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Physicochemical and comparative properties of pectins extracted from *Akebia trifoliata* var. *australis* peel

Yan Jiang[a], Yanxue Du[a], Xuemei Zhu[a], Hua Xiong[a,*], Meng Wai Woo[b], Juwu Hu[a,c]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, Jiangxi 330047, PR China
[b] Department of Chemical Engineering, Monash University, Clayton, Victoria 3800, Australia
[c] Jiangxi Academy of Sciences, Jiangxi 330029, PR China

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ABSTRACT

The physicochemical properties of the pectins extracted from *Akebia trifoliata* var. *australis* peel with hydrochloric acid and citric acid, namely HEP and CEP, were evaluated as compared with citrus pectin (CP). X-ray diffraction confirmed that CP had more well defined crystal than HEP and CEP. The DE values of HEP, CEP and CP were 59.46%, 76.64% and 71.03%, respectively. CP exhibited the highest viscosity-average molecular weight of 64,848 Da, followed by HEP (45,353 Da) and CEP (28,877 Da). In general, the emulsion activity of HEP and CEP increased as oil concentration was increased, while HEP showed the strongest emulsion activity among the three pectins. Textural analysis demonstrated that the gelling properties of three pectins decreased with increase in pH, and CP displayed superiority in hardness (9.03 g), while CEP was the poorest (1.45 g). All results suggested that *A. trifoliata* var. *australis* had the potential in producing pectin for commercial food industry application.

1. Introduction

The backbone of pectin molecules is composed of poly-α-(1→4)-D-galacturonic acid with carboxyl groups in the methyl ester form. Pectin is a family of complicated variable heteropolysaccharides that has been widely used in food industry as stabilizer, thickener, emulsifier and gelling agent (Pilnik, 1990). Furthermore, it has been reported that pectin is able to defend against a large number of diseases, such as obesity, diabetes, constipation, gall stone and colon cancer (McCleary & Prosky, 2001). Pectins are mostly characterized by their physicochemical characteristics, which include the degree of esterification, galacturonic acid, neutral sugar contents, molecular weight and side chain of pectin. These parameters are greatly influenced by the extraction conditions (Fishman, Pfeffer, Barford, & Doner, 1984; O'Donoghue & Somerfield, 2008). Although pectin exists in many plant tissues, its production is conventionally based on only a few sources (apple pomace and citrus peel) around the world (Thakur, Singh, & Handa, 1997; Willats, Knox, & Mikkelsen, 2006). Consequently, more potential sources should be explored to produce commercial pectins from the endogenous species and by-products of food processing, particularly in developing countries.

Akebia trifoliata (Thunb.) Koidz. var. *australis* (Diels) Rehd., a woody climbing vine, belonging to the genus *Akebia*, is widespread in Asia, especially in China, Korea and Japan (Liu, Ma, Zheng, Zhang, & Lin, 2007). *A. trifoliata* var. *australis* is commonly referred to as an important medicinal herb in traditional Chinese and Japanese medicine for activating blood circulation, subduing inflammation and facilitating diuresis. Besides medicinal uses, many parts of this plant are conventionally consumed as edible materials in China. The dried young leaves can serve as a tea substitute, and the fruits are used for making fruit vinegar, sweet replacement and juice (Jiang, Shi, Cao, Gao, & Tu, 2008; Kitaoka et al., 2009). Three new lignan glycosides were firstly isolated from the stem of *A. trifoliata* var. *australis* (Guan et al., 2007). Triterpenes and triterpene saponins were also shown to be present in the stems, leaves, dried pericarps and seeds of the *A. trifoliata* var. *australis* (Liu et al., 2007). Although the weight of the peel is comprised 60% total weight of the *A. trifoliata* var. *australis* fruit, it is still regarded as a waste material, discarded after eating, decocting and processing. Moreover, up to now, the study of pectins from *A. trifoliata* var. *australis*, as well as their extraction conditions, has not been reported. Therefore, it is possible to extract pectin from the *A. trifoliata* var. *australis* peel as a novel functional carbohydrate.

The objective of present work was to extract pectins from the *A. trifoliata* var. *australis* peel with hydrochloric acid and citric acid, as compared with CP. The physicochemical properties of the pectins were then characterized by scanning electron microscopy, FT-IR spectroscopy, X-ray diffraction, intrinsic viscosity,

* Corresponding author. Tel.: +86 791 6634810/13607911660; fax: +86 791 6634810.
E-mail address: huaxiong100@yahoo.com.cn (H. Xiong).

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viscosity-average molecular weight, emulsion activity and gels textural analysis.

2. Materials and methods

2.1. Materials

Ripe *A. trifoliata* var. *australis* fruits were harvested in October 2010 from a hill of Lushan Mountain (Jiangxi, China) and then immediately transferred to our lab. The peels of the fruits were removed, washed in tap water and cut into pieces of 1–2 cm^2. The peels were then blanched in boiling water for 5 min and dehydrated in a hot air drier at 60 °C. The dried peels were crushed into powders with a disintegrator and then passed through a 60-mesh sieve. Collected powders were sealed and stored in a refrigerator for pectin extraction. Citrus pectin (CP) from Shanghai Jingchun Reagent Co., Ltd. (Shanghai, China) was purchased for comparison. All other chemical reagents were of analytical or chromatographic grade.

2.2. Pectin extraction

Twenty grams of peel powders were soaked in 600 ml of distilled water, pH was adjusted to 1.5 with 2 M hydrochloric/citric acids, respectively. Two resulting slurries were stirred in a water bath at 85 °C for 1.5 h, and then filtered through a 200-mesh filter cloth. The filtrates were centrifuged at 4800 rpm for 20 min to remove the solid particles. The pectins were precipitated with two volumes of 95% ethanol and kept for 1 h prior to filtration. The precipitates were successively washed three times with 65%, 85% and 100% ethanol, and obtained pectins were lyophilized and then ground to powders. Two samples were stored in hermetically sealed bags prior to analysis.

2.3. Scanning electron microscopy (SEM)

The pectins were mounted onto a specimen stub with a double-sided tape and sputter coated with gold. Subsequently, the morphological features of the samples were observed by SEM (Quanta 200 F, FEI, Hillsboro, OR, USA) with an accelerating voltage of 20 kV.

2.4. Determination of the physicochemical composition

The length, diameter, total weight and the contents (w/w) of the peel, seed, and pulp were determined using average measures of 15 ripe fruits. The protein ($N \times 6.25$), moisture, ash, fat, total sugars and fiber contents of the fruit peels and pectins were determined by approved AOAC methods (1995) and their results are presented in Tables 1 and 2, respectively. The pH of the pectins was determined with a pH meter (PB-10, Sartorius, German). The determination of galacturonic acid (GlaA) contents was performed by the carbazole-sulphuric acid method (Bitter & Muir, 1962), using D-galacturonic acid as a standard.

2.5. Determination of degree of esterification (DE)

The degree of esterification (DE) was assessed by a direct titrimetric method described by Pinheiro et al. (2008). Briefly, pectins (200 mg) in 50 ml conical flasks were moistened with ethanol and dissolved in 20 ml of deionized water at 40 °C for 2 h. After the pectins were completely dissolved, one drop of phenolphthalein was added. The solutions were then titrated with 0.1 M sodium hydroxide and the results were recorded as V_1. Then, 10 ml of 0.1 M sodium hydroxide was added, with the conical flasks covered with glass stopples, the solutions were stirred at room temperature for 2 h. Another 10 ml of 0.1 M hydrochloric acid was added and the solutions were shaken until the pink colour disappeared. The solutions were titrated with 0.1 M sodium hydroxide again, and the final results were recorded as V_2. The DE was calculated according to the following formula:

$$\text{DE}\ \ (\%) = \frac{V_2}{V_1 + V_2} \times 100 \quad (1)$$

Table 1
Physicochemical composition of ripe *Akebia trifoliata* var. *australis* fruit and peel.

Length of fruit (cm)	8.15 ± 1.84
Fruit diameter (cm)	4.81 ± 1.72
Fruit weight (g)	78.08 ± 15.83
Peel content (%)	63.05 ± 2.75
Seed content (%)	15.21 ± 1.58
Pulp content (%)	20.89 ± 1.06
Moisture (%)[a]	85.63 ± 0.04
Ash (%)[a,b]	6.35 ± 0.12
Protein (%)[a,b]	2.59 ± 0.23
Fat (%)[a,b]	0.32 ± 0.53
Total sugars (%)[a,b]	18.21 ± 0.11
Total fiber (%)[a,b]	18.45 ± 0.56
Pectin (%)[a,b]	13.26 ± 0.41

Fruit length, diameter, weight, peel, seed and pulp contents are an average of 15 fruits. Each value is expressed as mean ± standard deviation of triplicate tests with at least three measurements.
[a] Chemical composition of *Akebia trifoliata* var. *australis* peel.
[b] Chemical composition is given in percentage of dry basis.

2.6. FT-IR spectroscopy

The sample was incorporated with KBr and pressed into a pellet prior to analysis using an infrared spectrometer (Nicolet 5700, USA). The spectrum was recorded in the transparent mode from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}.

2.7. X-ray diffraction analysis (XRD)

The X-ray diffraction patterns of the pectins were recorded with the aid of an X-ray diffractometer (D8-focus, Bruker, Karlsruhe, Germany). The pectins in powder form were scanned from 5° to 60° diffraction angle (2θ) with Cu Kα radiation at voltage of 40 kV, current of 40 mA, step-scan mode with a step size of 0.02° (2θ) and counting time of 0.2 s/step.

2.8. Monosaccharide composition analysis

The sample (10 mg) was hydrolyzed in 2 M trifluoroacetic acid (2 ml) at 100 °C for 8 h. The hydrolysate was cooled to ambient temperature and dried by nitrogen in a water bath (70 °C). Hydroxylamine hydrochloride (10 mg) and pyridine (0.5 ml) were then added, the mixture was incubated at 90 °C for 30 min with shaking. After cooling, 0.5 ml of acetic anhydride was added to the mixture and shaken at 90 °C for 30 min again. The derivatives were obtained after filtering through the organic filter membrane. Quantification was performed by gas chromatography (6890N, Agilent Technologies Co., USA) equipped with a hydrogen flame ionization detector. The column used was DB1701 (30 m × 0.25 mm, 0.25 μm) and nitrogen served as carrier gas. The temperature program of initial temperature 170 °C, with a hold of 2 min, followed by a temperature rise of 10 °C/min to 250 °C, with a final hold of 10 min was conducted.

2.9. Intrinsic viscosity ([η]) and viscosity-average molecular weight (M_v)

The pectin intrinsic viscosity was assessed using the procedure described by Kar and Arslan (1999) with a slight modification and

Table 2
Characterization and sugar composition of pectins.

Characteristics	Pectin samples		
	HEP	CEP	CP
Moisture (%)	7.84 ± 0.23[a]	10.29 ± 0.35[b]	10.64 ± 0.27[b]
Ash (%)	2.59 ± 0.15[c]	1.03 ± 0.10[a]	1.75 ± 0.22[b]
Protein (%)	0.76 ± 0.09[a]	0.69 ± 0.11[a]	1.84 ± 0.31[b]
pH	2.96 ± 0.06[a]	3.40 ± 0.03[b]	4.12 ± 0.05[c]
GlaA (%)	80.06 ± 2.15[c]	71.43 ± 1.57[b]	61.24 ± 1.62[a]
Individual neutral sugars (%)			
Rhamnose	1.52 ± 0.33[a]	1.64 ± 0.2[a]	19.72 ± 0.97[ab]
Fucose	–	0.05 ± 0.02	–
Xylose	1.59 ± 0.29[b]	1.30 ± 0.14[ab]	1.09 ± 0.17[a]
Mannose	–	–	0.07 ± 0.01
Glucose	0.16 ± 0.03[a]	0.34 ± 0.10[b]	0.06 ± 0.02[a]
Degree of esterification (%)	59.46 ± 1.48[a]	76.64 ± 2.01[b]	71.03 ± 2.46[b]
$[\eta]$ (m^3/kg)	0.158 ± 0.002[b]	0.109 ± 0.001[a]	0.212 ± 0.007[c]
M_v (Da)	45,353 ± 698[b]	28,877 ± 322[a]	64,848 ± 2604[c]

Each value is expressed as mean ± standard deviation of triplicate tests with at least three measurements. Means within the same row with different letters are significantly different ($p < 0.05$), according to Duncan's Multiple-Range Test.

calculated by joint extrapolation with the Huggins equation. The viscosity-average molecular weight was obtained according to the Mark–Houwink–Sakuarda equation relating to the intrinsic viscosity and molecular weight (M_v):

$$[\eta] = K[M_v]^{\alpha} \quad (2)$$

where K and α are constants depending on the temperature, solute and solvent characteristics. In this paper, the following values were assumed $K = 2.34 \times 10^{-5}$ and $\alpha = 0.8224$.

Samples were dispersed in 0.1 M sodium phosphate buffer (pH 7.0) with the solution concentration ranging from 1.0 to 10 kg/m^3. The viscosities of pectin solutions at different concentrations were determined at 20 °C by means of an Ubbelohde viscometer No. 13 (I.D.: 0.84 mm).

2.10. Emulsion activity

Solutions, containing 10 ml of citrate buffer (0.1 M, pH 5.0), 0.5% (w/v) pectin and 0.02% NaN_3 as a bacteriocide were prepared with stirring at ambient temperature overnight. Oil-in-water (O/W) emulsions prepared by adding 5%, 10%, 15%, 20%, 25%, 30%, 35%, and 40% (oil volume/total volume) soybean oil to pectin solutions were homogenized using a high-speed homogenizer (Jiangsu Huanyu Instrument Co., China) at 15,000 rpm for 1 min, then 50 μl of emulsions were mixed with 5 ml of 0.1% (w/v) sodium dodecyl sulphate (SDS). The emulsion activity was assessed as turbidity T (Pearce & Kinsella, 1978):

$$T = \frac{2.303 \times A \times D}{L} \quad (3)$$

where T is the turbidity in 1/cm, A is the absorbance at 500 nm, D is the dilution factor, and L is the path length of the cuvette = 1 cm. The absorbance was measured immediately at 500 nm after preparing the diluted emulsions with an UV spectrophotometer (TU-1901, Purkinje General Instrument Co., Ltd., Beijing, China), the 0.1% SDS solution was used as a blank.

2.11. Pectin gels preparation

To form pectin gels, sucrose (60%) was added to the pectin solution at 1% (w/v), pH was adjusted to 2.0 and 4.0, the mixture was then heated at 100 °C for 30 min with magnetic stirring. The gels were allowed to stand overnight at 4 °C for 12 h prior to textural analysis.

2.12. Texture profile analysis

Texture profile analysis (TPA) was performed using a CT3 Texture Analyzer (Brookfield Engineering Labs, Inc., USA). The gels were pierced by compression at a constant speed of 1.0 mm/s to a distance of 3 mm from the gel surface with a cylindrical probe (TA 3/100, diameter 12.7 mm). The parameters including hardness, springiness, adhesiveness, chewiness, gumminess, cohesiveness and resilience were derived from the analysis of the stress–strain curve.

2.13. Statistical analysis

All experiments were conducted in triplicate and the results were expressed as the mean value ± standard deviation of three measurements. Significant differences between means ($p < 0.05$) were analyzed with ANOVA using SPASS 16.0 (SPASS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Composition of A. trifoliata var. australis fruits

As presented in Table 1, the average peel content of the ripe *A. trifoliata* var. *australis* fruit was found to be 63.05%, seed and pulp content were 15.21 and 20.89%, respectively. Furthermore, the total sugar content of the peel (up to 18.21%) was higher than that of the passion fruit peel (10.2%) reported by Kulkarni and Vijayanand (2010), while its pectin content (13.26%) was similar with the apple pomace consisting of 10–15% pectin (Endress, 2000). The data suggested that the *A. trifoliata* var. *australis* peel is a rich source of sugars, fiber and pectin worthy of future development and utilization.

3.2. Morphology of the pectins

The pictures of HEP, CEP and CP showed in detail the distinct surface structures. HEP (Fig. 1A) consisted of monostratal, lamellate and irregular-shaped particles with relatively smooth surfaces. However, CEP (Fig. 1B) particles were more irregular and lumpish in shape with rough surfaces, which differed greatly with HEP. Comparatively, CP (Fig. 1C) particles presented multi-laminate and flaky structures, which looked like flowers blooming in profusion. Therefore, the analysis indicated that the pectin formation was greatly influenced by the extraction process, pectins extracted with different extractants could exhibit significantly different morphologies.

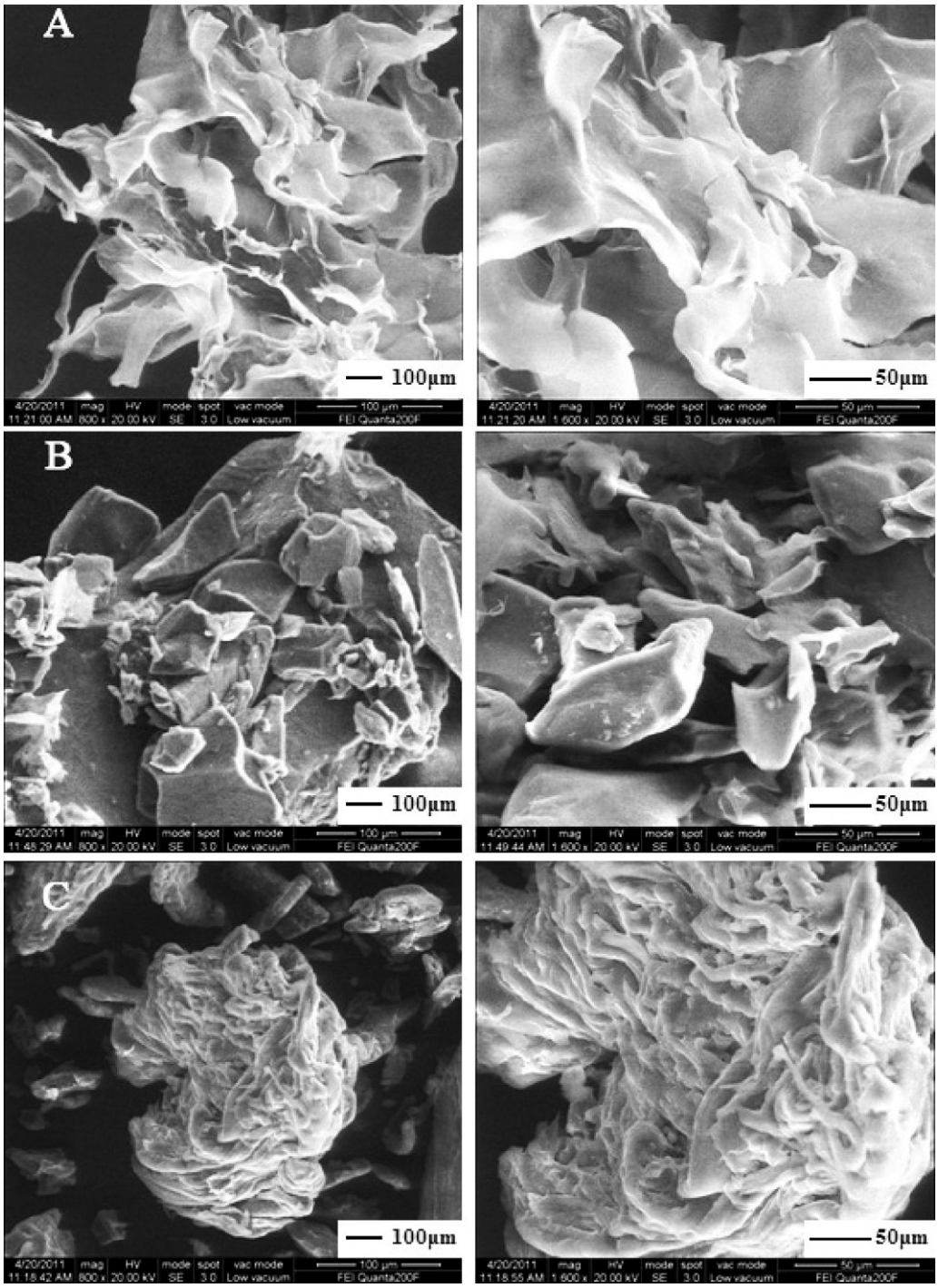

Fig. 1. Morphology of the pectins: (A) HEP, (B) CEP, and (C) CP at magnifications 800× (left) and 1600× (right).

3.3. Characterization and sugar composition of pectins

The characterization and sugar composition of HEP, CEP and CP are shown in Table 2. The three pectins exhibited GlaA contents of 80.06, 71.43 and 61.24%, respectively, which were significantly ($p < 0.05$) different from each another. In particular, the GlaA content of HEP was the highest, followed by those of CEP and CP. The contents of xylose, glucose and arabinose were also determined, data indicated that HEP and CEP were extremely low in total neutral sugar contents (3.27 and 3.33%, respectively), whereas CP exhibited a considerably high neutral sugar content of 20.94%.

Above all, those results demonstrated that the polysaccharides extracted by hydrochloric acid and citric acid were mostly composed of GlaA and a small number of neutral sugars, initially suggesting that the two polysaccharides were both pectins. The degree of esterifications of the pectins were 59.46%, 76.64% and 71.03%, respectively, which were confirmed and compared later by FT-IR spectroscopy.

3.4. FT-IR spectroscopy

Analysis of the FT-IR spectra was performed in order to identify the major functional groups of the pectins extracted by

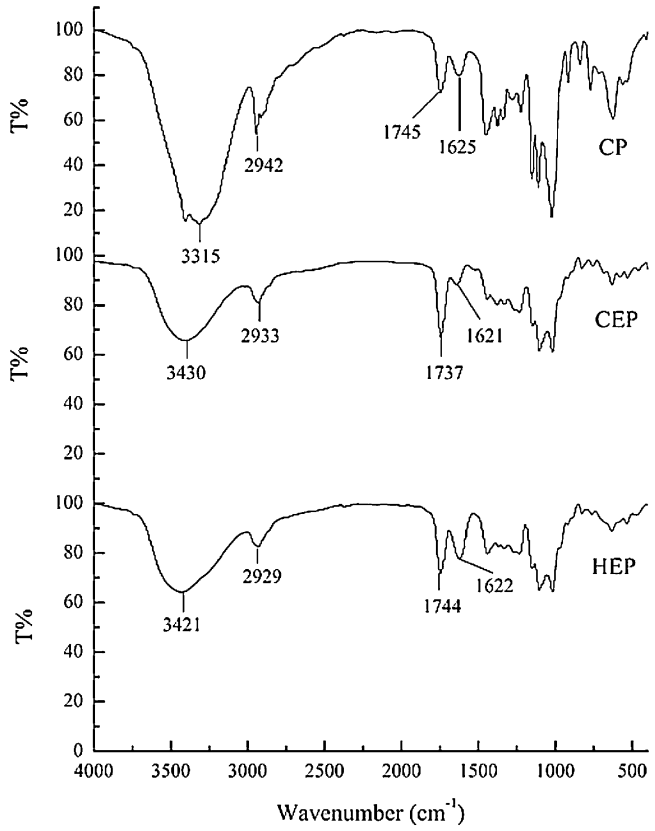

Fig. 2. FT-IR spectra of HEP, CEP and CP.

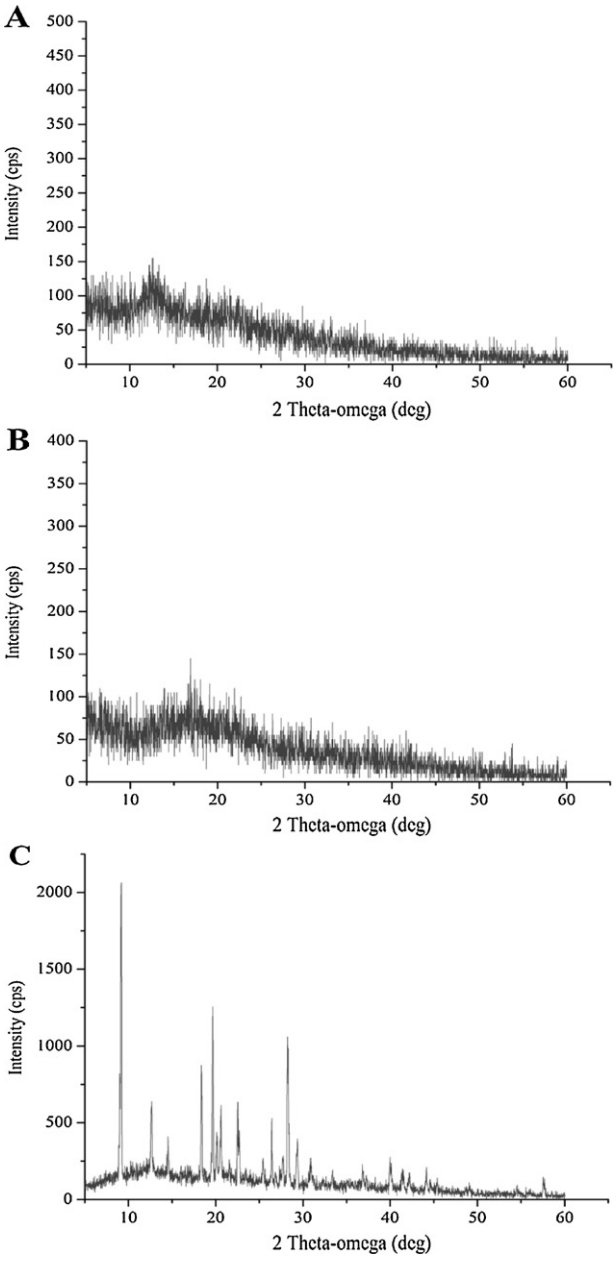

Fig. 3. X-ray diffraction patterns of HEP (A), CEP (B), and CP (C).

hydrochloric acid and citric acid. These spectra were also compared with the commercial citrus pectin (Fig. 2). It was apparent that the FT-IR spectra of HEP and CEP exhibited similarities of the %T patterns to that of CP, further confirming that the polysaccharides extracted from *A. trifoliata* var. *australis* peel were pectins. A broad band at around 3400 cm^{-1} was due to stretching of hydroxyl groups whereas an absorption at around 2900 cm^{-1} was attributed to C–H stretching of the CH_2 groups. The strong absorption observed at 1730–1760 cm^{-1} and at 1600–1630 cm^{-1} were caused by C=O stretching vibration of ester carbonyl groups and ionic carboxyl groups, respectively (Pappas et al., 2004; Silverstein, Bassler, & Morril, 1991). These two distinct bands were used to assess the DE value of pectin. The rationale for this is that the absorbance intensity of the ester carbonyl groups increases with the increase in DE whereas the intensity of the ionic carboxyl stretching band decreases (Singthong, Cui, Ningsanond, & Goff, 2004). According to calculation, HEP gave the 59.10% of DE, CEP gave the 81.42% of DE and CP exhibited 66.83% of DE. Compared with the DE values determined by the direct titrimetric method (HEP: 59.46%; CEP: 76.64%; CP: 71.03%), the differences were less than 5%. The results suggested that both HEP and CEP were high methoxyl (DE > 50%) pectins. It was further observed that the DE value of HEP was much lower than that of CEP, which might be ascribed to the less deesterifying action of citric acid on pectin solubilization, indicating that different extraction methods can strongly influence the degree of esterification of pectins. The similar trend for the effect of hydrochloric acid and citric acid on the degree of esterification was reported elsewhere (Kumar & Chauhan, 2010; Yapo, 2009).

In addition, the moderately intense absorption patterns ranging from 800 to 1300 cm^{-1} were collectively regarded as the finger print region of C–O–C stretching, OH bending and CH_3 deformation. The other bands occurring at 400-800 cm^{-1} were corresponding to C–O=O bending, O–C–O bending, O–C=O bending and CH deformation (Gan, Manaf, & Latiff, 2010; Shurvell, 2002).

3.5. X-ray diffraction

X-ray diffraction was used to provide more information of the pectin structure (amorphous or crystalline). The X-ray diffraction patterns of the pectins are displayed in Fig. 3. HEP (Fig. 3A) presented similar X-ray diffraction spectra to that of CEP (Fig. 3B), and both of them were typical of amorphous polymers with the characteristic peaks observed at 12.58° and 16.92° (2θ). However, the peak intensity of HEP was slightly greater than CEP. CP showed more extremely sharp and intense diffraction peaks of 2θ between 5° and 60° (Fig. 3C), especially at 2θ of 9.18°, 18.36°, 19.64°, and 28.24°, which might indicate that CP had more well defined crystal in nature than HEP and CEP (Khodzhaeva et al., 2003). The differences of the X-ray diffraction spectra between *A. trifoliata* var. *australis* pectins and citrus pectin were attributed to their sources.

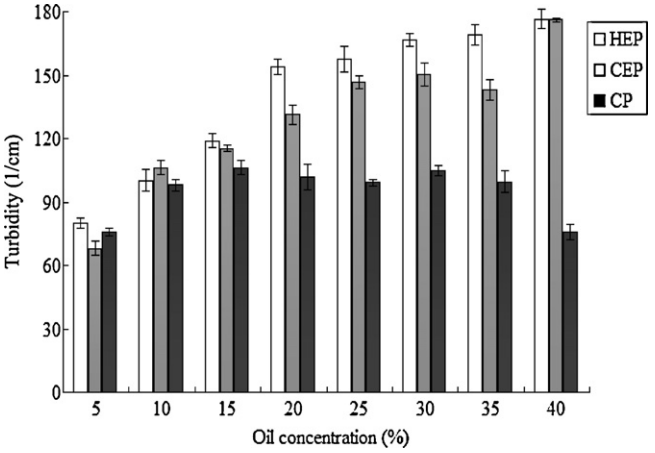

Fig. 4. Emulsion activity (turbidity) of HEP, CEP and CP at different oil concentrations. Data shown was performed in triplicate with at least three times.

3.6. Molecular weight (M_v) analysis

The intrinsic viscosity and viscosity-average molecular weight of HEP, CEP and CP solutions measured in 0.1 M sodium phosphate buffer (pH 7.0) are shown in Table 2. It could be observed apparently that CP exhibited the highest intrinsic viscosity of 0.212 m^3/kg, followed by HEP (0.158 m^3/kg) and CEP (0.109 m^3/kg). The corresponding viscosity-average molecular weight was 64,848 Da, 45,353 Da and 28,877 Da for CP, HEP and CEP, respectively. The molecular weight of HEP was much lower than those of mango and lime pectins (245,000 Da and 123,000 Da, respectively) extracted with hydrochloric acid at pH 1.5 reported by Koubala et al. (2008), but it approximated to that of sunflower pectin (35,300–48,000 Da) extracted with sodium hexametaphosphate followed by acidized alcohol precipitation according to Iglesias and Lozano (2004). Additionally, the molecular weights of HEP and CEP were much lower than most pectins that had been reported, this could be due to the great influences of the plant source and extraction condition used on the intrinsic viscosity and molecular weight of pectins. With respect to the same extraction condition of pH 1.5, the molecular weight of HEP was almost twice of that of CEP. The reason was that citric acid (2 M) may act as a more severe extractant which could have led to some de-polymerisation of *A. trifoliata* var. *australis* pectic macromolecules while hydrochloric acid conditions had a relatively slight degradative effect on the molecular weight.

3.7. Emulsion activity

Fig. 4 showed the turbidity of emulsions determined immediately after preparation (zero time), a higher turbidity was an indication of greater emulsion activity (Yadav, Johnston, Hotchkiss, & Hicks, 2007). As shown, the turbidity of HEP increased with the oil concentration increasing, with particularly fast increment when the oil concentration was between 5 and 20% ($p < 0.05$). This may be attributed to the impedance of the flow of water molecules through the high oil concentration mixture compared with the low oil concentration (Sharma, Liptay, & Maguer, 1997). Referring to CEP, generally, when the level of oil concentration was increasing, the turbidity of CEP increased similarly with HEP. Although the turbidity of CEP was lower than that of HEP at lower oil concentration (except 10%), it was not significantly different ($p > 0.05$) with HEP at oil concentration of 40%. However, the turbidity of CP was highest at 15% oil concentration and was much lower than those of HEP and CEP (except oil concentration of 5%). Therefore, the emulsion activities of HEP and CEP were much higher than that of CP. It has been reported that the pectins can increase the viscosity of the aqueous phase and decrease the tendency of the dispersed oil globules to move and coalesce. Moreover, they can also absorb at the interface to form a coating around the dispersed particles and depend on electrostatic repulsion to stabilize the emulsions (Kokini & Carrillo, 1989; Zouambia, Moulai-Mostefa, & Krea, 2009). Leroux, Langendorff, Schick, Vaishnav, and Mazoyer (2003) reported that the emulsifying properties of pectin were most probably due to the protein residues present within the pectin. The protein contents of HEP and CEP were only 0.76 and 0.69%, respectively, thus, whether the emulsifying properties of *A. trifoliata* var. *australis* pectin is also related with protein is required to be confirmed in the further work.

3.8. Textural analysis

The high methoxyl pectins are prone to form gels under the conditions of low water activity, low pH and high sucrose concentration, whose gel structures are stabilized by hydrogen bonding and hydrophobic interactions (Crandall & Wicker, 1986). The texture profile analysis of HEP, CEP and CP gels at different pH is presented in Table 3. Obviously, the hardness, adhesiveness, chewiness and gumminess of CEP gel were much lower than those of HEP and CP at pH 2.0, the reason was that the gel-forming characteristics of the pectins were also greatly influenced by molecular weight. High molecular weight not only strengthens the interactions between pectin chains but also enlarges the junction areas to make pectin gels stronger (Yoo et al., 2009). As presented in Table 2, molecular weight of CEP was lower than that of HEP and CP, which leaded to a weak gel property. Since the gel strength of high methoxyl pectins increases with the degree of esterification increasing at constant pH (BeMiller, 1986), therefore, the textural characteristics of CP with a high DE (Table 2) were higher than that of HEP. When the pH increased to 4.0, the three pectins were all difficult to form gels. Compared with pH 2.0, the main characteristics of the three pectin gels such as hardness, adhesiveness were decreasing significantly ($p < 0.05$). The reason was that at higher pH, the electrostatic repulsion which was augmented as a result of the de-protonation of the great number of carboxyl groups on pectin backbone can prevent the formation of pectin gel. Consequently, the lower pH can decrease the quantity of negative charges to increase the attraction and reduce the repulsive force between pectin molecules by increasing the formation of the hydrophobic

Table 3
Texture profile analysis of HEP, CEP and CP.

		Hardness (g)	Springiness	Adhesiveness	Chewiness (g)	Gumminess (g)	Cohesiveness	Resilience
pH 2.0	HEP	4.16 ± 0.21^{c}	0.76 ± 0.05^{d}	4.78 ± 0.18^{c}	2.03 ± 0.09^{c}	3.63 ± 0.18^{c}	0.94 ± 0.03^{d}	0.52 ± 0.07^{a}
	CEP	1.45 ± 0.15^{b}	0.62 ± 0.05^{c}	0.50 ± 0.10^{b}	0.62 ± 0.04^{b}	0.42 ± 0.11^{b}	0.67 ± 0.05^{c}	0.96 ± 0.02^{cd}
	CP	9.03 ± 0.31^{d}	0.14 ± 0.03^{a}	4.89 ± 0.26^{c}	3.09 ± 0.13^{d}	5.09 ± 0.25^{d}	0.54 ± 0.03^{b}	0.57 ± 0.03^{a}
pH 4.0	HEP	0.88 ± 0.11^{a}	0.54 ± 0.02^{b}	0.35 ± 0.04^{ab}	0.50 ± 0.07^{b}	0.39 ± 0.06^{b}	0.51 ± 0.02^{b}	0.86 ± 0.02^{b}
	CEP	0.79 ± 0.13^{a}	0.57 ± 0.06^{bc}	0.12 ± 0.02^{a}	0.16 ± 0.01^{a}	0.13 ± 0.01^{a}	0.21 ± 0.01^{a}	0.91 ± 0.01^{bc}
	CP	0.77 ± 0.09^{a}	0.51 ± 0.03^{b}	0.53 ± 0.05^{b}	0.63 ± 0.02^{b}	0.12 ± 0.01^{a}	0.72 ± 0.08^{c}	0.99 ± 0.04^{d}

Each value is expressed as mean ± standard deviation of triplicate tests with at least three measurements. Means within the same column with different letters are significantly different ($p < 0.05$), according to Duncan's Multiple-Range Test.

interactions between ester groups of pectins and hydrogen bonds between adjacent galacturonan (Oakenfull & Scott, 1984; Walkenstrom, Kidman, Hermansson, Rasmussen, & Hoegh, 2003).

4. Conclusion

A. trifoliata var. *australis* peel was rich in pectin and sugars, which were 13.26 and 18.21%, respectively. Pectins extracted with hydrochloric acid and citric acid were of high methylation, low neutral sugar contents and high GlaA contents. Furthermore, both pectins had typical amorphous characteristics, particularly the pectin extracted with hydrochloric acid exhibited a higher molecular weight, emulsion activity and gelling properties, but a lower degree of esterification. The results indicated that the type of acid extractant strongly influenced the physicochemical parameters of pectins, especially the degree of esterification, molecular weight and gelling properties. Further research should be performed to investigate the toxicology of the pectins and the structural analysis in the application of gels in order to utilize the *A. trifoliata* var. *australis* pectins to the maximum extent.

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