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Original research

Optimization of microwave assisted acid extraction and characterization of pectin from saffron flower waste (*Crocus sativus* L.)

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A B S T R A C T

Saffron flower waste is a new source of pectin and response surface methodology (RSM) was used to optimize the microwave-assisted acid extraction process. The optimal extraction conditions of pectin obtained from saffron flower waste were in microwave power of 700 W, irradiation time of 2.43 min, and pH of 1.5. The physicochemical and functional properties of pectin was evaluated and the results demonstrated that pectin extracted under optimal conditions was low methoxyl with emulsifying activity of 67.97%, surface activity of 36.89 mN/m and 33.24 mN/m at 0.10 and 0.50 % w/v, total phenolic content (TPC) of 2.86 mg EGA/g pectin and degree of esterification (DE) of 40.99%. In addition, the FT-IR and ^{1}H-NMR spectra were used to identify the functional groups existing in the structure of extracted pectin.

Keywords: Saffron flower; Pectin; Optimization; Microwave-assisted extraction; RSM

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1. Introduction

Saffron (*Crocus Sativus Linnaeus*) is an herbaceous and perennial herb belonging to the *Iridaceae* family. The most important part of this plant is a red color stigma, which is sold as the most expensive spice in the world (Melnyk et al., 2010; Garavand et al., 2019). Saffron is cultivated in several countries including Iran, Greece, Turkey, Spain, Morocco, and India (Pitsikas, 2016; Shahi et al., 2016). Annual saffron production across the world is more than 220 tons. After the separation of the stigma, other parts of the flower like petals, sepal, and anther are discarded as a by-product. Typically, to produce 1 kg of dried stigmas, about 80 kg of petals is discarded as waste (Gracia et al., 2009; Kakouri et al., 2017). Recently, studies have focused on antioxidant activity, anti-microbial and therapeutic properties, as well as the use of anthocyanins of petal saffron to make natural pigment in the food and pharmaceutical industries (Jafari et al., 2016; Khazaei et al., 2016).

Pectin is a complex combination of polysaccharides that is present in the wall and middle lamella of cell plants. The major structure of pectin consists of homogalacturonan and rhamnogalacturonan, and mainly composed of D-galacturonic acid, L-rhamnose and neutral sugars including L-ramnose, L-arabinose, and D-galactose (Adetunji et al., 2017). Homogalacturonan consists of liner-linked galacturonic acid α (1→4), some of which are methylated or acetylated (Marić et al., 2018). Depending on the degree of esterification (DE), pectin is categorized into two sets, high methoxyl (DE> 50%) and low methoxyl (DE <50%) (Lu et al., 2019). It is known as a hetero polysaccharide (heteroglycans) that is broadly applied in food systems, such as stabilizing, emulsifying and thickening agents (Ren et al., 2019). The most important commercial origins for extracting pectin are apple pomace, citrus peel, and sugar-beet pulp. To this point, pectin has also been extracted from a large number of non-commercial sources to meet the industrial need, including mango peel, watermelon rind, banana peels, and kiwi fruit pomace (Rehman et al., 2019).

Recently, some new methods including ultrasound and microwave-assisted extraction or a combination of two techniques have been developed for pectin extraction (Grassino et al., 2016; Swamy & Muthukumarappan, 2017; Yang et al., 2019).

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Microwave-assisted extraction is the new techniques of pectin extraction from agricultural products. The main advantages of this method in comparison with traditional ones (acidified hot water) are shorter process time, less solvent consumption, higher production efficiency, higher quality of product and lower cost. The factors affecting the extraction efficiency in this method are microwave power, processing time (radiation), pH, and liquid-to-solid ratio (LSR) (Maran et al., 2013; Košťálová et al., 2016).

Response surface methodology (RSM) is used as an efficient method to optimize processes in food industries. It is particularly important in situations that multiple independent variables affect the desired responses. Optimal conditions can be achieved with minimal tests and the lowest cost. RSM is applied to design the experiments, build the model and predict and optimize the effect of different conditions (factors) on the response (Pasandide et al., 2017; Niknam et al., 2020).

In recent years, the particular focus has been made on pectin extraction from agricultural by-products. In this case, saffron flower waste (SFW) can be considered as a novel source. A significant approach in this study compared to other studies conducted in recent decades is the use of a new source of pectin from saffron flower waste, while in other researches the extraction of pectin from fruits and vegetables or peel waste has been conducted and so far, research in the field pectin was not extracted from the petals. Therefore, current study aims to optimize the conditions for the microwave-assisted extraction of pectin from SFW via RSM and then to investigate the physico-chemical aspects of extracted pectin under optimal conditions.

2. Material and Methods

2.1. Materials and Chemicals

Saffron flowers were harvested from Torbat-e-Heydarieh, Iran. After separation of the stigmas, other components of the flower (petal, anther, stem, and sepal) were dried using a hot air dryer (60°C, 1.5 ms^{-1}) to obtain equilibrium. Then the samples were powdered using the electrical milling (IKA-Werke M20, Germany) and screened with a 50 mesh sieve. The obtained powder stored in polyethylene bags and stored in dark and freeze (-18°C) condition. All chemicals, including citric acid, sodium carbonate, hydrochloric acid, sodium hydroxide, phenolphthalein, sulfuric acid, phenol, sodium azide, Folin-ciocalteu reagent, and DPPH, were analytical grade and purchased from Merck and Sigma companies.

2.2. Microwave-assisted extraction process

The extraction process of pectin from SFW was conducted according to a method described by Swamy and Muthukumarappan (2017). The extraction conditions are shown in Table 1. After extraction, samples were filtered and centrifuged at 10000×g for 15 min. Then, the ethanol (96%) was added (with the ratio of 1:1) to the samples and stored in a refrigerator (5°C for 24 h). The obtained pectin was filtered and washed 4 times with ethanol 80% to remove impurities (Due to the high levels of anthocyanins in the petals and its extraction under acidic conditions, the color of pectin was slightly dark red, which was removed by washing several times with alcohol to omit impurities. The washing process was continued until the color of the alcohol became completely clear). Finally, the samples were dried at 45°C for 24 h. The extraction yield was measured using the following equation (Eq. 1):

$$\text{Extaction yield } (\%) = \frac{X}{X_0} \times 100 \quad (1)$$

where X is the weight (g) of the purified pectin, and X_0 is the weight (g) of the initial dry powder.

2.3. Experimental design

To get to the best LSR, the one factor at a time experimental design was used. The effect of variation in LSR (10, 15, 20 and 25 v/w) was examined when the others including microwave power, irradiation time and pH were kept constant at 500 W, 2 min and 2, respectively. After selecting the best LSR, this factor was kept constant at 20 v/w in all experiments. To evaluate the effect of other variables including power (W), irradiation time (min) and pH on extraction yield, a Box-Behnken design (BBD) with three variables in three levels (-1, 0, +1) was applied (Table 1). It must be noted that the range of values selected for each variable was determined by primary experiments that were done before starting the main evaluation (data not shown).

Table 1. Levels and values for independent variables.

Variables		Assigned code and true values for variables		
		-1	0	+1
Power (watt)	(X_1)	300	500	700
Time (min)	(X_2)	1	2	3
pH	(X_3)	1	2	3

2.4. Degree of esterification (DE)

The DE of pectin was measured using the method defined by Sayah et al. (2016) with some modifications. Briefly, 100 mg of dried pectin was wetted with 2 mL of ethanol 96%. Next, 20 mL of CO_2 – free water (40°C) was added to the solution. Then, three drop of phenolphthalein reagent was added to the solution and titrated with sodium hydroxide (NaOH, 0.1 N) until the appearance of pink color. The consumed volume was recorded as V_1. Then, 10 mL of sodium hydroxide was added to the solution and stirred for 20 min. At this point, 10 mL of hydrochloric acid (HCl, 0.1 N) was added up to it and mixed until the pink color vanished completely. Consequently, the remained hydrochloric acid was titrated with sodium hydroxide and the consumed volume was recorded as V_2. The DE of pectin was calculated according to the following equation (Eq. 2):

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

2.5. Chemical composition of dried pectin

Moisture content was determined using an oven at 105 ± 2ºC for 24 h. Ash content measured according to standard method (AOAC, 1995). Protein content of pectin was estimated using the Kjeldahl method (AOAC, 1995). The carbohydrate content was calculated via phenol-sulfuric acid technique (Fishman et al., 1999).

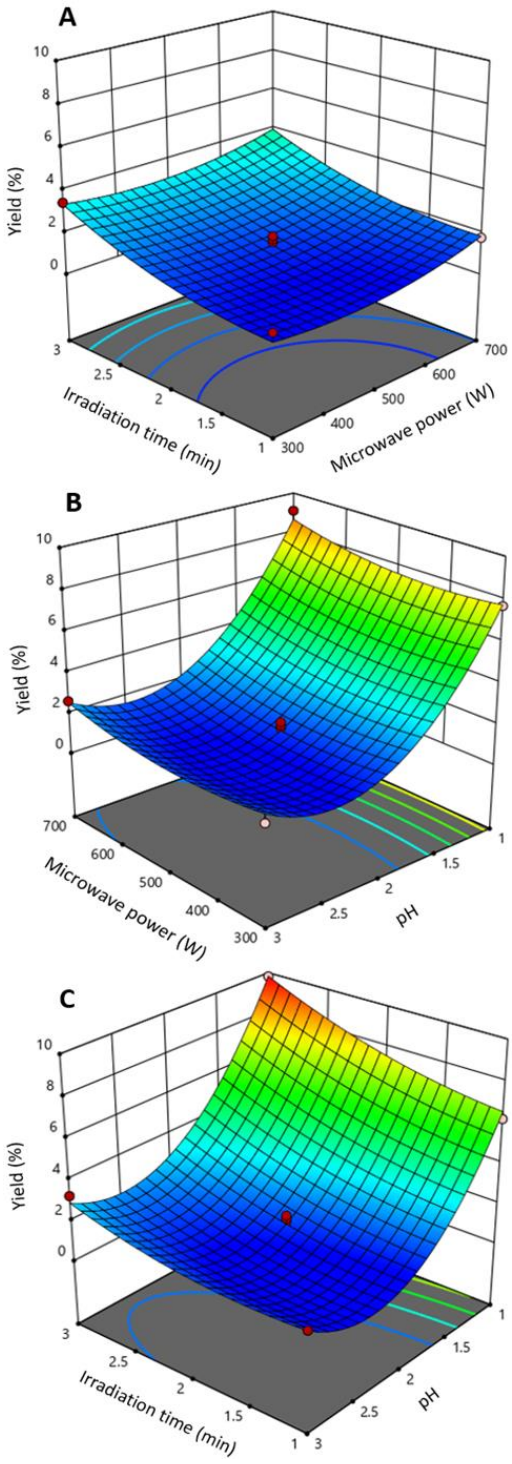

Fig. 1. 3D response plots for the effect of variables on the extraction yield of SFW pectin.

2.6. Emulsifying activity (EA) and emulsion stability (ES)

To do these, the technique defined by Hosseini et al. (2019) was applied. To prepare an emulsion, 5 mL of sunflower oil was added to a 5 mL pectin solution of 0.5% (w/v) containing 0.02% sodium azide (to prevent the growth of microorganisms). Next, the pre-emulsions were mixed by a rotary homogenizer (T25 Ultra-Turrax, IKA, Germany) at 10000 rpm for 4 min and centrifuged at 2000×g for 10 min. The EA was calculated as follows:

$$\text{EA (\%)} = \frac{\text{Height of the emulsion layer}}{\text{Total height}} \times 100 \quad (3)$$

Also, to study the stability of the emulsion, a similar technique was applied for preparation of emulsion. Afterward, the samples were stored for one and 30 days at a temperature of 5 and 25 °C, respectively. The ES was calculated by the following equation:

$$\text{ES (\%)} = \frac{\text{The height of the remaining emulsion layer}}{\text{The initial emulsion layer height}} \times 100 \quad (4)$$

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

Evaluation of these parameters was performed according to the technique designated by Bayar et al. (2017) and Niknam et al. (2020). Briefly, 10 mL of distilled water or sunflower oil (with a density of 0.9 at 25°C) were added to the 100 mg of pectin powder in graduated tubes. The samples were incubated for 15 min at room temperature and then every 5 minutes, the tubes were vortexed for 15 s. Next, the samples were centrifuged for 20 min at 2000×g. Finally, the supernatant was separated and the remaining residue (wet solid) was weighted. The value of WHC and OHC was estimated by dividing the weight of the sludge (sediment) from the centrifuge to the original weight.

2.8. Surface tension

The surface tension of pectin solution (0.1, 0.5, and 1.0% w/v) was estimated by a tensiometer machine (Nanometric-Pazhoh, Iran) with a Wilhelmy plate method. Distilled water was used for calibration of the device and as a reference (Munoz et al., 2007).

2.9. Viscosity measurement

Different concentrations of the pectin (0.1, 0.5, 1.0, and 1.5% w/v) were prepared in distilled water. Then the solutions incubated at 7°C for 24 h for complete hydration. The viscosity of different concentrations of pectin was measured using rotating viscometer (Brookfield, Pro II-LVDV., USA) with LV spindle at room temperature. Experiments were conducted at shear rates from 0 to 100 s^{-1} with 5 s intervals. Finally, the viscosity and shear stress were plotted against shear rate and the obtained results were fitted with power law model as follow:

$$\sigma = k(\gamma^{\cdot})^n \quad (5)$$

where σ is the shear stress (Pa), $\gamma^{\cdot}$ is the shear rate (s^{-1}), k is the consistency index ($Pa.s^n$), and n is the flow behavior index (Saberian et al., 2017).

2.10. DPPH radical scavenging activity

The method reported by Sridhar et al. (2019) was used to investigate the antioxidant activity. In summary, 2 mL of the solution at different concentrations (1.0-6.0 mg /mL) was added to 3 mL ethanolic DPPH solution (0.0039 g in 100 mL ethanol 96%). The mixtures were vortexed and incubated for about 30 min at room temperature. Finally, the absorption of the samples was

measured at 517 nm via spectrophotometer (SP-UV 500DB, Germany). The DPPH radical activity was estimated as follows:

$$\text{DPPH}^{\cdot}\ \text{scavenging activity}\ (\%) = \frac{A_0 - A_s}{A_0} \times 100 \quad (6)$$

where A_0 is the absorbance of the DPPH solution (blank), and A_s is the absorbance of the sample.

2.11. Total phenolic content (TPC)

The Folin-Ciocalteu method was used according to Katirci et al. (2018) with some modifications. In summary, 500 µL of pectin solution (1.0 %w/v) was prepared and 2.5 mL of Folin-Ciocalteu reagent (diluted with distilled water 1:10) was added and incubated for about 3 min at room temperature. Then 2 mL of sodium carbonate solution 20% was added and incubated for 1 h, at ambient temperature. The absorbance was measured at 760 nm. Gallic acid was used as standard (R^2 = 99.97) and the TFC of pectin was expressed as milligram Gallic acid equivalents/gram of pectin (mg GalAE/g).

2.12. FT-IR analysis

Pectin extracted under optimal conditions was applied for FT-IR analysis. FT-IR spectroscopy of pectin was conducted using Bruker FT-IR Tensor 27 spectrometer with potassium bromide (KBr) method in the wavenumber range of 400 – 4000 cm^{-1}.

2.13. Nuclear magnetic resonance (NMR) spectrum

Pectin extracted under optimal conditions was used for NMR analysis. Briefly, 20 mg of powdered pectin was transferred to the glass flask, then 1 mL of D_2O was added. The mixture was stirred for 1 h at 40°C using a magnetic stirrer. Then, the prepared solution with low viscosity was transferred to the specific tube of NMR. ^{1}H-NMR analysis was carried by the Varian Unity Inova 500 MHz spectrometer in internal temperature of 25°C. 128 scans were collected with a relaxation delay of 1s and an acquisition time of 2.04 s.

2.14. Statistical analysis

All experiments were performed in triplicates under optimal conditions, and results were expressed as mean ± standard deviation. All figures in this study were plotted using the statistical software Design Expert 7.0 and OriginPro.

3. Results and Discussion

3.1. Optimization of microwave-assisted extraction

3.1.1. Analysis of regression model

Optimization by Box-Behnken design was done to evaluate the most appropriate conditions for maximizing extraction yield. Experimental and predicted values for the response are demonstrated in Table 2. This table indicates that the pectin extraction yield was ranged between 0.94 and 10.82%. Additionally, by resolving the suggested model for the extraction yield (Eq. 7), the highest extraction yield of SFW pectin was 10.27% under optimum extraction conditions (microwave power of 700 W, time of 2.43 min and pH of 1.5). The second-order equation of the extraction yield based on the codded values was:

$$\begin{aligned}\text{Yield}\ (\%) &= 1.41 + 0.365\,X_1 \\ &+ 1.13\,X_2 - 2.97\,X_3 - 0.07\,X_1X_2 - 0.09\,X_1X_3 - 0.50\,X_2X_3 \\ &+ 0.56\,X_1^2 + 0.55\,X_2^2 + 3.28\,X_3^2 \end{aligned} \quad (7)$$

Table 2. BBD design and corresponding results of extraction yield of SWF pectin (A: microwave power, B: irradiation time, C: pH).

Standard order	A (W)	B (min)	C	Actual value	Predicted value	Residual
1	500	2	2	1.04	1.41	-0.3660
2	500	1	3	1.66	1.64	0.0150
3	500	3	1	10.82	10.83	-0.0150
4	500	2	2	1.84	1.41	0.4340
5	700	2	3	2.62	2.56	0.0600
6	700	3	2	3.54	3.95	-0.4150
7	500	3	3	3.26	2.90	0.3550
8	700	2	1	9.10	8.67	0.4300
9	500	2	2	0.94	1.41	-0.4660
10	500	2	3	1.58	2.01	-0.4300
11	500	2	2	1.67	1.41	0.2640
12	500	1	1	6.22	6.57	-0.3550
13	700	1	2	1.76	1.83	-0.0750
14	500	2	2	1.54	1.41	0.1340
15	300	2	1	7.70	7.76	-0.0400
16	300	1	2	1.38	0.96	0.4150
17	300	3	2	3.44	3.36	0.0750

Table 3. Analysis of variance (ANOVA) and regression coefficients of calculated model.

Source coefficient	Sum of squares	df	MS	F-value	p-value
Model	132.70	9	14.74	64.02	<0.0001
A – Microwave power	8.07	1	8.07	34.78	0.0085
B – Irradiation time	10.22	1	10.22	44.35	0.0003
C - pH	70.33	1	70.33	305.34	<0.0001
AB	0.0196	1	0.0196	0.0851	0.7790
AC	0.0324	1	0.0324	0.1407	0.7187
BC	0.9568	1	0.9568	4.34	0.0757
A^2	1.35	1	1.35	5.88	0.0458
B^2	1.31	1	1.31	5.67	0.0488
C^2	45.22	1	45.22	196.31	<0.0001
Residual	1.61	7	0.2303		
Lack of fit	0.9852	3	0.3284	2.09	0.2437
Pure error	0.6271	4	0.1568		
Total	134.31	16			
R^2	0.9880				
Adj-R^2	0.9726				
Pred-R^2	0.9053				

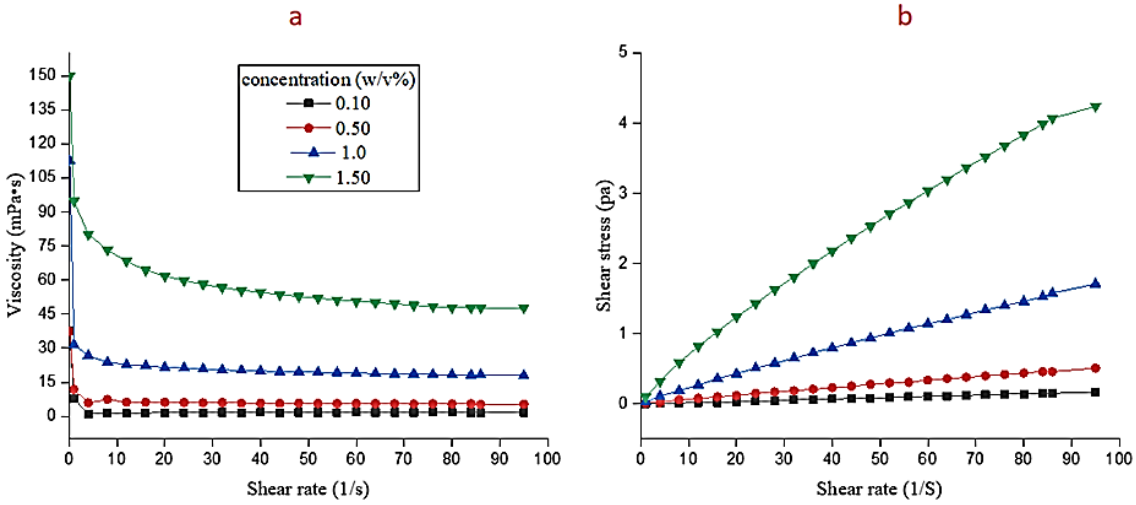

Fig. 2. The flow behavior of different concentrations of SFW pectin.

To ensure that the obtained equation is sufficiently accurate, the optimal extraction conditions were repeated three times. The mean of these three replicates was 10.27 ± 0.74%. Therefore, this result demonstrated that the suggested model is actually appropriate for prediction of the extraction yield. Additionally, this value was higher than pectin obtained from sources like banana peel (2.18%) (Swamy & Muthukumarappan, 2017) and dragon fruit peel (7.5%) (Thriugnanasambandham et al., 2014) and lower than orange peel pectin (19.24%) (Maran et al., 2013). The experimental data was assessed by ANOVA and the significance of the regression coefficients were computed by their corresponding p-values (Table 3). The high F-value (64.02) and the low p-value ($p < 0.001$) indicate that the proposed model was extremely significant. The R^2 and adj-R^2 values were 0.9880 and 0.9726 respectively, which showed adequacy of developed models. Additionally, lack of fit value was insignificant (0.243), which confirmed that the experimental data was well fitted with the quadratic polynomial model.

3.1.2. The effect of extraction variables on extraction yield of SFW pectin

As can be seen in Fig. 1, all three variables were notably affecting the extraction yield but pH plays a greater role and therefore choosing a suitable pH is crucial. The results showed that extraction process was more effective at lower pH value, which is confirmed by previous studies (Kazemi et al. 2019; Maran et al., 2013). It can be explained by higher solubility of pectin at lower pHs, which led to increase of pectin entry into the extraction medium (Maran et al., 2013). As can be seen in the Fig. 1, by increase in the microwave power from 300 to 700 W, the extraction yield is increased. It can be stated that, microwave power is one of the crucial factors that governs the extraction efficiency. The higher extraction yield at increased microwave power could be related to the destruction of the plant matrix wall especially middle lamella, which might increase the pectin release to the medium (Kazemi et

al. 2019). Also, by elevating the time up to 180 s, the extraction yield was increased which could be associated with increasing the expose time of SFW against microwave irradiation (Thriugnanasambandham et al., 2014).

Table 4. Chemical composition of SFW pectin extracted under optimum conditions.

Chemical composition (%)	Values (%)
Moisture	7.61±0.14
Ash	1.72±0.29
Protein	8.79±0.24
Total sugar	60.77±1.99
Surface tension (mN/m)	
0.1 (%w/v)	36.89±0.08
0.5 (%w/v)	33.24± 0.23
1.0 (%w/v)	34.31±0.16

3.2. *Investigation of the chemical composition of dried pectin*

The results of these parameters are demonstrated in Table 4. The moisture content and ash content of pectin obtained from SFW were lower than pistachio green hull pectin (12.2% and 5.16%) (Kazemi et al., 2019) and orange peel pectin (8.81% and 1.89%) (Hosseini et al., 2019). While the amount of protein was much higher than pistachio green hull (6.3%) and also pectin extracted from eggplant peel (2.53%) (Kazemi et al., 2019). The protein content depends on many factors such as extraction method and the source of pectin. Additionally, the main part of protein present in the SFW is highly soluble in acidic pH (Yuliarti et al., 2015; Venkatanagaraju et al., 2019). Also, applying microwave and destruction of the cell wall during extraction could cause the release of protein compounds into the solution. The amount of protein that is allowed in 100 g of pectin is 15.6 g which is reported by Food and Agriculture Organization (FAO JECFA, 2009). The total sugar content of obtained pectin was 66.77±1.99%, which was lower than that from *Opuntia ficus indica* cladodes pectin (89.94%) and higher than that from pomace of eleven apple cultivars (39.13%) respectively (Bayar et al., 2018; Sato et al., 2011).

3.3. *Degree of esterification (DE)*

One of the factors useful in determining the suitability of pectin for use in food formulation is the DE value. DE of pectin depends on the extraction conditions and its source (Azad et al., 2014). Among the extraction parameters, pH is one of the most important parameters affecting the DE of the obtained pectin in which by decreasing pH value, the degree of esterification is also reduced. It must be noted that, in particular extraction conditions such as long irradiation time, low pH, and high microwave power, desertification of the galacturonic acid chain occurs (Kazemi et al., 2019). In current study, DE of extracted pectin under optimal conditions was 40.99 ± 0.31%, which indicates that it could be classified as low DE pectin. By comparing the results of other studies, DE of pectin obtained from SFW is higher than that for pectin extracted from orange peel (37.5%) (Hosseini et al., 2016), *Opuntia ficus-indica* cladodes (35.04%) (Bayar et al., 2018), pistachio green hull (12.1%) (Kazemi et al., 2019), and also potato pulp (32.58%) (Yang et al., 2019).

3.4. *Emulsifying properties of SFW pectin*

The value of emulsifying activity, which, by definition, is the volume of the emulsion layer on total volume, was 67.99 ± 2.26%. This value was greater than that of pistachio green hull (58.30%), eggplant peel (56.16%) (Kazemi et al., 2019), and *Citrus medica* peel (46.5%) (Pasandide et al., 2017), respectively. This could be due to the presence of protein in structure (polysaccharide – protein interaction). The stability of emulsion in 1 and 30 days is shown in Table 5. According to the results, it can be concluded that the stability of the emulsions is higher at 5°C than the ambient temperature. These results are similar to the values reported by (Yapo et al., 2007).

3.5. *WHC and OHC of SFW pectin*

These parameters are defined as the ability of hydrocolloids to absorb and hold water and oil. They are influenced by the total charge density of surface and the hydrophilic- hydrophilic characteristics of hydrocolloid. The water absorption capacity of a hydrocolloid depends on the structural constituents, physical properties, porosity, and particle size (Kazemi et al., 2019; Bayar et al., 2018). In current study, the measured WHC was 8.77 ± 0.27 g of water / g of pectin under optimal conditions, which is much higher than those reported for pectin extracted from *Opuntia ficus indica* cladodes (5.64) (Bayar et al., 2018) and eggplant peel (6.22) (Kazemi et al., 2019). This could be due to the presence of free hydroxyl groups in the pectin structure and also high protein content of sample (Elleuch et al., 2011). The ability of absorbing and holding oil is another important characteristic of polysaccharides. Density, thickness, and particle size are the factors that could affect oil holding capacity (Chau & Huang, 2003). The results indicated that the OHC of SFW pectin was 2.58±0.1 g of oil /g of pectin, which was higher than those reported for pectin extracted from *Opuntia ficus indica* cladodes (Bayar et al., 2018) and eggplant peel (Kazemi et al., 2019) that were 1.23 and 2.12 g of oil /g of pectin, respectively.

Table 5. Emulsifying activity and emulsion stability of pectin extracted under optimum conditions.

Emulsification activity (%)		Emulsion stability (%)			
Storage time	0	1 day		30 day	
Temperature (°C)	25	5	25	5	25
	67.97±2.26	68.56±3.76	66.82±5.03	63.23±1.27	57.41±3.21

3.6. Surface properties of SFW pectin

As shown in the Table 4, by increasing concentration of the pectin from 0.1 to 0.5% w/v, the surface tension decreased from 36.89 to 32.24 mN/m. The surface tension of solution slightly increased from 33.24 ± 0.23 to 34.31 ± 0.16 mN/m when the pectin concentration increased from 0.5 to 1.0% w/v. This could be explained by the viscosity elevation in solution and also the ability of pectin to reduce surface tension. The presence of acetyls and proteins in pectin structure is one of the reasons that cause pectin to be expressed as a surface-active agent (Yapo et al., 2007). Compared to other studies, the surface tension of SFW pectin was lower than pistachio green hull (49.75 and 46.23 mN/m at 0.1 and 0.5 %w/v) and watermelon rind (40.17 mN/m at 4.0 % w/v) (Petkowicz et al., 2017).

3.7. Flow properties of SFW pectin

Viscosity can be defined as the resistance of a fluid against the shear stress and the resistance of the fluid layers against sliding (Khan et al., 2015). Molecular weight, degree of esterification, methyl ester groups concentration, and pH are significant determinants elements of the viscosity of the pectin solutions (Do Nascimento Oliveira et al., 2018). In current research, various concentrations of pectin (0.1, 0.5, 1.0, and 1.5% w/v) were prepared. According to Fig. 2a, by increasing pectin concentration, the viscosity also increased; all solutions showed the shear thinning behavior and their viscosity decreased as shear rate was increased. The well-known pseudoplastic behavior was more evident for the solution containing higher amounts of pectin. As demonstrated in Fig. 2b and Table 6, the flow index values were less than 1 for all solutions, which authenticated the obtained results about the shear-thinning behavior. Similar results were reported by Hosseini et al. (2016) and Chen et al. (2014). Formation of weak physical and chemical bounds among the pectin chains and destruction of such bounds at high shear rates can explain the pseudo plastic behavior of the pectin solution at higher concentrations. However, at low concentrations, the distance between the pectin biopolymers is high, and thus, the aforementioned bounds are much lower, therefore they show the Newtonian behavior (Hosseini et al., 2016; McClements, 2004).

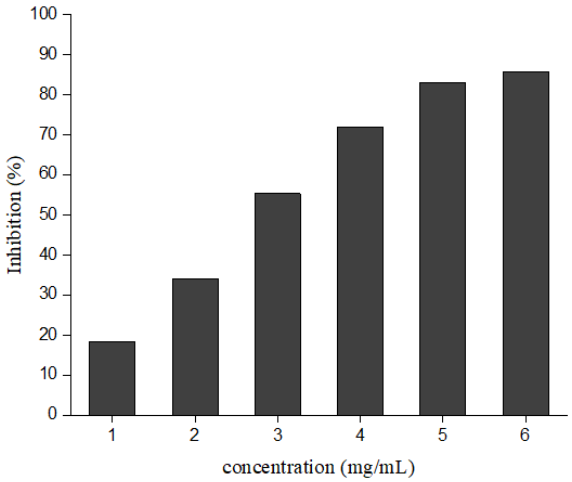

Fig. 3. DPPH free radical scavenging activity of different concentrations of SFW pectin.

3.8. Antioxidant activity and TPC of SFW pectin

Phenolic compounds with antioxidant properties may have a vital role in preserving food products and maintaining human health. Saffron petals contain flavonoids, phenolic, and anthocyanin, which are well-known natural antioxidant (Ahmadian-Kouchaksaraie et al., 2016; Karimi et al., 2010). Based on the results shown in Fig. 3, the DPPH free radical scavenging activity of different concentrations of pectin was in the range of 18.41-85.74% and IC_{50} of 2.93 mg/mL was also obtained. The IC_{50} value of SFW pectin was lower than value reported for pectin obtained from *Opuntia ficus indica* cladodes (8 mg/mL) (Bayar et al., 2017) and more than pectin extracted from eggplant peel (1.39 mg/mL) (Kazemi et al., 2019), respectively. The high antioxidant activity of the solutions can be attributed to the existence of the phenolic compounds of obtained pectin under optimal conditions (2.86 ± 0.20 mg GalAE/g pectin). High concentration of phenolic compounds directly enhances the ability of different extracts to inhibit free radicals.

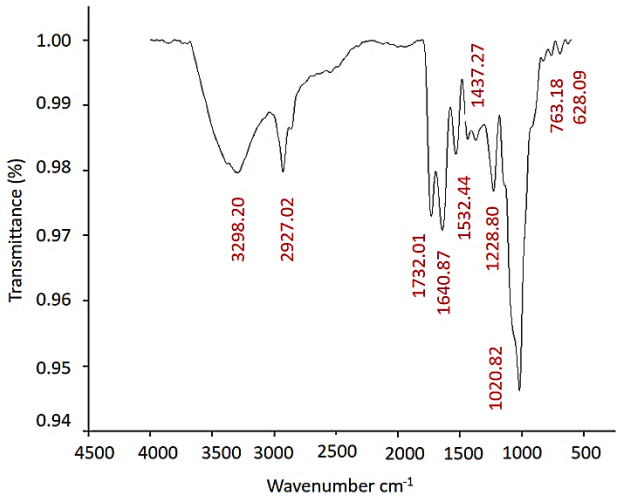

Fig. 4. FT-IR spectrum of SFW pectin.

Table 6. The power low parameters for SFW pectin at different concentrations.

Concentration (%w/v)	K (Pa.s^n)	n	R^2
0.1	0.002	0.941	0.974
0.5	0.008	0.905	0.991
1.0	0.030	0.883	0.999
1.5	0.103	0.826	0.999

3.9. FT-IR spectroscopy

FT-IR spectroscopy is one of the most common techniques to identify the structure (functional groups) of pectin. The FT-IR spectrum of extracted SFW pectin (pectin extracted under optimal condition, including, power of 700 W, irradiation time of 2.43 min, and pH of 1.5) is shown in Fig. 4. The peak at 3298.20 cm^{-1} is related to the stretching absorption of the OH group (Wang et al., 2016). The resulting peak at the 2927.02 cm^{-1}, actually relates to

the aliphatic stretching vibration of C-H, which includes the bending and stretching vibrations of $–CH_2$. Also, the peak appearing at 1732.10 is connected with the vibration of $O\text{-}CH_3$ (Pappas et al., 2004). The stretching vibrations at 1640.87 cm^{-1} are related to the C=C groups. The absorption peaks in the region of 1437.27, 1371.84, and 1228.80 cm^{-1} are associated with the bending vibrations of CH_2, OH, and CH_3CO. The fingerprint area is considered for a range from 900 - 1200 cm^{-1} (Kpodo et al., 2017). A strong absorption peak at 1020.82 cm^{-1} region is associated with the linked glycosides in D-arabinofuranose (Bayar et al., 2017). The band with peak at 628.09 cm^{-1} is associated with low frequency vibrations of pyrenoid ring, i.e., pectin ring skeletal C–C deformation vibrations (Grassino et al., 2016). Absorption at the region of 1000 to 1250 cm^{-1} attributes to C-O-C vibrations of glycoside bonds (Kazemi et al., 2019).

3.10. ^{1}H-NMR spectrum analysis of SFW pectin

The ^{1}H-NMR spectrum of the SFW pectin is demonstrated in Fig. 5. As can be seen there, the most chemical shifts appear in the area of 2.84-4.18 ppm. The strong peak detected in the 3.75-3.84 ppm area is the probability the proton from the esterified units of galacturonic acid (-OCH3) (Kpodo et al., 2017). In this spectrum, the signal appeared at 3.67 ppm could be known as remaining galacturonate (Hosseini et al., 2019). It is also suggested that the signal in about 2-3 ppm is due to the presence of the proton group of $R_2N\text{-}CH_3$ (C is attached to N). The signals of 1.18 ppm were probably derived from branched and unbranched methyl group links of rhamnose (Kazemi et al., 2019). Probably signals at 1.19 ppm were derived from methoxyl groups O-2,4 linked L-rhamnose and the signal are found at 1.29 ppm in this spectrum, is methylene groups ($R\text{-}CH_2\text{-}R$) (Wang et al., 2016).

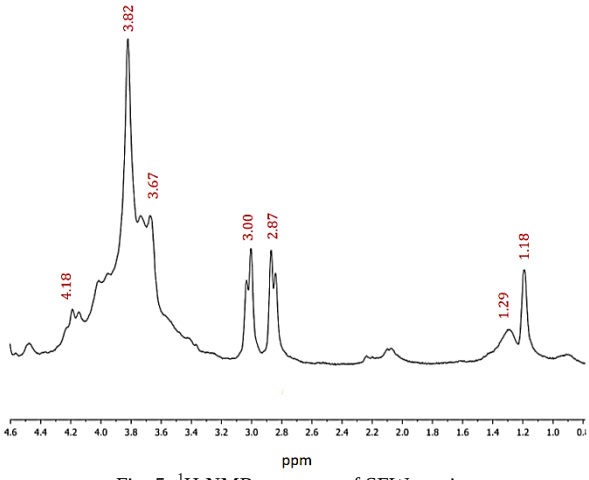

Fig. 5. ^{1}H-NMR spectrum of SFW pectin.

4. Conclusion

The maximum extraction yield of SFW pectin was 10.27% that has obtained at LSR of 20 v/w, microwave power of 700 W, pH of 1.5 and 2.43 min irradiation. The extraction yields successfully modeled using RSM methodology, which showed that pH was the most determining factor for pectin extraction. The pectin solutions indicated pseudo plastic behavior at all concentrations. The ^{1}H-NMR and FT-IR spectrum confirmed the presence of esterified pectin in obtained samples. Additionally, the WHC and OHC of the obtained pectin were 8.77 g of water/g of pectin, 2.58 g of oil/g of pectin, respectively. Consequently, pectin extracted from saffron flower waste may be proposed as a proper, cheap and new source of pectin with appropriate functional and chemical properties that could be used in food formulations.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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