JFBE 1(1): 57-66, 2018

Journal of Food and Bioprocess Engineering

Journal homepage: https://jfabe.ut.ac.ir

Original research

Optimization of microwave assisted extraction (MAE) of pectin from black mulberry (*Morus nigra* L.) pomace

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ABSTRACT

Microwave assisted extraction (MAE) tech-nique was employed for the extraction of pectin from black mulberry (*Morus nigra* L.) pomace in this research. The effects of process variables namely microwave power (300-900 watt), irradiation time (10-30 min) and liquid to solid ratio (15-30 mL/g) on the yield, degree of esterification (DE) and galacturonic acid content (GalA) of pectin were investigated and optimized by using response surface methodology (RSM) with a face canter central composite design. Depending on the extraction conditions, the yield, DE and GalA of pectins varied in the range of 8.87-14.47% (dry weight basis), 44.44-57.06%, and 29.17-43.13%, respectively. The results showed that both the microwave power and irradiation time had highly significant effects on the pectin yield, while the effect of liquid to solid ratio was found to be not significant, in this regard. The optimal extraction conditions for maximum yield, DE and GalA of pectin were power of 900 watt, irradiation time of 18.17 min and liquid to solid ratio of 15 mL/g. Under these conditions, 13.16% of pectin with DE of 55.07% and GalA of 36.94% was extracted. The intrinsic viscosity and viscosity average molecular weight of a representative pectin sample was also determined and it was found to be 1.22 dL/g and 32.78 kDa, respectively. The black mulberry pomace pectin showed a viscoelastic behavior in the frequency sweep test and the crossover between G' and G'' occurred at low frequency region (~ 0.07 rad/s).

Keywords: Black mulberry pomace, Pectin, Microwave assisted extraction, Yield, RSM

Received 27 May 2017; Received 8 August 2017; Accepted 27 August 2017

1. Introduction

Pectin is a structural heteropolysaccharide contained in the primary cell walls and middle lamella of terrestrial plants. It consists of a backbone of α-D-(1→4)-galacturonic acid residues which are partially esterified with methyl alcohol at the carboxylic acid groups (Wang et al., 2007). Some neutral sugar side chains have been attached to this linear backbone (Liu et al., 2010). Pectin is widely used in the food industry as a thickener, emulsifier, texturizer and stabilizer and also it is added in jams and jellies as a gelling agent (Thirugnanasambandham et al., 2014). Although most plant tissues contain pectin, the citrus peels and apple pomace are the main source materials for commercial pectin production (May, 1990).

In fruit juice production from berry fruits, press resi-dues (cakes) are formed in large quantities, which contain several valuable nutrients, ingredients and compounds. These residues are suitable for pectin recovery, though their pectin content is somewhat lower than other typically pectin-rich fruits (Belafi-Bako et al., 2011). The black mulberry (*Morus nigra*), which originates from Iran, is cultivated for its fruits in Southern Europe and southwest Asia, and is the most important species in the Mediterranean countries (Lin & Tang, 2007; Ercisli & Orhan, 2008). The black mulberry fruits can be consumed either as fresh or processed (Kamiloglu et al., 2013). The black mulberry has a brief harvest season, after which fresh fruits can only be stored refrigerated for a maximum of six weeks. Thus, further processing is desirable to allow storage (Hojjatpanah et al., 2011). Black mulberry fruit can be processed into many forms such as syrup, jam, pulp, ice cream, vinegar, concentrate, alcohol and several other products (Kamiloglu et al., 2013).

Various extraction techniques have been investigated and used for the extraction of pectin from different kinds of plant materials in recent years. Commercial pectin is usually extracted using hot water acidified with a mineral acid (the so-called conventional acid extraction), under the PH, temperatures, and durations conditions generally in the range of 1.3-3.0, 60-100°C, and 0.5-6.0 hour, respectively (Koubala et al., 2008). This process is time-consuming and leads to pectin degradation, so the conventional method has both quantitative and qualitative disadvantages for pectin extraction (Liu et al., 2006). Therefore, in order to achieve pectin with the

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desired characteristics, alternative methods of extraction are required to be explored or the existing ones are modified (Guo et al., 2012). MAE is an interesting alternative to conventional extraction methods and it has many advantages such as shorter extraction time (a few minutes or seconds), less solvent demand, higher extraction rate, better products with lower cost (Prakash Maran et al., 2013).

Microwave-assisted extraction (MAE) technique has been recently used by several researchers for the ex-traction of pectin from different plant source materials, in which the overall outcome was a considerable increase in the yield and quality of extracted pectin. For instance, extraction of pectin from press residues of fresh berry fruits (red and black currant, raspberry and elderberry) was accomplished by Belafi-Bako et al. (2011), who found that MAE resulted in pectin with higher yield and better rheological properties compared with conventional extraction method. Response surface methodology (RSM) was also used to study and optimize the effects of extraction parameters of MAE on pectin yield (Wang et al., 2007; Li et al., 2012; Lv et al., 2013; Prakash Maran et al., 2013; Seixas et al., 2014; Thirugnanasambandham et al., 2014). To the best of our knowledge, MAE has not been used for the extraction of pectin from black mulberry pomace yet. Thus, the main objective of this research was to study and optimize the effects of MAE process variables (namely microwave power, time of irradiation, and liquid to solid ratio) on the yield, DE and GalA of pectin from black mulberry pomace. Besides this, the intrinsic viscosity, average molecular weight and rheological behavior and of pectin were also determined.

2. Material and Methods

2.1. Raw materials and reagents

Fresh and thoroughly ripe black mulberry (*M.nigra*) fruits were purchased from a local fruit market in Karaj, Iran. The fruits were immediately pressed in a domestic squeezer to be separated into juice and pomace. The resulting pomace was passed through a double layer filter cloth to more juice be removed as possible as. The wet pomace obtained in this way, was dried in a vacuum oven (Townson & Mercer Ltd, England) at 50 °C until its moisture content was reduced to 6.4% (measured according to the method of Imran et al. (2010). The dried pomace was then ground by using a laboratory grinder and the resulting powder was packed into polyethylene containers and stored at refrigerator (4°C) until pectin extraction.

The m-hydroxydiphenyl reagent and standard D-galacturonic acid monohydrate used in this study were purchased from Sigma-Aldrich Company (Sigma-Aldrich, St. Louis, USA). All the other solvents and chemicals were of analytical grade and purchased from Merck Chemicals Co. (Darmstat, Germany).

2.2. Pectin extraction

A household microwave oven (Samsung, Model CE-280, Malaysia) to which two condensers were attached (for recirculating of evaporated solvent), was used for the extraction of pectin. The power of microwave oven was selectable from four values of 300, 450, 600 and 900 watt and the irradiation time was arbitrary. About 20 gram of black mulberry pomace powder was transferred into a glass flask and different volumes of distilled water (200, 400 and 600 mL) was added. The pH of mixture was adjusted to constant value of 2.0 with 1.0 M HCl solution in all the extractions. The flask was placed in the middle of the oven and was exposed to microwave irradiation at different powers and durations as specified in the experimental design (Table 1). When the extraction completed, the contents of flask was initially cooled to room temperature and then centrifuged at 4000 rpm for 10 min. The resulting supernatant was filtered one more time using Buchner funnel (Whatman filter paper no.1) and then evaporated under the vacuum at 50°C up to one-fifth of its initial volume. The pH of the concentrated extract was adjusted to 3.5 by adding 2 M NaOH solution. Next, ethanol 96% in the volume ratio of 1.5:1 (1.5 part ethanol 96% and 1 part pectin extract) was added to this clear extract and stirred for 10 min. The mixture was allowed to stand at room temperature for about 8 hour in order to pectin completely precipitated. The precipitated pectin was separated by vacuum filtration on a filter paper (Whatman no.1), washed twice with ethanol 96% (totally 250 mL), and dried in a vacuum oven at 45°C until its weight remained constant. Afterwards, it was ground, sieved (mesh no. 80) and finally the powdered pectin specific viscosity (η_{rel}) were calculated. The intrinsic viscosity ([η]) of pectin was estimated was stored at refrigerator (4°C) until further experiments.

The extraction yield of pectin (PY) was calculated as follows:

$$\text{PY}\ (\%\text{w/w}) = \frac{W_P}{W_{BMPP}} \times 100 \qquad (1)$$

Where W_P is the mass of dried extracted pectin and W_{BMPP} is the dry mass of black mulberry pomace powder used as raw material.

2.3. Analytical methods

2.3.1. Determination of degree of esterification (DE) and galacturonic acid (GalA) content

The degree of esterification of pectins was determined by potentiometric titration, as described by Bochek et al. (2001). The galacturonic acid content was measured colorimetrically at 520 nm by the m-hydroxydiphenyl method adapted from Ibarz et al. (2006) with a Cecil UV-Vis spectrophotometer (model CE 2502, England). A standard curve of absorbance versus concentration was prepared by fitting the absorbance values of D-galacturonic acid monohydrate solutions (20-100µg/mL) at 520 nm. All analysis was performed in duplicate.

2.3.2. Intrinsic viscosity ([η]) and viscosity average molecular weight (Mv)

The intrinsic viscosity of a pectin sample (Std. no 3 in table 2) was determined based on the method of Pagan et al. (2001). Four pectin concentrations in the range from 0.1 to 0.4 g/dL were prepared by dissolving dried pectin in aqueous solution containing 5mM Na_2EDTA, 0.155 M NaCl and 0.04% NaN_3 as preservative, at pH adjusted to 5.0 with acetic acid, and agitating for 24 h at room temperature. Pectin solutions and solvent were filtered by passing through 0.45 µm membrane filter and then their passage times were measured by an Ostwald U-tube viscometer (Schott Gerate, Germany) immersed in a water bath (25°C). From these passage times, the relative viscosity (η_{rel}) and by the combined

extrapolation of Huggin's and Kraemer's equations to "zero" concentration (Iglesias & Lozano, 2004) and finally the viscosity average molecular weight (Mv) of pectin was calculated from the Anger–Berth relationship (Anger & Berth, 1986).

2.4. Rheological Measurement

Dynamic oscillatory test was performed using a controlled-stress rheometer (Physica MCR 300, Anton Paar, GmbH, Austria) with a single gap concentric cylinder geometry (outer radius: 14.46 mm, gap: 1.13 mm). Solutions of 0.5, 1.0 and 1.5 %w/v were prepared by dissolving dried pectin sample (Std. no 3 in Table 2) in deionized water and stirring for 8 hour at room temperature. Strain sweep test (0.01-100% at 10 Hz) was performed to set the upper limit of the linear viscoelastic region (LVR) and subsequently, the strain of 1% (well within LVR) was selected for further tests. Frequency sweep test was performed over the range of 0.01-100 rad/s at 25 °C to determine the viscoelastic properties (i.e. elastic modulus (G') and viscous modulus (G") of the pectin solution. The data obtained were analyzed using Origin Pro 8.6.0 software (OriginLab Corporation, Northhampton, MA, USA).

2.5. Experimental design and statistical analysis

Response surface methodology (RSM) was used for optimization of microwave assisted extraction (MAE) conditions of pectin from black mulberry pomace. A face centered central composite design (FCCCD) with totally 20 runs was applied to investigate the individual and interactive effects of process variables on the yield, degree of esterification (DE), and galacturonic acid (GalA) content of pectin. The process variables and their low (-1), central (0) and high (+1) levels which were selected based on the literature survey and preliminary experiments, are shown in Table 1. Six times replication in the center point of the design were performed to estimate the pure error. The experimental data were analyzed by analysis of variance (ANOVA) and the adequacy of the developed response surface model was determined by evaluating the lack of fit and coefficient of determination (R^2). The p-values < 0.05 were considered to be statistically significant. The experimental design and statistical analysis were performed using Design-Expert 8.0.7.1 software (Stat-Ease Inc., Minneapolis, MN, USA).

Table 1. Independent variables and their levels used for face centered central composite design (FCCCD).

Independent variables	Factor levels		
	-1	0	+1
A : extraction time (min)	10	20	30
B : Microwave power (watt)	300	600	900
C : Liquid/solid ratio (mL/g)	15	22.5	30

3. Results and Discussion

The experimental results of MAE of pectin from black mulberry pomace according to a face centered central composite design (FCCCD) are listed in Table 2.

Table 2. Results of MAE extraction of pectin according to face centered central composite design (FCCCD).

Std. no	Independent variables			Dependent variables		
	A (min)	B (watt)	C (mL/g)	Yield (%)	DE (%)	GalA (%)
1	10	300	15.0	10.03	56.41	36.59
2	30	300	15.0	11.96	46.66	31.38
3	10	900	15.0	12.90	56.75	34.38
4	30	900	15.0	13.66	52.78	33.05
5	10	300	30.0	8.87	46.26	43.13
6	30	300	30.0	12.33	44.44	36.92
7	10	900	30.0	14.26	50.00	32.63
8	30	900	30.0	14.19	57.06	31.59
9	10	600	22.5	12.73	48.38	29.63
10	30	600	22.5	14.00	49.00	29.17
11	20	300	22.5	11.65	44.52	35.96
12	20	900	22.5	14.47	47.50	36.09
13	20	600	15.0	11.75	54.83	33.34
14	20	600	30.0	12.98	47.15	34.05
15	20	600	22.5	12.40	47.14	31.71
16	20	600	22.5	13.00	46.42	33.46
17	20	600	22.5	13.18	48.38	32.84
18	20	600	22.5	12.37	46.66	32.13
19	20	600	22.5	12.68	45.83	32.50
20	20	600	22.5	12.21	49.00	30.25

3.1. Pectin Yield

Based on the experimental results shown in Table 2, the extraction yield of pectin ranged from 8.87% to 14.47% on a dry weight basis. The highest yield of pectin (14.47%) was obtained when the black mulberry pomace was exposed to the microwave power, irradiation time and liquid to solid ratio of 900 watt, 20 min and 22.5 g/mL, respectively.

Table 3. Results of ANOVA for response surface reduced quadratic model.

Source	Sum of squares	DF	Mean square	F-value	P-value
Yield					
Model	32.70	6	5.45	25.43	< 0.0001
A	5.40	1	5.40	25.21	0.0002
B	21.43	1	21.43	100.03	< 0.0001
C	0.54	1	0.54	2.53	0.1354
AB	2.76	1	2.76	12.89	0.0033
BC	0.90	1	0.90	4.19	0.0614
C^2	1.66	1	1.66	7.74	0.0155
Residual	2.79	13	0.21		
Lack of fit	2.05	8	0.26	1.73	0.2823
Pure error	0.74	5	0.15		
Cor total	35.48	19			
R^2	0.9215				
Adj-R^2	0.8853				
CV (%)	3.68				
DE					
Model	285.56	7	40.79	17.33	< 0.0001
A	6.18	1	6.18	2.62	0.1312
B	66.56	1	66.56	28.28	0.0002
C	50.72	1	50.72	21.55	0.0006
AB	26.86	1	26.86	11.41	0.0055
AC	44.94	1	44.94	19.09	0.0009
BC	12.25	1	12.25	5.21	0.0416
C^2	78.05	1	78.05	33.16	< 0.0001
Residual	28.24	12	2.35		
Lack of fit	20.84	7	2.98	2.01	0.2297
Pure error	7.40	5	1.48		
Cor total	313.80	19			
R^2	0.9100				
Adj-R^2	0.8575				
CV (%)	3.11				
GalA					
Model	169.71	8	21.22	12.96	0.0001
A	20.31	1	20.31	12.40	0.0048
B	26.37	1	26.37	16.11	0.0020
C	9.18	1	9.81	5.61	0.0373
AB	10.24	1	10.24	6.25	0.0295
BC	29.22	1	29.22	17.85	0.0014
A^2	20.03	1	20.03	12.24	0.0050
B^2	42.39	1	42.39	25.89	0.0004
C^2	7.00	1	7.00	4.28	0.0629
Residual	18.01	11	1.64		
Lack of fit	11.89	6	1.98	1.62	0.3067
Pure error	6.12	5	1.22		
Cor total	187.72	19			
R^2	0.9041				
Adj-R^2	0.8343				
CV (%)	3.81				

3.2. Model fitting

By applying multiple regression analysis on the experimental data, a reduced second-order polynomial model was generated by Design Expert software to predict the pectin yield as a function of process which can be expressed by the following equation in terms of coded units:

$$PY = +12.87 + 0.73A + 1.46B + 0.23C - 0.59AB + 0.34BC - 0.58C^2 \quad (1)$$

where PY is the extraction yield of pectin (%), and A, B and C are irradiation time, microwave power and liquid to solid ratio,

respectively. The insignificant terms of AC, A^2, and B^2 were eliminated from the model by backward elimination regression (BER) procedure with setting alpha exit to 0.100, however, the insignificant and hierarchical term of C was re-added to the model. Analysis of variance (ANOVA) was performed to determine the adequacy of the developed models and the statistical significance of each model term was evaluated by F-test and p-value. The results of ANOVA for reduced quadratic model developed for experimental data of pectin yield are listed in the upper part of Table 3. Based on the ANOVA results the developed model was highly significant, as it is evident from the model F-value and a low probability value ($p < 0.0001$).The non-significant lack of fit ($p = 0.1972$) also confirmed the validity of this model.

The goodness of fit of the model was further checked by the coefficient of determination (R^2) and adjusted coefficient of determination (adj-R^2) which was 0.9215 and 0.8853, respectively, and consequently there was a good correlation between the experimental and predicted values. At the same time, a low value (3.68%) of variation coefficient (CV), clearly indicated a high degree of precision and a good deal of reliability of the experimental values. According to the ANOVA results, the linear term of microwave power B) had the largest effect on pectin yield ($p<0.0001$), followed by linear term of irradiation time (A), the interaction term of irradiation time and microwave power (AB) and quadratic term of liquid to solid ratio (C2). The all other terms including C, AC, BC, A^2 and B^2 did not show significant effects on the pectin yield.

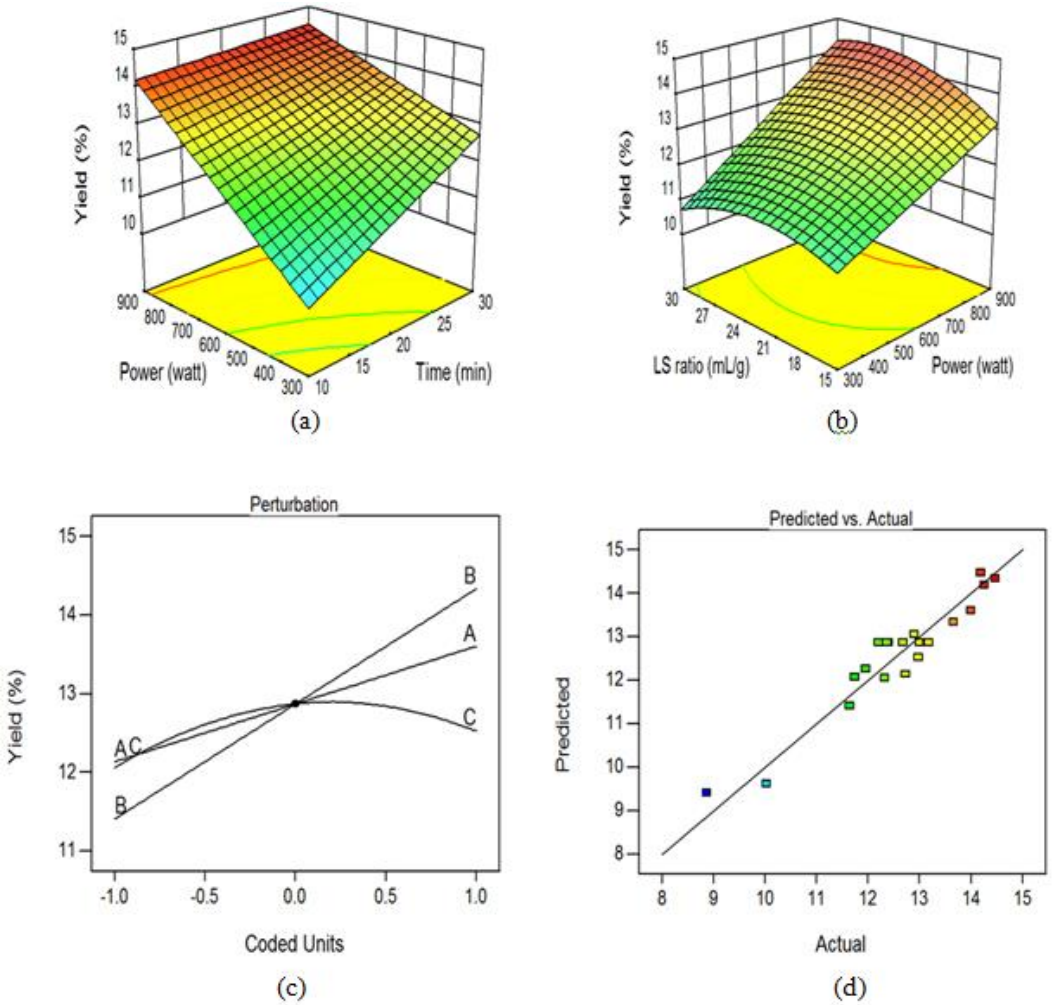

Fig. 1. Response surface plot representing the effect of (a) irradiation time and microwave power at constant liquid to solid (LS) ratio of 22.5 mL/g (b) microwave power and liquid to solid ratio at constant irradiation time of 20 min on the yield of pectin and (c) perturbation plot (d) predicted versus actual values.

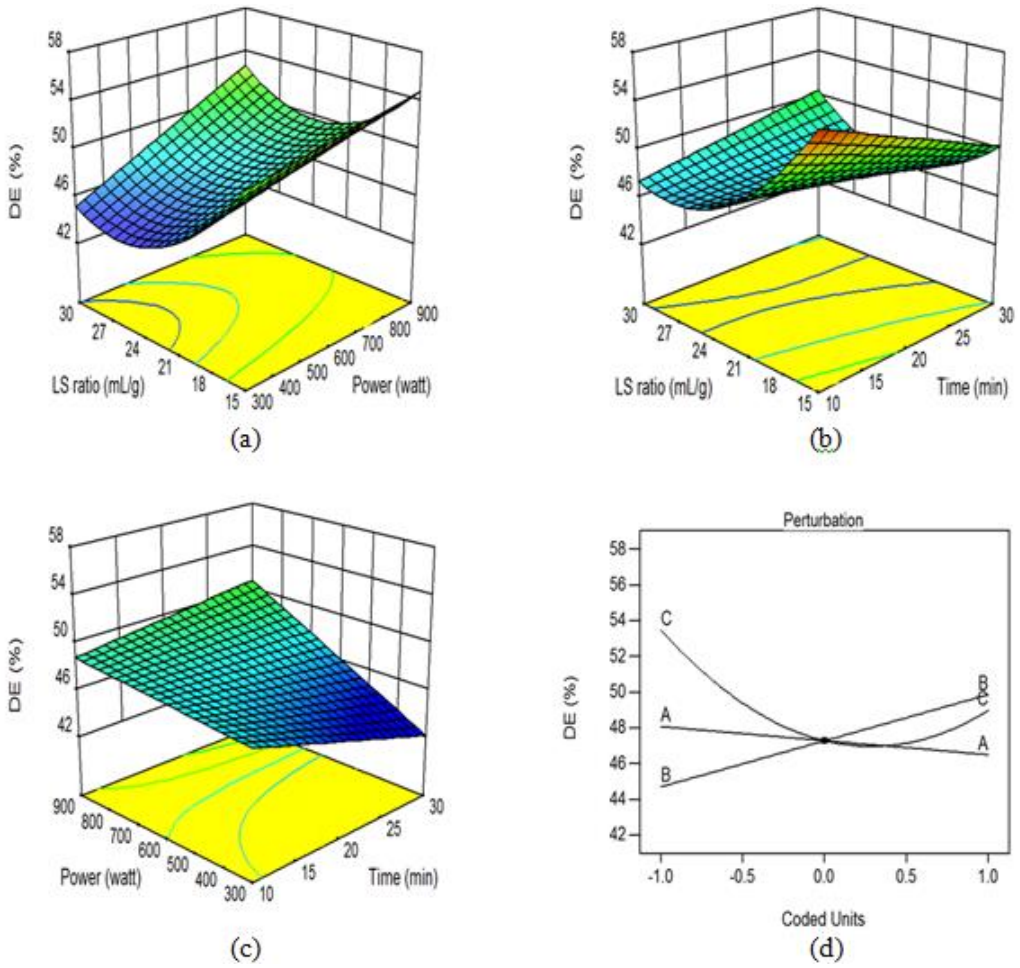

Fig. 2. Response surface plot representing the effect of (a) irradiation time and microwave power at liquid to solid ratio of 22.5 g/mL (b) irradiation time and liquid to solid ratio at microwave power of 600 watt and (c) microwave power and irradiation time irradiation time of 20 min on the DE of pectin and (d) perturbation plot.

3.3. Effects of MAE conditions on the pectin yield

The individual effects of irradiation time (A), microwave power (B) and liquid to solid ratio (C) on the extraction yield of pectin are shown in Fig. 1(c). In this so-called perturbation plot the effect of changing one factor over its range on the response is plotted, while the all other factors are kept constant by default in their corresponding center levels. It can be seen that both the irradiation time and microwave power showed a positive linear effect on the yield of pectin ($p < 0.001$).

On the contrary, the liquid to solid ratio showed a predominantly quadratic effect on the pectin yield and its main effect was found to be not significant ($p = 0.1354$).

The best way to visualize the effect of independent variables on a dependent variable is to draw response surface plots of the model describing the relationship between the variables (Chen et al., 2014). Fig. 1(a) depicts the three-dimensional (3-D) response surface plot for pectin yield as a function of irradiation time and microwave power at a fixed liquid to solid ratio of 22.5 mL/g. As can be seen the yield of pectin increased remarkably with increasing both irradiation time and microwave power, so that the highest yield was obtained when the highest level of irradiation time (30 min) and microwave power (900 watt) were used. It has been ratiocinated that irradiation of microwaves and subsequently increase of temperature within the plant material ruptures the cell wall matrix of parenchymal cells, causing the formation of capillary holes. In this way, the solvent penetrates easily and greatly to the plant tissue and subsequently leaks more pectin out (Seixas et al., 2014; Bagherian et al., 2011). The damage to the plant tissue increased with increasing intensity of the microwave field, which is explained by an increase in intracellular spaces (Seixas et al., 2014). Results showed that at lower levels of microwave power, the yield of pectin increased considerably with prolonging the irradiation time. Whereas, at higher power levels, the major part of the pectin was extracted in the first ten minutes of process and prolonging the irradiation time, did not result in a substantial change in pectin yield.

Fig 1(b) shows the combined effect of microwave power and liquid to solid ratio on the yield of pectin at a constant irradiation time of 20 min. The results showed that at any given level of microwave power, the yield of pectin increased with increasing liquid to solid ratio up to 22.5 g/mL, as seen in Fig 1(b). However,

further increase in the liquid to solid ratio not only did not increase the pectin yield, but instead slightly decreased it. This is in accordance with the previous result of Thirugnanasambandham et al. (2014). Generally in conventional extraction techniques a higher volume of solvent will increase the recovery, but in MAE higher solvent volume may give lower recoveries (Guo et al., 2001; Chen et al., 2007; Ping et al., 2010). This decrease in the pectin yield at larger volumes of extraction solvent was attributed to the inadequate stirring of solvent by microwaves (Wang & Weller, 2006).

3.4. Degree of esterification (DE)

The effect of MAE conditions on the degree of esterification (DE) of pectin from black mulberry pomace was also evaluated. The experimental results of DE values have been given in Table 2 which was in the range of 44.44-57.06%. It is worth mentioning that we recently extracted pectin with DE values from 51.78% to 74.4% from black mulberry pomace by using conventional acid extraction method (data not published yet). Therefore, the pectin extracted from black mulberry pomace can be considered as the high methoxyl pectin (HMP).

3.5. Model fitting

A reduced quadratic response surface model was fitted to the experimental values of DE which can be represented by the following equation in terms of coded values (Eq. 2):

$$\mathrm{DE} = +47.28 - 0.79\mathrm{A} + 2.58\mathrm{B} - 2.25\mathrm{C} + 1.83\mathrm{AB} + 2.37\mathrm{AC} + 1.24\mathrm{BC} + 3.95C^2 \quad (2)$$

where DE is the degree of esterification of pectin (%); and A, B, and C are the coded values of irradiation time, microwave power and liquid to solid ratio, respectively. The non-significant terms of A^2 and B^2 were deleted from the model and not included in the analysis of variance. The results of ANOVA for the reduced quadratic model representing the pectin DE are listed in the middle part of Table 3. ANOVA showed that the developed model was highly significant ($p < 0.0001$) and its lack of fit was not significant ($p = 0.2297$). The values of R^2 and adj-R^2 of this model were 0.91 and 0.8575, respectively, which indicates that the developed quadratic model was adequate in representing the observed values of DE. According to the ANOVA results, all the models terms were significant ($p < 0.05$) on the DE of pectin except for the linear and quadratic terms of irradiation time (A and A^2) and quadratic term of microwave power.

3.6. Effect of MAE conditions on the degree of esterification (DE) of pectin

The perturbation plot and 3-D response surface plots indicating the effects of process variables of MAE on the degree of esterification (DE) of pectin are shown in Fig 2(a-c). From these plots it is evident that changes in irradiation time did not lead to a significant change in DE values of pectin. However, it was observed a linear increase in DE value due to the increasing of microwave power. This result is in accordance with the previous results reported by Kratchanova et al. (1994), Fishman et al. (2006), Bagherian et al. (2011), and Seixas et al. (2014), who found that the DE of pectin extracted from citrus and passion fruit peels significantly increased with increasing microwave power.

3.7. Galacturonic acid content (GalA)

The galacturonic acid content (GalA) of pectins extracted by MAE from black mulberry pomace is given in Table 2, which was varied from 29.17% to 43.13% depending on the extraction conditions. These GalA contents are in consistence with the range of 20-50% reported for pectin from some berry fruits (Hilz et al., 2005; Cserjesi et al., 2011). These relatively lower GalA contents may be due to the presence of other monosaccharides such as rhamnose, arabinose, glucose and galactose in larger amounts in pectin structure, as stated by Kiss (2009).

3.8. Model fitting

The experimental data obtained for GalA content of pectin were fitted to a second order polynomial model to give the GalA value as a function of process variables. The developed model was then reduced by backward elimination regression procedure with alpha out set to 0.10. The final reduced quadratic model in which the insignificant term of AC was eliminated, can be represented by the following equation (Eq. 3):

$$\mathrm{GalA} = +32.13 - 1.43\mathrm{A} - 1.62\mathrm{B} + 0.96\mathrm{C} + 1.13\mathrm{AB} - 1.91\mathrm{BC} - 2.70A^2 + 3.93B^2 + 1.60C^2 \quad (3)$$

where GalA is the galacturonic acid content of pectin (%), and A, B, and C are coded values of process variables i.e. irradiation time (min), microwave power (watt), and liquid to solid ratio (g/mL), respectively. Analysis of variance for the model is given in lower part of the Table 3, showing that the generated model was significant ($p=0.0001$) and the lack of fit was not significant ($p=0.3067$). The R^2, adj-R^2 and CV values were 0.9041 and 0.8343, and 3.81, respectively, indicating that the model explained the response adequately. The results of ANOVA showed that the linear effect of all the variables were significant ($p < 0.05$). The interaction terms of irradiation time and microwave power (AB) as well as microwave power and liquid to solid ratio (BC) and the quadratic terms of irradiation time (A2) and microwave power (B2) were also significant ($p < 0.05$).

3.9. Effects of MAE conditions on the galacturonic acid content (GalA) of pectin

The effects of MAE conditions on the GalA content of pectin have been depicted in the response surface plots and perturbation plot in Fig. 3(a-c). Fig. 3(a) shows the interaction effect of irradiation time and microwave power on the GalA content of pectin at a fixed liquid to solid ratio of 22.5 g/mL. As can be seen both irradiation time and microwave power showed quadratic effect on the GalA content. The center level of irradiation time (20 min) combined with low (300 watt) or high (900 watt) level of microwave power, were conditions in which the highest value of GalA content was obtained. The combined effect of microwave

power and liquid to solid ratio on the GalA content of pectin at a fixed irradiation time of 20 min is shown in Fig. 3(b).

As be seen the GalA content markedly increased with increasing liquid to solid ratio from 15 to 30 g/mL, specifically at lower levels of microwave power.

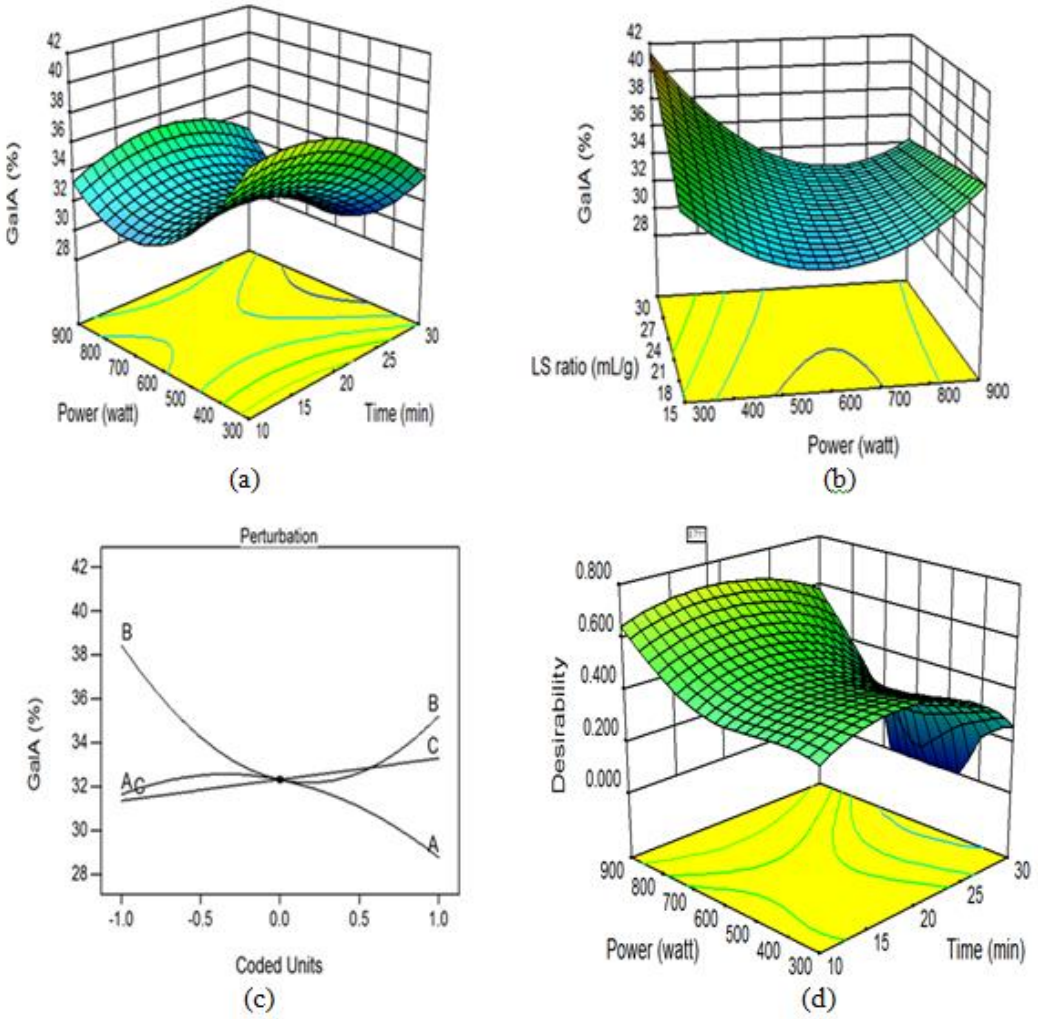

Fig 3. Response surface plot for the effect of (a) irradiation time and microwave power at a constant liquid to solid (LS) ratio of 22.5 g/mL (b) microwave power and liquid to solid ratio at a constant irradiation time of 20 min on the galacturonic acid content (GalA) of pectin and (c) the perturbation plot and (d) 3-D plot for overall desirability.

3.10. Optimization of MAE conditions

The numerical optimization technique with desirability function approach was used to optimize the MAE of pectin from black mulberry pomace. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimization finds a point that maximizes the desirability function. In this study, the criteria for optimization were maximizing the yield, degree of esterification (DE), and galacturonic acid content of pectin within the experimental range of operating parameters.

The optimum extraction conditions based on desirability function were irradiation time of 18.17 min, microwave power of 900 watt, and liquid to solid ratio of 15 g/mL with the desirability value of 0.71. Under these conditions the values of yield, DE, and GalA of pectin were predicted to be 13.16%, 55.07%, and 36.94%, respectively. The 3-D representation of desirability in the .Real experiments (two replicates) were carried out under the optimum conditions in which the mean value of 13.23% was obtained for pectin yield, suggesting that there was no significant difference between the predicted and real values.

3.11. Comparison between MAE and conventional extraction method (soxhlet)

The extraction of pectin from the same raw material (black mulberry pomace) used for MAE was carried out three times by using conventional (soxhlet) method at the extraction time of 1.5 hour, extraction temperature of 90°C, liquid to solid ratio of 30

g/mL, and pH = 2. The mean values obtained by conventional method along with MAE results at Std. no 7 and 8 for comparison are given in Table 4. As can be seen, MAE not only gave more pectin yield but at the same time reduced the extraction time up to ninety percent compared with the conventional method.

Table 4. The comparison between conventional and MAE method on the extraction of pectin from black mulberry pomace.

Method	Conditions	Yield (%)	DE (%)	GalA content (%)
Conventional*	90 min, 90 °C, 30 mL/g	13.46	51.45	34.03
MAE	10 min, 900 watt, 30 mL/g	14.26	50.00	32.63
MAE	30 min, 900 watt, 30 mL/g	14.19	57.06	31.59

3.12. *Intrinsic viscosity ([η]) and viscosity average molecular weight (Mv)*

The Huggins and Kraemer plots for determination of the intrinsic viscosity of pectin are shown in Fig. 4. By combined extrapolation of Huggins and Kraemer equations, the mean value of 1.22 dL/g was obtained for [η] of pectin, and the M_v of pectin calculated from Anger-Berth equation was found to be 32.78 kDa.

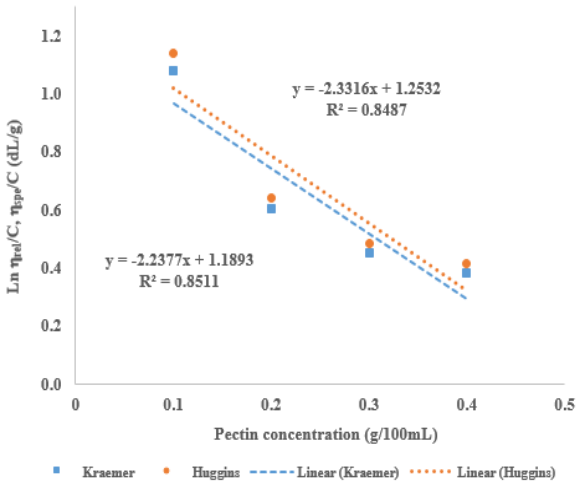

Fig. 4. Huggins and Kraemer plots for determination of intrinsic viscosity.

3.13. *Rheological properties*

The frequency sweep test was performed on a dispersion of MAE pectin sample (Std. no 3 according to Table 2) at the concentrations of 0.5 and 1.5 %w/v and the result is shown in the Fig. 5. As can be seen at both concentrations, pectin dispersion behaved as a viscoelastic material and the crossover of G' (elastic modulus) and G" (viscous modulus) occurred at low frequency region (about 0.07 rad/s). The crossover of G' and G" values provides a good indication of the viscoelastic behavior of a material (Lagher et al., 2002) and defines the beginning of the elastic behavior or approaching gel state (Norziah et al., 2001). The lower the crossover value is, the greater the elastic contribution becomes (Lagher et al., 2002).

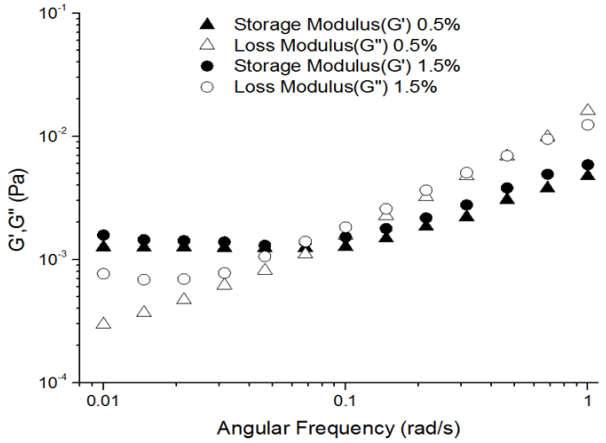

Fig. 5. Frequency dependence of G' (elastic modulus) and G" (viscous modulus) of pectin dispersions at 0.5 and 1.5 %w/v.

4. Conclusion

In this study, MAE technique was used for the extraction of pectin from black mulberry pomace and response surface methodology (RSM) was employed for the optimization of extraction conditions. The individual and interactive effects of three process variables namely irradiation time, microwave power, and liquid to solid ratio each at three levels on the yield, degree of esterification (DE) and galacturonic acid (GalA) content of extracted pectin were investigated by using a face canter central composite design. The yield, DE and GalA content of pectin varied in the range of 8.87-14.47%, 44.44-57.06%, and 29.17-43.13%, respectively. A response surface reduced quadratic model was developed to describe the experimental results of each response. Based on the ANOVA results, microwave power was the most significant factor effecting the yield, DE and GalA content of the extracted pectin. The optimal conditions for MAE extraction of pectin from black mulberry pomace were determined to be irradiation time of 18.15 min, microwave power of 900 watt and liquid to solid ratio of 15 mL/g. Under such conditions 13.23% pectin was extracted. The black mulberry pomace pectin showed a viscoelastic behavior in the rheological test and a crossover occurred between G' and G" in the lower frequencies (~0.07 rad/s).

References

Alistair, M. S., Glyn, O. P., & Peter, A. W. (2006) Food Polysaccharide and their Applications, 2nd ed., CRC Press, New York.

Anger, H., & Berth, G. (1986). Gel permeation chromatography and the Mark-Houwink relation for pectins with different degrees of esterification. *Carbohydrate Polymers*, *6*(3), 193–202.

Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave-and ultrasound-assisted methods for extraction of pectin from grapefruit. *Chemical Engineering and Processing: Process Intensification*, *50*(11), 1237–1243.

Bélafi-Bakó, K., Cserjési, P., Beszédes, S., Csanádi, Z., & Hodúr, C. (2012). Berry pectins: microwave-assisted extraction and rheological properties. *Food and Bioprocess Technology*, *5*(3), 1100–1105.

Bochek, A. M., Zabivalova, N. M., & Petropavlovskii, G. A. (2001). Determination of the esterification degree of polygalacturonic acid. *Russian Journal of Applied Chemistry*, *74*(5), 796–799.

Chan, S.-Y., & Choo, W.-S. (2013). Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. *Food Chemistry*, *141*(4), 3752–3758. https://doi.org/10.1016/j.foodchem.2013.06.097

Chen, Y., Zhang, J.-G., Sun, H.-J., & Wei, Z.-J. (2014). Pectin from *Abelmoschus esculentus*: Optimization of extraction and rheological properties. *International Journal of Biological Macromolecules*, *70*, 498–505.

Cserjési, P., Bélafi-Bakó, K., Csanádi, Z., Beszédes, S., & Hodúr, C. (2011). Simultaneous recovery of pectin and colorants from solid agro-wastes formed in processing of colorful berries. *Progress in Agricultural Engineering Sciences*, *7*(1), 65–80.

Ercisli, S., & Orhan, E. (2008). Some physico-chemical characteristics of black mulberry (*Morus nigra* L.) genotypes from Northeast Anatolia region of Turkey. *Scientia Horticulturae*, *116*(1), 41–46.

Guo, X., Han, D., Xi, H., Rao, L., Liao, X., Hu, X., & Wu, J. (2012). Extraction of pectin from navel orange peel assisted by ultra-high pressure, microwave or traditional heating: A comparison. *Carbohydrate Polymers*, *88*(2), 441–448.

Hager, A., Howard, L. R., Prior, R. L., & Brownmiller, C. (2008). Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed black raspberry products. *Journal of Food Science*, *73*(6), H134–H140.

Hilz, H., Bakx, E. J., Schols, H. A., & Voragen, A. G. J. (2005). Cell wall polysaccharides in black currants and bilberries—characterisation in berries, juice, and press cake. *Carbohydrate Polymers*, *59*(4), 477–488.

Hojjatpanah, G., Fazaeli, M., & Emam-Djomeh, Z. (2011). Effects of heating method and conditions on the quality attributes of black mulberry (*Morus nigra*) juice concentrate. *International Journal of Food Science & Technology*, *46*(5), 956–962.

Iagher, F., Reicher, F., & Ganter, J. (2002). Structural and rheological properties of polysaccharides from mango (*Mangifera indica* L.) pulp. *International Journal of Biological Macromolecules*, *31*(1–3), 9–17.

Iglesias, M. T., & Lozano, J. E. (2004). Extraction and characterization of sunflower pectin. *Journal of Food Engineering*, *62*(3), 215–223.

Imran, M., Khan, H., Shah, M., Khan, R., & Khan, F. (2010). Chemical composition and antioxidant activity of certain Morus species. *Journal of Zhejiang University Science B*, *11*(12), 973–980.

Kamiloglu, S., Serali, O., Unal, N., & Capanoglu, E. (2013). Antioxidant activity and polyphenol composition of black mulberry (*Morus nigra* L.) products. *Journal of Berry Research*, *3*(1), 41–51.

Kiss, K. (2009). *Extraction and enzymatic hydrolysis of pectins*. Ph. D. dissertation. Veszprem, Hungary: University of Pannonia.

Koubala, B. B., Kansci, G., Mbome, L. I., Crépeau, M.-J., Thibault, J.-F., & Ralet, M.-C. (2008). Effect of extraction conditions on some physicochemical characteristics of pectins from "Amelioree" and "Mango" mango peels. *Food Hydrocolloids*, *22*(7), 1345–1351.

Lapasin, R. (2012). *Rheology of industrial polysaccharides: theory and applications*. Springer Science & Business Media.

Li, D., Jia, X., Wei, Z., & Liu, Z. (2012). Box–Behnken experimental design for investigation of microwave-assisted extracted sugar beet pulp pectin. *Carbohydrate Polymers*, *88*(1), 342–346.

Lv, C., Wang, Y., Wang, L., Li, D., & Adhikari, B. (2013). Optimization of production yield and functional properties of pectin extracted from sugar beet pulp. *Carbohydrate Polymers*, *95*(1), 233–240.

Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2013). Optimization of microwave assisted extraction of pectin from orange peel. *Carbohydrate Polymers*, *97*(2), 703–709.

May, C. D. (1990). Industrial pectins: sources, production and applications. *Carbohydrate Polymers*, *12*(1), 79–99.

Methacanon, P., Krongsin, J., & Gamonpilas, C. (2014). Food Hydrocolloids Pomelo (*Citrus maxima*) pectin : Effects of extraction parameters and its properties. *Food Hydrocolloids*, *35*, 383–391. https://doi.org/10.1016/j.foodhyd.2013.06.018

Norziah, M. H., Kong, S. S., Karim, A. A., & Seow, C. C. (2001). Pectin–sucrose–Ca^{2+} interactions: effects on rheological properties. *Food Hydrocolloids*, *15*(4–6), 491–498.

Pagan, J., Ibarz, A., Llorca, M., Pagan, A., & Barbosa-Cánovas, G. V. (2001). Extraction and characterization of pectin from stored peach pomace. *Food Research International*, *34*(7), 605–612.

Pérez-Gregorio, M. R., Regueiro, J., Alonso-González, E., Pastrana-Castro, L. M., & Simal-Gándara, J. (2011). Influence of alcoholic fermentation process on antioxidant activity and phenolic levels from mulberries (*Morus nigra* L.). *LWT-Food Science and Technology*, *44*(8), 1793–1801.

Ridley, B. L., O'Neill, M. A., & Mohnen, D. (2001). Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry*, *57*(6), 929–967.

Santos, J. D. G., Espeleta, A. F., Branco, A., & de Assis, S. a. (2013). Aqueous extraction of pectin from sisal waste. *Carbohydrate Polymers*, *92*(2), 1997–2001.

Seixas, F. L., Fukuda, D. L., Turbiani, F. R. B., Garcia, P. S., Carmen, L. de O., Jagadevan, S., & Gimenes, M. L. (2014). Extraction of pectin from passion fruit peel (*Passiflora edulis* f. *flavicarpa*) by microwave-induced heating. *Food Hydrocolloids*, *38*, 186–192.

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin-a review. *Critical Reviews in Food Science & Nutrition*, *37*(1), 47–73.

Thirugnanasambandham, K., Sivakumar, V., & Maran, J. P. (2014). Process optimization and analysis of microwave assisted extraction of pectin from dragon fruit peel. *Carbohydrate Polymers*, *112*, 622–626.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, *17*(6), 300–312.

Wang, S., Chen, F., Wu, J., Wang, Z., Liao, X., & Hu, X. (2007). Optimization of pectin extraction assisted by microwave from apple pomace using response surface methodology. *Journal of Food Engineering*, *78*(2), 693–700.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry*, *100*(4), 1356–1364.

Yeoh, S., Shi, J., & Langrish, T. A. G. (2008). Comparisons between different techniques for water-based extraction of pectin from orange peels. *Desalination*, *218*(1), 229–237.