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Research Article

Optimization of ultrasound-microwave assisted acid extraction of pectin from potato pulp by response surface methodology and its characterization

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ABSTRACT

The ultrasound-microwave assisted HCl extraction of pectin from potato pulp was optimized using the response surface methodology. Effects of extraction temperature, pH, and time on the yield were evaluated, and structural characteristics of pectin extracted under optimal conditions were determined. The yield was 22.86 ± 1.29% under optimal conditions of temperature 93 °C, pH 2.0, and time 50 min. The obtained pectin was rich in branched rhamnogalacturonan I (61.54 mol%). Furthermore, the pectin was a low-methoxyl (degree of methylation, 32.58%) but highly acetylated (degree of acetylation, 17.84%) pectin and the molecular weight was 1.537×10^5 g/mol. Fourier transform infrared spectroscopy and ^{1}H nuclear magnetic resonance indicated that pectin had a linear region of α-1, 4-linked galacturonic acids which could be methyl and acetyl-esterified, and rhamnose linked with galacturonic acid to form rhamnogalacturonan which was branched with side chains. Scanning electron microscopy showed most of pectin had a lamellae structure.

1. Introduction

Pectin is an acidic macromolecular polysaccharide, which exists in the middle lamella and primary cell walls and usually acts as the hydrating agent and cementing material for the cellulosic network (Muralikrishna & Tharanathan, 1994). The structure of pectin mainly consists of homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). The HG is a linear region of α-(1,4)-linked galacturonic acids (GalA), and the GalA residues can be methyl-esterified at C-6 and acetyl-esterified at O-2 and/or O-3 in different plant species (Yapo, 2011). The backbone of branched RG-I is composed of repeating disaccharide units [→4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →] and the neutral sugar side chains (including galactan, arabinan, and arabinogalactan) are linked at C-4 of the rhamnosyl (Rha*p*) residues. The GalA residues of the RG-I backbone may be *O*-acetylated at the C-2 and/or C-3 positions (Pellerin & O'Neill, 1998). The RG-II is a structurally complex galacturonan, which is substituted with disaccharides and octasaccharides, and mainly exists in the form of a dimer cross linked by a borate ester in cell walls (O'Neill et al., 1996; Pellerin & O'Neill, 1998). The proportions of HG, RG-I, and RG-II in pectin vary according to plant sources. In general, HG accounts for about 65% of pectin, while RG-I and RG-II make up around 20–35% and 10%, respectively (Mohnen, 2008).

As a natural food additive, there is a growing demand using pectin as stabilizer, gelling agent, and emulsifier in food products. Commercial pectin is usually obtained from citrus peel, apple pomace and sugar beet pulp, in addition, in order to enrich the varieties of pectin products, other fruit processing wastes, such as grapefruit peel (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011), grape pomace (Minjares-Fuentes et al., 2014), pomegranate peel (Moorthy, Maran, Surya,

Abbreviations: HG, homogalacturonan; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; GalA, galacturonic acid; Gal, galactose; Rha, rhamnose; Ara, arabinose; Glc, glucose; Xyl, xylose; Man, mannose; GlcA, glucuronic acid; UMAE, ultrasound-microwave assisted extraction; BBD, Box–Behnken design; D_2O, deuterium oxide; DM, degree of methylation; DA, degree of acetylation; NMR, nuclear magnetic resonance; MALLS, multi-angle laser light scattering; SEC, size-exclusion chromatography; RI, refractive index; FTIR, Fourier transform infrared spectroscopy; SEM, scanning electron microscopy; ANOVA, analysis of variance; SD, standard deviation; R^2, coefficient of determination; CV, coefficient of variation; Mw, weight-average molecular weight; MF, mass fraction

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Naganyashree, & Shivamathi, 2015), and banana peel (Swamy & Muthukumarappan, 2017) have also been gradually developed as new raw materials for pectin extraction.

Potato (*Solanum tuberosum*) is one of the most important food crops in China. In 2017, the production quantity of fresh potato reached to 99 million tonnes (FAO, 2017), making China the world's largest producer of potato. In addition to being used as food, potatoes are often used for processing starch, with 4.5–5.0 tonnes of fresh potato pulp by-product generated for 1 tonne of processed starch. However, only a small amount of potato pulp is exploited, and much is discarded, resulting in serious environmental pollution and wasting of resources. It was reported that potato pulp was rich in pectin (17%, dry basis) (Mayer, 1998); therefore, it is an excellent material for pectin extraction. In our previous study, potato pectin was obtained from pulp by five kinds of acids (HCl, H_2SO_4, HNO_3, citric acid and acetic acid) and the effects of the different acid extractants on the structure and emulsifying properties of pectin were analyzed. The results showed that the potato pectin was rich in a highly branched RG-I region (60.77–65.54 mol%) and its degree of acetylation (DA) was high at 9.21–15.38%. Despite potato pectin extracted by HCl exhibiting better emulsifying properties than that of citrus pectin and apple pectin, its yield (9.72%) was relatively low and extraction time (60 min) was long (Yang, Mu, & Ma, 2018). Therefore, new extraction technologies are needed to shorten the time and increase yield.

Ultrasound-microwave assisted extraction (UMAE) is a promising technology that combines the advantages of ultrasound and microwave extraction and is increasingly used for extracting some kinds of pectin. The cavitation phenomena caused by propagation of ultrasound can promote the breakdown of cell walls and increase the mass transfer rate, while the microwave can directly interact with water molecules inside cells to rapidly increase the temperature and the consequent evaporation of moisture can accelerate the rupture of cell walls to release pectin (Mandal, Mohan, & Hemalatha, 2007; Vilkhu, Mawson, Simons, & Bates, 2008). Xu et al. (2018) extracted jackfruit pectin and, compared with the conventional heating method, found that UMAE increased the pectin yield and the pectin from UMAE had stronger antioxidant abilities. The UMAE also increased the yield and improved emulsifying properties of pectin from sugar beet (Peng et al., 2015). In addition, compared with grapefruit pectin extracted only by microwave heating, when ultrasound was used to pretreat the grapefruit solution, the obtained pectin had higher yield, degree of esterification and intrinsic viscosity (Bagherian et al., 2011). These results confirmed that UMAE is an effective method to improve the yield and properties of pectin; however, to the best of our knowledge, this technology has not been applied to pectin extraction from potato pulp, and information on the structural characteristics of the potato pectin obtained from UMAE is also lacking.

In the present study, UMAE combined with HCl was used for extracting pectin from potato pulp in order to improve the extraction efficiency. The Box–Behnken design (BBD) was applied to optimize the extraction conditions of temperature, pH, and time. Furthermore, the chemical composition and structural characteristics of potato pectin extracted under the optimized conditions were investigated. The purposes of this study were to evaluate the possibility of using UMAE for pectin extraction from potato pulp and to analyze the structural features of the prepared pectin, which might make potato pulp to be exploited efficiently and rationally.

2. Materials and methods

2.1. Materials and reagents

Potatoes (Kexin No. 1) were purchased from Inner Mongolia Huaou Starch Industry Co., Ltd. (Inner Mongolia, China). Thermostable α-amylase was purchased from Novozymes A/S (activity was 120 KNU/g; Bagsværd, Denmark). The citrus pectin, the carbazole reagent (GC reagent), and monosaccharide standards including GalA, D-galactose, L-rhamnose, L-arabinose, D-glucose, D-xylose, D-mannose, and glucuronic acid were all bought from Sigma–Aldrich (St. Louis, MO, USA). The H_2SO_4 (guaranteed reagent), HCl (analytical reagent), sodium bisulfite (analytical reagent), and NaOH (analytical reagent) were bought from Beijing Chemical Works (Beijing, China). Chromatographic grade trifluoroacetic acid was bought from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Deuterium oxide (D_2O, 99 atom% D) was bought from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Sodium-3-(trimethylsilyl) propionate-2, 2, 3, 3-d4 (TSP) (> 98 atom% D) was purchased from J&K Scientific Ltd. (Beijing, China).

2.2. Potato pectin obtained by UMAE

The preparation and residual starch hydrolysis of potato pulp by thermostable α-amylase were according to the method of Yang et al. (2018). After removing the remaining starch, the final pulp was dried and ground into powder that was used for pectin extraction. The moisture content of final pulp powder was 3.98 ± 0.25%. The powder (5 g) was dissolved in ultrapure water (1:20, w/v) and the pH (1–2) of the solution was adjusted using HCl. Afterwards, the solution was transferred to a sample bottle and placed in the ultrasound-microwave cooperative extractor (CW-2000; Shanghai XTrust Analytical Instruments Co., Ltd., Shanghai, China) for 30–50 min at a temperature of 87–93 °C. The extractor could be operated at fixed ultrasound power of 50 W and adjustable microwave power of 10–800 W. The mixture was cooled to room temperature before centrifuging at 7000 × *g* for 30 min (GL-21M Refrigerated Centrifuge; Hunan Xiangyi Centrifuge Instrument Co., Ltd., Changsha, China). For the main purpose of removing salts and low molecular weight free sugars from pectin solution, the supernatant was then treated three times using the ultrafiltration membrane (molecular weight cutoff value of 10 kDa; Shanghai Diqing Filtration Technology Co., Ltd., Shanghai, China). The purified solution was collected and treated with three volumes (v/v) of absolute ethanol overnight. After that, the pectin precipitate was separated by recentrifuging the mixture under the same conditions. The precipitate was washed twice with 70% (v/v), 80% (v/v), and 90% (v/v) ethanol, respectively. Finally, the washed pectin was dispersed in ultrapure water and freeze dried (SIM-FD5, Gold SIM China Co., Ltd., Beijing, China). The yield (%) of pectin was determined as follows:

$$\text{Yield}\,(\%) = \frac{mo}{m} \times 100\% \quad (1)$$

where m_o is the mass of dried potato pectin, and m is the mass of dried pulp powder after hydrolyzing the residual starch using thermostable α-amylase.

2.3. Design of experiment

The BBD with three independent variables [extraction temperature (X_1), pH (X_2) and extraction time (X_3)] at three levels was carried out to optimize the extraction conditions of potato pectin and the yield of pectin was the response. The relationship between the coded and uncoded values of variables is as follows:

$$xi = \frac{\mathrm{X}i - \mathrm{X}0}{\Delta \mathrm{X}} \quad (2)$$

where x_i is the coded value of the variable, X_i is the actual value of the variable, X_0 is the actual value of X_i at the center point, and ΔX is the step change.

The total number of experiments (N) for BBD were determined by the formula of $N = 2p\,(p - 1) + C_p$, where p is the number of variables and C_p is the number of center points (Swamy & Muthukumarappan, 2017). Accordingly, the design consisted of 17 experiments: 12 factorial experiments and five replicates at the center point (Gan & Latiff, 2011).

Table 1
Experimental design and results of extraction yields of potato pectin.

Run	Independent variables						Experimental results	Predicted results
	x_1	X_1 (°C)	x_2	X_2	x_3	X_3 (min)	Yield (%)	Yield (%)
1	0	90.00	0	1.50	0	40.00	16.94 ± 0.83	17.29
2	−1	87.00	0	1.50	−1	30.00	18.00 ± 0.62	17.78
3	0	90.00	1	2.00	−1	30.00	15.35 ± 0.49	15.69
4	0	90.00	0	1.50	0	40.00	17.85 ± 2.31	17.29
5	1	93.00	−1	1.00	0	40.00	12.39 ± 0.30	12.51
6	−1	87.00	−1	1.00	0	40.00	13.06 ± 0.32	13.20
7	0	90.00	−1	1.00	1	50.00	12.26 ± 0.89	11.92
8	0	90.00	0	1.50	0	40.00	17.92 ± 2.46	17.29
9	0	90.00	−1	1.00	−1	30.00	12.07 ± 0.94	12.15
10	−1	87.00	1	2.00	0	40.00	16.33 ± 1.56	16.21
11	0	90.00	0	1.50	0	40.00	16.91 ± 2.69	17.29
12	0	90.00	0	1.50	0	40.00	16.85 ± 1.86	17.29
13	0	90.00	1	2.00	1	50.00	19.53 ± 3.05	19.45
14	1	93.00	0	1.50	−1	30.00	16.10 ± 2.54	15.90
15	1	93.00	0	1.50	1	50.00	21.16 ± 1.51	21.38
16	1	93.00	1	2.00	0	40.00	20.70 ± 1.27	20.56
17	−1	87.00	0	1.50	1	50.00	15.62 ± 3.01	15.82

The experiments at the center point were used to evaluate the experimental error. In order to minimize the effect of unexplained variability in the observed responses as a result of systematic errors, all experiments were performed in random order. The design and levels of uncoded and coded variables are shown in Table 1.

The second-order polynomial equation explaining the relationship between the response and variables is shown below:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (3)$$

where Y is the response; β_0 is the constant term; β_1, β_2 and β_3 are coefficients of linear terms; β_{11}, β_{22} and β_{33} are coefficients of quadratic terms; β_{12}, β_{13} and β_{23} are coefficients of interaction terms; and x_1, x_2 and x_3 are the coded values.

2.4. Characterization of potato pectin extracted under optimal conditions

2.4.1. Moisture, ash, and protein content determination

The moisture and ash content of pectin were determined through AOAC (2000). The protein content was analyzed by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978; Peterson, 1977). The BSA solutions (10–100 μg/mL) were used as standards and the concentration of potato pectin solution was 1 mg/mL. The results of ash and protein content are presented on a dry basis.

2.4.2. Monosaccharide composition of pectin

The content of neutral sugars and glucuronic acid (GlcA) was determined according to the method of Yang et al. (2018). The ICS-3000 Ion Chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a CarboPac™ PA20 analytical column (3 mm × 150 mm, Dionex) was used for quantification. Different concentrations (0.01–5 ppm) of sugar mixtures including rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), mannose (Man), and GlcA were used as standards.

The GalA content of pectin was analyzed by the carbazole–sulfuric acid method (Taylor, 1993). Different concentrations (10–90 μg/mL) of GalA solutions were used as standards and the concentration of potato pectin solution was 0.01% (w/v). The standard or sample solution (200 μL) was placed in a stoppered test tube, and 3 mL of concentrated H_2SO_4 and 100 μL of carbazole reagent (0.1%, w/v) were added. Then, the tube was mixed well and incubated in a water bath at 60 °C for 60 min. The mixture was cooled to room temperature before reading the absorbance at 530 nm against the water blank.

The molar percentages of the HG and RG-I domain in potato pectin from UMAE were calculated according to the formula of M'sakni et al. (2006).

2.4.3. Determination of degree of methylation (DM) and DA

The DM and DA of pectin were analyzed according to the method of Müller-Maatsch, Caligiani, Tedeschi, Elst, and Sforza (2014). Potato pectin (30 mg) was finely mixed with 1 mL of 0.4 mol/L NaOH solution and kept at room temperature for 2 h. The mixture was centrifuged at 3000 × *g* for 30 min to obtain the supernatant, and 100 μL of 2 mg/mL TSP solution (in D_2O) as the internal standard was added to the supernatant. The thoroughly mixed solution was passed through a 0.45-μm filter before transferring to a nuclear magnetic resonance (NMR) tube. The ^{1}H NMR spectra were acquired by Bruker-Spectrospin 300 UltraShield NMR spectrometer (Bruker Corporation, Fällanden, Switzerland) at 300.13 MHz. Sixteen scans were obtained at the sweep width of 5995.20 Hz and acquisition time of 5.47 s. The methanol and acetic acid content of pectin were quantified by the integration of the peaks of methanol (δ = 3.36 ppm), acetic acid (δ = 1.92 ppm), and TSP (δ = 0 ppm), and the DM and DA of potato pectin were calculated according to the formula in Müller-Maatsch et al. (2014).

2.4.4. Molecular mass distribution

The molecular mass distribution of potato pectin was analyzed by the combination of multi-angle laser light scattering (MALLS) and size-exclusion chromatography (SEC) as mentioned in Yang et al. (2018). The system included an Optilab rEX refractive index (RI) detector (Wyatt Technology, Santa Barbara, CA, USA), a DAWN HELEOS II multi-angle light scattering detector (Wyatt Technology), an L-2400 UV detector (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA), and a TSK gel G4000PWXL column (7.8 mm × 300 mm, Tosoh Corporation, Tokyo, Japan). The concentration of potato pectin solution was 1 mg/mL and the value of dn/dc was 0.135 mL/g. The data were collected and analyzed using the Astra 5.3.4 software (Wyatt Technology).

2.5. Analysis of Fourier transform infrared spectroscopy (FTIR)

The spectra were collected using the TENSOR 27 Fourier transform infrared spectrometer (Bruker Corporation, Ettlingen, Germany). The potato or citrus pectin powder was mixed evenly with KBr powder (spectroscopic grade, 1:150, w/w) and pressed into the pellet. Each sample was scanned 64 times at the recording range of 4000–400 cm^{-1} and resolution of 4 cm^{-1}. The data were analyzed using Origin 8.0 software (OriginLab Corporation, Northampton, MA, USA) and the spectrum of citrus pectin was used for comparison.

2.6. ^{1}H NMR spectroscopy

The ^{1}H NMR spectrum was collected according to Grassino et al. (2016) with some modifications. The potato pectin powder was dissolved in D_2O and the ^{1}H NMR spectrum was recorded at 25 °C using an Agilent DD2 600 MHz NMR spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) at 599.76 MHz. The spectral width was 9615.4 Hz and the sample was scanned 64 times. TSP was used as an internal standard and chemical shifts (δ, ppm) in the ^{1}H spectrum were relative to the chemical shift of TSP (δ = 0 ppm).

2.7. Morphological analysis of pectin

The surface structural features of potato pectin were observed by scanning electron microscopy (SEM, SU8010; Hitachi, Tokyo, Japan). Pectin powder was fixed on the sample table and coated with gold under the vacuum condition. The image was taken at an accelerating potential of 10 kV and magnification of 300×.

2.8. Statistical analysis

All experiments were carried out in triplicate and the data were expressed as the mean ± standard deviation (SD). Design Expert 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA) was used for BBD and analysis of variance (ANOVA).

3. Results and discussion

3.1. Model fitting and statistical analysis

The BBD was applied to optimize the UMAE process for potato pectin and evaluate the effects of temperature, pH and time on pectin yields. The experimental and predicted values of yields obtained from different extraction conditions are shown in Table 1. The actual yields of pectin were in the range of 12.07–21.16% and the quadratic polynomial model developed by Design Expert 8.0.6 software is as follows:

$$Y(\%) = 17.29 + 0.92x_1 + 2.77x_2 + 0.88x_3 + 1.26x_1x_2 + 1.86x_1x_3 + x_2x_3 + 0.62x_1^2 - 2.3x_2^2 - 0.2x_3^2 \quad (4)$$

where Y is the yield of pectin and x_i represents the coded variable (x_1 is temperature, x_2 is pH and x_3 is time).

ANOVA was performed to evaluate the reliability of the quadratic polynomial model (Table 2). The high F-value (57.10) and low p-value (< 0.0001) showed that the model was highly significant. The coefficient of determination (R^2) of the model was 0.9866, meaning that only 1.34% of the total variation was not explained by this model; the adjusted R^2 (0.9693) was quite close to the R^2, indicating that the developed model fitted well. The high values of R^2, adjusted R^2, and predicted R^2 (0.9218) confirmed the accuracy of the model in showing the relationship between yield and variables (Moorthy et al., 2015). The coefficient of variation (CV) is the ratio of SD to the mean, and the low value of CV suggested high reproducibility of results. In this model, the CV% (2.97%) was much lower than 10%, so the variation in the mean value was low and the response model developed was adequate (Karazhiyan, Razavi, & Phillips, 2011). Moreover, the high p-value (0.6703) of lack of fit indicated that the lack of fit was not significant relative to the pure error, which also showed that the developed model was good. Meanwhile, the value of adequate precision was 25.318, which was much higher than 4, so the model was reliable within the range of independent variables (Minjares-Fuentes et al., 2014).

The significance of each term in the model can be determined according to their p-values and the term with $p < 0.05$ will have a remarkable effect on potato pectin yield. Therefore, all linear terms (x_1, x_2, and x_3) and interaction terms (x_1x_2, x_1x_3, and x_2x_3) significantly affected the yield. The quadratic terms of temperature (x_1^2) and pH (x_2^2) were also significant, but the quadratic term of extraction time (x_3^2) was not (Table 2). In conclusion, pH was the most significant variable affecting pectin yield, followed by the temperature and time.

Table 2
The results of analysis of variance (ANOVA) for the fitted quadratic polynomial model.

Source	Sum of squares	DF	Mean Square	F-value	p-value
Model	121.94	9	13.55	57.10	< 0.0001
x_1	6.73	1	6.73	28.38	0.0011
x_2	61.22	1	61.22	257.99	< 0.0001
x_3	6.21	1	6.21	26.18	0.0014
x_1x_2	6.35	1	6.35	26.76	0.0013
x_1x_3	13.84	1	13.84	58.32	0.0001
x_2x_3	3.98	1	3.98	16.77	0.0046
x_1^2	1.63	1	1.63	6.86	0.0345
x_2^2	22.19	1	22.19	93.52	< 0.0001
x_3^2	0.16	1	0.16	0.68	0.4368
Residual	1.66	7	0.24		
Lack of fit	0.49	3	0.16	0.56	0.6703
Pure Error	1.17	4	0.29		
Cor Total	123.60	16			
R^2	0.9866				
Adjusted R^2	0.9693				
Predicted R^2	0.9218				
CV %	2.97				
Adequate Precision	25.318				

3.2. Response surface analysis

The effects of independent variables (temperature, pH, and time) on yield were illustrated by three dimensional response surface plots. When investigating the effects of two variables on potato pectin yield, the third variable was kept constant (Swamy & Muthukumarappan, 2017).

Extraction temperature was a significant variable affecting pectin yield. As temperature increased from 87 to 93 °C, the yield increased (Fig. 1(a) and (b)). This might because the cells of plant tissue were destroyed at high temperature, thus the diffusivity of solvent into the cell walls would be accelerated and more pectin substance released and dissolved in the solvent, leading to a notable increase in yield.

The pH of the solution was another variable that remarkably affected the yield. As can be seen from Fig. 1(a) and (c), the pectin yield increased sharply as pH increased from 1.0 to 2.0. When the plant material was heated in acid solution, the cell structure tended to break down and insoluble pectin was hydrolyzed into soluble pectin. However, under highly acidic conditions, some pectin might be degraded into low molecular weight substances or free sugars which could not be precipitated with the ethanol (Garna et al., 2010), therefore, the highest yield was at pH 2.0. This is in accordance with the results of Swamy and Muthukumarappan (2017), in which the yield of banana pectin increased with increase of pH from 1.0 to 3.0. In contrast, Jafari, Khodaiyan, Kiani, and Hosseini (2017) extracted pectin from carrot pomace and found that the yield increased as pH increased from 1.0 to 1.5; however, the yield decreased due to the pectin aggregation at pH > 1.5, which hindered the release of pectin.

Prolonging the extraction time from 30 to 50 min increased the yield of potato pectin (Fig. 1(b) and (c)). This was probably because sufficient reaction time accelerated the mass transfer of pectin from plant tissue into solution, so more pectin was released (Jafari et al., 2017). Minjares-Fuentes et al. (2014) also found that increasing time from 20 to 60 min (75 °C) significantly increased the yield of grape pectin.

3.3. Optimization of extraction conditions and verification experiments

From the developed quadratic polynomial [Eq. (4)], the optimal extraction conditions of potato pectin were obtained: extraction temperature of 93 °C, pH of 2.0 and extraction time of 50 min. Under the optimal conditions, the predicted yield was 24.11%. To ensure reliability of the model, verification experiments of optimum conditions were applied in triplicate and the actual yield was 22.86 ± 1.29%, which did not significantly differ with the predicted value. Thus, the developed model was reliable to optimize the extraction process of potato pectin. Moreover, the yield of pectin from UMAE was much higher than that of the pectin obtained from conventional HCl extraction of 60 min (9.72%) in our previous study (Yang et al., 2018), so the UMAE resulted in a remarkable increase in yield and shortened the extraction time.

3.4. Protein, ash, and moisture content of potato pectin

Protein was found existing in potato pectin and the content (4.14%, dry basis; Table 3) was higher than that of apple pectin (1.6%, dry basis) and citrus pectin (3.0–3.3%, dry basis) (Kravtchenko, Voragen, & Pilnik, 1992). In addition, potato pectin from UMAE had higher protein

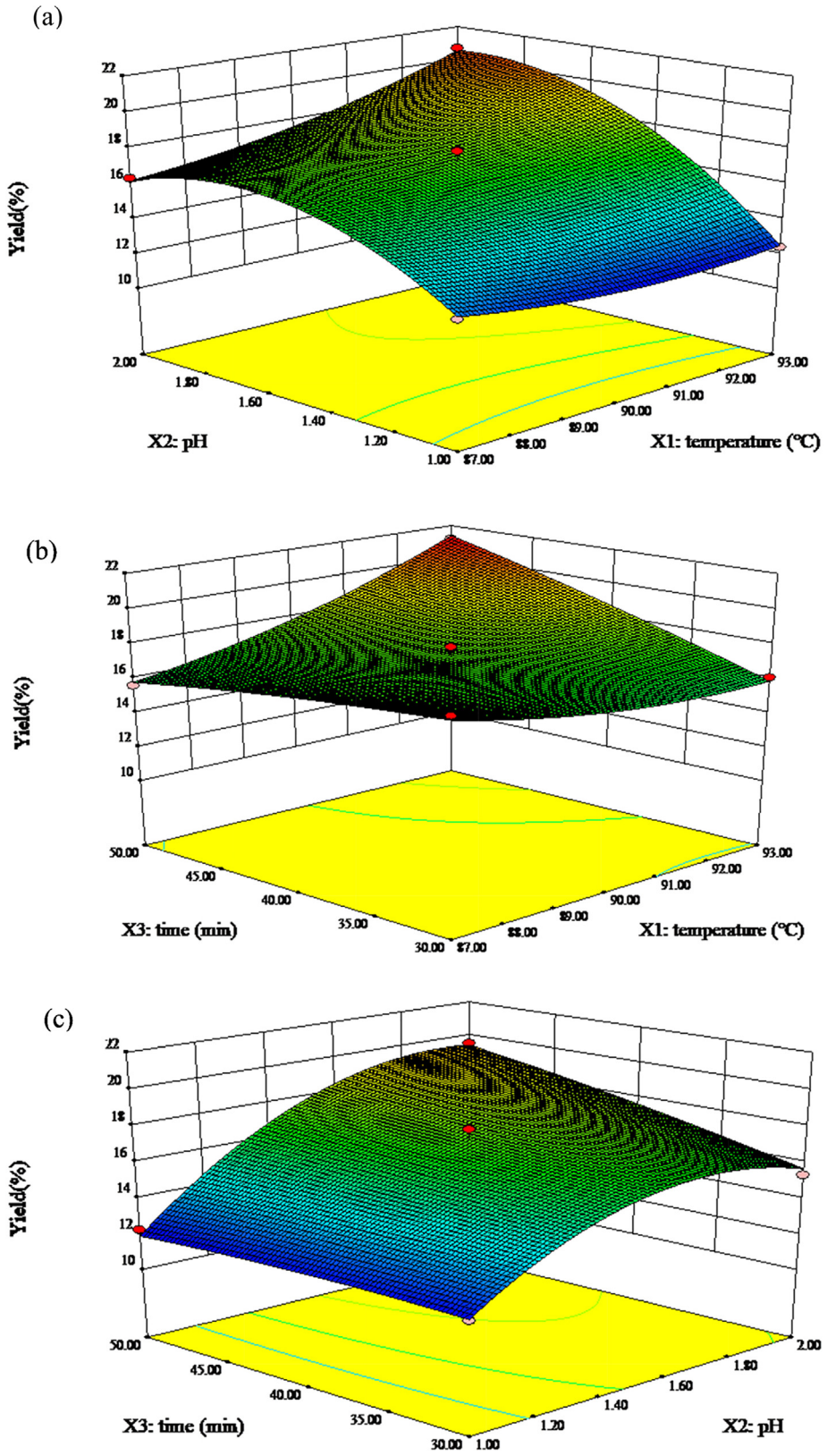

Fig. 1. Response surface showing effects of independent variables (X_1: temperature, X_2: pH and X_3: time) on the yield of potato pectin. (a) temperature vs pH, (b) temperature vs time, (c) pH vs time.

content than potato pectin from conventional HCl extraction in our previous study (2.78%, dry basis) (Yang et al., 2018). The protein was the most positive factor influencing the emulsifying activity of pectin. Due to the hydrophobicity of protein, it can act as an anchor for pectin to adsorb onto an oil droplet surface, thus decreasing the interfacial tension of the oil–water interface (Chen, Fu, & Luo, 2016; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). The increase of protein content may enhance the emulsifying properties of potato

Table 3
Composition and structural characteristics of potato pectin obtained under the optimized conditions.

Component	Content
Moisture (%)	5.66 ± 0.22
Protein (%, dry basis)	4.14 ± 0.15
Ash (%, dry basis)	1.42 ± 0.068
DM (%)	32.58 ± 0.84
DA (%)	17.84 ± 0.38
Monosaccharide composition (%)	
GalA	41.78 ± 0.42
Gal	49.38 ± 0.27
Rha	3.80 ± 0.079
Ara	2.047 ± 0.013
Glc	2.60 ± 0.085
Xyl	0.26 ± 0.015
Man	0.064 ± 0.001
GlcA	0.072 ± 0.042
Sugar molar ratios	
HG (mol%)	35.27
RG-I (mol%)	61.54
Rha/GalA	0.11
(Gal + Ara)/Rha	12.65

pectin.

The ash content of potato pectin was only 1.42% (dry basis; Table 3), indicating that pectin had a low content of inorganic impurity. Moreover, the ash content of potato pectin was at the same level as the apple pectin (1.89%, dry basis), citrus pectin (1.96–2.38%, dry basis), sugar beet pectin (1.35–1.68%, wet basis), and tomato pectin (1.21–1.25%, wet basis) in previous studies (Grassino et al., 2016; Kravtchenko et al., 1992; Peng et al., 2015).

3.5. Monosaccharide composition of potato pectin

The monosaccharide composition of potato pectin obtained from optimal conditions is shown as percentages in Table 3. Gal and GalA were the two main monosaccharides in potato pectin and represented 49.38% and 41.78% of total monosaccharides, respectively. In addition, potato pectin also contained Rha (3.80%) and Ara (2.047%). The GalA and Rha are from the backbone of HG and RG-I, and Gal and Ara comprise the neutral sugar side chains (galactan, arabinan and arabinogalactan) linked to the backbone of RG-I. The GalA content was below 50%, which might indicate a relatively low proportion of HG in potato pectin; furthermore, the Gal content was significantly higher than that of Ara, suggesting that the RG-I region might be highly branched with galactan or arabinogalactan. In addition to the monosaccharides (GalA, Gal, Rha and Ara) from the pectin structure, low levels of Glc (2.60%), Xyl (0.26%), Man (0.064%) and GlcA (0.072%) also occurred in pectin, and this might be due to the residual starch in the pulp or the co-extraction of cellulose and hemicellulose connected with the pectin.

To further illustrate the structural characteristics of potato pectin extracted by UMAE, the molar proportions of HG and RG-I of pectin were determined according to the method of M'sakni et al. (2006) and the sugar molar ratios of Rha/GalA and (Gal + Ara)/Rha were also calculated. The molar percentages of HG and RG-I were 35.27 and 61.54 mol%, respectively, indicating that the main domain of potato pectin was RG-I. The molar ratio of Rha/GalA can also illustrate the relative ratio of RG and HG in pectin. The citrus and apple pectin whose HG regions were dominant over the RG-I regions had Rha/GalA ratios within 0.017–0.027; however, potato pectin had a Rha/GalA ratio of 0.11, confirming the predominance of RG-I (Besson, Yapo, Beugre, Koffi, & Gnakri, 2014).

The molar ratio of (Gal + Ara)/Rha evaluates the branching extent of the RG-I domain. This ratio was much higher for potato pectin (12.65) than for commercial apple pectin (4.81), illustrating that the RG-I region of potato pectin was branched with more neutral sugar side chains than that of apple pectin (Zhang et al., 2013). However, the branching extent of RG-I from UMAE was lower than that of RG-I extracted by conventional HCl extraction in our previous study ((Gal + Ara)/Rha, 28.45), possibly because the intense effects of ultrasound and microwave led to a partial degradation of neutral sugar side chains of RG-I (Yang et al., 2018; Zhang et al., 2013).

3.6. DM and DA of potato pectin

DM and DA are the molar ratios of methanol or acetic acid to GalA, respectively, and are important structural characteristics influencing functional properties, such as, emulsifying properties of pectin. Potato pectin extracted with UMAE had DM of 32.58% and DA of 17.84%, thus it can be considered as low methoxyl pectin with a high degree of acetylation. The DA of potato pectin was notably higher than that of apple pectin (2–4%) and citrus pectin (1–2%), despite being lower than that of sugar beet pectin (23.9%) (Chen et al., 2016; Voragen, Schols, & Pilnik, 1986). It was reported that reducing the amounts of methyl ester and acetyl groups in pectin decreased the surface activity of pectin, and as a result, the interfacial tension and particle size of the emulsion stabilized by pectin increased (Chen et al., 2016). Additionally, chemically acetylated citrus pectin also showed better emulsifying properties than non-acetylated citrus pectin (Leroux et al., 2003). The use of UMAE in the potato pectin extraction process resulted in increased DM and DA of pectin comparing with pectin from conventional HCl extraction, with DM of 28.61% and DA of 11.92% (Yang et al., 2018), which might positively affect the emulsifying properties of pectin.

3.7. Molecular mass distribution of potato pectin

The elution profile of potato pectin extracted under the optimal conditions is presented in Fig. S1. The RI detector was used to determine the relative concentrations of different molar mass distributions in pectin, and the signal of MALLS detector revealed its molar mass distribution. The RI elution profile included three obvious peaks at the elution time of 10–12, 13–17, and 17–21 min. For the MALLS elution profile, peaks also appeared at the same range of elution time as the signals for RI elution profile, and the peak at 10–12 min had the lowest intensity of RI signal, but the peak at 17–21 min had the highest intensity of RI signal. Therefore, the high molar mass pectin was at low concentration and low molar mass pectin was dominant. The wavelength of the UV detector was set at 280 nm to detect the presence of protein in pectin. The UV elution profile showed two peaks in the ranges of 10–12 and 17–19 min, which were in accordance with the RI signals. This result suggested the existence of protein in pectin, as confirmed by the protein result in Table 3.

The values of weight-average molecular weight (Mw), Mw/Mn, and mass fraction (MF, %) of potato pectin are shown in Table S1. The Mw of pectin was in the range of 2.038×10^4–9.449×10^5 g/mol, and the lowest Mw pectin (Peak 3) had the highest MF (52.1765%), which were consistent with the results of the elution profile for potato pectin. Considering the whole range of the distribution, the Mw of potato pectin was 1.537×10^5 g/mol, which was lower than that of potato pectin from conventional HCl extraction (2.799×10^5 g/mol). The decrease of Mw may be because parts of the neutral sugar side chains of RG-I were degraded by the high intensity of ultrasound and microwave (Xu et al., 2018; Yang et al., 2018; Zhang et al., 2013). In addition, the Mw/Mn of 5.303 was much higher than 1, indicating that potato pectin from UMAE had a wide molecular weight distribution and was a heterogeneous natural polysaccharide.

3.8. FTIR and ^{1}H NMR spectra of potato pectin

The FTIR spectrum was used to determine main functional groups in

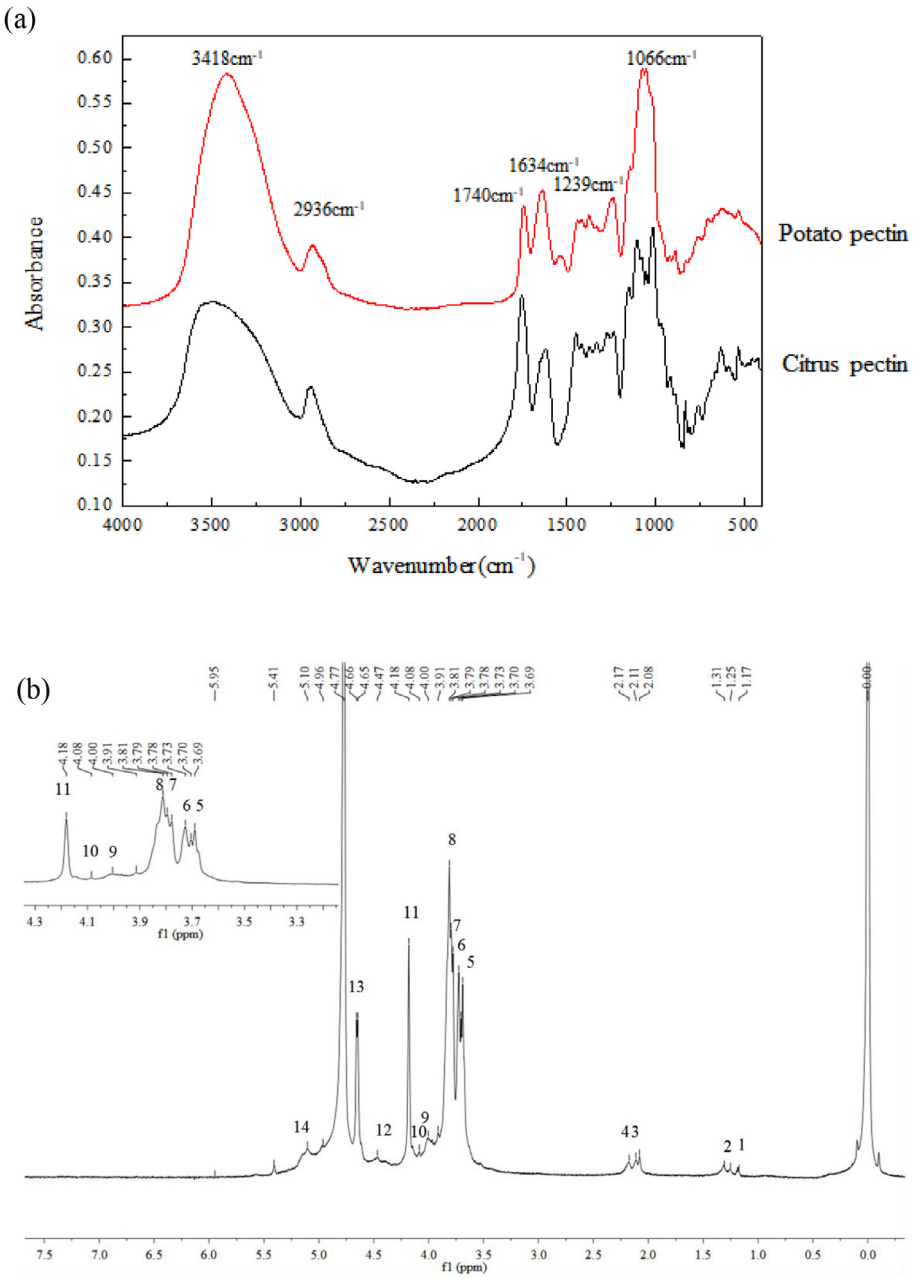

Fig. 2. (a) FTIR spectra of potato pectin and citrus pectin and (b) ^{1}H NMR spectrum of potato pectin obtained under the optimal conditions.

potato pectin, with citrus pectin as the standard. As is shown in Fig. 2(a), the spectrum of potato pectin was similar to that of citrus pectin, indicating that ultrasound and microwave heating had little effect on the main chemical structure of potato pectin. For the spectrum of potato pectin, the strong absorption peak at about 3418 cm^{-1} was attributed to the stretching vibration of O—H groups which were from the inter- and intra-molecular hydrogen bonding of the GalA backbone. The peak at around 2936 cm^{-1} corresponded to absorption of the stretching and bending vibrations of C—H groups (CH, CH_2 and CH_3) in pectin (Singthong, Cui, Ningsanond, & Goff, 2004). The peaks at around 1740 and 1634 cm^{-1} were assigned to absorptions of esterified carboxyl (–COOR) and ionized carboxyl ($–COO^-$), respectively. The DM of pectin is related to the absorption peak areas of 1740 and 1634 cm^{-1} and can be quantified by the ratio of the absorption peak area of 1740 cm^{-1} to the sum peak areas of 1740 and 1634 cm^{-1} (Pappas et al., 2004; Singthong et al., 2004). For potato pectin, the intensity of the peak at 1634 cm^{-1} was stronger than that at 1740 cm^{-1}, suggesting that the DM of potato pectin was low and this was confirmed by the result of DM = 32.58% (Table 3). Additionally, it is worth noting that there were also several peaks in the range of 1300–800 cm^{-1}, which is often considered to be the fingerprint region and is unique for different kinds of pectin. The spectrum of potato pectin had a peak at about 1239 cm^{-1}, meaning the presence of $–O–CH_3$ groups (Torralbo, Batista, Di-Medeiros, & Fernandes, 2012). In addition, unlike the fingerprint region of citrus pectin, potato pectin had the strong peak at about 1066 cm^{-1}, which probably because the acetylation resulted in the drastic structure changes in potato pectin (Synytsya, ČopíKová, Matějka, & Machovič, 2003).

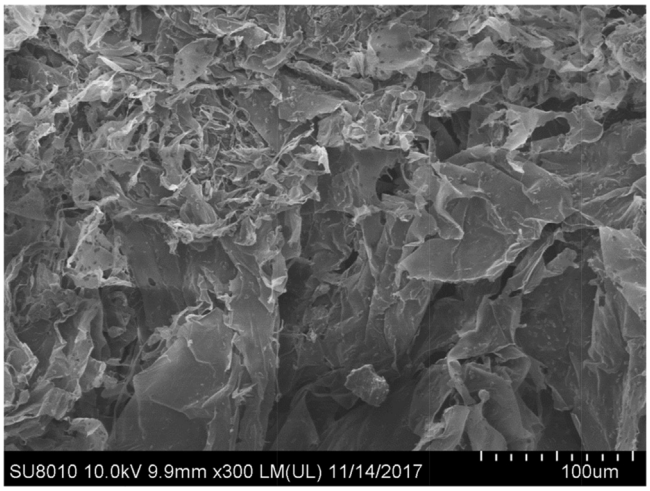

Fig. 3. The SEM image of potato pectin obtained under the optimal conditions, 300× magnifications.

In order to further analyze the structural characteristics of potato pectin obtained by UMAE, the ^{1}H NMR spectrum of pectin was also determined (Fig. 2(b)). The peaks at 1.17 and 1.25 ppm were from methyl rhamnoses and were related to the O-2-linked and O-2, 4-linked Rha*p* residues, respectively (Vignon & Garcia-Jaldon, 1996). The signal at 2.11 ppm was attributed to the acetyl groups binding at the O-3 of GalA residues of HG, and the signal at 2.17 ppm corresponded to acetyl groups linking at the O-2 of GalA residues of HG (Renard & Jarvis, 1999). A very strong signal at 3.81 ppm was caused by methyl groups linking with the carboxyl groups of GalA. The peaks at 3.78, 4.47, and 5.10 ppm were related to the H-2, H-4, and H-1 of α-1, 4-linked D-GalA residues (Grassino et al., 2016). According to data of Khodaei and Karboune (2013), several signals related to the β-1, 4-linked D-galactose and α-1, 5-linked L-arabinose were also observed in the spectrum. The signals at 4.65, 3.69, 4.00, and 3.73 ppm were attributed to the H-1, H-2, H-4, and H-5 of D-galactose, respectively; and peaks at 4.08 and 4.18 ppm were from H-2 and H-4 of L-arabinose, respectively.

The results of FTIR and ^{1}H spectra indicated that the potato pectin extracted with UMAE had a linear backbone of α-1, 4-linked GalA residues. The GalA residues were methyl esterified at a low level and could also be esterified by acetyl groups at O-2 and O-3 positions. The Rha linked with GalA to form the backbone of RG-I and the neutral sugar side chains composed of galactose and arabinose were linked at O-4 of Rha.

3.9. Morphological characteristics of potato pectin

The SEM was applied to investigate the surface morphology of potato pectin (Fig. 3). Most of the potato pectin had an irregular lamella structure, which was packed closely. Among the packed-lamella pectin, there was also a small amount of pectin with a filamentous structure. Interestingly, some pectin lamellae had scattered particles on their surface.

4. Conclusions

The BBD was applied to optimize the UMAE process for potato pectin extraction and evaluate the effects of three variables (extraction temperature, pH, and time) on the yield. The optimal extraction conditions were temperature 93 °C, pH 2.0, and time 50 min. Under optimal conditions, the maximum yield (22.86 ± 1.29%) did not have significant difference from the predicted yield, indicating high reliability of the developed model. Gal (49.38%) and GalA (41.78%) were the main monosaccharides of potato pectin extracted under optimal conditions, and the branched RG-I region (61.54 mol%) was dominant in pectin. The obtained potato pectin was classified as a low methoxyl (DM, 32.58%), but highly acetylated (DA, 17.84%) pectin with protein content of 4.14% (dry basis). Furthermore, the potato pectin was a heterogeneous polysaccharide and its Mw was 1.537×10^{5} g/mol. The FTIR and ^{1}H NMR results revealed that the obtained pectin had the linear region composed of α-1, 4-linked GalA residues and parts of them were methyl- or acetyl-esterified at O-2 and O-3. The Rha and GalA consisted of RG and the O-4 of Rha was linked with neutral sugar side chains. The SEM showed that the surface morphology of pectin was mainly in the lamellae structure. The results suggested that UMAE was an effective way to increase the extraction efficiency of potato pectin extraction.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.03.027.

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