

Optimization and characterization of pectin extracted from sour orange peel by ultrasound assisted method

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ABSTRACT

In this work, a Box-Behnken design (BBD) with three variables (ultrasound power, irradiation time and pH) in three levels was applied for pectin extraction optimization. The optimization process showed that the maximum extraction yield was $28.07 \pm 0.67\%$ in ultrasound power of 150 W, irradiation time of 10 min and pH of 1.5 (as optimum conditions). In these conditions, ash, moisture and protein contents of SOPP were 1.89 ± 0.51 , 8.81 ± 0.68 and $1.45 \pm 0.23\%$, respectively. HPLC analysis indicated that 65.3% of the extracted pectin was galacturonic acid and approximately 72% of total neutral sugars was galactose. The optimized pectin had a total phenolic content of 39.95 ± 3.13 mg gallic acid equivalents/g pectin, the surface tension of 46.56 ± 0.23 and 42.14 ± 0.61 mN/m in concentrations of 0.1 and 0.5%w/v, water holding capacity and oil holding capacity of 3.10 ± 0.12 and 1.32 ± 0.21 g water or oil/g pectin with a suitable emulsifying and antioxidant properties. In addition, SOPP with degree of esterification of $6.77 \pm 0.43\%$ was classified as low methoxyl pectin, which confirmed by FTIR and ^1H NMR analysis.

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1. Introduction

Pectin is a complex heteropolysaccharide including a backbone of α -1,4-galacturonic acids which are partially esterified at the carboxylic acid groups [1]. Based on the esterified carboxylic acid groups, pectin divide to two categories with different applications: high methoxyl pectin (HMP, esterification degree higher than 50%) and low methoxyl pectin (LMP, esterification degree lower than 50%) [2]. The presence of the esterified groups and also neutral sugars, as the side chains, lead to create several parts covalently connected to each other, which homogalacturonan, rhamnogalacturonan I and II, and xylogalacturonan are the most important of them [3]. These polysaccharides, which are extensively distributed in cell wall of the plant tissues, have many applications in food (as gelling agent, emulsifier and stabilizer) and drug (as anti-tumor, antioxidant, anti-diabetic and anti-cancer) industries [4,5].

Traditionally, pectin is obtained from citrus peel by acidified water, which leads to the destruction and the change of its structure and natural properties, respectively. Therefore, the different non-conventional methods such as microwave-assisted extraction, supercritical fluid extraction and ultrasound-assisted extraction (UAE) were developed. Among these methods, UAE by the cavitation effect of ultrasound waves improves the destruction of plant cell wall and increases the rate of mass transferring which leads to the higher efficiency and quality of the recoverable pectin with a shorter extraction time. Also, this

method is a green technique due to the lower consumption of solvents and energy. Thus, UAE can be a suitable alternative to conventional approach [6].

Sour orange or bitter orange (*Citrus aurantium* L.) is a member of citrus family, which is used as sedative, hypnotic and appetizer in traditional Iranian medicine. Sour orange peel, as a native product of Iran, is a rich source of pectin, which is unfortunately discarded as waste of fruit juice industry. In previous studies, we extracted sour orange peel pectin (SOPP) by aqueous and microwave-assisted extraction methods [7,8]. These papers focused more on the pectin extraction optimization from this byproduct and therefore, the structural and functional properties of SOPP are still completely unclear. Therefore, the aim of this study is as follows: (1) UAE (as a new method) optimization of SOPP and investigation of the effect of various extraction factors on the yield and properties of SOPP, (2) identification of the structure, monosaccharide and chemical compositions of the extracted pectin and its relationship with extraction method and also, (3) investigation of physicochemical and functional properties of SOPP.

2. Materials and methods

2.1. Raw material and chemicals

The fresh sour orange (*Citrus aurantium* L.) peels were obtained from a fruit store in Fasa, Fars, Iran. The peels were washed, finely cut to the small pieces and dried (50 °C) in an oven. The dried peels were blended in a blender and sieved using 40-mesh screen to remove non-powdered

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particles. The obtained powder was kept in a dark and dry place for next experiments.

Citric acid (99%), hydrochloric acid (37%), sulfuric acid (95–97%), TFA (trifluoroacetic acid) ($\geq 99\%$), sodium hydroxide ($\geq 99\%$), Folin-Ciocalteu's phenol reagent, DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and standard monosaccharides (galacturonic acid, arabinose, galactose, rhamnose, xylose, glucose, and fructose) were bought from Merck Chemical Co. (Darmstadt, Germany). Also, ethanol (96%) was bought from Ghadir Co. (Tehran, Iran).

2.2. Experimental design

First, one factor at a time design was used for selection of the best liquid/solid ratio (LSR). In this design, the LSR was variable (10, 15, 20, 25, 30 and 35 v/w), while the other three factors were kept constant on the center point (ultrasound power of 100 W, irradiation time of 20 min and pH of 2.25). After selection of the best LSR (20 v/w), this factor was considered as a constant in Box-Behnken design (BBD) for the evaluation of the effect of the three other factors (ultrasound power, irradiation time and pH) in three levels (Table 1) on the extraction yield and degree of esterification (DE). It should also be stated that the levels were chosen based on preliminary tests [9–11]. All calculations and graphics were carried out using the statistical software Design Expert 7.0 and Excel.

2.3. Pectin production and recovery

In order to ultrasound-assisted extraction of SOPP, the best level of LSR (20 v/w) was first selected using one factor at a time design and then, the pectin extraction was done according to the conditions shown in the Table 1. Briefly, 5 g of sour orange peel powder was mixed with acidified distilled water (using acid citric) in different pH and then the probe of ultrasonic device (Ultrasonic Co., Tehran, Iran), in a frequency of 20 kHz and temperature lower than 30 °C, was submerged into the mixture and finally the time and power of ultrasound was set up based on BBD (Table 1). After extraction, the mixture was centrifuged (10000×g, 15 min) to remove impurities and then, ethanol (96%) was added to it. To complete the pectin precipitation process, the prepared mixture was placed at 4 °C for 24 h. Afterward, the coagulated

pectin was separated using centrifugation (10000×g, 20 min) and dried (45 °C, 16 h). In final, the SOPP yield was calculated as following equation (Eq. 1):

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

2.4. DE (degree of esterification)

DE, as one of the most important properties of pectin, was determined for all pectin samples using the method described by Hosseini et al. [7]. Briefly, 100 mg of pectin sample was dissolved in 20 mL deionized water and 2 mL ethanol (96%). Then, 3 drops of phenolphthalein was added to the solution and titrated with NaOH solution (0.1 M) till the solution colour changed to pink (V_1). Afterward, 10 mL of NaOH solution (0.1 M) was added and the mixture was stirred for 20 min. 10 mL of HCl solution (0.1 M) was added and stirred until the disappearance of pink colour. At last, the solution was again titrated with NaOH (0.1 M) (V_2). The DE of SOPP was calculated as follows (Eq. 2):

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

2.5. Characterization of the pectin extracted at the optimized conditions

2.5.1. Chemical composition

In this section, chemical parameters of SOPP extracted in optimum point were determined. Moisture content of SOPP was evaluated based on losing weight in an oven at 105 °C for 24 h [12]. Ash content was estimated by incinerating SOPP in furnace at 550 °C for 6 h and weighing after cooling in a desiccator. Also, protein content of SOPP was measured using Kjeldahl method ($N \times 6.25$) [10].

2.5.2. Monosaccharides composition

Monosaccharides composition of SOPP extracted in optimum point were evaluated using high performance liquid chromatography (HPLC) method. Briefly, SOPP was hydrolyzed by TFA (2 M) solution at 85 °C for 3 h. Afterward, the solution was immediately cooled in an ice-water bath and centrifuged (2500×g for 15 min) to separate impurities. The prepared solution was freeze-dried to remove TFA from the hydrolysates. At last, the obtained hydrolysates were dissolved in deionized water and injected to a HPLC apparatus equipped with an Eurokat H column and a smartline RI detector 2300 (Knauer, Berlin, Germany). It should also be noted that 0.005 M sulfuric acid was applied as mobile phase (the flow rate and temperature of mobile phase were 0.8 mL/min and 70 °C, respectively). The monosaccharides composition of SOPP was calculated by considering the retention time of the standard monosaccharides (galacturonic acid, arabinose, galactose, rhamnose, xylose, glucose and fructose).

2.5.3. Total phenolic content (TPC)

TPC of SOPP extracted under optimal condition was estimated using Folin-Ciocalteu method [14]. In brief, 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 0.5 mL of SOPP solution (1% w/v). The prepared solution was incubated at room temperature for 1 h and then, its absorbance was measured at 750 nm. The standard curve (with a R^2 of 99.37%) was obtained using gallic acid. TPC of pectin was expressed as milligram gallic acid equivalents/g of SOPP (mg GAE/g pectin).

2.5.4. Determination of surface tension

Surface tension of SOPP extracted under optimal conditions was analyzed by the method described by Yapo et al. [13]. For this purpose, a tensiometer (Nanometric-pazhoh Co. Tehran, Iran) with the Wilhelmy

Table 1
Box-Behnken design for independent factors with experimental and predicted responses.

Factors		Unit	Actual levels				
			−1	0	+1		
Ultrasound power (X_1)		W	50	100	150		
Irradiation time (X_2)		min	10	20	30		
pH (X_3)		–	1.5	2.25	3		
Run no.	X_1	X_2	X_3	EEY ^A	EDE ^A	PEY ^A	PDE ^A
1	50	10	2.25	13.5	26.31	13.95	28.83
2	150	10	2.25	17.2	10.71	17.75	10.58
3	50	30	2.25	13.0	30.48	12.42	30.61
4	150	30	2.25	14.0	28.00	13.55	25.48
5	50	20	1.50	21.2	10.42	21.29	8.07
6	150	20	1.50	26.6	5.63	26.56	5.93
7	50	20	3.00	8.6	45.45	8.64	45.15
8	150	20	3.00	8.4	21.58	8.31	23.92
9	100	10	1.50	23.8	31.14	23.26	30.97
10	100	30	1.50	26.2	27.04	26.69	29.26
11	100	10	3.00	14.6	50.67	14.11	48.45
12	100	30	3.00	4.4	66.67	4.94	66.84
13	100	20	2.25	13.2	25.80	13.20	23.70
14	100	20	2.25	12.9	23.08	13.20	23.70
15	100	20	2.25	13.5	22.22	13.20	23.70

^A EEY, EDE, PEY and PDE are experimental extraction yield, experimental DE (degree of esterification), predicted extraction yield and predicted DE, respectively.

plate method (with a mica plate) was applied to measure the surface tension of SOPP solutions (0.1 and 0.5% w/v).

2.5.5. Emulsifying properties

Emulsion activity (EA) and emulsion stability (ES) of SOPP (extracted under optimal conditions) was measured as the method described by Hosseini et al. [8]. In brief, the mixture of 5 mL sunflower oil, 5 mL SOPP solution (0.5% w/v) and 0.02% sodium azide (NaN_3) was treated with ultrasound for 5 min and centrifuged ($4000\times g$ for 5 min). At last, EA was calculated as follows:

$$\text{EA}(\%) = \frac{V_E}{V_T} \times 100 \quad (3)$$

where V_E was the volume of emulsion layer and V_T was the total volume.

To evaluate ES, a similar procedure was used for producing emulsion. Then, after 1 and 30 days at 4 °C and room temperature, ES was calculated as follows:

$$\text{ES}(\%) = \frac{V_R}{V_I} \times 100 \quad (4)$$

where V_R was the remained emulsion volume and V_I was the initial emulsion volume.

2.5.6. Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC of SOPP was evaluated using the method described by Bayar et al. [15]. Briefly, 10 mL distilled water or sunflower oil was mixed with 1 g of SOPP in a 15 mL tube and then, vortexed strongly for 1 min. In the next stage, the mixture was centrifuged ($3000\times g$ for 15 min), the supernatant was removed and the residual was weighted, respectively. At last, WHC and OHC was revealed as gram of water or oil retained by 1 g SOPP.

2.5.7. DPPH radical scavenging capacity

Antioxidant activity of SOPP was determined according to the method described by Chaouch et al. [16]. Briefly, 1 mL of SOPP aqueous solution (in different concentrations of 1–50 mg/mL) was mixed with 4 mL of DPPH methanolic solution (0.1 mM). After vortexing, the mixture was incubated in darkness (25 °C for 30 min). At last, the absorbance of the mixture was measured using a UV-Vis spectrophotometer (Spectrum SP-UV500DB) at 517 nm. Ascorbic acid in same concentrations (AA) was applied as standard. The activity of DPPH radical scavenging was calculated as follows:

$$\text{DPPH scavenging activity}(\%) : \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100 \quad (7)$$

2.5.8. Nuclear magnetic resonance (NMR)

SOPP extracted under optimal extraction condition was dried and then dissolved in D_2O . Afterward, ^1H NMR spectroscopy was performed using a Varian Unity Inova 500 MHz spectrometer (Palo Alto, CA, United States). It should be stated that, the internal temperature was 23 °C and 32 scans were collected with a relaxation delay of 1 s.

2.5.9. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectroscopy of SOPP obtained under optimal conditions was carried out using a Bruker FT-IR Tensor 27 spectrometer (Billerica, Massachusetts, USA) with KBr method in the wavenumber range of $4000\text{--}500\text{ cm}^{-1}$ and resolution of 4 cm^{-1} .

2.5.10. X-ray diffraction (XRD)

The XRD pattern of SOPP extracted under optimal conditions was recorded using an X-ray diffractometer (PHILIPS, Amsterdam, Netherlands). The SOPP sample was scanned with diffraction angle (2θ)

between 10° to 80° . It should also be noted that the step size and time per step were 0.05° (2θ) and 1 s/step, respectively.

3. Results and discussion

3.1. Optimization and experimental data analysis

At first, the best LSR level (20 v/w) for ultrasound-assisted extraction of SOPP was selected and then 15 runs (BBD) were carried out to optimize the other three factors (ultrasound power, irradiation time and pH), while the LSR was fixed at the selected level (Table 1). According to the Table 1, the highest and lowest extraction yield was related to the run number 6 (26.6%) and run number 12 (4.4%), respectively. Also, in the optimum extraction point (ultrasound power of 150 W, irradiation time of 10 min and pH of 1.5), the extraction yield of 28.74% was achieved using predicted model for SOPP extraction yield (Eq. 8). This model was evaluated by three runs on the mentioned conditions and the obtained results insured that the predicted model for extraction process was in high accuracy (the extraction yield of SOPP under optimal condition was $28.07 \pm 0.67\%$). The UAE yield of SOPP was approximately similar to the obtained result from microwave-assisted extraction ($28.8 \pm 0.3\%$) and higher than aqueous extraction ($17.95 \pm 0.3\%$) in their optimum extraction conditions [7,8]. This result shows that the UAE technique is a highly efficient method because, in addition to the high extraction yield, this method has shorter process time and

Table 2

Analysis of variance (ANOVA) for SOPP (sour orange peel pectin) extraction yield and DE (degree of esterification).

Source	DF	Some of squares	Mean square	F-value	p-value
(A) Yield					
Model	9	590.112	56.568	141.46	0.000
Linear	3	506.187	168.729	364.03	0.000
Power	1	12.251	12.251	26.43	0.004
Time	1	16.531	16.531	35.67	0.002
pH	1	477.405	477.405	1030.00	0.000
Square	3	34.572	11.524	24.86	0.002
Power $\times$ Power	1	0.028	0.028	0.06	0.815
Time $\times$ Time	1	4.777	4.777	10.31	0.024
pH $\times$ pH	1	31.321	31.321	67.57	0.000
Interaction	3	49.352	16.451	35.49	0.001
Power $\times$ Time	1	1.823	1.823	3.93	0.104
Power $\times$ pH	1	7.840	7.840	16.91	0.009
Time $\times$ pH	1	39.690	39.690	85.63	0.000
Error	5	2.318	0.464		
Lack-of-fit	3	2.138	0.713	7.92	0.114
Pure error	2	0.180	0.090		
Total	14	592.429			
R ²			0.9961		
Adj R ²			0.9890		
Pred R ²			0.9416		
(B) DE					
Model	9	3503.11	389.23	47.74	0.000
Linear	3	1928.54	642.85	78.85	0.000
Power	1	273.08	273.08	33.49	0.002
Time	1	139.11	139.11	17.06	0.009
pH	1	1516.35	1516.35	185.99	0.000
Square	3	1339.52	446.51	54.77	0.000
Power $\times$ Power	1	622.12	485.55	59.55	0.001
Time $\times$ Time	1	448.27	500.48	61.39	0.001
pH $\times$ pH	1	269.13	108.40	33.01	0.002
Interaction	3	235.05	78.35	9.61	0.016
Power $\times$ Time	1	43.03	43.03	5.28	0.070
Power $\times$ pH	1	91.01	91.01	11.16	0.021
Time $\times$ pH	1	101.00	101.00	12.39	0.017
Error	5	40.77	8.15		
Lack-of-fit	3	33.78	11.26	3.22	0.246
Pure error	2	6.98	3.49		
Total	14	3543.87			
R ²	9		0.9885		
Adj R ²	3		0.9678		
Pred R ²	3		0.8431		

lower energy consumption. It is noteworthy that the extraction yield of SOPP in this study was higher than the reported results for the obtained pectin from apple pomace by microwave-assisted extraction method (15.75%), durian rind by conventional method (8.80%), dragon fruit peel by microwave-assisted extraction method (7.50%), pistachio green hull by microwave-assisted extraction method (18.13%), pomegranate peel by ultrasound-assisted extraction method (23.87%), *Artocarpus heterophyllus* fruit peel by ultrasound-assisted extraction method (14.48%) and some other of this polymer sources [17–22].

The results of ANOVA (analysis of variance) for the models of extraction yield and DE are listed in Table 2. The high F-value (141.46 and 47.74 for extraction yield and DE, respectively), low p -value (<0.001 for both factors) and also insignificant lack-of-fit clearly indicated that the predicted models are significant and the square polynomial models were well-fitted with the obtained data [19]. On the other hand, the determination coefficient for both responses (99.61 and 98.85% for extraction yield and DE of SOPP, respectively) was very high and exhibited that the relationship between variables and responses can be explained

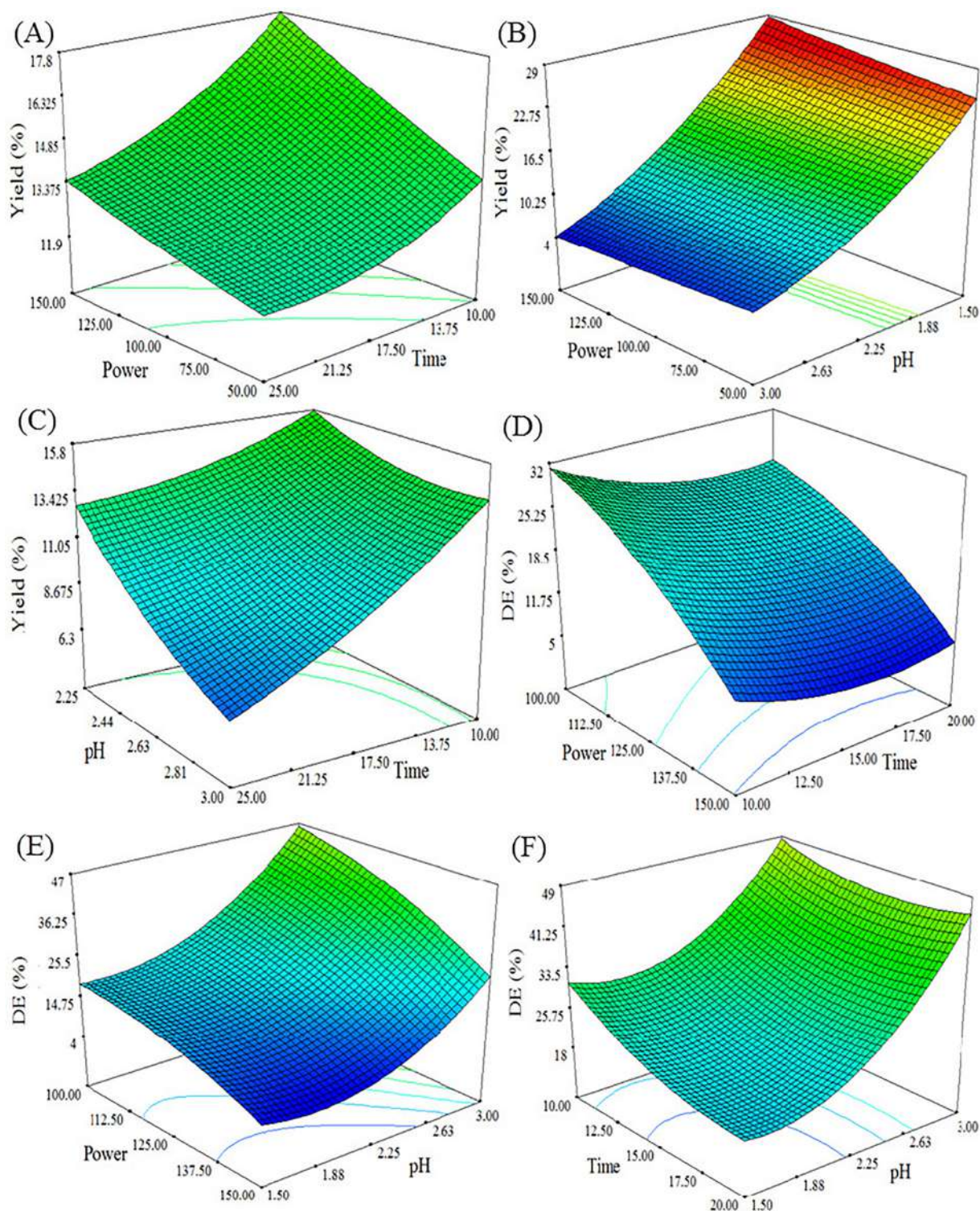


Fig. 1. Independent factors effects of UAE (ultrasound-assisted extraction) on SOPP (sour orange peel pectin) yield and DE (degree of esterification).

Table 3

Chemical and physicochemical parameters of SOPP (sour orange peel pectin) extracted in optimum conditions.

Chemical composition (%)		Molar ratio ^C	
Ash	1.89 ± 0.51	HG	62.5%
Moisture	8.81 ± 0.68	RG I	33.2%
Protein	1.45 ± 0.23	MR ₁	1.91
DE	6.77 ± 0.43	MR ₂	0.04
TPC (mg GAE/g pectin) ^A	39.95 ± 3.13	MR ₃	10.22
Monosaccharides composition (%) ^B		Surface tension (mN/m)	
GalA	65.3	0.1% (w/v)	
Gal	25.1	0.5% (w/v)	
Rha	2.8		
Ara	2.5		
Xyl	2.0		
Fru	1.8		
Glu	0.4		

^A GAE was gallic acid equivalent.^B GalA, Gal, Rha, Ara, Xyl, Fru and Glu were galacturonic acid, galactose, rhamnose, arabinose, xylose, fructose and glucose, respectively.^C HG (homogalacturonan) = GalA-Rha; RG I (rhamnogalacturonan I) = 2Rha + Ara + Gal; MR₁ = GalA/(Rha + Ara + Gal + Xyl + Fru); MR₂ = Rha/GalA; MR₃ = (Ara + Gal)/Rha.

precisely and accurately by the proposed models [20]. According to the above-mentioned results, the proposed square polynomial models are perfectly suitable for prediction of the relationship between independent variables and responses and there is no need for higher-order models. These square polynomial models are shown in below:

$$\text{Yield}(\%) = 13.20 + 1.24X_1 - 1.44X_2 - 7.72X_3 + 0.09X_1^2 + 1.14X_2^2 + 2.91X_3^2 - 0.67X_1X_2 - 1.40X_1X_3 - 3.15X_2X_3 \quad (8)$$

$$\text{DE}(\%) = 23.70 - 5.84X_1 + 4.17X_2 + 13.77X_3 - 11.47X_1^2 + 11.64X_2^2 + 8.54X_3^2 + 3.28X_1X_2 - 4.77X_1X_3 + 5.02X_2X_3 \quad (9)$$

3.2. Effect of process variables on SOPP yield

The obtained consequences in Table 2 and Eq. (8) showed that all of the applied factors were highly effective on extraction yield of SOPP. The most influential factor on extraction process was pH, thus this factor should be considered more than the other factors. Also the interaction between irradiation time and pH had a great effect on the extraction yield. For more exploration in the relationship between responses and variables, three-dimensional (3D) plots were established in Fig. 1.

According to the Fig. 1(B and C), the highest extraction yield was achieved in the lowest pH value. In another word, high acidic condition increased pectin leakage from the plant material and in consequence, increased the extraction yield of SOPP [8,19,21,22]. In this study, irradiation time of ultrasound was one of the other important factors which had a vise versa effect on the extraction yield (Fig. 1A and C). This observation may be due to the fragmentation of the pectin structure to its oligosaccharides by the cavitation effects of ultrasound waves in the high exposing times [23]. The ultrasound power was another effective factor which had a direct effect on the extraction yield. Fig. 1A and B reveals that ultrasound power was very efficient in this study and by increase in the factor, the extraction yield was increased [21,23]. This result

may refer to the cavitation effects of ultrasound waves. This cavitation enhanced the solvent penetration into plant matrix and thereby improved pectin leakage and extraction yield [22,23].

3.3. The effects of extraction variables on DE

DE of pectin is an important property in food processing systems due to its effect on functional properties such as gelling, solubility and etc. [24]. For this reason, DE of all BBD runs was measured and the results were shown in Table 1. As can be seen, the highest and lowest DE values were related to the run number 12 (66.67%) and run number 6 (5.63%). Also, the extracted SOPP under optimal condition (ultrasound power of 150 W, irradiation time of 10 min and pH of 1.5) had a DE value of 6.77 ± 0.43%. In addition, it is to note that the extraction yield was not related to DE. The highest extraction yield was due to optimum extraction conditions (ultrasound power of 150 W, irradiation time of 10 min and pH of 1.5), while DE was quite low in these conditions. In general, although the use of harsh extraction conditions (high power and low pH) leads to a high yield, the DE for these conditions is low which is probably due to deesterification of pectin in these conditions [8]. In comparison with the previous studies on the SOPP, DE in ultrasound assisted extraction method was lower and higher than aqueous extraction (DE of 23%) and microwave-assisted extraction (DE of 1.5 ± 0.2%) [7,8]. These results show that de-esterification of SOPP by ultrasound waves was lower than microwave.

Fig. 1 indicated that DE had a direct relationship with pH and a reverse relationship with ultrasound power and extraction time. However, in the many studies were proved that the use of harsh conditions such as low pH and high power and extraction time can lead to de-esterification of poly-galacturonic chains [25].

3.4. Chemical composition

Ash, moisture and protein contents of SOPP extracted under optimal conditions (ultrasound power of 150 W, irradiation time of 10 min and pH of 1.5) were evaluated as reported in Table 3. It could be seen that the Ash, moisture and protein contents of SOPP were 1.89 ± 0.51, 8.81 ± 0.68, 1.45 ± 0.23%, which were lower than the reported data about microwave-assisted extracted pistachio green hull pectin (5.16, 12.2 and 6.3%, respectively) by Kazemi et al. [26]. The protein content was higher than the *Opuntia ficus indica* cladodes pectin extracted by UAE (0.32 ± 0.03%) [27]. The use of different sources could be the most effective reason for these differences. It should be noted that lower amount of protein and ash content shows a higher purity of sample.

3.5. Monosaccharides composition and molar ratio

The backbone of homogalacturonan (HG) and rhamnogalacturonan I and II (RG I and II) regions in pectin structure composed by GalA (galacturonic acid). The neutral sugars like Gal (galactose), Rha (rhamnose), Ara (arabinose), Xyl (xylose) and Fru (fructose) are the main constituents of pectin side chains. According to the Table 3, GalA was the main monosaccharide of SOPP with 65.3% of the total monosaccharides. This value shows that the obtained supernatant could be considered as pectin (GalA content >65%) according to the FAO (food and agriculture organization) regulations. In the case of neutral sugars, Gal was the most important with approximately 72% of total neutral sugars and

Table 4

Emulsifying properties of sour orange peel pectin (SOPP) solutions.

Storage time	Emulsion activity (%)	Emulsion stability (%)			
		1 day		30 day	
Temperature (°C)	23	4	24	4	24
Pectin*	53.42 ± 2.14	93.14 ± 3.45	85.17 ± 1.25	90.25 ± 2.31	79.14 ± 1.91

* SOPP extracted under optimal extraction conditions.

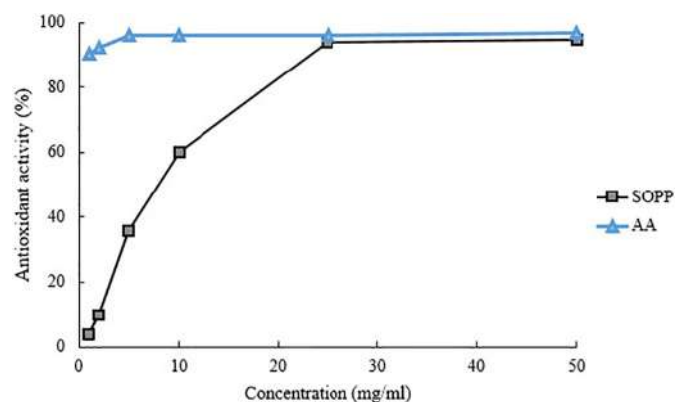


Fig. 2. DPPH radical scavenging activity of SOPP (sour orange peel pectin) and AA (ascorbic acid) as a standard.

Rha and Ara with 8.0 and 7.2% were in the next ranks. Higher amounts of these monosaccharides (Gal, Rha and Ara) are probably due to higher amounts of RG I regions in SOPP structure. Moreover, Xyl and Fru were found in a lower percentage which may refer to the presence of xylogalacturan (XG) and RG II in the SOPP structure. Also, very low amounts of glucose (Glu; 0.4%) could be due to the remnant of non-pectic polysaccharides like hemicellulose or cellulose bonded to the side chains of SOPP [28]. Consequently, it seems that the ultrasound-assisted method had a high efficiency and selectivity in SOPP production (see to amount of glucose).

To achieve the primary information about the structure of SOPP, molar ratio (MR) of these monosaccharides were provided in Table 3. According to this table, MR₁ (pectin linearity) of SOPP (1.91) was slightly lower than pistachio green hull (PGH) pectin extracted by microwave-assisted extraction (with MR₁ value of 1.97), which shows SOPP had a lower linear regions than PGH pectin [26]. MR₂ and MR₃,

which show the contribution of RG region in the pectin structure and the length of branches attached to RG-I, respectively, had values of 0.04 and 10.22. In addition, higher value of HG (62.5%) showed that the smooth regions are more in SOPP structure and the high MR₃ was a sign of the presence of long branches in it (mostly galactan). According to the obtained results, SOPP can recognize as a good emulsifier and stabilizer for use in food systems [28,29].

3.6. TPC (Total phenolic content)

TPC, as one of the important parameters of pectin properties, could have effect on functional properties such as antioxidant activity. Thus, TPC of SOPP extracted under optimum conditions were determined and the obtained result was illustrated in Table 3. According to this result, SOPP had a TPC value of 39.95 ± 3.13 mg GAE/g pectin. To the best our knowledge, very little studies were performed to evaluate this parameter of pectin. However, TPC of SOPP was higher than pectin extracted by microwave from pistachio green hull (18.18 mg GAE/g pectin) [14]. It is well-known that high temperature in conventional or microwave-assisted extraction methods can leads to degradation in phenolic compounds, while UAE with lower temperature efficiently breaks the cell wall, increases diffusion rate across the cell wall and promotes the release of compounds such as phenols [30].

3.7. Surface properties

Surface tension is one of the most important properties in different food products especially in aerated food products. Generally, polysaccharides with lower surface tension values are better for use in these products [31]. In the current study, the surface tension of SOPP extracted under optimum extraction conditions was evaluated and results showed in Table 3. Considering this results, SOPP aqueous solutions in 0.1 and 0.5%w/v had the surface tension values of 46.56 ± 0.23 and 42.14 ± 0.61 mN/m, respectively. This obtained result showed that by

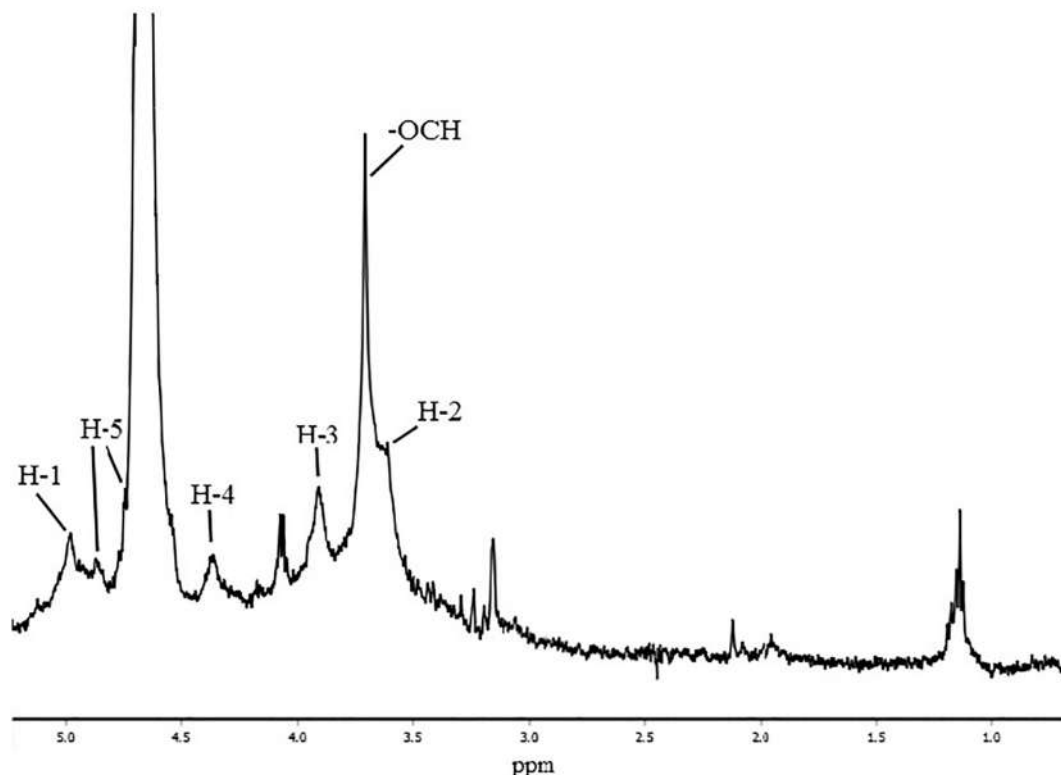


Fig. 3. ¹H NMR spectrum of SOPP (sour orange peel pectin) obtained by UAE (ultrasound-assisted extraction) method.

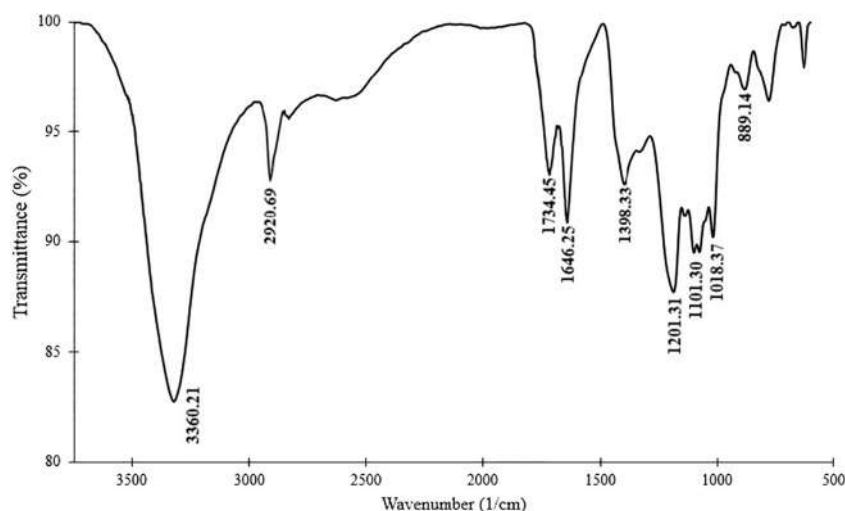


Fig. 4. FTIR spectrum of SOPP (sour orange peel pectin) obtained by UAE (ultrasound-assisted extraction) method.

increase in SOPP concentration from 0.1 to 0.5%w/v, the surface tension was decreased and thereby the surface activity was increased. This observation may be due to the increase in concentration of TPC and protein content in SOPP solution, because it has been proven that some phenolic compounds could accumulate at the air-water interface and increase the surface activity [32].

3.8. Emulsifying properties

The obtained results of emulsion activity (EA) and emulsion stability (ES) of SOPP were illustrated in Table 4. According to this results, SOPP had an emulsion activity value of $53.42 \pm 2.14\%$ which was higher than the both previous studies on SOPP extracted by aqueous (45.0%) and microwave-assisted (40.7%) methods [7,8]. Also, this result was higher than the obtained data from *Citrus medica* peel (46.5%) and sugar beet pulp (~43–47%) by Pasandide et al. [2] and Yapo et al. [13]. In addition, the results showed that the produced emulsions were more stable in lower temperature and the highest stability was due to the stored emulsion in 4 °C after 1 day ($93.14 \pm 3.45\%$). The molecular weight and galacturonic acid content of the extracted pectin are the important factors in emulsifying properties which both are affected by the extraction technique [13,27]. The use of UAE method usually increases the galacturonic acid content and leads to increasing emulsifying

properties. Besides, protein content of extracted pectin could be effective on these properties due to its hydrophobicity which can bind the oil droplets to the carbohydrate chains [33].

3.9. WHC (water holding capacity) and OHC (oil holding capacity)

WHC, as a fundamental property in many food systems, exhibits the capability of 1 g sample to hold the water. WHC of SOPP extracted under optimal conditions was 3.10 ± 0.12 g water/g pectin which was lower than the pectin extracted from pistachio green hull (4.11 ± 0.34 g water/g pectin) [26]. In a research, Bayar et al. [27] explained that many factors could effect on the WHC such as the amount of free hydroxyl groups in pectin structure, porosity of pectin powder and so on. On the other hand, SOPP had a OHC value of 1.32 ± 0.21 g oil/g pectin, which was higher and lower than that OHC value of the *Opuntia ficus indica* cladodes pectin (1.23 ± 0.42 g oil/g pectin) and pistachio green hull pectin (2.02 ± 0.19 g oil/g pectin), respectively [15,26]. This parameter can also be influenced by many factors such as the total charge density of surface [27].

3.10. DPPH radical scavenging

DPPH radical scavenging is the most practical method for evaluation of the antioxidant activity of a sample. In this method, the colour of samples changes from purple to yellow when the free radicals of DPPH are scavenged by antioxidant compounds [34]. Thus, the antioxidant activity of SOPP solutions in different concentrations (1–50 mg/ml) were determined and the obtained results were illustrated in Fig. 2. According to these results, with increase in SOPP concentration up to 25 mg/mL, the antioxidant activity was increased with a steep slope. Afterwards, the antioxidant activity of SOPP solution was approximately fixed. The highest antioxidant activity of SOPP was very close to antioxidant activity of AA (ascorbic acid). In many studies were showed that the high TPC, GaLA content and the low molecular weight of pectin can have positive effect on this parameter [27].

3.11. ¹H NMR spectrum analysis

The ¹H NMR spectrum of SOPP extracted under optimal conditions is illustrated in Fig. 3. A broad and sharp peak at 3.7 ppm refers to the methoxyl group protons of the esterified galacturonic acid units. The appeared peaks around 1 ppm were probably due to the methyl group links of rhamnose [35]. The signals around 2 ppm were probably

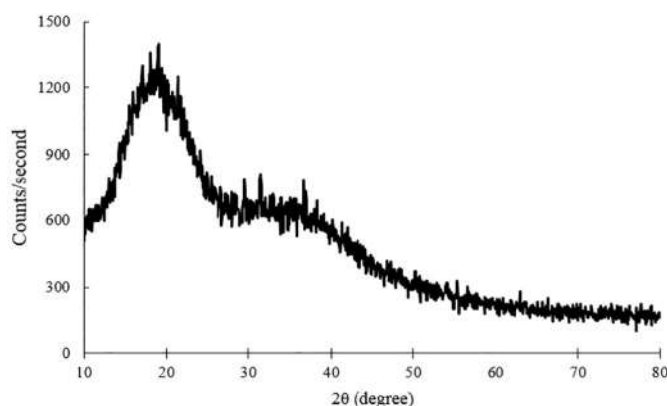


Fig. 5. XRD (X-ray diffraction) patterns of SOPP (sour orange peel pectin) obtained by UAE (ultrasound-assisted extraction) method.

derived from $-\text{CH}_3$ of the acetyl groups. The signals of 4.9 (H-5) and 5.0 (H-1) ppm belong to non-esterified galacturonic acid units [14]. The proton signals of H-1, H-2, H-3, and H-4 of galacturonate and methyl galacturonate residues were observed at 5.0, 3.6, 3.9 and 4.4 ppm, respectively. Thus, the above-mentioned results confirmed the presence of the pectin structure in extracted sample and were in agreement with previous studies [14,28,35,36].

3.12. FT-IR spectrum analysis

The infrared spectrum of SOPP extracted under optimal extraction conditions was illustrated in Fig. 4. The characteristic chemical shifts of 3360 , 2920 and 1201 cm^{-1} were due to inter and intramolecular hydrogen of $\text{O}-\text{H}$, $\text{C}-\text{H}$ of CH_2 and CH_3 , and $\text{C}-\text{O}-\text{C}$ of glycoside compounds [27,28]. The chemical shifts of 1646 and 1734 cm^{-1} , as the proprietary signals of pectin structure, are attributed to the free and esterified carboxyl groups, respectively. The average of the signal of 1734 cm^{-1} over the sum of the peaks of 1734 and 1646 cm^{-1} is considered as DE. Thus, in accordance with the results obtained from Section 3.3, the SOPP can be categorized as LMP. Also, the observations confirmed the presence of pectin in the extracted sample.

3.13. XRD pattern analysis

To provide more information about pectin structure (amorphous or crystalline), the XRD pattern of SOPP extracted under optimal extraction conditions was exhibited in Fig. 5. As can be seen, SOPP showed several sharp and intense peaks at 17° , 18° , 19° , 21° , 24° , 29° , 31° and 36° (2θ) which are due to crystallinity of it. However, the amorphous structure is also observed in this spectrum. Thus, it could be concluded that SOPP had both crystalline and amorphous portions in its structure. This result was in line with the XRD result of pectin extracted from *Musa sapientum* L. [37].

4. Conclusion

In this paper, BBD was used for pectin extraction optimization from sour orange peel by ultrasound waves. Also, the physicochemical, structural and functional properties of SOPP in optimum extraction point were evaluated. The results showed that the optimized yield ($28.07 \pm 0.67\%$) was achieved in ultrasound power of 150 W , irradiation time of 10 min and pH of 1.5 . In these optimal conditions, SOPP was categorized as LMP, which confirmed by FTIR and ^1H NMR results. Also, the galacturonic acid and glucose contents of pectin were 65.3 and 0.4% , which showed the SOPP has a suitable purity. In addition, the emulsifying activity of SOPP extracted by ultrasound waves was higher than some other pectin sources and emulsions were more stable in low temperature. The antioxidant activity of SOPP in concentration of 25 mg/mL was approximately similar to ascorbic acid. In final, it seems that the ultrasound waves had a high efficiency in terms of quantity and quality of the extracted pectin from sour orange peel. These waves by the cavitation effect improve the destruction of plant cell wall and increase the rate of mass transferring and thereby the extraction yield of pectin with a shorter extraction time. Thus, UAE can be a suitable alternative to conventional approach.

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