J. Appl. Glycosci., **56**, 159−164 (2009)

Regular Paper

Isolation and Structural Determination of Reducing Fructooligosaccharides Newly Produced in Stored Edible Burdock

(Received February 5, 2009; Accepted February 17, 2009)

Yojiro Ishiguro,[1] Keiji Ueno,[1] Masami Abe,[1] Shuichi Onodera,[1] Eri Fukushi,[2] Noureddine Benkeblia[3] and Norio Shiomi[1,*]

[1]*Department of Food and Nutrition Sciences, Graduate School of Dairy Science Research, Rakuno Gakuen University* (*582, Bunkyoudai, Midorimachi, Ebetsu 069−8501, Japan*)
[2]*Graduate School of Agriculture, Hokkaido University* (*Kita-9, Nishi-9, Sapporo 060−8589, Japan*)
[3]*Department of Life Sciences, The University of the West Indies* (*Mona Campus, Kingston 7, Jamaica*)

Abstract: Fresh edible burdock roots were stored in soil of 1 m depth underground from November to May. Three fructooligosaccharide derivatives without a terminal glucose residue, designated saccharides 1, 2 and 3, were generated in the stored burdock roots. They were purified from the sugar extract using carbon-Celite column chromatography. Saccharides 1, 2 and 3 have R-sucrose values (retention time of sucrose = 1) of 1.55, 2.15 and 2.73 by HPAEC, reducing terminal, molar ratios (reducing sugar to D-fructose) of 0.50, 0.33 and 0.25 and degrees of polymerization of 2, 3 and 4 by TOF-MS, respectively. Analyses by GLC and NMR confirmed the three different following structures: first was inulobiose [β-D-fructofuranosyl-(2→1)-β-D-fructopyranose], and the two others were inulotriose [β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructopyranose] and inulotetraose [β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructopyranose]. The NMR spectra showed that 70 to 80% of the terminal fructose residue of the three saccharides is pyranosyl form, while 20 to 30% is furanosyl form. The ^{13}C- and ^{1}H-signals were also assigned by 2D-NMR including COSY, HSQC, HSQC-TOCSY and HMBC. These saccharides could be synthesized by purified burdock fructan:fructan 1-fructosyltransferase from 1-kestose to free D-fructopyranose giving inulobiose and sucrose, while elongation of fructofuranosyl units occurred at this transferred fructofuranosyl residue to produce inulooligosaccharides having one, two and more additional units of fructofuranose.

Key words: reducing fructooligosaccharides, inulooligosaccharides, storage, burdock (*Arctium lappa* L), fructan:fructan 1-fructosyltransferase

Roots of burdock (*Arctium lappa* L.), a Compositae root vegetable, are known for their abundant inulin content. Inulin is a fructan consisting of straight chains composed of one molecule of D-glucose and about 30 molecules of D-fructose. There are referenced data neither on the different carbohydrates of burdock roots nor on their variation during storage period, except the work of Nagao[1)] (1996) who reported that inulin was hydrolyzed into low-molecular-weight sugars in burdock roots when stored at low temperature. However, the mechanism of the breakdown of inulin during storage at low temperature was not yet identified. There is little information on the postharvest biochemistry and physiology of carbohydrate components of raw burdock roots, and no referenced data are available on the different carbohydrates and their variation, including their metabolizing enzymes, during storage.

The present work is devoted to the isolation and structural analysis of newly-formed reducing oligosaccharides in stored burdock roots and attempts to synthesize them from 1-kestose and D-fructose with purified burdock fructan:fructan 1-fructosyltransferase (1-FFT). Moreover, we aimed to assess the variation of the different type of carbohydrates in burdock roots during storage under different temperature regimes, because carbohydrate components are of important consideration to maintain the high quality of fresh burdock roots.

* Corresponding author (Tel. & Fax. +81−11−388−4759, E-mail: n-shiomi@rakuno.ac.jp).

MATERIALS AND METHODS

Plant materials. Burdock roots (*Arctium lappa* L.) had been freshly harvested on November from an experimental field of Makubetsu Agricultural Co-operative, Hokkaido, Japan and then stored in soil 1 m deep underground until May. Samples were taken from freshly harvested burdock roots and from the six months stored roots.

Fructooligosaccharides and inulin standards. The standards 1-kestose [3a, 1^{F}-β-D-fructofuranosylsucrose] and nystose [4a, 1^{F}(1-β-D-fructofuranosyl)$_2$ sucrose] were prepared as described by Takeda *et al.*[2)] The standards 5a, 6a, 7a, 8a, 9a and 10p [1^{F}(1-β-D-fructofuranosyl)$_m$ sucrose, m=3, 4, 5, 6, 7 and 8～] were prepared from Jerusalem artichoke tubers by the method of Shiomi *et al.*[3)] Inulin (Wako Pure Chemical Ind., Ltd., Osaka, Japan) was

used as a standard for 10p.

Extraction of saccharides and fructooligosaccharides. Saccharides and fructooligosaccharides (FOS) were extracted by the method of Shiomi.[4] Briefly, tissues (10 g) were homogenized in 80 mL of aqueous ethanol (70%) containing a small amount of calcium carbonate (0.5 g/L). The homogenate was boiled under reflux in a water bath for 10 min and filtered. The residue was extracted three times with aqueous ethanol and one time with water under the same conditions. The extracts were combined and made up to 500 mL with distilled water. An aliquot of the solution (10 mL) was concentrated to dryness under vacuum at 35°C using a Büchi rotavapor (Büchi Labortechnik AG, Flawil, Switzerland). This dry concentrate was redissolved in 1 mL of water, filtered through a 0.45-μm filter and analyzed by high performance anion exchange chromatography (HPAEC Dionex, Dionex Corp., Sunnyvale, USA).[5,6]

Analysis of saccharides and FOS. D-Glucose, D-fructose, sucrose, FOS [1^F(1-β-D-fructofuranosyl)$_m$ sucrose, m=1−7], inulooligosaccharides and inulin were separated on an HPLC-carbohydrate column PA1, Carbo Pack (Sunnyvale, USA) with a Dionex Bio LC series HPLC (Sunnyvale, USA) and pulsed amperometric detector (PAD). The gradient was established by mixing eluent A (150 mM NaOH) with eluent B (500 mM acetate-Na in 150 mM NaOH). The concentration of sodium acetate was as follows: 0−1 min, 25 mM; 1−2 min, 25−50 mM; 2−20 min, 50−200 mM, 20−22 min, 500 mM; 22−30, 25 mM. The flow rate through the column was 1.0 mL/min. The applied PAD potentials for E1 (500 ms), E2 (100 ms) and E3 (50 ms) were 0.01, 0.60 and −0.60 V, respectively, and the output range was 1 μC.[7] Samples (25 μL) were injected using an auto-sampler (Intelligent Auto-sampler, model AS-4000, HITACHI Ltd., Tokyo, Japan).

Isolation of reducing FOS. The stored burdock roots (1 kg) were homogenized in 5 L of aqueous ethanol (70%) containing a small amount of calcium carbonate (0.5 g/L). The homogenate was boiled under reflux in a water bath for 15 min. Homogenate was filtered and the residue was extracted three times with aqueous ethanol under the same conditions. The filtrates were combined and the resulting extract was concentrated under vacuum at 35°C to 700 mL. After the concentrated extract was centrifuged (8000 rpm, 30 min), the supernatant (400 mL) was loaded onto a carbon-Celite column (6 × 74 cm) and eluted by successive elution with water (9.6 L) and 3 (9.7 L) and 5% ethanol (3.5 L). Saccharide 1 was eluted with 3% ethanol (1.3−2.1 L), and saccharides 2 and 3 were eluted with 5% ethanol (0.6−2.0 and 14.5−16.9 L). Saccharides fractions 1, 2 and 3 were concentrated under vacuum and lyophilized to give white powders, 220, 230 and 139 mg, respectively.

Purification of burdock 1-FFT. 1-FFT, which transfers a terminal D-fructosyl group from a (2-1)-β-linked fructooligosaccharide to HO-1 of another D-fructosyl group, was purified from an extract of burdock roots by successive chromatographies with DEAE-Sepharose CL-6 B, TOYOPEARL HW-55S and Sephadex G-100 up to disc-electrophoretical homogeneity as described previously.[8] The enzyme was free from sucrose:sucrose1-fructosyltransferase and fructan:fructan 6^G-fructosyltrasferase and catalyzed the D-fructosyl transfer from 1-kestose to saccharides of 1-kestose series [1^F(1-β-D-fructofuranosyl)$_m$ sucrose].

Isolation of reducing FOS synthesized by 1-FFT. Saccharides 1′, 2′ and 3′ were synthesized *in vitro* using purified 1-FFT.[8] A mixture of burdock 1-FFT (5 U), 0.2 M fructose and 0.2 M 1-kestose in 100 mM sodium phosphate buffer (pH 6.5, 40 mL) supplemented with 1 mL of toluene was incubated at 30°C for 168 h. After the reaction was stopped by heating at 100°C in a boiling water bath for 3 min, the reaction mixture was loaded onto a carbon-Celite column (6 × 74 cm) and eluted with water and 3 and 5% ethanol in the same way as described above. Saccharides fractions 1′, 2′ and 3′ were concentrated under vacuum and lyophilized to give white powders, 65.4, 33.2 and 5.7 mg, respectively.

Gas liquid chromatographic (GLC) analysis of methanolyzates of permethylated reducing FOS. Methylation of the oligosaccharides was carried out by the method of Hakomori.[9] The permethylated saccharides were methanolysed by heating with 1.5% methanolic HCl at 92°C for 10 min. The reaction mixture was treated with Amberlite IRA-410 (OH^-) to remove HCl, and evaporated under vacuum to dryness. The resulting methanolysate was dissolved in a small volume of methanol and analyzed using a gas chromatograph [Shimadzu GC8A; glass column (2.6 mm × 2 m) packed with 15% butane-1,4-diol succinate polyester on acid-washed Celite; flow rate of carrier nitrogen, 40 mL/min].

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). MALDI-TOF-MS spectra were measured using a Shimadzu-Kratos mass spectrometer (KOMPACT Probe) in positive ion mode with 2.5% dihydroxybenzoic acid as a matrix. Ions were formed by a pulsed UV laser beam (nitrogen laser, 337 nm). Calibration was done using nystose as an external standard.

Nuclear magnetic resonance (NMR) measurements of reducing FOS. Each reducing FOS (5 mg) was dissolved in 0.5 mL D_2O. NMR spectra were recorded at room temperature with a Bruker AMX 500 spectrometer (^{1}H 500 MHz, ^{13}C 125 MHz) equipped with a 5-mm diameter C/H dual (1D spectra) and TXI probe (2D spectra). Chemical shifts of ^{1}H (δ_H) and ^{13}C (δ_C) in ppm were determined relative to the external standard of sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)propanoate in D_2O (δ_H 0.00 ppm) and 1,4-dioxane (δ_C 67.40 ppm) in D_2O, respectively. ^{1}H-^{1}H COSY[10,11] and HSQC[12] were obtained using gradient selected pulse sequences. The phase sensitive HSQC-TOCSY spectra were determined with the sequence including inversion of directly resonance (IDR).[13] HMBC[14] spectra were obtained using the pulse sequences of CT-HMBC.[15]

RESULTS

FOS [(1-β-fructofuranosyl)$_m$ sucrose; m=1 (1-kestose, 3a), 2 (nystose, 4a), 3 (5a), 4 (6a), 5 (7a), 6 (8a), 7 (9a) and 8～(10p)] as well as glucose, fructose and sucrose in burdock roots freshly harvested are shown on the HPAEC

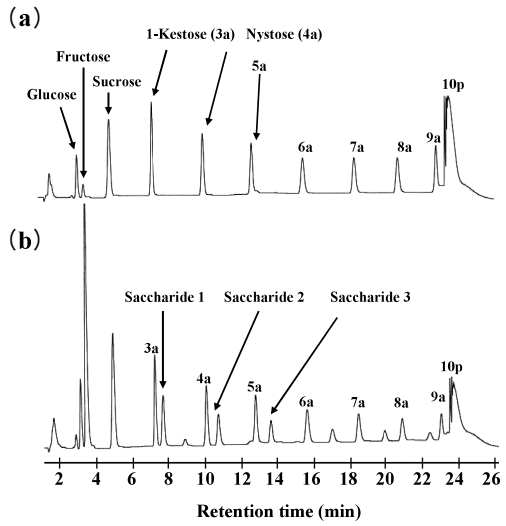


Fig. 1. HPAEC charts of fructo-oligo- and poly-saccharides separated from burdock roots (a) freshly harvested, and (b) burdock roots stored in soil for six months.

The standards 5a, 6a, 7a, 8a, 9a and 10p; 1^F (1-β-D-fructofuranosyl)$_m$ sucrose, m=3, 4, 5, 6, 7 and 8～, respectively.

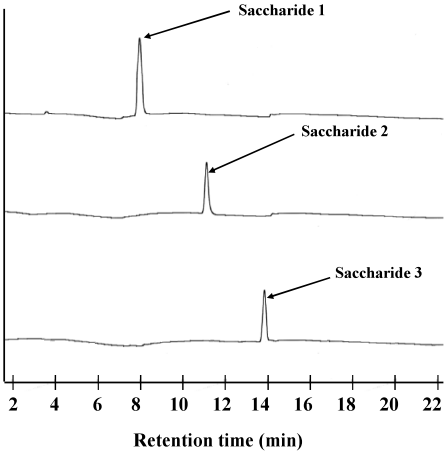


Fig. 2. HPAEC chart of saccharides 1, 2 and 3 isolated from the stored burdock roots using carbon-Celite column chromatography.

Table 1. GLC analysis of methanolysis products of permethylated saccharides 1, 2 and 3.

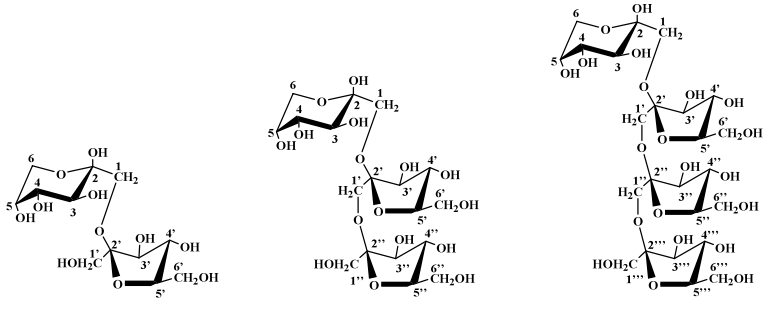
Methanolyzate	Relative retention time[a]			
Saccharide 1	1.05	1.26	2.68	3.88
Saccharide 2	1.06	1.26	2.66	3.94
Saccharide 3	1.04	1.24	2.65	3.87
Methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside	1.00			

[a]Retention time of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucoside = 1.

chart (Fig. 1 (a)). Several newly-formed saccharides were found in the roots stored in soil underground for six months after harvesting as shown by the chromatograms (Fig. 1 (b)). Their three saccharides were named saccharides 1, 2 and 3. R_S-values (R_f of sucrose = 1) of saccharides 1, 2 and 3 were 1.06, 0.78 and 0.69 by paper chromatography (data not shown), while relative retention times (retention time of sucrose = 1) were 1.55, 2.15 and 2.73 by HPAEC, respectively. Saccharide 1 was eluted after 1-kestose while saccharide 2 and saccharide 3 were eluted after nystose (4a) and fructosylnystose (5a), respectively. This order of elution seems to follow the degree of polymerization, since saccharides 1, 2 and 3 were eluted after DP 3, DP 4 and DP 5 short chain FOS of inulin series.

Saccharides 1, 2 and 3 were isolated from the stored burdock roots by carbon-Celite column chromatography and were shown to be homogenous by HPAEC (Fig. 2) and TLC. They had reducing power and gave only D-fructose by hydrolyses with 0.1 M hydrochloric acid and yeast invertase. The molar ratio of reducing sugar to D-fructose in saccharides 1, 2 and 3 were 0.50, 0.33 and 0.25, respectively. The degrees of polymerization of saccharides 1, 2 and 3 were 2, 3 and 4 as shown by measurements of $[M+Na]^+$ ions, m/z 365, 527 and 689 using TOF-MS (data not shown), respectively. The methanolyzates of saccharides 1, 2 and 3 showed four peaks corresponding to methyl 1,3,4,6-tetra-*O*-methyl-D-fructoside (relative retention time; 1.04−1.06 and 1.24−1.26) and methyl 3,4,6-tri-*O*-methyl-D-fructoside (2.65−2.68 and 3.88−3.94) on GLC chromatograms (Table 1), respectively. From these results, saccharides 1, 2 and 3 were confirmed to be D-fructosyl-(2→1)-D-fructose, D-fructosyl-(2→1)-D-fructosyl-(2→1)-D-fructose and D-fructosyl-(2→1)-D-fructosyl-(2→1)-D-fructosyl-(2→1)-D-fructose, respectively.

Based on the chemical shift values and the relative intensities of the ^{13}C-NMR signals of the C2 atoms, it was concluded that saccharides 1, 2 and 3 contain one reducing fructopyranosyl residue. The presence of typical signals for the C2, C3, C4 and C5 atoms of fructopyranose residues shows that the reducing end has no 6-OH group. The analyses of the saccharides by ^{13}C- and ^{1}H-NMR spectroscopy confirmed the presence of three different structures, as shown in Fig. 3. Thus, saccharides 1, 2 and 3 were determined to be β-D-fructofuranosyl-(2→1)-β-D-fructopyranose (inulobiose), β-D-fructofuranosyl-(2→1)-β-



Saccharide 1 Saccharide 2 Saccharide 3

Fig. 3. Chemical structures of saccharides 1, 2 and 3.

Table 2. ^{1}H- and ^{13}C-NMR chemical shifts of saccharides 1, 2 and 3.

		Saccharide 1				Saccharide 2				Saccharide 3			
		δC	δH		JHH	δC	δH		JHH	δC	δH		JHH
Fructopyranose	1	64.60	3.76	d	10.5	64.62	3.76	d	11.2	64.81	3.81	d	10.2
			3.66	d	10.5		3.67	d	11.2		3.71	d	10.2
	2	98.66				98.57				98.75			
	3	68.95	3.73	d	10.1	68.86	3.74	d	10.1	69.07	3.79	d	10.1
	4	70.28	3.88	dd	10.1, 2.0	70.23	3.88	dd	10.1, 3.7	70.41	3.91	dd	10.1, 3.0
	5	69.90	3.98	ddd	2.0, 1.9, 1.3	69.84	3.98	ddd	3.7, 1.6, 1.2	70.03	4.02	m	
	6	64.27	4.01	dd	12.8, 1.3	64.22	4.01	dd	12.8, 1.2	64.40	4.05	dd	12.9, 1.2
			3.66	dd	12.8, 1.9		3.67	dd	12.8, 1.6		3.70	dd	12.9, 1.5
Fructofuranose′	1	61.16	3.73	d	12.7	61.30	3.86	d	10.4	61.54	3.92	d	10.2
			3.68	d	12.7		3.69	d	10.4		3.75	d	10.2
	2	104.45				104.42				103.97			
	3	77.96	4.17	d	8.4	78.47	4.22	d	8.4	78.70	4.26	d	8.4
	4	75.07	4.14	dd	8.4, 7.3	74.95	4.14	dd	8.4, 8.1	75.12	4.19	dd	8.4, 7.9
	5	82.00	3.85	m		81.95	3.85	ddd	8.1, 6.2, 3.7	82.15	3.90	ddd	7.9, 6.2, 3.1
	6	62.79	3.81	dd	12.7, 1.2	62.64	3.79	m		62.84	3.84	dd	12.2, 3.1
			3.71	dd	12.7, 6.4		3.71	m			3.75	dd	12.2, 6.2
Fructofuranose″	1					61.22	3.74	d	11.1	61.71	3.91	d	10.2
							3.67	d	11.1		3.75	d	10.2
	2					104.42				103.94			
	3					78.47	4.16	d	8.8	78.42	4.25	d	8.4
	4					74.95	4.08	dd	8.8, 7.9	75.29	4.12	dd	8.1, 8.1
	5					81.95	3.85	m		82.01	3.89	m	
	6					63.02	6.82	m		63.14	3.86	dd	12.2, 3.1
							3.74	m			3.77	dd	12.2, 7.1
Fructofuranose‴	1									61.43	3.78	d	12.2
											3.71	d	12.2
	2									104.64			
	3									77.75	4.21	d	8.5
	4									75.29	4.13	dd	8.3, 7.9
	5									82.03	3.89	m	
	6									63.18	3.87	dd	12.2, 3.1
											3.78	dd	12.2, 7.1

Chemical shifts (δ) in ppm were determined relatively to the external standard of sodium [2,2,3,3-^{2}H$_4$]-3-(trimethylsilyl) propanoate (δH, 0.00 ppm) and 1,4-dioxane (δC 67.40) in D_2O.

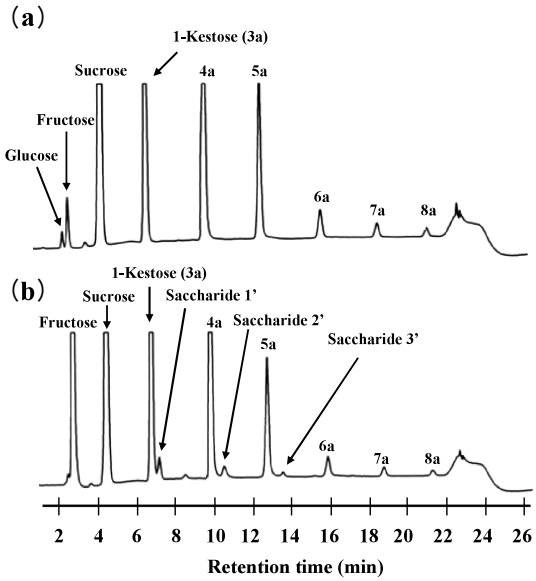


Fig. 4. HPAEC patterns of saccharides synthesized from 1-kestose (a) and from 1-kestose plus D-fructose (b) using purified burdock 1-FFT.

The standards 4a, 5a, 6a, 7a, and 8a; 1^{F}(1-β-D-fructofuranosyl)$_m$ sucrose, m=2, 3, 4, 5 and 6, respectively.

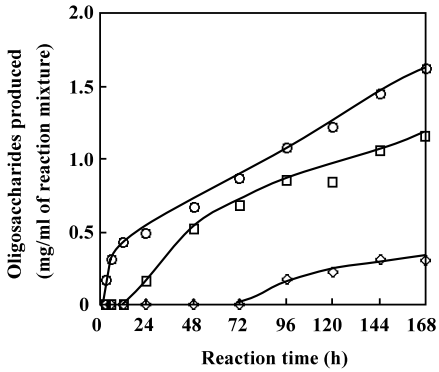


Fig. 5. Time course of the formation of saccharides 1′, 2′ and 3′ from 1-kestose and D-fructose in the presence of purified burdock 1-FFT. ○, saccharide 1′; □, saccharide 2′; ◇, saccharide 3′.

D-fructofuranosyl-(2→1)-β-D-fructopyranse (inulotriose) and β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructopyranose (inulotetraose), respectively (Fig. 3). The spectra also showed that 70 to 80% of the terminal (reducing) fructose residue of the three saccharides, inulobiose, inulotriose and inulotetraose, have a fructo-pyranosyl form, while 20 to 30% of them have a fructofuranosyl form. The ^{13}C- and ^{1}H-signals of saccharides 1, 2 and 3 were also assigned as shown in Table 2 by the method of 2D NMR analyses reported previously.[16)]

Synthesis of the same saccharides as newly-formed oligosaccharides in the stored burdock was tried from 1-kestose and D-fructose using purified burdock 1-FFT.

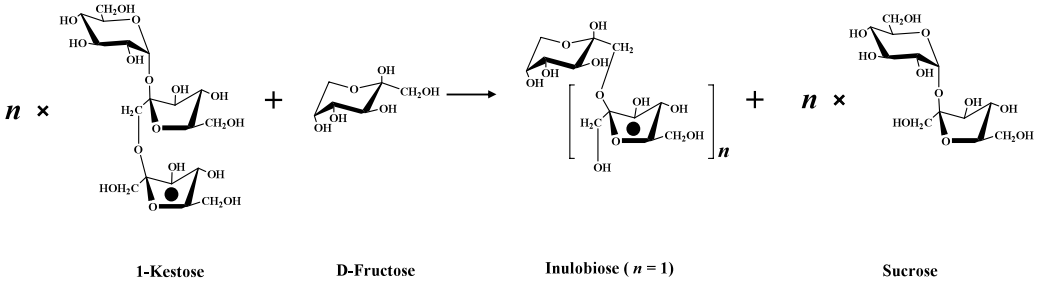


Fig. 6. Synthesis of inulooligosaccharides by fructosyl transfer from 1-kestose to D-fructose by burdock 1-FFT.

The FOS [1^F(1-β-D-fructofuranosyl)$_m$ sucrose, m=2, 3, 4, 5, 6 and 7～] were synthesized from 1-kestose or 1-kestose and D-fructose with the 1-FFT (Figs. 4 (a) and 4 (b)). Saccharides 1′, 2′, 3′ and several other saccharides were synthesized from 1-kestose and D-fructose with the 1-FFT (Fig. 4 (b)), while these saccharides were not synthesized without fructose (Fig. 4 (a)). The time course of enzymatic formation of saccharides 1′, 2′ and 3′ from 1-kestose and D-fructose was examined by HPAEC (Fig. 5). Synthesis of saccharide 1′ proceeded with a higher velocity than that of saccharides 2′ and 3′. The time course study showed that after saccharide 1′ was first synthesized from 1-kestose and D-fructose within 3 h, saccharides 2′ was synthesized from 1-kestose and saccharide 1′, and saccharide 3′ from 1-kestose and saccharide 2′. Elongation of fructofuranosyl units was estimated to occur in this transferred fructofuranosyl residue to produce the oligosaccharides having one, two and more additional fructofuranosyl units (Fig. 6).

Saccharides 1′, 2′ and 3′ were isolated from the reaction mixture containing 1-kestose, fructose and purified burdock 1-FFT using carbon-Celite column chromatography. Saccharides 1′, 2′ and 3′ were shown to be pure as judged by HPAEC and TLC, and have R-sucrose values of 1.55, 2.15 and 2.73 by HPAEC, reducing terminal, molar ratios (reducing sugar to fructose) of 0.50, 0.33 and 0.25, and degree of polymerization of 2, 3 and 4 by TOF-MS, respectively. The methanolyzates of saccharides 1′, 2′ and 3′ gave four peaks corresponding to methyl 1,3,4,6-tetra-*O*-methyl-D-fructoside and methyl 3,4,6-tri-*O*-methyl-fructoside on GLC chromatograms, respectively. ^{13}C- and ^{1}H-NMR spectra of saccharides 1′, 2′ and 3′ were identical to those of saccharides 1, 2 and 3 isolated from the stored burdock. From these results, saccharides 1′, 2′ and 3′ synthesized by 1-FFT were confirmed to be inulobiose, inulotriose and inulotetraose, respectively.

DISCUSSION

The formation of fructosyl-saccharides or fructo-inulinoses was reported first in chicory during growth, storage and forcing by Van den Ende *et al.*[17] and they observed some changes which consisted of a significant increase in fructose concentration; an increase in the concentration of low DP fructans, a decrease of high DP fructans, and the appearance of new short chain fructans without terminal glucose. Later, Van den Ende *et al.*[18] reported that three different reducing FOS, inulobiose (DP 2), inulotriose (DP 3) and inulotetraose (DP 4), were synthesized with inulin as a donor and fructose as an acceptor using a fructan:fructan fructosyltransferase purified from chicory root. These newly produced FOS were also isolated from chicory roots.[19] The same saccharides as inulooligosaccharide series of DP 2 and DP 3 were identified in either sprouted or unsprouted onion bulbs stored for two months.[20] However, although the degree of polymerization was determined, these authors[19,20] did not report the structural conformation of the fructosyl terminal of these isolated saccharides.

In this study, the saccharides 1′, 2′ and 3′ were firstly found to be synthesized from 1-kestose and D-fructose using purified burdock 1-FFT. The suggested reaction of biosynthesis of these three saccharides is as follow: 1-kestose acts as a donor of a fructosyl unit and D-fructopyranose as an acceptor (Fig. 6), although higher FOS (DP ≥ 4) and inulin may also play a significant role as the donor of the fructosyl unit. In fact, the acceptor D-fructose is under the pyranose form because in water almost all free fructose has the pyranose form. This biosynthesis may be induced by the quantity of free D-fructose found in the vacuoles, likely as D-fructopyranose. Because of the continuous release of D-fructose by the hydrolyzing activity of the fuctan 1-exohydrolase (1-FEH), this free D-fructose is used as a substrate to synthesis these saccharides, thus avoiding a high accumulation of fructose which could be detrimental to the metabolic activities in the vacuoles.

The reaction of forming these short-chain reducing FOS, inulobiose, inulotriose or inulotetraose, might be triggered by the accumulation of D-fructose released from the hydrolysis of fructans and/or the pyranosyl form of the free fructose in the vacuoles; the physiological process inducing this reaction is unclear and enigmatic. We suggest that formation of these inulooligosaccharides would play a role of "balance" between the different FOS and a "buffer" between the different tri- and tetra-saccharides, because of the low rate of fructose utilization in the metabolism.

The *in vivo* biosynthesis of new short reducing FOS composed by D-fructose molecules seems to occur under specific conditions such as forcing or storage. However, this reaction is unclear and the questions raised are: (i) what triggers this reaction? (ii) which physiological factor is behind this reaction? and (iii) what are the roles of these newly formed compounds? Further work will answer these questions and lead to a better understanding of the complex metabolism of fructan in plants.

We are indebted to Mr. Y. Takada at the Graduate School of Agriculture, Hokkaido University for ^{13}C- and ^{1}H-NMR measurements.

REFERENCES

1) Y. Nagao: Changes of carbohydrates and chlorogenic acid content in edible burdock during growth and storage. *J. Jpn. Soc. Hortic. Sci.*, **65** (Suppl. 2), 758–759 (1996).
2) H. Takeda, K. Sato, S. Kinoshita and H. Sasaki: Production of 1-kestose by *Scopulariopsis brevicaulis*. *J. Ferment. Bioeng.*, **79**, 242–246 (1994).
3) N. Shiomi, J. Yamada and M. Izawa: Isolation and identification of fructo-oligosaccharides in roots of asparagus (*Asparagus officinalis* L.). *Agric. Biol. Chem.*, **40**, 567–575 (1976).
4) N. Shiomi: Structure of fructo-polysaccharide (asparagosin) from roots of asparagus (*Asparagus officinalis* L.). *New Phytol.*, **123**, 263–270 (1993).
5) R.D. Rocklin and C.A. Pohl: Determination of carbohydrate by anion exchange chromatography with pulse amperometric detection. *J. Liq. Chromatogr.*, **6**, 1577–1590 (1983).
6) D.C. Johnson: Carbohydrate detection gains potential. *Nature*, **321**, 451–452 (1986).
7) N. Shiomi, S. Onodera, N.J. Chatterton and P.A. Harrison: Separation of fructooligosaccharide isomers by anion exchange chromatography. *Agric. Biol. Chem.*, **55**, 1427–1428 (1991).
8) M. Abe, K. Ueno, Y. Ishiguro, T. Omori, S. Onodera and N. Shiomi: Purification, cloning and functional characterization of fructan:fructan 1-fructosyltransferase from edible burdock (*Arctium lappa* L.). *J. Appl. Glycosci*., in press, (2009).
9) S. Hakomori: A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *J. Biochem.*, **55**, 205–208 (1964).
10) I. Brereton, S. Crozier, J. Field and D.M. Doddrell: Quadrature detection in F_1 induced by pulsed field gradients. *J. Magn. Reson.*, **93**, 54–62 (1991).
11) M. Kienlin, C.W. Woonen, A. Toorn and P.M. Zijl: Rapid recording of solvent-suppressed 2D COSY spectra with inherent quadrature detection using pulsed field gradients. *J. Magn. Reson.*, **93**, 423–429 (1991).
12) Y.-C. Li and G.T. Monterione: Solvent saturation-transfer effects in pulsed-field-gradient heteronuclear single-quantum-coherence (PFG-HSQC) spectra of polypeptide and proteins. *J. Magn. Reson.*, **B101**, 315–319 (1993).
13) W. Willker, D. Leibfrits, R. Kerssebaum and W. Bermel: Gradient selection in inverse heteronuclear correlation spectroscopy. *Magn. Reson. Chem.*, **31**, 287–292 (1993).
14) A. Bax and M.F. Summers: ^{1}H and ^{13}C assignment from sensitivity-enhanced detection of heteronuclear multiple-bound connectivity. *J. Am. Chem. Soc.*, **108**, 2093–2094 (1986).
15) K. Furihata and H. Seto: Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett.*, **39**, 7337–7340 (1998).
16) N. Takahashi, H. Okada, E. Fukushi, S. Onodera, T. Nishimoto, J. Kawabata and N. Shiomi: Structural analysis of six novel oligosaccharides synthesized by glucosyl transfer from β-D-glucose 1-phosphate to raffinose and stachyose using *Thermoanaerobacter brockii* kojibiose phosphorylase. *Tetrahedron Assym.*, **16**, 57–63 (2005).
17) W. Van den Ende, A. Mintens, H. Spelleers, A.A. Onuoha and A. Van Laere: The metabolism of fructans in roots of *Cichorium intybus* during growth, storage and forcing. *New Phytol.*, **132**, 555–563 (1993).
18) W. Van den Ende, J. De Roover and A. Van Laere: *In vitro* synthesis of fructosyl- only oligosaccharides from inulin and fructose by purified chicory root fructan : fructan fructosyl transferase. *Physiol. Plant.*, **98**, 346–352 (1996).
19) J.W. Timmermans, T.M. Slughek, M. Izuka, W. Van den Ende, J. De Roover and A. Van Laere: Isolation and structural analysis of new fructans produced by chicory. *J. Carbohydr. Chem.*, **20**, 375–395 (2001).
20) M.K. Ernst, N.J. Chatterton, P.A. Harrison and G. Mattschka: Characterization of fructan oligomers from species of the genus *Allium* L. *J. Plant. Physiol.*, **153**, 53–60 (1998).

ごぼう貯蔵中に新しく生成された還元性フルクトオリゴ糖の単離と構造決定

石黒陽二郎[1]，上野敬司[1]，阿部雅美[1]，小野寺秀一[1]，
福士江里[2]，Noureddine Benkeblia[3]，塩見徳夫[1]
[1] 酪農学園大学大学院酪農学研究科
食品栄養科学専攻
(069–8501 江別市文京台緑町 582)
[2] 北海道大学大学院農学研究科
(060–8589 札幌市北区北 9 条西 9 丁目)
[3]Department of Life Sciences, The University of the West Indies (Mona Campus, Kingston 7, Jamaica)

新鮮ごぼう根を地下 1m の土中で 11 月から翌年の 5 月まで 6 カ月間貯蔵した．この貯蔵ごぼうに末端グルコース残基を有しないフルクトオリゴ糖が新しく生成された．このごぼうから抽出した糖液について電気化学検出器付陰イオン交換高速液体クロマトグラフィー (HPAEC) 分析を行ったところ，糖 1 は 1-kestose (3a) のあとに溶出され，糖 2，3 は nystose (4a)，fructosylnystose (5a) のあとにそれぞれ溶出された．この糖抽出液について活性炭-セライトカラムクロマトグラフィーを行うことにより糖 1，2，3 を単離した．糖 1，2，3 は HPAEC による相対保持時間 (sucrose = 1)，1.55，2.15，2.73 を有し，還元末端をそれぞれもっていた．また，糖 1，2，3 のフルクトースに対する還元糖の比は 0.50，0.33，0.25 であることと TOF-MS 分析の結果から糖 1，2，3 の重合度は 2，3，4 であることがわかった．これらの結果と各糖の NMR 分析，糖メチル誘導体の GLC 分析結果から，糖 1，2，3 はそれぞれ β-D-fructofuranosyl-(2→1)-β-D-fructopyranose (inulobiose)，β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructopyranose (inulotriose)，β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructopyranose (inulotetraose)と同定された．^{13}C-NMR 分析により各糖の還元末端フルクトース残基の 70–80% がピラノース構造をとることがわかった．それぞれの糖の^{13}C-，^{1}H-シグナルの完全帰属も初めて行った．さらに上述のごぼう貯蔵中に生成された inulobiose，inulotriose，inulotetraose と同様の糖が，ごぼうから精製した fructan: fructan 1-fructosyltransferase (1-FFT) のフルクトシル転移作用により 1-kestose と D-フルクトピラノースから合成されることを初めて見出した．