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Note

Isolation and Characterization of Pectin from Peel of *Citrus tankan*

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A pectin was extracted from the peel of *Citrus tankan* with a yield of 2.75%. The uronic acid content was 80.0%, and the degree of methoxylation was 63.2%. The pectin was composed of D-GalA, D-Gal, L-Ara and L-Rha in the molar ratio of 100:11.3:3.6:2.6. The molecular weight was estimated to be approximately 9.2×10^4. The pectin formed a gel by conventional procedures.

Key words: pectin; *Citrus tankan*; peel; high methoxyl; slow set

Pectin is one of the most complex plant polysaccharides. It mainly constructs the primary cell wall and middle lamella in plant tissues and is covalently linked to other polymers. It is utilized in the food industry as a gelling agent and stabilizer of jam, jelly and acid milk product. The main constituent of pectin is D-GalA linked with α-(1→4) glycosidic linkages. Some of the carboxyl groups are methyl esterified, and the hydroxyl groups are partially acetylated. Rha residues are inserted into the main GalA chain. Arabinan, galactan, or arabinogalactan side chains are linked to the Rha residue. Other sugars such as D-Xyl, D-Man, L-Glc, L-Fuc, and D-GlcA are sometimes found in the side chains.[1)]

Citrus tankan (local name, Tankan) is a citrus plant of tangors that is mainly cultivated in Kagoshima and Okinawa, Japan. The fruit is as popular as that of *Citrus depressa* (local name, Shekwasha) and is one of the most significant agricultural products in Okinawa. The annual production of *C. tankan* is much higher than that of *C. depressa*. It is therefore required to be efficiently utilized. We have previously isolated and characterized the pectins from the fruit of *C. depressa*.[2,3)] We report here the isolation and characterization of the pectin extracted from the peel of *C. tankan*.

Fruit of *C. tankan* were collected in February 2002 from Motobu (Okinawa, Japan). The dry peel sample was suspended in 0.05 M HCl at 85 °C for 1 h, and then filtered and adjusted to pH 4.5. Ethanol was added to the solution, and the precipitate was washed with ethanol and dried *in vacuo*. The precipitate was dissolved in distilled water and passed through a column of Amberlite IR-120B. The eluate was adjusted to pH 4.5, dialyzed, and freeze-dried. The freeze-dried material was dissolved in a 0.05 M NaOAc buffer (pH 4.8) and then loaded into a DEAE-Sepharose column (2.6 × 28 cm) that had been equilibrated with the same buffer. The column was washed with the buffer, and the bound material was eluted with a 1 M NaOAc buffer (pH 4.8), dialyzed, and finally freeze-dried. The contents of total carbohydrate and uronic acid were determined by the phenol-sulfuric acid method[4)] and the carbazole-sulfuric acid method,[5)] respectively, using D-GalA as a standard. The moisture content was determined by drying at 110 °C for 2 h, and the ash content was determined by incinerating overnight in a muffle furnace at 550 °C. DM was calculated as the molar ratio from GalA and released MeOH which had been determined by the method of Inari and Takeuchi.[6)] The polysaccharide components were determined by hydrolyzing the polysaccharide with 1.5 M H_2SO_4 at 100 °C for 3 h, and then applying the neutralized hydrolyzate to DX 500 liquid chromatography (Dionex Co.), using a column of CarboPac PA1 equilibrated at a flow rate of 1 ml/min at 35 °C with 15 mM NaOH for neutral sugars and with 100 mM NaOH/150 mM NaOAc for uronic acids. The molecular mass was determined by HPLC in a column of TSK-gel $GMPW_{XL}$ at room temperature at a flow rate of 0.3 ml/min with 0.15 M sodium chloride in a 0.05 M sodium phosphate buffer (pH 7.2), using refractive index detection. Standard pullulans were used as molecular mass markers. Specific rotation was measured at 589 nm, and infrared spectra were recorded by KBr discs. The ^{1}H- and ^{13}C-NMR spectra were recorded at 500.00 MHz and 125.65 MHz in D_2O at 80 °C. Sodium 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid (TSP) was used as an internal standard. To form a gel of the high-methoxyl type, sucrose (60%) was added to the polysac-

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Abbreviations: GalA, galacturonic acid; Rha, rhamnose; GlcA, glucuronic acid; Fuc, fucose; DM, degree of methoxylation

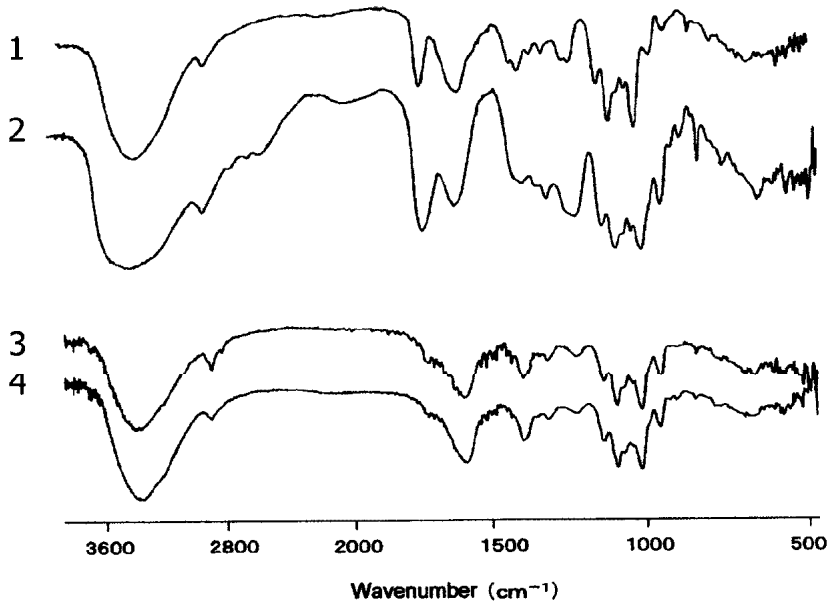

Fig. 1. Infrared Spectra of the Polysaccharide, De-Esterified Polysaccharide Extracted from *C. tankan*, Standard Pectin and De-Esterified Standard Pectin.

1, Polysaccharide from *C. tankan*; 2, Standard pectin; 3, De-esterified polysaccharide from *C. tankan*; 4, De-esterified standard pectin.

charide solution (1%) and heated at 100°C for 30 min while stirring, before a citric acid solution (0.05M) was added while stirring. To form a gel of the low-methoxyl type, a $CaCl_2$ solution (5%) was added to the de-esterified polysaccharide solution (1%) while heating. The polysaccharide was de-esterified in 0.05M NaOH at 4°C for 1 h with mild stirring. After the pH value had been adjusted to 4.5, the mixture was dialyzed and then freeze-dried.

The yield was 2.8% based on the amount of fresh material. The contents of total carbohydrate, uronic acid, ash and moisture were 94.0%, 80.0%, 7.0% and 11.0%, respectively. Liquid chromatography of the hydrolyzate of the polysaccharide identified D-GalA, D-Gal, L-Ara and L-Rha in a 100:11.3:3.6:2.6 molar ratio. This result indicates that the polysaccharide was mostly composed of D-GalA and a small quantity of neutral sugars, indicating that the polysaccharide was a pectin. DM was estimated to be 63.2%, indicating that the polysaccharide was of the high-methoxyl type (slow set).[7] The molecular mass was estimated to be approximately 9.2×10^4 with only one broad peak. The specific rotation of the polysaccharide was +201° at 25°C, indicating that the orientation of the GalA residues was α-linked. De-esterification was carried out to facilitate an analysis by IR and NMR spectroscopy. The IR spectra of the polysaccharide and of the de-esterified polysaccharide were sufficiently consistent with those of the standard pectin and de-esterified standard pectin (lemon) over a wide range of wave number, including the fingerprint region (Fig. 1). The ^{1}H-NMR spectrum (Fig. 2) of the de-esterified polysaccharide was substantially different from the polysaccharide spectrum on account of the ester-linked methyl groups.[8] Five major signals were derived from D-GalA, and were respectively assigned to H-1, 5.08 ppm; H-2, 3.74; H-3, 3.97; H-4, 4.43; and H-5, 4.76. In the ^{13}C-NMR spectrum (Fig. 3) of the de-esterified polysaccharide, six major signals were assigned as D-GalA to C-1, 101.8 ppm; C-2, 70.9; C-3, 71.8; C-4, 81.1; C-5, 73.9; C-6, 177.3. Other signals at 107.4, 77.6, 76.4, 75.0 and 63.6 ppm were assigned to C-1, C-5, C-3, C-2 and C-6 of D-Gal, respectively. The chemical shifts in ^{1}H- and ^{13}C-NMR spectra indicate that the polysaccharide contained α-1,4-linked GalA residues, constituting a homogalacturonan moiety, and β-1,4-linked Gal residues, comprising (arabino)galactan as the side chain.[9] It is known that the gelation of high-methoxyl pectin requires an acidic medium and a high content of soluble solids, and that low-methoxyl pectins form gels in the presence of divalent ions.[10] Adding sucrose and citric acid to the solution resulted in the polysaccharide solution turning into a gel. The de-esterified polysaccharide solution formed a gel in the presence of Ca ions.

In conclusion, the pectin obtained from the peel of *C. tankan* was similar to the pectins of *C. depressa* under the same extraction condition. These pectins from *C. tankan* and *C. depressa* had a high proportion of galactose in the neutral sugars and no xylose residues which almost all citrus fruits, except grapefruit, contain. They were almost the same in DM values, although the molecular mass of *C. tankan* pectin was larger than that of *C. depressa*. It is anticipated that the pectin of *C. tankan* could also be utilized as a gelling agent by the food industry. In addition to their gelation ability, the physiologically active aspects of citrus pectins have recently been studied.[11–13] It is possible that the pectin from *C. tankan* would have similar effects because of its

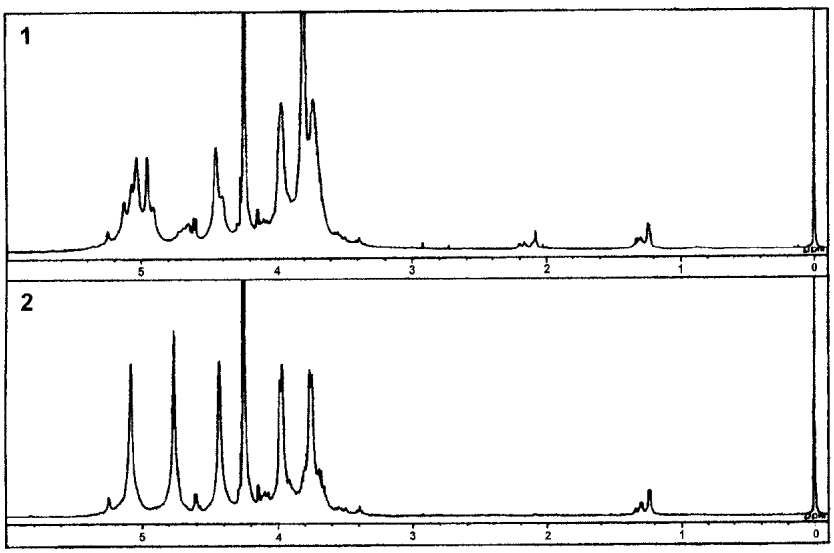

Fig. 2. ^{1}H-NMR Spectra of the Polysaccharide and De-Esterified Polysaccharide from *C. tankan* in D_2O at 80°C.
1, Polysaccharide; 2, De-esterified polysaccharide.

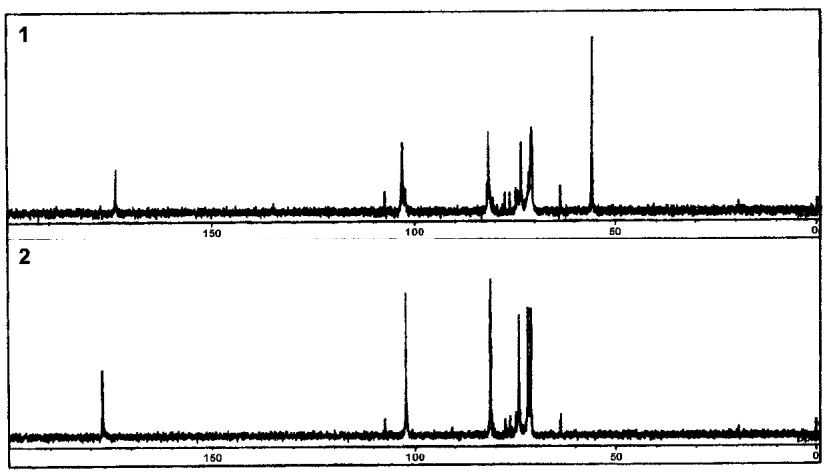

Fig. 3. ^{13}C-NMR Spectra of the Polysaccharide and De-Esterified Polysaccharide from *C. tankan* in D_2O at 80°C.
1, Polysaccharide; 2, De-esterified polysaccharide.

high galacturonic acid content, low neutral sugar content, high DM and relatively high molecular mass. Modified citrus pectin (MCP) is marketed as a supplement for treating cancer and heavy metal toxicity in the United States. Therefore, the pectin from *C. tankan* could be a good source of MCP because the main neutral sugar is Gal. We are continuing to study the effective utilization of the pectins from *C. tankan* and *C. depressa* for food, physiological and pharmaceutical applications.

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