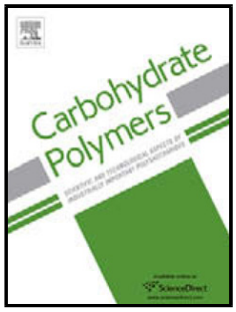


Title: Chemical characterization and prebiotic activity of fructo-oligosaccharides from *Stevia rebaudiana* (Bertoni) roots and *in vitro* adventitious root cultures

Author: Sheila Mara Sanches Lopes Mariane Grigio Francisco Bruna Higashi Rafaela Takako Ribeiro de Almeida Gabriela Krausová Eduardo Jorge Pilau José Eduardo Gonçalves Regina Aparecida Correia Gonçalves Arildo José Braz de Oliveira

PII: S0144-8617(16)30832-3
DOI: http://dx.doi.org/doi:10.1016/j.carbpol.2016.07.043
Reference: CARP 11342

To appear in:

Received date: 28-4-2016
Revised date: 5-7-2016
Accepted date: 12-7-2016

Please cite this article as: Lopes, Sheila Mara Sanches., Francisco, Mariane Grigio., Higashi, Bruna., Almeida, Rafaela Takako Ribeiro de., Krausová, Gabriela., Pilau, Eduardo Jorge., Gonçalves, José Eduardo., Gonçalves, Regina Aparecida Correia., & Oliveira, Arildo José Braz de., Chemical characterization and prebiotic activity of fructo-oligosaccharides from Stevia rebaudiana (Bertoni) roots and in vitro adventitious root cultures.*Carbohydrate Polymers* http://dx.doi.org/10.1016/j.carbpol.2016.07.043

Chemical characterization and prebiotic activity of fructo-oligosaccharides from *Stevia rebaudiana* (Bertoni) roots and *in vitro* adventitious root cultures

Sheila Mara Sanches Lopes[a], Mariane Grigio Francisco[b], Bruna Higashi[a], Rafaela Takako Ribeiro de Almeida[c], Gabriela Krausová[d], Eduardo Jorge Pilau[c], José Eduardo Gonçalves[e], Regina Aparecida Correia Gonçalves[a,b], Arildo José Braz de Oliveira[a,b*]

[a] Graduate Program in Pharmaceutical Sciences, State University of Maringá, Ave. Colombo 5790, 87.020-900, Maringá, Brazil.

[b] Department of Pharmacy, State University of Maringá, Ave. Colombo 5790, 87.020-900, Maringá, Brazil.

[c] Department of Chemistry, State University of Maringá, Ave. Colombo 5790, 87.020-900, Maringá, Brazil.

[d] Department of Microbiology and Technology, Dairy Research Institute, Ke Dvoru 12a, 160 00 Prague, Czech Republic.

[e] Program of Master in Health Promotion, University Center of Maringá, Ave. Guedner, 1610, 87.050-900, Maringá, Brazil.

*Corresponding author: State University of Maringá, Ave. Colombo 5790, 87.020-900, Maringá, Brazil. Tel.: +55 44-3011-4871; e-mail address: ajboliveira@uem.br

E-mail addresses: sanches_sheila@hotmail.com (S.M. Sanches Lopes), mariane_fco@hotmail.com (M. Grigio Francisco), brunahigashi@hotmail.com (B. Higashi), rafaela.takako@gmail.com (R.T. Ribeiro de Almeida) gabika.kunova@gmail.com (G. Krausová), epilau@gmail.com (E.J. Pilau) jose.goncalves@unicesumar.edu.br (E.J. Gonçalves), racgoncalves@uem.br (R.A. Correia Gonçalves).

FOSs, Fructo-oligosaccharides; DP, Degree of Polymerization; DP_n, Average Degree of Polymerization; MS, Murashige Skoog medium; GI, Growth Index; 1-SST, Sucrose: Sucrose 1-Fructosyltransferase; 1-FFT, Fructan: Fructan 1-Fructosyltransferases

Highlights

- *In vitro* adventitious root cultures of *Stevia rebaudiana* in a roller bottle system.
- Extraction of fructo-oligosaccharides (FOSs) from *Stevia rebaudiana* roots and adventitious root cultures.
- Similar chemical profiles of the extracts analyzed by NMR, GC-MS and off-line ESI-MS.
- FOSs from *Stevia rebaudiana* enhanced the growth of both bifidobacteria and lactobacilli with strains specificity.

ABSTRACT

Stevia rebaudiana (Bertoni) is widely studied because of its foliar steviol glycosides. Fructan-type polysaccharides were recently isolated from its roots. Fructans are reserve carbohydrates that have important positive health effects and technological applications in the food industry. The objective of the present study was to isolate and characterize fructo-oligosaccharides (FOSs) from *S. rebaudiana* roots and *in vitro* adventitious root cultures and evaluate the potential prebiotic effect of these molecules. The *in vitro* adventitious root cultures were obtained using a roller bottle system. Chemical analyses (gas chromatography-mass spectrometry, ^{1}H nuclear magnetic resonance, and off-line electrospray ionization-mass spectrometry) revealed similar chemical properties of FOSs that were obtained from the different sources. The potential prebiotic effects of FOSs that were isolated from *S. rebaudiana* roots enhanced the growth of both bifidobacteria and lactobacilli, with strains specificity in their fermentation ability.

Chemical compounds studied in this article: Inulin from chicory (PubChem CID: 16219508); Fructose (PubChem CID: 5984); Glucose (PubChem CID: 5793); Sucrose (PubChem CID: 5988); Agar (PubChem CID: 76645041); α-Naphthaleneacetic Acid (PubChem CID: 6862); Trifluoroacetic Acid (PubChem CID: 6422); Ethanol (PubChem CID: 702); Tween 80 (PubChem CID: 86289060); L-Cysteine Hydrochloride (PubChem CID: 60960).

Keywords: Fructo-oligosaccharides, *Stevia rebaudiana*, adventitious root cultures, prebiotic potential

1. Introduction

Stevia rebaudiana (Bertoni) is a perennial herb that belongs to the Asteraceae family. It is native to South America and widely known for its foliar diterpenoid steviol glycoside content, with a sweet flavor and low calories (Lemus-Mondaca, Vega-Gálvez, Zura-Bravo, & Ah-Hen, 2012; Moraes, Donega, Cantrell, Mello, & McChesney, 2013). Previous studies have reported the presence of fructan-type polysaccharides that have commercial applications, such as inulin with a high degree of polymerization (DP) (Lopes et al., 2015) and fructan fractions (DP = 17) from their roots (Oliveira et al., 2011).

Fructans are a group of non-digestible carbohydrates that have a linear structure comprising (2→1)-linked *β*-D-fructofuranosyl units, mostly ending with a glucose residue (Fig. 1) (Verspreet, Dornez, Van den Ende, Delcour, & Courtin, 2015a). These native molecules are a mixture of oligomer and polymer chains with a variable number of fructose molecules. Fructo-oligosaccharides (FOSs) are short-chain fructans (DP = 3-8). Fructan molecules with DP > 10 are denominated as inulin (Karimi, Azizi, Ghasemlouc, & Vaziri, 2015; Meyer, Bayarri, Tárrega, & Costell, 2011; Villegas, Tárrega, Carbonell, & Costell, 2010).

Inulin and FOSs have applications as functional food and prebiotic nutrients (Morris & Morris, 2012; Caleffi et al., 2015; Verspreet et al., 2015a). The *β*-configuration of anomeric carbon C2 in these molecules makes it a non-digestible carbohydrate in the upper portion of the human intestinal tract, but they can be fermented in the colon for a limited number of beneficial colonic bacteria that selectively influence the growth and/or activity of microbiota (Morris & Morris, 2012; Roberfroid et al., 2010).

The fermentation process that is associated with these molecules results in various beneficial effects, such as improvements in the intestinal absorption of minerals, increases in the cellularity and number of crypts that result from the production of short-chain fatty acids (Lobo et al., 2011; Pitaressi, Tripodo, Cavallaro, Palumbo, & Giammona, 2008), a reduction of triglyceride and cholesterol levels, the modulation of hyperglycemia (Nishimura et al., 2015), and improvements in the efficiency of the immune system (Dwivedi, Kumar, Laddha, & Kemp, 2016; Moreno-Vilet et al., 2014; Peshv & Van den Ende, 2015).

In addition to their health benefits, fructans have important technological properties as a replacement for fat and sugar in low-calorie food and as a texturizing agent that is related to the DP of these molecules (Aravind, Sissons, Fellows, Blazek, & Gilbert, 2012; Crispín-Isidro, Lobato-Calleros, Espinosa-Andrews, Alvarez-Ramirez, & Vernon-Carter, 2015). Fructo-oligosaccharides are used as dietary fiber and a sugar replacement because of their high solubility and characteristic sweetness (Sołowiej et al., 2015; Villegas et al., 2010).

In vitro plant cell, tissue, or organ cultures are alternative methods for the cultivation of whole plants with active metabolism and a high rate of biomass production. Adventitious roots can be used as raw materials in industry as an option that replace the extrativism of medicinal plants (Baque, Moh, Lee, Zhong, & Paek, 2012; Silja & Satheeshkumar, 2015; Thiyagarajan & Venkatachalam, 2012). These biotechnological methods are sustainable production techniques used for obtaining bioactive compounds with interest in pharmaceutical, food, and cosmetic industry (Baque et al., 2012; Cui et al., 2011; Lee & Paek et al., 2012; Lulu, Park, Ibrahim, & Paek, 2015).

The isolation of FOSs with a low DP from *S. rebaudiana* roots has not been

performed previously. Only inulin molecules with a high DP from *S. rebaudiana* roots have been characterized (Oliveira et al., 2011; Lopes et al., 2015). Considering the commercial interest of FOS molecules and importance of biotechnological methods as sustainable production techniques for bioactive compounds, the objective of the present study was to isolate and characterize FOSs from *S. rebaudiana* roots and *in vitro* adventitious roots that were cultured in a roller bottle system. We also evaluated the potential prebiotic effects of these molecules *in vitro*.

2. Materials and Methods

2.1 Plant and chemical materials

Stevia rebaudiana (Bertoni) specimens were identified by Jimi Naoki Nakagima (Federal University of Uberlândia) in March 2008. A voucher specimen (14301-HUEM) was deposited at the Herbarium of the State University of Maringá, Brazil. The *S. rebaudiana* seeds and cultivar roots were obtained at the Iguatemi Research Station of the State University of Maringá.

We used fructo-oligosaccharide (Orafti® P95, Beneo-Orafti, Belgium) with a DP < 10, product of the partial enzymatic hydrolysis of chicory inulin. Fructose, glucose, and a myo-inositol standard were purchased from Sigma-Aldrich. All of the other reagents and nutrients for the culture medium were of analytical grade.

2.2 Culture of S. rebaudiana adventitious roots in a roller bottle system

Aseptic cultures of *S. rebaudiana* adventitious roots were obtained by organogenesis from shoots originated by *in vitro* seed germination in Murashige and Skoog (1962) nutrient medium that was supplemented with 30 g/L D-sucrose, 1% agar (w/v), and 2.0 mg/L α-naphthaleneacetic acid (NAA; i.e., a phytoregulator that induces

adventitious root formation). The cultures were maintained at 28 ± 1°C with a 16 h photoperiod at a photon flux density of 20-50 mmol m^{-2} s^{-1} from daylight fluorescent lights (Reis et al., 2011).

Approximately 0.05 g of fresh roots that were obtained from *in vitro* shoots was transferred to Schott-type flasks (15 cm length, 6 cm diameter, and 250 ml volume) that contained 15 ml of liquid medium with 33.3% strength Murashige and Skoog (MS/3) medium that was supplemented with 30 g/L D-sucrose and 2.0 mg/L α-NAA. The *S. rebaudiana* adventitious roots were cultivated in a roller bottle system at 2 rotations per minute at 25 ± 1°C under dark and light conditions.

The growth curve of *S. rebaudiana* adventitious roots was determined over 5 weeks. Two independent experiments were performed to evaluate the reproducibility of the root growth profile. The growth index (GI) of the adventitious roots was calculated as the following, based on the fresh weight of final roots (FW_f) and fresh weight of inoculated roots (FW_i) (Silja & Satheeshkumar, 2015):

$$\text{Growth Index (GI)} = \frac{FW_f - FW_i}{FW_i}$$

2.3 Total sugar determination

The total carbohydrate content in the extracts was determined by the phenol-sulfuric acid method with D-fructose as the standard (Dubois, Gilles, Hamilton, Reberes, & Smith, 1956).

2.4 Fructo-oligosaccharide extraction

The *S. rebaudiana* roots were dried in a drying oven at 48 °C for 3 days, milled, and extracted with water under reflux conditions at 80 ºC for 5 h. The aqueous extract

was filtered and concentrated in a rotary evaporator. The crude aqueous extract was precipitated with ethanol PA (1:3; v/v). The material was refrigerated overnight at 4 ºC and then centrifuged at 6000 × *g* for 20 min. The ethanolic supernatant was collected, stored in freezer and lyophilized (Fig. S1), yielding the dry extract of the soluble fructan fraction (SFF).

The *S. rebaudiana* adventitious roots were lyophilized and milled separately each week and extracted with distilled water under reflux conditions at 80 ºC for 5 h. The aqueous extract was filtered and concentrated in a rotary evaporator up to a final volume of 20 ml. The extract was then lyophilized, resulting in the total fructan extract (TFE). The TFE from 28-day-old roots was used to characterize the fructan content of *S. rebaudiana* adventitious roots.

2.5 Monosaccharide composition

The sugar composition was determined by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A MSD mass spectrometer. The samples were hydrolyzed with 0.5 M trifluoroacetic acid at 60°C for 1 h, followed by an oxime-trimethylsilyl derivatization reaction (Lopes et al., 2015). Myo-inositol was added to the samples as the internal standard.

The oxime-silylated derivatives (dissolved in 200 µl of hexane) were analyzed by GC-MS using an HP5-MS UI-Agilent with a fused silica capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas, with an oven temperature of 170-210°C (2 °C/min). The injector and interface were kept at 280 ºC and 260 ºC, respectively. The injections were made in split mode with a split ratio of 1:40. The mass spectrometer was operated in electron impact (EI) mode at 70 eV. The quadrupole and

source temperatures were 150 ºC and 230 °C, respectively.

The compounds were identified using the NIST Mass Spectral Library Database (NIST 11) and with comparisons of the mass spectra and retention times of glucose and fructose standards that were analyzed under the same conditions. The monosaccharide concentrations were obtained by analyzing the relative area integration of the chromatographic peaks that were corrected to the internal standard (Lopes et al., 2015).

2.6 ^{1}H nuclear magnetic resonance spectroscopy

The nuclear magnetic resonance (NMR) spectra were recorded at 298 K using a Bruker Avance III HD spectrometer that was operated at 500.00 MHz for the ^{1}H nucleus using the standard pulse sequences that are available in the Bruker software. The fructo-oligosaccharide samples were dissolved in 99.95% D_2O (20 mg/0.7 ml). The chemical shifts (δ) are expressed in parts per million. The chemical shifts were compared with the FOS sample (Orafti® P95) and the literature data (Caleffi et al., 2015; Lopes et al., 2015).

2.7 Electrospray ionization-mass spectrometry

The SFF and TFE were introduced to the Quattro Micro API mass spectrometer (Waters) using a syringe pump (40 µl/min) for off-line electrospray ionization-mass spectrometry (ESI-MS) analysis. The mass spectra were obtained in positive ionization mode, with a capillary voltage of 3500 V, cone voltage of 70 V, source temperature or 120 °C, and desolvation temperature of 350 °C. Each spectrum was produced by the accumulation of data over 1 min (Oliveira et al., 2011).

2.8 Prebiotic effect

2.8.1 Bacterial strains

To test prebiotic effects *in vitro*, five strains of lactobacilli and five strains of bifidobacteria were used. Strains with a CCDM identification number were obtained from the Culture Collection of Dairy Microorganisms (CCDM, Laktoflora, Czech Republic). The JKM and JOV strains were obtained from the Czech University of Life Sciences (Prague, Czech Republic) and were isolates from infant feces. The other strains were obtained from the Dairy Research Institute (Tabor, Czech Republic). The commercial strain *Bif. animalis* subsp. *lactis* Bb12 was purchased from Chr. Hansen (Starovice, Czech Republic).

2.8.2 Bacterial growth

Basal medium (10 g tryptone, 10 g peptone, 5 g yeast extract, 1 ml of Tween 80, 0.5 g L-cysteine hydrochloride, and 1 L of distilled water) was autoclaved at 121 °C for 15 min. The FOS molecules that were obtained in the SFF from *S. rebaudiana* roots were added at the concentration of 2.0 g/L as the sole carbon source to the basal medium after sterile filtration (Puradisc FP 30 filter, 0.2 μm). Wilkins Chalgren anaerobic broth (Oxoid, Basingstoke, UK) was used as a positive control. Basal medium without the addition of sugar was used as a negative control. For comparisons, medium that contained Orafti® P95 (2.0 g/L) was used to represent commercially available chicory FOS-based prebiotic.

Fresh bacterial cultures were grown overnight in Wilkins Chalgren broth, centrifuged at 5000 × *g* for 7 min, and resuspended in saline. Bacterial suspensions were inoculated into each tested medium and incubated at 37 °C for 24 h under anaerobic conditions. All of the strains were grown in triplicate.

Bacterial growth was evaluated as the change in absorbance of the tested media at a wavelength of 540 nm (A_{540}) during 24 h of incubation using a densitometer (DEN-

1, Dynex). For the determination of bacterial metabolites, acetic acid and lactic acid the isotachophoretic method (IONOSEP 2003, Czech Republic) was used. The results were evaluated using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) and Statistica 10 (StatSoft, Prague, Czech Republic) software. Values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Fructo-oligosaccharide extraction and yield

In the extraction process for *S. rebaudiana* roots (Fig. S1) the ethanol precipitation of the aqueous extract was required to separate the fructan molecules of long-chain (precipitate fraction) and short-chain (supernatant fraction) produced by the plant. This step was not necessary to aqueous extract of adventitious root cultures because only fructo-oligosaccharides with short-chain were produced.

The FOS yield from the SFF that was obtained from *S. rebaudiana* roots was 24%, and the FOS yield from the TFE that was obtained from *S. rebaudiana* adventitious root cultures was 16% under dark conditions and 9% under light conditions. The two extracts with a better FOS yield (SFF and TFE in the dark) were similar to species that are currently used for commercial purposes like Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) (Chi et al., 2011; Meyer et al., 2011).

3.2 Culture of S. rebaudiana adventitious roots in a roller bottle system and fructan production

Preliminary experiments with the aqueous extract from *S. rebaudiana* adventitious roots that were cultured in a roller bottle system under dark and light

conditions showed that cultures that were grown in the dark had a higher yield and more capacity to accumulate carbohydrate (35.4%) than cultures that were grown under light conditions (26.6%). Therefore, all of the subsequent experiments that evaluated FOS production were performed under dark conditions.

The growth curve of *S. rebaudiana* adventitious roots that were cultivated in the dark (Fig. 2) showed a lag phase with slow growth during the first and second weeks. The fresh weight (FW) of these adventitious roots were 0.073 g and 0.132 g, corresponding to 0.43-fold and 1.54-fold increases, respectively. The exponential phase between the third week (root FW = 0.376 g) and fourth week (root FW = 0.492 g) reached a maximum growth index (8.5-fold) during the fourth week of cultivation. Growth deceleration occurred in the fifth week, with a decline in biomass production (root FW = 0.393 g).

The roller bottle system was chosen for the adventitious root cultures because it is a well-established methodology in our laboratory (Reis et al., 2011). It has the advantages of slow rotation that allows temporary immersion of the roots, which improves oxygenation and avoids disrupting young cells that are being formed.

The presence of FOS molecules in the TFE during the growth period of *S. rebaudiana* adventitious roots was monitored by off-line ESI-MS. The data (Fig. S2) showed the presence of FOS molecules only from the third week (TFE 21-day-old) of culture (i.e., the exponential phase), with the best accumulation profile for the TFE from 28-day-old roots, which had characteristic peaks of sodium and potassium adducts in the mass spectrum (Oliveira et al., 2011; Verspreet et al., 2015b).

The present data agreed with the literature, in which primary metabolites are produced in the exponential phase (*in vitro* root cultures) or vegetative stage (cultivar roots), periods during which carbon sources are required for plant growth (Machado et

al., 2006; Ould-Ahmed et al., 2014).

3.2 Chemical characterization of fructo-oligosaccharides

The GC-MS analysis of the FOS samples identified the presence of the monosaccharides fructose and glucose. The peaks were identified according the retention time in comparison with fructose (Fig. 3C) and glucose (Fig. 3D) standards, fragmentation profile at the mass spectra of oxime-silylated derivatives (Fig. S3), besides the NIST Mass Spectral Library Database.

The SFF chromatogram (Fig. 3A) showed a majority peak that corresponded to fructose units at retention times of 8.6, 9.5, and 9.8 min. Glucose peaks were identified at retention times of 10.1, 10.6, 12.5. The internal standard myo-inositol was observed at 11.9, 15.1 and 15.7 min. The TPE chromatogram (Fig. 3B) peaks at retention time 7.7, 7.9, 8.0, 9.4 and 9.7 min were assigned as fructose units and peaks with retention time of 9.8, 10.1, 10.5 and 12.4 min were identified as glucose.

The quantitative analysis of the SFF by CG-MS showed a higher content of fructose (11.2%) than glucose (2.2%), whereas the TFE showed similar fructose (6.5%) and glucose (5.2%) content.

The ^{1}H NMR spectra (Fig. 4) presented signals of mono-, di-, and oligosaccharides. Fructo-oligosaccharide signals of terminal glucose hydrogen of the fructan (**3**) chain (H1'-Glc) were assigned in the anomeric region with chemical shifts at (δ) 5.45 (J = 3.7 Hz). Signal referents of the residue (→2-β-Fru) were observed at δ 4.25 (H3-Fru, J = 8.0 Hz) and δ 4.11 (H4-Fru, J = 8.4 Hz) (Fig. 4). All chemical shift values (δ) (Table 1) are similar in the SFF (*S. rebaudiana* roots, Fig. 4A), TFE (*S. rebaudiana* adventitious roots, Fig. 4B) and FOS commercial (Orafti® P95, Fig. 4C) as well as with the literature data (Caleffi et al., 2015; Lopes et al., 2015; Oliveira et al., 2011).

Signals of α- and β-anomeric forms (H1') of free glucose molecules were observed in the SFF and TPE extracts at δ 5.25 and δ 4.66, respectively (Fig. 4) (Cérantola et al., 2004; Zhang et al., 2013). Signals of sucrose (**1**) molecules were also observed in the FOS extracts, their chemical shift values (δ) (Table 1) were assigned by comparison with sucrose standard (Fig. S4) and literature data (Matulová, Husárová, Capek, Sancelme & Delort, 2011; Zhang et al., 2013).

In the region between δ 3.65 and 3.95 ppm, were identified other fructosyl hydrogens (H1, H5, and H6) and glucosyl hydrogens (H3, H5, and H6) (Table 1) of FOSs and sucrose molecules (Matulová et al., 2011; Zhang et al., 2013).

The off-line ESI-MS mass spectrum for SFF (Fig. 5A) presented FOS molecules with characteristic peaks that indicated potassium adducts $[M + K]^+$ (Oliveira et al., 2011). The peaks at *m/z* 544, 706, 868, and 1030 corresponded to FOS molecules GF_2, GF_3, GF_4, and GF_5, respectively, where G represents glucose molecules, F represents fructose molecules, and *n* indicates the number of fructose units. Peaks with a low intensity at *m/z* 1192 and 1354 corresponded to GF_6 and GF_7 (Verspreet et al., 2015b; Oliveira et al., 2011; Zhao et al., 2011). These peaks had a characteristic mass difference of 162 Da, corresponding to hexose residues that are associated with FOS molecules with a DP of 3 to 8, respectively.

The off-line ESI-MS mass spectrum for TFE (Fig. 5B) presented FOS molecules with characteristic peaks that indicated potassium adducts $[M + K]^+$ as mentioned above and peaks that indicated sodium adducts $[M + Na]^+$ at *m/z* 528, 690, 852, 1014, 1176, and 1338, corresponding to FOS molecules of GF_2, GF_3, GF_4, GF_5, GF_6, and GF_7, respectively. This technique allowed the observation of the mixture of oligomer chain with variable number of fructose units. The FOS molecules in the both extracts showed an average degree of polymerization (DP_n) = 5.

For the SFF and TFE extracts, we observed the presence of FOS molecules with DP = 3 (GF_2), which was denominated 1-kestose (**2**), the first trisaccharide in the biosynthesis route of fructans that is catalyzed by the enzyme sucrose:sucrose 1-fructosyltransferase (1-SST; EC 2.4.1.99). The other FOS peaks that are represented by GF_n (Fig. 1) resulted in elongation of the FOS chain by adding *n* units of fructose that were catalyzed by the enzyme fructan:fructan 1-fructosyltransferase (1-FFT; EC 2.4.1.100) (Ould-Ahmed et al., 2014; Verspreet et al., 2015a; Zhao et al., 2011).

The SFF from *S. rebaudiana* roots and TFE from *S. rebaudiana* adventitious root cultures showed similar profiles in all of the chemical experiments, which confirmed the presence of FOS molecules in both extracts.

Considering recent work with *S. rebaudiana* roots (Lopes et al., 2015) and the present results, we propose that two products with different industrial applications can be obtained using the same extraction process. Therefore, FOS molecules with a low DP can be isolated from supernatant ethanolic fractions, and inulin with a high DP can be isolated from precipitate fractions.

3.3 Prebiotic effects

Prebiotics are oligo- and polysaccharides. They are non-digestible in the human gastrointestinal tract but selectively fermented by intestinal microbiota. Fructo-oligosaccharides are a well-described group of prebiotics that are used in functional foods. In the present study, FOSs from *S. rebaudiana* roots were assayed and their fermentability by bifidobacteria and lactobacilli was tested.

The tests with bifidobacteria strains (Fig. 6) demonstrated the limited ability to use FOSs from *S. rebaudiana* roots (FOS-R). Among the tested strains, *Bif. bifidum* CCDM 559 presented better growth in the tested medium. The bacterial density of this

strain in the medium that contained FOS-R increased 1.4-fold relative to the basal medium (i.e., without any saccharide). This strain was the only one for which the bacterial density was higher in the FOS-R medium than the medium that contained Orafti® P95 (i.e., a commercial FOS that is used as a prebiotic).

The bifidobacteria strains Bb12, JKM, and CCDM presented increases in growth compared with the basal medium, but these strains had a bacterial density that was lower than Orafti® P95. The *Bif. bifidum* JOV strain was the only one for which no significant difference ($p > 0.05$) in growth was found between the medium with FOS-R and the basal medium (Fig. 6). Acetic acid is the main metabolic product of bifidobacteria. The concentration of acetic acid in the medium containing FOSs from *S. rebaudiana* roots was higher than basal medium, however was lower than medium containing Orafti® P95 (Table 2). The *Bif. bifidum* CCDM 559 strain was only one where the bacterial growth and acid production was higher in the medium containing FOS-R than medium containing Orafti® P95.

The results for lactobacilli were generally more variable (Fig. 7). Lactobacilli presented better growth values than bifidobacteria. All five strains that were tested were able to utilize FOS from *S. rebaudiana* roots. The best ability to ferment FOS from *S. rebaudiana* roots was observed for the *Lbc. gasseri* PHM-7E1 and *Lbc. animalis* CCDM 382 strains, followed by the *Lbc. fermentum* RL25 strain.

The main fermentation products of FOS are lactic acid and acetic acid. The main fermentation product of inulin is butyric acid (Karimi et al., 2015; Rossi et al., 2005). The production of lactic acid by the tested strains corresponded to the aforementioned bacterial density results. The production of lactic acid (Table 2) was the highest for the PHM-7E1 and CCDM 382 strains (73 mg/100 ml), but these values did not overcome the lactic acid production in the medium containing Orafti® P95.

The fermentability of FOSs from chicory (Orafti® P95) and FOSs from *S. rebaudiana* roots was very similar for the two lactobacilli strains (RL25 and PE1TB-P), which were not significantly different ($p > 0.05$). The lactic and acetic acid production (Table 2) in these strains were also similar. For the other strains of lactobacilli, the fermentability of FOSs from chicory (Orafti® P95) was higher than FOSs from *S. rebaudiana*.

The rate of fermentability of various carbohydrates is related to the enzymatic system of bacteria. For example, *β*-fructofuranosidase is an enzyme that hydrolyzes fructose moieties from the terminal *β*-2,1 positions, contributing to fructan metabolization (Grajek & Olejnik, 2005; Karimi et al., 2015). Other polysaccharide-related factors that can influence fermentability include the saccharide structure (i.e., the degree of molecule branching and glycosidic linkage) and degree of polymerization (i.e., size of the chain) (Al-Sheraji et al., 2015; Ward, Ninonuevo, Mills, Lebrilla & German 2007; Van Loo, 2004).

The FOS-R and Orafti® P95 are chemically similar. As observed in the ^{1}H NMR profile (Fig. 4), gas chromatography-mass spectrometry and fragmentation profile at the mass spectra (Fig. S3). The degree of polymerization a determining factor in fermentative capacity (Moreno-Vilet et al., 2014) is similar between the samples. The Orafti® P95 has a $DP_n = 4$ and FOS from *S. rebaudiana* roots $DP_n = 5$. The carbohydrate content was significantly different between the samples; the total sugar determination showed higher carbohydrate content for Orafti® P95 (100%) than FOS from *S. rebaudiana* roots (78%).

Therefore, the factors involved in the fermentation capacity of these molecules by lactobacilli and bifidobacteria was probably related to bacterial strain and the best response observed for commercial FOS was due the industrial processing of the sample

that allows high carbohydrate content. Certain prebiotics are not suitable in combinations with a certain genus or even a certain bacterial species (Kadlec & Jakubec, 2014). Other studies also found that the ability to ferment fructan molecules is strain-specific for populations of lactobacilli and bifidobacteria (Huebner, Wehling, & Hutkins, 2007; Makras, Van Acker, & De Vuyst, 2005; Cummings, Macfarlane, & Englyst, 2001).

4. Conclusion

The present study isolated and chemically characterized FOS molecules from *S. rebaudiana* roots and adventitious root cultures, which had similar chemical profiles. The extracts showed the presence of FOS molecules with DP of 3 to 8, with an average degree of polymerization $DP_n = 5$. The two proposed sources showed to be promising for obtaining FOSs with a good yield. The *in vitro* culture of *S. rebaudiana* adventitious roots can be an interesting alternative to the use of whole plant roots for the production of FOS compounds. The FOS molecules that were isolated from *S. rebaudiana* roots enhanced the growth of populations of both bifidobacteria and lactobacilli, with strain specificity in their fermentation ability. The present findings emphasize the importance of investigating alternative and sustainable sources to obtaining molecules with prebiotic potential that can serve as food nutrients that promote health.

Acknowledgment

The authors thank the Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process n°. 481915/2013-3) as well as the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Complexo de Centrais de Apoio à Pesquisa (COMCAP) of the State University of

Maringá. Authors also thank the Ministry of Education, Youth and Sports of the Czech Republic (COST LD 14123) for financial support.

References

Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, 5(4), 1542-1553.

Aravind, N., Sissons, M. J., Fellows, C. M., Blazek, J., & Gilbert, E. P. (2012). Effect of inulin soluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. *Food Chemistry*, *132*(2), 993–1002.

Baque, M. A., Moh, S-H., Lee, E-J., Zhong, J-J., & Paek, K-Y. (2012). Production of biomass and useful compounds from adventitious roots of high-value added medicinal plants using bioreactor. *Biotechnology Advances*, *30*(6), 1255–1267.

Caleffi, E. R., Krausová, G., Hyršlová, I., Paredes, L. L. R., dos Santos, M. M., Sassaki, G. L., Gonçalves, R. A. C., & Oliveira, A. J. B. (2015). Isolation and prebiotic activity of inulin-type fructan extracted from *Pfaffia glomerata (Spreng)* Pedersen roots. *International Journal of Biological Macromolecules*, *80*, 392–399.

Cérantola, S., Kervarec, N., Pichon, R., Magné, C., Bessieres, M. A., & Deslandes, E. (2004). NMR characterization of inulin-type fructooligosaccharides as the major water-soluble carbohydrates from *Matricaria maritima* (L.). *Carbohydrate Research*, 339, 2445–2449.

Chi, Z. M., Zhang, T., Cao, T. S., Liu, X. Y., Cui, W., & Zhao, C. H. (2011). Biotechnological potential of inulin for bioprocesses. *Bioresource Technology*, *102*, 4295-4302.

Crispín-Isidro, G., Lobato-Calleros, C., Espinosa-Andrews, H., Alvarez-Ramirez, J., & Vernon-Carter, E. J. (2015). Effect of inulin and agave fructans addition on the rheological, microstructural and sensory properties of reduced-fat stirred yogurt. *LWT - Food Science and Technology*, *62*(1), 438–444.

Cui, X. H., Murthy, H. N., Jin, Y. X., Yim, Y. H., Kim, J. Y., & Paek, K. Y. (2011). Production of adventitious root biomass and secondary metabolites of *Hypericum perforatum L.* in a balloon type airlift reactor. *Bioresource Technology*, *102*, 10072–10079.

Cummings, J. H., Macfarlane, G. T., & Englyst, H. N. (2001). Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition*, 74, 415S-420S.

Dwivedi. M., Kumar, P., Laddha, N., C., & Kemp, E. H. (2016) Induction of regulatory T cells: A role for probiotics and prebiotics to suppress autoimmunity. *Autoimmunity Reviews*, *15*, 179-392

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*, 350–356.

Grajek, W., & Olejnik, A. (2005). Probiotics, prebiotics and antioxidants as functional foods. *Acta Biochimica Polonica*, 52, 665-671.

Huebner, J., Wehling, R. L., & Hutkins, R. W. (2007). Functional activity of commercial prebiotics. *International Dairy Journal 17*, 770–775.

Kadlec, R. & Jakubec, M. (2014). The effect of prebiotics on adherence of probiotics. *Journal Dairy Science*, 97, 1-8.

Karimi, R., Azizi, M. H., Ghasemlou, M., & Vaziri, M. (2015). Application of inulin in cheese as prebiotic , fat replacer and texturizer : A review. *Carbohydrate Polymers*, *119*, 85–100.

Lee, E. J., & Paek, K. Y. (2012). Enhanced productivity of biomass and bioactive compounds through bioreactor cultures of *Eleutherococcus koreanum* Nakai adventitious roots affected by medium salt strength. *Industrial Crops and Products*, *36*(1), 460–465.

Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L., & Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*, *132*(3), 1121–1132.

Lobo, A. R., Cocato, M. L., Borelli, P., Gaievski, E. H. S., Crisma, A. R., Nakajima, K., Nakano, E. Y., & Colli, C. (2011). Iron bioavailability from ferric pyrophosphate in rats fed with fructan-containing yacon (*Smallanthus sonchifolius*) flour. *Food Chemistry*, *126*(3), 885–891.

Lopes, S. M. S., Krausov, G., Rada, V., Gonçalves, J. E., Gonçalves, R. A. C., & Oliveira, A. J. B. (2015). Isolation and characterization of inulin with a high degree of polymerization from roots of *Stevia rebaudiana* (Bert.) Bertoni. *Carbohydrate Research*, *411*, 15-21.

Lulu, T., Park, S.-Y., Ibrahim, R., & Paek, K.-Y. (2014). Production of biomass and bioactive compounds from adventitious roots by optimization of culturing conditions of *Eurycoma longifolia* in balloon-type bubble bioreactor system. *Journal of Bioscience and Bioengineering*, *119*(6), 712–717.

Machado, F. A. P. S. A., Capelasso, M., de Oliveira, A. J. B., Gonçalves, R. A. C., Zamuner, M. L. M, Mangolin, C. A., & Machado, M. F. P. S. (2006). Fatty acids production from plants na callus culture of *Cereus peruvianus mill.* (Cactaceae). *Journal of Plant Sciences, 1*(4), 368 -373.

Makras, L., Van Acker, G., & De Vuyst, L. (2005). *Lactobacillus paracasei* subsp. *paracasei* 8700:2 Degrades Inulin-Type Fructans Exhibiting Different Degrees of Polymerization. *Applied And Environmental Microbiology*, *71(11)*, 6531–6537.

Matulová, M., Husárová, S., Capek, P., Sancelme, M., & Delort, A.-M. (2011). NMR structural study of fructans produced by *Bacillus* sp. 3B6, bacterium isolated in cloud water. *Carbohydrate Research*, *346*(4), 501–507.

Meyer, D., Bayarri, S., Tárrega, A., & Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids, 25*, 1881–1890.

Moraes, R. M., Donega, M. A., Cantrell, C. L., Mello, S. C., & Mcchesney, J. D. (2013). Effect of harvest timing on leaf production and yield of diterpene glycosides in *Stevia rebaudiana* Bert : A specialty perennial crop for Mississippi. *Industrial Crops & Products, 51*, 385–389.

Moreno-Vilet, L., Garcia-Hernandez, M. H., Delgado-Portales, R. E., Corral-Fernandez, N. E., Cortez-Espinosa, N., Ruiz-Cabrera, M. A., & Portales-Perez, D. P. (2014). *In vitro* assessment of agave fructans (*Agave salmiana*) as prebiotics and immune system activators. *International Journal of Biological Macromolecules, 63*, 181–187.

Morris, C., & Morris, G. A. (2012). The effect of inulin and fructo-oligosaccharide supplementation on the textural , rheological and sensory properties of bread and their role in weight management : A review. *Food Chemistry, 44*, 237-248.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologycal Plant, 15*, 473–495

Nishimura, M., Ohkawara, T., Kanayama, T., Kitagawa, K., Nishimura, H., & Nishihira, J. (2015). Effects of the extract from roasted chicory (*Cichorium intybus* L.) root containing inulin-type fructans on blood glucose, lipid metabolism, and fecal properties. *Journal of Traditional and Complementary Medicine, 5*(3), 161–7.

Oliveira, A. J. B., Gonçalves, R. A. C., Chierrito, T. P. C., dos Santos, M. M., de Souza, L. M., Gorin, P. A. J., Sassaki, G. L., Iacomini, M. (2011). Structure and degree of polymerisation of fructooligosaccharides present in roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni. *Food Chemistry, 129*(2), 305–311.

Ould-Ahmed, M., Decau, M. L., Morvan-Bertrand, A., Prud'homme, M. P., Lafrenièred, C., & Drouin, P. (2014). Plant maturity and nitrogen fertilization affected fructan metabolism in harvestable tissues of timothy (*Phleum pratense L.*) *Journal of Plant Physiology,171*, 1479–1490.

Peshev, D., & Van den End, W. (2014). Fructans: Prebiotics and immunomodulators. *Journal of Functional Foods, 8*(1), 348–357.

Pitarresi, G., Tripodo, G., Cavallaro, G., Palumbo, F. S., & Giammona, G. (2008). Inulin – iron complexes : A potential treatment of iron deficiency anaemia. *European Journal of Pharmaceutics and Biopharmaceutics, 68*, 267–276.

Reis, R. V., Borges, A. P. P. L., Chierrito, T. P. C., de Souto, E. R., de Souza, L. M., Iacomini, M., de Oliveira, A. J. B., & Gonçalves, R. A. C. (2011). Establishment of adventitious root culture of *Stevia rebaudiana* Bertoni in a roller bottle system. *Plant Cell, Tissue and Organ Culture (PCTOC), 106*(2), 329–335.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., et al. (2010). Prebiotic effects: Metabolic and health benefits. *British Journal of Nutrition, 104,* S1–S63.

Rossi, M., Corradini, C., Amaretti, A., Nicolini, M., Pompei, A., Zanoni, S. et al. (2005). Fermentation of fructooligosaccharides and inulin by bifidobacteria: A comparative study of pure and fecal cultures. *Applied and Environmental Microbiology, 71,* 6150-6158.

Silja, P. K., & Satheeshkumar, K. (2015). Establishment of adventitious root cultures from leaf explants of *Plumbago rosea* and enhanced plumbagin production through elicitation. *Industrial Crops & Products, 76,* 479–486.

Sołowiej, B., Glibowski, P., Muszyński, S., Wydrych, J., Gawron, A., & Jeliński, T. (2015). The effect of fat replacement by inulin on the physicochemical properties and microstructure of acid casein processed cheese analogues with added whey protein polymers. *Food Hydrocolloids, 44,* 1-11.

Thiyagarajan, M., & Venkatachalam, P. (2012). Large scale in vitro propagation of *Stevia rebaudiana* (bert) for commercial application : Pharmaceutically important and antidiabetic medicinal herb. *Industrial Crops & Products, 37*(1), 111–117.

Van Loo, J. (2004). The specificity of the interaction with intestinal bacterial fermentation by prebiotic determine their physiological efficacy. *Nutrition Research Reviews 17,* 89-98.

Verspreet, J., Dornez, E., Van den Ende, W., Delcour, J. A., & Courtin, C. M. (2015a). Cereal grain fructans: Structure, variability and potential health effects. *Trends in Food Science & Technology, 43*(1), 32–42.

Verspreet, J., Hansen, A. H., Dornez, E., Delcour, J. A., Van den Ende, W., Harrison, S. J., & Courtin, C. M. (2015b). LC-MS analysis reveals the presence of graminan- and neo-type fructans in wheat grains. *Journal of Cereal Science, 61,* 133–138.

Villegas, B., Tárrega, A., Carbonell, I., & Costell, E. (2010). Optimising acceptability of new prebiotic low-fat milk beverages. *Food Quality and Preference, 21*(2), 234–242.

Ward, R. E., Ninonuevo, M., Mills, D. A., Lebrilla, C. B., & German, J. B. (2007). *In vitro* fermentability of human milk oligosaccharides by several strains of bifidobacteria. *Molecular Nutrition & Food Research, 51,* 1398-1405.

Zhang, A. Q., Zhang, Y., Yang, J., Jiang, J., Huang, F. F., & Sun, P. L. (2013). Structural elucidation of a novel water-soluble fructan isolated from *Wedelia prostrata. Carbohydrate Research, 376,* 24–28.

Zhao, M., Mu, W., Jiang, B., Zhou, L., Zhang, T., Lu, Z., Jin, Z., & Yang, R. (2011). Purification and characterization of inulin fructotransferase (DFA III-forming) from *Arthrobacter aurescens* SK 8.001. *Bioresource Technology, 102*(2), 1757–1764.

Figure Caption

Fig. 1. Simplified scheme of the fructan biosynthesis: Sucrose (**1**), 1-Kestose (**2**) and general fructans formula (**3**) (*n* represent the number of fructose units). Enzymes of the process: 1-SST - sucrose:sucrose 1-fructosyltransferase, 1-FFT - fructan:fructan 1-fructosyltransferase.

Fig. 2. Growth curve and fresh weight of *S. rebaudiana* adventitious roots, cultivated in a in a roller bottle system during 5 weeks.

Fig. 3. GC-MS Chromatogram (TIC) of oxime-silylated residues of FOS from *S. rebaudiana* roots: soluble fructan fraction - SFF (**A**) and *S. rebaudiana* adventitious roots: total fructan extract - TFE (**B**).

Fig. 4. ^{1}H NMR spectra (500.00 MHz, D_2O at 298 K) of the FOS from *S. rebaudiana* roots (SFF) (**A**) and *S. rebaudiana* adventitious roots (TFE) (**B**).

Fig. 5. Off-line ESI-MS spectra in positive ion mode of FOS from *S. rebaudiana* roots (SFF) (**A**) and *S. rebaudiana* adventitious roots (TFE) (**B**).

Fig. 6. Fermentability of FOS from *S. rebaudiana* roots by different bifidobacteria strains.

Fig. 7. Fermentability of FOS from *S. rebaudiana* roots by different lactobacilli strains.

Table 1. ^{1}H chemical shifts (δ) of fructo-oligosaccharides extracts from *S. rebaudiana* root (SFF), adventitious root cultures (TPE) and commercial FOS.

Sample	Sugar Unit	^{1}H Chemical Shifts/δ					
		1	**2**	**3**	**4**	**5**	**6**
SFF	→2)-*β*-Fru*f*	3.93-3.71	-	4.25	4.11	3.89	3.78-3.73
TPE	→2)-*β*-Fru*f*	3.92-3.71	-	4.26	4.12	3.89	3.79-3.72
Orafti® P95	→2)-*β*-Fru*f*	3.92-3.71	-	4.25	4.11	3.87	3.78-3.76
Sucrose*	*α*-Glc*p*-(1→	5.42	3.58	3.75	3.48	3.87	3.83
	→2)-*β*-Fru*f*	3.68	-	4.21	4.06	3.87	3.80

Commercial FOS (Orafti® P95, Beneo-Orafti, Belgium)
*Chemical shifts (δ) of sucrose observed in the SFF, TPE and Orafti® P95 samples.

FOSs, Fructo-oligosaccharides; DP, Degree of Polymerization; DP_n, Average Degree of Polymerization; MS, Murashige Skoog medium; GI, Growth Index; 1-SST, Sucrose: Sucrose 1-Fructosyltransferase; 1-FFT, Fructan: Fructan 1-Fructosyltransferases

Table 2: Acid´s production by strains of lactobacilli and bifidobacteria cultivated in the media containing fructo-oligosaccharides from *S. rebaudiana* roots, basal medium without sugar source (negative control) and commercial FOS Orafti® P95 (measured using isotachophoresis, from triplicate determination, ± standard deviation).

Bacterial strains	***Stevia* FOS**		**Basal medium**		**Orafti® P95**	
	Lactic acid (mg/100 ml)	**Acetic acid** (mg/100 ml)	**Lactic acid** (mg/100 ml)	**Acetic acid** (mg/100 ml)	**Lactic acid** (mg/100 ml)	**Acetic acid** (mg/100 ml)
Bif. bifidum **CCDM 559**	59 ± 2	28 ± 2	35 ± 3	10 ± 2	38 ± 3	15 ± 3
Bif. animalis subsp. *lactis* **Bb12**	45 ± 4	21 ± 3	43 ± 4	17 ± 3	179 ± 5	39 ± 4
Bif. bifidum **JKM**	29 ± 3	16 ± 1	26 ± 3	11 ± 2	163 ± 4	34 ± 2
Bif. breve **CCDM 562**	28 ± 5	28 ± 4	19 ± 4	14 ± 3	43 ± 2	39 ± 3
Bif. bifidum **JOV**	30 ± 4	16 ± 2	25 ± 3	13 ± 3	160 ± 4	50 ± 2
Lbc. fermentum **RL25**	55 ± 5	12 ± 2	32 ± 3	8 ± 2	58 ± 3	17 ± 2
Lbc. animalis **CCDM 382**	73 ± 5	13 ± 1	38 ± 3	8 ± 3	120 ± 3	30 ± 3
Lbc. delbrueckii subsp. *bulgaricus* **CCDM 66**	35 ± 3	10 ± 2	24 ± 4	8 ± 2	39 ± 3	11 ± 2
Lbc. casei subsp. *paracasei* **PE1TB-P**	34 ± 2	15 ± 2	23 ± 3	14 ± 2	35 ± 3	13 ± 3
Lbc. gasseri **PHM-7E1**	73 ± 3	24 ± 2	39 ± 4	14 ± 3	195 ± 4	40 ± 2

Fig. 1

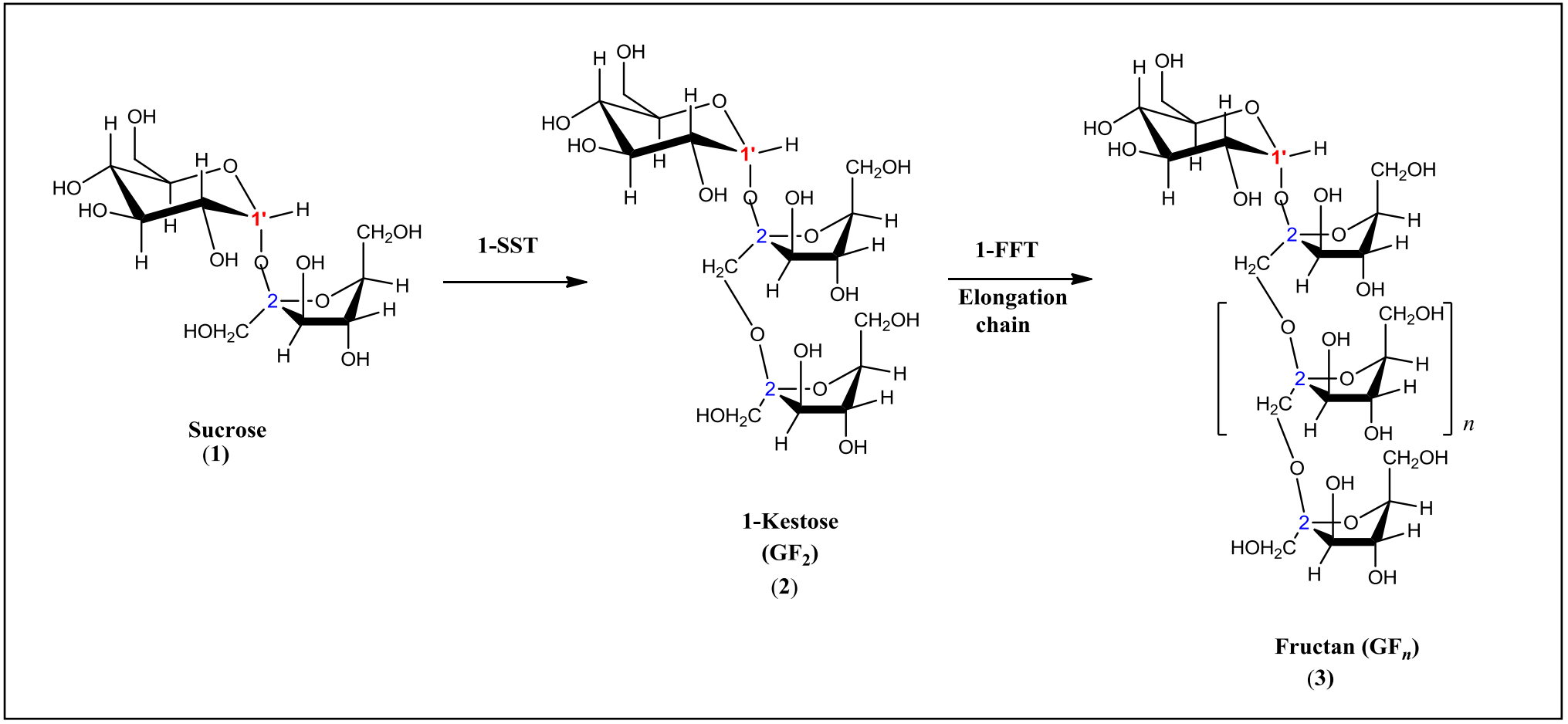


Fig. 2

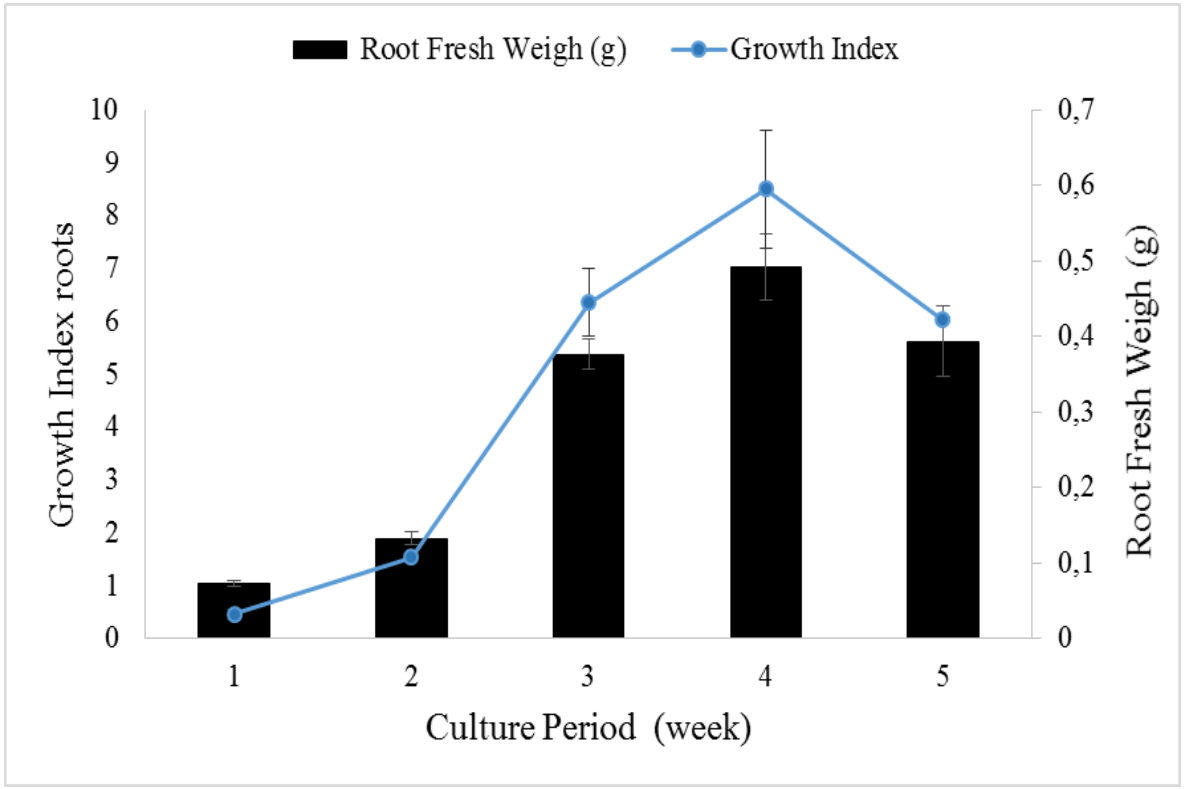


Fig. 3

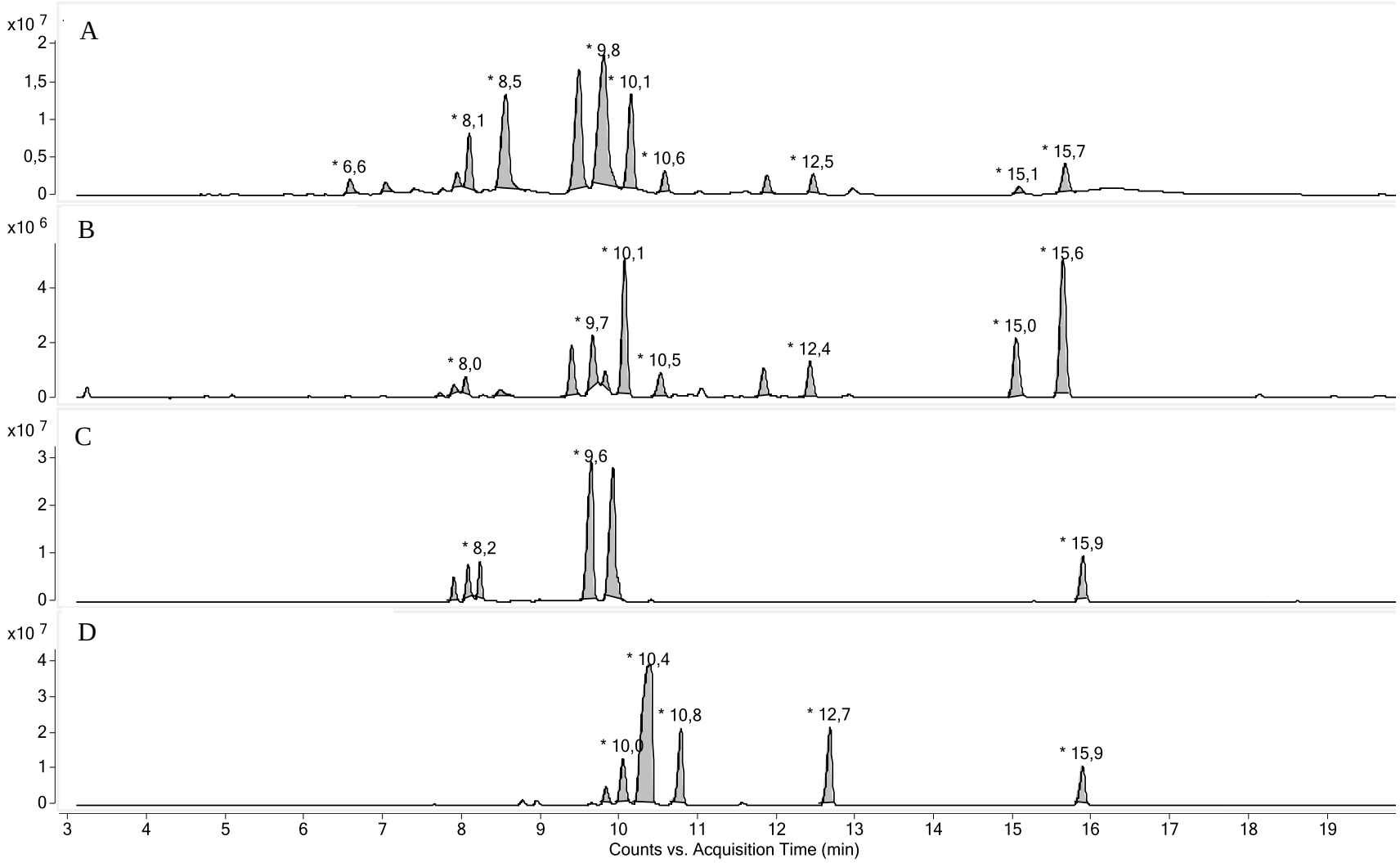


Fig. 4

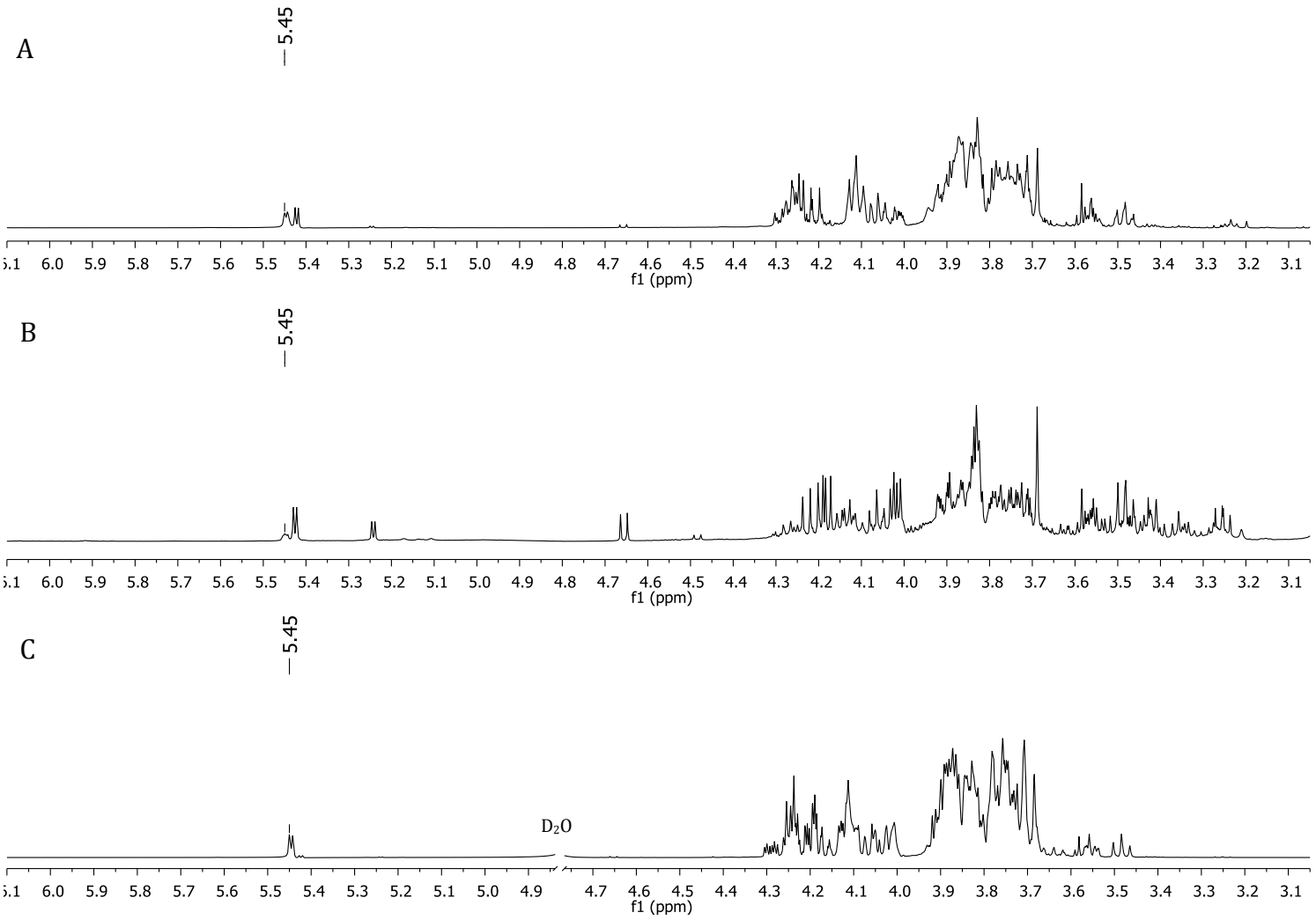
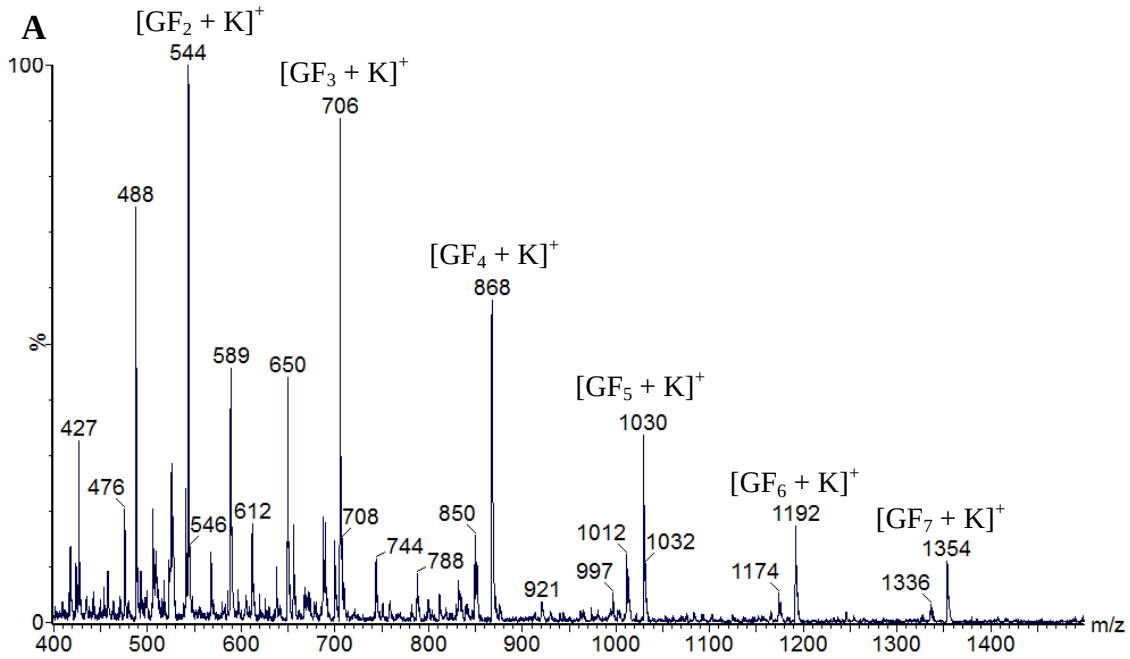


Fig. 5



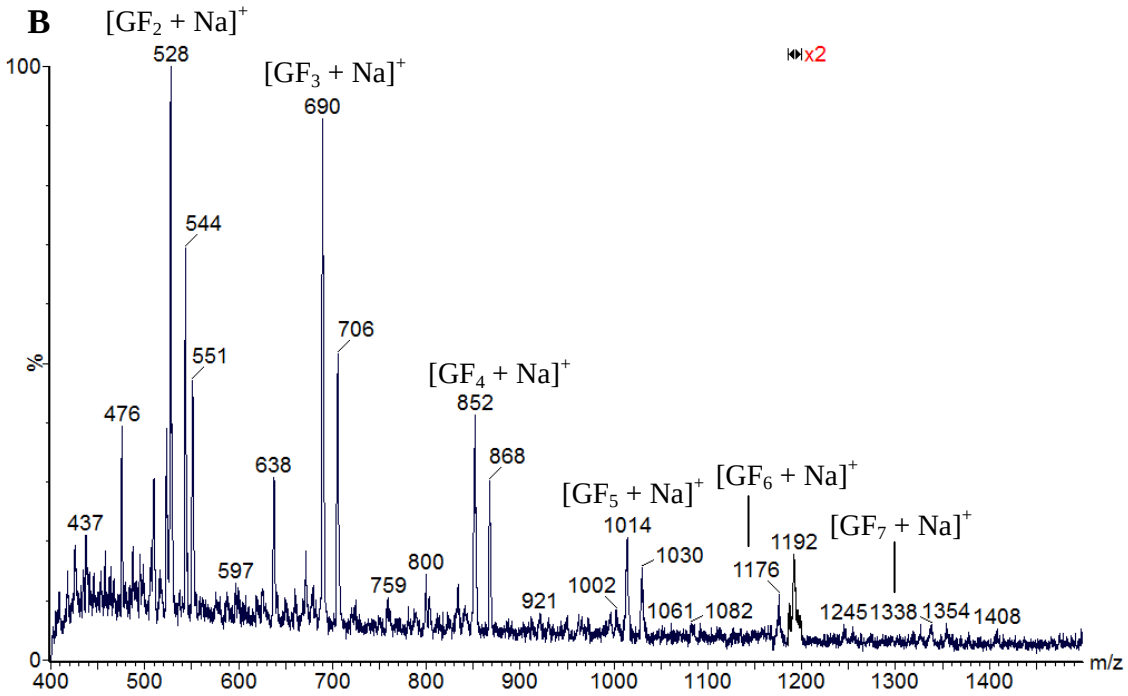
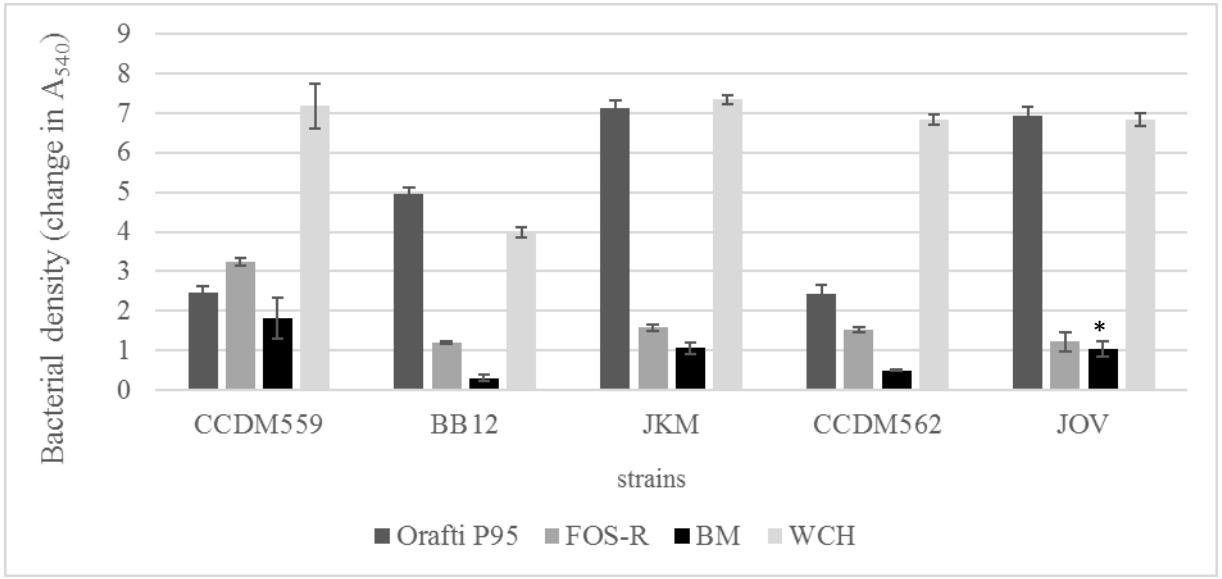


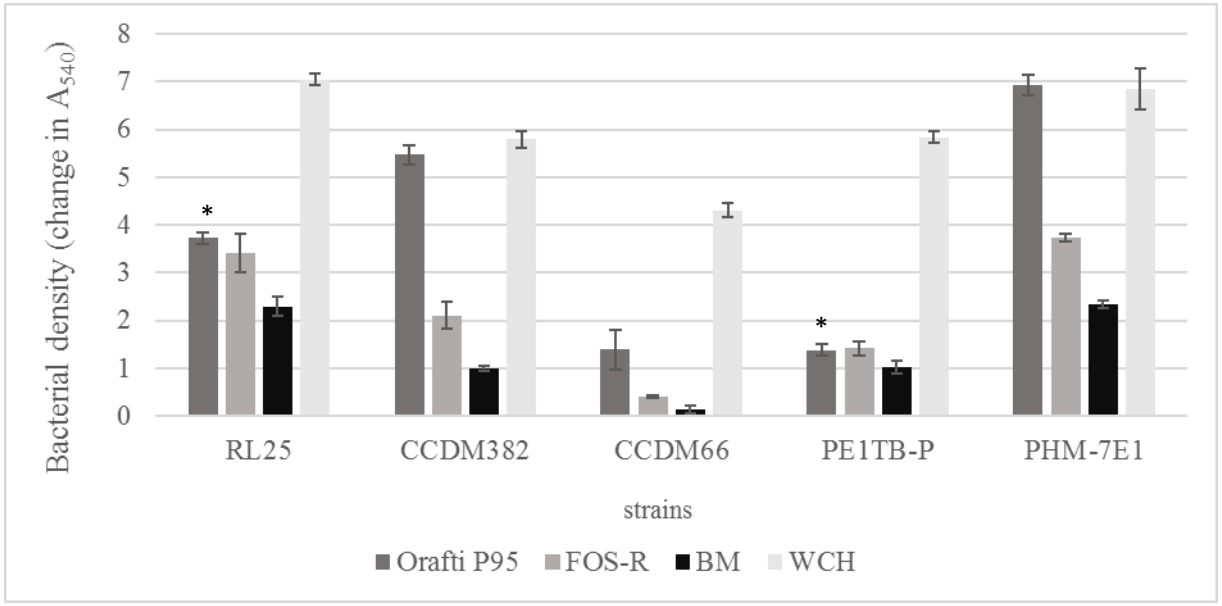
Fig. 6



*- statistically not significant difference ($p > 0.05$)

Orafti® P95 - medium containing commercial fructo-oligosaccharides, FOS-R – medium containing fructo-oligosaccharides from *S. rebaudiana* roots, BM – basal medium, without sugar (negative control), WCH – Wilkins-Chalgren broth, containing glucose as carbon source (positive control). Bars present mean ± SD.

Fig. 7



*- statistically not significant difference ($p > 0.05$)

Orafti® P95 - medium containing commercial fructo-oligosaccharides, FOS-R – medium containing fructo-oligosaccharides from *S. rebaudiana* roots, BM – basal medium, without sugar (negative control), WCH – Wilkins-Chalgren broth, containing glucose as carbon source (positive control). Bars present mean ± SD.