Extraction and physicochemical characterization of Krueo Ma Noy pectin

Jittra Singthong[a], Suwayd Ningsanond[a], Steve W. Cui[b,*], H. Douglas Goff[c]

[a]*School of Food Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[b]*Food Research Program, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ont., Canada N1G 5C9*
[c]*Department of Food Science, University of Guelph, Guelph, Ont., Canada N1G 2W1*

Abstract

A new pectin was extracted from the leaves of Krueo Ma Noy (*Cissampelos pareira*), a woody climbing plant grown in Thailand. Optimized condition for the extraction of pectin was using the solid:solvent ratio=1:50 in distilled water at 25–28 °C and natural pH (3.8–4.0). A crude pectin extract was obtained after alcohol precipitation and drying; the crude extract was dialyzed and lyophilization produced a dialyzed pectin fraction. The extracted Krueo Ma Noy pectin is a low methoxyl pectin which consisted mainly of uronic (galacturonic) acid ($\sim$70–75%) and small amounts of neutral sugars. Krueo Ma Noy pectin exhibited shear thinning flow behavior and the extent of shear thinning was concentration dependent. Gelation was observed when Krueo Ma Noy pectin (both crude extract and dialyzed fractions) concentration exceeded 0.5% (w/v); gel strength of the dialyzed fraction was much higher than that of the crude extract. The addition of NaCl significantly increased the Krueo Ma Noy pectin gel strength when salt concentration was below 0.4 M. Krueo Ma Noy pectin was also found to be sensitive to Ca^{2+} ions: the addition of 1 mM calcium chloride significantly increased the gel strength and excessive amount of Ca^{2+} ($>$3 mM) resulted in aggregation/precipitation of the pectin, possibly due to the calcium binding properties of the carboxyl group. It was also observed that addition of sugar increased the gel strength of Krueo Ma Noy pectin.
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1. Introduction

Krueo Ma Noy, *Cissampelos pareira*, is in the family of *Menispermaceae*. It is a woody climbing vine with leaves up to 30 cm in length. The plant is found throughout warm parts of Asia, East Africa and South America (Smitinand & Larsen, 1991). It is widespread in the northeast of Thailand.

Krueo Ma Noy is commonly referred to as a medicinal herb by indigenous people for treating a variety of ailments such as asthma, dysentery, diuretic and traumatic pain (Mukerji & Bhandari, 1959). The extracts from root of this plant exhibited some biological functions such as anti-tumor, antileukemia, diuretic and muscle relaxant (Caceres, Giron, & Martinez, 1987; Itokawa, Morita, Matsumoto, & Takeya, 1993; Kupchan, Patel, & Fujita, 1965; Tang & Eisenbrand, 1992) and these functionalities

have been largely attributed to the presence of a number of alkaloids, especially bisbenzylisoquinoline alkaloids (Dwuma-badu et al., 1975; Manske & Holmes, 1954). The water extracts from leaves can form gel in a short period of time and the gel has been consumed as a dessert by Northeastern Thais.

Polysaccharides obtained from different sources are widely used in food systems for various purposes, such as thickeners, stabilizers, gelling agents and texture modifiers. The gelling components in Krueo Ma Noy could be another source of natural polysaccharides. However, there is no information available in the literature on any chemical composition and rheological properties of this material. Preliminary experiments indicated that the water extract from Krueo Ma Noy leaves is primarily an acidic polysaccharide. The paper is for the first time to explore the potential use of this new material by examining the effect of extraction conditions on yield and viscosity and characterizing its physicochemical properties.

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* Corresponding author. Tel.: +1 519 780 8028; fax: +1 519 829 2600.
E-mail address: cuis@agr.gc.ca (S.W. Cui).

2. Materials and methods

2.1. Preparation of plant materials

Krueo Ma Noy leaves, procured from the farmer's market in the Northeast of Thailand, were cleaned and dried at 60 °C for 3 h. The dried leaves were ground and stored at room temperature in a vacuum packed container before use.

Dried Krueo Na Noy leaves were analyzed for moisture, ash, protein, lipid and dietary fiber contents according to the methods of AOAC (1997).

2.2. Extraction and purification

Dried Krueo Ma Noy leaf powder was extracted under conditions of varied pH (3–7) and temperature (25–90 °C) with distilled water (Table 1) and it was found that the most effective and economic condition was at room temperature (25–28 °C) and natural pH (3.8–4.0) with a solid:water ratio of 1:50 for 60 min. The crude extract was filtered through a vacuum filter and centrifuged at 10,000 rpm for 15 min. The supernatant was concentrated to about half of its volume in a rotary evaporator before being precipitated with ethanol 95% (w/v) (final ethanol concentration was ∼70%). The precipitate was dried in a vacuum oven and ground to obtained *crude extract* powder.

The crude extract was solubilized in distilled water (0.1% w/v) at 85 °C for 1 h, centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was dialyzed against distilled water for 3×24 h (15,000 molecular weight cutoff) at room temperature. The dialyzed fraction was concentrated by rotary evaporation before lyophilized to give a *dialyzed* Kreuo Ma Noy polysaccharide.

2.3. Chemical compositions

Moisture, ash, and mineral contents were determined according to the AOAC method (AOAC, 1997). Protein content was converted from nitrogen content (N×6.25), which was determined using an Automatic Elemental Analyzer (Strada Rivoltana, Milan, Italy).

Total sugars and uronic acid content were determined using the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956), and Blumenkrantz and Asboe-Hansen (1973), respectively.

Monosaccharide compositions were analyzed according to the procedure of Wood, Weisz, and Blackwell (1994) using a DIONEX HPAEC system with pulsed amperometric detector (PAD).

2.4. FT-IR spectroscopy

All samples were dried and stored in desiccators prior to FT-IR analysis. FT-IR spectra were recorded using a Golden-gate Diamond single reflectance ATR on a FTS 7000 FT-IR spectrophotometer with DTGS detector (DIGI-LAB, Randolph, MA). The spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1} (mid infrared region) at the resolution of 4 cm^{-1} and 128 scans were collected to obtain a high signal-to-noise ratio. At least triplicate spectra readings for each sample were performed.

2.5. Rheological properties

All rheological properties were determined on a Bohlin CVO Rheometer (Bohlin Instruments, East Brunswick, NJ) A parallell plate geometry (40 mm diameter, 1.0 mm gap) was used for both steady shear and oscillatory measurements. Steady shear viscosity was determined at various concentrations and temperatures. The viscoelastic properties, i.e. storage modulus (G') and loss modulus (G''), were determined by 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 was conducted to set the upper limit of the linear viscoelastic zone. All oscillatory tests were performed at a strain value of 0.02 (2%), which was within the linear viscoelastic region. A thin layer of low viscosity mineral oil was added to cover the sample in order to prevent solvent evaporation during measurements.

3. Results and discussion

3.1. Chemical and monosaccharide compositions

Dried Krueo Ma Noy leaves contained, on average, 6.43% moisture, 11.18% ash, 6.78% protein, 2.22% lipid and 65.98% total dietary fiber. Preliminary extraction optimization process found that higher temperature gives

Table 1
Effect of extraction conditions on the yield, viscosity and protein content of extracted Krueo Ma Noy polysaccharides

Extraction conditions			Results		
Temperature (°C)	Time (min)	pH	Yield (%)	Viscosity (cPs)[a]	Protein (%)
75	20	3	27.2	279	1.9
95	20	3	28.9	48	2.4
75	60	3	26.5	116	1.8
95	60	3	26.6	29	2.0
75	20	5	25.9	278	1.9
95	20	5	26.2	48	2.4
75	60	5	24.5	113	2.5
95	60	5	27.1	27	2.5
85	40	4	25.5	93	1.8
85	40	4	26.1	93	1.8
85	40	4	25.8	94	1.7
85	40	4	25.8	93	1.7
28 (RT)[b]	20	∼3.8	21.5	681	3.1

[a] Apparent viscosity was determined using a Brookfield Viscometer models RVT at a concentration of 0.5% (w/v).
[b] Measured at room temperature (in Thailand).

Table 2
Chemical composition of Krueo Ma Noy leaves pectins

Composition (%)	Crude extract	Dialyzed
Moisture	10.57 ± 0.54	8.53 ± 0.39
Ash	9.86 ± 0.01	8.40 ± 0.69
Protein	3.19 ± 0.10	0.29 ± 0.07
Total sugar (as glucose equivalent)	9.06 ± 0.02	8.56 ± 0.01
Uronic acid (as galacturonic acid equivalent)	70.56 ± 0.10	75.93 ± 0.83
Monosaccharide		
Rhamnose	1.07 ± 0.00	1.07 ± 0.00
Arabinose	0.60 ± 0.01	0.51 ± 0.00
Galactose	0.78 ± 0.01	0.64 ± 0.01
Glucose	0.61 ± 0.03	0.32 ± 0.01
Xylose	0.28 ± 0.00	ND
Mannose	0.58 ± 0.02	0.56 ± 0.00
Minerals		
Na	0.16 ± 0.00	0.26 ± 0.00
K	3.90 ± 0.00	1.60 ± 0.00
Ca	1.40 ± 0.00	2.70 ± 0.00
Mg	0.90 ± 0.00	0.71 ± 0.00
Fe (mg/g)	0.03 ± 0.00	0.03 ± 0.00
Zn (mg/g)	0.12 ± 0.00	0.14 ± 0.00

higher yield, but poor viscosity of the final product (Table 1). It was found that extraction at room temperature and natural pH ($\sim$pH 3.8, as exhibited by the leaves powders without any adjustment) gave relative high yield with very high viscosity (Table 1). Therefore, the crude extract used in this study was at room temperature and natural pH ($\sim$3.8).

The chemical and sugar compositions of *crude extract* as well as *dialyzed extract* from Krueo Ma Noy leaves are presented in Table 2. The yield of the dialyzed extract was

about 85% of the crude one. The total protein (derived from nitrogen) content of the crude extract was significantly reduced after dialysis. The dialysis process did not change the monosaccharide profile, however, the total uronic acid content was increased from 70.56% in crude extract to 75.93% in dialyzed material. The dialysis process also significantly reduced the level of potassium. In contrast, the levels of calcium and sodium were increased after dialysis, indicating stronger binding properties of the two cations with the acidic polysaccharides. The preliminary composition analysis indicated that the polysaccharides extracted from Krueo Ma Noy leaves could be a pectin, which was later confirmed by FT-IR spectroscopy (see Section 3.2).

3.2. FT-IR spectroscopy

FT-IR spectra in 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: the position and intensity of the bands are specific for every polysaccharide (Cernà et al., 2003; Kalapathy & Proctor, 2001). In order to confirm the identity of Krueo Ma Noy extracts, both *crude and dialyzed* Kreuo Ma Noy polysaccharides were analyzed by FT-IR and their spectra were compared against three commercial pectin standards (Fig. 1). It was found that the FT-IR spectra of both crude and dialyzed polysaccharides exhibited similarities of the absortion patterns to that of commercial pectin standards, confirming the preliminary conclusion derived from chemical composition analysis (Table 2) that the polysaccharide extracted from Krueo Ma Noy leaves is a pectin.
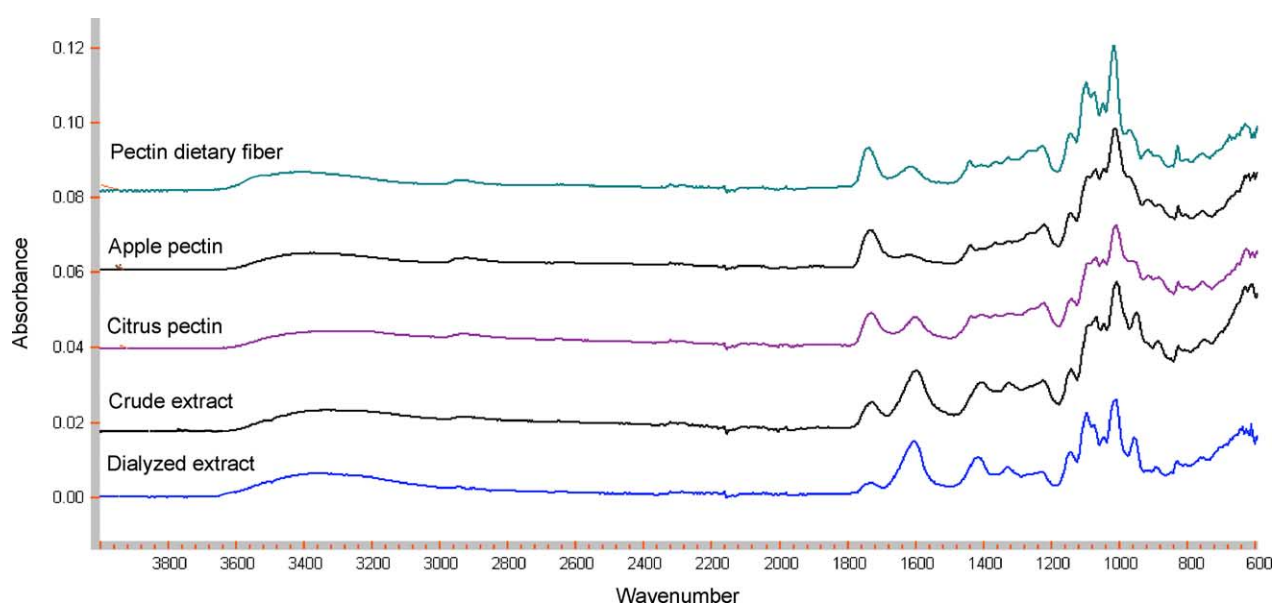

Fig. 1. Fourier transform infrared spectra of crude and dialyzed pectins from Krueo Ma Noy leaves and pectin standards (apple pectin, citrus pectin and pectin dietary fiber, respectively).

Further analysis of the FT-IR spectra revealed that the broader band of absorption between 3600 and 2500 cm^{-1} was due to O–H stretching whereas strong absorbance observed at 1730–1760 and 1600–1630 cm^{-1} were attributed to the ester carbonyl (C=O) groups and carboxyl ion stretching band (COO$^-$), respectively (Kamnev, Colina, Rodriguez, Ptitchkina, & Ignatov, 1998; Silverstein, Bassler, & Morril, 1991). In the FT-IR spectra of pectin standards, the weaker symmetric COO$^-$ stretching was followed by moderately intense absorption patterns between 800 and 1300 cm^{-1}; these characteristic absorbencies are collectively referred to as the finger print region. Other bands of lesser importance in pectin samples are C–H bending, occurring at 1380 cm^{-1} and C=O stretching occurring at 1000–1300 cm^{-1}. In order to determine whether Krueo Ma Noy pectin is a low methoxy pectin or a high methoxy pectin, the FT-IR spectra of both crude and dialyzed pectin were compared against three commercial citrus pectins of various degree of esterification, as shown in Fig. 2. The intensity of the absorbance of the ester carbonyl groups (1730–1760 cm^{-1}) increased with the increase in the degree of esterification; in contrast, the intensity of the free carboxyl stretching band decreased (Chatjigakis, Pappas, Proxenia, Kalantzi, Rodis, & Polissiou, 1998; Filippov, 1992; Manrique & Lajolo, 2002). The qualitative comparison of absorbencies at the two characteristic peaks of pectins from Krueo Ma Noy leaves suggest that it is probably a low methoxyl pectin. It is further observed that the degree of esterification of dialyzed pectin is lower than the crude extract suggesting that the dialysis process removed some higher methoxy pectin moieties probably having smaller molecular weights. Quantitative

determination of DE of Kreuo Ma Noy pectin is reported separately (Singthong, Cui, Ningsanond, & Goff, 2004).

3.3. Rheological properties

3.3.1. Flow behavior

3.3.1.1. Effect of concentration. The flow curves of crude and dialyzed Krueo Ma Noy pectin at concentrations of 0.1–2% (w/v) at 25 °C are shown in Fig. 3a and b. The higher concentration group (2.0 and 1.5%) exhibited shear thinning flow behavior with a more pronounced shear thinning observed at high shear rate region. Of the medium concentration flow curves (0.5 and 1.0%), Newtonian flow behavior was observed at low shear rate region whereas shear thinning flow behavior was observed at higher shear rate region. In the low concentration group (0.1 and 0.25%), significant lower viscosities were observed and the flow curves still exhibited some non-Newtonian characteristics (Figs. 3 and 4).

The changes of flow behaviors of Krueo Ma Noy pectin at different concentrations could be explained by the concentration effect of polysaccharides. Some polysaccharide chains are known be able to form ordered structures through chain–chain entanglements. For concentrated polymer systems (in this case, Krueo Ma Noy pectin gels), the application of a shear force to the system will cause the disruptions of ordered structures, which would lead to a decreased viscosity with increased shear rate. In semi-dilute solutions there is a constant formation and disruption of chain–chain entanglements. At lower shear rate, the rates of disruption and formation of the polymer chain entanglements are at equilibrium, as a result
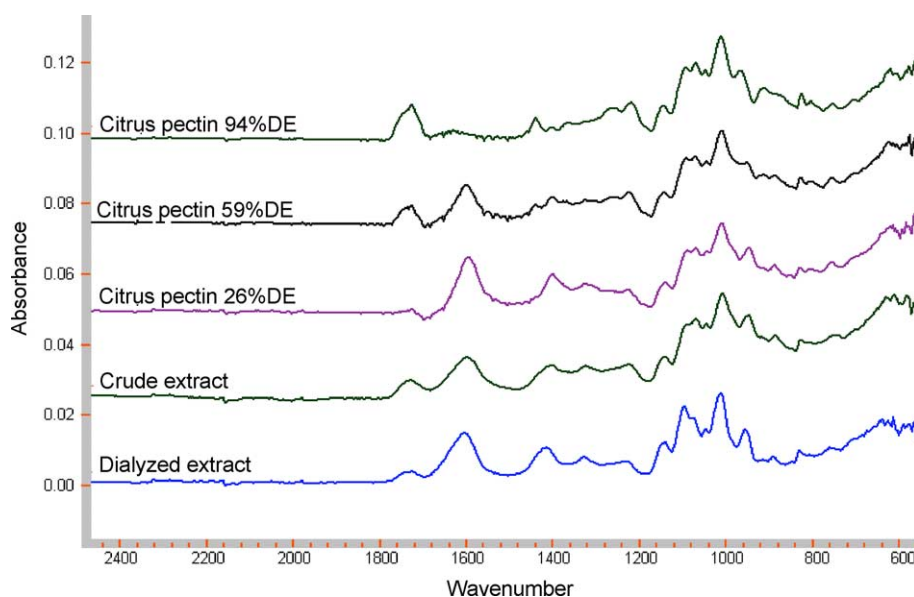

Fig. 2. Fourier transform infrared spectra of commercial pectin standards with different degree of esterifications and Krueo Ma Noy pectins (crude and dialyzed).
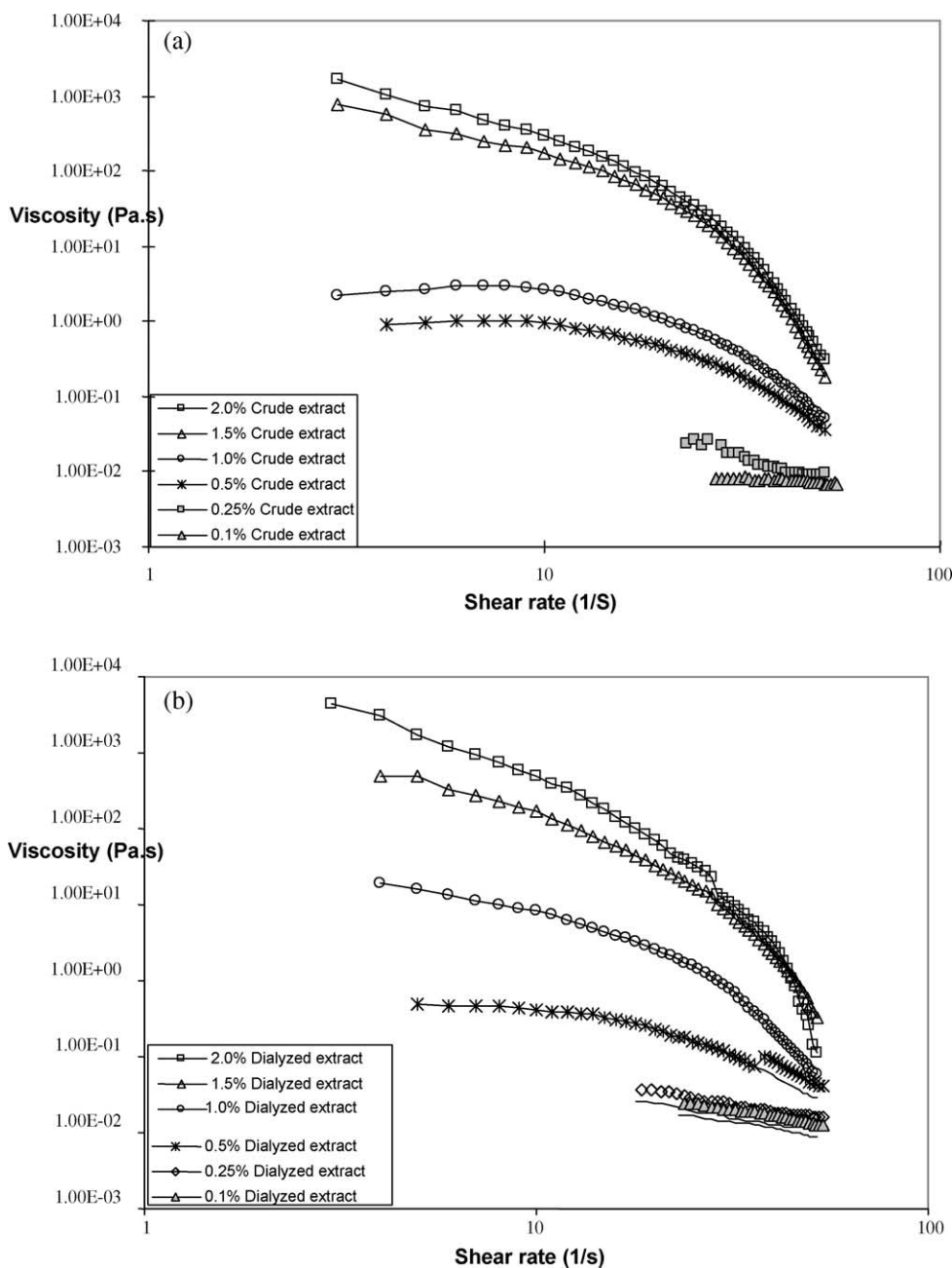
Fig. 3. Steady shear flow curves of Krueo Ma Noy pectins at different concentrations at 25 °C: (a) crude extract; (b) dialyzed.

the viscosity remained unchanged. As the shear rate increases, the rate of formation of the entanglements could not keep up with the rate of disruption of the entanglements, the net result is the decrease of viscosity with increase in shear rate. At dilute polymer systems, the formation of the entanglement is minimum; in other words, there is no entanglement to be disrupted, and the observed flow curve is the second plateau. The flow curve of 0.25% Krueo Ma Noy pectin falls between the semi-diluted solution and dilute solutions. The data missed from Fig. 3a for low concentration solutions at low shear rate is caused by the limitation of the instrument. A true strain controlled rheometer could be used to improve the measurement. The flow curves of

dialyzed Krueo Ma Noy pectin at the same concentrations are shown in Fig. 3b, which follows the trends discussed for the crude extract. However, at the same concentration, the dialyzed polysaccharides tend to exhibit more pronounced shear thinning flow behavior than the crude extract. The dialyzed Krueo Ma Noy pectin exhibited shear thinning flow behavior even at 0.1% (w/v) polymer concentration. This could be explained by the fact that the effective concentration of the dialyzed sample was higher than the crude extract due to their differences in purity (Table 2).

3.3.1.2. Effect of temperature. Temperature also had a significant effect on the flow behavior of Krueo Ma Noy
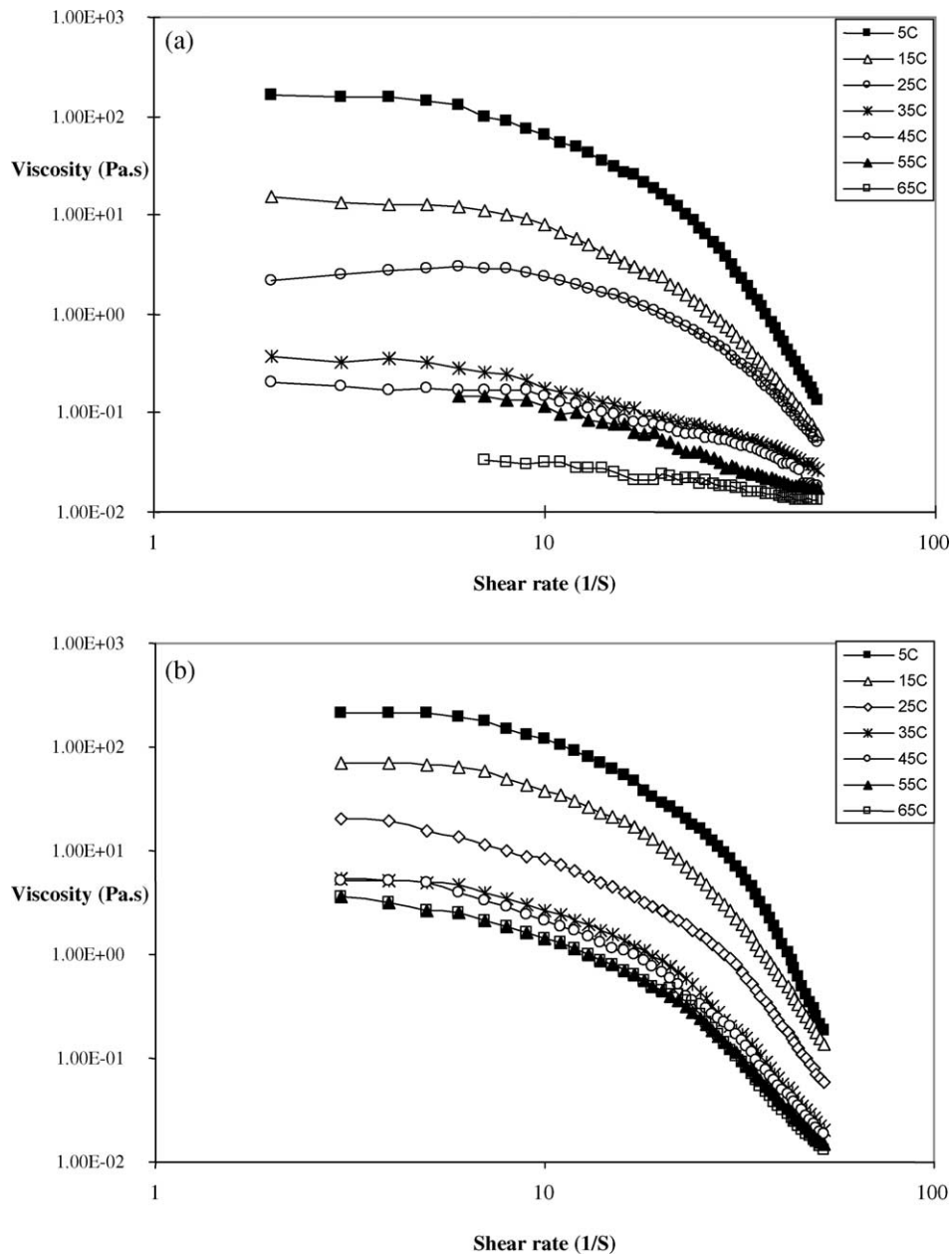
Fig. 4. Steady shear flow curves of Krueo Ma Noy pectins (0.5%, w/v) at different temperatures: (a) crude extract; (b) dialyzed.

pectin. The increase in temperature appeared to have the similar effect as of decreased concentration that both viscosity and shear thinning flow behavior decreased with increase in temperature (Fig. 4a and b). It was observed again that the dialyzed Krueo Ma Noy pectin exhibited much stronger viscosity and shear thinning flow behavior compared to the crude extract at same conditions; this observation is in agreement with the results observed for concentration effect.

3.3.2. Viscoelastic behavior

3.3.2.1. Effect of concentration. The viscoelastic properties of Krueo Ma Noy pectin were examined by oscillatory

experimental measurements. At concentration 0.1%, the mechanical response was predominantly liquid-like with loss modulus (G'') higher than storage modulus (G') over most of the accessible frequency range (data not shown). When polymer concentration was increased to 0.5%, the mechanical spectrum at 25 °C was typical of a semi-dilute to concentrated solution where the loss modulus was higher than the storage modulus at low frequencies and the reverse was observed at higher frequencies (Fig. 5a and b). It is worth noting that at same polymer concentration, the moduli of dialyzed Krueo Ma Noy pectin was much higher than that of the crude extract, although the nature of the mechanical spectrum remained the same. However, the mechanical spectra of both *dialyzed and crude* extracts samples changed
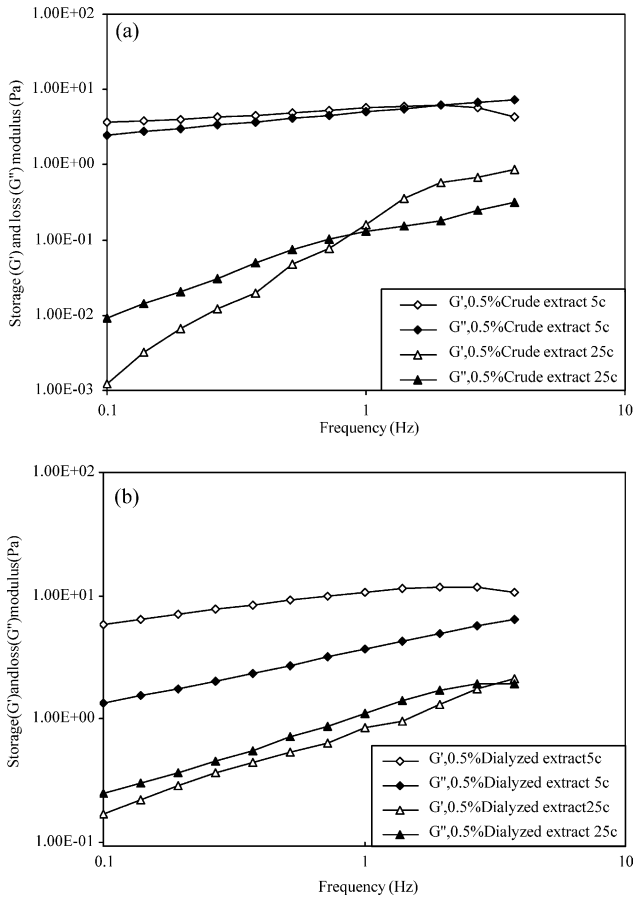
Fig. 5. Frequency dependence of storage (G') and loss (G'') modulus of Krueo Ma Noy pectin (0.5%, w/v) at 5 and 25 °C, respectively: (a) crude extract; (b) dialyzed.

significantly when they were measured at 5 °C: both mechanical spectra behaved like a gel, but with a significant difference in gel strength. In order to evaluate the gelling properties of Krueo Ma Noy pectin, both the crude and dialyzed Krueo Ma Noy pectins were monitored on the rheometer at constant temperature 5 °C over a period of time (Fig. 6). During the aging process, G' continued to increase with increasing time, and then started to flatten at $\sim$50 min for 1% crude extract and $\sim$40 min for 1% dialyzed extract. For 0.5% Krueo Ma Noy pectin, in both the crude and dialyzed samples, G' increased rapidly at the first 20–40 min, then increased slowly until 60–90 min when an apparent plateau was reached. It is apparent that the gel strength is highly concentration dependent. The mechanical properties of Krueo Ma Noy gels were examined at two temperatures after the gels formed at 4 °C for at least 12 h (Fig. 7). At both 5 and 25 °C, crude extract exhibited a strong gel structure as its storage modulus G' was much greater than the corresponding loss modulus G'' and the two moduli were independent of frequency. The dialyzed Krueo Ma Noy pectin exhibited similar mechanical properties, but the gel strength was much stronger at 2% (w/v). The stronger gel strength observed for the dialyzed pectin is consistent with the previous observation by steady shear measurements in which the apparent higher viscosity was attributed to the higher effective concentration of the dialyzed Krueo Ma Noy pectin.

3.3.2.2. Effect of pH. Decreasing pHs of Krueo Ma Noy pectin solutions caused increases in gel strength (G'; Fig. 8a). In the acidic region (pH 2–4), storage modulus showed the tendency to be a constant value. The mechanical spectra at pH 2–4 are typical of gels with storage modulus
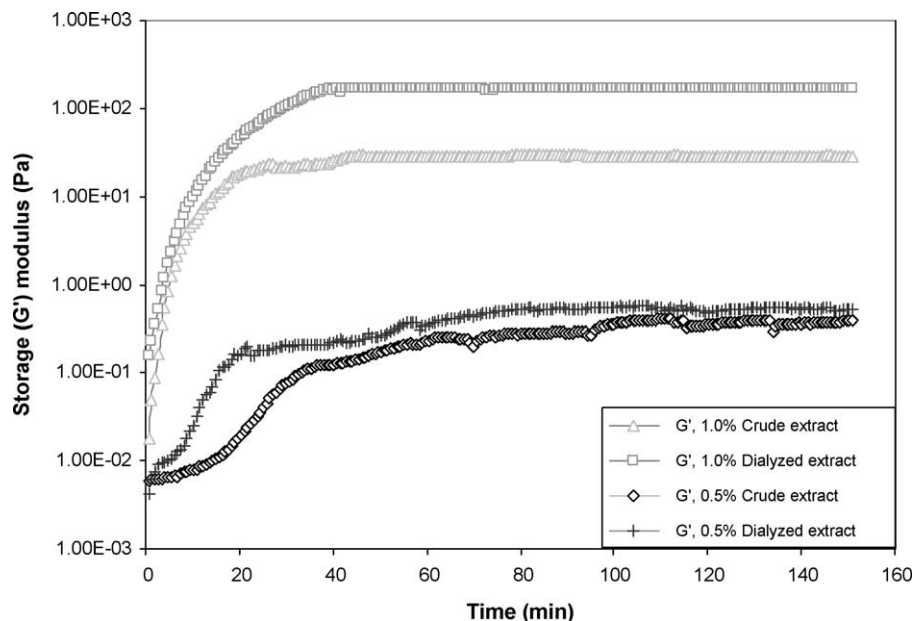

Fig. 6. Evolution of storage modulus (G') with time of crude and dialyzed Krueo Ma Noy pectin at 5 °C.
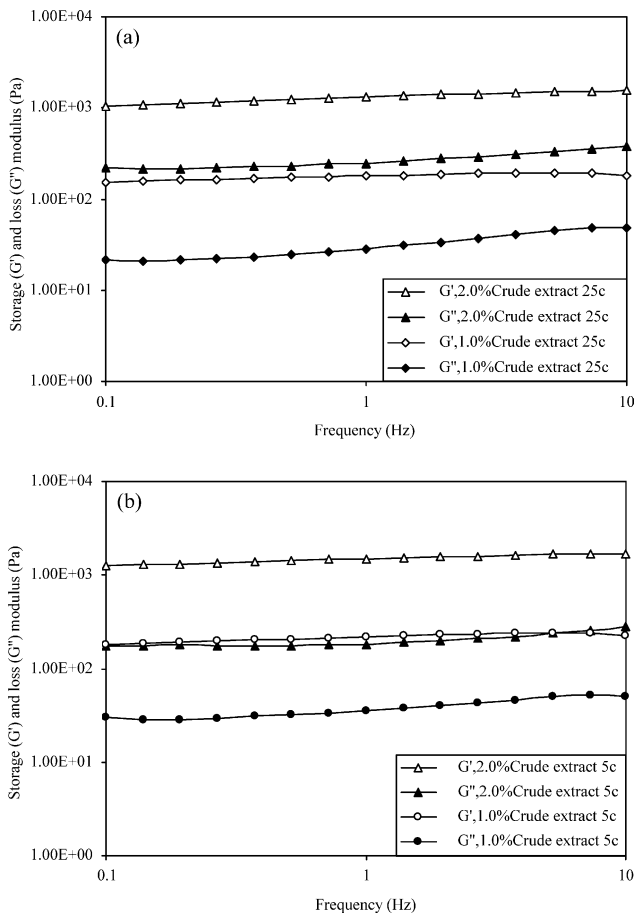
Fig. 7. Comparison of mechanical spectra of dialyzed Krueo Ma Noy pectin at 1.0 and 2.0% (w/v), respectively: (a) 25 °C; (b) 5 °C.

higher than loss modulus and the two moduli had little frequency dependence. However, when pH was increased to 5–8, the mechanical responses of Krueo Ma Noy pectin at 0.5% (w/v) were predominantly liquid-like, in which $G'' > G'$ was observed over most of the accessible frequency range (0.1–10 Hz). This result seems to be in agreement with earlier study on acid induced gelation of low methoxy pectins (Gilsenan, Richardson, & Morris, 2000). It is believed that the reduction of pH would reduce the charge density of the pectin chain, thereby reducing electrostatic repulsion and promoting inter-chain interaction.

3.3.2.3. Effect of co-solutes.
The effect of ionic strength and ion types on viscoelastic behavior is important not only to determine whether the sample behaves as polyelectrolyte but also to evaluate the functional rheological properties (Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000). Krueo Ma Noy pectin solutions formed gels at NaCl concentration between 0.2 and 0.4 M (Fig. 8b). The addition of small amount of Ca^{2+} significantly promoted the gelation of Krueo Ma Noy pectin (Fig. 8c). Additional increase of Ca^{2+} concentration up to 5 mM did not change the gel strength for crude extract. However, the increase of
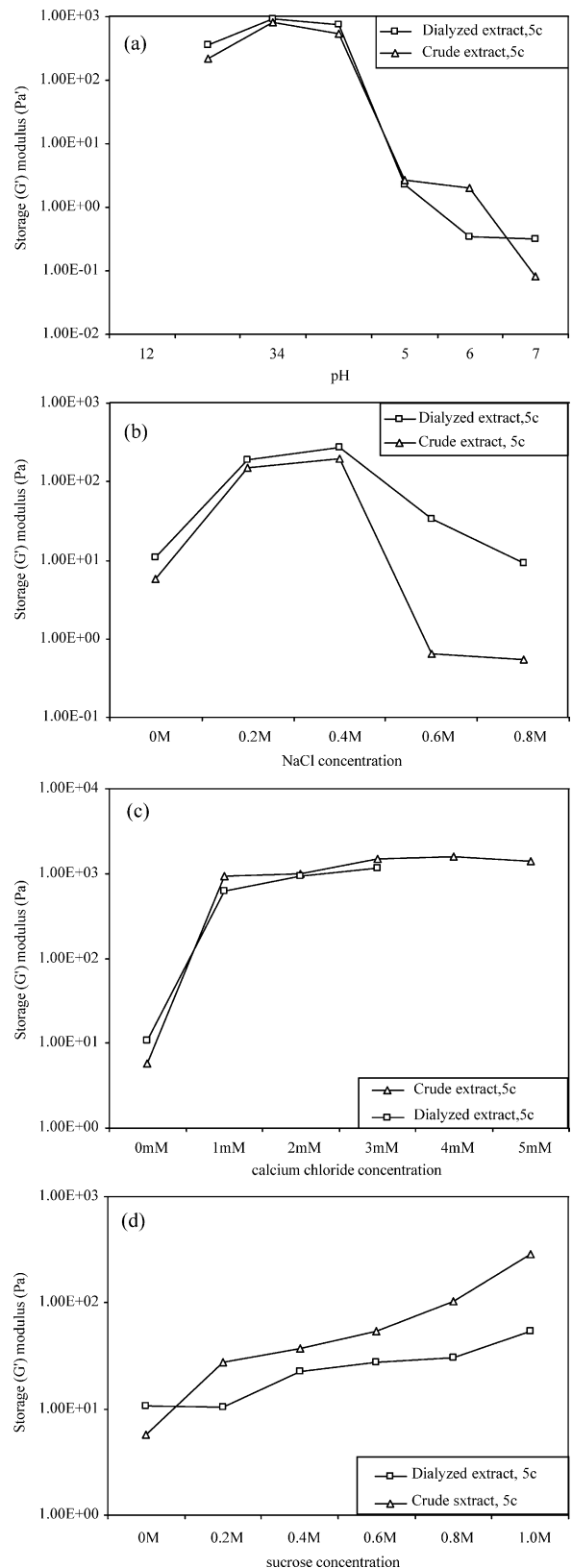

Fig. 8. Effect of pH and solutes on storage modulus (G') of 0.5% (w/v) Krueo Ma Noy pectin (crude and dialyzed, respectively) at 5 °C and 0.1 Hz. (a) Effect of pH. (b) Effect of sodium chloride. (c) Effect of calcium chloride. (d) Effect of sucrose.

Ca^{2+} concentration greater than 3 mM in dialyzed Krueo Ma Noy pectin resulted in precipitation. The precipitation could be caused by strong bindings of the Ca^{2+} with the pectin molecules, which favors aggregations and essentially the collapse of the three-dimensional network. The strong ability of dialyzed Krueo Ma Noy pectin with calcium ion is also evident in the composition analysis (Table 2) in which the Ca^{2+} content was about two times of that in the crude extract. The gel strength of the Krueo Ma Noy gels was further enhanced by sucrose; there is an apparent correlation between the concentration of sucrose and G' within the sugar concentrations examined: the higher the sucrose concentration, the stronger the gel (Fig. 8d).

4. Conclusion

Krueo Ma Noy pectin described in this paper has a great potential as a commercial hydrocolloid for the food industry. The material is extracted from a unique plant source, i.e. plant leaves, and the extraction process is simple and easy to be scaled up to industrial scale. The present paper reported the basic chemical and physical properties of this new material and found to exhibit unique gelling properties and broad applications. This report just started the exploration of the material, therefore, extensive research is needed in order to fully understand the new material and apply it in food and non-food systems. Some additional works on structural characterization and gelation of Krueo Ma Noy pectin have been published elsewhere (Cui, Singthong, Ningsanond, & Goff, 2004; Singthong et al., 2004). Further research is undergoing in order to fully explore the full potential of this new hydrocolloid.

Acknowledgements

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