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Controlled methyl-esterification of pectin catalyzed by cation exchange resin

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ABSTRACT

This study developed a new method to methyl-esterify pectin using a cation exchange resin. Homogalacturonan (HG)-type pectin (WGPA-3-HG) and rhamnogalacturonan (RG)-I-type pectin (AHP-RG) obtained from the roots of *Panax ginseng* and sunflower heads, respectively, were used as models. Compared to commonly used methyl-esterification methods that use either methyl iodide or acidified methanol, the developed method can methyl-esterify both HG- and RG-I-type pectins without degrading their structures via β-elimination or acid hydrolysis. In addition, by modifying reaction conditions, including the mass ratio of resin to pectin, reaction time, and temperature, the degree of esterification can be controlled. Moreover, the resin and methanol can be recycled to conserve resources, lower costs, and reduce environmental pollution. This new methodology will be highly useful for industrial esterification of pectin.

1. Introduction

Pectins are complex polysaccharides present in all plant primary cell walls (Ridley, O'Neill, & Mohnen, 2001). The predominant structure of pectin is homogalacturonan (HG), which is mainly composed of α-(1→4)-D-Gal*p*A (De Vries, Den Uijl, Voragen, Rombouts, & Pilnik, 1983; Thibault, Renard, Axelos, Roger, & Crépeau, 1993). The second major structural element of pectin is rhamnogalacturonan I (RG-I), which consists of repeating disaccharide units [→4)-α-D-Gal*p*A-(1→2)-α-**L**-Rha*p*-(1→] in the backbone and neutral side chains composed of arabinan, galactan, or arabinogalactan (AG) (Yapo, 2011). The Gal*p*A residues in pectin can be methyl-esterified at their carboxyl groups, and the percentage of esterified Gal*p*A residues per total Gal*p*A residues is defined as the degree of esterification (DE), one of the most important properties of pectin (Jiang, Liu, Wu, Chang, & Chang, 2005).

Pectin has been widely used in the food industry as a gelling and stabilizing agent (Gamonpilas, Krongsin, Methacanon, & Goh, 2015), and the gelling mechanisms and properties are closely related to its DE (Garnier, Axelos, & Thibault, 1993; Ngouémazong et al., 2012; Ralet, Dronnet, Buchholt, & Thibault, 2001). Industrial demand for pectin with tunable abilities to gel or stabilize fruit and dairy products has increased the need for pectin with controllable DE, which also has a significant impact on the biological activities of pectin. For example, esterified cross-linking in pectin impacts its ability to induce apoptosis in prostate cancer cells (Jackson et al., 2007). The inhibitory potency of de-esterified RG-I-4 on galectin-3-mediated hemagglutination is decreased 50-fold when compared to normal RG-I-4 (Gao et al., 2013). In addition, structural analysis of pectin via β-elimination requires esterification of Gal*p*A residues (Deng, O'Neill, Hahn, & York, 2009; Deng, O'Neill, & York, 2006). Therefore, it is crucial to develop effective methods to control methyl-esterification.

Several methods have been reported to methyl-esterify carboxylic acid groups in pectin. The most commonly used are reactions of tetrabutyl ammonium pectinate with methyl iodide (Matricardi, Dentini, Crescenzi, & Ross-Murphy, 1995; Renard & Jarvis, 1999a, 1999b) and the treatment of pectin with methanol acidified with sulfuric or hydrochloric acid (Rosenbohm, Lundt, Christensen, & Young, 2003; van Alebeek, Zabotina, Beldman, Schols, & Voragen, 2000; Willats et al., 2000). However, these approaches are also responsible for extensive depolymerization of pectin via β-elimination (Renard & Thibault, 1996) or acid hydrolysis of glycosidic linkages (Bertaud, Sundberg, & Holmbom, 2002; Rosenbohm et al., 2003; Willats et al., 2000). Furthermore, separation of the acid catalysts from the reaction mixture is very difficult and produces wastewater and equipment corrosion. Therefore, new methods for methyl-esterification of pectin need to be developed.

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The objective of this study was to develop a new way to methyl-esterify both HG- and RG-I-type pectins using a cation exchange resin as a catalyst. The efficiency of methyl-esterification by this new method was compared to those using methyl iodide and hydrochloric acid-acidified methanol. The effects of different conditions were also assessed on the degree of esterification, including varying the mass ratio of resin to pectin and reaction time, as well as temperature.

2. Materials and methods

2.1. Materials

Strongly acidic cation exchange resin (AG 50W-X8) was purchased from Bio-Rad (Hercules, California, USA). The functional group of the resin is sulfonic acid, and the mesh size is 100–200 with a mean particle size of 106–250 μm. Standard polygalacturonic acid (DE = 0%) and pectin (DE = 92%) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The roots of *P. ginseng* and sunflower heads were cultivated and collected from Changbai Mountain and Baicheng city in Jilin province of China, respectively. All other chemicals were of analytical grade.

2.2. Preparation of pectins

Ginseng pectin WGPA and its fraction WGPA-3-HG were prepared and characterized as described by Zhang et al. (2009). Briefly, *w*ater-soluble *g*inseng *p*olysaccharide (WGP) was extracted from the roots of *P. ginseng* using hot water and precipitated with 80% ethanol. WGP was then applied to a DEAE-cellulose column (8.0 cm × 20 cm, Cl^-) and eluted with distilled water to give the neutral fraction (WGPN), and the column was washed further with 0.5 M NaCl to give the acid fraction (WGPA). WGPA was loaded onto a DEAE-Cellulose column (8.0 cm × 20 cm, Cl^-) and eluted with a stepwise gradient of aqueous NaCl (0, 0.1, 0.2, 0.3 and 0.5 M) to give five fractions: WGPA-N, WGPA-1, WGPA-2, WGPA-3 and WGPA-4. WGPA-3 was applied to a semi-preparative Sepharose CL-6B column (3.0 cm × 90 cm) yielding two fractions: WGPA-3-RG and WGPA-3-HG.

The heads of sunflower (*Helianthus annuus* L.) were extracted with 0.2% oxalic acid (solid:liquid ratio 1:16, w/v) at 100 °C for 1 h and filtered through four sheets of gauze. The solid material was extracted again under the same conditions. The filtrates were combined, centrifuged to remove water-insoluble materials, concentrated to 1500 mL and precipitated with 60% aqueous ethanol. After centrifugation, the supernatant was precipitated with 80% aqueous ethanol. Following further centrifugation and drying by solvent exchange (95% ethanol, acetone, and ether), the polysaccharide fraction AHP-0.2-80% was obtained. AHP-0.2-80% was further fractionated using a preparative Sepharose CL-6B column (3.0 cm × 90 cm) to yield two fractions: AHP-RG and AHP-HG.

2.3. Methyl-esterification of pectin

2.3.1. Methyl-esterification of pectin with methyl iodide

Pectin was methyl-esterified by treating with methyl iodide (MeI) and tetrabutyl ammonium fluoride (TBAF) in DMSO containing 8% water, as described by Deng et al. (2006). Briefly, a suspension of pectin (100 mg) in water (1.6 mL) and DMSO (20 mL) containing TBAF (200 mg) and MeI (100 μL) in a 50-mL round-bottom flask was stirred at room temperature for 18 h. The reaction mixture was poured into ice-cold water (60 mL) and centrifuged to remove iodine. The resulting supernatant was dialyzed (MWCO 3500) against deionized water for 48 h and then lyophilized.

2.3.2. Methyl-esterification of pectin with acidified methanol

Methyl-esterification of pectin by methanol acidified with hydrochloric acid (HCl–MeOH) was performed as previously described (van Alebeek et al., 2000). Each pectin sample (100 mg) was added to anhydrous methanol (20 mL) containing 0.1 M HCl, and the suspension was stirred at room temperature (20 °C) for 3 days. The methyl-esterified pectin was filtered off and washed carefully with 80% aqueous ethanol until no more chloride was present in the washings. Finally, the product was washed with absolute ethanol and dried under reduced atmospheric pressure.

2.3.3. Methyl-esterification of pectin catalyzed by cation exchange resin in methanol

Methyl-esterification of pectin catalyzed by cation exchange resin was carried out as follows. Pectin (100 mg) and anhydrous methanol (200 mL) were placed in a 500-mL round-bottomed flask attached to a reflux condenser. The mixture was heated at reflux temperature (65 °C) in an oil bath and stirred with a magnetic stirring bar for 2 h until the swollen pectin formed a relatively homogeneous suspension. The cation exchange resin was then added as the catalyst (mass ratio of resin to pectin, 0–3.0), and the suspension was stirred at 65 °C or 20 °C for 0 h to 24 h. Upon completion of the reaction, the mixture was filtered to remove methanol, and the resulting residue was dissolved in distilled water, filtered, and washed carefully with distilled water. The solution was freeze-dried to yield methyl-esterified pectin, and the remaining resin was regenerated by activation at 105 °C.

2.4. Determination of degree of esterification (DE)

The DE of pectin was estimated by using FT-IR as previously described (Chatjigakis et al., 1998; Kyomugasho, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015; Singthong, Cui, Ningsanond, & Goff, 2004). To quantify the DE of the products, a calibration curve was constructed based on pectin standards of known DE (20, 40, 50, 60 and 80%) that were prepared by mixing the appropriate quantities of commercial standards. The mixed pectin samples were dissolved in deionized water, and the pH was adjusted to 6.0 with KOH to guarantee total ionization of the carboxylic acid groups. The standard pectins and products were dried and desiccated in a vacuum jar prior to FT-IR analysis. FT-IR spectra were obtained using a Nicolet magna 750 FT-IR spectrophotometer equipped with a DTGS detector covering the frequency range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} with 128 co-added accumulated transients. Specific bands at 1740 and 1630 cm^{-1} corresponded to the absorption of the esterified carbonyl groups and carboxylic ions, respectively. The DE was proportional to the ratio of the area from the band at 1740 cm^{-1} over the sum of the areas from the bands at 1740 and 1630 cm^{-1}. The regression equation used for the calibration curve was $DE = 138.15A_{1740}/(A_{1740} + A_{1630}) - 0.0705$ ($r^2 = 0.995$; where A_{1740} and A_{1630} are the areas from the bands at 1740 and 1630 cm^{-1}, respectively).

2.5. Sugar composition analysis

Sugar composition was analyzed using high-performance liquid chromatography (HPLC) as described previously (Yang, Zhao, Wang, Wang, & Mei, 2005). In brief, each pectin sample (2 mg) was first methanolyzed with anhydrous methanol (1.0 mL) containing 2 M HCl at 80 °C for 16 h, and the products were hydrolyzed with 2 M trifluoroacetic acid (TFA, 0.5 mL) at 120 °C for 1 h. The released monosaccharides were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) and analyzed on a DIKMA Inertsil ODS-3 column (4.6 mm × 150 mm) connected to a Shimadzu HPLC system (LC-10ATvp pump and UV–VIS detector). The derivative (20 μL) was injected, eluted with 82.0% PBS (0.1 M, pH 7.0) and