



Pectins from *Canna edulis* Ker residue and their physicochemical characterization

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ABSTRACT

Water-soluble pectin (WSP), chelated-soluble pectin (CSP) and acid-soluble pectin (ASP) were sequentially extracted from *Canna edulis* Ker residue, a by-product after the extraction of starch, and their physicochemical properties were studied. ASP was further separated through preparative column chromatography, leading to isolation of two fractions, ASPI and ASPII, which chemical features were analyzed by GC, GC–MS and NMR. Moreover, their behaviors in the simulated gastric and intestinal fluid were monitored. The results exhibit that *C. edulis* residue contains high amount of ASP derived from the heteropolysaccharide with rhamnogalacturonan as main chain. Furthermore, three pectins show gel formation and dissolution behaviors. It well suggests that *C. edulis* residue can be exploited for a good source of pectin for functional materials.

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1. Introduction

Pectin has been widely used in the food, pharmaceutical and cosmetic industries, with its technofunctional properties (gel-forming agents, stabilizers and emulsifiers so on) (Pilnik, 1990). Moreover, the application of pectin is determined by its chemical features, including galacturonic acid content, methoxyl content, degree of esterification and acetyl value. Therefore, it is important to study the chemical structure and physicochemical characteristics of the pectin with different origins.

Canna edulis Ker belonging to the genus *Canna* (Cannaceae) is largely cultivated in South America, Vietnam, Thailand and China (Chansri, Puttanlek, Rungsardthong, & Uttapap, 2005). The dry rhizome of *C. edulis* contains 70–80% starches which are reported more digestible than other kinds of starches (Pérez, Lares, & González, 1997). *C. edulis* residue, as waste discarded after starch extraction, is a potentially environmental problem because it is highly susceptible to putrefaction as a result of high moisture content (80%). To the best of our knowledge, this by-product might contain plenty dietary fiber composed of pectic polysaccharides. Consequently, the utilization of this residue could not only eliminate a possible source of pollution but also increase economic value. Pectins extracted from several plant by-products are widely used in the food industry as gelling agents (Pilnik & Voragen, 1992). However, up to now, it has no report on pectin from *C. edulis* residue.

Therefore, in this study, water-soluble pectin (WSP), chelated-soluble pectin (CSP) and acid-soluble pectin (ASP) were sequentially extracted from *C. edulis* residue and their physicochemical properties were characterized.

2. Materials and methods

2.1. Samples and reagents

Fresh rhizomes of *C. edulis* were obtained from Guizhou Ziyun Jiahe Chemical Co., Ltd in the Guizhou Province of China. *C. edulis* by-product was obtained from the residues of rhizomes after the extraction of starch. The by-product was washed with water several times and dried at ambient temperature for 24 h. The products were ground with a mortar and pestle to a fine powder passing through a 60 mesh sieve. The powder was transferred to airtight plastic bags and stored in a desiccator at room temperature for further analysis. Deuterated water (D₂O), deuterated sodium borohydride (NaBD₄) and methyl iodide (CH₃I) were obtained from Sigma–Aldrich Chemical Co., Ltd (USA). N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) was obtained from Fluka & RDH Co., Ltd (Switzerland). All other reagents were of chromatographic or analytical quality.

2.2. Extraction and purification

Pectins were extracted according to the method of Emaga, Robert, Ronkart, Wathélet, and Paquot (2008) with slight modification. The by-products (10 g) were added into distilled water (300 mL) adjusted pH to 5.0. WSP were extracted and further puri-

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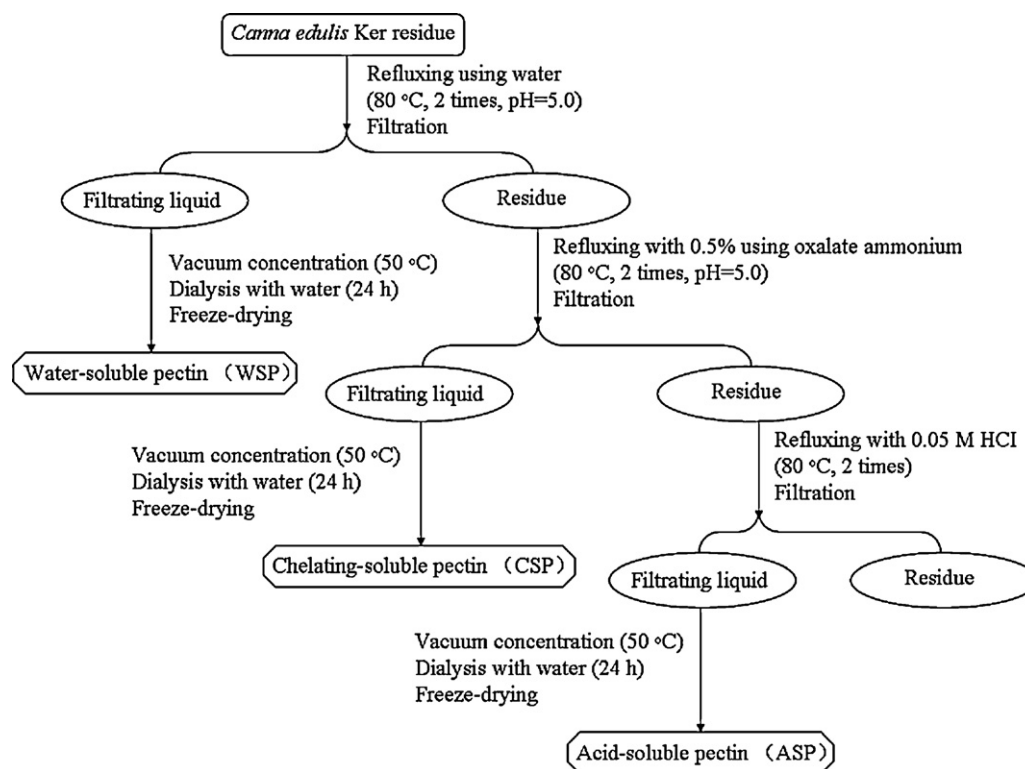


Fig. 1. Extracting scheme of pectin from *Canna edulis* Ker residue.

fied through concentration, dialysis and lyophilization. CSP and ASP were obtained through sequentially extracting residue using 0.5% oxalate ammonium and dilute HCl solution, respectively (Fig. 1).

Furthermore, ASP (100 mg) obtained was dissolved in 0.05 mol/L NaCl aqueous solution. The sample solution was separated repeatedly by gel column chromatography on Sepharose CL-4B, eluted with aqueous solution.

2.3. Galacturonic acid and methoxyl content

Galacturonic acid (GlaA) and methoxyl contents were determined photometrically by the carbazole and the chromotropic acid methods, respectively (Bauerle, Otterbach, Gierschner, & Baumann, 1977).

2.4. Ionic content

The ionic contents including kalium, sodium and calcium were determined using Inductively Coupled Plasma-Atomic Emission Spectroscopy (IRIS Advantage 1000, Thermo Electron, USA).

2.5. Structural characterization

2.5.1. Single sugar composition

The sample was hydrolyzed, and the released sugar was transformed into alditol acetates with acetic anhydride in the presence of 1-methylimidazol according to the method of Blakeney, Harris, and Stone (1983). Quantification was performed in a Shimadzu GC-2010 gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a hydrogen flame ionization detector. The column used was Rtx-5 (30 m × 0.25 mm × 0.25 μm) and nitrogen served as carrier gas. Temperatures of injector and detector were 290 and 280 °C, respectively. An oven temperature program of initial temperature 100 °C, with a hold of 5 min, followed by a temperature rise of 20 °C/min to 190 °C, 3 °C/min to 260 °C, and 10 °C/min to 280 °C,

with a final hold of 5.17 min was conducted. Data were collected and processed with an Agilent Chem Station software system (Agilent Technologies, Waldrom, Germany) and β-D-allose was used as internal standard.

2.5.2. Methylation

Reduction of uronic acids was carried out according to the method of Taylor and Conrad (1972) with modification. In brief, sample (5 mg) was dissolved in 5 mL of D₂O containing EDC (15 mg). The reaction was allowed to proceed for at least 2 h. Then 15 mg of NaBD₄ was added slowly to the reaction mixture at room temperature. After 4 h, the reacted solution was dialyzed against distilled water and then lyophilized.

The methylation of the reduced sample was carried out according to the method of Ciucanu and Kerek (1984). The sample (4–5 mg) was dissolved into dimethyl sulfoxide (DMSO) (0.3–0.5 mL) with the addition of finely powdered NaOH (20 mg) and CH₃I (0.1 mL). The mixture was stirred (1000 g) for 6 min in a closed vial at 25 °C, followed by the addition of H₂O (1 mL) and CH₂Cl₂ (1 mL). The CH₂Cl₂ layer was washed with water (3 × 10 mL) and dried at room temperature.

2.5.3. Preparation of partially O-methylated alditol acetates and GC–MS examination

The reaction product was hydrolyzed with 2 M H₂SO₄ for 1 h at 100 °C to obtain partially O-methylated aldoses. The released partially O-methylated aldoses were transformed into alditol acetates with acetic anhydride in the presence of 1-methylimidazol according to the method of Blakeney et al. (1983). Quantification was performed in an AutoSystem XL GC/TurboMass MS Gas Chromatography/Mass Spectrometry (Perkin Elmer Co., USA). The column used was Rtx-5 (30 m × 0.25 mm × 0.25 μm) and nitrogen served as carrier gas. Temperatures of injector and detector were 290 and 280 °C, respectively. An oven temperature program of initial temperature 100 °C with a hold of 5 min, followed by a temperature rise of

Table 1The content and molecular parameters of pectin from *Canna edulis* Ker residue, and dissolution time and pH value of its solutions.

Pectin	Content (%)	GlaA (%)	Methoxyl content (%)	K (mg/g)	Na (mg/g)	Ca (mg/g)	pH	Dissolution time (min)
Water-soluble pectin	3.81 ± 0.14 a	29.79 ± 0.32 a	4.05 ± 0.13 a	0.45 ± 0.01 a	0.13 ± 0.01 a	3.88 ± 0.01 a	5.85 ± 0.12 a	>90
Chelating-soluble pectin	3.41 ± 0.13 a	32.27 ± 0.46 b	7.08 ± 0.11 b	0.85 ± 0.02 b	4.97 ± 0.01 b	8.10 ± 0.02 b	4.58 ± 0.09 b	>90
Acid-soluble pectin	50.40 ± 1.07 b	27.31 ± 0.58 c	1.35 ± 0.07 c	0.02 ± 0.01 c	0.58 ± 0.00 c	1.52 ± 0.00 c	5.15 ± 0.34 c	75

All values are means of triplicate determinations ± S.D.

Means within columns with different letters are significantly different ($P \leq 0.05$).

GlaA: galacturonic acid content.

20 °C/min to 190 °C and 3 °C/min to 260 °C, and 10 °C/min to 280 °C with a final hold of 5.17 min was conducted.

2.6. ^1H nuclear magnetic resonance spectroscopy (^1H NMR)

The ^1H NMR data was measured on an Avance III 400 spectrometer (Bruker Co., Switzerland) at 400 MHz for ^1H . The chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS) using TMS as standard.

2.7. Dissolution properties

The sample was tested for their dissolution behavior in water and the pH of the solution was determined (Einhorn-Stoll & Kunzek, 2009).

2.8. Monitoring the behavior of sample in the simulated gastric fluid

The behavior of sample in the simulated gastric fluid was determined in a turbidimetric assay (Polovic et al., 2009). Sample (10%, w/w) was mixed with HCl (0.4 mol/L) containing NaCl (8 g/L) and diluted with HCl (0.1 mol/L) containing NaCl (2 g/L). After allowing 1 h for gel to form, the absorbance at 620 nm was recorded on a SunriseTM microplate reader (Tecan Co., Switzerland).

2.9. Monitoring the behavior of sample in the simulated intestinal fluid

The disappearance of acid-formed sample gel in slightly alkaline condition of intestinal fluid was monitored following the decrease of absorbance at 620 nm as a function of time. As described above, after 1 h when the gel was formed, 1 mol/L NaOH was added in order to neutralize the acid. Neutralized gel was mixed with phosphate buffer solution (100 mmol/L pH 7.40) and the absorbance at 620 nm was monitored for the next 16 h.

2.10. Texture analysis

Texture profile analysis (TPA) parameters were determined by using a texture analyzer TA-XT2i (Stable Micro Systems, Ltd, London, U.K.). A flat plate probe (P/0.5-Delrin cylinder probe) with 0.5 in. of diameter was attached to the moving crosshead. Samples

were prepared through mixing 50 g/L pectin solution in 400 g/L glucose solution and 0.4 mol/L HCl containing 8 g/L of NaCl with the ratio of 3:1. It was 40% of compression ratio from the initial height of the sample in two bites. Force–time curves were obtained at a crosshead speed of 1.0 mm/s during 2 compression cycles. The texture profile parameters were determined using the TPA curve.

2.11. Statistical analysis

All determinations were triplicates, and mean values and standard deviations were calculated. Analysis of variance (ANOVA) was performed and the mean separation was done by Fisher's Least Significant Difference ($P \leq 0.05$) using SPSS 13.0 program for windows (SPSS Inc., IL, USA).

3. Results and discussion

3.1. Extraction and isolation of pectin

Pectin was sequentially extracted from the by-product with water, oxalate ammonium aqueous solution and dilute acid solution to offer WSP, CSP, and ASP, respectively (Table 1). The contents of WSP (3.81%) and CSP (3.41%) are much lower than that of ASP (50.40%), in agreement with previous studies which reported that pectin macromolecules were hydrolyzed and extracted easily by acid (Faravash & Ashtiani, 2007). For three kinds of pectins, GlaA contents are low, in accordance with those from plantain at one stage of ripeness (Emaga et al., 2008). Moreover, GlaA and methoxyl contents are higher in the CSP than in the WSP or ASP fraction ($P < 0.05$). A possible reason for very low content of methoxyl group in ASP is that acidic demethoxylation can take place in the acidic solution. Additionally, in view of its high content (50.40%), ASP was separated repeatedly on Sepharose CL-4B to afford two single peaks named ASPI and ASPII.

3.2. Structural characterization

3.2.1. Three kinds of pectins

The chemical features of pectins are presented in Table 2. Galacturonic acid constitutes the main components of all samples and its content in the CSP is higher than in the WSP and ASP. For neutral sugar, three pectins are rich in glucose, as reported for water-soluble cell-wall material from apple and peach (Zhou et al., 2000). This phenomenon can be explained for co-extraction of other sub-

Table 2

Single sugar composition of water-soluble pectin, chelating-soluble pectin, acid-soluble pectin, acid-soluble pectins I and II (%).

Single sugar	WSP (%)	CSP (%)	ASP (%)	ASPI (%)	ASPII (%)
Rhamnose	0	0.66	0.07	5.15	1.57
Arabinose	0.25	2.48	0.44	9.64	3.77
Xylose	0.09	0.72	0.40	10.65	7.62
Mannose	0.10	0.74	0.37	10.53	0.53
Glucose	69.58	63.02	70.83	0.98	0.25
Galactose	0.13	2.4	0.58	29.03	38.51
Galacturonic acid	29.79	32.27	27.31	35.38	48.32

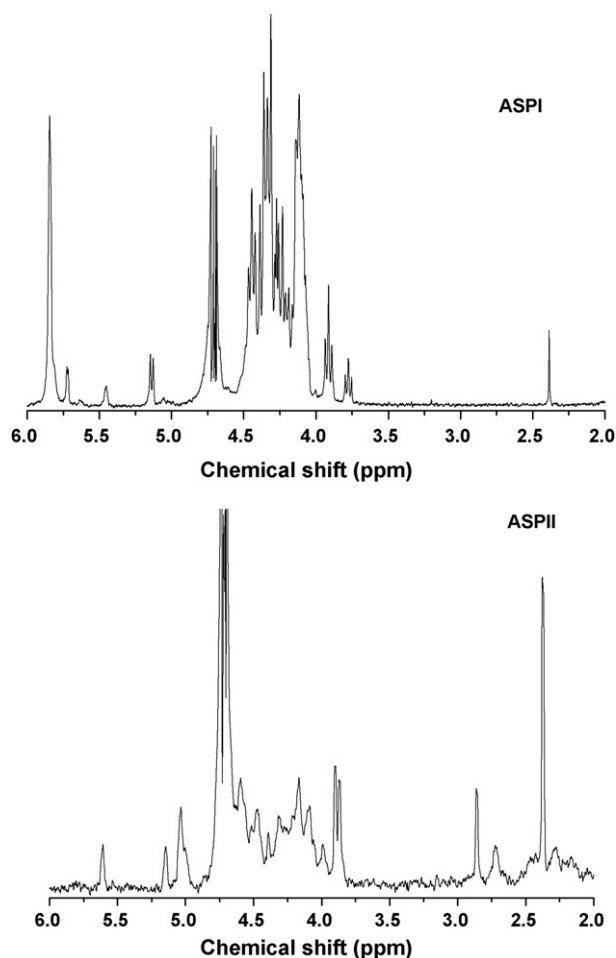


Fig. 2. ^1H NMR spectrum of acid-soluble pectins I and II.

stances with pectin, such as starch, cellulose, hemicellulose, and glucose, which were not purified in the process of dialysis. Moreover, arabinose, xylose, mannose, and galactose contents are higher in the CSP than in the WSP and ASP, suggesting that CSP may be highly branched.

3.2.2. Acid-soluble pectins I and II

For the former, galacturonic acid is dominated component and other neutral sugars consist of rhamnose, arabinose, xylose, mannose and galactose, in accordance with the results obtained in the measurement of ^1H NMR spectrometry, which exhibits five anomeric proton signals at δ_{H} 5.84, 5.82, 5.72, 5.45 and 5.14 (Fig. 2). For the latter, galacturonic acid also constitutes main component and xylose, arabinose, rhamnose and galactose occur in lower amounts, in agreement with the results from ^1H NMR spectrum which shows four anomeric proton signals at δ_{H} 5.61, 5.15, 5.03 and 5.01 (Fig. 2). It indicates that ASPI and ASPII derive from the heteropolysaccharide.

ASPI and ASPII were per-O-methylated and the distribution of the corresponding methyl ethers is shown in Table 3. Only trace of terminal 2,3,4-Me₃GalA and high amount of 2,3-Me₂GalA suggest that 1,4-linked galacturonosyl units as backbone are branched in position 3 and dominate in both ASPI and ASPII with varying ratios. For ASPI, arabinosyl, galactosyl and mannosyl residues locate at side chain, which could be deduced from minor amounts of terminal 2,3,5-Me₃Ara, 2,3,4,6-Me₄Gal and 2,3,4,6-Me₄Man, and large amounts of 2,3-Me₂Ara, 2,3,6-Me₃Gal and 2,3,6-Me₃Man, with mean degrees of polymerization of 7, 11 and 11, respectively.

Table 3

Partially O-methylated alditol acetates from acid-soluble pectins I and II.

OMe-alditol acetates	Linkage site	ASPI (%)	ASPII (%)
2,3,5-Me ₃ Ara	Ara (1→	1.32	–
2,3-Me ₂ Ara	→5) Ara (1→	8.32	3.68
2,3,4,6-Me ₄ Gal	Gal (1→	2.61	1.61
2,3,6-Me ₃ Gal	→4) Gal (1→	26.42	36.84
2,6-Me ₂ Gal	→3,4) Gal (1→	–	1.53
3,4-Me ₂ Rha	→2) Rha (1→	–	–
3-MeRha	→2,4) Rha (1→	5.12	1.58
2,3,4-Me ₃ Xyl	Xyl (1→	4.34	5.37
3,4-Me ₂ Xyl	→2)Xyl (1→	6.31	2.27
2,3,4,6-Me ₄ Man	Man (1→	0.98	–
2,3,6-Me ₃ Man	→4) Man (1→	9.51	+
2,3,4-Me ₃ GalA	GalA (1→	+	+
2,3-Me ₂ GalA	→4) GalA (1→	32.23	42.58
2-MeGalA	→3,4) GalA (1→	4.36	5.69

Moreover, a small quantity of 2-MeGalA suggests that rhamnose could occur at galacturonosyl main chain in the 1 and 2 linked positions. According to structural characteristics of pectin obtained (Caffall & Mohnen, 2009), in the view of equally total amounts of arabinosyl, galactosyl, mannosyl and xylosyl terminals (1.32, 2.61, 4.34 and 0.98%) and of branched arabinose and galacturonic acid (4.36 and 5.12%), it could well deduce that the main chain of ASPI is composed of 1,4-linked galacturonosyl and 1,2-linked rhamnosyl units with side chains at positions 3 and 4, respectively. For other single sugars, arabinose, galactose and mannose in 5-, 4- and 4-linked positions occur at branched rhamnose, and xylose in 2-linked position presents at branched galacturonic acid in the form of single sugar and oligosaccharide.

Similar to ASPI, main chain is composed of rhamnose and galacturonic acid in the ASPII. However, in the side chains, arabinose in 5-linked position occurs at 4-linked galactose branched at position 3 which links into branched rhamnose in the main chain, which is different from ASPI. In addition, for ASPII, mannose occurs in trace amount compared to ASPI.

3.3. Dissolution properties

As shown in Table 1, three kinds of pectins dissolve difficultly in distilled water. It may be explained that the pectins form a thin sticky layer on the surface resulting from a glass–gel transition of the outer layer of the particles and the layer is rather difficult to dissolve afterwards (Kravtchenko, Renoir, Parker, & Brigand, 1999). Moreover, the dissolvability of ASP is higher than those of WSP and CSP, attributing to the breakdown of this sticky layer during the purification. In addition, all pectins were freeze-dried to form the foam-like fibrous material structure with the bad wetting behavior. It accords with the previous study which reported that the lyophilized pectin dissolved relatively slowly and had a worse dispersibility and wetting behaviour in comparison with that dried at 50 °C (Einhorn-Stoll & Kunzek, 2009).

3.4. The behavior of sample in the simulated gastric fluid

Fig. 3 shows the behaviors of three different pectins in the simulated gastric fluid. In the initial stage, the clearing of pectin gel with high absorbance values can be attributed to the passage of pectin gel formed at a pH lower than 2.5 through a gel–sol transition point (Gilsenan, Richardson, & Morris, 2000). With the increase of dilution factors, the absorbance values decrease in the form of exponential decay function. It suggests that pectin aggregates disappear, as a consequence of the disruption of hydrogen bonds among and within the pectin polymer.

As reported, disappearance of the gel as a function of dilution degree conforms to the second-order exponential decay function

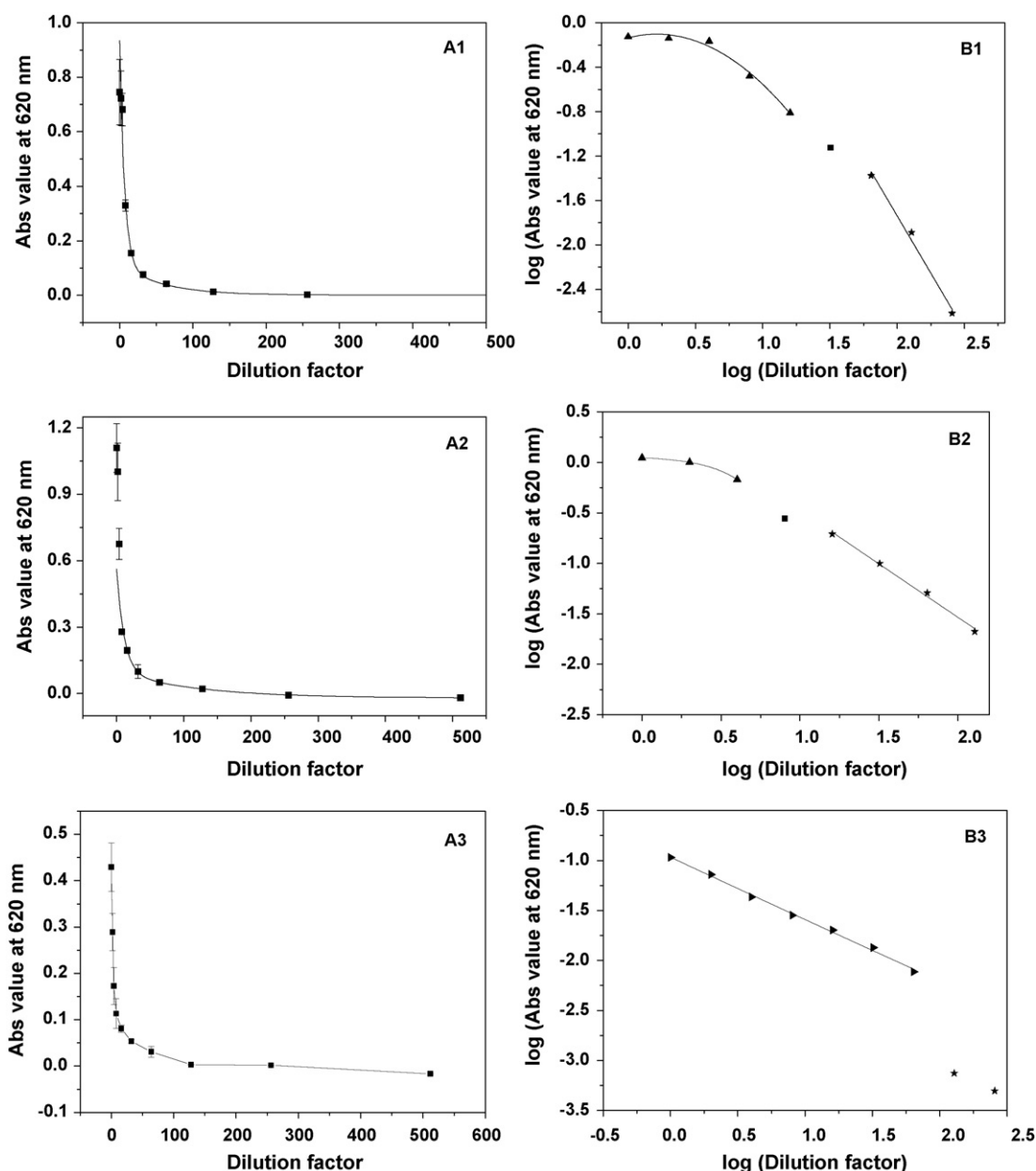


Fig. 3. (A) Gel disappearance in simulated gastric fluid as a decreasing exponential function ($R \geq 0.98$) of WSP (A1), CSP (A2) and ASP (A3) dilution. (B) Absorbance at 620 nm versus WSP (B1), CSP (B2) and ASP (B3) dilution plotted on a logarithmic scale.

which is exhibited as follows:

$$A = C_0 + C_1 \exp\left(\frac{-D}{b_1}\right) + C_2 \exp\left(\frac{-D}{b_2}\right)$$

where A is absorbance at 620 nm, D is the degree of dilution, C_0 , C_1 and C_2 are coefficients, and b_1 and b_2 are decay contents.

For WSP, CSP and ASP, the initial solutions (50 g/L) were separately diluted by 35.76 ± 3.89 , 41.61 ± 5.38 and 21.27 ± 2.67 times, the gels formed in the acidic liquid disappear. The minimal concentrations for gel formation were 1.40 ± 0.15 , 1.20 ± 0.16 , 2.35 ± 0.75 g/L for WSP, CSP and ASP, respectively. This result could be explained by the quantity of galacturonic acid in the three different pectins (29.79% for WSP, 32.27% for CSP and 27.31% for ASP, respectively), which directly correlates with the gel formation.

3.5. The behavior of sample in the simulated intestinal fluid

For three pectins, acid-formed gel dissolving in simulated intestinal fluid as a second exponential decay function of incubation time is shown in Fig. 4. With the prolong of time, the absorbance values decrease in exponential decay form, indicating that hydrogen bonds formed in the acidic condition are gradually disrupted under the alkaline circumstance. The times for gel dissolving were found to be 83 ± 0.07 , 74 ± 3.16 and 69 ± 1.82 min for WSP, CSP and ASP, respectively.

3.6. Textural analysis

WSP, CSP and ASP contain a certain amount of calcium ion (3.88 mg/g for WSP, 8.10 mg/g for CSP and 1.52 mg/g for ASP) determined by Inductively Coupled Plasma-Atomic Emission Spec-

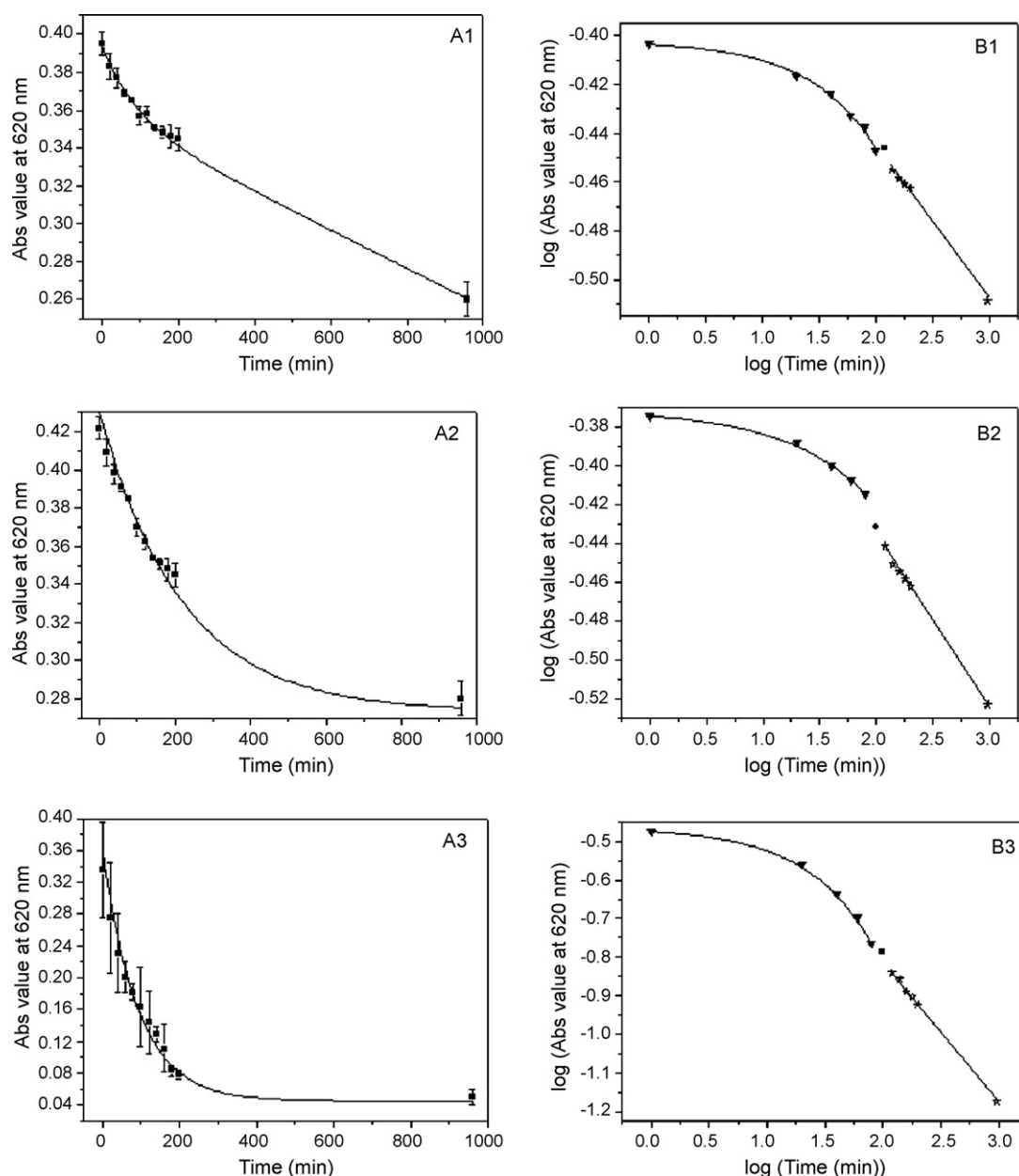


Fig. 4. (A) Acid-formed gels dissolving in simulated intestinal fluid as a second exponential decay function of incubation time ($R \geq 0.98$) for WSP (A1), CSP (A2) and ASP (A3). (B) Absorbance at 620 nm versus incubation time of WSP (B1), CSP (B2) and ASP (B3) plotted on a logarithmic scale.

troscopy (Table 1). Therefore, in view of low methoxyl contents (Table 1), the gelation in the three pectins might mainly take place by ionic linkages via calcium bridges between two carboxyl groups belonging to different chains in close contact (Willats, Knox, & Mikkelsen, 2006). Moreover, as a result of the addition of glucose solution with high concentration and hydrochloric acid, hydrophobic effect and hydrogen bond could also improve the formation of pectin gel.

Table 4 exhibits textural properties of WSP, CSP and ASP. According to the values of hardness, chewiness, adhesiveness, fracturability and gumminess, pectins used for investigation are placed in relative order: CSP > WSP > ASP ($P < 0.05$). It suggests that these values are positively related to methoxyl and calcium contents in the *C. edulis* pectin (Table 1). Moreover, the three pectins show low and similar values of cohesiveness ($P > 0.05$), implying that the internal structures are easily broken down.

Table 4

Texture profile analysis of water-soluble pectin, chelated-soluble pectin and acid-soluble pectin.

	WSP	CSP	ASP
Hardness (g)	7.95 ± 0.386 a	11.57 ± 2.471 b	5.23 ± 1.256 c
Springiness	0.48 ± 0.003 a	0.57 ± 0.005 b	0.69 ± 0.033 c
Cohesiveness	0.50 ± 0.022 a	0.50 ± 0.008 a	0.50 ± 0.021 a
Chewiness (g)	8.23 ± 0.248 a	10.18 ± 0.428 b	3.77 ± 0.391 c
Resilience	0.77 ± 0.161 a	0.75 ± 0.070 b	0.57 ± 0.034 c
Adhesiveness	19.71 ± 2.544 a	60.13 ± 0.003 b	11.34 ± 1.387 c
Fracturability (g)	8.50 ± 0.826 a	11.07 ± 10.889 b	3.63 ± 0.927 c
Gumminess (g)	3.95 ± 0.009 a	5.79 ± 0.021 b	2.59 ± 0.130 c

All values are means of triplicate determinations \pm S.D.

Means within rows with different letters are significantly different ($P \leq 0.05$).

4. Conclusion

C. edulis by-product contains abundant pectins, especially ASP (50.40%). Three kinds of pectins show good gelling and dissolution behavior in the simulated gastric and intestinal fluids, respectively. Further investigation should be done to characterize the structure and study structure–function relationships of pectic saccharides in order to utilize pectin to the greatest extent.

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