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Malva nut gum. (Part I): Extraction and physicochemical characterization

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Abstract

Malva nut is the seed of *Scaphium scaphigerum* and has long been used as a traditional medicine in South East Asia. This paper reports on the extraction and characterization of gums from malva nut seed. Sequential extraction with water, 0.05 M HCl, and 0.05 M NaOH solutions resulted in 1, 6 and 20% gum yield, respectively, based on the dry weight of the seeds. The alkaline extracted gum fraction was further characterized and revealed 62.0% carbohydrates, 8.3% ash and 8.4% protein. The major constituent monosaccharides of the gum were 31.9% arabinose, 29.2% galactose and 29.5% rhamnose, as measured by acid hydrolysis and ion exchange chromatography. The gum also contained 6.4% uronic acid and small amounts of glucose, xylose and mannose. Methylation analysis revealed that alkaline extracted malva nut gum was primarily composed of terminal L-Ara*f*, 1,3-linked L-Ara*f*, 1,4-linked D-Gal*p*, 1,4-linked D-GalA*p* with small amounts of branching units: 1,2,4-linked D-Gal*p* and 1,2,3,4-linked Rham*p*. The gum showed a single peak in the size exclusion chromatography from which the average molecular weight and intrinsic viscosity were determined to be 6.65×10^6 Da and 10.0 dl/g, respectively. These values are much higher than the many commercial hydrocolloid gums. Further purification of the gums by dialysis, against deionised water, and decoloring by hydrogen peroxide treatment reduced the molecular weight and intrinsic viscosity of the polysaccharides.
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Keywords: Extraction; Malva nut; *Scaphium scaphigerum* (G.Don); Polysaccharide gums; Molecular weight, Intrinsic viscosity

1. Introduction

Malva nut fruit [*Scaphium scaphigerum* (G. Don) Guib and Planch] is known in Thailand as Pungtalay or Sumrong. The plant belongs to the Sterculiaceae family, which includes other species such as *Scaphium macropodum* Beumee and *Sterculia lychnophora* Hance. *Scaphium scaphigerum* is growing in Vietnam, China, Malaysia, Indonesia as well as the east part of Thailand, especially in the drier regions (Yamada et al., 2000). Malva nuts are harvested from a native tall tree (20–40 m tall) and the dry fruit is about 25×15 mm, ellipsoid in shape and glabrous (Fig. 1). The seeds are known to contain a large amount of mucilaginous substance and have been used as a traditional medicine in South-East Asia. The jelly made from malva nuts is consumed, when sweetened, as dessert, but its principal use is for relief of canker sores and cough. However, malva nut mucilage is not commonly used as a stabilizer or thickening agent in food products due to lack of information on the physicochemical properties of the mucilage and the extraction process. Chen, Cao, and Song (1996) isolated and purified the extract of mucilage from malva nut (*Sterculia lychnophora* Hance) and reported that the molar ratio of galactose, arabinose and rhamnose was 1.00:1.67:1.01 with molecular weight of 162,200 Da. They reported that rhamnose, in the main chain, is linked by α-(1→3) glycosidic linkage. Its major fragment is composed of galactose and rhamnose with a molar ratio of 1.00:2.78 and molecular weight of 62,500 Da. The extraction method used by Chen produced low molecular weight gum extract, which is not suitable for the gum to be used as a thickening agent.

Thailand imports a substantial amount of hydrocolloid gums, mainly pectin and seed gums (locust bean and guar gums), with an annual value of $6,654,429 and $3,730,135, respectively, in 2004 (Thai Customs Department, 2004). Thus, there could be a substantial domestic market for Thai-produced mucilages, provided they have appropriate functional properties and low enough cost to substitute for some of the imported materials. In addition, many studies have shown that water-

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Fig. 1. Dry seeds of Malva nut fruit.

Fig. 2. Schematic flow diagram for the extraction of gums from malva nut seeds.

soluble polysaccharide gums may have beneficial effects on human health, including reducing serum cholesterol levels. Foods that include soluble fiber from oats have received an official approval by the US Food and Drug Administration and permit a claim to be made about their potential use in reducing the risk of heart disease (US Food & Drug Administration, 1994). Likewise, foods fortified with malva nut gum may be well accepted by the consumer since there is well-established knowledge of medicinal uses of malva nuts. The objective of the present study was to develop an extraction procedure that optimizes yield and functional properties of the polysaccharide gums. This paper reports on the results of the extraction procedure, and chemical and structural characterization of the major fraction of malva nut gum. A subsequent paper covers the results on the solution properties and gelation characteristics of the gum.

2. Materials and methods

2.1. Materials

Mature malva nut fruits were collected between March and April in Eastern Thailand, transported to the laboratory, dried and stored at room temperature.

2.2. Extraction of malva nut gum

Endogenous enzymes were inactivated by boiling the seeds in 80% ethanol for 1 h. The gum extraction followed a procedure described by Rombouts and Thibault (1986) with some modifications (Fig. 2). Briefly, the fruits were heated in water at 90 °C for 2 h to extract the water-soluble fraction. Insoluble solids were separated by filtration through a silk-screen cloth, while the extract (Fraction 1) was centrifuged to collect the supernatant. The remaining solids, after water extraction, were extracted with 0.05 M HCl at 85 °C for 30 min, filtered through a silk-screen cloth. The extract was centrifuged and the supernatant provided the acid soluble fraction (Fraction 2). The remaining solids were further
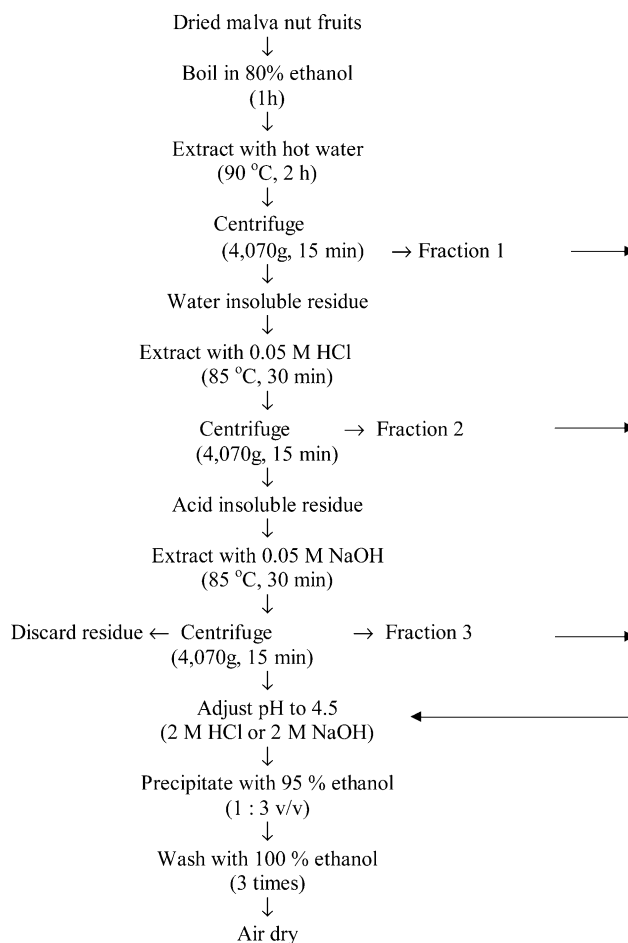
extracted by 0.05 M NaOH at 85 °C for 30 min, and filtered through a silk-screen cloth. The extract was centrifuged and supernatant collected to yield the alkaline soluble fraction (Fraction 3). The pHs of the acid soluble and alkaline soluble extracts were adjusted to 4.5 with 2 M NaOH or HCl. Finally, all three extracts were precipitated by adding three volumes of 95% ethanol and washed with 100% ethanol three times, followed by air-drying.

2.3. Chemical composition analysis

Ash and moisture contents were determined according to the AOAC methods (1996). Protein content was determined from the nitrogen content ($N \times 6.25$) using an Automatic Elemental Analyzer (ThermoQuest Italia S.P.A. EA/NA 1110, Strada Rivoltana, Milan, Italy). Total was determined according to the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956) and was measured as glucose equivalents. Uronic acid was determined according to Blumenkrantz and Asboe-Hansen (1973). Monosaccharide content was determined using a Dionex HPLC system (Dionex Canada Ltd, Oakville, Ont., Canada) equipped with pulsed amperometric detection as previously described by Wood, Weisz, and Blackwell (1994). In this method, the polysaccharide (2 mg/

ml) was hydrolyzed in 1 M H_2SO_4 for 2 h at 100 °C to give constituent monosaccharides. The hydrolysate was cooled, diluted 1:10 with water, filtered through a 0.45 μm filter and injected into the HPLC column.

2.4. Purification

2.4.1. Dialysis

The alkaline soluble fraction was further purified by dialysis against deionized water for 48 h at 22 °C, using a 3500 Da molecular weight cut off membrane (Spectra/Por® RC membrane, Spectrum Laboratories Inc., Rancho Dominguez, CA).

2.4.2. Decoloring

The decoloring of the gum followed a procedure described by Abdel-Aal, Sosulski, and Sokhansanj (1996) with a slight modification. Malva nut gum (2 g) was dissolved in water (800 ml) at 60 °C for 1 h, cooled to the room temperature (22 °C) and the pH was adjusted to 9 with 2 M NaOH. Then the gum solution was treated with 30% H_2O_2 (1: 62.5 v/v) for 3 h and neutralized to pH 7 with 2 M HCl. This was followed by dialysis against deionized water for 48 h at 22 °C, using a 3500 Da molecular weight cut off membrane (Spectra/Por® RC membrane, Spectrum Laboratories Inc., Rancho Dominguez, CA). The solution was concentrated by vacuum evaporation (Buchi Vacuum Controller V-805, Brinkmann Instruments (Canada) Ltd., Ont., Canada) at 40 °C (50 mbar, 35 rpm) until the volume of the solution decreased to 200 ml. The pH of the concentrated solution was adjusted to 4.5 with 2 M HCl and precipitated with 100% ethanol (1:3 v/v). The precipitate was separated by centrifugation ($9020 \times g$, 15 min), washed three times with 100% ethanol and air dried.

2.5. Methylation and GC–MS of partially methylated alditol acetates (PMAA)

2.5.1. Reduction of uronic acids

The reduction of uronic acid was performed following the procedure described by Taylor and Conrad (1972); York, Darvill, McNeil, Stevenson, and Albersheim (1986) with a few modifications. Malva nut gum (5 mg) was dissolved in deuterium oxide (2 ml). To the solution, 50 mg of 1-cyclohexyl-3-(2-morpholinoethyl)-carboiimidemethyl-p-toluenesulfonate (CMC, Sigma) was added while using 0.1 M HCl in D_2O to keep the pH at 4.75. The solution was left for 1 h under stirring, followed by adding 5 ml of sodium borodeuteride (160 mg/ml) drop wise. The pH was adjusted to 7.0 using 2 M HCl in D_2O and left to react for 0.5 h, then brought back to 4.0. The solution was dialysed against distilled water overnight at 22 °C (3.5 kDa molecular weight cut off), and lyophilized. The polysaccharide was re-dissolved in 1 ml distilled water, to which 1 ml of 10% acetic acid in methanol was added. The mixture was then dried under a stream of nitrogen to remove boric acid. Another 1 ml of 10% acetic acid in methanol was added to the residue and evaporated under nitrogen. This process was repeated 3–4 times. Finally, a few drops of methanol were added and the solution evaporated to remove any boric acid residue.

2.5.2. Methylation analysis

Methylation analysis of malva nut gums with and without uronic acid reduction was conducted according to the method of Ciucanu and Kerek (1984) with a few modifications. The samples (2–3 mg) were dried at 80 °C for 5 h and stored over P_2O_5 in a glass desiccator under vacuum. Dimethyl sulfoxide (DMSO) was added to the dried gum and the mixture sonicated for 3 h followed by stirring at 85 °C for 2 h. This was followed by another sonication for 3 h to improve sample solubility. Dry sodium hydroxide powder (20 mg) was added and the mixture was stirred at room temperature for 3 h. After adding methyl iodide (0.3 ml), the mixture was mixed for an additional 2.5 h. The partially methylated polysaccharides were extracted with methylene chloride, passed through a sodium sulphate column and dried under a stream of nitrogen. The dried, partially methylated, polysaccharides were hydrolysed in 4 M trifluoroacetic acid (0.5 ml) at 100 °C for 6 h and dried under a stream of nitrogen. The acid hydrolysate was reduced with deuterated sodium borohydride before it was acetylated with acetic anhydride (0.5 ml). Aliquots of partially methylated alditol acetates (PMAA) solution (0.5–1 μl) were injected onto a GC–MS system (ThermoQuest Finnigan, San Diego, CA) fitted with a SP-2330 column (Supelco, Bellefonte, PA) (30 m × 0.25 mm, 0.2 μm film thickness, 160–210 °C at 2 °C/min, then 210–240 °C at 5 °C/min) and equipped with an ion trap MS detector.

2.6. Molecular weight determination

The molecular weight, intrinsic viscosity and polydispersity of malva nut gums were determined using high performance size exclusion chromatography (HPSEC) according to a method described previously (Wang, Wood, Huang, & Cui, 2003). Two columns connected in series (Shodex Ohpak KB-806 M, Showa Denko K.K. Tokyo, Japan; Ultrahydrogel linear, Waters, Milford, MI) were kept at 40 °C during measurements. A Shimadzu SCL-10Avp pump unit (Shimadzu Scientific Instruments Inc., Columbia, MA) was used with the Viscotek Triple detectors (Viscotek Co., Houston, TX) consisting of a refractive index detector (Model 200), a viscometer (Model 250) and a right angle light scattering detector (Model 600). The mobile phase was 100 mM $NaNO_3$ with a flow rate of 0.6 ml/min. The injection volume of sample was 100 μl. Pullulan of known molecular weight and intrinsic viscosity was used as a standard and dn/dc of 0.146 ml/g was used for malva nut gum solution. The gum was dissolved in water (60 °C, 3 h), cooled and filtered through a 0.45 μm filter prior to injection onto the column. The weight average molecular weight, intrinsic viscosity and polydispersity index were calculated using the software TriSEC provided by Viscotek.

2.7. FT-IR spectroscopy

Malva nut gums, commercial pectin standards with known degree of esterification (DE) (26 and 59% DE) and gum arabic

Table 1

Normalized monosaccharide compositions of the alkaline extracted malva nut gum

Monosaccharide compositions	(%, w/w)
Arabinose	31.9±0.2
Galactose	29.2±0.2
Rhamnose	29.4±0.1
Glucose	2.7±0.2
Xylose	2.1±0.1
Mannose	4.8±0.3

from Sigma-Aldrich Co. (Steinheim, Germany) were dried in a vacuum oven at 80 °C for 4 h, and desiccated overnight in a vacuum jar with P_2O_5 prior to FT-IR analysis. FT-IR spectra of the gums 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 (Singthong, Ningsanond, Cui, & Goff, 2004). Triplicate spectra were recorded for each sample. The wave numbers corresponding to chemical species of interest included the ester region (1740–1720 cm^{-1}), carboxylate ion stretches (1600–1414 cm^{-1}) and amide peaks (1650 and 1550 cm^{-1}).

3. Results and discussion

3.1. Extraction of gum

The current sequential extraction procedure indicated that malva nut gum could not be solubilized effectively by water, nor by a dilute acid solution. The yields obtained by hot water extraction and dilute acid extraction were only ∼1.0±0.1% and ∼6.0±0.1% on a dry matter basis, respectively. Extraction with 0.05 M NaOH provided the highest yield (20±0.2% of dry matter). Clearly, most of the polysaccharides are either covalently linked to other components or physically trapped in the cell wall matrix, which can only be effectively released under alkaline condition. All the extracts have a dark brown color.

3.2. Chemical composition of alkaline extracted malva nut gum

The alkaline extracted malva nut gum was selected for further characterization. The monosaccharide compositions of the gum are summarized in Table 1, which mainly consisted of

galactose, arabinose, and rhamnose at a ratio of Gal:Ara:Rha= 1.0:1.1:1.0. Chemical analysis revealed that the gum contained 62.0% total carbohydrates plus 8.3% proteins, 8.4% ash, and 7.8% moisture. The rest of the components are still under investigation. This data are different from the data for aqueous extract of Sterculia lychnophora Hance seeds reported by Chen et al. (1996). The major component of the latter have a monosaccharide ratio of Gal:Ara:Rha=1.01:1.67:1.00, i.e. a significantly higher proportion of arabinose. There are a number of other plant polysaccharide gums bearing a similar monosaccharide composition profile as malva nut gum. BeMiller (1973) reported that flaxseed mucilage was composed primarily of polysaccharides which, on acid-catalyzed hydrolysis, yielded L-galactose, L-arabinose, L-rhamnose, D-galacturonic acid and traces of D-glucose. The major monosaccharides in gum arabic (Acacia senegal) were rhamnose (13%), arabinose (27%), galactose (44%) and glucuronic acid (15%) (Williams & Phillips, 2000). The level of uronic acid in malva nut gum reflects the relative amount of acidic portion in the gum. The uronic acid content of malva nut gum (6.4%) was much lower than that in flaxseed gum (21%) (Cui & Mazza, 1996), gum arabic (15%) (Anderson & Morrison, 1990) and water soluble yellow mustard gum (10.3%) (Cui, Eskin, & Biliaderis, 1993). The lower levels of detected monosaccharide composition, compared to the total carbohydrate content, was likely caused by the incomplete hydrolysis of the acid fraction of the polysaccharides. In summary, the results from the present study indicated that arabinose, rhamnose and galactose are the major monosaccharides present in malva nut gum.

3.3. Methylation analysis

Methylation analysis of the dialyzed and decolored gums from malva nut seeds revealed that they were primarily composed of terminal L-Araf, 1,3-linked L-Araf, 1,4-linked D-Galp, 1,4-linked D-GalAp with small amounts of branching units: 1,2,4-linked D-Galp and 1,2,3,4-linked Rhamp. The molar ratios are 1.97:1.00:1.56:0.25:0.36 for terminal L-Araf: 1,3-linked L-Araf: (sum of 1,4-linked D-Galp and 1,4-linked D-GalAp): 1,2,4-linked D-Galp: 1,2,3,4-linked L-Rhamp. (Table 2). It should be noted that the amount of 1,4-D-Galp was much lower than the galactose detected by the monosaccharide analysis before uronic acid reduction, and increased significantly after the reduction (0.21 vs 1.56). Since,

Table 2

Chemical names and deduced linkages of partially methylated alditol acetates (PMAA) of the alkaline extracted malva nut gum with and without uronic acid reduction

Chemical name	Deduced linkage	Molar ratio[a] (n)	Molar ratio[a] (y)
4-O-acetyl-(1-deuterio)-1,2,3,5-tetra-O-methyl-arabinotol	Terminal Araf	1.24	1.97
1,3,4-tri-O-acetyl-(1-deuterio)-2,5-di-O-methyl-arabinotol	1,3-L-Araf	1.00	1.00
1,4,5-tri-O-acetyl-(1-deuterio)-2,3,6-tri-O-methyl galactol	1,4-D-Galp	0.21	1.56
1,2,4,5-tetra-O-acetyl-(1-deuterio)-3,6-tri-O-methyl galactol	1,2,4-D-Galp	Nd	0.25
1,2,3,4,5-penta-O-acetyl-(1-deuterio)-rhamnitol	1,2,3,4-D-Rhamp	Nd	0.36

n, without uronic acid reduction; y, with uronic acid reduction; Nd, not detectable.
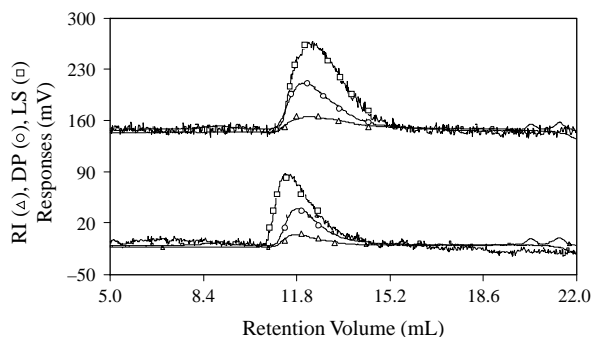[a] Relative molar ratio calculated from the ratio of peak area.

Fig. 3. High performance size exclusion chromatograms of the alkaline extracted malva nut gum before dialysis (bottom) and after dialysis (top).

the total uronic acid content was only about 6.4%, it is unlikely that all in the increase in 1,4-D-Gal$p$ was caused by the reduction of galacturonic acid. Meanwhile, the proportion of rhamnose also increased after uronic acid reduction. This indicates that both the Rham$p$ and Gal$p$ were not completely converted during the methylation process, possibly due to their presence near the galacturonic acid which prohibited the complete methylation and hydrolysis. After the reduction of the galacturonic acid to galactose, these sugar residues are readily methylated and hydrolyzed, giving higher proportions of galactose and arabinose. A branched pectin-like polysaccharide structure is proposed based on methylation analysis, which contains 1,4-linked D-Gal$p$, 1,4-linked D-GalAp and some 1,2-linked L-Rham$p$ in the backbone chain branched at the 2 position of the 1,4-linked D-Gal$p$ residues and the 3 and 4 positions of 1,2-linked L-Rham$p$. Most of the Ara$f$ were terminal sugar residues while substantial amounts of the Ara$f$ residues were 1,3-linked, most likely in the branching chains.

3.4. Molecular weight and intrinsic viscosity

Molecular weights of malva nut gum, both before and after dialysis against de-ionized water, were determined by size exclusion chromatography. The HPSEC chromatograms for the samples before and after dialysis are shown in Fig. 3. HPSEC coupled with three detectors permitted the determination of average molecular weight (M_w), intrinsic viscosity ($[\eta]$) and polydispersity index (P_d). The results are summarized in Table 3. The weight average molecular weight of the gum was 6.65×10^6 Da before dialysis. After dialysis, it was reduced to 3.30×10^6 Da. Chemical analysis indicated that the dialysis reduced the ash content of the gum from 8.40 to

Table 3
Molecular weights, intrinsic viscosities and polydispersity indices of the alkaline extracted malva nut gum solutions before and after dialysis and decoloring

Alkaline extracted malva nut gum	Mw (Da)	Intrinsic viscosity (dl/g)	Polydispersity index
Original gum	6.65×10^6	10.0	1.1
After dialysis	3.30×10^6	7.7	1.3
After decoloring	2.27×10^6	5.4	1.3

5.34% (dry basis). It is likely that the reduction in Mw is associated with the disruption of some structural associations among gum molecules. It is well known that divalent ions, such as Ca^{2+}, can interact with uronic acid moieties from two or more polysaccharide molecules to form supramolecular aggregates, or in the extreme case form gel networks. Removal of such ions by dialysis shifted the equilibrium towards the dissociation of these molecular aggregates, thus the measured molecular weight decreased. Cui, Kenaschuk, and Mazza (1994) also reported that dialyzed flaxseed gum exhibited lower apparent viscosity and much weaker viscoelastic responses compared to a crude flaxseed gum. Nevertheless, the Mw of malva nut gum obtained in the present study was much higher than the Mw of water extracted polysaccharide gum obtained from *Sterculia lychnophora* Hance (162,200 Da; Chen et al., 1996). This may be due to the differences in specie of malva nut, as well as in the extraction approaches. The intrinsic viscosity of the malva nut after dialysis was also lower than that before dialysis. This is consistent with the molecular weight results. The polydispersity index obtained indicated that the molecular weight distribution of malva nut gum after dialysis (1.3) was slightly broader than that before dialysis (1.1). It is worth noting that the molecular weight and intrinsic viscosity of malva nut gum are much higher than most of the polysaccharide gums that are currently available in the market, including guar gum, locust bean gum and pectin. This makes it a very promising viscosity enhancer.

As mentioned above, malva nut gums have a dark brown color. An attempt was made to decolour the gum by treatment with various concentrations of hydrogen peroxide. Treating the sample with 30% hydrogen peroxide for 3 h produced a white/yellow sample. Any treatment less severe than this condition did not significantly reduce the color. The molecular weight of the gum was reduced from 6.65×10^6 to 2.27×10^6 Da after this treatment. This is probably because hydrogen peroxide is a strong oxidizing agent which may cause structural degradation of polysaccharides, leading to reduction in molecular weight. The alkaline condition in the decoloring process might also have caused the degradation of the polysaccharides. Rombouts and Thibault (1986) reported that the average molecular weight and intrinsic viscosity of alkaline extracted pectin from sugar beet were lower than those of water extracted pectin. They ascribed this to the alkaline degradation of pectin molecules. The intrinsic viscosity of the malva nut gum after decoloring was also lower than that before decoloring. This is in accordance with the molecular weight result.

3.5. FT-IR spectroscopy

FT-IR spectroscopy was employed to further investigate the structure of malva nut gums in comparison to gum arabic and pectin standards with known degree of esterification (DE) (26 and 59% DE). In the FT-IR spectrum of pectic polysaccharides, bands in the 1000–2000 cm^{-1} region are independent of pectin sources and may be used to identify galacturonic acid moieties (Filippov & Shamshurina, 1972; Wellner, Kacurakova,
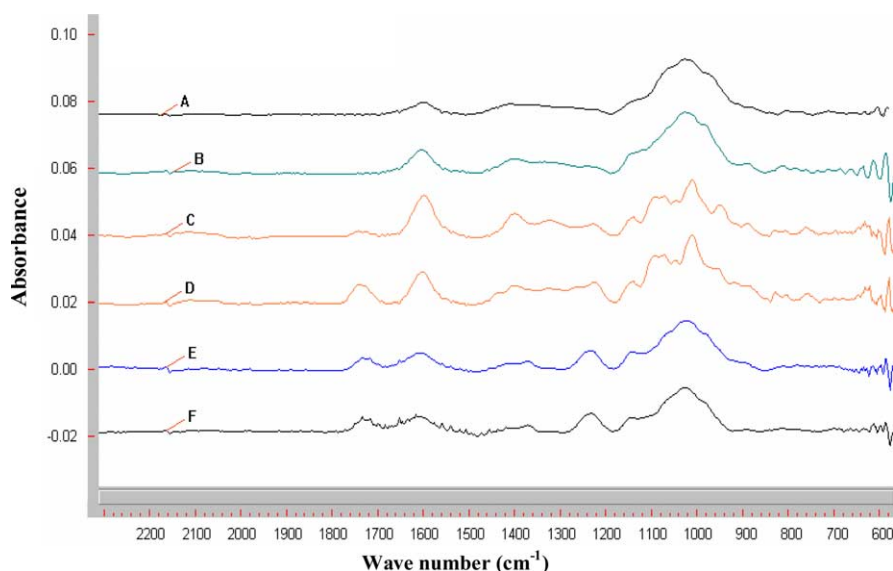
Fig. 4. FT-IR spectra of gum arabic (A), malva nut gum (alkaline extraction) (B), 26% DE citrus pectin (C), 59% DE citrus pectin (D), malva nut gum (acid extraction) (E), and malva nut gum (water extraction) (F).

Malovikova, Wilson, & Belton, 1998). The carbonyl absorption bands shown at 1600 and 1740 cm^{-1} were from free (COO–) and esterified (COO–R) carboxyl groups, respectively. It can be seen from Fig. 4 that the total carbonyl absorption band area increased as the polygalacturonic acid content increased. The spectrum also showed carboxylate ion peaks at 1600 and 1414 cm^{-1} and carbohydrate peaks at 1140, 1100, 1060 and 990 cm^{-1}. The carbohydrate peaks of the alkaline extracted malva nut gum were similar to gum arabic because they had a similar monosaccharides composition. This observation confirmed the results from the monosaccharide composition and methylation analysis. It is worth noting that the gum arabic like portion of malva nut gum was not solubilized by hot water and weak acid during extraction, but was only extractable by alkaline solutions. This appeared to indicate the existence of possible cross-links via covalent bonds, although the extremely high molecular weight may also contribute to the poor solubility of malva nut gum (>6 millions Da compared to 0.6 millions Da for gum arabic). In contrast, the spectra of water and acid extracted malva nut gums exhibited characteristic absorbances of high DE pectins in the carbonyl absorption region. It appeared that the alkaline extraction caused saponification of the methyl esterified carboxyl group as evidenced in Fig. 4A.

4. Conclusions

Malva nut gum can be effectively extracted by a dilute alkaline solution. The extracted gum contains high levels of carbohydrates (62.0%). The major constituent monosaccharides were arabinose, galactose and rhamnose together with small amounts of uronic acid, glucose and xylose. The polysaccharides contained terminal L-Ara*f*, 1,3-linked L-Ara*f*, 1,4-linked D-Gal*p*, 1,4-linked D-GalA*p* with small amounts of branching units: 1,2,4-linked D-Gal*p* and 1,2,3,4-linked

Rham*p*. The molecular weight (6.65×10^6 Da) of malva nut gum was very high, which was significantly reduced by the purification processing (dialysis and decoloring). FT-IR spectroscopy and methylation analysis revealed that malva nut gum had a fairly similar structure to gum arabic, yet much higher molecular weight. The rheological properties of malva nut gum will be reported separately.

References

Abdel-Aal, E. S. M., Sosulski, F. W., & Sokhansanj, S. (1996). Bleaching of wheat distillers' grains and its fibre and protein fractions with alkaline hydrogen peroxide. *Lebensmittel-Wissenschaft und-Technologie*, *29*, 210–216.

Anderson, D. W., & Morrison, N. A. (1990). The identification of Combretum gums which are not permitted food additives, II. *Food Additives and Contaminants*, *7*, 181–188.

AOAC (1996). *Official methods of analysis* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.

BeMiller, J. N. (1973). Quince seed, psyllium seed, flaxseed and okra gums. In R. S. Whistler, & J. N. BeMiller (Eds.), *Industrial gums* (pp. 331–337). New York: Academic Press.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Chen, J., Cao, P., & Song, H. (1996). Purification and properties of polysaccharide PPIII from *Sterculia lychnophora* Hance. *China Journal of Chinese Materia Medica*, *21*, 39–41.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Cui, W., Eskin, N. A. M., & Biliaderis, C. G. (1993). Chemical and physical properties of yellow mustard (*Sinapis alba* L.) mucilage. *Food Chemistry*, *46*, 169–176.

Cui, W., Kenaschuk, E., & Mazza, G. (1994). Flaxseed gum: Genotype, chemical structure and rheological properties. In *Proceeding of the 55th Flax Institute of the United States* (pp. 166–167). Fargo, North Dakota: Flax Institute of the United States.

Cui, W., & Mazza, G. (1996). Physicochemical characteristics of flaxseed gum. *Food Research International*, *29*, 397–402.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Filippov, M. P., & Shamshurina, S. A. (1972). Comparative study of pectin substances by IR spectroscopy. *Food Science and Technology Abstract*, *6*, 7A2601974.

Rombouts, F. M., & Thibault, J. F. (1986). Feruloylated pectic substances from sugar-beet pulp. *Carbohydrate Research*, *154*, 177–187.

Singthong, J., Ningsanond, S., Cui, S. W., & Goff, H. D. (2004). Structural characterization, degree of esterification and some gelling properties of of Krueo Ma Noy (*Cissampelos pareira*) pectin. *Carbohydrate Polymers*, *58*, 391–400.

Taylor, R. L., & Conrad, H. E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry*, *11*, 1383–1388.

Thai Customs Department. (2004). *Import/export statistics from Jan–Oct 2004* [online]. Available from: http://www.customs.go.th/statistic [2004, Dec 15].

US Food and Drug Administration (1994). *A food labeling guide—Appendix C: Health claims*. Rockville, MD: United States Food and Drug Administration.

Wang, Q., Wood, P. J., Huang, X., & Cui, W. (2003). Preparation and characterization of molecular weight standards of low polydispersity from oat and barley $(1 \rightarrow 3)$ $(1 \rightarrow 4)$-β-D-glucan. *Food Hydrocolloids*, *17*, 845–853.

Wellner, N., Kacurakova, M., Malovikova, A., Wilson, R., & Belton, P. S. (1998). FTIR study of pectate and pectinate gels formed by divalent cations. *Carbohydrate Research*, *308*, 123–131.

Williams, P. A., & Phillips, G. O. (2000). Gum arabic. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 155–168). New York: CRC Press.

Wood, P. J., Weisz, J., & Blackwell, B. A. (1994). Structural studies of (1, 3),(1, 4)- β-D-glucans by ^{13}C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chemistry*, *71*, 301.

Yamada, T., Itoh, A., Kanzaki, M., Yamakura, T., Suzuki, E., & Ashton, P. S. (2000). Local and geographical distributions for a tropical tree genus, *Scaphium* (Sterculiaceae) in the Far East. *Plant Ecology*, *148*, 23–28.

York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., & Albersheim, P. (1986). Isolation and characterization of plant cell wall components. *Methods in Enzymology*, *118*, 3–40.