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Structural and rheological properties of pectic polysaccharide extracted from *Ulmus davidiana* esterified by succinic acid

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ABSTRACT

The present study was carried out to investigate the physicochemical and structural properties of pectic polysaccharide extracted from *Ulmus davidiana* (UDP) and to determine the physicochemical, structural, and rheological properties of esterified UDP with succinic acid (ES-UDP). The results indicated that UDP had high amounts of galacturonic acids and various neutral sugars, such as galactose, rhamnose, and glucose. UDP was identified as a low methoxyl pectin, consisting of 1,4-linked α-D-Gal*p*A (the main backbone chain), supported by the results of Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction, and 1D Nuclear magnetic resonance (NMR) spectroscopy. In the FT-IR and XRD, no difference was detected between UPD and ES-UDPs. However, ^{1}H and ^{13}C NMR spectra revealed that the new ester bonds were formed between a hydroxyl group of UDP and a carboxyl group of succinic acid during esterification. In the steady shear rheological analysis, the consistency index (K) of ES-UDP was significantly higher than that of UDP and increased significantly with increasing concentration of succinic acid. In the dynamic rheological analysis, the tan δ values of all ES-UDP solutions were significantly lower than those of the UDP solution.

1. Introduction

Chemical modification is the most commonly investigated modification method due to the non-destructive nature of several selected processes and the potential enhancement in the polymer functionality [1]. Modification can enable enhancement or introduction of key properties to the polysaccharides. The number of reactive sites for chemical modification increases with the number of hydroxyl groups in polymers [2]. There are various methods of chemical modification of polymers, but the most important methods are cross-linking, esterification, and etherification [3]. Such modifications of polysaccharides can enhance pasting, gelatinization, swelling, and solubility properties [4].

Esterification is one of the most common modification techniques that can be used to improve viscosity and to enhance the resistance to machinability, heating, shearing, and acid exposure. In general, polymers can form esters either through intra- or intermolecular linkage during esterification [5]. Polymer esters are esterified by various reactants such as acid anhydrides, octenyl succinic anhydride (OSA), dodecenyl succinic anhydride (DDSA) fatty acids, and polycarboxylic acid [6,7]. Esterification with OSA is a commonly applied method of polymer esterification [8]. Modification with polycarboxylic acids such as citric, adipic, and glutaric acid have also been used widely for polymers [6]. However, esterification using succinic acid has not been studied so far.

Succinic acid has been used as a food additive, and as such is listed as a "Generally Recognized as Safe" food additive by the Food and Drug Administration (FDA). Succinic acid is a dicarboxylic acid and thus may promote esterification owing to the two carboxyl groups that can react with different polysaccharide chains. Thus, we hypothesized that succinic acid may play an important role for steady and dynamic shear rheological properties of polysaccharides owing to greater network development. The formation of adhesive and friction forces between succinic acid and polysaccharide surfaces were predicted, based on new intermolecular and/or intramolecular ester bridges between a hydroxyl group of polysaccharide and a carbxoylic group of the succinic acid after esterification. The physicochemical and rheological properties of polysaccharides esterified with succinic acid have not been reported so far.

Ulmus davidiana (Ulmaceae) is naturally growing in North-East Asia such as Korea, Japan, and China. Especially, root barks of *Ulmus davidiana* have been used in traditional medicine to prevent inflammation, cancer, edema, and other ailments [9,10]. According to Lee et al. [11], water extract from *Ulmus pumila* L. root bark contains pectic polysaccharides which consist of various monosaccharides such as galacturonic acid, rhamnose, galactose, and glucose. Previous studies mostly focused on extraction, antioxidant, and anti-inflammatory activities of pectic polysaccharide extracted from *Ulmus davidiana* (UDP) [11]. However, no studies have been performed on the rheological properties of UDP. Moreover, the structural and physicochemical properties

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of esterified pectic polysaccharide extracted from *Ulmus davidiana* (ES-UDP) are also unknown. Therefore, the present study aimed to produce ES-UDPs with different concentration of succinic acid usable for emulsifiers, gelling agents, and thickeners in food production. Thus, the objectives of the present study were to produce ES-UDPs using four different concentration of succinic acid (0.6, 1.2, 1.8, and 2.4 g succinic acid per 100 g of dry basis UDP, respectively) and to determine structural properties (^{1}H and ^{13}C NMR, FT-IR, XRD, and SEM), and rheological properties (steady shear, frequency sweep, time sweep, and temperature sweep).

2. Materials and methods

2.1. Materials

The root of *Ulmus davidiana* was obtained from the local market of Kyungdong in Seoul, Korea. Succinic acid and acetic anhydride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Monosaccharide standards (galactose, glucose, rhamnose, xylose and arabinose) and galacturonic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

2.2. Extraction of pectic polysaccharide from root bark of Ulmus davidiana

The extraction procedure of UDP was carried out following the methodology previously reported by Lee et al. [11] with slight modifications. Dried root bark of *Ulmus davidiana* (60 g) was crushed into small pieces of size (3 cm × 1 cm × 0.5 cm) and was mixed with distilled water (1:20, w/v). During extraction, temperature was maintained at 80 °C for 24 h using shaking water bath (BS-11, Jeio tech Co., Ltd., Daejeon, Korea). After cooling at room temperature, the suspension was centrifuged (Combi 408, Hanil BioMed Inc., Gwangju, Korea) at 3500 rpm for 10 min. To allow pectic polysaccharides precipitation, ethanol (95%) was added to the supernatant (3:1, v/v) and then kept at room temperature for 12 h. It was then separated by filtration using a Whatman No. 1 filter paper (GE Healthcare, Amersham, UK) and freeze dried to obtain pure pectic polysaccharide.

2.3. Characterization of UDP

2.3.1. Chemical composition

The yield of UDP was measured as percentage of the dry weight of sample, which was calculated by using the formula: yield of UDP (% dry weight) = (weight of polysaccharide / weight of dried sample) × 100. The chemical composition of UDP including moisture content, ash content, protein content, and fat content were determined according to A.O.A.C. methods [12] by 925.09B, 923.03, 979.09, and 920.39C, respectively. The content of total sugar was determined by the phenol sulfuric acid method described by Dubois et al. [13].

2.3.2. Monosaccharide composition

The content of GalA was measured by the m-hydroxyphenyl colorimetric method described by Blumenkrantz et al. [14] with D-galacturonic acid as the standard (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Degree of esterification was determined by titration method, as described by Wai et al. [15] with some modifications.

The neutral monosaccharide composition of UDP was analyzed by GC method of Zhu et al. [16] with slightly modification. Briefly, approximately 5 mg of the sample was dissolved in an ampoule containing 3 mL of 4 M trifluoroacetic acid. The mixture was hydrolyzed at 121 °C for 6 h. After hydrolysis, trifluoroacetic acid was evaporated to dryness at 40 °C. Neutral sugar was reduced to alditol using 25 mg of sodium borohydride with 3 mL of distilled water at room temperature for 2 h. Acetic acid was added to solution to decompose excess sodium borohydride until bubble formation stopped. A stream of nitrogen gas was used to dry solution and 3 mL of methanol was added to remove borohydrate. The procedure was repeated four times. Acetic anhydride (5 mL) was subsequently added to the mixture and incubated for an additional 60 min at 100 °C. The hydrolysate was then converted into the corresponding alditol acetates and analyzed with GC (G3440B, Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5 column (0.25 mm × 30 m × 0.25 μm, Agilent Technologies, Palo Alto, CA, USA) and a flame-ionization detector. Initial column temperature was held at 140 °C for 5 min, increased to 240 °C at 4 °C/min and maintained at 240 °C for 5 min. Nitrogen gas (N_2) was used as carrier gas with flow rate of 1.0 mL/min. The neutral monosaccharides were estimated by the standards (rhamnose, arabinose, xylose, glucose, and galactose) purchased from Sigma Aldrich (St. Louis, MO, USA).

2.4. Preparation of esterified pectic polysaccharide with succinic acid

Esterified pectic polysaccharides with succinic acid (ES-UDP) were carried out using the method of Šubarić et al. [17] with some modifications. The mixture of succinic acid and acetic anhydride (1:4, w/w) was prepared by hot-dissolving. 50 g of UDP (dry basis) were introduced to a beaker and soaked with distilled water (1:20, w/v). The slurry was thoroughly mixed and adjusted to pH 9 using 1 M NaOH solution. Under continuous stirring and pH kept at 9, the mixture of succinic acid and acetic anhydride (1.5, 3.0, 4.5 and 6.0 mL per 50 g of dry basis UDP) was drop-wise. After the addition of the succinic acid and acetic anhydride, the pectic polysaccharide suspension was stirred for 1 h at room temperature and then added with 1 M NaOH solution to pH 5.4. The esterified pectic polysaccharides were rinsed with distilled water to remove reagent residues and washed by ethanol aqueous solutions with a concentration of 50% until it was neutral. The washed pectic polysaccharides were air-dried at 50°C for 24 h, ground into powder, and passed through a 150 mesh. The esterified UDP with four different concentrations of succinic acid (0.6, 1.2, 1.8, and 2.4 g per 100 g of dry basis UDP) were denoted by ES-$UDP_{0.6}$, ES-$UDP_{1.2}$, ES-$UDP_{1.8}$, and ES-$UDP_{2.4}$, respectively.

2.5. Structural properties

2.5.1. Scanning electron microscopy (SEM)

SEM was carried out on a scanning electron microscope (S-4700, Hitachi CO., Tokyo, Japan). UDP and ES-UDPs were placed on double side carbon tape attached to a specimen holder and coated with platinum powder. The samples were examined at accelerating voltage of 10 kV and magnification of ×250.

2.5.2. Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of UDP and its esterified derivatives were obtained using a Fourier-transform infrared spectrophotometer (Spectrum GX, Perkin Elmer, Massachusetts, USA). Each of the samples were mixed with potassium bromide (KBr), compressed into pellets and analyzed between 500 and 4000 cm^{-1}.

2.5.3. X-ray diffraction (XRD) analysis

The XRD patterns of UDP and the ES-UDPs were detected by a X-ray diffractometer (X'Pert PRO MPD, PANalytica, Netherlands) using Cu Kα radiation (λ = 1.5418 Å) in the range (2θ = 10°–80°). The samples were scanned from 10° to 50° diffraction angle (2θ) (step size 0.02° 2θ, time per step: 38.4 s).

2.5.4. 1D nuclear magnetic resonance (NMR) spectroscopy

The dried UDP and ES-UDPs were dissolved in deuterium oxide (99.8 atom % D_2O, Sigma Chemical Co., USA) at 85°C for 3 h and freeze-dried three times to replace the exchangeable protons with deuterons before finally dissolving in D_2O for NMR analysis. The ^{1}H and ^{13}C NMR spectra of UDP and ES-UDPs were detected by a Bruker AMX 600 MHz spectrometer (Bruker BioSpin, Rheinstetten, Germany).