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Extraction, characterization and spontaneous gel-forming property of pectin from creeping fig (*Ficus pumila* Linn.) seeds

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ABSTRACT

Creeping fig (*Ficus pumila* Linn.) seeds can be manually rubbed and squeezed to produce a water extract (WE) that can gel at room temperature without any additives. Its gelling material, extraction behavior, and mechanism of spontaneous gel-formation were investigated. Gelling material was extracted from seeds using water, ammonium oxalate and hydrochloric acid, respectively. Results showed the main component of three extracts is low methoxyl pectin. The pectin locates in a transparent layer on the surface of seeds, as revealed by an inverted microscope. Hence, explained the feasibility of the squeezing and rubbing method in traditional handcraft. Comparing with the other methods, water-extracted pectin has high galacturonic acid content, viscosity-average molecular weight and intrinsic viscosity but low degree of methoxylation. This pectin forms the major component of WE, together with the high amount of calcium ions present in WE, it suggests the spontaneous gelation may be based on the 'egg-box' formation.

1. Introduction

Creeping fig (*Ficus pumila* Linn.) is a perennial plant belonging to the family of Moraceae. It grows vigorously on the mountain sides, or in adobe and concrete walls throughout the China (named "Bili"), Japan (named "Ooitabi"), Philippines and Taiwan. This plant is used for various purposes, for example, the fruit which contains sterol and triterpenoid components has been used in Chinese folk medicine as antitumor, anti-inflammatory and tonic medicament (Kitajima, Kimizuka, & Tanaka, 2000). The leaves which contain flavonoid glycosides have been traditionally consumed by some Okinawan elders either as a beverage or herbal medicine to treat diabetes, dizziness, high blood pressure, and neuralgia (Leong, Tako, Hanashiro, & Tamaki, 2008). However, the most remarkable characteristic of the plant is its seeds which can be rubbed and squeezed in water to yield a mucilaginous water extract (WE), capable of gelation at room temperature without addition of any sugars, acids or ions. The resulting gel is locally referred as "Liangfen" and sold as a summer drink in the local markets for hundreds of years in some oriental countries. However, till present, the mucilaginous material and the mechanism of spontaneous gel-formation of the WE have never been well reported. In our previous study, pectin may be the material for its gelling properties.

Pectins are complex heteropolysaccharides ubiquitously present in the cell wall of land plants, providing consistency and mechanical resistance to vegetable tissues (Taboada et al., 2010). It consists of a linear backbone of α-1,4-linked D-galacturonic acid (GalA) residues in which the carboxyl groups of the GalA can be free or methyl-esterified. Depending on their degree of methoxylation (DM), pectins are commonly categorized as high methoxyl pectin (HMP) or low methoxyl pectin (LMP), with DM > 50% and <50%, respectively. HMP can form a gel under acidic conditions in the presence of high sugar concentrations (Evageliou, Richardson, & Morris, 2000). On the other hand, LMP forms gels by interaction with divalent cations, particularly Ca^{2+}, according to the 'egg box' model (Durand, Bertrand, Clark, & Lips, 1990).

At the industrial level, pectins obtained are typically HMP while LMP are usually manufactured from HMP by controlled acid de-methoxylation (El-Nawawi & Heikal, 1995), alkali de-methoxylation (Renard & Thibault, 1996), or by demethoxylating reaction of pectin methyl esterase. An alternative approach to manufacturing LMP for food and health industries is to 'mine' plant cell walls that are enriched with LMP. Generally, pectins were obtained by extractions of the cell wall material using hot dilute acid, cold dilute sodium hydroxide or solutions of chelating agents (Levigne, Ralet, & Thibault, 2002). Extraction conditions such as temperature, pH, extractants used, and extraction time have a significant impact on the yield and the quality of pectin (Kulkarni & Vijayanand, 2010; May, 1990).

This paper is to reveal the gelling material of WE and to account for the feasibility of the squeezing and rubbing method in

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traditional handcraft. The mechanism of spontaneous gel-formation of WE will be investigated as well.

2. Materials and methods

2.1. Preparation of plant materials

Cut-opened and dried creeping fig fruits were procured from the farmer's market in Jinggang Mountains, Jiangxi province, China. The seeds were collected and dried at 60 °C for 3 h and stored at room temperature in a vacuum packed container before use.

2.2. Extraction and purification

Before extraction, the CFS was heated at 95 °C for 90 min in a drying oven to inactivate any pectolytic enzymes naturally present. After cooling, different experimental conditions were employed for the extraction of pectins from CFS according to method of Koubala et al. (2008) with some modifications: (1) distilled water at 25 °C for 30 min; (2) 80 mM ammonium oxalate, pH 4.5 (using oxalic acid) at 25 °C for 30 min; (3) 0.03 M hydrochloric acid (pH 1.5) at 85 °C for 30 min to yield water-extracted pectin (WEP), chelating agent-extracted pectin (ChEP) and acid-extracted pectin (HEP), respectively. Each sample was extracted thrice with the same solvent under magnetic stirring. A solid/liquid ratio of 1:20 (w/v) was applied in all cases. The extracts were then filtered to remove the solid residues and precipitated by the addition of ethanol to obtain a final concentration of 50% (v/v). The water-extracted and acid-extracted precipitates were washed successively with ethanol/water mixtures of volume ratios: 70/30, 80/20, 90/10, and absolute ethanol (Turquois, Rinaudo, Taravel, & Heyraud, 1999). While the ammonium oxalate-extracted precipitate was redissolved in water and centrifuged, the supernatant obtained was dialyzed against deionzed water to remove the ammonium oxalate that might precipitate simultaneously during precipitation. The obtained pectins were lyophilized and stored in hermetically sealed glass bottles prior to analysis.

2.3. Microscopic observations

The creeping fig seed before, during and after extraction was placed on glass slide and covered with water. The microscopic observation was performed on an inverted microscope (Olympus CKX 41, Olympus Co. Ltd., Tokyo, Japan).

2.4. Chemical and physical analysis

2.4.1. FT-IR spectrum and degree of methoxylation

The FT-IR spectra of the extracts were determined using FT-IR spectroscopy (Nicolet 5700, USA) and compared with those of citrus pectin P9135 and apple pectin P8471 (China Sigma–Aldrich, Shanghai, China). The degree of methoxylation was calculated from FT-IR spectra according to method of Gnanasambandam and Proctor (2000). First, a calibration curve was constructed between DM and ester carbonyl area from FT-IR spectra based on pectin standards of known DM. After which, the peak area of the ester carbonyl of sample was fit to the curve to calculate its DM.

2.4.2. Uronic acids

The uronic acid content was determined by the m-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) and by the method of Ahmed and Labavitch (1977) in the extracts and in the finely pulverized dry seeds, respectively. Galacturonic acid monohydrate (China Sigma–Aldrich, Shanghai, China) was used as standard.

In order to quantify the effectiveness of these extractions, the pectin extractability (expressed as the amount of galacturonic acids extracted from the seeds) was estimated according to the following Eq. (1) (Taboada et al., 2010):

$$\text{Pectin extractability (\%)} = \frac{m(\text{GalA})_{\text{extract}}}{m(\text{GalA})_{\text{seeds}}} \times 100 \quad (1)$$

where $m(\text{GalA})_{\text{extract}}$ and $m(\text{GalA})_{\text{seed}}$ are the amount of galacturonic acid in the extracts and seeds, respectively.

2.4.3. Neutral sugar content

Neutral sugars were analyzed by gas chromatography (GC) according to Liu, Cao, Huang, Cai, and Yao (2010) with some modification. Sample (3.3 mg) was hydrolyzed with 2 M trifluoroacetic acid (5 ml) for 12 h at 110 °C in a sealed tube. After neutralizing to pH of 6.5–7.0 with $BaCO_3$, the hydrolysate was filtrated, freeze-dried, and dissolved in 0.5 ml of 0.02% (w/v) pyridine–$NH_2OH{\cdot}HCl$ solution in a sealed test tube at 90 °C for 30 min. The solution was cooled to the room temperature and acetylated with 0.5 ml of acetic anhydride in a sealed test tube at 90 °C for 30 min. 1 μl of the sample was analyzed in a Agilent 6890 system GC (Agilent Technologies, Palo Alto, CA, USA) fitted with a HP-5 column (30 m, 0.25 mm I.D., 0.25 μm film thickness; 160–210 °C at 2 °C/min, 210–240 °C at 5 °C/min, then held at 240 °C for 10 min) with flame ionization detector (FID). Myo-inositol was used as the internal standard.

2.4.4. Intrinsic viscosity and viscosity-average molecular weight

To determine the intrinsic viscosities ($[\eta]$) of extracted pectins, viscosities (η) of pectin solutions were measured by recording flow time of pectin solution in an Ubbelohde dilution viscometer (diameter = 0.52 mm) at 25 ± 0.1 °C, immersed in a temperature controlled bath. The original pectin solution (0.2 g/dl) was prepared in an aqueous solution containing 0.1 M NaCl at pH 7 for 18 h with stirring at room temperature. Pectin solution and solvent were filtered using 0.45 μm membrane filters before viscosity measurements. The other concentrations were obtained by dilution with the solvent. The intrinsic viscosity ($[\eta]$) was estimated by extrapolation of Kraemer curves to "zero" concentration (Mead & Fuoss, 1942; Rao, 1999). After which, the viscosity-average molecular weight ($M\nu$) was calculated from the Mark–Houwink relationship, $M\nu = ([\eta]/k)^{1/\alpha}$, where the constants k and α are 4.36×10^{-4} and 0.78, respectively (Garnier, Axelos, & Thibault, 1993).

2.4.5. Moisture, ash and protein contents

Moisture was measured by using a halogen moisture analyzer (Model HR83, Mettler-Toledo, Switzerland), and ash content was determined according to AOAC method (AOAC, 1997). The amount of protein in the pectic substance was determined by the method of Bradford using bovine serum albumin as standard protein (Bradford, 1976).

2.5. Investigation on mechanism of spontaneous gel-formation of WE

2.5.1. Pectin and mineral contents in WE

CFS (5 g) was extracted in 100 ml deionized water for various periods. 50 ml of the extracts were used to determine the total galacturonic acid, which was measured by the m-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). The remaining 50 ml extracts were concentrated, lyophilized, and used to determine mineral contents in an inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer Optima 5300DV).

2.5.2. Gelling capacity

To simulate the formation of "Liangfen", solutions of pectins were rapidly mixed with $CaCl_2$ solution to give pectin solutions

with the desired pectin concentration (0.2%, 0.5% and 1%, w/w) and Ca^{2+} concentrations (1 mM) without heating. All solutions were added with 0.1 M NaCl in order to screen electrostatic interactions. After preparation, the gel samples were transferred into a Brookfield sample bottle (60 mm diameter × 60 mm height), sealed with matched rubber stoppers.

Gel strength of pectin gels after resting for various periods of time were determined by a Brookfield CT3-100 Texture Analyzer (Middleboro, USA). The gels were deformed by compression at a constant speed of 1.0 mm/s to a distance of 3 mm from the gel surface using a cylindrical probe TA10 (diameter = 12.7 mm) (Rascón-Chu et al., 2009).

2.6. Statistical analysis

All of the experiments were done in triplicate. Statistical analysis was carried out using SPSS (version 16.0, Chicago, United States). The results were expressed as mean ± standard deviations and compared using the Tukey test at 5% confidence level.

3. Results and discussion

3.1. FT-IR spectrum analysis and determination of the degree of methoxylation

The CFS polysaccharides were analyzed by FT-IR spectroscopy and their spectra were compared against two commercial pectin standards (Fig. 1). FT-IR spectra within the wave number between 950 and 1200 cm^{-1} is considered as the 'finger print' region for carbohydrates, which allows the identification of major chemical groups in polysaccharides as the position and intensity of the bands are specific for every polysaccharide (Cerná et al., 2003). It was found that all the FT-IR spectra of polysaccharides displayed a similar general pattern and exhibited similarities of the absorption patterns to that of commercial pectin standards (apple pectin and citrus pectin). The FT-IR spectra showed good match with the spectra of the commercial pectins suggested that the polysaccharide extracted from CFS is pectin. However, FT-IR spectra of CFS pectins were much like that of citrus pectin, but distinguishably different in comparison to apple pectin in absorbance. For example, two higher peaks for apple pectin are shown at wave number 1012 cm^{-1} and 1106 cm^{-1} than other pectins. In addition, its peak at 1740 cm^{-1} was higher than peak at 1630 cm^{-1} while the other pectins unanimously show lower peak at 1740 cm^{-1} than at 1630 cm^{-1}. The differences may be contributed by the biological variation in plant material used.

To determine the DM of extracted pectins, further analysis of the FT-IR spectra was carried out. The broader band of absorption about 3440 cm^{-1} is caused by O–H stretching vibration. While the 2929 cm^{-1} band refers to the C–H stretching of CH_2 groups. Bands occur at 1012 and 1106 cm^{-1} indicating vibration of C–C and vibration of backbone respectively. The band at 1630 cm^{-1} corresponds to vibration of the O=C–O structure. Then band that appears at 1744 cm^{-1} can be assigned to C=O stretching vibration of methyl esterified carboxylic group. The peak areas of the free carboxyl groups and esterified groups are usually used to determine the degree of methoxylation. According to Gnanasambandam and Proctor (2000), a linear correlation is established between ratio of the peak area at 1740 cm^{-1} ($\upsilon_{(C=O)COOMe}$) over the sum of the peak areas at 1740 and 1630 cm^{-1} ($\upsilon_{as(COO-)}$) and DM of pectins. The FT-IR spectrum of commercial apple pectin (73% DM) showed higher absorbance at 1744 cm^{-1} than at 1630 cm^{-1}, which is characteristic to that of high methoxyl pectin. While the FT-IR spectra of CFS pectins showed lower absorbance at 1744 cm^{-1} than at 1630 cm^{-1}. The degree of methoxylation of CFS pectin was estimated to be about 14.6%, 14.2%, and 42.6% for WEP, ChEP and HEP, respectively. Hence, all of the three extracted pectins had low DMs. However, even though there was no significant difference in DM between ChEP and WEP, both of them were distinctively lower than that of HEP due to the different extraction conditions. It is known that pectinesterase can hydrolyze the carboxymethyl esters, liberate methyl alcohol and free carboxyl groups on pectin backbone (Jolie, Duvetter, Van Loey, & Hendrickx, 2010). At room temperature (25 °C) and mild pH of water extraction and ammonium oxalate extraction in the study, residue endogenous pectinesterase may have played a role in releasing methyl ester groups from galacturonic acid residues and increasing the amount of COO^-. Such changes in groups led to the reduction in absorbance at 1744 cm^{-1} and increase in absorbance at 1630 cm^{-1} in IR spectra, thus subsequently a reduction in the derived DM value after calculation.

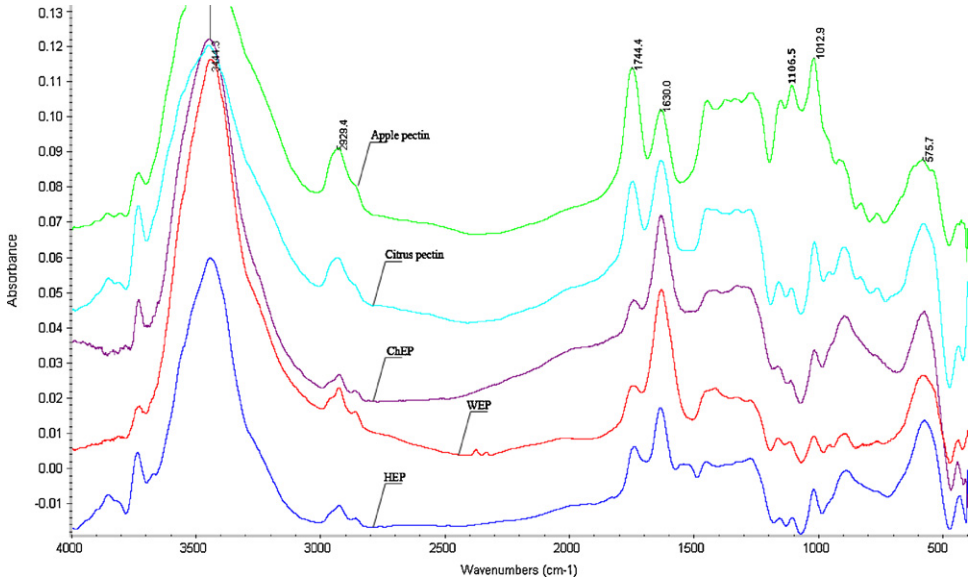

Fig. 1. Fourier transform infrared spectra of WEP, ChEP and HEP from creeping fig seeds and commercial pectin standards (apple pectin, citrus pectin).

3.2. Yield, extractability and physical–chemical characteristics of CFS pectins

3.2.1. Extraction yields and pectin extractability

In general, pectin extraction yield from different sources may vary depending on processing parameters and sample features. The yield of pectin extracted from CFS ranged from 5.25% to 6.07% (w/w) on a dry matter basis (w extract/w seeds), and the highest yield was obtained with HCl while the lowest yield with water. Acids are generally the strongest extracting agents with regards to the yield of extracted pectin (Yapo, 2009). However, the higher protein and ash content in HEP may have also contributed to its high yield, as shown in Table 1. All the yields were lower than those reported in major sources of pectin like apple fruit (16%) (Rascón-Chu et al., 2009) but higher than the pectin from linseed (0.35%) (Dıĭaz-Rojas et al., 2004). However, in contrast to the relatively low pectin yield, the total galacturonic acid content, a primary parameter used to determine the pectin content in a sample, was very high. The total galacturonic acid content of WEP, ChEP and HEP was 87.73%, 85.47% and 77.92%, respectively. The lower galacturonic acid content of HEP may due to the presence of more ash and proteins. For all the extracts, the GalA content was higher than the 65% limit established by the FCC purity specification for pectin (FCC, 2004). Therefore, the pectin extracts obtained from creeping fig seeds could be considered of a high purity.

In the seeds, the galacturonic acid content was 48.6 mg/g. Then the calculated pectin extractability of HEP, ChEP and WEP was 96.12%, 95.97% and 94.72%, respectively according to Eq. (1). There was no significant difference in pectin extractability among samples treated by different conditions. The high extractability of pectin by hot diluted acid, using either HCl or HNO_3, has been well demonstrated, and hot dilute acid has been suggested to be the best approach for large scale production of pectin at industrial level (May, 1990). Interestingly, the water extraction that commonly deemed as unsuitable method to directly extract pectin from many plant sources (Koubala et al., 2008; Yeoh, Shi, & Langrish, 2008) showed high pectin extractability in our study. In addition, the pectin extractability we obtained were considerably greater than the results of Taboada et al. (2010), where the pectin extractability of murta fruits is 78.6%, 10.6% and 6.1% for HCl-, $(NH_4)_2C_2O_4$-, and H_2O-extracted pectin, respectively.

To account for the differences, location of the pectin in CFS was established through microscopic examination by an inverted microscope. Microscopic examination revealed a transparent layer on the surface of seeds when soaked in water (Fig. 2A). Fig. 2B shows the release of the layer during extraction while Fig. 2C shows the image of one of the many seeds where the transparent layer was completely removed after an extraction period of 90 min. Finally, a mucilaginous solution and some hard cores were obtained after extraction. On the other hand, the amount of galacturonic acid released during extraction was detected by colorimetric method. The galacturonic acid content increased simultaneously along with extraction time (Fig. 3). For the initial 60 min, the release was very fast, and then the speed of the reaction decreased rapidly and almost stopped after 90 min. There was no significant difference in galacturonic acid content among samples after 90, 120 and 150 min of treatment. In addition, hard cores obtained after an extraction period of 150 min were pulverized and further extracted for another 30 min. No galacturonic acid was detected. A similar phenomenon was displayed for samples extracted by oxalic acid/ammonium oxalate solution and HCl. These results proved that the seeds consist of an inner hard core and a pectin-rich transparent outer layer. The layer can be easily removed during extraction, hence explaining the high pectin extractability and why CFS are manually rubbed and squeezed rather than ground to power to produce the "Liangfen" in traditional handicraft.

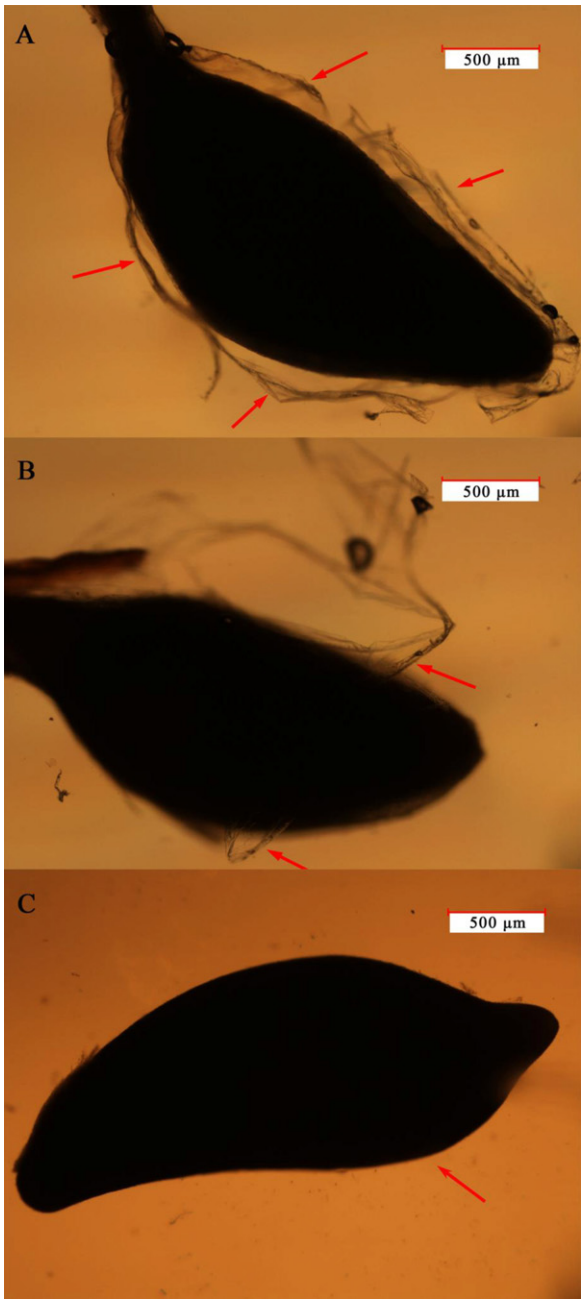

Fig. 2. Optical micrograph of a creeping fig seed suspended in water. (A) Untreated. (B) During extraction. (C) After extraction. Arrows in A and B indicate gel-forming polysaccharide layers, and arrow in C indicates the inner hard core.

3.2.2. Neutral sugars contents

The acetyl derivatives of hydrolyzed pectin were determined by GC analysis. The main neutral sugars present in all three kinds of pectin were mannose, arabinose and rhamnose (Table 1). Although the samples were qualitatively similar in the monosaccharides present, there are some differences in the relative abundance of each neutral compound in respect to the various extraction methods used. The relative amounts of neutral sugars in HEP were slightly higher than those in WEP and ChEP. It was reported that different kinds of pectins could be extracted according to the conditions. Levigne et al. (2002) found that at pH 1, the extracts from fresh sugar beet seemed to be richer in rhamnogalacturonans carrying neutral sugar as side chains. At pH 3, it is richer in homogalacturonans which consists of only D-GalA units. Rombouts and Thibault (1986) also reported that the total neutral sugar content of acid sol-

Table 1
Yield, extractability, chemical composition, degree of methoxylation (DM), intrinsic viscosity ([η]) and viscosity-average molecular weight ($M\nu$) of pectins extracted from creeping fig seeds.[a]

		Extraction conditions		
		H_2O	$(NH_4)_2C_2O_4$	HCl
Yield (%)		5.25 ± 0.28a*	5.46 ± 0.19ab	6.07 ± 0.34b
Galacturonic acids (%)		87.73 ± 2.21a	85.47 ± 1.93b	77.92 ± 2.52c
Pectin extractability (%)		94.72 ± 6.51a	95.97 ± 1.50a	96.12 ± 6.25a
Individual neutral sugars (%)	Rhamnose	0.74 ± 0.12a	0.79 ± 0.13a	1.56 ± 0.21b
	Arabinose	1.77 ± 0.15a	1.86 ± 0.14b	3.37 ± 0.16c
	Mannose	0.81 ± 0.13a	1.46 ± 0.02b	2.09 ± 0.17c
Protein (%)		0.17 ± 0.03a	0.28 ± 0.01b	3.23 ± 0.53c
Ash (%)		1.17 ± 0.32a	0.22 ± 0.04b	2.33 ± 0.31c
Moisture (%)		12.80 ± 0.11a	12.75 ± 0.23a	12.87 ± 0.10a
DM (%)		14.6 ± 1.0a	14.2 ± 0.9a	42.6 ± 0.8b
[η](dl/g)		9.38 ± 0.08a	7.10 ± 0.14b	2.85 ± 0.25c
$M\nu$ (kDa)		359.2 ± 4.0a	251.1 ± 6.5b	78.6 ± 8.9c

[a] Data are presented as means ± standard deviations of triplicate measurements.
* Mean values in the same row with different letters are significantly different (Tukey test, $p < 0.05$).

uble pectins (16.7%) is higher than those of water soluble pectins (15.6%) and oxalate soluble pectins (4.8%). The HEP (pH 1.5) in our study may also be richer in rhamogalacturonans, resulting in its relatively higher amounts of neutral sugar content. However, since the total neutral sugars in all types of pectin that were present are in low amount, it can be deduced that pectins extracted from the CFS consisted mainly of galacturonic acid forming the homogeneous backbone.

In the study, only mannose, arabinose and rhamnose but no galactose, glucose and xylose were determined. It has been reported that the neutral sugar component of pectin varies according to the plant sources. For example, Liu et al. (2001) reported that there are six kinds of neutral sugars in the citrus pectins, including rhamnose, mannose, galactose, glucose, arabinose and xylose, as compared to the four kinds of neutral sugars, rhamnose, galactose, glucose and mannose found in pectins from mulberry branch bark (Liu et al., 2010). The presence of neutral sugar side chains usually plays an important role in the structure and function of pectin. BeMiller (1986) stated that the sidechains of pectins might tend to limit the extent of interchain association, and thus, formation of junction zones for gelling may be inhibited. In this study, high content of galacturonic acid and low proportion of neutral sugars of the polysaccharide strongly suggest that the extracted pectins may have unique gelling capability.

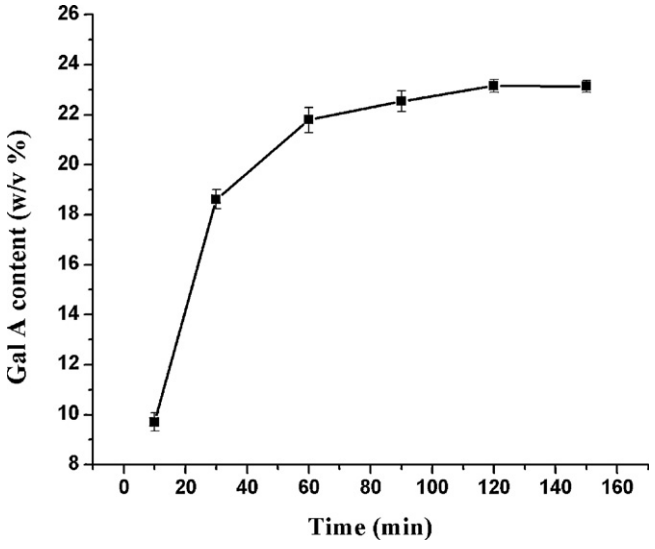

Fig. 3. The total galacturonic acid content of water-extract (WE) of creeping fig seeds as a function of extraction time.

3.2.3. Intrinsic viscosity and viscosity-average molecular weight of the extracts

The WEP showed the highest [η] of 9.38 dl/g, followed by ChEP (7.10 dl/g) and HEP (2.85 dl/g). The corresponding viscosity-average molecular weight ($M\nu$) was 359.2 kDa, 251.1 kDa and 78.6 kDa for WEP, ChEP and HEP, respectively. The different extraction conditions used allowed the recovery of different kinds of pectin with respect to molecular weight and conformation. Water and ammonium oxalate can thus be considered as smooth extractants due to the ability to solubilize pectins of high molecular weight. On the other hand, strong acid solution could lead to highly soluble smaller pectin molecules, due to partial hydrolysis. However, under the same acid conditions (HCl, pH 1.5 and 85 °C), the molecular weight of HEP in this study was similar with that of *Ceni* mango pectins (83 kDa) (Kratchanova, Bénémou, & Kratchanov, 1991) but lower than that of *Ambarella* peel pectin (303 kDa) (Koubala et al., 2008).

3.2.4. Ash, protein and moisture content of the extracts

The higher galacturonic acid and the lower ash content of pectin are the two criteria for its purity. Ash content affects the ability of pectin to gel (Miyamoto & Chang, 1992). The ash content of pectin was 1.17%, 0.22% and 2.33% for the WEP, ChEP and HEP, respectively. The low ash content of ChEP could be due to both the chelating and dialysis process. The ammonium oxalate used in extraction may have chelated many Ca^{2+}. Some metal ions may also have been dialyzed during the dialysis process after extraction. While the higher ash content of HEP could be due to increase of acid strength to solubilize indigenous minerals in the seed, where the solubilized mineral would then be precipitated along with pectin during alcohol precipitation. This result corresponds with those observed for extraction of soy hull pectin (Kalapathy & Proctor, 2001).

The protein content of WEP, ChEP, and HEP was 0.17%, 0.28% and 3.23%, respectively. The amount of protein in HEP was higher than that in WEP and ChEP, similar observations have previously been made for pectins isolated from yellow passion fruit rind (Yapo & Koffi, 2006). The HCl used in the extraction process was likely to "cosolubilize" more protein residues than those of water or ammonium oxalate.

The moisture was about 12.81% with no significant differences observed between various extraction methods used.

3.3. Gelling capability

There are many methods used to measure gel strength of pectin, such as SAG test, bloom test, rheological techniques and texture profile analysis (TPA). In this study, TPA was used. The initial maximum force, recorded when the probe had penetrated 3 mm into

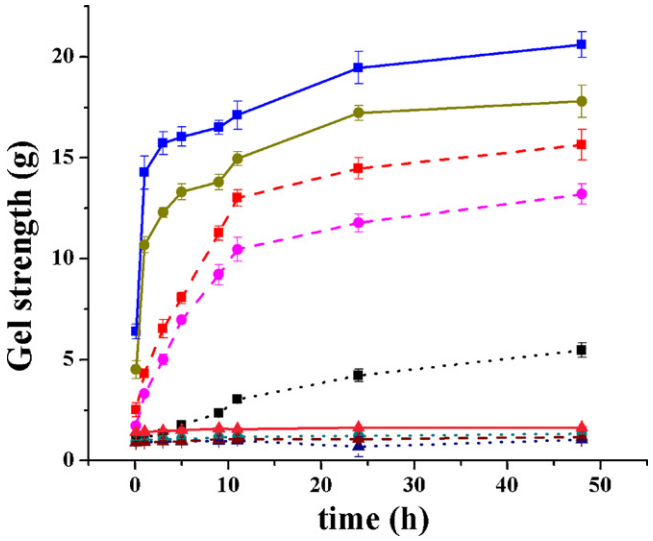

Fig. 4. Changes in gel strength of WEP (■), ChEP (●) and HEP (▲) as a function of storage time at pectin concentration 0.2% (•••), 0.5% (– – –), and 1% (–), w/w.

the pectin gels, was taken as gel strength (Montero, Fernádez-Díz, & Góez-Guillé, 2002). However, till now, there is no value to specify gelation or not. Based on visual observations, sensation of touch and TPA results in our experiments, force 1.70 g was established as the critical value, and values below 1.70 g showed that the solution did not gel at time of analysis.

Fig. 4 showed the changes in gel strength of extracted pectins as a function of storage time. Generally, the pectin gel strength increased with period of storage from 5 min to 48 h. Meanwhile, there was an increase in gel strength as the pectin concentration increased, which could be due to more pectin chains being held together by free carboxyl groups linkage with calcium ion, forming a strong gel (Elnawawi & Heikal, 1995). However, the gelation kinetics of CFS pectins were very different according to the extraction conditions. For WEP, it can gel at all three concentrations (0.2%, 0.5% and 1%, w/w), but at 0.2%, it took more than 5 h to form a weak gel. With respect to ChEP, it can form a gel at the various concentration levels except for 0.2%, and the values were slightly lower than those of WEP. It is notable that the gel strength of WEP and ChEP at 0.5% (15.6 g, 13.2 g) and 1% (20.6 g, 17.8 g) were considerably greater than those of pectin from Golden delicious apples at 2% (8.2 g) and 3% (15.3 g) after a 48 h rest (Rascón-Chu et al., 2009). This may suggest that the WEP and ChEP in this study had excellent gelling properties. On the other hand, no gelation was observed with HEP throughout the entire experiment, where gel strength for all concentrations is lower than the standard value of 1.7 g, even at 1% (w/w) concentration. Koubala et al. (2009) reported that the intrinsic viscosity of pectin tightly correlates with its gelling capacity, thus one of the reasons of the weak gelation of HEP could be its relatively low intrinsic viscosity (2.85 dl/g). This pectin also shows a relatively low content of galacturonic acid (77.92%) coupled with high ash content (2.33%) and high DM (42.6%), all these factors being known to have a detrimental effect on the gelation process of LMP.

3.4. Mechanism of spontaneous gel-formation of WE

The seeds of creeping fig are used to make home-made jelly cake in some oriental countries for a long history. The WE produced by rubbing and squeezing in water spontaneously forms a pudding-like gel at room temperature without any addition of additives. However, the mechanism of spontaneous gel-formation remains unknown. Low methoxyl pectin extracted using distilled water under stirring, precipitation by ethanol and purification by ethanol/water mixtures in our study (WEP) was found to be the major polysaccharide of WE. In addition, the gel-forming behavior displayed by WEP when Ca^{2+} concentration was 1 mM showed that the gel strength increased along with storage time and pectin concentration. This was similar to the production process of home-made jelly curd ("Liangfen") using WE. Therefore, it is speculated that the gelation of WE may be based on 'egg-box' formation of low methoxyl pectin.

However, WEP alone cannot gel without the addition of $CaCl_2$ solution. It is known that low methoxyl pectin forms a gel according to 'egg-box' model, where the carboxyl groups of galacturonic acid of low methoxyl pectin chelate with divalent cations (Gidley, Morris, Murray, Powell, & Rees, 1980; Grant, Morris, Rees, Smith, & Thom, 1973). Therefore, if the spontaneous gel-formation of WE was based on the 'egg-box' formation of low methoxyl pectin, high amounts of divalent cations would thus be necessary. To support this statement, water extract without precipitation and washing was used to detect the composition and amounts of metals released during extraction. The results were summarized in Table 2, where potassium (62.53–63.55 mg/g), calcium (15.21–15.62 mg/g), sodium (0.39–0.41 mg/g), and magnesium (0.28 mg/g) are the main metal components present in WE. Other metals, such as iron, copper, and zinc were also present, but in concentrations below 4 mg/100 g sample, while no lead was found. The calcium content is higher than the amount of calcium required (12.3 mg/g) to cause gelation of low methoxyl pectin, when calculated according to $R = 2[Ca^{2+}]/[COO^{-}]$ where R is equal to 0.2 (Fraeye et al., 2009). The high content of K, Na could be a factor that favors the gel formation. It was also reported that the affinity of LMP towards di- and polyvalent cations increases with ionic strength and with increasing polymer concentration (McKenna, Nicholson, Wehr, & Menzies, 2010).

Pectin extracted using HCl was unable to gel even at 1.0% (w/w) concentration, and gel strength of ChEP were significantly lower than those of WEP at 0.5% and 1.0% at the same conditions, thus indicates variations in the biochemical characteristics (molecular weight, DM and galacturonic acid content) among WEP, ChEP and HEP, would ultimately influence the gel-formation of WE. It was reported that molecular weight has a great influence on the

Table 2
Composition and amounts of metals in the water-extract (WE) of creeping fig seeds.[a]

Extraction time (min)	Metals (mg/g)						
	K	Na	Ca	Mg	Fe	Zn	Cu
10	62.53 ± 0.03a*	0.39 ± 0.01a	15.21 ± 0.01a	0.28 ± 0.01a	0.03 ± 0.00a	0.04 ± 0.00a	0.01 ± 0.00a
30	63.05 ± 0.02b	0.40 ± 0.01b	15.53 ± 0.01b	0.28 ± 0.00a	0.03 ± 0.00a	0.04 ± 0.00a	0.01 ± 0.00a
60	63.15 ± 0.01b	0.41 ± 0.01c	15.62 ± 0.02c	0.28 ± 0.00a	0.03 ± 0.00a	0.04 ± 0.00a	0.01 ± 0.00a
90	63.55 ± 0.03c	0.40 ± 0.02b	15.60 ± 0.02d	0.28 ± 0.01a	0.03 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.00b
120	63.50 ± 0.01c	0.41 ± 0.01c	15.61 ± 0.01c	0.28 ± 0.01a	0.03 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.00b
150	63.52 ± 0.02c	0.41 ± 0.01c	15.61 ± 0.01c	0.28 ± 0.01a	0.03 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.00b

[a] Data are presented as mean ± standard deviations of triplicate measurements.
* Mean values in the same column with different letters are significantly different (Tukey test, $p < 0.05$).

functional properties of pectin such as gelling, viscosity modifying, and thickening (May, 1990). High molecular weight promotes the interactions between pectin chains and increases the length of junction zones created by the associations of pectin chains, rendering pectin gels stronger. Therefore, the highest gel strength of WEP could be explained by its high molecular weight. In addition, results obtained showed that DM may also influence the gelling properties of pectins. Table 1 showed that WEP has a significantly low DM of 14.6% as compared to HEP with a DM of 42.6%. However, Fig. 4 showed a remarkable high gelling ability for WEP despite its low DM while HEP has weak gelling ability with a higher DM. It seemed that there were great interactions among the pectins with low DM. The absence of methyl esters allowed pectin chains to come closely together and contributed to the increased gel strength. This inverse relationship was also observed in the literature which confirmed that reduced DM increased gel strength of the pectin gels (Kim, Yoo, Kim, Park, & Yoo, 2008). To add on, the difference in galacturonic acid content, neutral sugars content as well as ash content may have also influenced the gel-forming characteristics of pectins as mentioned above.

Therefore, it could be concluded that the spontaneous gel-formation of WE was due to 'egg-box' formation through chelation of divalent cations to carboxyl groups of galacturonic acid located on the neighboring pectin chains. The high molecular weight, high galacturonic acid but low neutral sugars content, and low DM of WEP would also promote this spontaneous and extraordinary gel-forming property.

4. Conclusion

The present work described the extraction of cell wall polysaccharides from CFS with water, ammonium oxalate, and hot dilutes acid solutions. The main substance in all extractions is low methoxyl pectin. The resulting chemical and physical properties of the pectins hinge on the various extraction procedures employed. However, high pectin extractability (94.72–96.12%) was obtained regardless of the extraction conditions. Because the pectin is mainly located in the transparent layer on the surface of seeds, where it can be easily removed and dissolved in solvent during extraction, as revealed by an inverted microscope.

The extraction conditions have a significant impact on not just the yield and biochemical characteristics but also on the gelling properties of CFS pectins. WEP showed the best gelling capability when compared to ChEP and HEP. The fact that gel-forming behavior of WEP is closely similar to the spontaneous gel-formation of WE and high content of calcium ions present in WE would further substantiate the possibility that the spontaneous gel-forming property of WE was due to LMP interchain association and 'egg-box' network formation. The high viscosity-average molecular weight and intrinsic viscosity, high galacturonic acid but low neutral sugars content, and low DM of WEP also favor the spontaneous gel-forming property. The results suggest that CFS can be a very promising source of high quality low methoxyl pectin for food and non-food applications.

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