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Non-starch polysaccharides from American ginseng: physicochemical investigation and structural characterization

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ABSTRACT

Non-starch polysaccharides (GSP) from roots of American ginseng were extracted and purified. The physicochemical properties and detailed structure of GSP was systematically studied using high performance size-exclusion chromatography (HPSEC), Fourier transform infrared spectroscopy (FTIR), methylation analysis and 1D & 2D NMR spectroscopy. Weight average molecular weight (Mw) and intrinsic viscosity of GSP was 85.4 kDa and 0.41 dL/g, respectively. Monosaccharide composition analysis indicated that GSP consisted of rhamnose, arabinose, galactose, glucose and uronic acid with a weight ratio of 1:4:8:8:50. The dominate component in GSP was galacturonic acid (up to 70% in molar ratio) based on methylation analysis. FTIR demonstrated that GSP had pectin based structure and the degree of esterification (DE) was calculated to be 38%. Based on 1D & 2D NMR spectroscopy, the major sugar residue of GSP molecule was 4-α-D-Gal*p*A, other residues including 2-α-L-Rha*p*, 2,4-α-L-Rha*p*, α-L-Ara*f*, β-D-Gal*p*, 4-β-D-Gal*p* were also evidenced in GSP. As a pectin molecule, GSP contained mainly homogalacturonans structure, while a small portion of rhamnogalacturonan I character was also identified.

1. Introduction

Panax ginseng C.A. Meyer (*P. ginseng*), also known as North American ginseng, is a well-known herbal medicine. It has been used for several thousand years with functions of reducing stress, lowering blood sugar level and adjusting immunity (Assinewe, Arnason, Aubry, Mullin, & Lemaire, 2002; Chen, Xie, Fu, Lee, & Wang, 2007). *P. ginseng* comprises various classes of compounds, including ginsenosides, essential oil, peptidoglycans, carbohydrate, nitrogen-containing compounds, fatty acids and other phenolic compounds. Ginsenosides have been reported to be the major bioactive secondary metabolites of this herb. Previous studies mostly were focused on the extraction, quantification and the bioactive activity investigation of ginsenosides. (Chen et al., 2007; Du, Wills, & Stuart, 2004; Hu & Kitts, 2001; Liu et al., 2008; Mathur, Shukla, Pal, Ahuja, & Uniyal, 1994; Yang, Chen, Zhang, & Guo, 2004; Zhong, Bai, & Wang, 1996). However, non-starch polysaccharides from *P. ginseng* as biological response modifier has been drawing increasing attention due to the various bioactivities, e.g. immune-modulating and anti-cancer effects (Adamko, Ebeling, & Wu, 2009; Assinewe et al., 2002; Sun, 2011; Yeo, Lee, & Popovich, 2011; Zhang et al., 2012).

Although structural features of ginseng polysaccharides previously have been studied, e.g. those from different parts of *P. ginseng* (roots, leaves and fruit) have been reviewed by Sun (2011) in terms of their structural and bioactive properties, the fine structure characterization is still limited and the structure–function relationship has yet been established. Therefore, the main objectives of the present study were to uncover the physicochemical properties and characterize the detailed structure features of non-starch polysaccharides from ginseng roots using modern techniques including 1D & 2D NMR, and GC–MS in order to establish the structure–function relationships and to further facilitate its food and pharmaceutical applications.

2. Materials and methods

2.1. Materials

Four to five years dry roots of *Panax quinquefolius* were provided by Great Mountain Ginseng Co. Ltd, ON, Canada. All chemicals and reagents were of analytical grade otherwise specified.

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2.2. Extraction & purification methods

The extraction and purification procedures of water-soluble ginseng root polysaccharides (GSP) are demonstrated in Fig. 1a. Dried root powder was soaked twice by 80% (v/v) ethanol. Ten times (v/weight of dry seeds) of hot water (80 °C) was used for polysaccharides extraction and 3 volumes of ethanol was used to recover crude polysaccharides (CGSP) from solution. Protein and starch were further removed by protease and α-amylase, respectively to get purified sample GSP.

2.3. Pectinase hydrolysis

GSP water solution (10 mg/mL) was adjusted to pH 4.5 and incubated at 50 °C water bath. Pectinase (Sigma, P1746) was then added into the solution with amount of 100 unit/g GSP. Hydrolysis was carried out for 24 h under constant stirring (dilute HCl and NaOH solution was used during this process to control the pH) followed by elevating the temperature to 100 °C for 3 min to deactivate the enzyme. After GSP hydrolytes (EGSP) solution was cooled to room temperature, 3 volumes of ethanol was added to obtain EGSP precipitate (EGSPP) and EGSP supernatant (EGSPS), which was presented in Fig. 1a.

2.4. High performance size-exclusion chromatograph (HPSEC)

The molecular weight distribution was determined using HPSEC equipped with multiple detectors: a differential pressure viscometer (DP) for viscosity determination; a refractive index detector (RI) and a UV detector for concentration determination; a right angle laser light scattering detector (RALLS) and a low angle laser light scattering detector (LALLS) for direct molecular weight determination. Three columns in series: 2 Polyanalytik PAA-M mixed bed columns and one PAA-203 column were used. The columns, viscometer and RI detector were maintained at 40 °C. The eluent was 0.1 M $NaNO_3$ containing 0.03% (w/w) NaN_3 at a flow rate of 0.6 mL/min. Data was obtained and analyzed using the OmniSEC 4.6.1 software.

2.5. Total uronic acid and monosaccharide composition test

Total uronic acid was determined using the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973); Monosaccharide compositions were determined by treating sample (20 mg) in 1 mL 12 M H_2SO_4 at 30 °C for 30 min, then diluting to 6 mL (2 M H_2SO_4) followed by hydrolysis at 100 °C for 2 h. Analysis was carried out using a high performance anion-exchange chromatograph (HPAEC) with pulsed amperometric detection (PAD) (Wood, Weisz, & Blackwell, 1994). All the measurements were repeated three times.

2.6. Fourier transform infrared spectroscopy (FTIR) analysis

FT-IR spectra of GSP and other citrus pectin samples were recorded on a Golden-gate Diamond single reflectance ATR in an FTS 7000 FTIR spectrometer equipped with a DTGS detector (DIGILAB, Randolph, MA). The spectrum for each sample was recorded at absorbance mode from 1800 to 800 cm^{-1} at a resolution of 4 cm^{-1} with 128 co-added scans.

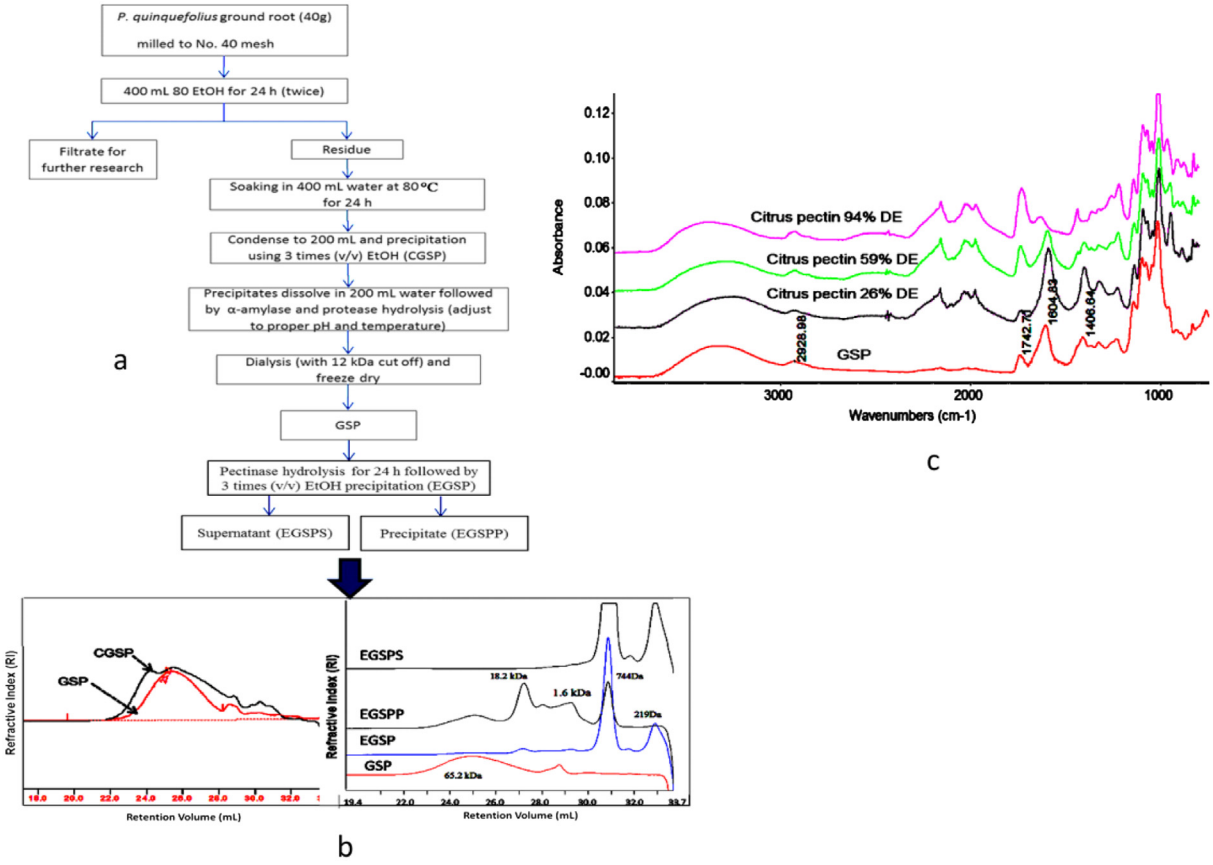

Fig. 1. Extraction procedure (a) and elution profiles of ginseng polysaccharides (GSP and its fractions) using HPSEC coupled with RI detector (b); FTIR spectra of commercial citrus pectins and GSP (c).