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Original Article

Optimization of Pectin Extractions from Walnut Green Husks and Characterization of the Extraction Physicochemical and Rheological Properties

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ABSTRACT

Background and Objectives: Walnut is a nutrient with green husks containing pectin. Extraction of this pectin is valuable due to economic and environmental aspects.

Materials and Methods: Effects of three variables of pH values (1, 1.5 and 2), extraction temperatures (60, 70 and 80°C) and process times (60, 90 and 120 min) were assessed on extraction efficiency rate, esterification degree and galacturonic acid of pectin extracted from walnut wastes using response surface statistical method. Furthermore, total ash, MW, emulsifier, rheological and Fourier transform infrared spectroscopy assessments were carried out on optimum samples.

Results: Based on the results, optimum conditions for pectin extraction from walnut green husks with the highest extraction efficiency rate (25.76%), esterification degree (54.28%) and galacturonic acid (64.49%) were associated to pH 1.75, process temperature of 80 °C and extraction time of 120 min. The most emulsion stability of the walnut waste of pectin was seen at 4 °C and on the first day of storage. Under optimal extraction conditions, MW of the walnut green husks was 38.88 kD. Optimum sample solution of the extracted pectin exhibited viscous and pseudoplastic behaviors.

Conclusions: Fourier transform infrared spectroscopy spectral diagrams of the optimal pectin samples have shown presence of galacturonic acid; thus, walnut wastes can be used as a rich source of pectin.

Keywords: Walnut green husk, Pectin, Esterification degree, Galacturonic acid

Introduction

Iran is well ranked for low price and good quality of its agricultural products. Favorable weather conditions contribute to quality and taste of these products (1). The average annual agricultural wastes are estimated as 35%. To decrease these wastes, methods such as appropriate harvesting, hygienic transportation, disinfection, packaging and optimal use of husks and leaves of the agricultural products can help (2). Walnut wastes include green husks, outer and inner wood husks and thin inner husks. The walnut green husk or pericarp is a byproduct and useful source of phytochemical compounds similar to those of walnut leaves, which increases values of walnut productions (3). The walnut green husk is a good source of fatty acids, citric and malic organic acids, phytosterol sugar emulsions, chlorophylls, joglones, polysaccharides such as pectin, vitamins and various minerals, including potassium (0.95%), nitrogen (0.84%), phosphorus (0.78%), calcium (0.49%) and magnesium (0.10%) (4). Hydrocolloid pectin includes a complex family of polysaccharides present in plant cell walls and is one of the most important biopolymers present in plant species (5, 6). The maximum quantity of pectin is found in premature fruits. During ripening, pectin is degraded by pectin esterase and pectinase enzymes, the middle lamella breaks and cells are separated from each other by decreasing the percentage of pectin and hence fruits gradually soften. Chemical compound, structure and percentage of pectin vary in various plants (7).

Pectin is widely used in food industries as colloidal additive with gel properties, immobilizer, condenser and emulsifier. Traditional use of pectin in marmalades, jams and fruit jellies is common,

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decreasing aquatic activity as well as creating favorable tissues (8). Pectin is extracted from pulp and agricultural product wastes such as peach (9), sugar beet (10), orange peel, sunflower oilseed (11), apple, lemon sour, banana peel (12), citrus peel, husk of blackberry tree branch (13), grapefruit peel (14), *Akebia trifoliata* husk (15), peanut and sugar beet roots (16), cocoa bean husk, grape pulp (17), golden kiwi, orange peel (18), tomato, carrot (19), pistachio green husk (20) and eggplant peel and cap. In recent years, recycling valuable compounds, controlling importing costs of these compounds and decreasing wastes have been great concerns discussed in country waste reduction panels. Use of walnut green husk wastes not only decrease environmental pollutions, but also includes the valuable pectin with special properties in food industries. To the best of the authors' best knowledge, no studies have been carried out on the extraction of pectin from walnut wastes. Therefore, the major aims of the present study were optimization of pectin extractions from walnut green husks and characterization of the extraction physicochemical and rheological properties.

Materials and Methods

Extraction and separation of pectin from walnut green husks

Fresh green walnuts were purchased from a local market in Tehran, Iran, and their green husks were separated and thoroughly washed with water. After drying, walnuts were cut into small pieces and dried in oven at 60 °C for 48 h to reach a constant weight and then flour-shaped granules were formed using mill. Powder was stored in cool and dry conditions (-18 °C) using capped containers. Pectin extraction process was carried out based on a method by Chaharbagh et al. (2017) with modifications. In this method, various extraction conditions such as pH (X_1), temperature (X_2) and process time (X_3) were set up to achieve optimal conditions for pectin extraction. Briefly, 10 g of the powder from the previous step were mixed with 200 ml of desired solvent such as acidified distilled water with citric acid at various pH of 1, 1.5 and 2. Heating process and stirring were carried out using water bath at various temperatures of 60, 70 and 80 °C and extraction times of 60, 90 and 120 min for both parts of the walnut wastes separately (Table 1). Range of the experiments for each agent was selected based on the results from previous studies and the initial experiments from the present study.

Table 1. Various factors affecting extraction of pectin from the walnut green husks

Factor	Unit	Code	Surface		
			-1	0	1
pH		X_1	1	1.5	2
Process temperature	Celsius degree	X_2	60	70	80
Process time	Minute	X_3	60	90	120

After cooling samples at ambient temperature, the pulp was separated from the solution accurately using mesh and centrifuge (4000 rpm, 20 min). Then, supernatant liquid was passed through filter papers and stored at refrigerator temperature (4 °C). To extract pectin and precipitate it in the isolated liquid phase, ethanol (96%) was added at a ratio of 1:2 until extracted pectin formed aggregation. Samples were stored at refrigerator temperature for 10 h to completely precipitate the pectin. To increase purity of the residual precipitate, several times washing with ethanol and methanol, centrifuging and filtering through filter papers were carried out. Then, oven was used to dry pectin samples at 55 °C for 24 h. After weighing, samples were powdered using shredder and laboratory mesh No. 60 and then stored in closed containers in cool and dry conditions.

Extraction efficiency rate

Efficiency rate of the pectin extraction from various treatments was assessed using Equation 1 (13).

Equation 1.

Extracted pectin efficiency rate (%) = pure pectin weight (g) / initial dry powder weight (g) × 100

Esterification degrees

In the present study, esterification degrees were assessed using potentiometric titration method. In this method, 0.1 g of dry pectin was first mixed with 3 ml of 96% ethanol. Then, 20 ml of deionized distilled water (D.W.) were added to the mixture at 40 °C and stirred using heater equipped with a magnetic stirrer until the pectin was completely dissolved. Three drops of phenolphthalein were added to the mixture and titrated with 0.1 M sodium hydroxide until a pale-pink appeared (initial volume). Then, 10 mL of 0.1 M sodium hydroxide were added to the neutralized solution and stirred for 30 min using magnetic stirrer until pectin was saponified. Then, 10 ml of 0.1 M hydrochloric acid were added to the mixture and stirred until the pink color was completely eliminated,

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the residual hydrochloric acid was titrated with 0.1 M sodium hydroxide until a pink color appeared (secondary volume). At the end of the process, the esterification degree was calculated based on the Equation 2 (21).

Equation 2.

Esterification degree (%) = secondary volume / secondary volume + initial volume × 100

Galacturonic acid content

The galactoronic acid content was assessed based on a method by Nateghi et al. (2017). Briefly, colorimetric method was carried out using meta-hydroxydiphenyl reagent. Furthermore, solutions were prepared and galacturonic acid calibration curve was plotted.

Quantity of ash

Total ash of the samples was assessed based on the AOAC standard methods using furnace system with standard No. 942/05. (23).

Molecular weight (MW) calculation

To assess parameters needed to calculate intrinsic viscosity of the pectin solutions, capillary glass viscometer at constant temperature and Mark Houwinke equation were used. The intrinsic viscosity was calculated by drawing diagrams of reduced viscosity numbers (vertical axis) versus concentrations (horizontal axis) and extrapolating them to zero concentration (Weska et al., 2007). Then using Equations 3–6, MW of the optimal samples was calculated.

Equation 3. $\rho / \eta = \nu$

Equation 4. $\eta_{Sp} = (\eta_{solution} - \eta_{solvent}) / \eta_{solvent}$

Equation 5. $\eta_{Sp} / C = [\eta] + K [\eta]^2 \times C$

Equation 6. $[\eta] = KM^{\alpha}_{w}$

Where, ν was cinematic viscosity (stoke), η was dynamic viscosity (poise), ρ was soluble density (g/ml), η_{sp} was specific viscosity, η_{sp} / C was reduced viscosity (ml g^{-1}), C was soluble concentration (g ml^{-1}), K was correction coefficient (constant number for each polymer, g ml^{-1}), [η] was intrinsic viscosity (ml g^{-1}), M_w was MW and α was correction coefficient (constant number for each polymer solution system).

Rheological properties

Of the extracted pectin samples at the optimum point, a solution with 1% concentration was prepared and stirred for approximately 9 h using magnetic stirrer. Dynamic rheology test was carried out at a constant temperature of 25 °C using rotary rheometer (Physica MCR 300, Germany). In this method, the strain scanning test (0.01–100% at 1 Hz) was carried out first to calculate the linear viscoelastic range. The storage modulus (G') and dissipation (G'') were assessed using oscillation test with low oscillation amplitude at frequencies of 0.1–100 Hz at 1% strain (25).

Emulsifier property

To assess the emulsifier activity and emulsion stability, Nateghi et al. (2017) method was used with modifications. In this method, the emulsion was prepared by adding 5 ml of sunflower oil to 5 ml of the pectin solution (5% v/w) and 0.02% sodium azide was used to inhibit bacterial growth. Selected sample was mixed at 10,000 rpm for 4 min using homogenizer. The new emulsion prepared at 4000 rpm min^{-1} was centrifuged at room temperature for 5 min. At the end of the process, the emulsifier activity was calculated using Equation 7.

Equation 7.

Emulsifier activity (%) = volume of the emulsified layer / total volume of the sample × 100

To investigate stability properties of the emulsion, prepared sample was stored at 23 and 4 °C for 1 and 30 d, then the property was calculated based on the Equation 8.

Equation 8.

Emulsion stability (%) = volume of the remaining emulsion layer / volume of the initial emulsion × 100

Fourier transform infrared spectroscopy (FTIR) spectroscopy property

To prepare solid samples for use in FTIR spectrometer (Vector 22), dry and fully powdered sample was compressed with potassium bromide in a glass tube and then inserted into the spectrometer and a desired spectrum in the range (400–4000 cm^{-1}) was reported (21).

Statistical method

First, data were tested for normality and if were normal, analysis of variance was used. Pectin samples extracted under various extraction conditions using response surface methodology in form of box benkan design, 15 treatments were assigned to the initial material (Equation 9). Each treatment was carried out in two replications (30 samples). In the next step,

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sequence of dependent variables (total ash, MW, emulsifier and rheological) to independent variable changes of the pectin extraction from walnut green husks was investigated using Equation 9.

Equation 9.

$$Y=\beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i=1}^{3}\sum_{j<i}\beta_{ij}X_iX_j$$

In this study, Minitab 16, Design Expert 7 and MS Excel were used for the statistical analysis.

Results

Optimization of the pectin extraction efficiency rate

Schematic matrix and experimental and predicted results of pectin extraction efficiency rate for each trial unit are presented in Table 2. These results showed that the pectin extraction efficiency rate in walnut green husks ranged 13.01–26.02%.

Table results of variance efficiency analysis, esterification degree and galacturonic acid proportion showed that lack-of-fit (non-processing test) at 0.05 was not significant and laboratory data were in good conditions based on the model. Furthermore, calculation of r-sq (adj) R^2 and R^2 indicated that the model was in a satisfactory condition. In Table 3, *p* values showed importance of the equation coefficients, which showed that linear and quadratic effects of all independent variables were significant (Table 3). After converting results of the t-test table to equation form, all sentences with no statistical significance were removed and equations were transformed to 10, 11 and 12, respectively.

Equation 10. $Y_1 = 0.16 + 3.73\ X_2 + 2.53\ X_3 - X_1^2 - 0.65\ X_2^2 + 0.52\ X_1X_3 + 1.20\ X_2X_3$

Equation 11. $Y_2 = 58.69 + 1.80\ X_1 + 2.46\ X_1^2 - 2.80\ X_2^2 - 3.02\ X_3^2 + 1.11\ X_1X_2 - 1.21X_2X_3$

Equation 12. $Y_3 = 68.32 + 1.15\ X_2 - 3.01\ X_1^2 - 1.94\ X_2^2 - 1.12\ X_1X_2$

Where, Y_1, Y_2 and Y_3 were the efficiency rate, esterification degree and pectin galacturonic acid proportion of the green husks, respectively. Moreover, Independent Variables X_1, X_2 and X_3 were responses of the coded levels.

Effects of various levels of variables on the quantity of walnut husk wastes can be shown using Equation 10 (Table 3) (Figures 1a, b). Figure 1a shows effects of increasing pH and process time on extracted pectin efficiency rate at constant temperature and central point (X_2, 70 °C). By increasing pH, quantity of the extracted pectin increased to a certain point. Then, process was reversed and declined. Based on the model equation, it can be concluded that the pectin efficiency rate increased and then decreased when pH increased from 1 to 2.

Table 2. Test design based on the models using results of optimal extraction conditions on efficiency rate, esterification degree percentage and galacturonic acid of the pectin from walnut green husks (mean ±standard error)

Galacturonic acid proportion (%)		Esterification degree (%)		Efficiency rate (%)		Walnut green husk	Code		
(Predicted)	(Real)	(Predicted)	(Real)	(Predicted)	(Real)	Treatment	X_1	X_2	X_3
61.36	61.75±0.02	57.246	56.74±1.05	13.13	13.12±0.02	1	-1	-1	-1
63.02	62.31±0.07	58.639	58.50±2.01	13.85	14.03±0.09	2	1	-1	1
65.92	66.64±0.10	55.861	56.00±1.07	21.01	20.84±0.02	3	-1	1	-1
63.09	62.71±0.03	61.694	62.20±0.70	20.92	20.94±0.10	4	1	1	1
64.39	64.12±0.05	55.505	55.92±3.02	15.79	16.05±0.05	5	-1	0	-1
63.26	64.09±0.03	60.153	60.20±1.02	15.05	15.12±0.01	6	1	0	1
64.75	63.93±0.02	57.168	57.12±0.07	19.80	19.74±0.06	7	-1	0	-1
64.70	64.98±0.01	59.745	59.33±1.08	21.17	20.92±0.02	8	1	0	1
63.11	63.00±0.05	50.929	51.02±0.70	13.24	13.01±0.01	9	0	-1	0
66.68	66.24±0.01	54.194	53.64±1.05	18.30	18.22±0.08	10	0	1	0
65.26	65.71±0.03	53.986	54.54±1.62	15.89	15.98±0.06	11	0	-1	0
66.33	66.45±0.07	52.391	52.30±0.09	25.78	26.02±0.07	12	0	1	0
68.32	68.23±0.05	58.693	58.88±0.60	18.89	18.64±0.01	13	0	0	0
68.32	68.01±0.04	58.693	58.65±0.90	18.89	19.00±0.05	14	0	0	0
68.32	68.72±0.03	58.693	58.55±1.00	18.89	19.05±0.04	15	0	0	0

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Table 3. Analysis of variance from the quadratic model for efficiency rate, esterification degree and percentage of the galacturonic acid pectin

Galacturonic acid proportion (%)			Esterification degree (%)			Efficiency rate (%)			
P number	F number	Mean squared	P number	F number	Mean squared	P number	F number	Mean squared	Walnut green husk waste
<0.009[a]	10.50	7.35	< 0.000[a]	45.27	14.3709	< 0.000[a]	229.14	19.48	
0.367	0.99	0.69	0	82.22	26.1003	0.187	2.33	0.19	X_1
0.011	15.33	10.74	0.09	4.39	1.3945	0.000	1312.60	111.60	X_2
0.187	2.34	1.63	0.176	2.48	0.7875	0.000	603.46	51.30	X_3
0.001	48.02	33.64	0	70.79	22.4732	0.001	44.02	3.75	X_1^2
0.007	20.01	14.02	0	91.22	28.9563	0.007	18.75	1.59	X_2^2
0.066	5.50	3.85	0	105.93	33.6289	0.673	0.20	0.01	X_3^2
0.044	7.19	5.04	0.011	15.53	4.9284	0.224	1.93	0.16	$X_1 X_2$
0.547	0.42	0.29	0.126	3.37	1.0712	0.015	13.09	1.11	$X_1 X_3$
0.196	2.23	1.35	0.008	18.6	5.9049	0.000	68.60	5.83	$X_2 X_3$
		0.70			0.3174			0.08	Error remaining
		0.13			0.0286			0.05	Pure error
0.111[b]	8.17	1.07	0.054[b]	17.81	0.51	0.331[b]	8.17	0.10	R-sq (adj)
Justified R^2	Predicted R^2	R^2	Justified R^2	Predicted R^2	R^2	Justified R^2	Predicted R^2	R^2	
85.93%	84.8%	94.97%	96.61%	81.2%	98.79%	85.93%	84.80%	94.97%	

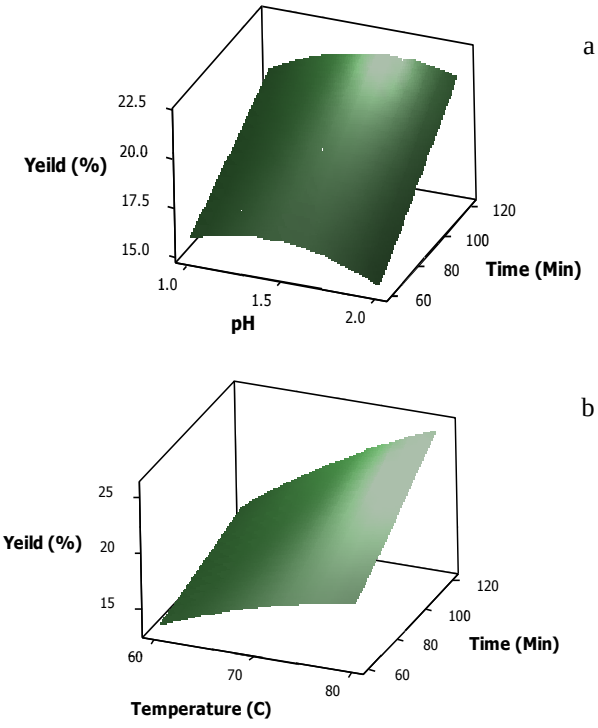

Figure 1. Three-dimensional diagrams of the response surface based on the independent factor changes in walnut husk pectin efficiency rate

The optimum values of each extraction variable were calculated using the equation as the highest efficiency rate of walnut green husks was 25.84% at pH 1.62, 80 °C and 120 min.

Optimization of pectin esterification degree

This factor represents the number of moles of methanol per 100 moles of galacturonic acid. The pectin esterification degree is one of the key factors in describing properties of the pectin gels. Solubility of pectin in water and its gelation strength depend on the pectin esterification degree (21). Based on Table 2, esterification proportion of the samples from walnut green husks ranged 52.30–60.20%. Effects of various levels of variables on pectin esterification degree can be described using Equation 11 (Table 3) (Figure 2a, b). Figure 2a shows effects of increasing temperature and time on esterification degree of the extracted pectin under conditions; in which, pH was constant at the central point (X_1, 1.5). By increasing these two target factors, esterification degree of the extracted pectin increased to a certain point and then decreased. Figure 2b shows effects of the temperature and pH changes on pectin esterification degrees with time constant at the central point (X_3, 90 min). This shows that pH increased with increased esterification degrees, but this increase was seen with increases in temperature. Based on the model equation, pectin esterification degree increased when pH increased from 1 to 2. However, increased process temperature and time initially increased the esterification degree and then decreased it. Rate of the pectin extraction increased with increased temperature and time. However, the lowest pectin esterification degree was

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achieved at the optimum points of the extraction temperature and time. The optimum values of each extraction variable were achieved using equation; where, the highest pectin esterification degree was achieved from walnut green husks (63.19%) linked to pH 2, process temperature of 72.92 °C and extraction time of 87.27 min.

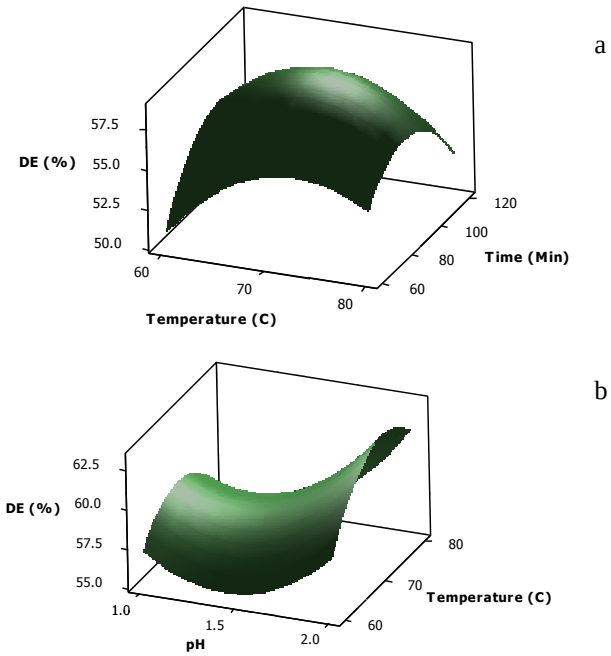

Figure 2. Three-dimensional diagrams of the response surface corresponding to changes of the independent factors in degree of walnut husk pectin esterification

Optimization of galacturonic acid proportion

Since galacturonic acid is a major constituent of pectin. Quantitation of galacturonic acid demonstrates purity of the extracted pectin, which is important for biological studies and pharmaceutical and nutritional applications. Concentration of galacturonic acid varies in various plants (19). Effects of various variables on galacturonic acid pectin can be demonstrated using Equation 12 (Table 3, Figure 3). Figure 3 shows effects of increasing process temperature and pH on proportion of the extracted galacturonic acid pectin under conditions; where, the time was constant at the central point (X_1, 90 min). By increasing these two target factors, proportion of the extracted galacturonic acid pectin increased to a certain point and then decreased. Esterification rate of the samples achieved from walnut green husks ranged 61.75–68.72 (Table 2). Proportion of the galacturonic acid pectin increased when pH increased from 1 to 1.44. However, the factor decreased with increased pH. Furthermore, increased temperature increased and then decreased the galacturonic acid proportion. The optimum values for the highest proportion of galacturonic acid pectin from walnut green husks (68.53%) included pH 1.44, process temperature of 72.92 °C and extraction time of 93.33 min.

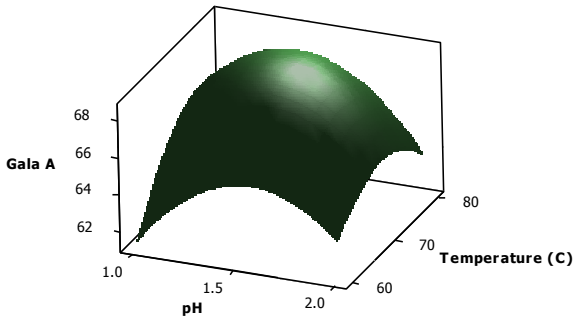

Figure 3. Three-dimensional response surface diagram of the independent factor changes in galacturonic acid proportion of the walnut husk pectin

Optimization conditions of the pectin from walnut green husks

Figure 4 shows the optimum diagrams of the efficiency rate, esterification degree and galacturonic proportion of pectin extracted from walnut green husks. It can be seen that the optimum conditions for pectin extraction from walnut green husks with the highest efficiency rate (25.76), esterification degree (54.28) and galacturonic acid proportion (64.94) linked to pH 1.75, process temperature of 80 °C and extraction time of 120 min.

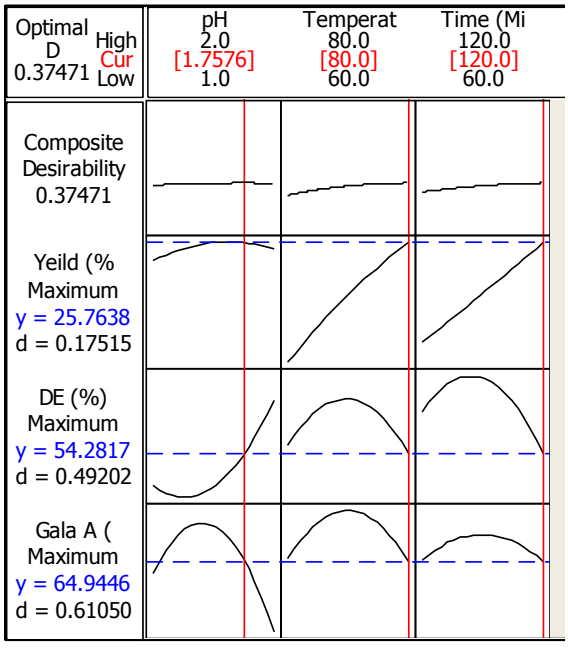

Figure 4. Optimization diagram of the pectin extraction conditions based on the efficiency rate, esterification degree and galacturonic percentage of the pectin samples extracted from walnut green husks

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Quantity of the ash

Ash is one of the most important factors in gel strength. Studies have shown that high ash contents result in formation of the junction and gel network; therefore, pectin with less ash contents is able to form further gels (26). Effects of ash are usually found in low-ester pectins because further carboxyl groups are available to react with the ash. In the present study, this test was carried out since the extracted pectin included low ester contents. Results showed that ash contents of the pectin powder included 1.05%.

Molecular weight calculation

The intrinsic viscosity value of a polymer is a measure of the hydrodynamic volume of polymer molecules and shows the molecular capacity of polymers to increased viscosity. Intrinsic viscosity is affected by MW, esterification degree, temperature and solution concentration and pH. The average MW of pectin is a key parameter in gel formation by pectin (27). In this study, intrinsic viscosity of the extracted pectin from walnut husks included 1.38 (mg g^{-1}) at optimum conditions, with K and α values of 9.55×10^{-2} and 0.73 from previous studies, including raji et al. (2017) study. The MW of pectin was calculated using Equation 13.

Equation 13. $\eta = 9.55 \times 10^{-2} (MW)^{0.73}$

In this study, MW of the extracted pectin samples at optimum conditions included 38.88 kDa.

Rheological properties

Gels are viscoelastic materials and hence dynamic rheological tests are useful for the assessment of gel properties and its gelation process.

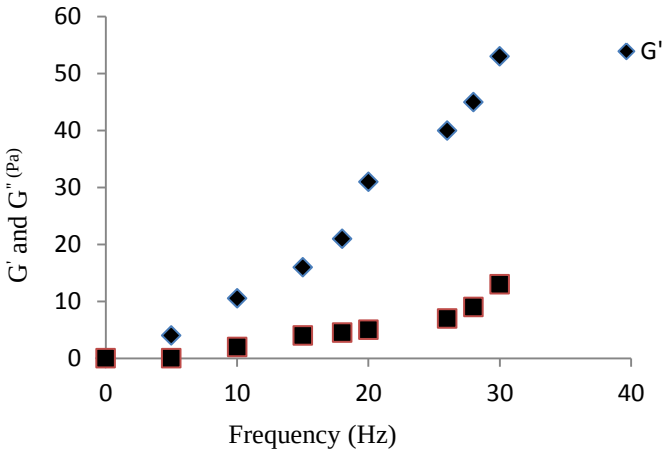

Figure 5. Variation diagram of the elastic (G') and viscose (G") coefficients for the optimal samples of walnut husk pectin at 1% concentrations

Figure 5 shows variation diagram of G' and G" modulus for the optimal pectin sample at 1% concentrations. The near-gel optimum behavioral pectin sample showed that the elastic coefficient (G') was larger than the reserve (G") coefficient at all frequency amplitudes, indicating a pseudo-solid behavior similar to that of elastic network (28).

Emulsifier properties

Pectin increases the stability of emulsions by increasing viscosity of the aqueous phase as well as absorption at interface of the phases and formation of coats around dispersed particles, depending on electrostatic repulsions to stabilize the emulsion. Many factors affect the emulsion activity of pectin, low MW is one of the most important factors, which is itself influenced by the extraction conditions such as extraction temperature and time (16). Emulsifier activity and emulsion stability of the emulsion prepared from the optimum sample of extracted pectin-oil solution (0.5% w/w) are shown in Table 4.

Table 4. Emulsifier activity and stability of oil emulsion / 0.5% volumetric solution percentage / weight solution of optimum walnut green husk pectin sample

	Emulsifier activity percentage	Emulsion stability percentage			
Temperature (celsius degree)	23	4		23	
Shelf life time (day)		1	30	1	30
Optimum sample of walnut green husk pectin	52.3	84.2	83.1	81.5	80.4

The emulsion stability rate did not change one day after sample storage at cold and ambient temperatures and after 30 days of storage.

Fourier transform infrared spectroscopy properties

Fourier transform infrared spectroscopy has been suggested as a useful technique for the assessment of pectin uric acids. In this study, infrared spectra were used to identify the main pectin functional groups extracted from the optimal sample. The infrared spectrum absorbed by the sample is shown in Figure 6. Table 5 shows the most important peaks introduced for the functional groups (wavelengths and intensities of the functional groups) of the commercial pectin (19) and the optimal pectin sample extracted from walnut husks.

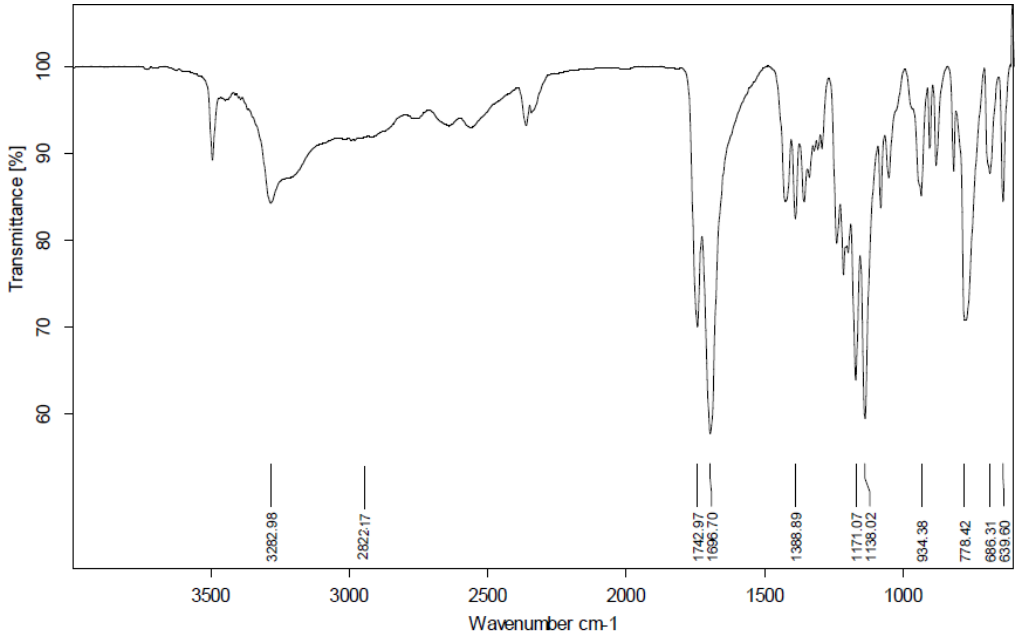

Figure 6. FTIR absorption spectrum of the optimal pectin sample isolated from walnut green husk

Table 5. FTIR absorption spectrum, wavelengths and intensities of commercial pectin functional groups and the optimal sample desired

Power	Functional groups	(cm^{-1})	
		Optimum sample of walnut green husk pectin	Commercial pectin
Broad, strong	Tensile O- H	3282	3389
Sharp, occasionally overlap with O - H	Tensile, symmetrical and asymmetrical C- H	2822	2940
Strong	Esterified C=O	1742	1753
Strong and weak	Asymmetrical tensile C=O	1388 and 1695	1441 and 1630
Weak	Finger print	1200- 1000	1200- 1000

Based on the Five-Peaks Table, 1 cm range for the optimal pectin sample was linked to the adsorption of

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tensile hydroxyl groups caused by intermolecular hydrogen bonds and various carbon groups (CH_1, CH_2, CH_3) at wavelengths of 2822–3282. Region between the bands 1640–1800 is a special region because it provides structural information and can be used to compare various types of pectins in the optimal pectin sample. Target range of this peak region at 1742 and 1692 cm^{-1} wavelengths have shown that these adsorptions can be linked to the vibration of tensile carboxyl groups (C=O) methyl ester and non-esterified carboxyl groups (29). Absorption peaks at the wavelengths of 1388 and 1695 in the optimal pectin sample was linked to the free carboxyl groups (19). Absorption peaks at wavelengths of 1000–1200 cm^{-1} in both samples can be attributed to the presence of various sugar compounds such as furanose and pyranose in pectin structure.

Discussion

In general, pectin accumulation may occur with increasing pH from 1.5 to 2, which slows its release from the plant cell wall, resulting in reduced pectin efficiency rate (Figure 1a). Low pH decreases MW of the pectin attached to the cell wall of the plant and force it to dissolve and separate from the plant matrix without molecular chain degradation. This results in deposit of the pectin molecules. Furthermore, high acidic strength hydrolyzes insoluble pectin and converts it into soluble pectin that increases the precipitation of pectin, enhancing pectin recovery (19). Although high acidic strength enhances pectin efficiency, but too low pH can result in formation of very small pectin particles. This changes pectin solubility to a point that cannot be precipitated by the addition of alcohol. Increases in process time and temperature affected the extracted pectin efficiency (Figure 1b). As the extraction time increased, the extraction efficiency rate increased. This was seen because by increasing the extraction time, the insoluble pectin density became solubilized and extraction efficiency rate increased. Researchers have reported temperature as one of the most important factors in extraction of polysaccharides such as pectin (18). As temperature increases, efficiency of the pectin extraction increases; hence, the most efficient pectin extraction was achieved at 80 °C in the current study. High temperature increased the solubility of the extracted pectin. As a result, pectin extraction rate, diffusion coefficient and extraction rate increased. Increased temperature also increased solubility of insoluble polysaccharides in alcohol and increased the efficiency rate. Use of temperatures above 95 °C in pectin extraction processes is not recommended due to increased solubility and mass transfer (18). It can be concluded that the rate of pectin extraction increased with simultaneous increases in temperature and time. As the model equation has shown, the lowest pectin extraction efficiency was achieved at the lowest extraction temperature and time, similar to that reported by other studies (22). As pH increased, this factor increased; therefore, the optimal conditions for pectin extraction efficiency and its esterification degree are different; possibly due to the breakdown of pectin ester bonds in acidic conditions (Figure 2). It is suggested that higher esterification degrees demonstrate less damaged pectins because ester bonds are less resistant to acid hydrolysis than galacturonic acids between alpha 1 and alpha 4 glycosidic bonds. This finding is similar to findings by other researchers, including Nateghi and Ansari (2018). They reported that with increasing pH, efficiency rate and esterification degree respectively decreased and increased (30). Based on the model from the pectin esterification degrees of both compounds, temperature included the second power in the equation and hence the effective factor was identified. The most important result includes the esterification degree of pectin type, which is industrially used for the preparation of ordinary jams of pectin with a esterification degree above 70% (rapid) or less than 68% (slow) in highly acidic fruit jams and jellies. In the present study, pectin included low or slow esterification types used to remove heavy metals from the body and decrease activity of the cancer cells (31). Results of pectins from orange peel, carrot pulp, walnut green husk, melon peel, sunflower and eggplant cap in slow pectin group are similar to the results of those from this study and other samples, including pumpkin pectin and eggplant peel in rapid pectin group.

Decreases in galacturonic acid proportion possibly occur because basic hydrolysis of the pectin neutral sugars occurs at pH above 2 and high temperatures (Figure 3). Previous studies have reported pH as the most important factor in galacturonic acid proportion, similar to reports from the present study (18). Suggestions have been made about the effects of pH on pectin purity. One suggestion describes that at pH

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below 1.5, pectin is extracted because non-pectic compounds dissolve the cell wall and precipitate with alcohol. At the lowest pH, extracted pectin is decomposed into low molecular weight (LMW) compounds and does not precipitate with ethanol (22). However time did not affect the quantity of pectin, the two other factors, temperature and pH, and their interactions included greater effects on this factor. Comparison of results from the esterification degree and galacturonic acid proportion showed that the esterification degree was further sensitive to extraction conditions and the rate of variation was higher, similar to results reported by Fathi et al. (2012), These possibly occurred due to the higher resistance of glycosidic bonds of galacturonic acid to acidic hydrolysis, compared to that of ester bonds. According to the Food and Agriculture Organization of the United Nations (FAO) and the European Union (EU), galacturonic acid proportion in pectin demonstrates pectin purity which must be at least 65% (18). In the current study, galacturonic acid proportion was reported in a similar range. Results of the ash assessment showed that the ash content of the pectin powder was 1.05%, which is similar to those from previous studies. In the present study, ash content of the sunflower pectin sample was reported as 1.5% (26). However, results of the ash content assessment in this study were less than the maximum allowed content and the extracted compounds were introduced as edible pectin. The MW of the extracted pectin sample at optimum conditions was 38.88 kDa. The MWs of the pectin extracted from pistachio husks and melon peels were 12.87 and 67.6 kDa, respectively (20, 27). The MW of various sources can be strongly affected by the types of plants and extraction conditions during the process. Low intrinsic viscosity of the pectin could be due to the presence of strongly bonded hemoglacturonic regions, whereas other types of high intrinsic viscosity pectins seem to include regions of helical structures (7).

Dynamic rheological tests in the linear viscoelastic range can include (elastic) storage coefficient G', modulus of dissipation (viscose) (G") and dissipation factor tanδ (G' / G'). Value of the storage coefficient is an indicator of the energy stored in the sample during the shear process and describes the elastic behavior of the sample. The dissipation coefficient is an indicator of the energy consumed in the sample during shearing and the energy wasted in the sample, explaining viscous behavior of the sample (28). If the elastic coefficient (G') is greater than the viscosity coefficient (G"), compound behaves like a solid which means that deformation of the material is essentially elastic or recyclable and if the viscosity coefficient (G") is greater than the elastic coefficient (G'). The energy used to change the material form is wasted and the material will behave like a liquid (Figure 5). Moreover, results showed that the elastic coefficient (G') and storage coefficient (G") increased with increasing frequency, similar to pectin rheology results of the pistachio husks (25). In Table 4, emulsifier activity of the pectin sample extracted from walnut green husks at 0.5% concentration and assessed immediately after the test at 23 °C was 52.3%. This was extracted from the pectin emulsifier activity of sugar beet pulps by citric acid, which was 32.2% higher and lower than the pectin emulsifier activity of carrot pulps (19). Emulsion stability decreased a little and results showed that the low storage temperature included further emulsifying properties, similar to results from other studies (18, 22). Citrus pectin emulsifier activity at 0.5% concentration was reported as 49.6% (32). Pectin from walnut green husks could exhibit a similar activity to citrus pectin under similar conditions. The emulsifier activity and relatively good stability of the resulting pectin can be attributed to the presence of polyphenols (e.g. caffeic acid), as the predominant phenolic acids, as well as hydroxycinnamic chlorogenic acid, as the major acid in tissues. The FTIR results demonstrated appropriate purity of the extracted pectin, as previously demonstrated by other studies (Figure 6) (22, 27, 33).

Conclusion

The present study has shown that the highest efficiency rate of walnut green husks (25.84%) occurs at optimal conditions of pH 1.62, process temperature of 80 °C and extraction time of 120 min. Furthermore, the highest esterification degree of pectin from walnut green husks (63.19%) occurs at optimal conditions of pH 2, process temperature of 72.92 °C and extraction time of 87.27 min. Esterification degree of the walnut green husk samples includes 52.30–60.20. As galacturonic acid proportion shows purity of pectin, galacturonic acid proportion in these samples was within the standard range. The optimum conditions for achieving the highest proportion of the pectin

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galacturonic acid from walnut green husks (68.53%) include pH 1.44, process temperature of 72.92 °C and extraction time of 93.33 min. The optimum extracted samples include good emulsifier and viscosity properties. In conclusion, walnut green husks are good sources for pectin extraction.

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