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Quantification of Inulo-oligosaccharides Using High pH Anion Exchange Chromatography with Pulsed Amperometric Detector (HPAEC-PAD)

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Inulo-oligosaccharides (IOS, F_n, n=2-6) were purified from enzymatic hydrolysates of water-soluble extract of Jerusalem artichoke tubers. Quantification of inulo-oligosaccharides was done using high pH anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) at the concentration range of 10-100 mg/L, which was compared with that of fructo-oligosaccharides (FOS, GF_n, n=1-7). Peak areas per mg IOS were higher than FOS at the same degree of polymerization (DP). Specific peak areas of IOS increased proportionally as DP increased up to six, in contrast to FOS showing no linearity.

Key words: *inulin, inulo-oligosaccharides, fructo-oligosaccharides, high pH anion exchange chromatography with pulsed amperometric detector.*

Inulin, a linear β-2,1-linked fructan ending with a sucrose residue, has been found mainly in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers and chicory (*Cichorium intybus* L.) roots. Hydrolysis of inulin by an endoinulinase produced two types of oligosaccharides, i.e. FOS ending with a sucrose residue and IOS lacking terminal glucose moiety. Quantification methods for water-soluble carbohydrates of Jerusalem artichoke tubers and chicory roots are essential for the development of new applications of inulin. While FOS have been quantitatively analyzed using high pH anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD),[1-2] the quantification of IOS has not yet been reported. It is necessary to develop a rapid and reliable method for the separation and quantification of oligosaccharides in water-soluble extracts or its enzymatic hydrolysates of Jerusalem artichoke tubers. In this paper, we describe the separation and the quantification of IOS with a DP up to six using HPAEC-PAD.

Materials and Methods

Materials. Fructose, glucose, and sucrose were purchased from Sigma (St. Louis, MI). Silica gel 60 F254 plate for TLC and Bio-Gel P2 extra-fine for colum chromatography were purchased from Merck (Darmstadt, Germany) and Bio-Rad (Richmond, CA), respectively. Other reagents were used of analytical grade. Jerusalem artichoke tubers were harvested from a field of Seulak Chicory Co. (Inje, Korea) in October 1996. The samples were washed with distilled water and stored as frozen. Endoinulinase was purified from *Arthrobacter* sp. S37.[3] Water was filtered by Mili-Q Watering System.

Preparation of standard oligosaccharides. Jerusalem artichoke tubers were sliced 1 cm thick and boiled for 1 h. The extract was then filtered by cheese-gauze and centrifuged at 16,000×g for 10 min. For the preparation of inulo-oligosaccharides, the water-soluble extract (1.60 g) was hydrolyzed by a purified endoinulinase (25 units) at 40 for 12 h. After the enzyme reaction, the hydrolysates were heated at 100°C for 10 min and centrifuged at 16,000×g for 10 min. Oligosaccharides were fractionated by Bio-Gel P2 extra-fine column chromatography (1.2×180 cm) with elution of hot water (70°C) at a flow rate of 0.1 mL/min.[4] The fractions of each peak were applied on Amide-80 HPLC column (4.6×250 mm, TOSOH, TSK-GEL) at 70°C with a refractive index detector.[5] The column was eluted with a mixture of CH_3CN and H_2O (75:25, v/v) at a flow rate of 1 mL/min. The fractions were concentrated using Integrated SpeedVac System (Savant Instrument ISS100). For the preparation of FOS, the water-soluble extract of Jerusalem artichoke tubers was directly fractionated using Bio-Gel P2 column chromatography and HPLC.

Quantification of standard sugars. Fructose, glucose, sucrose, five FOS (GF_n, n=1-7), and five IOS (F_n, n=2-6) were mixed at the concentration range of 10-100 mg/L. Separation and quantification were done using HPAEC-PAD.

HPAEC-PAD. The system for HPAEC-PAD consists of a

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Abbreviations: DP, degree of polymerization; FOS, fructo-oligosaccharides; HPAEC-PAD, high pH anion exchange chromatography with pulsed amperometric detector; IOS, inulo-oligosaccharides.

controller (Waters 600S), a pump (Waters 620), a Carbopak PA1 column (4×250 mm, Dionex), Carbopak PA guard column (0.45 mm, 4×50 mm, Dionex), and pulsed electrochemical detector (Waters 464). The applied potential of a pulse was kept at 0.1, 0.6, and -0.8 V for 0.299, 0.166, and 0.299 sec, respectively, with 50 mA of output range. The signal was integrated with a Waters 746 integrator (AT=4, PT=100). Prefiltered samples were eluted with a linear gradient of sodium acetate (0~0.6 M) in 0.1 M sodium hydroxide solution for 60 min at a flow rate of 1 mL/min.

Determination of total sugar. Total sugar amount was determined by modifying the anthrone method using fructose as a standard.[6] Two and a half milliliter of 0.2% (w/v) anthrone in 70% (v/v) H_2SO_4 solution was added to 0.5 mL of sample. After boiling for 10 min, the absorbance of the mixture was measured at 620 nm.

TLC. Development solvent was composed of n-propanol, ethyl acetate, and water (3:1:1, v/v).[4] Spots were detected by urea-metaphosphoric acid at 125°C.

Results and Discussion

Preparation and separation of standard sugars. Each purified oligosaccharide (FOS and IOS) showed more than 95% purity based on the ratio of the peak area to the total area of peaks by HPAEC-PAD analysis. Each peak was analyzed with TLC, which showed that it contained one type of sugar or oligomer. The sugar concentration of each fraction was measured by the anthrone method to compare the peak area of HPAEC-PAD. The glucose, fructose, sucrose, and oligosaccharides in a mixture were effectively separated by HPAEC-PAD (Fig. 1).

Quantification of standard sugars. Quantification of each standard sugar at the concentration range of 10-100 mg/L was done using HPAEC-PAD. The peak areas (or integration of PAD response) of each standard sugar increased linearly as the concentration of each sugar increased with its own characteristic slope (or peak area per mg sugar) (Fig. 2). Each peak area per mg IOS was higher than that of FOS at the same DP. Both peak areas per μg of

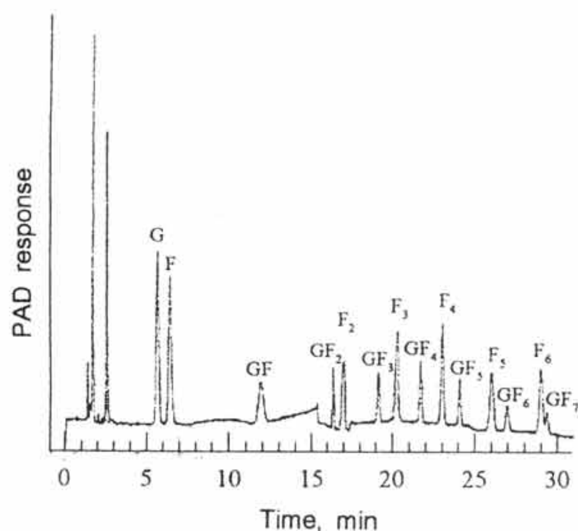

Fig. 1. High pH anion exchange chromatogram of standard oligosaccharides.

G, glucose;
F, fructose;
IOS: F2, inulo-biose;
F3, inulo-triose;
F4, inulo-tetraose;
F5, inulo-pentaose;
F6, inulo-hexaose;
FOS: GF, sucrose;
GF2, 1-kestose;
GF3, 1-nystose;
GF4, 1-F-1-β-D-fructofuranosyl-nystose;
GF5, 1-F-(1-β-D-fructofuranosyl)2-nystose;
GF6, 1-F-(1-β-D-fructofuranosyl)3-nystose;
GF7, 1-F-(1-β-D-fructofuranosyl)4-nystose.

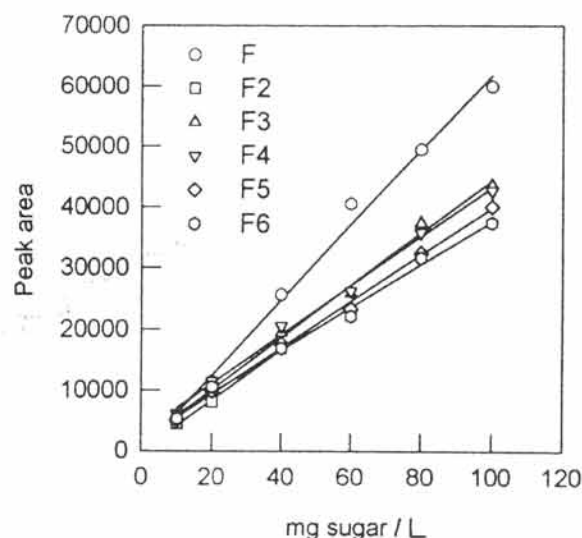

Fig. 2. Standard curves of fructose and IOS.

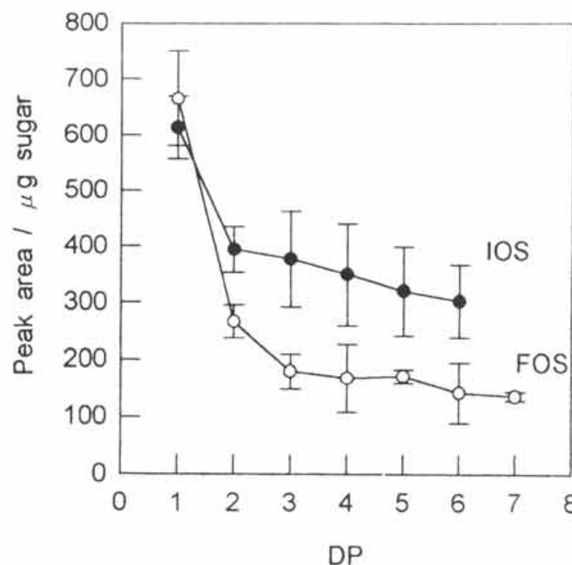

Fig. 3. Relationship between peak area per μg of oligosaccharides and DP.

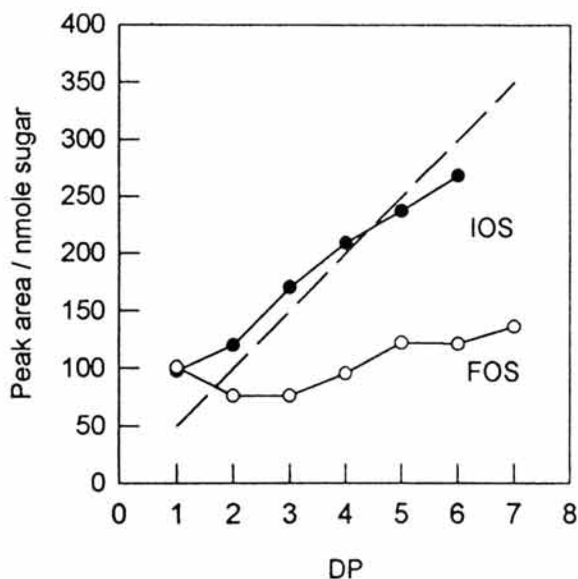

Fig. 4. Relationship between peak area per nmole of oligosaccharides and DP.

IOS and FOS decreased with increasing DP (Fig. 3).

Effects of a fructose unit on PAD response. The series of IOS showed a unique relationship between peak area per nmole of oligosaccharide and DP, i.e. each fructose unit in the series of IOS contributed almost the same net increase in the detector signal (Fig. 4). This projected response of IOS series seemed to be similar with that of malto-oligosaccharide series.[1] However, in the case of FOS series, a discrepancy from the projected response was observed. These data suggest that the different effect of each fructose unit on the detector response between IOS and FOS series depends on the presence of reducing activity of oligosaccharides. As the detector measures electrons released from the hydroxyl group of oligosaccharides in high pH condition, the reducing activity of oligosaccharides such as IOS and malto-oligosaccharide series contributes to the access of electrons from ionized hydroxyl groups to the gold electrode of PAD.

HPAEC-PAD provides a sensitive and convenient method to separate and quantify IOS as well as FOS with the same DP. As long as the pure oligosaccharides with higher DP are available, the application of HPAEC-PAD for the quantification of oligosaccharides could be expanded to IOS and FOS with higher DP in water-soluble extracts or enzymatic hydrolysates of Jerusalem artichoke tubers.

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