



An integrated valorization of industrial waste of eggplant: Simultaneous recovery of pectin, phenolics and sequential production of pullulan

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ABSTRACT

Based on a bio-refinery concept, the valorization of eggplant peel wastes (EPW) in the production of multiple value-added products was aimed. The acid-free extraction process was applied in the simultaneous recovery of pectin and phenolic compounds. The extraction variables were optimized by response surface methodology using a Box-Behnken design and the maximum yield of pectin (26.1%) and phenolic compounds (20.2%) was obtained in the extraction temperature of 90 °C, time of 90 min and liquid/solid ratio of 40 mL/g. After recovery of pectin and phenolic compounds from EPW, the solid leftovers were enzymatic hydrolyzed and the hydrolysates were used as a carbon source in the microbial production of pullulan by *Aureobasidium pullulans*. The produced pectin and pullulan were characterized through the chemical and structural features. The results of FT-IR and H-NMR analysis approved the predominant presence of these two polysaccharides in the isolated samples. On the other hand, the antioxidant activity of the recovered phenolic compounds extract was evaluated by DPPH and ABTS radical scavenging activity and reducing power assay.

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1. Introduction

The food processing industry produces a high volume of agricultural wastes composed of rejected or inedible plant tissues such as peels with a high amount of water content and organic matter (Philippi et al., 2016). The disposal of these wastes in landfills causes a severe risk to the local watercourses, production of uncontrolled greenhouse gas and other serious environmental problems. In addition, the increasingly tighter regulations regarding the handling of these wastes and also the demand for sustainable food processing procedures, are changing the food processing sector toward eco-friendly strategies (Arancon et al., 2013).

Food waste valorization is one of the high value options which gained great attention as a high potential alternative for the disposal of agri-food wastes in landfills. This procedure is based on the use of wastes as an inexpensive, rich in organic materials and abundant source in the production of high value-added products (Nizami et al., 2017).

Eggplant (*Solanum melongena* L.) is a widespread vegetable crop with dark purple skin known as top 10 vegetables in term of antioxidants content (Philippi et al., 2016). The eggplant peel waste (EPW) is the major by-product of this vegetable which mostly gen-

erated by food processing sectors such as vegetable canning factories (during production of various canned eggplant products such as eggplant meal, pickled eggplant, stuffed eggplant, etc.) and disposed in landfills as a waste, while it is a great source of polyphenolic substances and carbohydrates such as pectin and cellulose (Kazemi et al., 2019a).

Antioxidant components are microconstituents present in the food products with the ability to scavenge free radicals, inhibit lipid oxidation and oxidizing chain reactions. Phenolic compounds have redox properties that allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating agents (Boulekbache-Makhlouf et al., 2013). The use of these components in food formulations has beneficial effects on human health such as antioxidant, anticancer, anti-atherosclerosis, anti-inflammatory, anti-influenza, and prebiotic activities. Pectin is a commercial heteropolysaccharide widely used in food (like gelling, emulsifying, stabilizing and thickening agent), cosmetic (such as anti-aging agent) and pharmaceutical (like drug encapsulating agent) industries. Beyond the technological benefits, it is very useful in human health, as it binds to the cholesterol and reduces its absorption in the blood. Also, inhibiting metastasis, inducing apoptosis of human cancer cell and stimulating the immune response are other positive effects of it on human health. These technological and health benefits lead to a rise in the worldwide demand for it (4–5% growth per annum) (Hosseini et al., 2019). However, the

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majority of sugars of EPW are composed of cellulosic glucose that makes it an interesting source for the production of biochemical materials such as pullulan (Talekar et al., 2018). Pullulan is an exopolysaccharide produced by yeast-like fungus *Aureobasidium pullulans*. The previous studies exhibited that it can have very applications in food industry as starch replacer (Hassannia-Kolae et al., 2016), edible coating agent and stabilizing agent (Hilares et al., 2019), pharmaceutical products as sustained-release formulations, and cosmetic emulsions (Singh et al., 2008).

Thus, EPW represents a potential resource for the production of multiple value-added products. Also, this procedure provides many advantages including the production of multiple value-added materials, maximum use of EPW and minimum generated wastes

during the process. In fact, the use of EPW in the production of multiple value-added materials will promote innovation in food waste bio-refineries, which serves the food processing sector due to economic and environmental benefits by giving a sustainable process for the valorization of the wastes (Boukroufa et al., 2015). Besides, the use of EPW in the production of pullulan after recovery of pectin and phenolic compounds will be much more efficient because these two substances prevent the access of enzymes to the cellulosic part of the plant cell wall and the microbial growth, respectively (Talekar et al., 2018).

In this work, for the first time, an integrated bio-refinery concept was used to produce multiple value-added products from EPW. Thus, simultaneous extraction optimization of pectin and

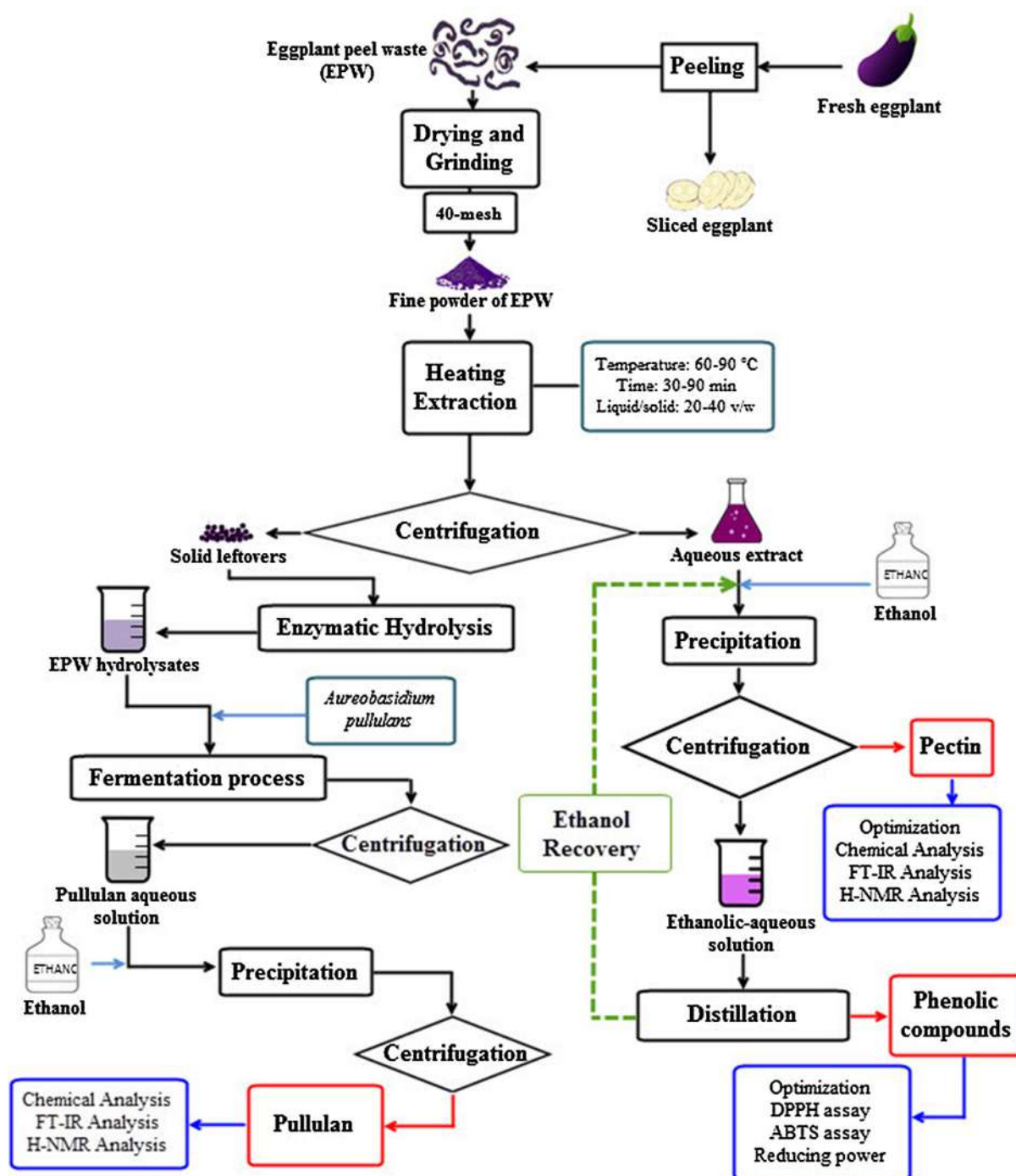


Fig. 1. Graphical scheme of EPW (eggplant peel waste) valorization process in this study.

phenolic compounds from EPW was performed using Box-Behnken design and response surface methodology. After this step, the leftovers of EPW were enzymatically hydrolyzed and used for the production of pullulan using *A. pullulans*. Also, the produced value-added materials were characterized (See Fig. 1).

2. Materials and methods

2.1. Raw material (EPW) and chemicals

The dark skinned eggplant peels were obtained from Kadbano Co. (Iran). The peels were washed by tap water, cut into small pieces and dried in an oven (50 °C, 48 h). The dried peels were grounded using a lab mill and sieved through a 40-mesh sieve. The obtained fine powder was homogenized and stored in a dark dry place until next uses.

Hydrochloric acid (37%), sulfuric acid (95–97%), sodium hydroxide (≥99%), Folin-Ciocalteu reagent, DPPH (2,2-Diphenyl-1-Picrylhydrazyl), ABTS reagent and sodium tetraborate were purchased from Merck Chemical Co. (Darmstadt, Germany). Ethanol (96%) was obtained from Ghadir Co. (Tehran, Iran).

2.2. Simultaneous extraction of pectin and phenolic compounds

For this purpose, the heating extraction method was used for the recovery of pectin and phenolic compounds from EPW. Response surface methodology (RSM) was applied to optimize the extraction conditions of heating extraction method (temperature, extraction time and liquid/solid ratio (LSR)) using Box-Behnken design (BBD) (Hamidi et al., 2019; Talekar et al., 2018; Pasandide et al., 2018). The extraction factors and their levels were selected in the best extraction range after several pre-tests, aiming the maximum yield of pectin and phenolic compounds. The studied factors were temperature (60, 75 and 90 °C), extraction time (30, 60 and 90 min) and LSR (20, 30 and 40 mL/g), which performed in a 15 experiment design including 12 factorial and 3 centering points (Table 1). The extraction process for each condition was repeated three times and the mean value was considered as response. It is noteworthy that the solvent (distilled water) was acidified using citric acid and fixed at pH of 2.5 during the extraction process. The extraction yields (based on dry weight) of pectin

and phenolic compounds were considered as responses to the design. The heating extraction process was performed in a conical flask in a water bath with continues stirring under various extraction conditions according to Table 1. After extraction process, in each run the samples were centrifuged (10,000g, 20 min) to separate the solid (EPW leftovers) and liquid phase. The solid phase was dried (45 °C), sieved (40-mesh) and stored in a dry place until next uses. The liquid phase was precipitated by ethanol (96%) at a volume ratio of 1:1 (ethanol:liquid phase) and placed at 4 °C for 18 h. The coagulates were separated by centrifugation (10,000g, 20 min), washed three times with ethanol and dried in an oven (45 °C, 16 h). Then, the remained water-ethanol solution was used for the total phenolic content (TPC) measurement using Folin-Ciocalteu reagent (Ainsworth and Gillespie, 2007) and a UV-Vis spectrophotometer device (Spectrum SP-UV500DB). Gallic acid was used as standard (R^2 of the standard curve was 0.9847) and the phenolic compound was expressed as gallic acid equivalent (GAE). The extraction yield of pectin and phenolic compounds was expressed as grams of pectin or phenolic compound per 100 g of EPW powder.

The experimental data were fitted to a quadratic polynomial mathematical equation in order to reveal the interaction between extraction variables and to optimize the extraction process considering the pectin and phenolic compounds as responses. The quadratic polynomial model in generalized form was given as follow:

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

where Y is the predicted response, X_i and X_j are independent variables. β_0 , β_j , β_{jj} and β_{ij} are the model coefficient of linear, quadratic and interaction terms, respectively, and k is the number of independent variables (in this study, $k = 3$). All the statistical analysis of the experimental data were carried out using Design Expert 7.0 software (Stat-Ease Inc., Minneapolis, USA) and Excel software.

2.3. Enzymatic hydrolysis of EPW and pullulan production

The leftovers of EPW obtained after recovery of pectin and phenolic compounds at optimal extraction condition were used for pullulan production by *A. pullulans*. At first, the solid leftovers of

Table 1
Experimental and predicted results for the optimization of pectin and phenolic compounds (TPC) extraction.

Factors			Unit	Actual levels			
				−1	0	+1	
Temperature (X_1)			°C	60	75	90	
Time (X_2)			min	30	60	90	
LSR (X_3)			mL/g	20	30	40	
				Pectin yield (%)		TPC yield (%)	
Run No.	X_1	X_2	X_3	Exp.	Pred.	Exp.	Pred.
1	60	30	30	4.2	4.4	14.8	15.0
2	90	30	30	1.6	1.7	4.7	4.9
3	60	90	30	7.0	6.9	8.8	8.7
4	90	90	30	15.3	15.1	14.4	14.2
5	60	60	20	9.4	9.0	8.2	8.2
6	90	60	20	8.6	8.3	5.0	5.1
7	60	60	40	8.4	8.7	11.8	11.7
8	90	60	40	14.6	15.0	10.2	10.2
9	75	30	20	8.6	8.8	8.6	8.4
10	75	90	20	9.6	10.1	2.2	2.4
11	75	30	40	5.8	5.3	5.5	5.3
12	75	90	40	20.1	19.9	14.1	14.2
13	75	60	30	3.2	3.0	3.7	3.2
14	75	60	30	2.3	3.0	2.4	3.2
15	75	60	30	3.4	3.0	3.4	3.2

EPW were subjected to enzymatic hydrolysis using Viscozyme® L (Sigma-Aldrich, St. Louis, Missouri, US) cellulolytic enzyme mixture (the enzymatic activity of Viscozyme® L was 125 FBG/ml). Briefly, an aqueous mixture of powdered EPW leftovers (3% w/v) was prepared in a shaking flask and the pH and temperature of the mixture were adjusted to 5.0 and 50 °C, respectively. Then, the enzymatic hydrolysis was performed using enzyme loading of 12.5 FBG/g of EPW and treatment time of 24 h (Talekar et al., 2018). After the hydrolyzation process, the mixture was centrifuged (10,000g, 20 min) and the supernatant was employed as a carbon source for microbial production of pullulan.

A wild strain of *Aureobasidium pullulans* KY767024 was donated from the Department of Food Science and Engineering, University of Tehran (Karaj, Iran) (Hamidi et al., 2019). The microorganism was stored in 4 °C on potato dextrose agar medium until use. The inoculum was grown in a rotary shaker at 28 °C and 180 rpm for 48 h in 250 mL Erlenmeyer flasks containing 100 mL of medium composed of 20 g/L of glucose, 20 g/L of peptone, 10 g/L yeast extract and 20 g/L malt extract.

Fermentation processes were carried out in 150 mL Erlenmeyer flasks containing 50 mL of enzymatic hydrolysate of EPW leftovers (3% w/v), supplemented with 5 g/L of KH_2PO_4 , 1 g/L of NaCl, 0.6 g/L of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g/L of ZnSO_4 , 0.01 g/L of MnSO_4 , 0.01 g/L of FeSO_4 and 4 g/L of yeast extract, inoculated with a specific volume of inoculum (5% v/v) at the temperature of 28 °C, pH of 5.5 and different fermentation periods (1–10 days). In the next step, the centrifuged broth samples were precipitated with 1:1 vol of ethanol and placed at 4 °C for 18 h. The coagulated pullulan was isolated by centrifugation (10,000g, 20 min), and dried in an oven (45 °C, 16 h). The pullulan production by *A. pullulans* was investigated during 10 days and the best fermentation period was selected considering the production yield. Afterward, the recovered pullulan at the best period was used in the next experiments.

2.4. Characterization of pectin and pullulan

2.4.1. Chemical composition

The ash and moisture content of the isolated pectin and pullulan was evaluated as the method described by Hosseini et al. (2019). The protein content of pectin and pullulan was determined using the Kjeldahl method considering $\text{N} \times 6.25$.

2.4.2. Galacturonic acid (GalA) content of pectin

GalA content of recovered pectin in the optimum extraction conditions was estimated by the meta-hydroxydiphenyl method using a UV–Vis spectrophotometer (Blumenkrantz and Asboe-Hansen, 1973). In brief, 1 mL of pectin solution (200 µg/mL) was prepared in 10 mL tube and mixed with 6 mL of H_2SO_4 containing 0.0125 M sodium tetraborate. The tube was cooled in an ice-water bath, agitated with a vortex mixer and heated in a boiling water bath for 5 min. After cooling, 20 µL of the meta-hydroxydiphenyl reagent was added to the mixture. The tube was vortexed for 5 min and the absorbance of the mixture was measured at 520 nm. The GalA content of pectin was estimated using the standard curve of standard GalA solution (with an R^2 value of 0.9974).

2.4.3. DE measurement of the isolated pectin

DE of pectin samples was measured using the titrimetric method (Kazemi et al., 2019a). Briefly, 20 mL of pectin solution (0.5% w/v) was mixed with 2 mL of ethanol and 3 drops of phenolphthalein reagent. The mixture was titrated with NaOH (0.1 M) (V_1). Then, the mixture was mixed with 10 mL of NaOH (0.1 M) and stirred for 20 min. Afterward, 10 mL of HCl (0.1 M) was added to the mixture and stirred until the pink color disap-

peared. Then the prepared mixture was titrated with NaOH (0.1 M) (V_2). The DE of pectin was estimated as follow:

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

2.4.4. Fourier transform-infrared (FT-IR) spectroscopy

The isolated pectin and pullulan were characterized using FT-IR spectroscopy and compared with the commercial pectin and pullulan. FT-IR spectra of samples were collected in KBr pellets on a Bruker FT-IR Tensor 27 spectrometer (Billerica, Massachusetts, USA) over a wavelength ranged from 600 to 4000 cm^{-1} and resolution of 4 cm^{-1} .

2.4.5. Hydrogen-1 nuclear magnetic resonance (^1H NMR) spectroscopy

^1H NMR spectra of pectin and pullulan were recorded by a Varian Unity Inova 500 MHz NMR spectrometer (Palo Alto, CA, United States). 8 scans were recorded for each sample in the internal temperature of 24 °C and acquisition time of 4.00 s.

2.5. Characterization of phenolic compounds

2.5.1. DPPH radical scavenging activity

The DPPH radical activity of the extract sample was evaluated using the method described by Bayar et al. (2017). Briefly, 1 mL of the extract sample in the different concentrations (5–250 µg GAE/mL) was added to the 4 mL of DPPH methanolic solution (0.1 mM). The prepared mixture was vortexed and incubated for 30 min in room temperature. The absorbance of the mixture was recorded using a UV–Vis spectrophotometer at 517 nm. For better comprehension, the standard samples of ascorbic acid (AA) and butylated hydroxyanisole (BHA) in similar concentrations (5–250 µg/mL) were used and compared to the antiradical activity of the extract sample. The DPPH radical scavenging activity of samples was estimated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} : \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100 \quad (3)$$

2.5.2. ABTS⁺ radical scavenging activity

The ABTS⁺ radical scavenging activity of the extract sample in different concentrations (5–250 µg GAE/mL) was evaluated (Chang et al., 2019). Briefly, the ABTS stock solution (7.4 mM) was mixed with potassium persulfate (2.6 mM) in phosphate buffered saline (pH 7.4) and kept at room temperature for 18 h. Then, the prepared ABTS⁺ solution was diluted with distilled water until its initial absorbance at 734 nm was 0.7 ± 0.02 . In the next step, 500 µL of the extract sample was mixed with 500 µL of diluted ABTS⁺ and incubated for 10 min at 37 °C. The absorbance of the mixture was recorded at 734 nm using a UV–Vis spectrophotometer. For better understanding, standard samples of AA and BHA in the same concentrations (5–250 µg/mL) were used and compared to the antiradical activity of the extract sample. The ABTS⁺ radical scavenging activity of samples was calculated by the following equation:

$$\text{ABTS radical scavenging activity (\%)} : \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100 \quad (4)$$

where A_{sample} and A_{control} was the absorbance of ABTS⁺ solution mixed with sample and distilled water, respectively.

2.5.3. Reducing power assay

Reducing power of the extract sample in different concentrations (5–250 µg GAE/mL) was evaluated (Mohammadian et al., 2019). Briefly, 0.5 mL of extract sample was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL potassium ferricyanide (1% w/v). The prepared mixture was incubated (50 °C, 20 min), charged with 1.25 mL of trichloroacetic acid (10% v/v) and centrifuged (1500g, 10 min). Then, 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of ferric chloride (0.1% w/v) and incubated for 10 min in room temperature. The absorbance of the mixture was recorded at 700 nm using a UV–Vis spectrophotometer. In this method, higher absorbance indicates a higher capacity of sample to reduce the ferric iron (Fe^{3+}) to its ferrous form (Fe^{2+}). For comparison, the standard samples of AA and BHA in the same concentrations (5–250 µg/mL) were used.

3. Results and discussion

3.1. Simultaneous optimization of pectin and phenolic compounds extraction

The response surface function of Box-Behnken design was used to optimize the various factors (each in three levels) of pectin and phenolic compounds extraction including temperature (60–90 °C), extraction time (30–90 min) and LSR (20–40 mL/g). All of the 15 experiments performed to recover pectin and phenolic compounds from EPW and the obtained results were exhibited in Table 1. The highest recovery yield of pectin and TPC was observed in run number 12 (20.1%) and 1 (14.8%), while the lowest recovery was related to run number 2 (1.6%) and 13 (2.4%), respectively. The optimum extraction conditions for maximizing the yield of pectin and TPC were estimated at temperature of 90 °C, extraction time of 90 min and LSR of 40 mL/g. The predicted optimum point of pectin and TPC, which obtained by the suggested models (Eqs. (5) and (6)) had a pectin yield of 27.4% and TPC yield of 21.8%. To verify the prediction of these models, the extraction process was repeated three times in the optimum conditions and the experimental mean yields for pectin and TPC were $26.1 \pm 2.7\%$ and $20.2 \pm 1.8\%$, respectively. The obtained results assured that the proposed quadratic polynomial models for pectin and TPC yield were very suitable and the mean experimental yields were in good accord with the predicted yields (Kazemi et al., 2019c). The quadratic polynomial models of pectin and TPC yield (based on dry weight) including the main and interaction effects were shown below in Eqs. (5) and (6), respectively.

$$\begin{aligned} \text{Yield of pectin (\%)} = & 2.97 + 1.39X_1 + 3.98X_2 + 1.59X_3 \\ & + 1.64X_1^2 + 2.42X_2^2 + 5.64X_3^2 \\ & + 2.73X_1X_2 + 1.75X_1X_3 + 3.33X_2X_3 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Yield of TPC (\%)} = & 3.18 - 1.15X_1 + 0.75X_2 + 2.17X_3 \\ & + 4.36X_1^2 + 3.16X_2^2 + 1.26X_3^2 + 3.90X_1X_2 \\ & + 0.405X_1X_3 + 3.74X_2X_3 \end{aligned} \quad (6)$$

The analysis of variance (ANOVA) for the proposed regression models of pectin and TPC were represented in Table 2. Both of the quadratic models of pectin and TPC were significant at low p-value (<0.0001) and high F-value (119.39 and 112.77). For the models of pectin and TPC, high value of R^2 (0.9954 and 0.9951, respectively) demonstrates the high precision of the proposed models (Maran et al., 2017) and imply that only 0.46 and 0.49% of all variations could not be explained by these models based on the obtained R^2 . In addition, the insignificant p-value (0.516 and

0.902) of lack of fit test for the models of pectin and TPC exhibits the exactness of the models and imply that the proposed models were extremely well fitted. Considering the given results, it should be stated that both of the quadratic polynomial models were suitable representing the relationship between extraction factors and responses (pectin and TPC yield) (Moorthy et al., 2017).

According to Table 2, all of the independent variables of the extraction process were effective on the dependent responses. Due to the high coefficient value (Eqs. (5) and (6)) of extraction time (X_2) and LSR (X_3) in linear term; LSR (X_3^2) and temperature (X_1^2) in quadratic term; interaction between time and LSR (X_2X_3) and interaction between temperature and time (X_1X_2) in interaction term were the most effective variables of pectin yield and TPC yield, respectively. For better understanding, three dimensional (3D) plots were provided (Fig. 2) to show the effect of independent process variables on the dependent responses (pectin and TPC yield). The increase in temperature during the extraction process enhances solubilizing of cell wall pectin in the medium by breaking the hydrogen bonding between pectin and plant cell wall components and thereby, increases pectin yield (Maran et al., 2017). It is noteworthy that, the leak of pectin from cell wall with increasing temperature, causes disruption in cell structure, open up the cell wall, improve the release of phenolic compounds and finally, increase in TPC yield (Talekar et al., 2018). Also, the swelling and loosening effects caused by the thermal extraction process, could increase the diffusivity and penetration of water into the matrix of EPW and in result, improve pectin and TPC yield (Moorthy et al., 2017). In this study, the extraction yield is affected by the extraction time, thoroughly with an increase in extraction time, the extraction yield was increased. The increase in heating time improves the swelling and hydration of EPW tissue, enhances the mass transferring rate and consequently, increases the yield of pectin and phenolic compounds (Hosseini et al., 2016). Fig. 2 showed that The LSR had a direct effect on the yield of pectin and TPC. Increasing this factor increases the concentration gradient of solvent (water), improves the distribution of plant material into the solvent, enhances the liberation rate of pectin and TPC into the medium, and leads to higher extraction yield (Maran et al., 2017).

3.2. Pullulan production by *A. pullulans*

In this study, the hydrolysates of EPW leftovers from simultaneous extraction of pectin and TPC were used as a carbon source in microbial production of pullulan as described in Section 2.3. The fermentation period of the pullulan production by *A. pullulans* was investigated for 10 days. According to Fig. 3, with an increase in the fermentation time up to seven days, the pullulan production was increased gradually up to a maximum production of 16.8 g/L, and then was decreased which was probably due to the hydrolysis of pullulan by the endogenous glucoamylase-A action (West and Strohfus, 1996). A similar result was observed for pullulan production by *A. pullulans* from Asian palm kernel (Sugumaran et al., 2013). The obtained pullulan in the mentioned time was isolated and characterized.

3.3. Characterization of pectin and pullulan

Chemical compositions of isolated pectin and pullulan were estimated and the results were listed in Table 3. The ash and protein content were found 3.2 and 3.4% for the recovered pectin, and 1.8 and 2.1% for the isolated pullulan, respectively. The obtained ash content of pullulan was similar to the data of produced pullulan using sucrose (1.7% and 1.8–1.9%; Vijayendra et al., 2001; Wu et al., 2010) and jiggery media (1.83%; Vijayendra et al., 2001) as a carbon source. Considering the recommendation of FAO for the protein content of pectin (lower than 15.6%; JECFA, FAO, 2009),

Table 2

Analyze of variance for the proposed model of pectin and phenolic compounds (TPC) yield.

Source	DF	Some of squares	Mean square	F-value	p-value
Pectin yield					
Model	9	384.507	42.723	119.39	<0.0001
Linear	3	161.967	53.989	150.88	<0.0001
X ₁	1	15.401	15.401	43.04	0.001
X ₂	1	126.405	126.405	353.25	<0.0001
X ₃	1	20.161	20.161	56.34	0.001
Square	3	136.364	45.455	127.03	<0.0001
X ₁ ²	1	9.951	9.951	27.81	0.003
X ₂ ²	1	21.564	21.564	60.26	0.001
X ₃ ²	1	117.520	117.520	328.42	<0.0001
2-Way interaction	3	86.175	28.725	80.27	<0.0001
X ₁ X ₂	1	29.703	29.703	83.01	<0.0001
X ₁ X ₃	1	12.250	12.250	34.23	0.002
X ₂ X ₃	1	44.222	44.222	123.58	<0.0001
Error	5	1.789	0.358		
Lack-of-Fit	3	1.103	0.368	1.07	0.516
Pure Error	2	0.687	0.343		
Total	14	386.296			
R ²			0.9954		
Adj R ²			0.9870		
Pred R ²			0.9503		
TPC yield					
Model	9	271.702	30.189	112.77	<0.0001
Linear	3	52.891	17.630	65.86	<0.0001
X ₁	1	10.615	10.615	39.65	0.001
X ₂	1	4.479	4.479	16.73	0.009
X ₃	1	37.797	37.797	141.19	<0.0001
Square	3	101.405	33.802	126.27	<0.0001
X ₁ ²	1	70.118	70.118	261.93	<0.0001
X ₂ ²	1	36.522	36.522	136.43	<0.0001
X ₃ ²	1	5.869	5.869	21.93	0.005
Interaction	3	117.407	39.136	146.19	<0.0001
X ₁ X ₂	1	60.934	60.934	227.62	<0.0001
X ₁ X ₃	1	0.642	0.642	2.40	0.182
X ₂ X ₃	1	55.831	55.831	208.56	<0.0001
Error	5	1.338	0.268		
Lack-of-Fit	3	0.285	0.095	0.18	0.902
Pure Error	2	1.053	0.527		
Total	14	273.041			
R ²			0.9951		
Adj R ²			0.9863		
Pred R ²			0.9746		

the results showed that the isolated pectin had good purity due to the low ash and protein content. The ash and protein content of isolated pectin from EPW was significantly lower than eggplant peel pectin extracted by microwave-assisted method (9.03 and 9.13%; [Kazemi et al., 2019b](#)) and slightly higher than eggplant peel pectin extracted using ultrasonic method (2.41 and 2.53%; [Kazemi et al., 2019a](#)) in the previous studies. In addition, the isolated pectin had a GalA content of 69.7%, which was slightly higher than the obtained value for eggplant peel pectin extracted by ultrasound-assisted (66.08%; [Kazemi et al., 2019a](#)) and microwave-assisted method (67.4%; [Kazemi et al., 2019b](#)). According to the FAO recommendations, the pectin with a GalA content lower than 65% should not be used in the food and pharmaceutical systems. Therefore, the resulting pectin, in addition to its high purity due to low ash and protein content, also has an acceptable level of GalA based on FAO recommendation ([Khamsucharit et al., 2018](#)). The recovered pectin under optimum condition had a DE value of 60.2%, which was categorized as pectin with high methoxyl groups (HM). It should be noted that similar data were observed in the previous studies for eggplant peel pectin obtained by ultrasonic (61.22%; [Kazemi et al., 2019a](#)) and microwave method (68.18%; [Kazemi et al., 2019b](#)). In addition, the results of DE measurement for obtained pectin were confirmed by FT-IR and ¹H NMR spectroscopy.

The dried powder of pectin and pullulan were analyzed by FT-IR spectroscopy and compared with the commercial pectin and pullulan. The obtained spectra for pectin and pullulan were exhibited in [Fig. 4\(A and C\)](#). The characteristic signals ranged at 3000–3500 cm⁻¹ were attributed to O—H stretching and the signal at 2924 cm⁻¹ was due to the C—H stretching. The appeared signals at the range from 1000 to 1250 cm⁻¹ were originated from C—O—C stretching of glycoside bonds ([Wu et al., 2012](#)). The comparison of the isolated and commercial pullulan ([Fig. 4C](#)) showed FT-IR spectra of them were almost identical, indicating that the major components of the isolated samples was pullulan ([Sugumaran et al., 2013](#)). In the case of pectin, same results were also observed by comparing the isolated and commercial pectin ([Fig. 4A](#)) and the predominantly presence of the esterified pectin structure was confirmed in the obtained samples. In addition, the characteristic signals appeared at 1735 and 1637 cm⁻¹, which were related to the esterified and free carboxyl groups of GalA units in the pectin structure, illustrated that the DE value of isolated pectin was higher than 50% ([Kazemi et al., 2019a](#)) and confirmed the obtained DE value using titrimetric procedure ([Grassino et al., 2016](#)).

The ¹H NMR spectra of isolated pectin and pullulan were shown in [Fig. 4B and D](#), respectively. In the case of pullulan, by comparison the ¹H NMR spectra of the isolated samples with commercial pullulan (see supplementary data, [Fig. S2](#)), the presence of the

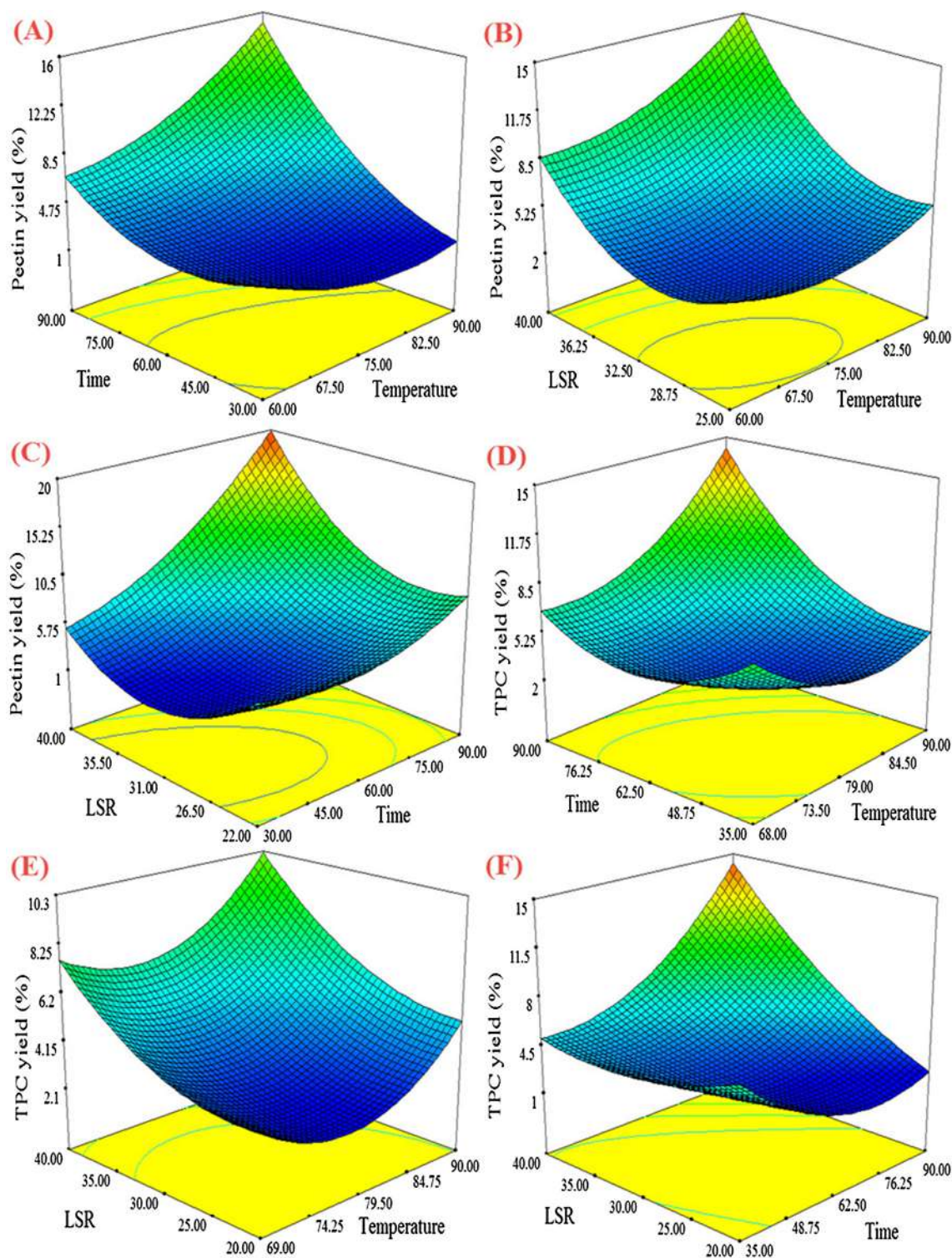


Fig. 2. 3D plots of the independent variables effect on the responses.

chemical structure of pullulan in the obtained supernatant was confirmed (Sugumaran et al., 2013; Chen et al., 2017). Also, the appeared two signals of α -1,4 glycoside bonds at 5.3 and 5.4 ppm, and one signal of α -1,6 glycoside bonds at 4.8 ppm were originated from the repeating maltotriose units in the pullulan structure (Fig. 4D) (Manitchotpisit et al., 2009). In the case of pectin spectrum, all of the signals appeared in the range between 2.1 and 5.2 ppm. The signals of the proton on C-2, C-3, C-4, C-5 and C-1

of the GalA units were detected at 3.4, 3.8, 4.1, 4.9 and 5.1 ppm, respectively. In addition, the two characteristic signals at 3.7 and 2.1 ppm were derived from the methyl ($-\text{OCH}_3$) and acetyl ($-\text{COCH}_3$) groups of the esterified GalA units in the structure of pectin and higher intensity of these two signals shows higher DE value for pectin (Khatib et al., 2017). Thus, the obtained spectrum confirmed the presence of pectin with high esterified GalA units in the produced sample (Grassino et al., 2016).

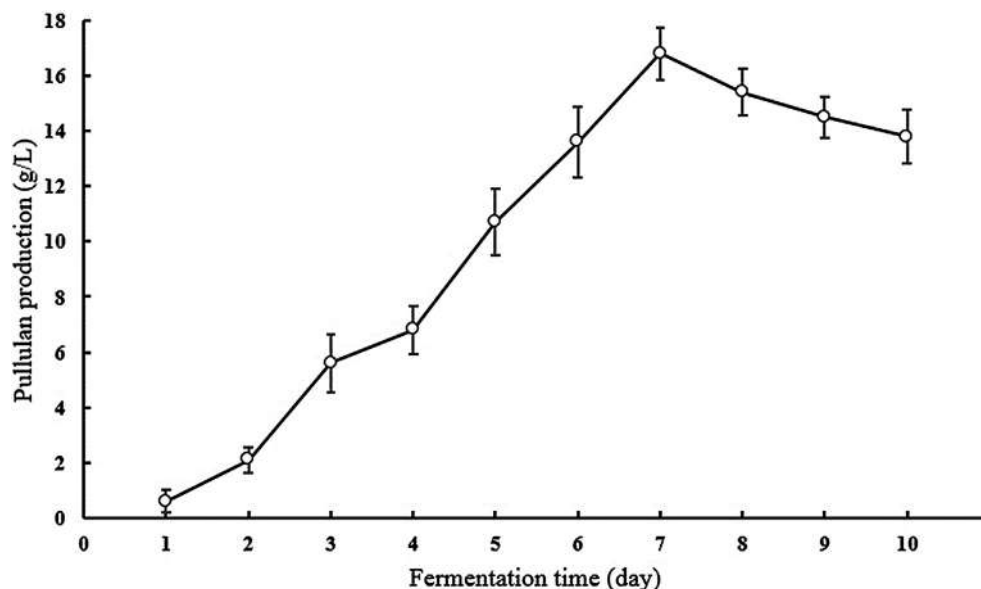


Fig. 3. The production of pullulan by *A. pullulans* from EPW leftovers during 10 days.

Table 3
Chemical composition of the isolated pectin and pullulan.

Chemical parameter	Samples	
	Pectin	Pullulan
Moisture content (%)	7.7 ± 0.9	3.5 ± 0.7
Ash content (%)	3.2 ± 0.1	1.8 ± 0.3
Protein content (%)	3.4 ± 0.8	2.1 ± 0.6
GalA content (%)	69.7 ± 2.9	–
DE (%)	60.2 ± 3.5	–

According to the mentioned results, both of the isolated samples (pectin and pullulan) are comparable with the commercial ones and both could be used as a functional ingredient in the formulation of various food systems (such as jams, dairy products and low-fat products), in the pharmaceutical purposes (such as in drug encapsulation) or cosmetic applications (Xu et al., 2014; Singh et al., 2008).

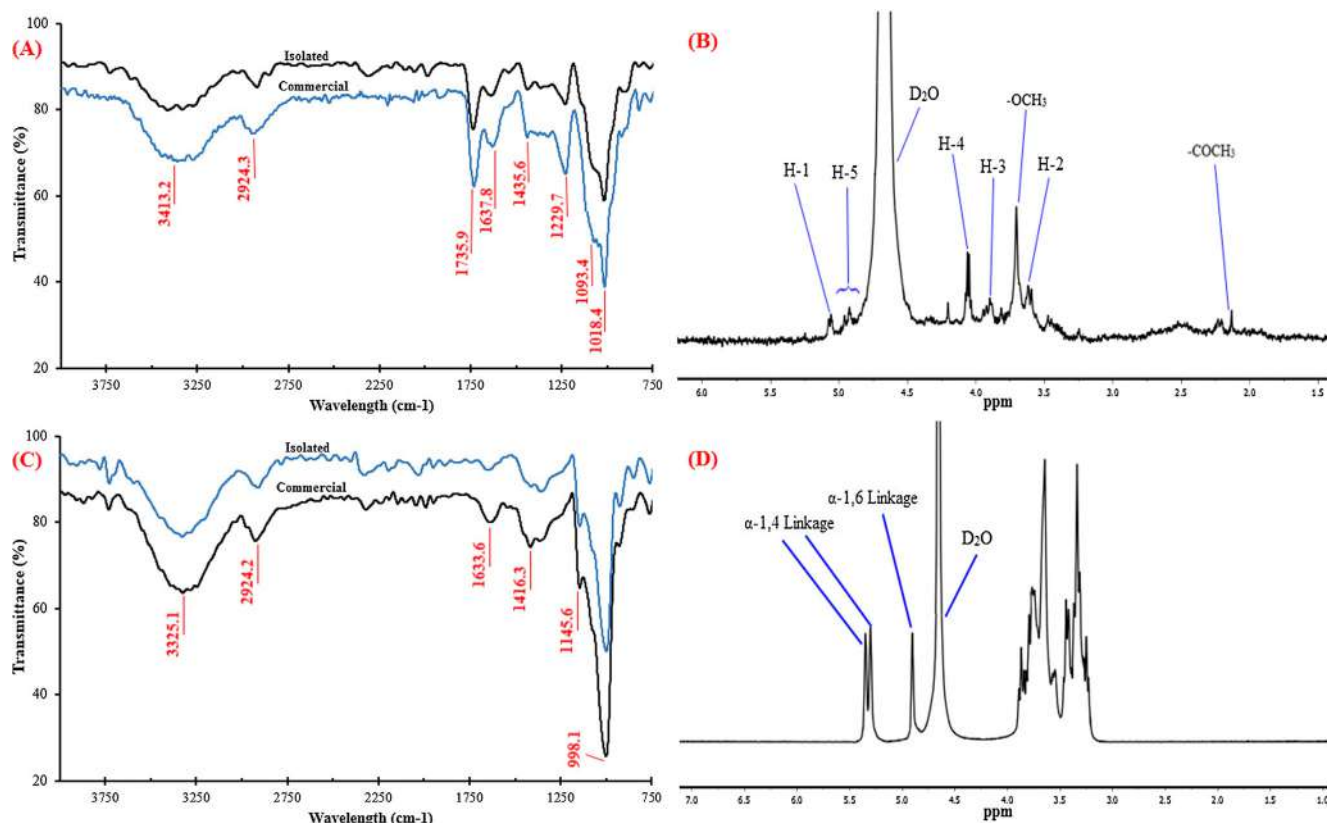


Fig. 4. FT-IR spectra of pectin (A) and pullulan sample (C); H-NMR spectra of pectin (B) and pullulan sample (D).

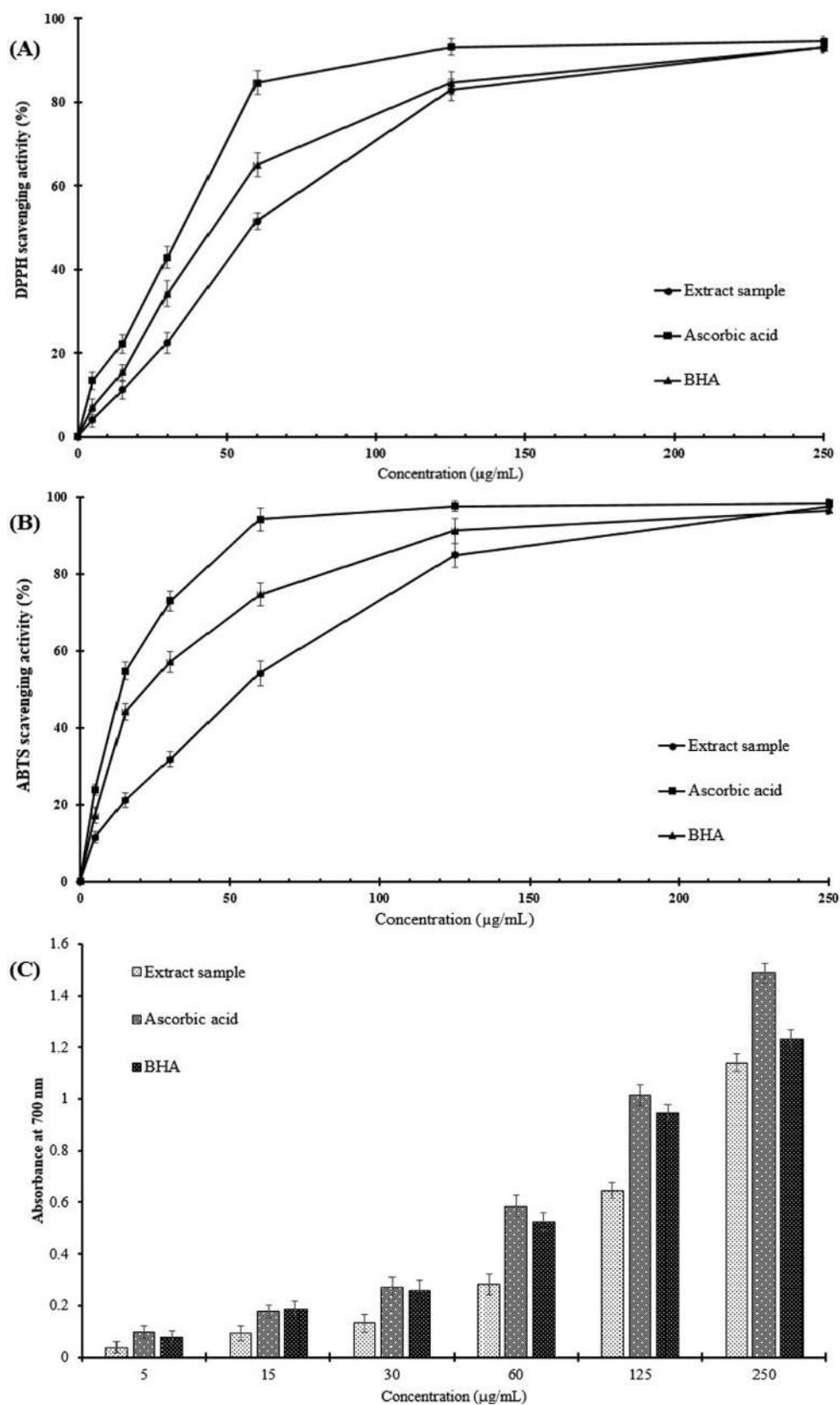


Fig. 5. DPPH radical scavenging activity (A), ABTS radical scavenging activity (B), and reducing power (C) of EPW extract sample, ascorbic acid and BHA.

3.4. Characterization of phenolic compounds extract

DPPH radical scavenging activity of extracted phenolic compounds from EPW was evaluated. In this method, DPPH molecules

(in the radical form) were scavenged by the sample and converted to its non-radical form and the color changes from dark purple to light yellow. Fig. 5A shows that with an increase in the sample concentration, the radical scavenging activity was increased and

reached its maximum activity (93.23%) in the concentration of 250 µg/mL. In addition, the DPPH radical scavenging activity of the extract sample was compared with two strong standard antioxidants including ascorbic acid (AA) and BHA (Sharma et al., 2015). The highest antiradical activity of the extract was very close to the antiradical activity of standard antioxidants, indicating notable quenching ability of the extract sample. Both of the antioxidants behaved similarly to the extract sample by an increase in concentration, but AA was the strongest. The IC₅₀ value of extract sample (70.56 µg/mL) was higher than AA (48.84 µg/mL) and BHA (61.88 µg/mL), means both standard antioxidants required a lower amount than the extract sample to scavenge 50% of the initial DPPH concentration (Sharma et al., 2015).

To further verify the antioxidant activity of the EPW extract sample, the ABTS radical scavenging activity of the EPW extract was investigated. The AA and BHA were used as standard antioxidants. The results of the ABTS radical scavenging activity were constituent with the DPPH antiradical activity and had the same dose-dependent behavior (Wang et al., 2014) as exhibited in Fig. 5B. The antiradical activity of extract sample was comparable to AA and BHA at concentration of 250 µg/mL. Also, the highest antiradical activity of the extract sample, AA and BHA were 97.63, 98.36 and 96.52%, respectively. The estimated IC₅₀ for the ABTS radical scavenging activity of the extract sample, AA and BHA were found to be 64.07, 28.40 and 43.15 µg/mL, respectively.

On the other hand, the reducing power of the extract sample, AA and BHA in the similar concentrations (5–250 µg/mL) was measured. According to Fig. 5C, all of the samples showed a dose-dependent behavior and the highest absorbance of all samples was observed in the concentration of 250 µg/mL. The strongest sample was AA and then BHA, while the extract sample showed lower reducing power in all concentrations.

The results suggest that the obtained extract sample from EPW is comparable with strong commercial antioxidants (AA or BHA) meaning the recovered phenolic compounds of EPW could be used as a strong natural antioxidant in the formulation of health-promoting products such as beverages (Dranca and Oroian, 2016).

3.5. Practical implications

Various food processing sectors globally generate food waste accounting for multi ton scale every year. Such residues create two great challenges for food industry: (i) depilation of natural resources and (ii) waste accumulation leading to rapidly escalating raw material costs, and increasingly expensive and restrictive waste disposal legislation (Pfaltzgraff et al., 2013). The key to overcome these challenges is to make use all of the valuable components in the generated waste. The re-use of food waste as an inexpensive resource for production of marketable value-added materials could help to achieve a circular economy process toward zero waste. To make it viable, the business, scientific communities and policy makers should cooperate to start a joint venture into the business of food waste valorization (Karmee and Lin, 2014). In the current study, It was demonstrated a sustainable zero waste bio-refinery process for valorization of EPW to minimize the production costs and the generated waste, and maximize the use of valuable components in EPW. However, the suggested process and results have not been tested on larger scales in the field, and therefore the future work can be mainly focused on large scale process.

4. Conclusion

This study presented an effective valorization of industrial food-waste (eggplant peel) as a considerable source of high methylated pectin and phenolic compounds and a potential low-cost carbon

source for production of pullulan using *A. pullulans*. Pectin and phenolic compounds were successfully recovered from EPW with an optimum extraction yield of 27.4% and 21.8%, respectively. Also, the solid leftovers of EPW were enzymatically hydrolyzed and then used as a carbon source in microbial production of pullulan (16.8 g/L). With respect to the obtained results, the simultaneous recovery and production of multiple value-added products could allow the maximum usage of wastes, decrease the overall cost of production and minimize the generated wastes. Thus, the suggested procedure, which is in line with bio-refinery concept, could be a promising solution for valorization of EPW. In addition, the resulting products could also be used as a food ingredient in the formulation of various food systems.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2019.09.013>.

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