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Alkylated pectin: Synthesis, characterization, viscosity and emulsifying properties

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Recently there has been an increased interest in modification of pectins. Pectins were alkylated with alkyl chain of various lengths (hexyl, dodecyl or octadecyl) at different degrees of substitution (0.69, 2.43 or 3.60) by reaction of TBA-pectin with alkyl bromide in this study. Their structures were characterized by GC, FTIR and ^{1}H NMR, which confirmed the formation of ester linkages between hydrophobic alkyl segment and carboxyl group of galacturonic acid. Alkylated pectins produced interesting rheology properties. They showed higher apparent viscosities but lower intrinsic viscosities than the original one. The higher apparent viscosities may be due to intermolecular association of hydrophobic segments in the molecule. The lower intrinsic viscosities would be ascribed to more compact conformation. In addition, the modified pectins showed an enhancement of emulsifying properties. These properties were significantly influenced by the degree of substitution and alkyl chain length. Such amphiphilic derivatives open new perspectives for improving or altering viscosity and emulsifying properties of pectin.

1. Introduction

Pectin is an important natural anionic polysaccharide extracted from cell walls of higher plants. It is based on (1 → 4)-linked α-D-galacturonic acid units, interrupted by L-rhamnose residues with side chains of neutral sugars, mainly L-rhamnose, L-arabinose and D-galactose. The galacturonic acid residues may be partially methyl-esterified on the carboxyl group and O-acetylated at C-2 or C-3. This polysaccharide is known as gelling agent, thickener, texturizer, emulsifier and stabilizer to modify properties of food systems (Mohare, Pratapwar, Sakarkar, & Sheikh, 2013). Moreover, characters of good biocompatibility, non-toxicity, and biodegradability as well as high nutritional values (e.g., mineral binding, prebiotic effect, cholesterol regulation, and anti-cancer action) entitle pectin to be an attractive biopolymer material, which can be employed in the pharmaceutical industry, health promotion and cosmetic applications (Srivastava & Malviya, 2011). However, functionality of pectin sometimes needs to be strengthened (Sila et al., 2009) or altered to satisfy the demand of market for innovative and functional products. A number of hydroxyl and carboxyl groups distributed along the backbone as well as a certain amount of neutral sugars presented as side chains make pectin capable of preparing a broad spectrum of derivatives (Chen et al., 2014). In recent years, the interest in studying modification of pectin has increased. For example, Rha et al. (2011) synthesized a pectin hydroxamic acid derivative by hydroxamation. Fan et al. (2012) prepared a quaternary ammonium derivative by reacting pectin with 3-chloro-2-hydroxypropyltrimethylammonium chloride in the presence of sodium hydroxide.

Introduction of alkyl chains into polymers is a commonly used strategy for hydrophobic modification of hydrocolloids. For example, chitosan was alkylated with octanal (Petit, Reynaud, & Desbrieres, 2015), starch was esterified with long chain fatty acid chlorides (C_8, C_{12}, or C_{16}) (Namazi, Fathi, & Dadkhah, 2011), and carboxylic groups of sodium alginate and sodium hyaluronate were esterified by long alkyl chains (C_{12} or C_{18}) (Pelletier, Hubert, Lapicque, Payan, & Dellacherie, 2000). Pectin was also tentatively alkylated with long alkyl chains (C_{12} or C_{18}), and their intramolecular associations (Fischer et al., 1998) together with some physicochemical properties (Miralles-Houzelle, Hubert, & Dellacherie, 2001) were investigated. However, the effects of alkylation ratio and alkyl chain length on functionalities and applications (e.g., viscosity and emulsifying properties) of such pectin derivatives were not fully investigated yet.

In this study, we reported a successful preparation of alkyl derivatives of pectin with various alkyl chain lengths at different

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degrees of substitution by reaction of TBA-pectin with alkyl bromide in dimethylsulfoxide (DMSO). The effects of alkyl chain length and substitution ratios on their viscosities (apparent viscosity and intrinsic viscosity) and emulsifying properties were subsequently evaluated.

2. Materials and methods

2.1. Materials

High-methoxyl citrus pectin was bought from Sigma (P9135, Shanghai, China). Bromohexane, bromododecane, bromooctadecane, hexanol, dodecanol, octadecanol, cyclopentane and tetrabutylammonium hydroxide (TBA^+OH^-) (25 wt.% solution in water) were purchased from Aladdin (Shanghai, China). All other chemicals were analytical reagents and used as received.

2.2. Synthesis of alkyl-pectin derivatives

Synthesis was carried out according to the method of Pelletier et al. (2000). An aqueous solution of pectin was first neutralized by TBA^+OH^-. After freeze-drying, TBA salt of pectin was dissolved in DMSO and alkyl bromide (C_6, C_{12} or C_{18}) was introduced, then the mixture was left to react for 24 h under stirring. DMSO was removed by dialysis against water for 7 days, and then the samples were concentrated to one third of their original volume by a rotary evaporator (N-1001, Eyela, Tokyo, Japan). The resulting products were left in contact with sodium chloride (1 M in 70% aqueous ethanol) to exchange the residual TBA^+ by Na^+ ions. Their filtration was acquired, and filtrates were washed with absolute ethanol until chloride ions were removed and finally washed with acetone. The resulting polymer was left to dry out under ambient temperature and pressure. The extent of substitution was controlled by introducing alkyl bromide at suitable stoichiometry.

2.3. Determination of degree of substitution

Degree of substitution (DS, defined as the mol alkyl chain per 100 mol galacturonic acid unit) was determined by gas chromatography using HP-5 column (30 m × 0.32 mm, 0.25 μm, Agilent Technologies, USA) according to Pelletier et al. (2000). Pectin derivatives were alkaline hydrolyzed in 0.4 M NaOH for 4 h at room temperature. Dodecane used as an internal reference was added and the mixture was vigorously stirred. After centrifugation (Feige, TDL-5-A, Beijing Feige Instrument Co., Beijing, China) at 1300 g for 15 min, the organic phase containing the internal reference and the released hexanol, dodecanol or octadecanol was pipetted off and analyzed by gas chromatography using a calibration curve. The injection temperature was 280 °C, and column temperatures were 200, 230, and 280 °C for C_6, C_{12}, and C_{18}, respectively.

Nomenclature PC_nS^x was used, where P stood for pectin, C_n was the covalently bound long alkyl chain ($n = 6$, 12 or 18), and x was the degree of substitution (S). Pectin treated in the same way as PC_nS^x except adding alkyl bromide was used as control (Pctrl).

2.4. High performance size exclusion chromatography (HPSEC)

HPSEC system consists of an Agilent 1200 pump unit, an automatic injector (Agilent Technologies, Waldbroon, Germany), a refractive index (RI) detector (Brookhaven Inc., New York, USA), and a linear mix column with a guard column. The columns were maintained at 40 °C. 0.5 mg/mL pectin solutions were filtered through 0.45 filters before injection, and eluted at 0.7 mL/min 0.05 M $NaNO_3$ containing 0.02% NaN_3 was used as mobile phase (Chen et al., 2014). Dextrans of T-10, T-40, T-70, T-150, T-500, T-1000, and T-2000 were used as standards to construct a standard curve.

2.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were recorded on a Nicolet 5700 spectrometer (Thermo Co., Madison, USA) according to our previous method (Chen et al., 2014). The dried samples were ground with KBr powder (spectroscopic grade) and pressed into pellets for spectra measurement in the frequency range of 4000–400 cm^{-1}. The data were analyzed with Ominic 7.2 software.

2.6. Determination of degree of methoxylation (DM)

The DM of samples was determined by taking the ratio of moles of methoxyl groups to the moles of anhydrous galacturonic acid. The galacturonic acid content was determined colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973). The content of methanol was determined by the colorimetric method of Inari and Takeuchi (1997).

2.7. ^1H NMR spectroscopy

For ^1H NMR analysis, 40 mg sample was deuterium exchanged by successive freeze-drying steps and then dissolved in 0.5 mL of 99.98% D_2O. ^1H NMR spectrum of the solution was recorded at 25 °C using a Bruker Avance 600 MHz NMR spectrometer (Brucker, Rheinstetten, Germany).

2.8. Determination of intrinsic viscosity

Intrinsic viscosities ([η]) of the original pectin and alkyl-pectin derivatives were measured by recording flow time of pectin solution in an Ubbelohde viscometer (diameter = 0.52 mm) at 25 ± 0.1 °C, immersed in a temperature controlled bath. Solutions were prepared by dissolution in 0.1 M NaCl solutions (Morris et al., 2008), previously filtered through 0.45 μm membrane filters. They were stirred for 24 h before diluted to appropriate concentrations, and then stirred for a further 24 h [η] was estimated by extrapolation of Kraemer curves to “zero” concentration.

2.9. Determination of apparent viscosity

Pectin solutions were prepared at 20 g/L by dissolving samples in 0.025 M NaCl (Charlot & Auzély-Velty, 2007) for gentle stirring 18 h at room temperature, then centrifuged (3000 g, 15 min) in order to devoid of entrapped air bubbles. Afterward, polymer solutions were stored at 4 °C overnight before measurements were carried out.

Experiments at imposed shear stress were performed on a Discovery DHR-2 Rheometer (TA, New Castle, USA), fitted with a parallel plate geometry (40 mm diameter, 1.0 mm gap). Temperature control (25 ± 0.1 °C) was achieved with a Peltier system in the bottom plate.

2.10. Emulsifying properties

Emulsifying capacity (EC) and emulsion stability (ES) were assessed according to the work of Baississe, Ghannem, Fahloul, and Lekbir (2010). Oil-in-water (O/W) emulsions were prepared in 50 mL graduated centrifuge tubes by adding vegetable oil (40 wt.% final oil level) to pectin solution (0.5%, w/w), containing 0.02% sodium azide as a bactericide. The whole system was then treated vigorously by a vortex mixer and followed by an Ultra-Turrax T25