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Composition and Characterization of Water Extractable Pectin from Pulp in Pods of Riang (*Parkia timoriana* (DC.) Merr.)

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Abstract

The purpose of this study was to preliminary investigate the extraction condition and composition of purified pectin from pulp in pods of Riang (*Parkia timoriana* (DC.) Merr.), a native tree in southern Thailand. Dry pulp of Riang was extracted using water or citric solvent at different concentrations under the same condition (90 ℃ for 2 h). It was found that the highest yield of pectin (21.6 % dry basis) was obtained by hot water extraction with an esterification degree of 48.3 %. It mainly contained 62.3 % galacturonic acid, followed by 18.5 % total sugar. The monosaccharides found in pectin were arabinose (2.6 %), galactose (2.3 %), glucose (1.5 %), mannose (0.5 %), fructose (0.5 %) and xylose (0.1 %). The finding pectin had a high average molecular weight of 2,070 KDa with a broad polydispersity index of 4.31, determined by high performance size exclusion chromatography (HPSEC) and it was classified as low methoxyl pectin confirmed by Fourier transform infrared spectroscopy (FT-IR) and rheological properties.

Keywords: Riang (*Parkia timoriana* (DC.) Merr.), Pectin, Chemical composition, Average molecular weight

Introduction

Pectin is a water soluble polysaccharide found in the middle lamella of plant cell walls, which is bound to cell wall and other materials to create cellulosic networks for firmness of plant tissue [1]. It can be commercially extracted from citrus peels and apple pomance with acid solution. Also, many sources of pectin have been found in other fruits and vegetables such as papayas, cocoa pod husks, okras and cabbages [1-5]. Pectin is widely used to form gel and increase viscosity and stability in many food and cosmetic industries. It is used in the production of jams, jellies and marmalades and other products to improve the texture. Pectin is also a beneficial dietary fiber, preventing diseases such as diabetes and colon cancer in human body, and lowering cholesterol and serum glucose [6,7].

The units of galacturonic acid constitute the main chain of pectin which is partially linked to neutral sugar side chains. The common sugars found on the branch chain are arabinose, galactose and glucose, and to a lesser extent xylose, mannose and rhamnose [8,9]. In general, pectin can be classified into 2 types based on the degree of esterification (DE): High methoxyl pectins (HMP) (≥ 50 % DE) and low methoxyl pectins (LMP) (< 50 % DE). The gelling formation obtained from HMP at low pH (~3) in the presence of high amount of sugar is commonly used in high sugar jam and jelly-like foods, while the gel obtained from LMP in a wide pH range (2 - 6) with or without a small amount of sugar needs Ca^{2+} for polymer interaction and is employed in low calorie and non-sugar added foods [10]. The quantities and characteristics of pectin vary depending on sources, extraction methods and extraction steps in the processes. Hot water or acid extractions generally affected both yield and quality of extracted pectin [11,12]. It was found that the optimal and friendly extraction conditions to obtain the highest pectin yield from citrus fruits were hot water

at 90 - 100 ℃ for 1 - 3 h [13,14]. Moreover, it was reported that organic acids such as citric acid at a pH between 2.0 - 2.5 or 6 % (w/v) at high temperature were good solvents for pectin extraction in other fruits [15-17].

Riang or *Parkia timoriana* (DC.) Merr. is a big tree locally found in the southern part of Thailand. It is also found in North-Eastern states of India and other Southeast Asian countries. Pods of Riang are elongate and composed of many seeds, which are arranged in a row. The mature seeds are typically planted until sprouted with a 1 - 2 cm long root and have been consumed in Southern Thailand. In addition, the naturally soft-dry pulp of the pods has been used as an ingredient in a traditional jelly sweet dish. Previous studies have been investigated the pectin-like and the gel formation ability with Ca^{2+} of the pulp of Riang pods. Some chemicals and properties were also separately investigated [18]. However, the characteristics and composition of purified pectin in its pulp have been less described.

Therefore, this research aimed to preliminary study on the extraction conditions for the highest yield of pectin from Riang pulp and to determine the composition of extracted pectin and its characteristics. The information obtained may be beneficial for the application of pectin to other products apart from food.

Materials and methods

Materials

Dry Riang pods were obtained from a gardener in Suratthani, Thailand. Bovine serum albumin standard was purchased from Merck (Germany). Citrus pectin, galacturonic acid and monosaccharide standards were purchased from Sigma-Aldrich (USA). Citrus pectins with the degree of esterification 55 - 70 and 85 % as specified by the supplier were used. Other chemicals not specified in this experiment were of analytical grade.

Preparation of dry Riang pulp

Dry Riang pods naturally fallen ripe fruits were collected, washed and wiped up with a clean cloth. The dry pulp (10 % moisture was determined) in the pods was scraped, powdered and passed through a 0.500 mm mesh sieve, and then stored in a sealed plastic bag at 5 ℃ until further used for pectin extraction. (The dry pulp was composed of 10.7 % moisture, 27.9 % dietary fiber, 5.5 % ash, 5.0 % protein and 0.8 % fat previously reported and published elsewhere.)

Water and acid extraction of pectin

Pectin in dry pulp of Riang was extracted using pure distilled water or a solution of citric acid at different concentrations of 5, 7 and 10 % (w/v) at 90 ℃ for 2 h (Dry pulp to water 1:10 w/v). The extracted samples were then centrifuged at 3,000 rpm for 15 min and the supernatant obtained was dialyzed against distilled water for 24 h, using dialysis tube with molecular weight cut off 12,400 Da (Sigma-Aldrich). The dialyzed samples were centrifuged (3,000 rpm) and the supernatants were collected and precipitated with 3 volumes of 95 % ethanol (1:3 v/v), and left overnight at room temperature (25 ℃). After centrifugation, the supernatant was discarded and the gelatinous precipitates were collected and washed with 95 % ethanol and then centrifuged again. Lastly, the precipitates were hot air-dried at 50 ℃, ground and passed through a 0.500 mm mesh sieve to obtain pectin powder. The weight of the pectin extract was calculated as follows:

$$\%\ Pectin\ =\ \frac{weight\ of\ pectin\ (g)}{weight\ of\ dry\ pulp\ (g)} \times 100$$

Determination of the degree of esterification (DE)

Titration method

The titration method adapted from USP 26-NF 21 [19] was applied to determine the DE of pectin. The pectin powder of 50 mg was dissolved in 30 mL of carbon dioxide-free deionized water at 60 ℃ for 30 min and then left to cool. After 3 drops of phenolphthalein were added, the sample solution was titrated with 0.1 N NaOH solution (It). The additional 30 mL of 0.1 N NaOH was added and stirred at room temperature for 30 min. Next, 30 mL of 0.1 N HCl solution was added and mixed until the pink was faded. The additional 3 drops of phenolphthalein were added and then titrated with 0.1 N NaOH (Ft) until the endpoint was observed. % DE was calculated from the equation as follows:

$$\%\ DE\ =\ \frac{Ft}{(Ft\ +\ It)} \times 100$$

Fourier transform infrared spectroscopy (FT-IR) method

The pectin powder and citrus pectin standards were dried for 24 h and mixed with spectroscopic-grade KBr. The test pectin was then put in the KBr-disk. The infrared spectra were measured on a Bruker-Tensor 27 FT-IR spectrometer, using MIR-ATR mode. The 64 scans at 4 cm^{-1} resolution were recorded from 4,000 - 400 cm^{-1}. The ratio of the peak area at 1,725 cm^{-1} over the sum of the 2 peak areas at 1,725 and 1,600 cm^{-1} (assigned to the absorption of esterified and non-esterified carboxyl groups, respectively) was calculated to % DE using OPUS software version 7.2 [20].

Moisture and ash contents

Moisture and ash contents were determined according to the method of AOAC [21]. Moisture content: 1 g of sample was weighed and dried by using a hot air oven (Binder, FD115, Germany) at 105 ℃ for 3 h to a constant weight. The weight loss was then recorded and calculated to % moisture. Ash content: 1 g of sample was weighed and burned in a furnace (Protherm, 442T, Turkey) at 600 ℃ for 3 h and the remaining was weighed and calculated to % ash.

Protein content

Protein content was analyzed according to the Bradford method [22]. The sample was prepared and diluted with distilled water to obtain a concentration of 5.0 mg/mL. The Bradford buffer solution was added (including bovine serum albumin standard solution) and mixed well before measuring the absorbance at a wavelength of 595 nm.

Galacturonic acid content

The galacturonic acid content was determined according to the method of Blumenkrantz and Asboe-Hansen [23] using a UV-Visible spectrophotometer (PG Instrument, T60, England) at the wavelength of 520 nm. The sample concentration of 5.0 mg/mL was prepared in distilled water and stirred at 50 ℃ for 3 h, and then diluted to fit the range of galacturonic acid standard. The diluted sample and standard solutions were mixed with 12.5 mM tetraborate in concentrated H_2SO_4 and then cooled immediately with ice bath. All solutions were heated at 100 ℃ for 5 min and left in an ice bath to cool down. The 0.15 % m-hydroxydiphenyl reagent was added and the absorbance was read after color development for 20 min.

Total sugar content

The pheno-sulfuric acid assay was used to determine the total sugar content as described by Dubois *et al*. [24]. A sample of 10 mg was weighed and hydrolyzed by 1 mL of 72 % H_2SO_4 for 30 min at room temperature. After dilution with 9 mL of distilled water, the 2 mL of diluted sample was pipetted into a test tube and mixed with 50 µL of 80 % phenol. The concentrated H_2SO_4 was added rapidly and left for 30 min before reading the absorbance at 490 nm, using a UV-Visible spectrophotometer. The glucose standard curve was plotted to compare the total sugar as glucose equivalents.

Mineral determination

The mineral content was determined by inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer, Optima 8000, USA) and CHNS/O analyzer (Thermo Scientific, Flash 2000, Italy).

Monosaccharide analysis

The pectin sample was hydrolyzed with 1 M H_2SO_4 solution at 100 ℃ for 2 h and diluted with distilled water. The monosaccharide hydrolysates were filtered through a 0.45 µm membrane and the volume of 25 µL was injected onto a high performance anion exchange chromatography (HPAEC) machine (Dionex, ICS-3000, USA) separated by CarboPac PA20 (150×3 mm^2) column with electrochemical detector. The 250 mM NaOH and deionized water ratio was 8/92 to 0/100 to gradient elute at the flow rate of 0.2 mL/min for 60 min. The type and concentration of monosaccharides were identified by comparison with a mixture of monosaccharide standards (arabinose, galactose, glucose, mannose, fructose and xylose).

Molecular weight distribution

The sample was prepared by dissolving in deionized water to the concentration of 5 mg/mL at 50 ℃ for 3 h and added with sodium chloride (NaCl) and ammonium acetate ($NH_4CH_3CO_2$) to meet 100 mM of each. The resulting solution was filtered by a filter membrane (0.45 µm) and measured the molecular weight by high performance size exclusion chromatography (HPSEC, Water e2695, Separations module, Waters corporation, USA) using multi angle laser light scattering (MALLS, Dawnheleos, Wyatt technology

corporation, CA) and refractive index (RI, Waters 2414, Waters corporation, USA) detectors. The volume of 20 µL was injected onto Ultrahydrogel 250 and guard columns (Waters Corporation, USA). A mixture of 100 mM NaCl and 100 mM $NH_4CH_3CO_2$ was used as a mobile phase with the flow rate of 0.3 mL/min. All data from MALLS and RI were analyzed by Astra software (Version 5.3.4, Wyatt technology corporation, CA) and dn/dc (0.145 mL/g) was used for molecular weight calculation.

Rheological measurement

The samples were determined on a Modular Compact Rheometer: MCR52 (Anton Paar, Germany), using RheoPlus Rheometer software version 3.61. A cone and plate geometry with diameter 50 mm was used for oscillatory measurements. The measurements were maintained at 25 ºC and subjected to frequency of 1.0 Hz with strain of 2.0 %. The 4 % (w/v) of samples were prepared by dissolving in distilled water at 60 ℃ for 20 min. After cooling down, 0.2 M $CaCl_2$ was added and thoroughly stirred. All samples (with and without 0.2 M $CaCl_2$ addition) were kept at room temperature (25 ℃) for 12 h before measurement.

Statistical analysis

Analysis of variance was conducted and Duncan's new multiple range test (DMRT) was used to compare the mean difference ($p < 0.05$) by SPSS program version 21. All determinations were made in triplicate and the results were expressed as mean values with standard deviation.

Results and discussion

Water and acid extraction of pectin

The effect of water and acid extraction on yield of Riang pectin and degree of esterification (DE) are presented in **Table 1**. The results indicated that the higher yield (21.6 %) and DE (48.3 %) of extracted pectin was mainly obtained by water extraction than those of acid extractions. It was observed that acid extractions (5, 7 and 10 %) significantly decreased the DE values to 44.7, 31.4 and 24.3 %, respectively. It was reported that the high pectin content was obtained from fruits and leaves by acid treatment at high temperature because this condition could release pectin materials from plant cell wall [15,25,26] while other authors found that acidic solvents at high temperature degraded pectin [27-29]. In this experiment, our result was in agreement with the last finding. The decrease in pectin yield and methoxyl group was found as a result of acid depolymerization and de-esterification probably due to too high temperature and long-time procedure. In comparison, the yield of water extractable pectin (21.6 %) exhibited a similar amount reported in ambarella peels varying from 16 to 22 %, based on the conditional extraction [30]. Therefore, the extract obtained from water extraction was further analyzed with respect to the highest yield.

Table 1 Effect of water and acid extraction on yield and DE of Riang pectin.

Solution (at 90 ℃)	Yield of pectin (%, dry basis)	DE (%)
Distilled water	21.6 ± 0.82^{d}	48.3 ± 0.29^{d}
5 % Citric acid	12.5 ± 0.54^{c}	44.7 ± 0.89^{c}
7 % Citric acid	9.8 ± 0.08^{b}	31.4 ± 1.86^{b}
10 % Citric acid	7.0 ± 0.54^{a}	24.3 ± 1.11^{a}

The different letters a, b, c and d in the same column are significantly different ($p < 0.05$).

Composition and characterization of water extractable pectin

The pectin obtained from water extraction was analyzed for chemical composition as shown in **Table 2**. It was found that the extracted pectin in dry pulp of Riang consisted of 5.3 % moisture, 3.8 % ash and 2.7 % protein. It contained a high value of 62.3 % galacturonic acid and a low sugar value of 18.5 %. The result of total sugar content was similar to citrus pectin in the region of 15.45 to 19.40 % reported by Georgiev *et al*. [31]. Some monosaccharides such as arabinose, galactose, glucose, mannose, fructose and xylose, were also found with some minerals. The experimental results explained that polysaccharides in the extract were mainly galacturonic acid and a small amount of neutral sugar, generally found in pectin structure. Based on DE values (48.3 %) and composition, the water extractable of Riang pectin in experiment indicated that it could be categorized as LMP (< 50 % DE) [6]. The low DE result was not consistent with the determination of 66 % DE as identified by Buathongjan *et al*. [32]. Although the DE of pectin depends on source and extraction conditions, it generally decreases with the increase of maturity stages [1].

The FT-IR spectrum illustrates the characteristics of water extractable Riang pectin by comparison to purified citrus pectins. **Figure 1** shows that the wave number range of 1,680 - 1,760 and 1540 - 1680 cm^{-1} were the stretching of the ester carbonyl group (C=O) and carboxyl ion groups (COO^{-}), respectively when compared with the data reported [26,33-35]. It was observed that the citrus pectin covered the area under the spectrum of C=O and COO^{-}. The peak area of C=O decreased, in accordance with a decrease of DE, while the peak area of COO^{-} increased. The spectrum of Riang pectin was similar to that of citrus pectin with 55 - 70 % DE, suggesting that the extracted pectin might have a similar percentage of DE. However, when determined by FT-IR technique, the intensity of the peak area of C=O was predicted and calculated as 46.2 % DE, approximately close to 48.3 % DE analyzed by titrimetric method (**Table 1**), which confirmed that pectin in pulp of Riang should be LMP.

Table 2 Chemical composition of water extractable pectin.

Chemical composition	**Content (%, dry basis)**
Moisture	5.3 ± 0.34
Ash	3.8 ± 0.02
Protein	2.7 ± 0.21
Galacturonic acid	62.3 ± 0.84
Total sugar	18.5 ± 0.79
Monosaccharides	
Arabinose	2.6 ± 0.10
Galactose	2.2 ± 0.00
Glucose	1.5 ± 0.08
Mannose	0.5 ± 0.02
Fructose	0.5 ± 0.06
Xylose	0.1 ± 0.00
Minerals	
K	1.15 ± 0.02
N	0.83 ± 0.02
Ca	0.70 ± 0.01
Si	0.07 ± 0.00
P	0.01 ± 0.00
Mn	0.01 ± 0.00
S	< 0.01

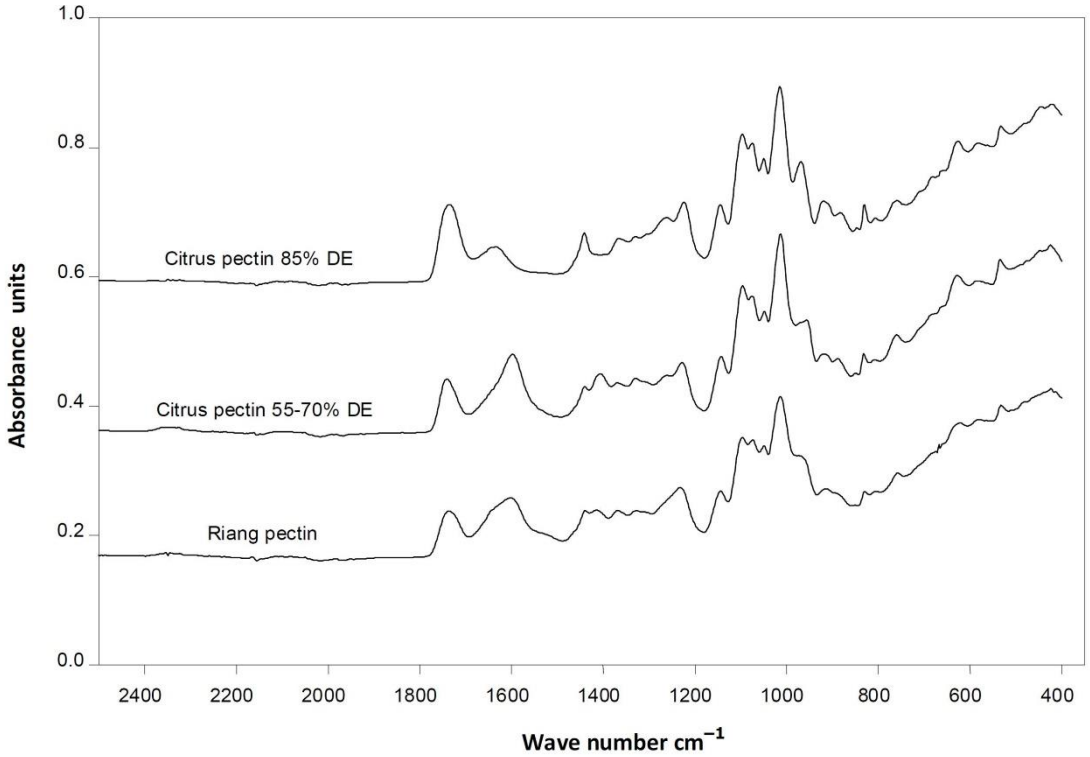

Figure 1 FT-IR Spectrum of citrus pectin and Riang pectin.

Molecular weight distribution

The molecular mass distribution chromatogram of Riang pectin run by HPSEC with double detectors is illustrated in **Figure 2**. The light scattering (LS) showed only one large distribution fraction as a function of time, while the refractive index (RI) presented 2 fractions of different molecular size. This is due to a common reason that LS signal is less sensitive to small molecular distribution. The high intensity signal detected by LS was possibly a predominant high molar mass component of galacturonic units of pectic polysaccharides in coincidence with a high peak by RI at a retention time of approximately 20 min. The smaller elution peak observed by RI around 34 min could be contributed to a small amount of protein molecules that are normally combined in polysaccharides. It was noticed that the average molecular weight (Mw) of Riang pectin was 2,070 KDa much greater than that of the low methoxyl citrus pectin ranging from 103 - 288 KDa and the polydispersity index (Mw/Mn) was 4.31, also greater than 1.81 - 2.40 in that report measured by HPSEC chromatography techniques [36]. The Mw was higher than previously reported in LMP extracted from okra pods, ranging between 700 - 1,700 KDa [37]. The broad molecular mass with Mw/Mn of 4.31 of extracted Riang pectin was probably due to the environmental growth and the effect of extraction conditions.

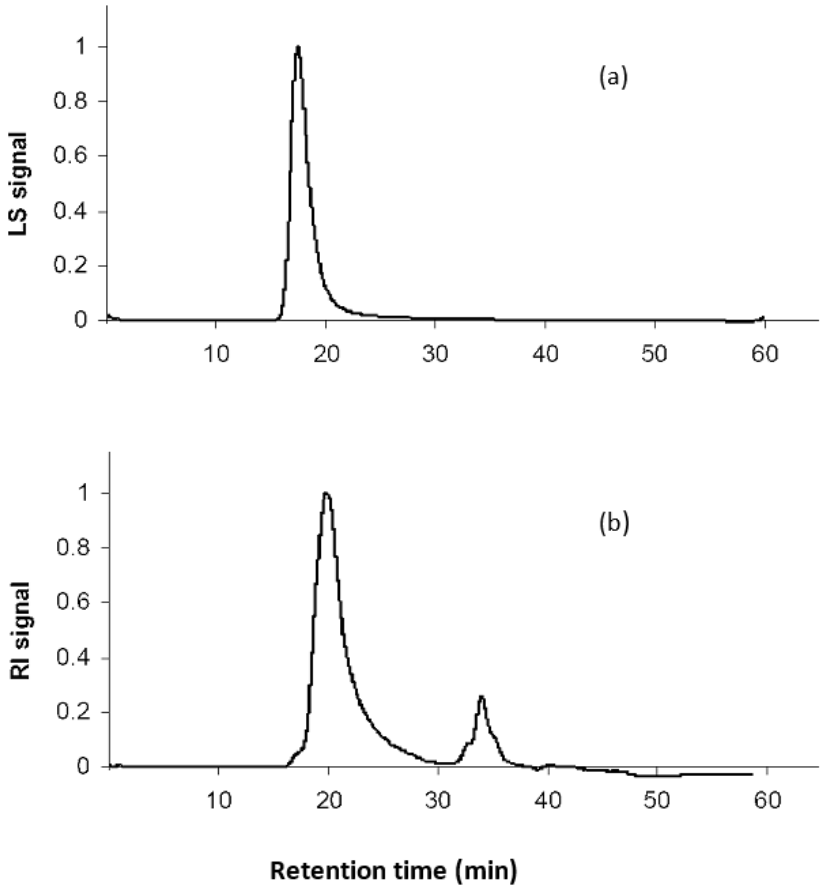

Figure 2 HPSEC chromatogram of Riang pectin from 2 detectors: (a) Light scattering (LS) (b) Refractive index (RI).

Rheological properties

The rheological characteristics of Riang pectin (4 % w/v) with and without addition of $CaCl_2$ are shown in **Figure 3**. In **Figure 3(a)**, the rapid decrease in complex viscosity was observed with increasing frequency, explaining strong shear thinning flow characteristics. A higher complex viscosity of Riang pectin with $CaCl_2$ was recorded as a function of specific frequency than that without $CaCl_2$, which displayed that the presence of Ca^{2+} influenced intermolecular networks of pectic substances. When further increased in frequency, the dramatically decreased in complex viscosity to nearly 0 value was found probably due to shear deformation within the constant period.

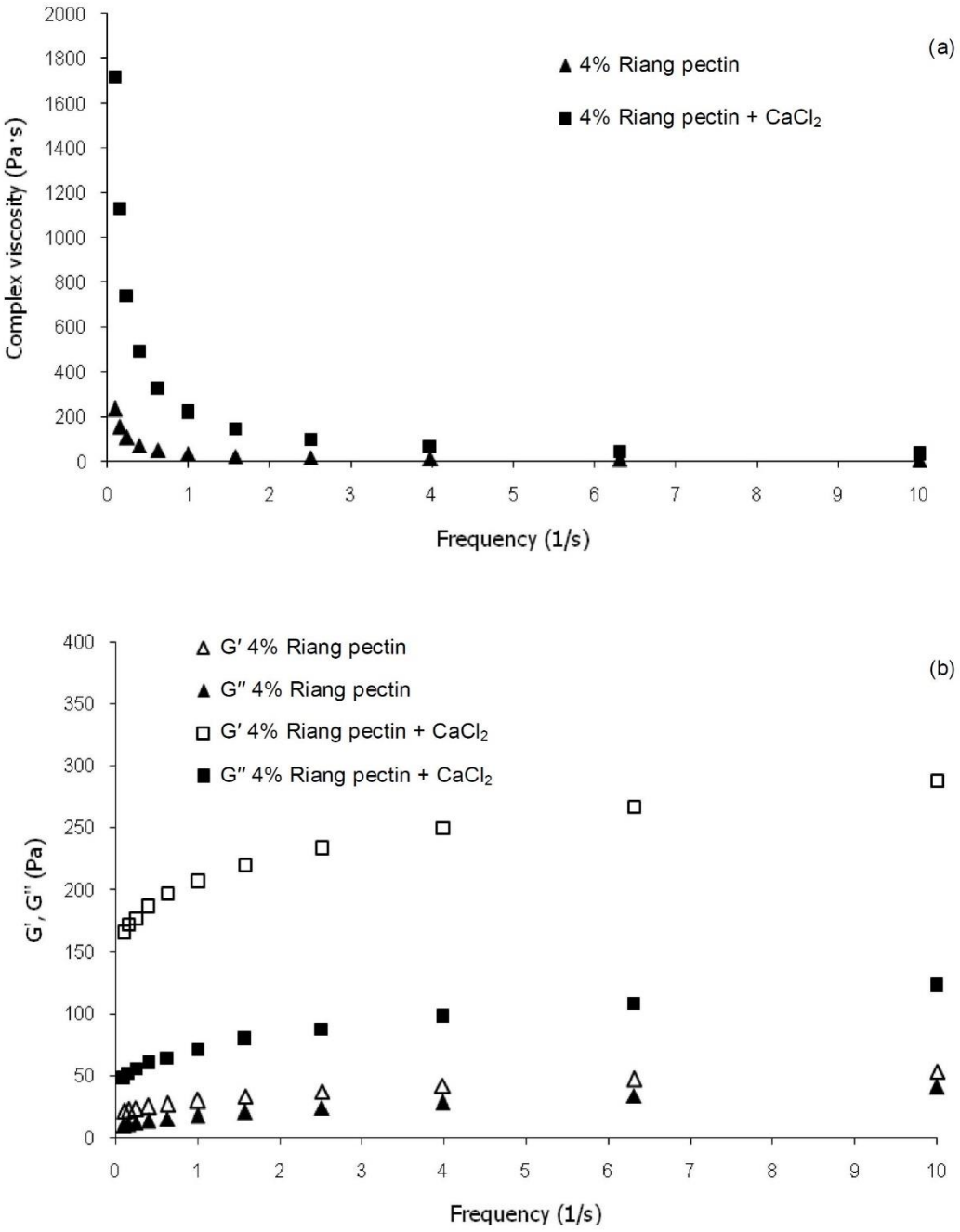

Figure 3 Rheological properties of Riang pectin: (a) Effect of $CaCl_2$ on complex viscosity (b) Effect of $CaCl_2$ on G′ and G″.

Figure 3(b) illustrates the storage (G′) and loss (G″) moduli in Riang pectin with and without addtion of $CaCl_2$. The frequency sweep tests showed a higher G′ than G″ without crossover, in the dynamic range between 0 and 10 Hz (1/s), supporting the phenomenon of gel-like behavior at low frequencies. It was observed that Riang pectins (with and without Ca^{2+}) were supposed to be weak-gels with respect to G' cross all over G" and dependent on the weak frequency in the experiment [38,39]. The interactions between carboxyl groups on the main chain of LMP and divalent cations (Ca^{2+}) are important for crossbridge gelation through the mechanism called “egg box” due to the association of intermolecular junction zones between the smooth homogalacturonan regions of different chains [1]. The rheological results supported that the extractable pectin in Riang pulp was LMP, which pectic polymers ionically cross-linked via free carboxyl group and divalent cations.

Conclusions

In this work, the composition and characteristics of water extractable pectin obtained from Riang pulp were studied. The hot water extraction gave better results compared to a hot acidic extraction using citric acid since it provided the highest yield of pectin. The water extractable pectin was mainly composed of galacturonic acid and considered as LMP, based on low DE values as it was confirmed by FT-IR fingerprint. The HPSEC analysis elucidated that pectic polysaccharides had high molecular weight, which enabled interaction with Ca^{2+} ions as observed by rheological analysis. These results suggest that Riang pulp is a good source of gelling pectin, which could be applied in food and non-food products.

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