Food Chemistry 244 (2018) 197–205

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Extraction, structure, and emulsifying properties of pectin from potato pulp

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ARTICLE INFO

Chemical compounds studied in this article:
Hydrochloric acid (PubChem CID:313)
Sulfuric acid (PubChem CID:1118)
Nitric acid (PubChem CID:944)
Citric acid (PubChem CID:311)
Acetic acid (PubChem CID:176)
Sodium hydroxide (PubChem CID:14798)
Sodium bisulfite (PubChem CID:23665763)
D-galactose (PubChem CID:6036)
D-galacturonic acid (PubChem CID:439215)
Ethanol (PubChem CID:702)

Keywords:
Potato pulp
Pectin
Acid extraction
Structure
Emulsifying properties

ABSTRACT

Effects of HCl, H_2SO_4, HNO_3, citric acid, and acetic acid on the yield, structure, and emulsifying properties of potato pectins were investigated. Results showed that the highest yield (14.34%) was obtained using citric acid, followed by HNO_3 (9.83%), HCl (9.72%), H_2SO_4 (8.38%), and acetic acid (4.08%). The degrees of methylation (37.45%) and acetylation (15.38%), protein content (6.97%), and molecular weight (3.207×10^5 g/mol) were the highest for pectin extracted using acetic acid, and (galactose + arabinose)/rhamnose was 33.34, indicating that it had a highly branched rhamnogalacturonan I domain. Fourier transform infrared spectroscopy showed a specific absorbance peak at 1064 cm^{-1}, which corresponds to the acetyl groups in potato pectins. SEM showed that all potato pectins are morphologically different. The emulsifying activity (EA, 44.97%–47.71%) and emulsion stability (ES, 36.54%–46.00%) of the pectins were influenced by acid types, and were higher than those of commercial citrus and apple pectin.

1. Introduction

China is the largest producer of potato in the world. In 2014, the yield of fresh potato in China reached 96 million tonnes (FAO, 2014). Potato is commonly used in starch processing, which results in a large quantity of waste pulp. In China, approximately 4.5–5.0 tonnes of fresh potato pulp are generated for every tonne of starch produced. However, while a small amount of the potato pulp byproduct is used as low-value animal feed, most of it is disposed, which means that it is a major contributor to environmental pollution. Previous studies have indicated that potato pulp consists of starch (37%), pectin (17%), cellulose (17%), hemicellulose (14%), and protein (4%) (dry basis) (Mayer, 1998), and potato pulp is rich in pectin which can be used as a good raw material for pectin extraction, however, there is little information about potato pectin.

Pectin is a complex acidic macromolecular polysaccharide found in primary cell walls and the middle lamella. It is generally composed of homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). HG is a linear polymer that consists of α-(1, 4)-linked galacturonic acids (GalA) and is a major domain of pectin (Caffall & Mohnen, 2009). The GalA residues in the HG backbone can be methyl esterified at C-6 and can also be acetylated at O-2 and/or O-3. According to the degree of methylation (DM), pectin can be classified as high methoxyl pectin (HMP) (DM > 50%) or low methoxyl pectin (LMP) (DM < 50%) (Yapo, 2011). The RG-I region is composed of 100 repeating disaccharide units ([→4)-α-D-Gal*p*A-(1 → 2)-α-L-Rhap-(1→]), and 20%–80% of the rhamnosyl residues in the backbone may be substituted with neutral sugar side chains (galactan, arabinan, and arabinogalactan) at O-4; further, GalA can also be acetylated at C-2 and/or C-3 (Albersheim, Darvill, Oneill, Schols, & Voragen, 1996; Ridley, Oneill, & Mohnen, 2001). The RG-I domain contains at least seven α-(1, 4)-linked GalA in the backbone, and the side chains are mainly composed of sugars such as apiose, rhamnose, xylose, galactose, and fucose (Pellerin & Oneill, 1998). The proportions of HG, RG-I, and

Abbreviations: HG, homogalacturonan; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; GalA, galacturonic acids; DM, degree of methylation; HMP, high methoxyl pectin; LMP, low methoxyl pectin; DA, degree of acetylation; HPP, pectin extracted by HCl; SPP, pectin extracted by H_2SO_4; NPP, pectin extracted by HNO_3; CPP, pectin extracted by citric acid; APP, pectin extracted by acetic acid; Mw, weight-average molecular weight; MF, mass fraction; FTIR, Fourier transform infrared spectroscopy; SEM, scanning electron microscopy; EA, emulsifying activity; ES, emulsion stability

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http://dx.doi.org/10.1016/j.foodchem.2017.10.059
Received 10 July 2017; Received in revised form 28 September 2017; Accepted 9 October 2017
Available online 12 October 2017
0308-8146/

RG-I are related to the pectin source; for example, the proportions of the HG and RG-I regions in commercial citrus and apple pectins are 65% and 20%–35%, respectively, whereas potato pectin has a high proportion of the RG-I region (75%) and a relatively low proportion of the HG region (20%). Furthermore, β-(1, 4)-linked galactan side chains are abundant in the RG-I domain of potato pectin. The degree of acetylation (DA) of potato pectin is about 14%, which is higher than that of commercial citrus pectin (2%) and apple pectin (4%) (Mohnen, 2008; Sorensen et al., 2000; Vincken et al., 2000; Voragen, Schols, & Pilnik, 1986). Commercial citrus and apple pectins are often used as gelling agents in the food processing industry because of their high DM, molecular weight, and high proportion of the HG region. In comparison, potato pectin is richer in acetyl groups and neutral sugar side chains but HG domain is shorter than commercial citrus and apple pectins, and thus, potato pectin does not have good gelling ability. On the other hand, previous studies have showed that acetyl groups and neutral sugar side chains have positive effects on the emulsifying properties of pectin (Funami et al., 2011; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). Therefore, the unique structure of potato pectin may allow it to be used as an emulsifier. However, little attention has been paid to the properties of potato pectin which limits its application in the food industry.

The alkaline, enzymatic, and acid methods are the most common ways to extract pectin from sources such as potato, apple, citrus fruits, sugar beet, and cocoa husk. The alkaline extraction method can retain the neutral sugar side chains in pectin; however, the methyl ester and acetyl groups of pectin are hydrolyzed by the β-elimination reaction (Rombouts & Thibault, 1986). The enzymatic extraction method can decrease the emission of waste acid or alkali solutions, and results in pectins with a higher molecular weight and degree of esterification (DE) than the acid method, but it takes longer time than acid method (Wikiera, Mika, Starzynskajaniszewska, & Stodolak, 2016). The acid extraction method is often used to extract pectin in the food industry because of its convenient and easy operation. Several studies have indicated that the use of different acid extractants can have different effects on pectin yield, structure, and physicochemical properties (Chan & Choo, 2013; Ma et al., 2013). In recent years, some studies have mainly focused on the application of endo-polygalacturonase and KOH/NaOH to extract the RG-I domain from potato pulp (Khodaei & Karboune, 2016; Khodaei, Karboune, & Orsat, 2016). As far as we know, few studies have reported the effects of different types of acids on the yield, structure, and emulsifying properties of potato pectin.

In the present study, the effects of three mineral acids (HCl, H_2SO_4, and HNO_3) and two organic acids (citric acid and acetic acid) on the yield, structure, and emulsifying properties of potato pectins were clarified. The purpose of this study was to provide a theoretical basis for the industrial extraction of pectin from potato pulp, and to evaluate the potential of potato pectin as a natural emulsifier in the food industry.

2. Materials and methods

2.1. Materials and reagents

Potatoes (Kexin No. 1) were bought from Inner Mongolia Huaou Starch Industry Co., Ltd. (Inner Mongolia, China). The commercial citrus and apple pectins and the carbazole reagent (GC reagent) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The rhamnose, arabinose, galactose, glucose, xylose, mannose, GalA, and glucuronic acid were HPLC grade, and were also purchased from Sigma–Aldrich. H_2SO_4 was guaranteed reagent, and was bought from Beijing Chemical Works (Beijing, China). The trifluoroacetic acid was chromatographic grade, and was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Deuterium oxide (99 atom % D) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Sodium-3-(trimethylsilyl) propionate-2, 2, 3, 3-d4 (TSP) was bought from J & K Scientific Ltd. (Beijing, China), and the purity was > 98 atom % D. The corn oil was food grade, and was bought from a local supermarket. All other reagents were of analytical grade.

2.2. Preparation and extraction of pectins from potatoes

The preparation and extraction process of pectins from potatoes was shown in Fig. S1. Briefly, fresh potatoes were washed, peeled, cut into pieces, and mashed with water (1:1, w/v), afterwards, 0.05% (w/v) sodium bisulfite solution was added to prevent the potato pulp from browning. The wet pulp was dried at 60 °C overnight, and then ground and sieved with an 80-mesh screen. To remove the high content of residual starch, the above potato pulp powder was dissolved in distilled water (1:30, w/v) and enzymatically hydrolyzed using thermostable α-amylase (100 μL/g, enzyme activity was 120 KNU; Novozymes, Bagsværd, Denmark) at pH 6.25 ± 0.02, 95 °C for 30 min. The resulting slurries were cooled down to room temperature and centrifuged at 7000 × *g* for 10 min (GL-21M Refrigerated Centrifuge, Changsha Xiangyi Centrifuge Instrument Co., Ltd., Changsha, China). The precipitate was washed once with 85% (v/v) ethanol and recentrifuged, followed by drying at 50 °C overnight. The final dried potato pulp was ground and passed through a 40-mesh screen before pectin extraction.

The potato pectin was extracted according to the method of Wan (2008), with some modifications. Five grams of potato pulp was dissolved in distilled water (1:15, w/v) and adjusted to pH 2.04 ± 0.02 with HCl, H_2SO_4, HNO_3, citric acid, or acetic acid, respectively. The resulting solutions were heated at 90 °C for 60 min, and centrifuged at 7000 × *g* for 30 min. The supernatants were collected and treated with three volumes (v/v) of absolute ethanol at 4 °C overnight. The pectin precipitates were collected by recentrifugation, and then were washed twice with 70% (v/v), 80% (v/v), and 90% (v/v) ethanol, respectively. Finally, the pectin was dispersed in distilled water and freeze dried (SIM-FD5, the Siemon Company, Los Angeles, America).

Hereafter, the potato pectins extracted by HCl, H_2SO_4, HNO_3, citric acid, and acetic acid are referred to as HPP, SPP, NPP, CPP, and APP, respectively. The pectin yield (%, wet basis) was calculated as the ratio of the mass of dried pectin (Wp) to the mass of potato pulp after enzymatic treatment (We):

$$\text{Yield (\%, wet basis)} = \frac{Wp(g)}{We(g)} \times 100\% \quad (1)$$

2.3. Proximate composition of potato pulp

The protein content was determined by the Kjeldahl method, with a nitrogen conversion factor of 6.25 (AOAC 955.04). Crude fat (AOAC 920.39), total and soluble dietary fiber (AOAC 991.43), moisture (AOAC 925.09), ash (AOAC 942.05), and starch (AOAC 996.11) contents were analyzed using AOAC methods. The pectin content was determined according to the method described by Donaghy and Mckay (1994) with slight modifications.

2.4. Pectin characterization

2.4.1. Moisture and protein content of potato pectin

The moisture content of the pectin was analyzed according to AOAC 925.09. The protein content was determined by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978; Peterson, 1977). Different concentrations of BSA solutions (10–100 g/mL) were used as standards. The concentration of the potato pectin solutions was 1 mg/mL, and the protein contents are presented on a dry basis.

2.4.2. Neutral sugars and galacturonic acid content of potato pectin

The individual neutral sugars of potato pectin were quantified by

the ion chromatography. Ten milligrams of pectin powder was mixed with 4 mL of 2 mol/L trifluoroacetic acid and hydrolyzed at 120 °C for 60 min. After cooling to room temperature, the trifluoroacetic acid was removed by nitrogen blowing. The remaining material was redissolved and made up to 10 mL with ultrapure water. Before analysis, the sample was diluted and passed through a 0.45-μm filter. The ICS-3000 Ion Chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a CarboPac™ PA20 (3 mm × 150 mm) and analytical column (ICS-3000; Dionex) were used for the analysis. The eluent was 250 mM NaOH and 1 M NaAc, which passed through the column at a flow rate of 0.5 mL/min at 35 °C. Different concentrations of neutral sugar mixtures (0.01–5 ppm; L-rhamnose, L-arabinose, D-galactose, D-glucose, D-xylose, D-mannose, and glucuronic acid) were used as standards.

The GalA content was determined by the carbazole–sulfuric acid method (Taylor, 1993), and GalA solutions (10–90 g/mL) were used as standards. Two hundred microliters of the standard or sample solutions (0.01%, w/v, g/mL) were placed in stoppered test tubes, followed by the addition of 3 mL of concentrated H_2SO_4 and 100 μL of 0.1% (w/v) carbazole reagent. The mixtures were mixed thoroughly and incubated in a water bath at 60 °C for 60 min. After cooling, the absorbance was read at 530 nm against water blank.

The molar percentages of the HG and RG-I regions of pectin were calculated according to Sakni et al. (2006) method. The formulas used are as follows:

$$\text{HG (mol\%)} = \text{GalA (mol\%)} - \text{Rha (mol\%)} \quad (2)$$

$$\text{RG-I (mol\%)} = [\text{GalA (mol\%)} - \text{HG (mol\%)}] + \text{Rha (mol\%)} + \text{Gal (mol\%)} + \text{Ara (mol\%)} \quad (3)$$

2.4.3. DM and DA of potato pectin

The DM and DA of pectin were analyzed according to the method described by Müller-Maatsch, Caligiani, Tedeschi, Elst, and Sforza (2014). Thirty milligrams of potato pectin was mixed with 1 mL of 0.4 mol/L NaOH solution and kept at room temperature for 2 h. The supernatant was obtained by centrifuging the samples at 3000 × *g* for 30 min, and then 100 μL of the internal standard solution (10 mg TSP in 5 mL D_2O) was added to the supernatant. The thoroughly mixed solution was passed through a 0.45-μm filter and transferred to an NMR tube to determine the methanol and acetic acid contents. The ^{1}H NMR spectra of the samples were collected using a Bruker Avance 300 MHz spectrometer (Bruker Corporation, Fällanden, Switzerland). The sample was scanned for 16 times and spectra were acquired at the sweep width of 5995.20 Hz and the acquisition time of 5.47 s. The contents of methanol and acetic acid were determined by the manual integration of the peaks of methanol (3.36 ppm), acetic acid (1.92 ppm), and TSP (0 ppm).

2.4.4. Molecular mass distribution of pectin

The molar mass distribution of pectin was analyzed by the combination of multi-angle laser light scattering (MALLS) and size-exclusion chromatography. The system consisted of an Optilab rEX differential refractometer (RI) (Wyatt Technology, Santa Barbara, CA, USA), a DAWN HELEOS II multi-angle light scattering detector (Wyatt Technology), and an L-2400 UV detector (Wyatt Technology), with a TSK Gel G4000PWxl column. The pectin solution (1 mg/mL) was passed through a 0.2-μm filter before analysis. The eluent was 0.1 M NaCl, and the flow rate was 0.5 mL/min at room temperature. A dn/dc value of 0.135 mL/g was used for the molar mass analysis of the potato pectin. The weight-average molecular weight (Mw), mass fraction (MF), and Mw/Mn values were analyzed using ASTRA 5.3.4 software (Wyatt Technology).

2.5. Analysis of Fourier transform infrared spectroscopy (FTIR)

Dried potato pectin samples were mixed well with KBr powder (spectroscopic grade) at a ratio of 1:150 (w/w) and then pressed into pellets for FTIR analysis. The spectra were collected using a TENSOR 27 Fourier transform infrared spectrometer (Bruker, Karlsruhe, Germany). For each sample, the recorded range was from 4000 cm^{-1} to 400 cm^{-1}, with 64 scans, and the resolution was 4 cm^{-1}. For comparison, commercial citrus and apple pectins were also prepared and analyzed under the same conditions. The data and spectra were analyzed using Origin 8.0 software (OriginLab Corporation, Northampton, MA, USA).

2.6. Morphological analysis of potato pulp and potato pectin

Scanning electron microscopy (SEM; SU8010; Hitachi, Tokyo, Japan) was conducted to observe the surface structural features of the potato pulp and potato pectins. SEM was used to illustrate the morphological differences of the potato pulp (before and after enzymatic treatment) and the pectins extracted using different types of acids. Dried pectin powder was fixed to the sample table with double-sided adhesive carbon tape, and each sample was coated with gold powder under vacuum conditions. The images were taken at an accelerating potential of 10 kV, at a magnification of 300×.

2.7. Emulsifying properties of pectin

The emulsifying activity (EA) and emulsion stability (ES) of the potato pectins and commercial citrus and apple pectins were analyzed according to the method by Dalev and Simeonova (1995), with some modifications. The emulsions were prepared by mixing 5 mL corn oil with 5 mL pectin solution (0.5%, w/w). The oil/water mixtures were then homogenized in a high-shear emulsifying machine (I25; Shanghai IKN Machinery Equipment Co., Ltd., Shanghai, China) at 10,000 rpm for 3 min. Finally, the emulsions were centrifuged at 3000 × *g* for 5 min. The EA was calculated as follows:

$$\text{EA (\%)} = (\text{EPV}_1/\text{WV}_1) \times 100 \quad (4)$$

where EPV_1 is the volume of the emulsion phase, and WV_1 is the total volume of the system.

ES was determined using emulsions prepared in the same way as above. The emulsions were heated at 80 °C for 30 min and cooled in a water bath for 15 min. Then, the emulsions were centrifuged at 3000 × *g* for 5 min. ES was determined as follows:

$$\text{ES (\%)} = (\text{EPV}_2/\text{WV}_2) \times 100 \quad (5)$$

where EPV_2 is the volume of the emulsion phase, and WV_2 is the total volume of the system.

2.8. Statistical analysis

The data were expressed as the mean ± standard deviation (SD), and one-way analysis of variance was conducted by Duncan's multiple range test using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Each test was carried out in triplicate. The means were considered to be significantly different at $p < .05$.

3. Results and discussion

3.1. Proximate composition of potato pulp before and after enzymatic treatment

Table S1 shows the proximate composition of the potato pulp; all results, except for the moisture content, are expressed as g/100 g (dry basis). Before enzymatic treatment, the potato pulp contained 56.62% starch, 7.83% soluble dietary fiber, 14.63% insoluble dietary fiber, 5.47% protein, 1.50% ash, and 0.24% fat. As a kind of soluble dietary fiber, pectin only accounted for 4.58% on the basis of potato pulp. After the enzymatic treatment, the starch content declined from 56.62% to 3.47%, and the dietary fiber became the main component, increasing

Table 1
Chemical composition of potato pectins obtained by five different acids.

	HPP	SPP	NPP	CPP	APP
Yield (%, wet basis)	9.72 ± 0.12^{b}	8.38 ± 0.36^{b}	9.83 ± 0.98^{b}	14.34 ± 1.34^{a}	4.08 ± 0.47^{c}
Monosaccharide composition (mg/g)					
Galacturonic acid	298.18 ± 36.72^{ab}	311.36 ± 4.46^{a}	275.38 ± 15.02^{bc}	258.80 ± 5.31^{c}	243.40 ± 10.81^{c}
Rhamnose	14.95 ± 0.92^{a}	15.79 ± 4.90^{a}	16.04 ± 2.73^{a}	14.08 ± 0.19^{a}	12.91 ± 0.64^{a}
Arabinose	28.81 ± 0.44^{bc}	32.25 ± 4.93^{b}	22.99 ± 4.26^{c}	24.25 ± 1.20^{bc}	49.89 ± 1.27^{a}
Galactose	438.77 ± 18.28^{a}	390.39 ± 42.81^{ab}	432.13 ± 45.72^{a}	341.28 ± 3.89^{b}	407.69 ± 7.18^{ab}
Glucose	24.69 ± 3.57^{bc}	27.95 ± 1.70^{bc}	23.18 ± 1.57^{c}	30.85 ± 3.14^{b}	46.03 ± 0.78^{a}
Xylose	0.99 ± 0.30^{a}	1.16 ± 0.49^{a}	0.86 ± 0.25^{a}	0.91 ± 0.053^{a}	0.96 ± 0.14^{a}
Mannose	0.58 ± 0.031^{a}	0.45 ± 0.34^{a}	0.56 ± 0.34^{a}	0.57 ± 0.12^{a}	0.65 ± 0.027^{a}
Glucuronic acid	0.89 ± 0.28^{b}	1.00 ± 0.14^{ab}	0.65 ± 0.36^{b}	0.95 ± 0.071^{b}	1.54 ± 0.094^{a}
Sugar molar ratios (%)					
Rha/GalA	0.061	0.060	0.070	0.066	0.061
(Gal + Ara)/Rha	28.45	25.13	26.01	23.95	33.34
HG (mol %)	32.5	35.13	31.07	33.44	28.48
RG-I (mol %)	63.94	60.77	65.54	61.49	65.03
DM (%)	28.61 ± 1.34^{b}	26.68 ± 0.15^{bc}	23.91 ± 0.049^{cd}	21.51 ± 0.18^{d}	37.45 ± 2.73^{a}
DA (%)	11.92 ± 0.66^{b}	10.51 ± 0.13^{bc}	10.34 ± 0.28^{bc}	9.21 ± 0.0071^{c}	15.38 ± 1.17^{a}
Protein (%, dry basis)	2.78 ± 0.017^{d}	1.85 ± 0.026^{e}	3.055 ± 0.073^{c}	3.64 ± 0.075^{b}	6.97 ± 0.076^{a}

Results were presented in the form of means ± standard deviation (SD). Data with different letters in the same row were significantly different, $p < .05$. HPP, SPP, NPP, CPP and APP were the pectins extracted by HCl, H_2SO_4, HNO_3, citric acid, and acetic acid.

from 22.46% to 79.57%. The content of soluble dietary fiber also increased to 23.18%. Furthermore, the pectin content reached 22.66%, which indicates that potato pulp is an excellent source for pectin extraction after enzymatic treatment. At the same time, the protein content in the potato pulp was also high, reaching 9.31%. However, the ash and fat contents were still low after enzymatic treatment, at 2.91% and 0.18%, respectively.

3.2. Extraction yields of potato pectins

The yields of the potato pectins obtained by HCl, H_2SO_4, HNO_3, citric acid, and acetic acid extraction are shown in Table 1. The results show that the types of acids had effects on yields of potato pectin, which ranged from 4.08% to 14.34% (wet basis). The highest yield (14.34%) found in this study was obtained by citric acid extraction, which is in agreement with the results of Kumar and Chauhan (2010) and Kermani et al. (2014), whose studies also showed that citric acid can extract higher contents of pectin than HCl or H_2SO_4, and explained that due to the chelating property of citric acid, more chelator–soluble pectin fractions could be extracted, therefore, the yields of citric acid extraction were much higher than that of other acids lacking chelating nature. Conversely, the lowest yield (4.08%) was obtained by acetic acid extraction. The extraction efficiencies were significantly different for citric acid and acetic acid even though they are both organic acids. For HCl, H_2SO_4, and HNO_3, which are mineral acids, the extraction yields ranged from 8.38% to 9.83%, were not significantly different. These results indicate that the yields of potato pectin are not only influenced by the nature of acids but also affected by the relative strength of the extractants.

3.3. Neutral sugars and uronic acid content of potato pectins

The contents of neutral sugars and uronic acids in pectin depend on both the raw materials and extraction methods. Table 1 shows the neutral sugar and uronic acid contents of HPP, SPP, NPP, CPP and APP. Galactose (341.28–438.77 mg/g) and GalA (243.40–311.36 mg/g) were the main components in all the potato pectins, followed by other neutral sugars, including arabinose (22.99–49.89 mg/g) and rhamnose (12.91–16.04 mg/g). In addition, it is worth noting that the galactose content was much higher than the GalA content in all the potato pectins. However, for the commercial citrus and apple pectins, the GalA contents were more than 50% (500 mg/g), whereas the galactose contents were less than 10% (100 mg/g), although these commercial pectins were also obtained by acid extraction (Besson, Yapo, Beugre, Koffi, & Gnakri, 2014; Kravtchenko, Voragen, & Pilnik, 1992).

The main component of the HG domain was GalA, and the low GalA content in all the potato pectins illustrated the short backbone of the HG domain. Galactose and arabinose are neutral sugars found in the side chains (galactan, arabinan, and arabinogalactan) of the RG-I domain, and the predominant content of galactose shows that the galactan side chains are rich. Compared with galactose, the arabinose content was much lower, which is possibly related to the lower content of arabinan or arabinogalactan side chains in the RG-I region. The high neutral sugars content in the potato pectins shows that the RG-I region is highly branched, and this could also be confirmed by the high molar ratios of (Gal + Ara)/Rha (23.97–33.34), which are often used as indicators of the branching extent of the RG-I domain. Compared with potato pectin, the average molar ratio (9.96) of apple pectin was much lower (Sato et al., 2011). The (Gal + Ara)/Rha molar ratio of CPP (23.97) was the lowest among all the pectin samples, which suggests that CPP has the least branched RG-I domain. On the other hand, APP had the high galactose (407.69 mg/g) and arabinose (49.89 mg/g) contents and the highest (Gal + Ara)/Rha molar ratio (33.34), which implies that its RG-I region is highly branched. It seems that compared with the other acids, acetic acid had the lowest hydrolyzing ability and could retain more side chains of the RG-I domain in potato pectin. Galactose, rhamnose, and arabinose were the original sugar components in the pectin polymers, and apart from these sugars, low contents of glucose (23.18–46.03 mg/g), xylose (0.86–1.16 mg/g), mannose (0.45–0.65 mg/g), and glucuronic acid (0.65–1.54 mg/g) were also found in all the potato pectin samples, which could be related to the residual starch from the potato pulp or the co-extraction of hemicellulose and cellulose connected with the pectin polymers.

Furthermore, the molar ratio of Rha/GalA reflects the contribution of the RG-I domain in pectin. If this molar ratio ranges from 0.05 to 1, then the main constituent of the pectin is the RG-I region (Schols & Voragen, 1996). In the present study, the molar ratio of Rha/GalA in all the potato pectins ranged from 0.06 to 0.07, which indicates high proportions of the RG-I domains (60.77%–65.54%) in potato pectin, whereas the HG region contents were much lower (28.48%–35.13%). However, the commercial citrus and apple pectins exhibited lower Rha/GalA (0.017–0.027) ratios, which suggest that the HG domains are predominant in pectins (Besson et al., 2014).

3.4. DM, DA, and protein content of potato pectins

DM and DA, which are defined as the molar ratios of methanol or acetic acid to GalA, respectively, were determined by ^{1}H NMR. Table 1 shows that the DM and DA values of all the potato pectins were in the ranges of 21.51%–37.45% and 9.21%–15.38%, respectively. These results are consistent with those obtained by HPLC, which showed DM and DA values of 31% and 14% for potato pectin, respectively (Voragen et al., 1986).

The pectin with the highest DM and DA was obtained by acetic acid extraction. For HCl, H_2SO_4, and HNO_3 extraction, the DM and DA values of HPP were much higher, followed successively by SPP and NPP. Interestingly, the pectin extracted using citric acid had the lowest DM and DA values. The above results might be related to the structure of potato pectin which is affected by acid types. For example, some studies have showed that the HG could be methyl esterified and acetylated, the highly acetylated RG-I domain was also abundant in potato pectin (Schols & Voragen, 1996; Willats, McCartney, Mackie, & Knox, 2001). The strength of acetic acid is the lowest among these five acids, so it could retain more methyl esters although its HG was not the highest (Table 1). Furthermore, the RG-I domain in APP was much higher (Table 1), thus the DA was the highest. The results in Table 1 also showed that the RG-I domain in NPP was a little higher than that of APP, however, the strength of HNO_3 is much greater and could destroy the acetyl groups and methyl esters, thus reducing the DM and DA.

In this study, the DM values of all the potato pectins were much lower than 50%; thus, potato pectin is classified as low methoxyl pectin. The DA values higher than 8% are considered as high acetylated pectins (Chan & Choo, 2013), therefore, the potato pectins are highly acetylated and the DA values are much higher than that of commercial citrus (1.4%–1.6%) and apple pectin (5.0%) (Kravtchenko et al., 1992). High degree of acetylation can alter the solubility and gelation properties of pectin (Willats et al., 2001), however, acetyl groups may act as a positive role in the emulsifying process.

Protein was also found in all the potato pectins whose content ranged in 1.85%–6.97% (dry basis), and this is similar to that of sugar beet pectin (0.9%–6.8%) extracted with H_2SO_4 under different acidic conditions (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). The protein content of pectins extracted using organic acids is much higher than that of pectins extracted with mineral acids. It is probably due to the weak hydrolyzing abilities of organic acids, thus more protein was recovered by ethanol precipitation. The highest protein content was found in the pectin extracted using acetic acid (6.97%), whereas SPP had the lowest protein content (1.85%). The protein content of all the potato pectins was much higher than that of the commercial apple pectin (~1.6%, dry basis), and except for SPP, the other potato pectins had similar or much higher protein contents than the commercial citrus pectin (3.0%–3.3%, dry basis) (Kravtchenko et al., 1992).

3.5. Molar mass distributions of potato pectins

The molar mass distributions of HPP, SPP, NPP, CPP and APP are shown in Fig. 1. The MALLS detector was used to determine the molar mass distribution of the pectin, and the RI detector was used to ascertain the relative concentrations of the different molar mass distributions. In addition, the wavenumber for the UV detector was set at 280 nm, and the detector could detect the presence of protein.

As can be seen in Fig. 1, all the potato pectins had similar elution profiles. For the RI elution profiles, all the pectins had three peaks, at 10–12 min, 13–16 min, and 17–20 min, and the peak at 17–20 min was the highest. However, for the MALLS detector, the highest peak always appeared at 10–12 min which was related to the high molecular mass pectin. These results indicate that the pectin with the highest molar mass always had lower concentrations, and the pectin with lower molecular weight was predominant in all the samples. In addition, we also detected protein in the sample because the UV detector showed peaks at 10–12 min and 16–20 min, which were consistent with the RI peaks. This is also consistent with the results given by the modified Lowry method (see Table 1). The protein might exist in pectin in a free or conjugated form.

Furthermore, Mw, MF, and Mw/Mn are presented in Table 2. All the potato pectins also showed three clear peaks with different mass fractions, and the lowest molar mass always had the highest distribution percentage (> 50%).

Considering the whole range of the elution profiles, APP and CPP exhibited the highest Mw (3.207×10^5 g/mol) and the lowest Mw (2.319×10^5 g/mol), respectively, whereas the HPP, SPP and NPP showed medium Mw in the range of 2.58×10^5 g/mol to 3.18×10^5 g/mol. Citric acid is a kind of tricarboxylic acid which has a stronger hydrolyzing ability than that of acetic acid, therefore, comparing with APP, CPP exhibited a lower RG-I domain (61.49%) and a higher proportion of HG (33.44%) (Table 1). Furthermore, the RG-I domain of CPP is the least branched as mentioned above. Previous researches reported that the Mw of potato RG-I domain which was substituted by long neutral sugar side chains was much higher than that of linear HG region (Khodaei et al., 2016; Thomassen, Vigsnaes, Licht, Mikkelsen, & Meyer, 2011). Therefore, CPP had much lower Mw than APP. Whereas, acetic acid had the lowest hydrolyzing ability among the five acids in this study, and thus, the molecular weight of the APP was the highest compared to the other pectins. In addition, HCl, H_2SO_4, and HNO_3 were shown to be strong hydrolyzers that could break down cell walls and release the pectin easily; however, compared with APP, the resultant pectins had medium Mw, and this may be because they were hydrolyzed by acids. These results were similar to those of Seixas et al. (2014), who extracted pectin from passionfruit peel using two organic acids (tartaric acid and acetic acid, respectively) and HNO_3, and found that higher Mw pectins were obtained using HNO_3 (4.966×10^5 g/mol) and acetic acid (4.625×10^5 g/mol) than with tartaric acid (2.298×10^5 g/mol). On the contrary, other studies found that pectins extracted with organic acids, such as citric acid, always had a higher Mw than pectins extracted using mineral acids. Previous study attributed this result to the weak strength of organic acids, which leaded to a low extent of pectin hydrolysis (Kermani et al., 2014). As for the opposite effects of citric acid and acetic acid on the potato pectin Mw, further studies are necessary to explain the reasons.

Mw/Mn, which is also known as the polydispersity index, reflects the width of the molar mass distributions of polymers. The Mw/Mn value of monodispersive polymers is 1, and higher Mw/Mn values indicate a wider molar mass distribution (Rogosic, Mencer, & Gomzi, 1996). The Mw/Mn values of all the potato pectins in this study ranged from 5.249 to 8.000, which are much higher than 1. Therefore, these values suggest that potato pectins are highly heterogeneous polysaccharides with broad molecular weight distributions.

3.6. FTIR spectra of different kinds of pectins

Fig. 2 shows the FTIR spectra of the potato pectins and commercial citrus and apple pectins. The spectra of the different potato pectins were similar, which indicates that the main structure of the pectic polymers was not greatly affected even with different acid extractants. The typical absorption areas of the pectic polysaccharides in the spectra of the commercial citrus and apple pectins were also found in all the potato pectin spectra.

For the potato pectins, strong and broad peaks were found at 3435 cm^{-1}, which can be attributed to O—H stretching vibration, and the peaks at 2938 cm^{-1} can be attributed to C—H stretching from the CH_2 groups (Chatjigakis et al., 1998). The bands at about 1748 cm^{-1} and 1626 cm^{-1} can be attributed to absorptions by esterified and free carboxyl groups, respectively. Further, the DM of pectin is related to the ratio of the peak area at 1748 cm^{-1} to the sum of the peak areas at 1748 cm^{-1} and 1626 cm^{-1} (Pappas et al., 2004). For all the potato pectins, the absorption area at 1748 cm^{-1} was weaker than that at

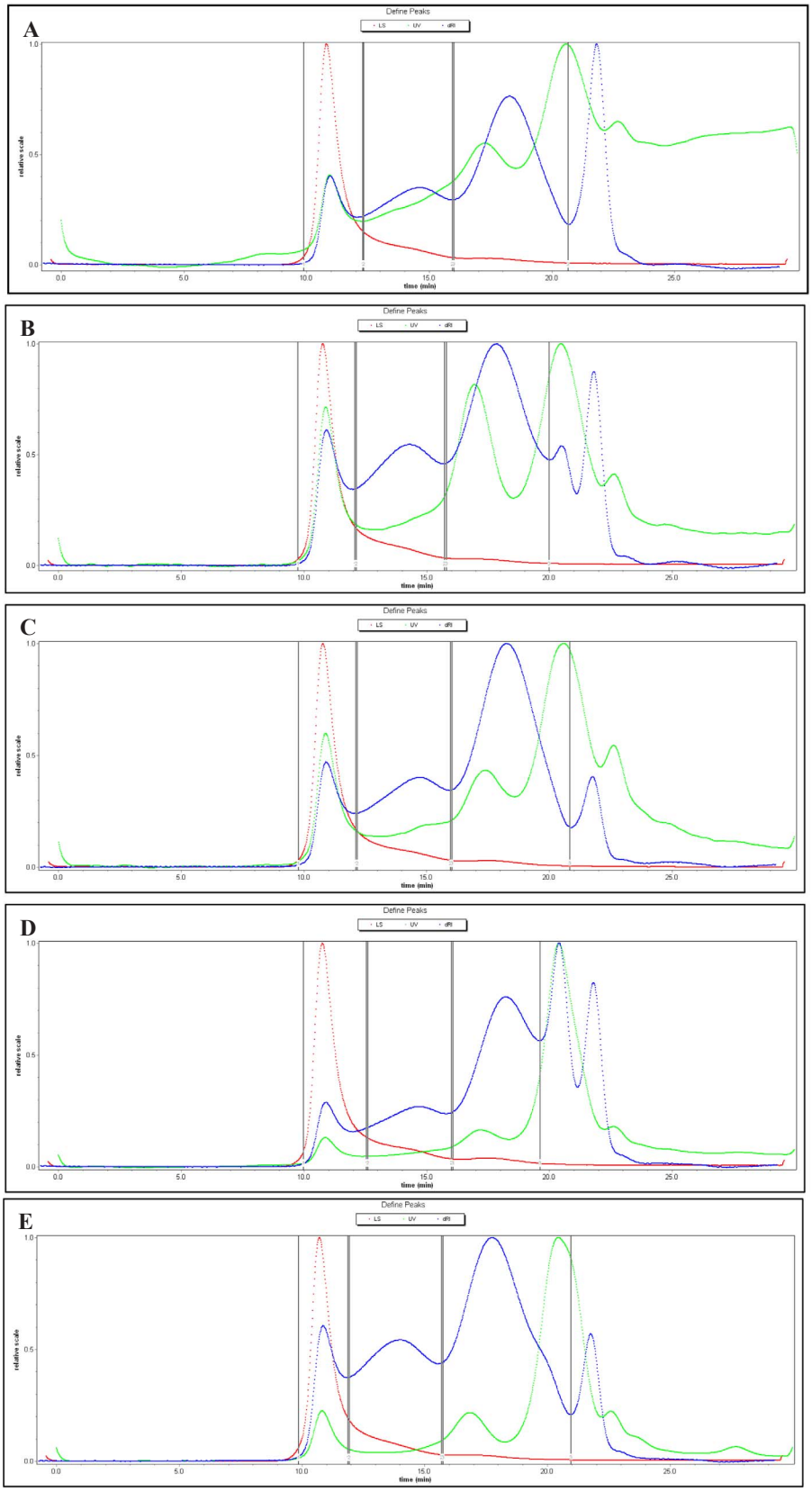

Fig. 1. The elution profiles of different potato pectins. (A–E were potato pectins extracted by HCl, H_2SO_4, HNO_3, citric acid and acetic acid.)

1626 cm^{-1}, which shows that they are low methoxyl pectins. This finding is also in accordance with the DM results shown in Table 1. The range of the absorption bands from 800 to 1300 cm^{-1} is often referred to as the fingerprint region of pectins. Different kinds of pectic substances can be distinguished throughout this region (Gan, Manaf, & Latiff, 2010). It is worth noting that, unlike the spectra of the commercial citrus and apple pectins, the potato pectins exhibited a unique and strong absorption peak at 1064 cm^{-1}, which can be attributed to acetyl groups; acetylation of pectin may result in structural changes of the pyranoid ring (Synytsya, Copikova,

Table 2
The weight-average molecular weight (Mw), Mw/Mn and mass fraction (MF) of different potato pectins.

	Whole range		Peak 1		Peak 2		Peak 3	
	Mw (g/mol)	Mw/Mn	Mw (g/mol)	MF (%)	Mw (g/mol)	MF (%)	Mw (g/mol)	MF (%)
HPP	2.799×10^5	5.520	1.528×10^6	13.3999	1.879×10^5	27.7554	3.929×10^4	58.8446
SPP	3.176×10^5	5.832	1.681×10^6	13.7737	2.145×10^5	30.9612	3.753×10^4	55.2651
NPP	2.580×10^5	8.000	1.485×10^6	11.9480	1.768×10^5	26.4600	2.429×10^4	61.5921
CPP	2.319×10^5	5.249	1.261×10^6	13.4801	1.605×10^5	24.8124	3.249×10^4	61.7075
APP	3.207×10^5	6.234	1.874×10^6	12.1301	2.240×10^5	30.5072	3.681×10^4	57.3627

HPP, SPP, NPP, CPP and APP were the pectins extracted by HCl, H_2SO_4, HNO_3, citric acid, and acetic acid.

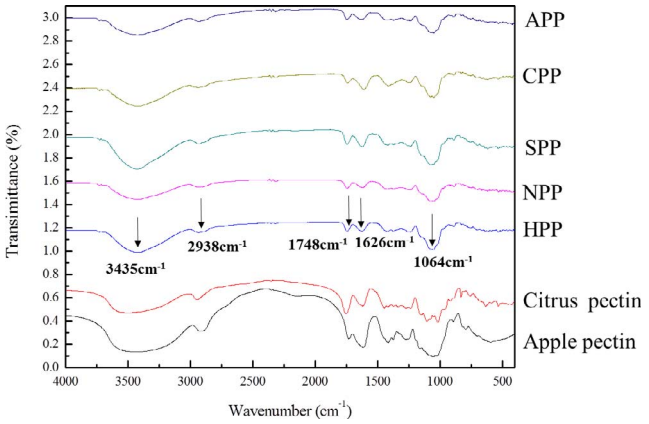

Fig. 2. FTIR spectra of citrus pectin, apple pectin and potato pectins. (HPP, SPP, NPP, CPP and APP were the pectins extracted by HCl, H_2SO_4, HNO_3, citric acid and acetic acid.)

Matějka, & Machovic, 2003). The high DA values (Table 1), as well as the spectra results confirm the existence of acetyl groups in potato pectins.

3.7. Morphological characteristics of potato pulp and potato pectin

Fig. 3 shows the SEM results of potato pulp (before and after enzymatic treatment) and potato pectins. Fig. 3A and B show the effect of thermostable α-amylase treatment on the removal of starch from potato pulp. Fig. 3A shows an image of the original potato pulp, and many round starch granules can be seen, which indicates that starch was still a main component in the pulp. Fig. 3B shows fewer starch granules in the pulp after enzymatic hydrolysis; instead, a large amount of fibrous material remains.

Fig. 3C–G show significantly different morphologic features among the potato pectins obtained using different acid extractants. HPP had a filamentous and loose structure with some scattered particles on the surface (Fig. 3C). The structure of SPP was similar to that of HPP, although its surface was more compact and much smoother (Fig. 3D). Fig. 3E shows the structure of NPP, which had a small lamellar structure that was closely packed, with an uneven surface. Fig. 3F shows the structure of CPP, which was soft, and could curl easily. The structure of APP was unique among the potato pectins because it had a highly compact surface that appeared to be very hard (Fig. 3G).

3.8. Emulsifying properties of pectins

The emulsifying properties of the potato pectins, commercial citrus and apple pectins are shown in Table 3. The EA of the potato pectins ranged 44.97%–47.71%, which is a little higher than that of the commercial citrus pectin (44.87%) and apple pectin (45.34%). And this data is similar to the range of EA (43.2%–47.1%) from sugar beet pectin extracted by acids (Yapo et al., 2007). Previous studies showed that EA is related to the protein and acetyl group contents, as well as the branching extent of RG-I domain. For the potato pectins, the EA of HPP was the highest (47.71%), and this may be due to the protein moiety (2.78%) and the high DA (11.92%). The protein and hydrophobic acetyl groups of pectin can act as anchors on the oil particle surface, thus decreasing the surface tension (Leroux et al., 2003). Although APP had the highest protein (6.97%) and acetyl group contents (DA, 15.38%), its EA (44.97%) was the lowest, and this is likely because of the steric hindrance from the highly branched RG-I domain caused by the abundance of neutral sugar side chains, which prevent the protein and acetyl groups from approaching the oil droplets, thus decreasing the EA.

The ES of the potato pectin emulsions ranged 36.54%–46.00%, which is higher than the ES of the commercial citrus pectin (36.14%) and apple pectin (19.15%) emulsions. The HPP and APP emulsions had the highest ES (46.00% and 45.80%, respectively), and the CPP emulsion had the lowest ES (36.54%). The higher ES for HPP and APP could be explained by the higher DA and rich neutral sugar side chains of the RG-I domain. Some interchain associations of pectin may be induced by calcium binding. However, high contents of acetyl group may reduce calcium sensitivity, thus minimizing pectin flocculation and allowing free pectin to react with oil particles to stabilize the emulsion (Leroux et al., 2003). Although an abundance of neutral sugar side chains would hinder the emulsifying process, it could improve the ES of HPP and APP by forming a thick hydrated layer on the oil droplets, thus preventing the oil particles from aggregating (Funami et al., 2011). For CPP, the DA was the lowest and its RG-I domain was the least branched; therefore, the hydrated layer may be too thin to stabilize the emulsion. Compared with the commercial citrus and apple pectins, potato pectin had better emulsifying properties, and it could potentially be used as a natural emulsifier in the food processing industry.

4. Conclusions

HCl, H_2SO_4, HNO_3, citric acid, and acetic acid significantly affected the extraction yield, structure, and emulsifying properties of potato pectin. The results showed that potato pectin extracted by citric acid had the highest yield (14.34%), although it had the lowest DM (21.51%), DA (9.21%), and molecular weight (2.319×10^5 g/mol). The yield of potato pectin obtained by acetic acid extraction was the lowest (4.08%); however, its DM (37.45%), DA (15.38%), and molecular weight (3.207×10^5 g/mol) were the highest. In addition, the potato pectins had high proportions of the RG-I domain (> 60%), which is highly branched with neutral sugar side chains. The FTIR spectra revealed acetyl groups in all the potato pectins, and SEM also showed significantly different morphology among the potato pectins. The results for the emulsifying properties indicated that potato pectins have better EA and ES than commercial citrus and apple pectins. Our findings suggest that potato pectin may be useful as a potential emulsifier in emulsified food products.

Acknowledgements

This study was supported by the earmarked fund for the National

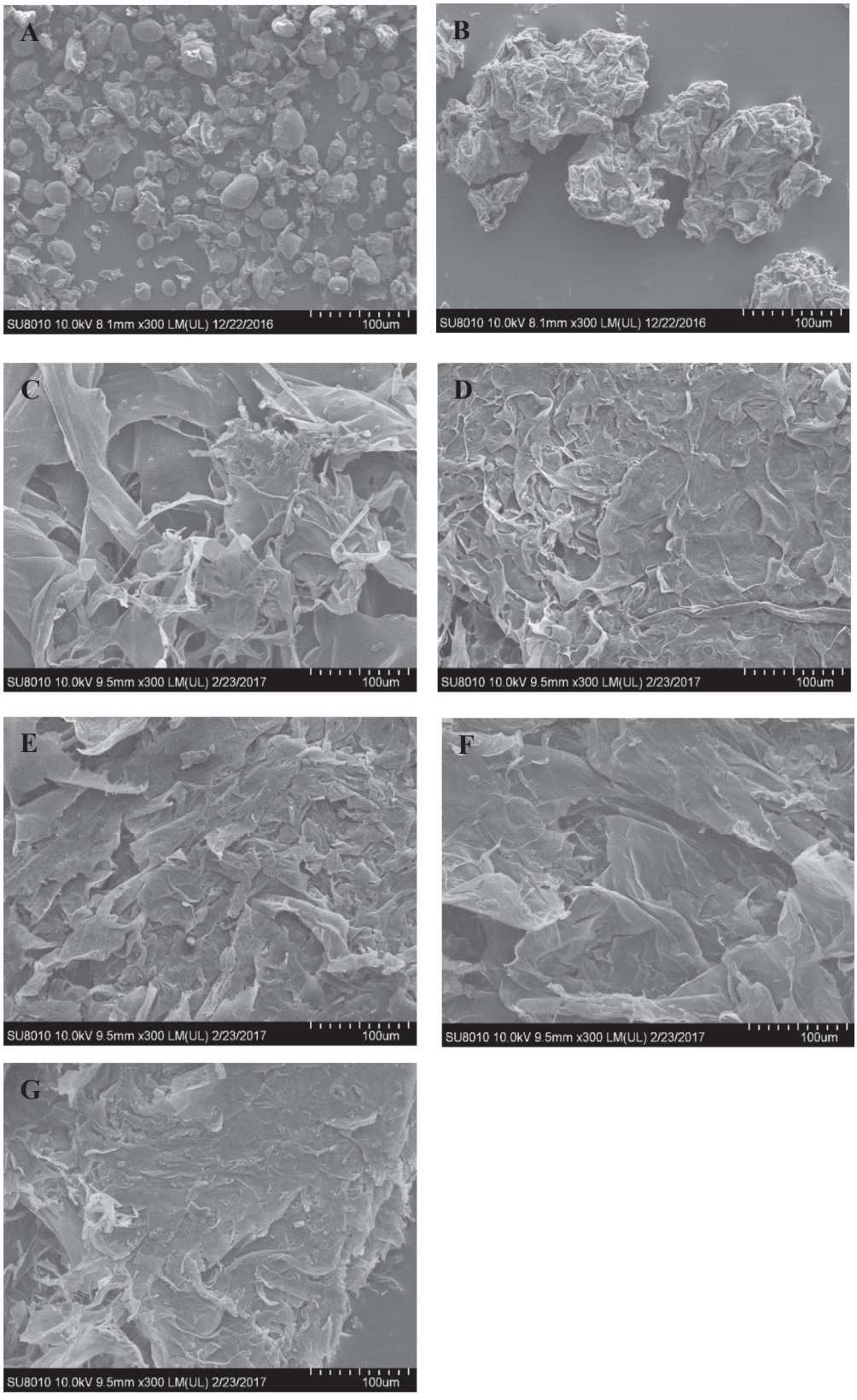

Fig. 3. The SEM images of potato pulp and potato pectins. A and B were potato pulp before and after enzyme treatment; C–G were the pectins extracted by HCl, H_2SO_4, HNO_3, citric acid and acetic acid; 300× magnifications.

Key R & D Program of China (2016YFE0133600) and the Public Welfare Industry (Agriculture) Research Project of China (201503001-2). The authors are grateful for the support provided by the Institute of Agro-Products Processing Science and Technology of the Chinese Academy of Agricultural Sciences.

Conflicts of interest

The authors have no conflicts of interest to declare.

Table 3
Emulsifying activity and emulsion stability of potato pectin, commercial citrus pectin and apple pectin.

	Emulsifying activity (%)	Emulsion stability (%)
HPP	47.71 ± 0.62^{a}	46.00 ± 0.78^{a}
SPP	45.99 ± 0.63^{ab}	43.52 ± 1.31^{ab}
NPP	45.37 ± 0.12^{b}	44.21 ± 1.51^{ab}
CPP	46.055 ± 1.86^{ab}	36.54 ± 2.72^{b}
APP	44.97 ± 0.19^{b}	45.80 ± 0.90^{a}
Citrus pectin	44.87 ± 0.33^{b}	36.14 ± 4.93^{b}
Apple pectin	45.34 ± 0.93^{b}	19.15 ± 6.87^{c}

All results were shown in the form of means ± standard deviation (SD). Data with different letters in the same column were significantly different, $p < .05$. HPP, SPP, NPP, CPP and APP were the pectins extracted by HCl, H_2SO_4, HNO_3, citric acid and acetic acid.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.10.059.

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