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Detection of Inulin, a Prebiotic Polysaccharide in Maple Syrup

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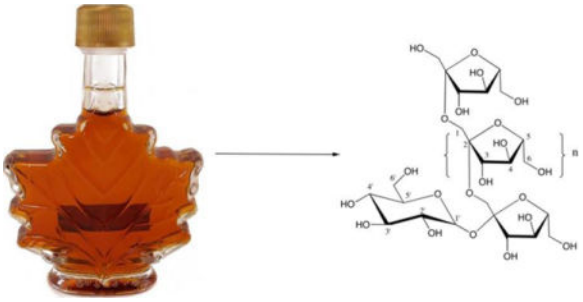
Abstract

Maple syrup is a widely consumed plant-derived natural sweetener produced by concentrating xylem sap collected from certain maple (*Acer*) species. During thermal evaporation of water, natural phytochemical components are concentrated in maple syrup. The polymeric components from maple syrup were isolated by ethanol precipitation, dialysis, and anion exchange chromatography and structurally characterized by glycosyl composition analysis, glycosyl linkage analysis, and nuclear magnetic resonance spectroscopy. Among the maple syrup polysaccharides, one neutral polysaccharide was characterized as inulin with a broad molecular weight distribution, representing the first isolation of this prebiotic carbohydrate from a xylem sap. In addition, two acidic polysaccharides with structural similarity were identified as arabinogalactans derived from rhamnogalacturonan type I pectic polysaccharides.

TOC image



Keywords

maple syrup; polysaccharide; inulin; arabinogalactan

INTRODUCTION

Maple syrup is a natural sweetener produced from concentrated xylem sap collected from certain maple (genus *Acer*) species, primarily the sugar maple (*Acer saccharum* Marsh).[1,2] The sugar maple is widely distributed in the northeastern region of North America with the

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Notes

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majority of maple syrup being commercially produced in this region, primarily in Quebec, Canada.[2] Maple syrup is of great economical importance to the eastern North American region since it is the largest commercially produced and consumed tree-sap derived food product worldwide.[3] As a commercial product that requires minimal processing (thermal evaporation), maple syrup contains several phytochemicals, primarily (poly)phenolics, which have been reported to impart a variety of biological effects. While sucrose is the major component in maple syrup, this plant-derived natural sweetener also contains simple sugars (glucose and fructose), amino acids, minerals (e.g. potassium, calcium, and magnesium), vitamins (e.g. B2 and niacin), organic acids (e.g. succinic and fumaric acid), and complex carbohydrates.[1] Among the various chemical constituents reported from maple syrup, biological evaluation has primarily focused on its diverse phenolic components. For instance, phenolic-enriched extracts of maple syrup have been reported to show anti-oxidant, anti-mutagenic, anti-cancer, anti-inflammatory and anti-neurodegenerative effects.[3–7]

In contrast to the phenolic components of maple syrup, molecules of larger molecular weight, such as oligosaccharides and polysaccharides, have been less investigated. Previous reports have identified dextran, arabinogalactan and rhamnogalacturonan in maple syrup.[8,9] These biopolymers are derived from primary cell wall components of maple trees.

In this study, an inulin type fructan has been isolated for the first time from maple syrup. Inulins are commercially extracted from chicory root and used as dietary fiber in the food industry.[10,11] As one type of poly-fructan, the inulin structure features a polydisperse molecular size and is a non-branched polymer consisting exclusively of β-1,2-frucosyl linkages between fructosyl residues and a terminal glucose.[12,13] Inulin is generally believed to be synthesized from sucrose by fructosyltransferases and to serve as energy storage.[10] Because the linkage type within inulin is resistant to hydrolysis by human digestive enzymes, inulin is considered a non-digestive polysaccharide, which makes its biological effects reside in its interactions with the human lower gastrointestinal tract, primarily in the colon.[10,14,15] Inulin-type fructans have been reported to benefit immune systems by interacting with gut lymphoid tissues,[16,17] colon health by selectively promoting the growth of bifidobacteria and lactobacilli probiotic bacteria,[18,19] and cardiovascular systems by decreasing cholesterol and triglyceride levels in serum.[10,20]

MATERIALS AND METHODS

Materials

Maple syrup (grade C, 40 L) was shipped frozen to our laboratory by the Federation of Maple Syrup Producers of Quebec (Longueuil, Quebec, Canada) and stored at −20 °C. Solvents for nuclear magnetic resonance (NMR) spectroscopy were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

Total carbohydrate content determination

Total carbohydrate content assay was performed in microtiter plates using a colorimetric assay as previously described.[21] Briefly, in each well of a 96-well microtiter plate, 30 μL of

each fraction was added to 100 μL concentrated sulfuric acid and 20 μL 5% phenol solution. The microtiter plates were then incubated at 90 °C for 5 min and recorded for absorbance at490 nm using a Spectramax M2 microplate reader (Molecular Devices, Sunnyville, CA). Glucose was used as the standard for the total carbohydrate content assay.

Extraction of maple syrup polysaccharides

Maple syrup (300 mL, 412 g) was first diluted with 450 mL distilled water followed by 95% ethanol to achieve a 60% ethanol/water solution. Crude polysaccharides were precipitated at −20 °C overnight. Precipitates were centrifuged and dried by sequential solvent exchanges with 100% ethanol and ether. The supernatants were combined and diluted with 95% ethanol to create an 80% ethanol/water solution, resulting in additional precipitation of polysaccharides as described above. Dry precipitates were re-dissolved in 50 mL distilled water and dialyzed through a membrane with molecular weight cut-off (MWCO) of 12,000-14,000 (Spectrum Laboratories, Inc., Rancho Dominguez, CA) against distilled water with stirring for 48 h. The retained polysaccharides were lyophilized to obtain Fr.1 (30 mg) from the 60% ethanol precipitation and Fr.2 (23 mg) from the 80% ethanol precipitation, respectively.

Purification of polysaccharides

Polysaccharides were fractionated on a Hiprep Q Sepharose anion exchange column (100 mm × 16 mm i.d., GE Healthcare Life Sciences, Pittsburgh, PA). 20 mg of Fr.1 were firstly eluted with 65 mL distilled water and then a linear gradient of NaCl (0-1 M) for 200 mL at 5 mL/min (Figure 1). 20 mg of Fr.2 were subjected to the same anion exchange chromatography. The eluate was collected in 5 mL fractions and pooled by total carbohydrate content. All fractions were dialyzed (MWCO 12,000-14,000) against distilled water with stirring for 48 h and lyophilized to obtain Fr.1-1 (10.5 mg, 0.004%, w/w), Fr.1-2 (5.5 mg, 0.002%, w/w), Fr.2-1 (1.1 mg, 0.0003%, w/w) and Fr.2-2 (8.3 mg, 0.002%, w/w).

Homogeneity and molecular size

The homogeneity and molecular sizes of polysaccharides were analyzed using a high performance size exclusion column TSK-gel G3000PW (300 mm × 7.5 mm i.d., TOSOH, Tokyo, Japan) at 40 °C on a Hitachi LaChrom Elite HPLC (Hitachi, Tokyo, Japan) equipped with a refractive index (RI) detector. Standard curves for molecular size determinations were generated using dextran standards (1000, 5000, 12,000, 25,000, 50,000, 80,000 and 150,000 Da).

Glycosyl composition analysis

Glycosyl composition analysis was accomplished with polysaccharide acid hydrolysates using high performance anion exchange chromatograph with pulsed amperometric detection (HPAEC-PAD) as previously described.[22] Briefly, 500 μg of Fr.1-1, Fr.1-2, Fr.2-1 and Fr.2-2 were hydrolyzed with 200 μL of 2M trifluoroacetic acid (TFA) in sealed ampoules at 120 °C for 2 h. The resulting hydrolysates were dried with a stream of nitrogen gas. 200 μL of isopropanol was added and dried by nitrogen gas. This process was repeated twice to remove residual TFA. Due to the fragility of fructose under acidic conditions, an alternate mild acid

hydrolysis for Fr.1-1 was performed.[23] 200 μg Fr.1-1 were hydrolyzed with 200 μL 0.2% TFA in sealed ampoules at 90 °C for 1 h.

Hydrolysates from both hydrolytic reactions were separated on a Hamilton RCX-30 column (250 mm × 4.6 mm i.d., Metrohm, Herisau, Switzerland) on a 9600 Professional IC Vario ion chromatograph with a 945 Professional Vario IC pulsed amperometric detector (Metrohm, Herisau, Switzerland). Monosaccharide standard curves for fucose, arabinose, xylose, rhamnose, galactose, glucose, fructose, galacturonic acid and glucuronic acid were created by HPAEC-PAD on the same column. Each monosaccharide and uronic acid was dissolved in distilled water at six concentrations (10, 20, 50, 100, 500, and 1000 ppm) and an aliquot of 10 μL was injected. Standard curves were created by plotting area under curves (AUCs) with concentrations. For analyzing fucose, arabinose, xylose, rhamnose, galactose, and glucose, the column was eluted with isocratic 16 mM NaOH over 40 min. For analyzing fructose, the column was eluted with isocratic 100 mM NaOH over 20 min. For analyzing glucuronic and galacturonic acids, the column was eluted with isocratic 20 mM sodium acetate in 100 mM NaOH over 20 min.

Glycosyl linkage analysis

Glycosyl linkage analysis was performed as previously described.[24] 800 μg Fr.1-1, Fr.1-2, Fr.2-1 and Fr.2-2 were stirred overnight in 300 μL dimethyl sulfoxide (DMSO). Intact polysaccharides were thrice permethylated in anhydrous DMSO solution of NaOH and methyl iodide for 20 min. The permethylated polysaccharides were extracted with methylene chloride and then hydrolyzed with 200 μL of 2M TFA at 120 °C for 2 h. Permethylated Fr. 1-1 was hydrolyzed with 200 μL 0.2% TFA at 90 °C for 1 h. Hydrolysates were dried with N_2 gas and residual TFA was removed by repeatedly drying with isopropanol. The partially methylated hydrolysates were reduced with $NaBD_4$ and then acetylated with acetic anhydride. The resulting partially methylated acetic acetates (PMAAs) were analyzed on a Series 6890 gas chromatography (Agilent Technologies, Santa Clara, CA) coupled with a mass selective detector (MSD) on a Supelco SP-2330 capillary column (30 m × 0.25 mm i.d., Sigma-Aldrich). The oven temperature was held at 80 °C for 2 min and increased to 170 °C at 30 °C/min, then to 240 °C at 4 °C/min, and held for 5 min.

Nuclear Magnetic Resonance (NMR)

The ^{1}H and ^{13}C NMR spectra and two-dimensional COSY, TOCSY, HSQC and HMBC data were recorded on a Varian 500 MHz NMR spectrometer equipped with a 5 mm OneNMR probe (Agilent Technologies). The samples were D_2O exchanged twice and dissolved in D_2O (0.5 mL, 99.96%). All spectra were recorded at 25 °C.

RESULTS AND DISCUSSION

Crude polysaccharides Fr.1 and Fr.2 were fractionated using a semi-preparative Q Sepharose anion exchange column and polysaccharide- enriched fractions were pooled based on a total carbohydrate content assay.[21] While subject to few interferences, this colorimetric assay is highly sensitive and has wide applicability for estimating the carbohydrate contents of plant natural products.[22] The elution profiles (Figure 1) showed that Fr.1 contained a neutral

polysaccharide fraction Fr.1-1 and an acidic polysaccharide fraction Fr.1-2. Similarly, Fr.2 also contained a neutral polysaccharide fraction Fr.2-1 and an acidic polysaccharide fraction Fr.2-2.

The molecular sizes of the purified polysaccharide components were analyzed by HPSEC-RI. The dispersal of Fr.1-1 across a broad region of elution volume indicated that Fr.1-1 was comprised of polydisperse and large molecular weight (>12,000 Da) polymers. Elution profiles of Fr.1-2 and Fr.2-2 showed symmetric peaks, indicating the molecular weights of Fr.1-2 and Fr.2-2 to be approximately 140,000 Da and 70,000 Da, respectively. Due to the limited quantity of Fr.2-1 and sensitivity of the RI detector, the molecular size of Fr.2-1 was not successfully measured.

The glycosyl and linkage compositions of the purified polysaccharides were evaluated as follows. Because fructose is easily degraded, Fr.1-1 was hydrolyzed with two hydrolytic conditions. Hydrolysis with 2M TFA at 120 °C for 2 h totally degraded fructose, leaving only glucose detectable in the HPAEC profile. Mild acid hydrolysis of Fr.1-1 using 0.2% TFA at 90 °C for 1 h was performed to hydrolyze the glycosidic bonds between fructose residues and preserve the resulting monosaccharides. By comparison to the standard curves, the glycosyl composition of Fr.1-1 (Table 1) contained primarily fructosyl (96%) and glucosyl (4%) residues, which suggested that Fr.1-1 is a poly-fructan with minor inclusion of glucose. The presence of 2-fructosyl residues indicated that the fructosyl residues were linked at position 2, which is characteristic for inulin. Inulin contains a non-branched 1,2-linked fructosyl backbone and terminates with a glucosyl residue (GF_n) (Figure 2).[12] The neutral monosaccharide compositions of Fr.1-2, Fr.2-1 and Fr.2-2 were analyzed on HPAEC-PAD and eluted with isocratic 16 mM NaOH for improved resolution. Neutral polysaccharide Fr.2-1 was composed of arabinosyl (25%), galactosyl (35%) and glucosyl (40%). Together with the presence of a terminal arabinosyl, 3- and 3,6-galactosyl, and 6-glucosyl linkages, Fr.2-1 may be a mixture of arabinogalactan and dextran, which has been speculated to be a microbial derived polysaccharide.[8] Acidic polysaccharides Fr.1-2 and Fr. 2-2 hydrolysates were also analyzed for their uronic acids (Table 1). Together with their neutral monosaccharide compositions, Fr.1-2 and Fr.2-2 appear to share structural similarity. The two polysaccharides were primarily composed of terminal and 5-arabinosyl and 3- and 3,6-galactosyl with a lower percentage of 2,4-rhamnosyl and 4-galacturonic acid residues. These results suggest that Fr.1-2 and Fr.2-2 are arabinogalactans derived from degradation of acidic rhamnogalacturonan type I (RG-I) pectic polysaccharides. These molecules are important plant cell wall components and often contain alternate rhamnosyl and galacturonic acid backbones and arabinogalactan side chains.[25]

The structures of the polysaccharides were characterized by NMR analyses. The ^{1}H NMR spectrum of Fr.1-1 (Figure 3) revealed seven major protons between 3.3 to 4.2 ppm and a very small anomeric proton signal at 4.8 ppm. The ^{13}C NMR and HSQC spectra showed one quaternary carbon at 104.1 ppm, three CH and two CH_2, which suggested characteristic structural features of the ketonic monosaccharide fructose, which lacks an anomeric proton but contains two methylenes at positions 1 and 6. The chemical shifts of Fr.1-1 were assigned using two-dimensional NMR experiments (Table 2). Briefly, with the correlations in COSY, the chemical shifts of proton 3–6 in the 2-fructosyl were assigned as H-3 at δ 4.02,

H-4 at δ 3.93, H-5 at δ 3.79 and H-6 at δ 3.40/3.74. The chemical shifts of carbon 3–6 in the 2-fructosyl were assigned accordingly with correlations in HSQC spectra as C-3 at δ 76.4, C-4 at δ 75.3, C-5 at δ 80.2 and C-6 at δ 63.3. The two doublets at δ 3.52 and δ 3.60 correlate to one carbon at δ 60.0, which were assigned as the proton and carbon chemical shifts of the H-1/C-1 methylene in 2-fructosyl. A correlation was observed between C2 and H1 in the HMBC spectrum, which further confirmed that the fructosyl residues were linked at position 2. The small proton signal at 4.8 ppm was attributed to the anomeric proton of glucose. The ^{1}H NMR spectra of Fr.1-2, Fr.2-1 and Fr.2-2 showed only proton resonances belonging to carbohydrates (anomeric protons, 4.4-5.5 ppm; clustered resonances between 3.4 ppm and 4.0 ppm)[26], suggesting that only polysaccharides were present in the samples. The proton resonances appearing near 1.1 ppm for Fr.1-2 and Fr.2-2 are characteristic for the rhamnose methyl group at position 5. However, further investigations on these molecules by 2D NMR analyses are needed to better define their complete structural features.

Maple syrup is widely consumed as a functional food in large part because of its phenolic constituents which are believed to impart health benefits. For example, quebecol is a phenolic compound from maple syrup[27] that possesses anti-inflammatory[28] and anti-proliferative properties.[29] A variety of phenolic derivatives from maple syrup demonstrate free radical scavenging activity superior to that of vitamin C.[3] Several polyphenolic molecules present in maple syrup show anti-proliferative effects through arresting cell cycles, suggesting potential effects in cancer prevention.[30] In addition, *in vivo* studies on phenolic-enriched maple syrup extracts have shown promising therapeutic potential for liver protection,[31] anti-inflammation[32] and anti-diabetes.[33,34]

The polysaccharides dextran, arabinogalactan, and rhamnogalacturonan have been previously identified from maple syrup.[8,9] Dextran is a common polysaccharide synthesized from sucrose and prominently features repeating units of α-6-linked glucose.[35] Previous studies[8,9] have attributed the glycosyl compositions and glycosydic linkages of arabinogalactans and rhamnogalacturonans to partially degraded pectins. Pectins are heteropolysaccharides and are one of the major structural components of plant cell walls.[36] The heterogeneous backbone of pectin is composed of homogalacturonan (HG) with repeating 4)-α-Gal*p*A-(1, and rhamnogalacturonan (RG) with the alternating disaccharide 4)-α-Gal*p*A-(1,2)-α-L-Rha*p*-(1,.[37] Arabinogalactan, a major side chain branched at C-4 of rhamnosyl residues, contains a linear 6-linked galactan substituted at C-3 with arabinoses.[38]

Herein, inulin is being reported from maple syrup for the first time. Inulins are widely present in several plant families including Liliaceae, Amaryllidaceae, Gramineae, and Compositae. Many edible fruits and vegetables such as banana, onion, artichoke, chicory roots, garlic, and leek contain inulin.[39] However, this study is the first report of inulin-type fructooligosaccharides isolated from xylem sap of higher plants. The identification of inulin in maple syrup, along with its presence in other natural plant-derived sweeteners including agave[40] and stevia,[41] raises the possibility of this prebiotic polysaccharide potentially contributing to the overall health benefits reported for these foods.

Ecologically, besides serving as an energy storage carbohydrate, plant cells regulate osmotic potential by quickly altering the degree of polymerization of inulin. This function allows

plants to withstand cold temperatures and dehydration.[42,43] This phenomenon also encourages future investigations on the quantity of inulin present in maple sap collected at various times during the tapping season to potentially maximize its inclusion in maple-derived food products.

In addition to its low caloric value, the prebiotic effects of inulin have been reported.[19,44–46] Because inulin passes through the human upper gastrointestinal tract (GIT) intact, it interacts primarily with human gut microbiota. Prebiotic molecules such as inulin[47,48] have been shown to selectively enhance the growth of beneficial bacteria, such as species of *Bifidobacterium* and *Lactobacillus*, while suppressing the growth of pathogenic strains.

In this study, the presence of inulin in maple syrup potentially supports an investigation by Cochu *et al.*[49] who compared the growth of probiotic *Lactobacillus* sp. when using raw maple sap and sucrose as carbon sources. Results showed that maple sap based media increased the viable cell counts of two strains of lactobacilli, *Lactobacillus helveticus* R0052 and *Lactobacillus acidophilus* AC-10, and enhanced their lactic acid production. This study suggested that molecules in the raw maple sap possess prebiotic effects and potentially provide health benefits to the GIT. Evidence[44,46] shows that bifidobacteria and lactobacilli have different preferences for utilizing inulin-type fructooligosaccharides of different chain lengths. This suggests that despite having the same repeating unit, maple syrup-derived inulin could potentially have unique effects on GIT bacteria. Thus, while our finding that maple syrup contains inulin adds to the potential health benefits of this food, the overall contribution of this inulin polysaccharide remains unclear given the lack of supporting in vivo data. Further investigation to clarify the contributions of inulin to the overall benefits of maple syrup is currently being pursued by our group.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ball DW. The chemical composition of maple syrup. J Chem Educ. 2007; 84:1647.
2. Perkins TD, van den Berg AK. Maple syrup-production, composition, chemistry, and sensory characteristics. Adv Food Nutr Res. 2009; 56:101–143. [PubMed: 19389608]
3. Li L, Seeram NP. Maple syrup phytochemicals include lignans, coumarins, a stilbene, and other previously unreported antioxidant phenolic compounds. J Agric Food Chem. 2010; 58:11673–11679. [PubMed: 21033720]

4. Legault J, Girard-Lalancette K, Grenon C, Dussault C, Pichette A. Antioxidant activity, inhibition of nitric oxide overproduction, and *in vitro* antiproliferative effect of maple sap and syrup from *Acer saccharum*. J Med Food. 2010; 13:460–468. [PubMed: 20132041]
5. Thériault M, Caillet S, Kermasha S, Lacroix M. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. Food Chem. 2006; 98:490–501.
6. Hawco CLA, Wang Y, Taylor M, Weaver DF. A maple syrup extract prevents β-amyloid aggregation. Can J Neurol Sci. 2016; 43:198–201. [PubMed: 26336085]
7. Aaron C, Beaudry G, Parker JA, Therrien M. Maple syrup decreases TDP-43 proteotoxicity in a *Caenorhabditis elegans* model of amyotrophic lateral sclerosis (ALS). J Agric Food Chem. 2016; 64:3338–3344. [PubMed: 27071850]
8. Storz G, Darvill AG, Albersheim P. Characterization of polysaccharides isolated from maple syrup. Phytochemistry. 1986; 25:437–441.
9. Adams GA, Bishop CT. Constitution of an arabinogalactan from maple sap. Can J Chem. 2011; 38:2380–2386.
10. Flamm G, Glinsmann W, Kritchevsky D, Prosky L, Roberfroid M. Inulin and oligofructose as dietary fiber: a review of the evidence. Crit Rev Food Sci Nutr. 2001; 41:353–362. [PubMed: 11497328]
11. Apolinário AC, de Lima Damasceno BPG, de Macêdo Beltrão NE, Pessoa A, Converti A, da Silva JA. Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. Carbohydr Polym. 2014; 101:368–378. [PubMed: 24299785]
12. Cérantola S, Kervarec N, Pichon R, Magné C, Bessieres M-A, Deslandes E. NMR characterisation of inulin-type fructooligosaccharides as the major water-soluble carbohydrates from *Matricaria maritima* (L.). Carbohydr Res. 2004; 339:2445–2449. [PubMed: 15388360]
13. Mancilla-Margalli NA, López MG. Water-soluble carbohydrates and fructan structure patterns from *Agave* and *Dasylirion* species. J Agric Food Chem. 2006; 54:7832–7839. [PubMed: 17002459]
14. Mussatto SI, Mancilha IM. Non-digestible oligosaccharides: A review. Carbohydr Polym. 2007; 68:587–597.
15. Mensink MA, Frijlink HW, van der Voort Maarschalk K, Hinrichs WLJ. Inulin, a flexible oligosaccharide. II: Review of its pharmaceutical applications. Carbohydr Polym. 2015; 134:418–428. [PubMed: 26428143]
16. Seifert S, Watzl B. Inulin and oligofructose: review of experimental data on immune modulation. J Nutr. 2007; 137:2563S–2567S. [PubMed: 17951503]
17. Pool-Zobel BL. Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. Br J Nutr. 2005; 93:S73–S90. [PubMed: 15877900]
18. Kolida S, Tuohy K, Gibson GR. Prebiotic effects of inulin and oligofructose. Br J Nutr. 2002; 87:S193–S197. [PubMed: 12088518]
19. Kolida S, Gibson GR. Prebiotic capacity of inulin-type fructans. J Nutr. 2007; 137:2503S–2506S. [PubMed: 17951493]
20. Kritchevsky D. The rationale behind the use of diet and dietary ingredients to lower blood cholesterol levels. Trends Food Sci Technol. 1994; 5:219–224.
21. Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S-I, Lee YC. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal Biochem. 2005; 339:69–72. [PubMed: 15766712]
22. Sun J, Marais JPJ, Khoo C, LaPlante K, Vejborg RM, Givskov M, Tolker-Nielsen T, Seeram NP, Rowley DC. Cranberry (*Vaccinium macrocarpon*) oligosaccharides decrease biofilm formation by uropathogenic Escherichia coli. J Funct Foods. 2015; 17:235–242. [PubMed: 26613004]
23. Nguyen SK, Sophonputtanaphoca S, Kim E, Penner MH. Hydrolytic methods for the quantification of fructose equivalents in herbaceous biomass. Appl Biochem Biotechnol. 2009; 158:352–361. [PubMed: 19333562]
24. York WS, Darvill AG, McNeil M, Stevenson TT. Isolation and characterization of plant cell walls and cell wall components. Methods Enzymol. 1986; 118:3–40.
25. Yapo BM. Rhamnogalacturonan-I: a structurally puzzling and functionally versatile polysaccharide from plant cell walls and mucilages. Polym Rev. 2011; 51:391–413.

26. Bubb WA. NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity. Concepts Magn Reson. 2003; 19A:1–19.
27. Li L, Seeram NP. Quebecol, a novel phenolic compound isolated from Canadian maple syrup. J Funct Foods. 2011; 3:125–128.
28. Cardinal S, Azelmat J, Grenier D, Voyer N. Anti-inflammatory properties of quebecol and its derivatives. Bioorg Med Chem Lett. 2016; 26:440–444. [PubMed: 26691759]
29. Pericherla K, Shirazi AN, Kameshwara Rao V, Tiwari RK, DaSilva N, McCaffrey KT, Beni YA, González-Sarrías A, Seeram NP, Parang K, Kumar A. Synthesis and antiproliferative activities of quebecol and its analogs. Bioorg Med Chem Lett. 2013; 23:5329–5331. [PubMed: 23953195]
30. González-Sarrías A, Li L, Seeram NP. Anticancer effects of maple syrup phenolics and extracts on proliferation, apoptosis, and cell cycle arrest of human colon cells. J Funct Foods. 2012; 4:185–196.
31. Watanabe Y, Kamei A, Shinozaki F, Ishijima T, Iida K, Nakai Y, Arai S, Abe K. Ingested maple syrup evokes a possible liver-protecting effect-physiologic and genomic investigations with rats. Biosci, Biotechnol Biochem. 2011; 75:2408–2410. [PubMed: 22146721]
32. Kamei A, Watanabe Y, Shinozaki F, Yasuoka A, Kondo T, Ishijima T, Toyoda T, Arai S, Abe K. Administration of a maple syrup extract to mitigate their hepatic inflammation induced by a high-fat diet: a transcriptome analysis. Biosci, Biotechnol Biochem. 2015; 79:1893–1897. [PubMed: 25982262]
33. St-Pierre P, Pilon G, Dumais V, Dion C, Dubois M-J, Dubé P, Desjardins Y, Marette A. Comparative analysis of maple syrup to other natural sweeteners and evaluation of their metabolic responses in healthy rats. J Funct Foods. 2014; 11:460–471.
34. Nagai N, Ito Y, Taga A. Comparison of the enhancement of plasma glucose levels in type 2 diabetes Otsuka Long-Evans Tokushima Fatty rats by oral administration of sucrose or maple syrup. J Oleo Sci. 2013; 62:737–743. [PubMed: 24005018]
35. Hehre EJ. Studies on the enzymatic systhesis of dextran from sucrose. J Biol Chem. 1946; 163:221–233. [PubMed: 21023643]
36. Ridley BL, O'Neill MA, Mohnen DA. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry. 2001; 57:929–967. [PubMed: 11423142]
37. Caffall KH, Mohnen D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr Res. 2009; 344:1879–1900. [PubMed: 19616198]
38. Cipriani TR, Mellinger CG, de Souza LM, Baggio CH, Freitas CS, Marques MCA, Gorin PAJ, Sassaki GL, Iacomini M. A Polysaccharide from a tea (Infusion) of *Maytenus ilicifolia* leaves with anti-ulcer protective effects. J Nat Prod. 2006; 69:1018–1021. [PubMed: 16872136]
39. Coussement PA. Inulin and oligofructose: safe intakes and legal status. J Nutr. 1999; 129:1412S–7S. [PubMed: 10395609]
40. López MG, Mancilla-Margalli NA, Mendoza-Diaz G. Molecular structures of fructans from *Agave tequilana* Weber var. azul. J Agric Food Chem. 2003; 51:7835–7840. [PubMed: 14690361]
41. de Oliveira AJB, Gonçalves RAC, Chierrito TPC, Santos dos MM, de Souza LM, Gorin PAJ, Sassaki GL, Iacomini M. Structure and degree of polymerisation of fructooligosaccharides present in roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni. Food Chem. 2011; 129:305–311.
42. Niness KR. Inulin and oligofructose: What are they? J Nutr. 1999; 129:1402S–1406s. [PubMed: 10395607]
43. Boeckner LS, Schnepf MI, Tungland BC. Inulin: a review of nutritional and health implications. Adv Food Nutr Res. 2001; 43:1–63. [PubMed: 11285681]
44. Rossi M, Corradini C, Amaretti A, Nicolini M, Pompei A, Zanoni S, Matteuzzi D. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. Appl Environ Microbiol. 2005; 71:6150–6158. [PubMed: 16204533]
45. Barboza M, Sela DA, Pirim C, LoCascio RG, Freeman SL, German JB, Mills DA, Lebrilla CB. Glycoprofiling bifidobacterial consumption of galacto-oligosaccharides by mass spectrometry reveals strain-specific, preferential consumption of glycans. Appl Environ Microbiol. 2009; 75:7319–7325. [PubMed: 19801485]

46. Saulnier DMA, Molenaar D, de Vos WM, Gibson GR, Kolida S. Identification of prebiotic fructooligosaccharide metabolism in *Lactobacillus plantarum* WCFS1 through microarrays. Appl Environ Microbiol. 2007; 73:1753–1765. [PubMed: 17261521]

47. Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P. An overview of the last advances in probiotic and prebiotic field. LWT-Food Sci Technol. 2013; 50:1–16.

48. Tako E, Glahn RP, Welch RM, Lei X, Yasuda K, Miller DD. Dietary inulin affects the expression of intestinal enterocyte iron transporters, receptors and storage protein and alters the microbiota in the pig intestine. Br J Nutr. 2008; 99:472–480. [PubMed: 17868492]

49. Cochu A, Fourmier D, Halasz A, Hawari J. Maple sap as a rich medium to grow probiotic lactobacilli and to produce lactic acid. Lett Appl Microbiol. 2008; 47:500–507. [PubMed: 19120917]

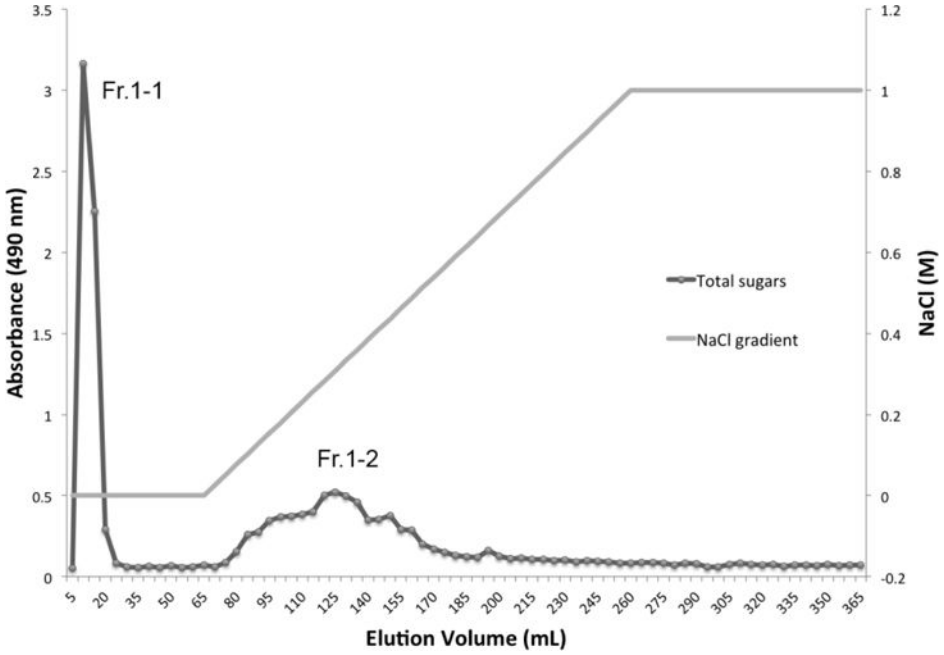

Figure 1.
The elution profile of Fr.1 by anion exchange chromatography.

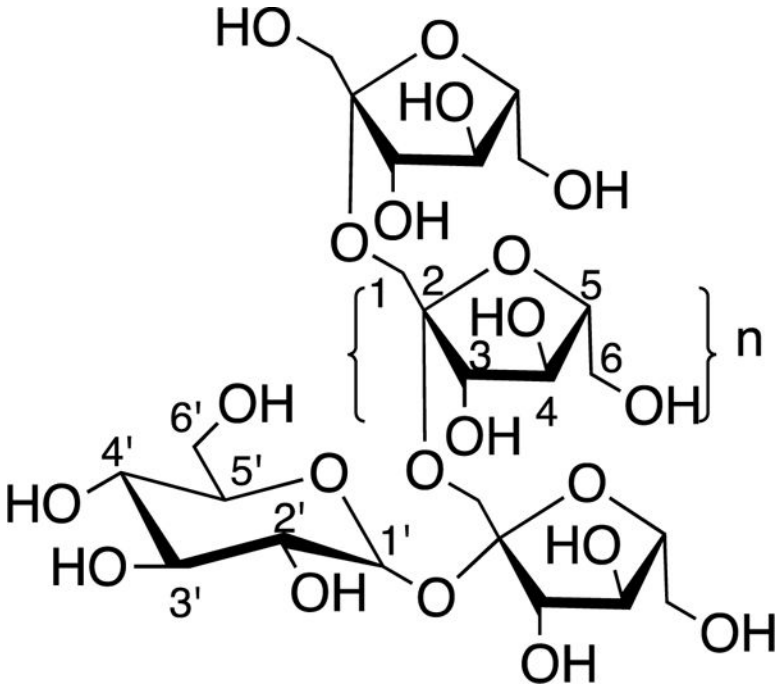

Figure 2.
General chemical structure of inulin.

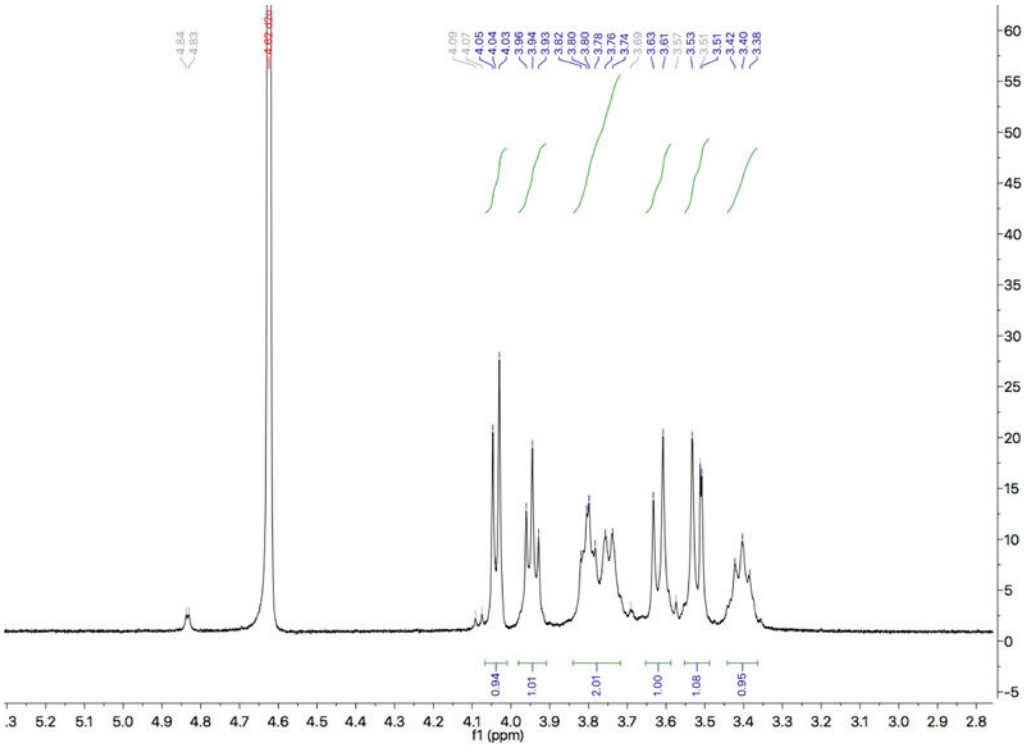

Figure 3.
^{1}H NMR spectrum of Fr.1-1.

Table 1

Glycosyl compositions of Fr.1-1, Fr.1-2, Fr.2-1 and Fr.2-2.

Glycosyl residue	Fr.1-1	Fr.1-2	Fr.2-1	Fr.2-2
		Mole %		
Rhamnosyl	0	9	0	7
Arabinosyl	0	35	25	42
Galactosyl	0	47	35	40
Glucosyl	4	1	40	2
Mannosyl	0	1	0	2
Fructosyl	96	0	0	0
Galacturonic acid	0	7	0	7

Table 2

^{1}H and ^{13}C NMR chemical shift assignments of Fr.1-1 in parts per million (ppm).

Residue	H-1/C-1	H-2/C-2	H-3/C-3	H-4/C-4	H-5/C-5	H-6/C-5
2-Fructosyl	3.52/3.60	-	4.02	3.93	3.79	3.40/3.74
	60.0	104.1	76.4	75.3	80.2	63.3
Terminal Glucosyl	4.82	3.42	3.73	-	-	-
	-	-	-	-	-	-