



Structural characterization, degree of esterification and some gelling properties of Krueo Ma Noy (*Cissampelos pareira*) pectin

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Abstract

Pectins extracted from Krueo Ma Noy (*Cissampelos pareira*) leaves mainly consisted of galacturonic acid with trace amount of neutral sugars. The dominant structure of Krueo Ma Noy pectin was established as a 1,4-linked α -D-galacturonan by a combination of carboxyl reduction and methylation analysis, and confirmed by FT-IR spectroscopy. The degree of esterification of Krueo Ma Noy pectins was 41.7 and 33.7% for crude and dialyzed pectins, respectively. Krueo Ma Noy pectin has an average molecular weight of 55 kDa, radius of gyration of 15.2 nm and intrinsic viscosity of 2.3 dl/g. Krueo Ma Noy pectin exhibited gelling properties in aqueous solutions at 0.5% (w/v) at 5 °C. Gels were formed at concentrations of 1.0% (w/v) and above even at room temperature. The gel strength, melting point, and melting enthalpy of Krueo Ma Noy pectin increased with polysaccharide concentration.

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Keywords: Krueo Ma Noy pectin; *Cissampelos pareira*; FT-IR spectroscopy; Degree of esterification; HPSEC; DSC

1. Introduction

Pectin is a complex heterogeneous polysaccharide found in the primary cell walls of most plants, in which it provides mechanical strength and flexibility due to its interaction with other cell wall components. The dominant structural feature of pectin is a linear 1 → 4-linked chain of poly- α -D-galacturonic acid with varying degrees of esterification of the carboxylic groups. However, pectins also contain α -L-rhamnopyranosyl residues in the backbone chain and branch chains of arabinan and galactan and their fine structure vary considerably. In the food industry, pectin is an important gelling agent and thickener. Its gelling properties are primarily dependent on the degree of esterification and the molecular weight of the polysaccharides (Barros et al., 2002; Manrique and Lajolo, 2002).

Krueo Ma Noy, *Cissampelos pareira*, is a woody, climbing vine from the family *Menispermaceae*. It is found throughout the warm regions of Asia, East Africa, and South America (Smitinand & Larsen, 1991). It is widespread in the northeast of Thailand. Krueo Ma Noy leaves (up to 30 cm in length) are commonly used as a herb by indigenous people due to their analgesic properties and they have been used for many years for ailments of women. The dark green gel formed after cold extraction of the leaves with water has been used by indigenous peoples as cooling medicine for treating fever. Local medicine also uses this plant for a number of ailments such as asthma and dysentery, as a diuretic and for treatment of traumatic pain (Mukerji & Bhandari, 1959). It is interesting to note that the formation of the gel occurs in a very short period of time after water extraction of the leaf. Singthong, Ningsanond, Cui, and Goff (2004) have recently shown that the polysaccharide responsible for gelation of Krueo Ma Noy extract is a pectin, but the structure of this pectin has not been determined.

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Enzymatic hydrolysis, NMR, and FT-IR are proven to be an effective method for determining structures of polysaccharides, including pectins (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). The degree of esterification (DE) and the percent of the total number of carboxyl groups esterified has a significant effect on the strength of the gel and the gelling mechanisms of pectins (Walter, 1991). Several methods for determining the DE have been reported, e.g. a titrimetric method adopted by Food Chemical Codex (FCC, 1981) and USP 26 NF 21 (2003). The DE of pectins can also be determined by HPLC (Levigne, Thomas, Ralet, Quemener, & Thibault, 2002; Plöger, 1992) and $^1\text{H-NMR}$ spectroscopy (Grasdalen, Bakøy, & Larsen, 1988). Recently, FT-IR becomes a preferred technique for determining the DE due to its ease of use (Filippov, 1992; Manrique & Lajolo, 2002). In previous paper, we reported the chemical composition and solution properties of the pectic polysaccharide extracted from Krueo Ma Noy (Singthong et al., 2004). The objectives of the present paper were to determine the structural features of Krueo Ma Noy pectin, its DE, molecular characteristics and gelling properties.

2. Materials and methods

2.1. Preparation of standard samples

Pectin standards with known DE, 26, 59 and 94%, were obtained from SIGMA (Steinheim, Germany). Standards with known DE, such as 42.5 and 76.5%, were prepared by mixing appropriate amounts of the three commercial standards.

2.2. Preparation of pectin from Krueo Ma Noy

Krueo Ma Noy leaves, procured from the farm market in the Northeast of Thailand, were cleaned with water then dried at 60 °C for 3 h. The dried leaves were ground and stored at room temperature (25 °C) in vacuum. Krueo Ma Noy pectins were extracted according to a method described previously (Singthong et al., 2004). To extract pectin, the dry powder (2% solids) was stirred in distilled water at 25–28 °C and natural pH (3.8–4.0). Alcohol precipitation and drying produced a crude extract; further dialysis against distilled water and lyophilization produced a purified extract.

2.3. Enzyme assay for identification of pectin

The identification and confirmation of pectins from Krueo Ma Noy leaf extract was carried out using an assay available in a kit from Megazyme (Megazyme International Ireland Ltd, Ireland). The sample was dissolved in de-ionized water, and the pH adjusted to 12 to catalyze the deesterification of the 6-methyl galacturonic acid.

The pectate was then incubated with pectate lyase, which cleaves the polygalacturonic acid and releases unsaturated (4,5-ene) oligosaccharides that gave an absorbance at 235 nm.

The amount of unsaturated product produced was calculated as:

$$\text{Unsaturated product} = \Delta\text{Abs} \times 1/L \times 1/\epsilon$$

where ΔAbs is the change of reaction absorbance minus blank absorbance measured after 30 min, L is the cuvette path length (=1 cm) and ϵ is the molar extinction coefficient of the reaction product ($4600 \text{ M}^{-1}\text{cm}^{-1}$). A value of more than 0.5×10^{-5} of the unsaturated product indicates the presence of pectin and conversely an unsaturated product concentration less than 0.5×10^{-5} indicates the absence of pectin (Hansen, Thuesen, & Soderberg, 2001).

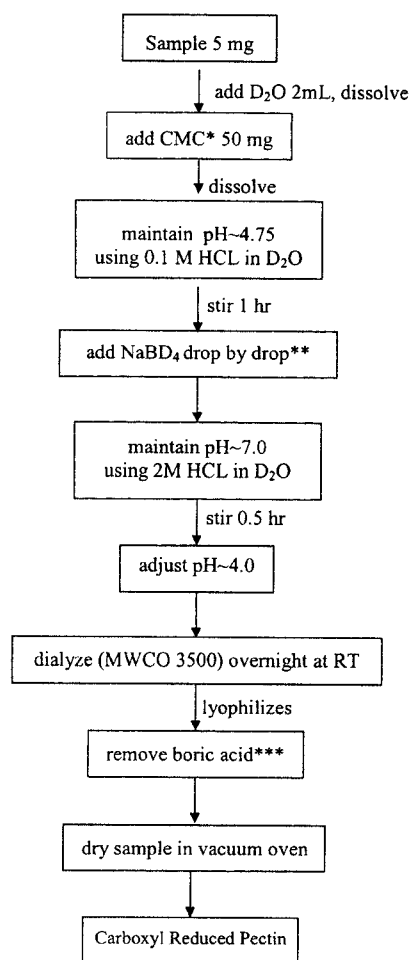
2.4. Methylation and GC-MS of partially methylated alditol acetate (PMAA)

Methylation analysis of pectic substances has been a difficult task due to the presence of large quantities of uronic acids. In this study, we first reduced the uronic acid into neutral sugars then carried out the normal methylation analysis for neutral sugars. The two steps are described in the following sections.

2.4.1. Reduction of uronic acids

The reduction of the uronic acid was conducted following a procedure described by Taylor and Conrad (1972) and York, Darvill, McNeil, Stevenson, and Alberheim (1986) with slight modification (Fig. 1). Sample (5 mg) was dissolved in deuterium oxide (2 ml) and 50 mg of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide methyl-p-toluenesulfonate (CMC, Sigma) was added. The pH was adjusted and maintained at 4.75, using 0.1 M HCl in deuterium oxide. After 1 h, 800 mg of sodium borodeuteride dissolved in 5 ml of deuterium oxide was added over a period of 0.5 h, and the pH of the reaction mixture was maintained at 7.0, using 2.0 M HCl in deuterium oxide during the reduction reaction.

The reaction was allowed to continue with constant stirring for 0.5 h at pH 7.0, after the addition of sodium borodeuteride. After titration of the solution to pH 4.0, the reduced polysaccharide was separated from salts by dialysis against distilled water overnight at 22 °C (3500 molecular weight cut off), and the solution was lyophilized. The polysaccharide was dissolved in distilled water and 10% acetic acid in methanol was added. The mixture was dried with a stream of nitrogen to remove boric acid. This process was repeated 3–4 times to ensure that most of the boric acid was removed. Finally, a few drops of methanol were added and the solution evaporated (two times) to remove any boric acid remained.



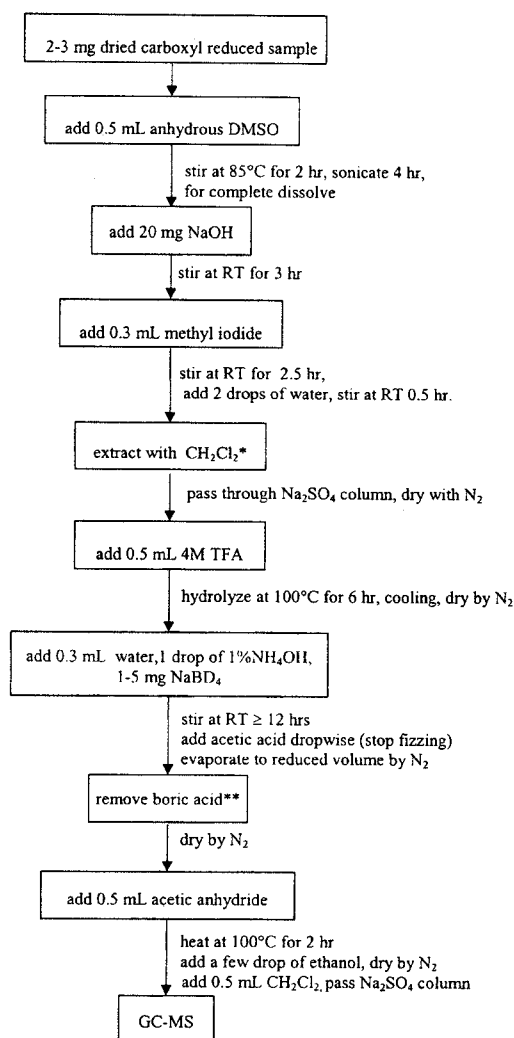
- * 1-Cyclohexyl-3-(2-morpholino-ethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC, Sigma)
- ** 800 mg of sodium borodeuteride dissolve in 5 mL of D₂O is added over 30 min. NaBD₄ can be replaced with NaBH₄.
- *** Add 0.5 mL distilled water and 0.5 mL 10% acetic acid in methanol, evaporate; repeat 3 times. Finally, add 0.5 mL methanol, evaporate (2 times).

Fig. 1. Flow chart of the carboxyl reduction procedure of uronic acids.

Samples of reduced uronic acid (2–3 mg) were dried at 80 °C for 4–5 h and then stored over night under vacuum over phosphorus pentoxide (P₂O₅) in desiccators.

2.4.2. Methylation analysis

The methylation analysis of the pectin samples after reduction of the uronic acid was carried out according to the method of Ciucanu and Kerek (1984) with slight modification (Fig. 2). The dried samples were dissolved in anhydrous DMSO at 85 °C for 2 h with constant stirring and then sonicated for 4 h to ensure that the samples were completely dissolved. Dry sodium hydroxide (20 mg) was added, and the mixture was stirred for 3 h at room temperature (22 °C). The mixture was stirred for additional



- * Transfer the mixture to flat bottom test tube using glass pipette. Wash reaction vial with 1 mL CH₂Cl₂ 3 times. Wash test tube with deionized water 3–5 mL 3 times
- ** Add 0.5 mL 5% acetic acid in methanol, evaporate. Add 0.5 mL methanol, evaporate (many times).

Fig. 2. Flow chart of methylation analysis procedure.

2.5 h after adding 0.3 ml methyl iodide. The methylated polysaccharide was then extracted with 1 ml methylene chloride. The methylene chloride extract was passed through a sodium sulphate column (0.5 × 15 cm) to remove water, and then evaporated by a stream of nitrogen. The dried methylated polysaccharide was hydrolyzed in 0.5 ml of 4.0 M trifluoroacetic acid (TFA) in a sealed test tube at 100 °C for 6 h and the TFA was removed by evaporation under a stream of nitrogen and dissolved in 0.3 ml distilled water. The hydrolysate was reduced, using sodium borodeuteride (1–5 mg) and acetylated with acetic anhydride (0.5 ml). Aliquots of the resultant partially methylated alditol acetates (PMAA) were injected on to GC–MS system (ThermoQuest Finnigan, San Diego, CA) fitted with

a SP-2330 (Supelco, Bellefonte, Pa) column (30 m × 0.25 mm, 0.2 μm film thickness, 160–210 °C at 2 °C/min, and then 210–240 °C at 5 °C/min) equipped with an ion trap MS detector.

2.5. Determination of the degree of esterification

2.5.1. Titrimetric method

The DE of pectin from Krueo Ma Noy pectin was determined by the titrimetric method of Food Chemical Codex (FCC, 1981) and USP 26 NF 21 (2003) with slight modification. Dried sample (500 mg) was transferred to a 250 ml flask, moistened with 2 ml of ethanol and dissolved in 100 ml of carbon dioxide-free water. After the sample was completely dissolved, five drops of phenolphthalein were added, the sample was titrated with 0.5 M sodium hydroxide and the result was recorded as the initial titer. Then, 10 ml of 0.5 M sodium hydroxide were added, the sample was shaken vigorously, and allowed to stand for 15 min; 10 ml of 0.5 M hydrochloric acid were added and the sample was shaken until the pink color disappeared. Phenolphthalein (five drops) were added and the solution was titrated with 0.5 M sodium hydroxide to a faint pink color that persisted after vigorous shaking (end-point). This volume of titration was recorded as the saponification titer (the final titer).

The DE was calculated from the following formula:

$$\%DE = \frac{\text{the final titer}}{(\text{the initial titer} + \text{the final titer})} \times 100$$

2.5.2. FT-IR spectroscopic method

Pectin standards and Krueo Ma Noy pectin were dried and desiccated in a vacuum jar prior to FT-IR analysis. FT-IR spectra of pectins were obtained using a Golden-gate Diamond single reflectance ATR in a FTS 7000 FT-IR spectrometer equipped with a DTGS detector (DIGILAB, Randolph, MA). The spectra were recorded at the absorbance mode from 4000 to 400 cm⁻¹ (mid infrared region) at a resolution of 4 cm⁻¹ with 128 co-added scans. At least triplicate spectra were recorded for each sample. Because the DE is defined as (number of esterified carboxylic groups/number of total carboxylic groups) × 100, it is inferred that the ratio of the area of the band at 1730 cm⁻¹ (corresponding to the number of esterified carboxylic groups) over the sum of the areas of the bands at 1730 and 1600 cm⁻¹ (corresponding to the number of total carboxylic groups) should be proportional to the DE, i.e. $DE = A_{1730} / (A_{1730} + A_{1600})$ (Manrique & Lajolo, 2002; Chatjigakis et al., 1998).

2.6. Molecular characterization

Molecular weight, molecular weight distribution, radius of gyration and intrinsic viscosity of Krueo Ma Noy pectin were determined by high performance size exclusion chromatography (HPSEC, Shimadzu SCL-10Avp,

Shimadzu Scientific Instruments Inc., Columbia, MA, USA) according to a method described by Wang, Wood, Huang, and Cui (2003). The column set consisted of two columns in series, a Shodex OhPak KB-806M (Showa Denko K.K., Tokyo, Japan) and an Ultrahydrogel linear (Waters, Milford, CT, USA) maintained at 40 °C during measurements. The mobile phase was 50 mM NaNO₃ (pH 5.8) with 0.03% (w/w) NaN₃ with a flow rate of 0.6 ml/min. Triple detectors, a right angle laser light detector, a refractive index detector and a viscosity detector, were used for characterizing the molecular weight and molecular weight distribution.

2.7. Rheological properties

All rheological properties were determined on a Bohlin CVO Rheometer (Bohlin Instruments, East Brunswick, NJ). A parallel plate geometry (40 mm diameter, 1.0 mm gap) was used for oscillatory measurements. The viscoelastic properties, storage modulus (G') and loss modulus (G''), were determined through small amplitude oscillatory test at frequencies from 0.1 to 10 Hz. Prior to any dynamic experiments, a strain sweep test at a constant frequency of 0.1 Hz determined the linear viscoelastic region. All oscillatory tests were performed at a strain value of 0.02 (2%) (within the linear viscoelastic region). A thin layer of low viscosity mineral oil was used to cover the sample in order to prevent solvent evaporation during measurements. Temperature sweeps were performed between 5 and 80 °C. Samples were loaded onto the rheometer in a gel state at 5 °C and the heating rate was 1 °C/min.

2.8. Differential scanning calorimetry (DSC)

Thermal analyses were performed using a differential scanning calorimeter (2920 modulated DSC; TA Instruments, New Castle, DE, USA). Sample size was about 80–90 mg, and the scanning rate was 5 °C/min. The reported values are means of duplicate measurements.

Table 1
Determination of content of unsaturated oligosaccharides in pectin and non-pectin polysaccharides

Polysaccharide type	Unsaturated oligosaccharides × 10 ⁻⁴
Carrageenan	0.009
Amidated low ester pectin	1.660
Low ester pectin	2.738
Sugar beet pectin	0.928
High ester pectin	1.522
Dialyzed extract	2.362

Average of duplicate results. Calculations: blank absorbance = enzyme blank + sample blank, Δ absorbance = reaction absorbance - blank absorbance, unsaturated product = Δ Abs × 1/L × 1/ε, where: L, cuvette path length (= 1 cm); ε, molar extinction coefficient (4600 M⁻¹ cm⁻¹).

3. Results and discussion

3.1. Pectin identification assay

Preliminary tests indicated the extracts from Krueo Ma Noy leaves were acidic polysaccharides, and possibly a pectin. A pectin identification assay was used to confirm

the identity of the polysaccharide. When pectin is demethylated and treated with pectate lyase, the glycosidic bonds of the galacturonide chain are cleaved by β -elimination, introducing a 4,5 double bond in the galacturonosyl moiety. The double bond absorbs at 235 nm. The contents of unsaturated oligosaccharides in the dialyzed extract of Krueo Ma Noy was much greater than 0.5×10^{-5}

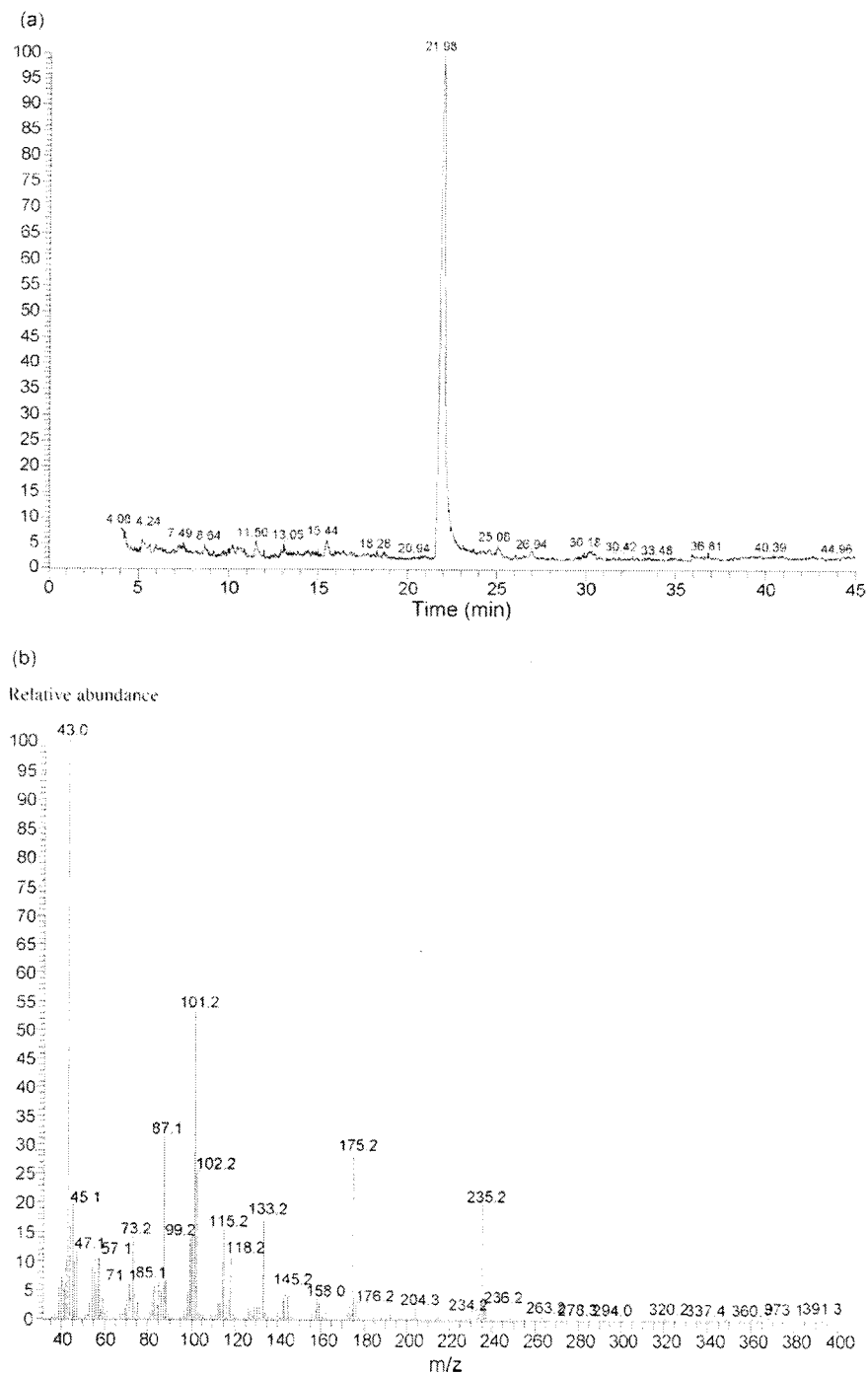


Fig. 3. Chromatogram of GC and Mass spectrum of dialyzed extract. (a) GC, (b) MS.

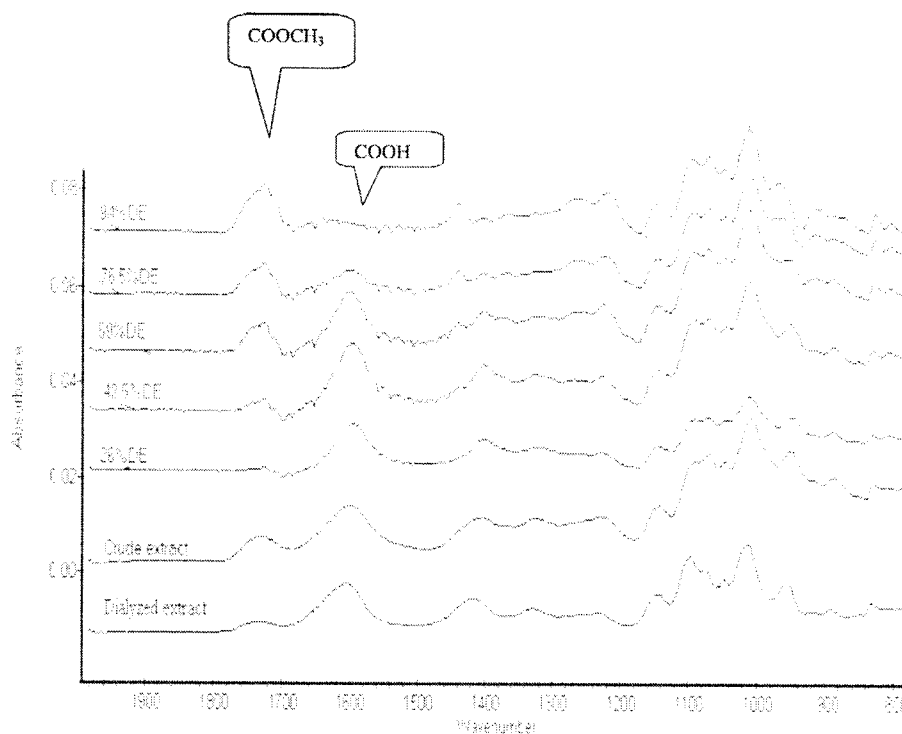


Fig. 4. Fourier transform infrared spectra of commercial pectin standards and Krueo Ma Noy pectins.

(Table 1), suggesting the presence of pectin (Hansen et al., 2001). For comparison purposes, commercial pectin standards and non-pectin samples were also examined (Table 1).

3.2. Methylation analysis

Carbodiimide-activated reduction of the carboxyl groups of glycosyluronic acids with sodium borodeuteride (NaBD_4) resulted in an easily identified sugar (deuterized). There was only one major peak detected from the GC–MS analysis of the partially methylated alditol acetate (PMAA) derived from the carboxyl reduced Krueo Ma Noy pectin (Fig. 3a), and its corresponding mass spectrum is displayed in Fig. 3b. The combination of the fragmentation pattern and retention time of the PMAA suggested that the reduced polysaccharide is made of 1,4-linked D -galactosyl residues. The diagnostic fragment m/z 235 is shifted two mass units higher than the m/z 233 expected from a 4-linked hexopyranosyl unit (Biermann & McGinnis, 1989). Because there were no GC peaks detected from the non-reduced Krueo Ma Noy extract, there were no D -galactosyl residues in the polymer. The major peak in Fig. 3a represents 4-*O*-substituted D -galacturonic acid. This result indicates that Krueo Ma Noy extract is a pectin that has a linear backbone chain of 1→4-linked α - D -galacturonic acid units (Walter, 1991).

3.3. FT-IR spectrum and the degree of esterification of pectins

The FT-IR spectra of Krueo Ma Noy pectin and commercial pectin standards are presented in Fig. 4. The functional groups of pectins and their corresponding frequencies and the nature of the bands are presented in Table 2. The broad, strong areas of absorption between 3600 and 2500 cm^{-1} are caused by O–H stretching absorption due to inter- and intra-molecular hydrogen bonds. The O–H stretching vibrations occur within a broad range of

Table 2
FT-IR spectrum of pectin: wave numbers and intensities of functional groups

Wave number (cm^{-1})	Functional groups	Intensity
3600–2500	O–H stretching	Broad, strong
3000–2800	C–H stretching, symmetric, asymmetric	Sharp, occasionally double overlapping with O–H
1760–1730	C=O, esterified	Strong
1630–1600	COO-asymmetric stretching	Strong
1400	COO-symmetric stretching	Weak
1380	C–H bending	Weak
1300–1000	C=O stretching	Weak

Adopted from Gnanasambandam and Proctor (2000) and Filippov (1992).

frequencies and indicate several features of a compound, including free hydroxyl groups stretching bonds that occur in samples in vapor phase and bonded O–H bands of carboxylic acid (Silverstein, Bassler, & Morrill, 1991). In the case of pectin samples, absorption in the O–H region was due to the inter- and intra-molecular hydrogen bonding of the galacturonic acid backbone. Bands around 2950 cm^{-1} ($3000\text{--}2800\text{ cm}^{-1}$) refer to C–H absorption, these include CH, CH₂ and CH₃ stretching and bending vibrations. Typically, two moderately intense bands are observed in the C–H region of aliphatic compounds. In pectin samples, the C–H stretching and bending vibrations are seen, usually, as a band superimposed upon the broader O–H band that ranges from $2500\text{ to }3600\text{ cm}^{-1}$ (Filippov, 1992; Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000).

These absorbencies can be observed with all pectin standards studied. In the case of esterified pectins, an O–CH₃ stretching band would be expected between $2950\text{ and }2750\text{ cm}^{-1}$ due to methyl esters of galacturonic acid. However, due to a large O–H stretching response occurring in a broad region ($3600\text{--}2500\text{ cm}^{-1}$), the O–CH₃ activity is masked and therefore is not a reliable indicator of methoxylation. Stronger bands occurring between $1760\text{--}1730$, and $1630\text{--}1600\text{ cm}^{-1}$ are derived from the ester carbonyl groups and carboxylate ion stretching band, respectively (Chatjigakis et al., 1998; Manrique & Lajolo, 2002). It was observed that the intensity of the absorbance or band area of the ester carbonyl groups increased with the increase in DE, in contrast, the absorbance intensity or the band area of the carboxylate stretching band ($1730\text{--}1760\text{ cm}^{-1}$) decreased (Fig. 4). In a similar manner, the intensity of the absorbance or band area of the free carboxylate groups ($1630\text{--}1600\text{ cm}^{-1}$) increased with the decrease in DE. These observations established the basis for quantitative analysis of DE of pectins by FT-IR: the $1760\text{--}1730\text{ cm}^{-1}$ bands represents ester carbonyl groups while the $1630\text{--}1600\text{ cm}^{-1}$ band represents the free carboxylate groups.

It is also worth noting that the carboxylate groups showed two bands, an asymmetrical stretching band near $1650\text{--}1550\text{ cm}^{-1}$, and a weaker symmetric stretching band near 1400 cm^{-1} . In pectin samples, the weaker symmetric (COO⁻) stretching is followed by moderately intense absorption patterns between $1300\text{ and }800\text{ cm}^{-1}$; these collectively are referred to as the fingerprint region for pectins. Other bands of lesser importance in pectin samples are C–H bending, occurring at 1380 cm^{-1} , and C=O stretching occurring at $1300\text{--}1000\text{ cm}^{-1}$ (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998; Gnanasambandam & Proctor, 2000).

In order to quantify the DE of pectins, a calibration curve was constructed based on pectin standards of known DE. The calibration curve was established from the ratio of $A_{1730}/(A_{1730}+A_{1600})$, as presented in Fig. 5. For every triplet measurements of pectin standards, the coefficients of

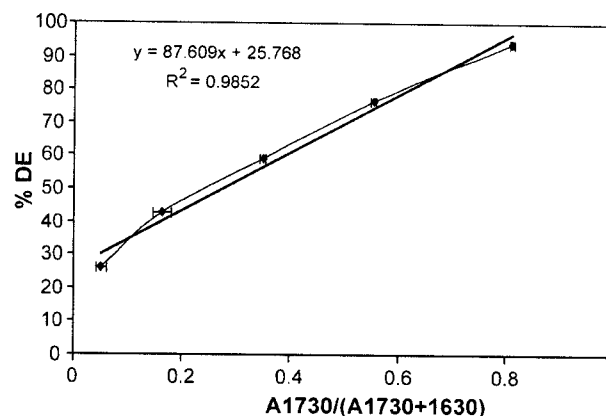


Fig. 5. Calibration curve of absorbance from the FT-IR spectra of pectin standards: ratio of the peak area at 1730 cm^{-1} over the sum of the peak areas at $1730\text{ and }1600\text{ cm}^{-1}$ versus degree of esterification of pectins (%).

variation of the ratios were less than 2%, indicating an excellent reproducibility. The high value of the square of the linear correlation coefficient ($r^2=0.98$) indicated a highly linear relationship between the DE and the absorbent area at $1730\text{ and }1600\text{ cm}^{-1}$. Using this calibration curve, the DE of pectin from Krueo Ma Noy extracts were calculated and the results are presented in Table 3. The DE of Krueo Ma Noy pectins were $41.65\text{ and }33.69\%$ for crude and dialyzed pectin samples, respectively. The DE of dialyzed extract was lower than that of crude extract; this observation suggests that some small molecules of pectin with higher DE were removed during the dialysis process.

To evaluate the validation of the FT-IR method, the DE values obtained by FT-IR method were compared against those obtained from the titrimetric method (Table 3). The results confirmed that FT-IR is a reliable method for determining the DE of pectins since there was no significant difference observed between the DE from the two methods for both samples.

3.4. Molecular characterization

Molecular weight, molecular weight distribution, radius of gyration and intrinsic viscosity of Krueo Ma Noy pectin were determined by high performance size exclusion chromatography (HPSEC) according to a method described by Wang et al. (2003) (Table 4). The weight average molecular weight (Mw) of dialyzed Krueo Ma Noy pectin

Table 3

The degree of esterification of pectins from crude and purified extracts of Krueo Ma Noy obtained from different methods

Method	Degree of esterification ^a (%)	
	Crude extract	Dialyzed extract
FT-IR spectroscopy	41.7 ± 0.0^b	33.7 ± 0.2^b
Titrimetric	43.2 ± 1.0^b	36.2 ± 3.0^b

^a Values are means of triplicate measurements.

^b Values with different letters in each column are significantly ($P \leq 0.05$) different from each other.

Table 4

Averages (number average, weight average and z-average) of molecular weight, radius of gyration, intrinsic viscosity and polydispersity of dialyzed extract

Molecular characterization	Means \pm SD ^a
Number average ($M_n \times 10^{-3}$ Da)	29.08 \pm 2.45
Weight average ($M_w \times 10^{-3}$ Da)	55.08 \pm 2.62
Z-average ($M_z \times 10^{-3}$ Da)	99.00 \pm 7.55
Radius of gyration (R_g , nm)	15.21 \pm 0.28
Intrinsic viscosity ($[\eta]$, dl/g)	2.30 \pm 0.04
Polydispersity (Pd, M_w/M_n)	1.90 \pm 0.11

^a Each result represents the average of three determinations \pm SD.

was 55 kDa, which is significantly lower than the literature values from 85 to 103 kDa reported for citrus pectin but in the general range of 10^4 – 10^5 Da for pectins from various fruit sources (Corredig, Kerr, & Wicker, 2000). The polydispersity parameter of 1.9 indicated a broad molecular weight distribution of dialyzed Krueo Ma Noy pectin. The HPSEC method also provided the radius of gyration (R_g) and intrinsic viscosity $[\eta]$ of dialyzed Krueo Ma Noy pectin (15.2 nm and 2.3 dl/g, respectively), which reflect the conformation of the polymer in the solvent system. The R_g (15.2 nm) value of dialyzed Krueo Ma Noy pectin was much lower than that of four commercial pectins

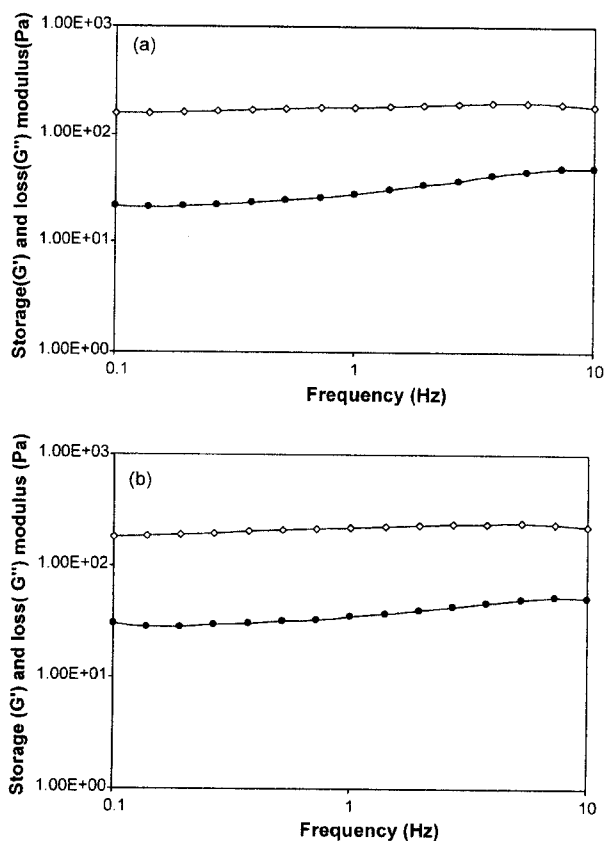


Fig. 6. Frequency dependence of storage (G' , \diamond) and loss (G'' , \blacklozenge) modulus of 1% (w/v) of Krueo Ma Noy pectin. (a) Crude extract, (b) dialyzed extract.

(50–59 nm) with molecular weights ranging from 90,000 to 200,000 Da (Corredig et al., 2000). Although the flow rate and solvent had some influence on the values of R_g , the experimental data from different laboratories are still comparable.

3.5. Rheological properties

The viscoelastic properties of Krueo Ma Noy pectin were examined by oscillatory experimental measurements (Fig. 6). Crude extract exhibited a strong gel structure at 1.0% (w/v) as its storage modulus G' was much greater than the corresponding loss modulus G'' and the two moduli were independent of frequency (Fig. 6a). The dialyzed Krueo Ma Noy pectin exhibited similar rheological behavior, but the gel strength was stronger than that of crude extract (Fig. 6b). The stronger gel strength of the dialyzed pectin may be caused by a higher effective polymer concentration. The gel

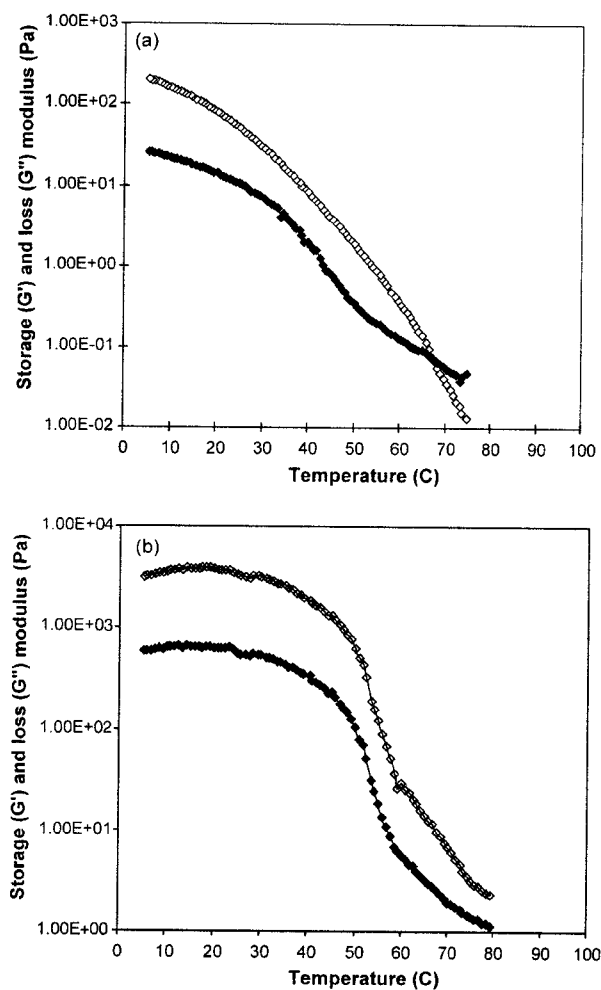


Fig. 7. Temperature dependence of storage (G' , \diamond) and loss (G'' , \blacklozenge) modulus during heating from 5 to 80 °C at rate of 1 °C/min for 1% (w/v) of Krueo Ma Noy pectin (pH 3.8). (a) Crude extract, (b) dialyzed extract.

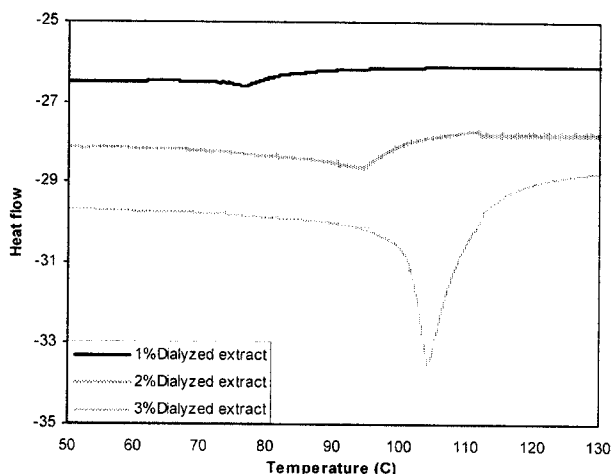


Fig. 8. DSC thermograms of dialyzed Krueo Ma Noy pectin.

strength increased with increasing sample concentration (data not shown).

A Krueo Ma Noy pectin gel did not show a clear melting point when it was heated at 1 °C/min at constant frequency (0.1 Hz) (Fig. 7). At the beginning of the heating, G' decreased with the increase of temperature. The storage modulus G' and loss modulus G'' showed a crossover around 67 °C (Fig. 7a), which indicated a melting point for crude extract. The rate of decrease of G' was much faster at temperatures > 40 °C, this is especially true for dialyzed extract (Fig. 7b). The melting point of Krueo Ma Noy pectin gels was difficult to determine due to the sensitivity and operation limitations of the rheometer.

3.6. Differential scanning calorimetry

The effect of Krueo Ma Noy pectin concentration on the melting temperature and melting enthalpy involved was determined by DSC (Fig. 8, Table 5). The increase of polymer concentration had a positive influence on the melting enthalpy (ΔH) and melting temperature (T_m). For crude extract, the ΔH values increase from 0.11 to 10.84 J/g and T_m increased from 75.15 to 113.49 °C when polymer concentration was increased from 2 to 4%. The ΔH and T_m values of dialyzed extract increased from 0.56 to 5.85 J/g and 76.10 to 104.21 °C, respectively, when the polymer concentration was increased from 1% to 3% (Table 5).

Table 5
Thermal properties of crude and dialyzed extracts determined by DSC

Krueo Ma Noy pectin	T_m (°C) ^a	ΔH (J/g) ^a
2% Crude extract	75.2 ± 0.5	0.11 ± 0.02
3% Crude extract	105.1 ± 0.9	2.48 ± 0.13
4% Crude extract	113.5 ± 1.6	10.84 ± 1.37
1% Dialyzed extract	76.1 ± 0.8	0.56 ± 0.15
2% Dialyzed extract	92.7 ± 2.4	0.65 ± 0.05
3% Dialyzed extract	104.2 ± 0.5	5.86 ± 1.77

T_m , peak temperature of melting (°C); ΔH , enthalpy (J/g).

^a Each result represents the average of duplicate values ± SD.

At the same polymer concentration, the ΔH values of dialyzed extract were higher than that of crude extract, this might be due to the higher effective concentration of the dialyzed pectin sample.

4. Conclusion

The polysaccharide extracted from Krueo Ma Noy (*C. pareira*) leaves consisted mainly of pectin. The dominant structure of the dialyzed pectin was a 1→4 linked α -D-galacturonan. FT-IR spectroscopy confirmed the pectin structure. Two characteristic peaks, 1730 and 1600 cm^{-1} , absorption of the esterified and non-esterified carboxyl groups of pectin, respectively, were used to quantify the DE of Krueo Ma Noy pectin using standards of known DE to construct the calibration curve. The DE of Krueo Ma Noy pectins were estimated to be 41.7 and 33.7% for crude extract and dialyzed pectin samples, respectively; these results were confirmed by titration. The weight average molecular weight, radius of gyration and intrinsic viscosity of the dialyzed extract were determined to be 55 kDa, 15.2 nm and 2.3 dl/g, respectively. This study also showed that Krueo Ma Noy pectin forms gels from aqueous solutions and the gel strength is a function of pectin concentration (0.5–3.0%). In addition, the melting point and melting enthalpy of Krueo Ma Noy pectin gels also increased with increasing polymer concentration (1.0–3.0%). Since pectin from Krueo Ma Noy leaves is easy to extract and the raw material is readily available at low cost and the material exhibited unique gelling properties and has a long history for food consumption in Southern East Asia (Singthong et al., 2004), there is significant commercial potential for this new pectin.

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