (0.1 mM) was added to 1 mL of the solution sample. The mixture was vortexed and was incubated at room temperature in dark for 30 min. The prepared reaction mixture was centrifuged to separate impurities and to avoid the possible interference. Then, the absorbance of the mixture was recorded at 517 nm. Lower absorbance of mixture presents higher antiradical activity. The DPPH radical scavenging activity was calculated using the following equation (Eq. (5)):

$$\text{DPPH radical scavenging activity (\%)} : \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100 \quad (5)$$

where A_{sample} : the absorbance of 1 mL of sample with 4 mL of DPPH solution; A_{control} : the absorbance of 1 mL of distilled water with 4 mL of DPPH solution.

3. Results and discussion

3.1. Model fitting and optimization process

Four independent variables of ultrasound power (100–200), sonication time (10–30 min), pH (1.5–3) and LS ratio (15–25 v/w), each at three levels was optimized by response surface methodology Box-Behnken design (BBD). The experimental and predicted data for each experiment are listed in Table 1. The extraction yields of WP ranged from 1.80 to 10.60% and the empirical second-order polynomial model

Table 2

Analyze of variance for the developed model of WP (walnut green husk pectin) yield.

Source	DF	Some of squares	Mean square	F-value	p-value
WP yield					
Model	14	192.350	13.739	212.28	<0.0001
Linear	4	129.193	32.298	499.03	<0.0001
X ₁	1	7.363	7.363	113.77	<0.0001
X ₂	1	2.253	2.253	34.85	<0.0001
X ₃	1	117.813	117.813	1820.29	<0.0001
X ₄	1	1.763	1.763	27.24	<0.0001
Square	4	56.547	14.137	218.42	<0.0001
X ₁ ²	1	1.613	1.613	24.93	<0.0001
X ₂ ²	1	0.750	0.750	11.59	0.005
X ₃ ²	1	52.083	52.083	804.72	<0.0001
X ₄ ²	1	0.853	0.853	13.18	0.003
2-Way interaction	6	6.610	1.102	17.02	<0.0001
X ₁ X ₂	1	0.090	0.090	1.39	0.261
X ₁ X ₃	1	0.250	0.250	3.86	0.073
X ₁ X ₄	1	0.250	0.250	3.86	0.073
X ₂ X ₃	1	5.760	5.760	89.00	<0.0001
X ₂ X ₄	1	0.010	0.010	0.15	0.701
X ₃ X ₄	1	0.250	0.250	3.86	0.073
Error	12	0.777	0.065		
Lack-of-Fit	10	0.730	0.073	3.13	0.266
Pure Error	2	0.047	0.023		
Total	26	193.127			
R ²			0.9960		
Adj. R ²			0.9913		
Pred. R ²			0.9777		
Adequate Precision			46.056		
CV %			6.04		

in terms of coded factors was developed as follow:

$$\begin{aligned} \text{Yield of WP (\%)} = & 2.23 + 0.78X_1 - 0.43X_2 - 3.13X_3 - 0.38X_4 \\ & + 0.55X_1^2 + 0.38X_2^2 + 3.13X_3^2 + 0.40X_4^2 \\ & - 0.15X_1X_2 - 0.25X_1X_3 + 0.25X_1X_4 \\ & + 1.20X_2X_3 + 0.05X_2X_4 + 0.25X_3X_4 \end{aligned} \quad (6)$$

Analysis of variance (ANOVA) was employed to analyze the experimental data and to study the reliability of the developed model (Table 2). The high F-value (212.28) and low p-value (<0.0001) of the model represented that the developed model was highly significant. The R² value of the model was found to be 0.9960, indicating that 99.60% of all variations could be explained by the developed model and the adjusted R² (0.9913) was close to the R² value, meaning that the developed model was well-fitted. The high value for the R², adjusted R² and predicted R² (0.9777) confirmed the precision of the developed model for explaining the interaction of extraction process variables [20]. The coefficient of variation (CV) for the developed model was found to be 6.04, which was much lower than 10%, implying the high reproducibility of the obtained data [17]. Furthermore, the obtained adequate precision value (46.056) for the developed model was much higher than 4, suggesting that the model was reliable within the range of independent variables. Also, the p-value for the lack-of-fit was not significant (0.266), indicating the best fit of the model [21].

As can be seen in Table 2, all of the independent variables in linear (X₁, X₂, X₃, and X₄), quadratic (X₁², X₂², X₃², and X₄²), and the interaction term of X₂X₃ were significantly effective on the WP yield. Among the variables, pH (X₃) was the most effective extraction factor and it is necessary selecting an appropriate level of pH to obtain higher extraction yield. Three dimensional (3D) plots were established from the developed model to evaluate and visualize the relationship between independent variables and WP yield (Fig. 1).

An increase in the yield with an increase in ultrasound power was observed (Fig. 1A and B). Ultrasound waves enhanced the extraction yield by disrupting the cell wall and promoting the cell content liberation into the extraction medium. The beneficial effects of ultrasound

waves are related to the formation of expansion-compression cycles in the extraction medium [10,15].

Fig. 1A clearly indicated that the pectin yield was decreased by an increase the sonication time. This observation is probably due to the over-exposure to ultrasound waves, which leads to structural destruction of polysaccharides. In addition, consuming low time for the extraction is reasonable from the economic point of view [14].

As mentioned previously, pH was the most effective variable in the extraction process. Fig. 1B, D and F, obviously depicted that the acidic pH of the extraction medium improved the extraction yield. Acidic pH of the solvent could hydrolyze the insoluble pectin of the cell wall into the soluble form, reduce its molecular weight and promote the extraction yield of pectin [10,13–15].

The increase in the LS ratio from 15 to 25 v/w, had a negative effect on the liberation of pectin from plant material and decreased the extraction yield (Fig. 1C and E). At low LS ratio the cavitation intensity imposed on the plant tissue was higher, which promoted the fragmentation of raw material [22]. This observation was in line with previously reported data for the extraction of pectin from pomegranate peel [23], *Artocarpus heterophyllus* fruit peel [14], industrial waste of *Musa balbisiana* [13] and eggplant peel [15]. This observation is also in line with economic efficiency [14].

The optimal conditions of ultrasound-assisted extraction process were predicted using the developed polynomial model (Eq. (6)) as ultrasound power = 200 W, sonication time = 10 min, pH = 1.5, and LS ratio = 15 v/w. In these conditions, the predicted yield of WP was 13.06%. To compare the predicted results with the experimental value, three experiments were carried out at the optimal conditions and the obtained results confirmed the predicted model because the mean yield of WP (12.78 ± 0.83%) had no significant difference with the predicted yield.

3.2. Characterization of WP

3.2.1. Chemical parameters

The protein content of pectin is one of the most effective factors in its emulsifying properties due to the hydrophobic nature of proteins. However, FAO recommends that the protein content of pectin should not be higher than 15.6% [15]. According to Table 1, the protein content of WP (1.46%) was lower than the commercial citrus (3–3.3%) and apple pectin (1.6%) [24]. In fact, the higher protein content of pectin may enhance the emulsifying properties, but it also shows higher impurity in the isolated sample, which is not desirable for analytical purposes. The ash content of WP (4.54%) was similar to the citrus peel pectin (3.7–4.9%) [25], which revealed that the purity of WP is comparable to pectin of commercial source. It was also lower than several non-commercial pectin sources such as sunflower head (6.1%) [26], and pistachio green hull (5.16%) [27]. These different contents are probably related to the plant source, extraction technique, and extraction conditions.

Galacturonic acid (GalA) units constitute the main backbone of the pectin structure. According to the FAO regulations, the GalA content should be at least 65% w/w of pectin [15]. The GalA content of WP was found to be 69.44%, meaning that WP can be used as a food ingredient. It should be stated that higher GalA content of pectin is effective on diverse functional properties such as emulsifying properties and radical-scavenging activity [28]. The degree of esterification (DE) corresponds to the percentage of carboxyl groups of GalA units esterified with methyl or acetyl groups. The DE of WP was found to be higher than 50% (59.21%), meaning that WP should be placed in the category of HM pectin. It can gel in the acidic pH and in the presence of sugar, thus, it could be used in the formulation of high sugar products such as jam and jellies as a thickening and gelling agent. The presence of phenolic compounds in the pectin structure could have a positive effect on the functionality of pectin such as emulsifying and antioxidant properties. Total phenolic content (TPC) of WP was found to be 29.97 mg GAE/g pectin, which was higher than pectin from grapefruit peel

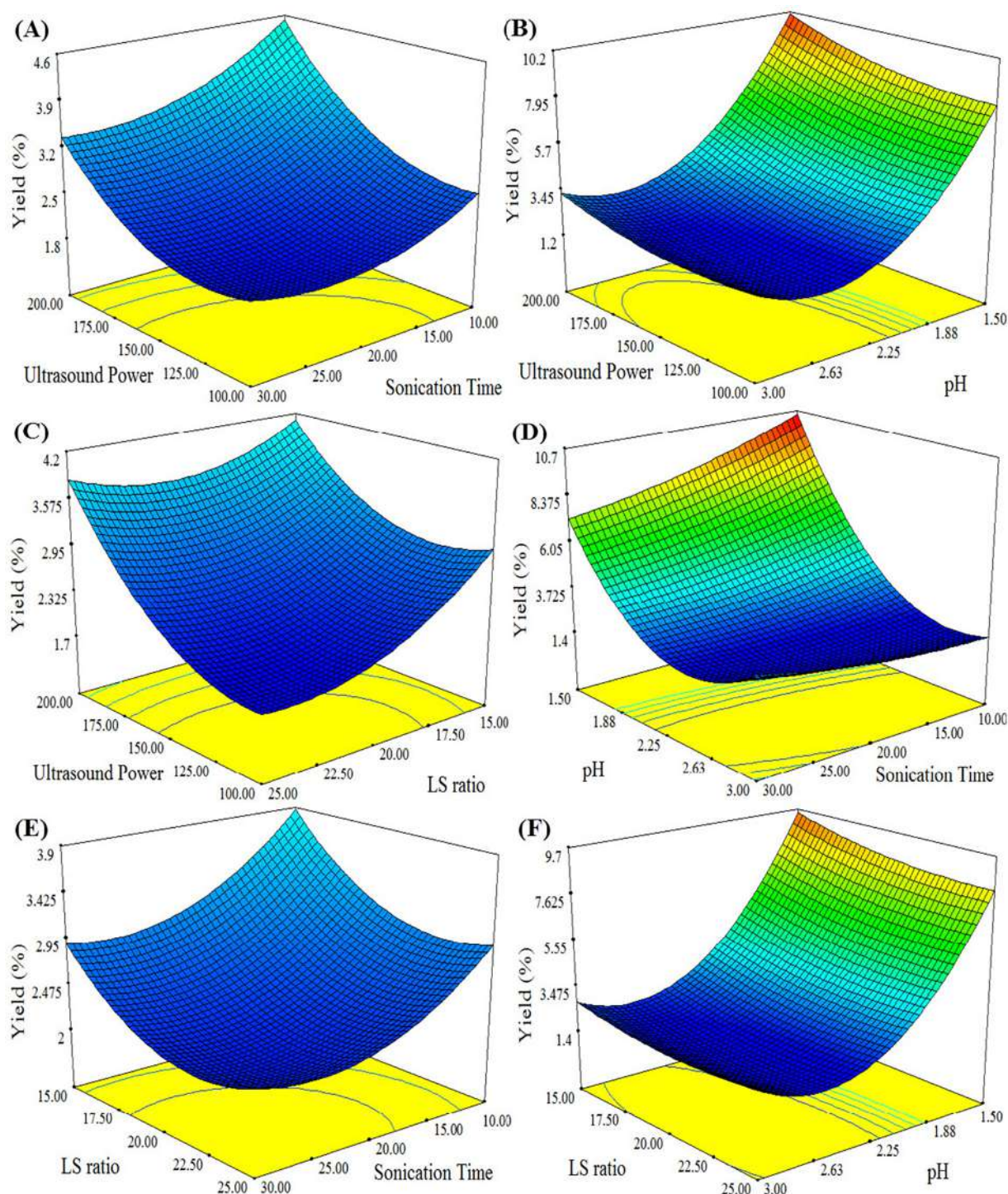


Fig. 1. Response surface plots representing extraction process variables effect on WP (walnut green husk pectin) yield.

(4.21–7.06 mg GAE/g) [29], pistachio green hull (18.18 mg GAE/g) [27], and *Averrhoa bilimbi* (13.9 mg GAE/g) [30]. Probably, the applied plant source is the main reason for these differences and a higher TPC value of WP is due to this fact that walnut green husk is a considerable resource for phenolic compounds as previously reported by several studies [3,4,31–33].

3.2.2. Molecular weight of WP

The Mw is a key factor for the functional properties of pectin and the ability of a pectin sample to be an effective emulsifier and stabilizer is greatly dependent on it [34]. Previously, Yapo et al. [19]

reported that high-Mw beet pulp pectin fraction exhibited poor emulsifying properties whereas the low-Mw fraction displayed a good emulsifying ability. Also, a low-Mw pectin sample could be effective on the antiradical activity. The molecular weight distribution of WP was in the range from 6.3096 to 158.4893 kDa with a weight-average molecular weight (Mw) of 93.550 kDa and a number-average molecular weight (Mn) of 27.277 kDa. The higher value of Mw/Mn (3.429) suggested that the isolated WP was a heterogeneous natural polysaccharide. In addition, the Mw value of WP was lower than potato pulp pectin (153.7 kDa) [17] and the okra plant pectin (791–1693 kDa) [35].

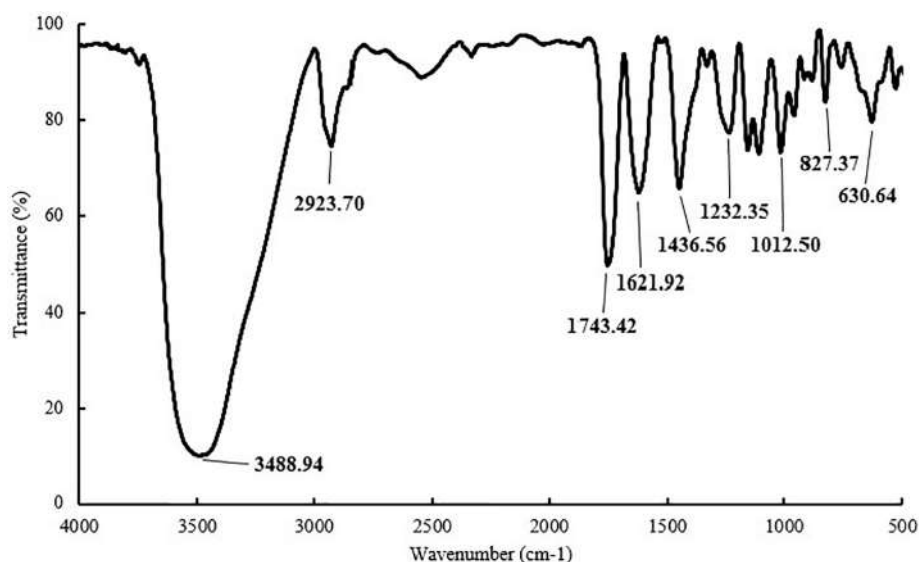


Fig. 2. FTIR spectrum of WP (walnut green husk pectin) isolated at the optimal point of ultrasound-assisted extraction.

3.2.3. Spectroscopy analysis

In this part, FTIR, H-NMR and XRD spectroscopies were used to illuminate the structural information of WP. According to the FTIR spectrum in Fig. 2, the strong and wide signal around 3488 cm^{-1} was originated from the stretching vibration of the hydroxyl group. The vibrations of C-H groups of GalA units (CH , CH_2 , and CH_3) were found around 2923 cm^{-1} [10]. The presence of two signals at 1743 and 1621 cm^{-1} was due to the esterified ($-\text{COOR}$) and unesterified carboxyl groups ($-\text{COO}^-$) [35,36]. The absorption areas of these two peaks are related to the DE value of WP and the DE value could be estimated using the ratio of absorption bond at 1743 cm^{-1} over the sum of areas at 1743 and 1621 cm^{-1} [37,38]. According to Fig. 2, it is obvious that

the DE value of WP was higher than 50% (HM), which was in accordance with the titrimetric measurement of DE [15,36,38]. The appeared signals in the range of $1200\text{--}1450\text{ cm}^{-1}$ were assigned to the C-O-H bending and C-O stretching vibrations [15].

H-NMR spectroscopy was employed to achieve more information about the structural characteristics of WP (Fig. 3). The strong signal at 3.70 ppm was attributed to the methyl esterified of GalA units and two relatively weak signals at 1.95 and 2.1 ppm was due to the acetyl esterified carboxyl groups of GalA units. The intensity of these peaks revealed that the isolated WP sample was HM pectin and also, the methyl esterified GalA units were higher than acetyl esterified. In addition, according to the previous studies, the protons on the C-2, C-3, C-4,

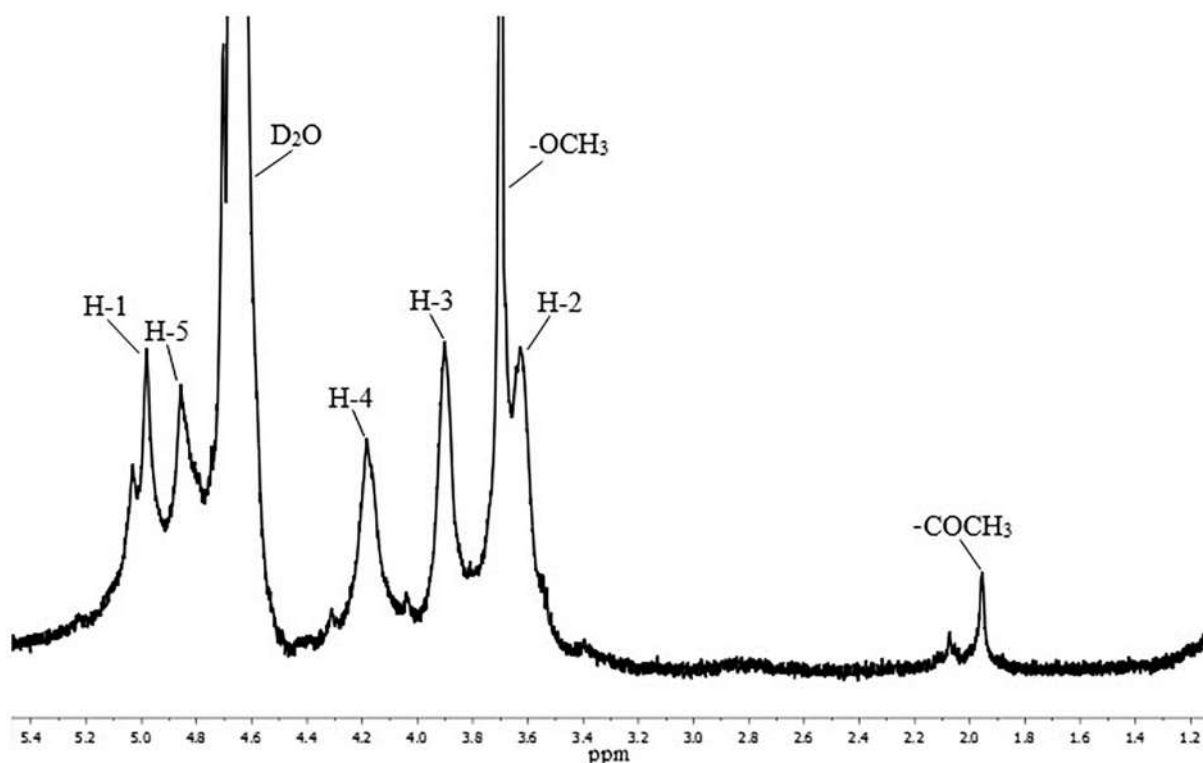


Fig. 3. ^1H NMR spectrum of WP (walnut green husk pectin) isolated at the optimal point of ultrasound-assisted extraction.

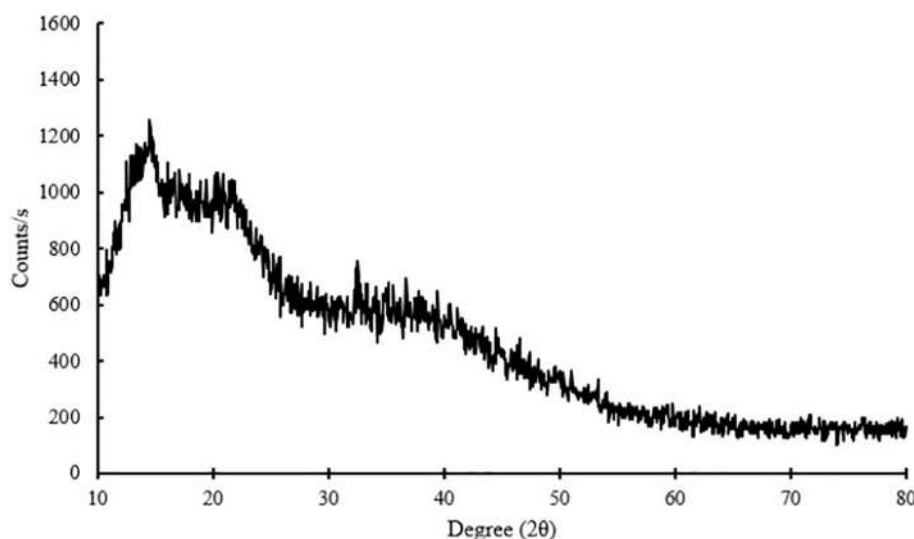


Fig. 4. XRD pattern of WP (walnut green husk pectin) isolated at the optimal point of ultrasound-assisted extraction.

C-5, and C-1 were observed at 3.6, 3.9, 4.2, 4.8 and 4.95 ppm, respectively (10, 15, 16, 35–38).

XRD spectroscopy was applied to analyze the crystallinity and/or non-crystallinity of the WP structure. The XRD spectrum revealed an amorphous structure for the WP sample (Fig. 4). However, several crystalline portions were detected in the WP structure due to the appearance of several sharp peaks at 14.61, 17.19, 20.86, 32.36 and 36.66° (2Theta). Similar results were reported for pectin extracted by the ultrasonic method from grapefruit peel [16] and sour orange peel [10].

3.2.4. Functional properties

The measured values for WHC, OHC, EA, and ES of WP are listed in Table 3. WHC is a key property from the point of view of technological and psychological, due to its ability to increase the bulk volume of foods, and to improve sensorial and textural properties [40]. The WHC value of WP (5.84 g/g) was found to be significantly higher and lower than commercial apple pectin (2.00 g/g) and commercial citrus pectin (10.35 g/g) [40]. It was also higher than pectin samples extracted from

non-commercial sources such as *Averrhoa bilimbi* (3.70 g/g) [30], olive-oil by-products (0.34–1.87 g/g) [40], and sour orange peel (3.10 g/g) [10]. The WHC value of pectin is related to its chemical structure, molecular weight, GalA content, and other factors such as its porosity, particle size, and pH [28].

OHC is another important property of pectin in high-fat products. In fact, pectin with high OHC can allow the stabilization of high-fat emulsions and food products [28]. The OHC value of WP was similar to the commercial apple pectin (2.22 g/g) and commercial citrus pectin (2.59 g/g) [40]. It was higher than several pectin samples from non-commercial sources such as sour orange peel pectin (1.32 g/g) [10] and *Opuntia ficus indica* cladodes (1.23 g/g) [5]. The high OHC value of pectin could be related to the high DE value and the hydrophobic nature of some constituents [28].

To evaluate further functionality of WP, emulsifying activity (EA) and emulsion stability (ES) of the WP sample was studied. The EA value of WP (54.26%) was similar to commercial apple pectin (50–54%) [40], and therefore could be considered as a good emulsifying agent because its EA value was higher than 50% [41]. The previous studies showed that the high value of protein content, DE value, GalA content in the pectin structure and low value for Mw of pectin could have a positive effect on EA [28,40]. In addition, the use of ultrasonic extraction process could be effective on EA of pectin, since it can increase the GalA content of extracted pectin [42]. In the case of ES, the emulsions were more stable at lower temperature, because the highest ES was observed after 1 day storing at 4 °C (95.12%) and minimum stability was observed in the stored emulsion at 24 °C for 30 days (70.31%) [43]. WP exhibited high values for both WHC, OHC and also good values for emulsion properties, therefore, the application of WP in various products could be comparable to other commercial or non-commercial pectin sources.

DPPH radical-scavenging activity is a general and simple method to evaluate the antioxidant activity of natural compounds. The DPPH radical-scavenging activity of WP is presented in Fig. 5. The result revealed that the WP sample showed significant antiradical activity in a dose-dependent manner. This behavior was also observed for several pectin samples extracted from various sources such as sour orange peel [10], *Opuntia ficus indica* cladodes [5], jackfruit peel [44] and *Premna microphylla* Turcz leaves [45]. However, the ascorbic acid sample, as a standard antioxidant, showed stronger radical-scavenging activity than the WP sample at a similar concentration. In addition, the IC₅₀ value of WP and ascorbic acid was found to be 3.89 and 0.329 mg/mL. In comparison with other sources, the radical-scavenging activity of WP was higher than pectin from *Opuntia ficus*

Table 3

Physicochemical and functional parameters of WP (walnut green husk pectin) extracted under the optimal point of ultrasound-assisted extraction.

Physicochemical parameters			
Moisture (%)			5.11 ± 0.63
Ash (%)			4.54 ± 0.34
Protein (%)			1.46 ± 0.29
GalA content (%)			69.44 ± 3.18
DE (%)			59.21 ± 3.04
TPC (mg GAE*/g pectin)			29.97 ± 1.51
Mw (kDa)			93.550
Mn (kDa)			27.277
Mw/Mn			3.42969
Functional parameters			
Water holding capacity (g water/g pectin)			5.84 ± 0.41
Oil holding capacity (g oil/g pectin)			1.21 ± 0.38
Emulsion capacity (%)	–	24 °C	54.26 ± 3.15
Emulsion stability (%)	After 1 day	4 °C	95.12 ± 1.67
		24 °C	83.57 ± 3.15
	After 30 days	4 °C	89.28 ± 2.54
		24 °C	70.31 ± 3.42
IC ₅₀ value of DPPH scavenging activity (mg/mL)			3.89

* GAE was gallic acid equivalent.

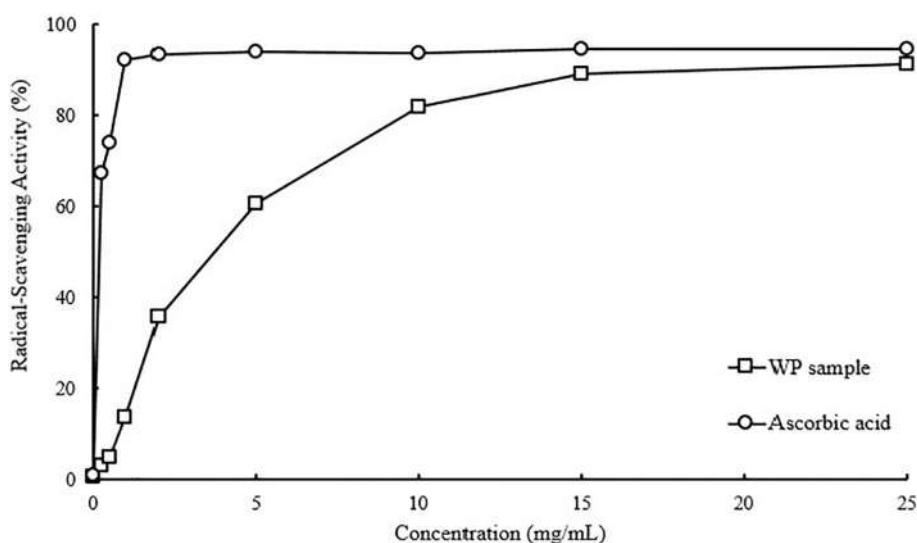


Fig. 5. Comparison of radical-scavenging activity of WP (walnut green husk pectin) and ascorbic acid as a strong antioxidant.

indica cladodes [46] and sour orange peel [10]. Given that the hydroxyl groups can scavenge the DPPH free radical, it can be concluded that this result is probably due to the different content of the hydroxyl group in the various pectin samples [45]. In addition, the chemical composition of isolated pectin could have a significant effect on this parameter, for instance, a higher TPC value and GalA content of isolated pectin probably suggest higher radical-scavenging activity for pectin sample [38].

4. Conclusion

The four variables of the ultrasound-assisted extraction process were optimized by BBD for pectin recovery from waste of walnut processing. The highest extraction yield (12.78%) was achieved at ultrasound power 200 W, sonication time 10 min, pH 1.5 and LS ratio 15 v/w. At these conditions, WP was examined for its physicochemical, structural and functional properties and the results were compared to other commercial and non-commercial pectin samples from diverse resources. The results revealed that WP had a high content of methylated GalA (69.44%) with a DE value of 59.21%, and low molecular weight (93.55 kDa). Also, WP presented good functional properties such as emulsifying properties, water and oil holding and antiradical activity. In addition, the structural properties of WP were studied using FTIR, H-NMR and XRD spectroscopy. As a result, the utilization of walnut processing waste in the production of pectin can reduce the pollution of the environment, as well as provide financial benefits. In addition, WP can be used, as a natural ingredient with numerous technological and health-promoting benefits, in the formulation of various food, cosmetic and pharmaceutical products.

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