Isolation and prebiotic activity of inulin-type fructan extracted from *Pfaffia glomerata* (Spreng) Pedersen roots

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ABSTRACT

Pfaffia glomerata (Amaranthaceae) is popularly known as "Brazilian ginseng." Previous studies have shown that fructose is the major carbohydrate component present in its roots. Inulin-type fructans, polymers of fructose, are the most widespread and researched prebiotics. Here, we isolated and chemically characterized inulin extracted from *P. glomerata* roots and investigated its potential prebiotic effect. Fructans were isolated and their structures were determined using colorimetric, chromatography, polarimetry, and spectroscopic analysis. The degree of polymerization (DP) was determined, and an *in vitro* prebiotic test was performed. The structure of inulin was confirmed by chromatography and spectroscopic analysis and through comparison with existing data. Representatives from the genera *Lactobacillus* and *Bifidobacterium* utilized inulin from *P. glomerata*, because growth was significantly stimulated, while this ability is strain specific. The results indicated that inulin extracted from *P. glomerata* roots represents a promising new source of inulin-type prebiotics.

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1. Introduction

Pfaffia glomerata, Amaranthaceae, occurs in Guiana, Bolivia, Argentina, and Brazil. In Brazil, it mainly occurs in the states of São Paulo, Paraná, Mato Grosso, and Goiás [1,2]. This plant is popularly known as "Brazilian ginseng," and it is used commercially as a substitute for Asian ginseng, *Panax ginseng. P. glomerata* roots are used in folk medicine as a tonic, aphrodisiac, anti-diabetic, and antiulcer gastric, with a number of studies demonstrating its effectiveness [1–6].

To date, the main components isolated from *P. glomerata* roots are glomeric acid (triterpenoid), pfameric acid (nortriterpenoid), rubrosterone, and β-ecdysone [3,7]. Among the active ingredients contained in this plant, the steroid β-ecdysone is the most important compound extracted from the roots [8]. However, studies about *P. glomerata* remain limited, with no studies focusing on its primary metabolites, such as polysaccharides.

Previous studies using species from the Amaranthaceae family, such as *Gomphrena macrocephala*, have shown that the tuberous roots constitute approximately 50% of carbohydrates, with polymeric fructose being the main component [9]. Polysaccharides are of great interest because they are easy to obtain, low cost, biodegradable, and have a diversity of applications [10]. Fructans are reserve carbohydrates, which are classified as fructooligosaccharides or inulins, depending on the degree of polymerization (DP). In addition, fructans are classified into different families based on their glycosides linkages. Examples include $(2 \to 1)$-linked β-D-fructofuranosyl units, such as inulin which are usually derived from vegetable sources; or $(2 \to 6)$-linked β-D-fructofuranosyl units, such as levans that are typically found in monocot plants and bacterial sources, and structures composed of both $(2 \to 1)$- and $(2 \to 6)$-linked β-D-fructofuranosyl units, such as graminans and are more scarce (Fig. 1) [11–14]. Because they are derived from vegetable sources, inulins are widely found in roots and tubers, especially the Liliaceae family, Asteraceae and Poaceae [15,16]. Inulins exhibit a range of properties, acting as thickeners and fat substitutes in low-calorie products and prebiotic activity, which are of particular interest for the food industry. At present,
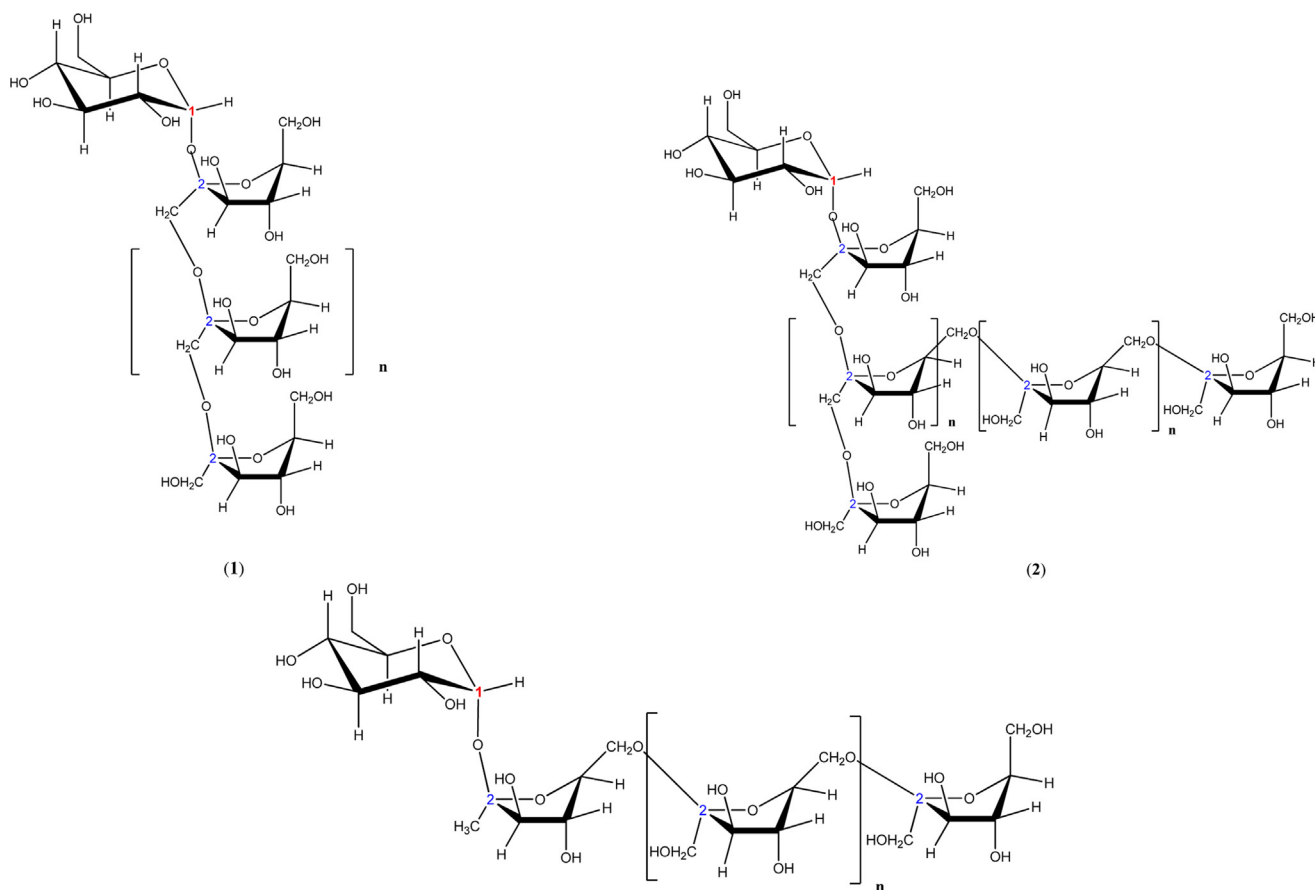
Fig. 1. Chemical structure of fructans: inulin-like (1), levan (2) graminan (3).

inulin-type fructans are the most widespread and researched prebiotics [17–20].

Prebiotics are non-digestible food ingredients that have a beneficial effect on the consumer by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [21,22]. According to Morris and Morris [12], desirable bacteria (bifidobacteria and lactobacilli) become more prominent in the gut, which benefits the human host. Thus, this work aimed to isolate and chemically characterize inulin extracted from the roots of *P. glomerata* and to investigate their potential prebiotic effect on lactobacilli and bifidobacteria under *in vitro* conditions.

2. Materials and methods

2.1. General experimental procedures

Optical rotation was measured in distilled H_2O at 20 °C using a 1 cm light path length cell in a Perkin-Elmer® 343 polarimeter (Perkin-Elmer, Waltham, MA) [23,24]. The analysis by nuclear magnetic resonance (¹H and ¹³C NMR) was performed on a Varian® Mercury Plus spectrometer (Varian, Palo Alto, CA) operating at 300.05 MHz for 1H nucleus, 75.45 MHz for 13C, and using deuterated water as the solvent. Tetradeuterium 2,2,3,3-3-(trimethylsilyl)-propionic acid (TMSP) was used as the internal reference (δ 0 ppm). Two-dimensional NMR methods were conducted to confirm the structure. The DP was calculated, with the mean ratio of the protons signals integral (H3-Fru and H4-Fru) by the integral of the anomeric hydrogen glucose signal (H1′-Glc) in the ¹H NMR spectrum, according to Cérantola et al. [38] and Yang et al. [40]. The MALDI-TOF analyses were conducted using a spectrometer Autoflex II (Bruker Daltonics, Billerica, MA). The spectra were obtained in positive-mode. The sample was dissolved at 1 mg/mL in deionized H_2O and ionized by a pulsed nitrogen laser (337 nm) accelerated between 20 and 60 kV. The compound 2,5-dihydroxybenzoic acid (2,5 DHB) was used as the matrix, at concentrations ranging from 2.5 to 10 mg/mL [25]. For the High Pressure Size Exclusion Chromatography (HPSEC-RI-MALLS) analysis (GPC), the samples were solubilized with sodium nitrite solution 0.2 mol/L containing 0.02% sodium azide (w/v). The sample was filtered through a membrane of cellulose acetate 0.22 µM and applied to chromatograph steric exclusion high pressure (HPSEC) equipped with a Wyatt DAWN DSP light scattering and Waters differential refractive index detector, model 2410. Four Waters Ultrahydrogel columns were used in sequence and with different exclusion limit: 7×106 (column 2000); 4×105 (column 500); 8×104 (column 250) and 5×103 (column 120). The eluent used was sodium nitrite solution 0.2 mol/L with sodium azide 0.02% (w/v) with a flow of 0.6 ml/min, monitored by the peristaltic pump 515 WATERS.

2.2. Plant material

The roots of *P. glomerata* were provided by the Association of Brazilian Ginseng Small farmers (termed ASPAG) from Querência do Norte, Paraná, Brazil (S 23° 05′ 07.0″, W 53° 29′ 06.4″). The roots were planted in December 2010 and harvested in May 2012 through organic production.

The samples provided form part of a collection that was previously identified in a comparison with herbarium specimens by Professor Dr. Lin Chau Ming from Paulista State University campus

in Botucatu, São Paulo, Brazil, in partnership with Dr. Cirino Corrêa Junior from EMATER, Paraná, Brazil.

2.3. Isolation of inulins

P. glomerata dried roots (100 g) were reduced to sections of approximately 1–2 cm in size, and the compounds were extracted with distilled water (1:15 w/v) at 100 °C for 2 h. This procedure was repeated 3 times to improve the extraction of compounds of interest. After filtration, the aqueous extract was concentrated, and polysaccharides were precipitated with 3 volumes of ethanol 96° GL [28]. The precipitate was filtered through a Büchner funnel, lyophilized, and weighed to calculate the yield.

2.4. Colorimetric analysis

Analyses of water-soluble carbohydrates and proteins were performed by colorimetric techniques. Analyses were performed in triplicate and mean values, standard deviations and coefficients of variation were calculated, Total sugar content was determined by the phenol–sulfuric acid method [29]. Total protein content was determined by the modified Lowry method [30,31]. Free reducing sugars content was determined by the *p*-hydroxybenzoic acid hydrazide (PAHBAH) assay [32]. Fructose content was determined by the resorcinol method [33]. DP was calculated from the data obtained by colorimetric analysis according to the method of Moerman et al. [12].

2.5. Glycoside linkage composition of fructans

Methylation of fructans was carried out according to method described by Ciucanu and Kerek [27]. The sample (5 mg) was dissolved in dimethylsulfoxide and per-*O*-methylated with iodomethane. Subsequently, the sample was sonicated for 30 min and allowed to stand overnight [26,27]. The permethylated polysaccharide was hydrolyzed with 2.0 M trifluoroacetic acid (TFA) for 30 min at 60 °C and evaporated to dryness. It was then reduced with ammonium hydroxide 2.0 M and a solution of sodium borohydride deuterated (10 mg/mL) for 1 h. Subsequently, it was acetylated with acetic anhydride, 2.0 M TFA and kept in oven for 10 min at 50 °C [25]. The methylation of fructans was analyzed by GC–MS using a Varian® 3800 gas chromatograph coupled to a Varian Ion-Trap2000R mass spectrometer (Varian, Palo Alto, CA). The column was DB-225MS (30 m × 0.25 mm) programmed from 50 to 215 °C at 40 °C/min, using helium as the carrier gas at a flow rate of 1 mL/min. The inlet temperature was 250 °C and the MS transfer line was set at 250 °C.

2.6. Bacterial strains

Table 1 shows the list of strains (6 strains of lactobacilli and 6 strains of bifidobacteria) assayed in this study. For storage purposes, all strains were grown in Wilkins Chalgren (WCH) anaerobic broth (Oxoid, Basingstoke, UK) and stored at −44 °C in WCH broth supplemented with 20% (v/v) glycerol as a cryoprotectant.

2.7. Bacterial growth in tested substrates

To assess bacterial growth, the following media were used: (a) medium containing inulin from *P. glomerata* roots and (b) medium containing prebiotic formula Orafti® GR (Beneo, Belgium) with inulin from chicory, DP 2–60 (on average > 10). Basal medium (tryptone 10 g, peptone 10 g, yeast extract 5 g, Tween 80 1 mL, L-cysteine hydrochloride 0.5 g, distilled water 1 L, pH 6.5) was supplemented with inulin from *Pfaffia* roots and Orafti® GR (each 2 g/L) as a sole carbon source. They were added to the cooled autoclaved (121 °C,

Table 1
Bacterial strains used for testing.

Strain	Origin	Source
Lactobacillus fermentum RL 25	Human feces	DRI[a]
Lactobacillus casei subsp. *paracasei* PE1TB-P	Biopsy sample	DRI[a]
Lactobacillus animalis CCDM 382	Raw goat milk	CCDM[b]
Lactobacillus acidophilus CCDM 151	Pill Biolacta	CCDM[b]
Lactobacillus gasseri PHM-7E1	Biopsy sample	DRI[a]
Lactobacillus gasseri CCDM 214	Human feces	CCDM[b]
Bifidobacterium bifidum CCDM 559	Human feces	CCDM[b]
Bifidobacterium animalis subsp. *lactis* CCDM 94	Dairy culture isolate	CCDM[b]
Bifidobacterium breve CCDM 562	GIT of a child	CCDM[b]
Bifidobacterium bifidum JOV	Infant feces	CULS[c]
Bifidobacterium bifidum JKM	Infant feces	CULS[c]
Bifidobacterium adolescentis AVNB3-P1	Biopsy sample	DRI[a]

[a] DRI – Dairy Research Institute (Tábor, Czech Republic).
[b] CCDM – Culture Collection of Dairy Microorganisms Laktoflora (Prague, Czech Republic).
[c] CULS – Czech University of Life Sciences (Prague, Czech Republic), Department of Microbiology, Nutrition and Dietetics.

15 min) basal media after sterile filtration (Puradisc FP 30 filter 0.2 μm, Whatman, Germany). Inoculated medium without saccharide represented the negative control (BM – basal medium). Wilkins Chalgren anaerobic broth (Oxoid, Basingstoke, UK) was used as a positive control. To prepare the inoculum, Wilkins Chalgren broth for both lactobacilli and bifidobacteria was used. Overnight bacterial cultures in the exponential growth phase were centrifuged (5000 × g, 7 min) and resuspended in saline to limit the introduction of residual sugars from the WCH broth into the tested media. Subsequently, bacterial suspensions were inoculated into the tested media and incubated at 37 °C for 24 h in anaerobic chambers (Oxoid, Basingstoke, UK). Fermentability was evaluated throughout bacterial growth detection by measuring the optical density of the tested media (densitometer DEN-1, Dynex, Czech Republic). The growth of lactobacilli and bifidobacteria was evaluated as the change in absorbance (A) at a wavelength of 540 nm at 24 and 48 h, respectively. The results are expressed as media turbidity increase estimated from an increase in A_{540}.

2.8. Determination of bacterial metabolites

Concentrations of lactic and acetic acids, as the main fermentation products of lactobacilli and bifidobacteria, were measured using the isotachophoretic (ITP) method. After fermentation, the samples were subjected to isotachophoretic separations using IONOSEP 2003 device (Recman, Czech Republic). Prior to analysis, the samples were diluted with 150 volumes of deionized water, and then purified using the Puradisc FP 30 filter with a pore size 0.2 μm (Whatman, Germany). Solution containing 10 mM HCl, 22 mM ε-aminocaproic acid and 0.1% 2-hydroxy-ethylcellulose (pH 4.5) as leading electrolyte (LE) was used. As trailing electrolyte (TE), 5 mM caproic acid was used. All chemicals were obtained from Sigma–Aldrich (Czech Republic). The values of the initial and final stream used were 80 μA and 30 μA, respectively.

2.9. Statistical analyses

To evaluate the results, Statgraphics® Centurion XV (StatPoint, Inc., Warrenton, USA), the multiple range comparison – LSD test was used. A statistically significant difference was designated at the level of $P < 0.05$.
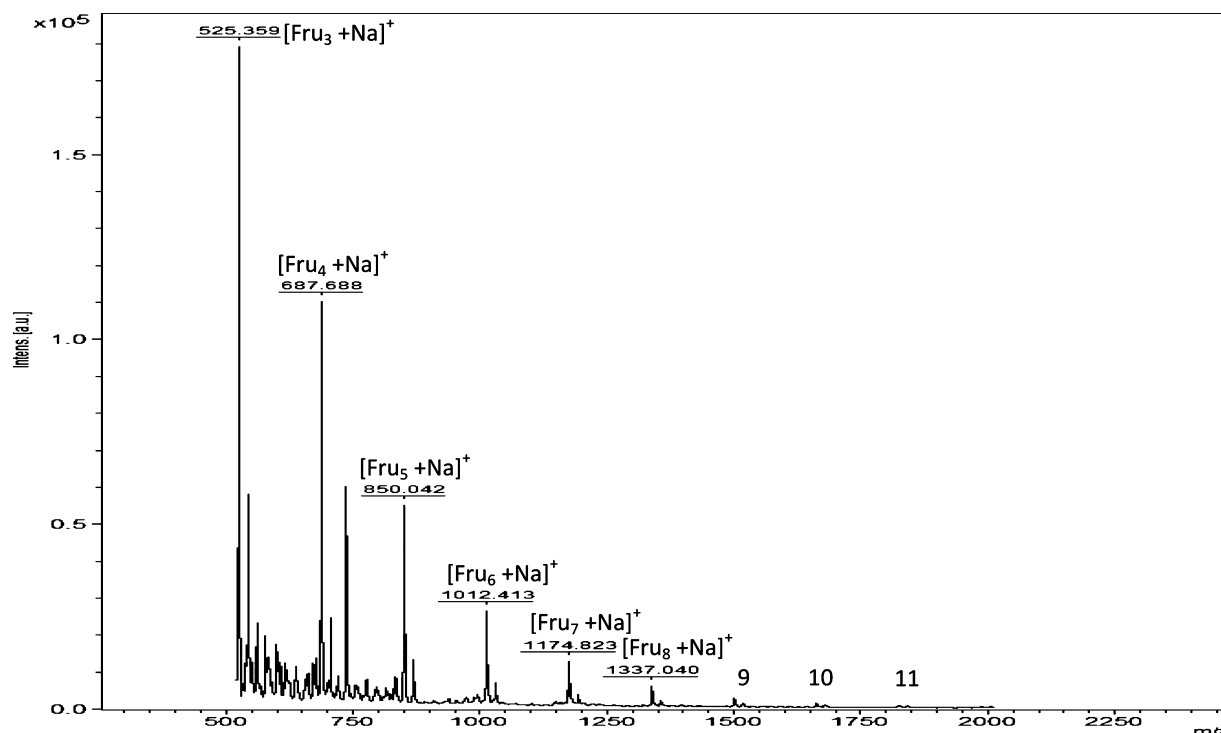
Fig. 2. MALDI-TOF-MS spectra of inulin from *P. glomerata* roots.

3. Results and discussion

3.1. Isolation and chemical characterization

The dry extract of *P. glomerata* roots that was obtained by hot water extraction and ethanol precipitation showed a yield of 11.45% (w/w). The water-soluble carbohydrate and protein content of the dry extracts were estimated by colorimetric methods. Total sugar concentration was $68.86\% \pm 3.50\%$ which was composed of fructose $59.66\% \pm 1.69\%$, and free reducing sugars $6.76\% \pm 0.46\%$. Protein concentration was $8.76\% \pm 1.34\%$. These results indicate that the dry extract was primarily composed of fructose-type carbohydrates in linked form. The calculated DP was 13.

To identify the monosaccharides and the glycosidic linkage, partially methylated alditol acetates (PMAA) were analyzed by GC–MS, following previously published methods in the literature [34–36]. The results showed the presence of derivatives 2,5-*O*-acetyl-1,3,4,6-tetra-*O*-methyl-mannitol, 2,5-*O*-acetyl-1,3,4,6-tetra-*O*-methyl-glucitol, 1,2,5-*O*-acetyl-3,4,6-tri-*O*-methyl-mannitol, and 1,2,5-*O*-acetyl-3,4,6-tri-O-methyl-glucitol. The major derivative of fructose is 1,2,5-*O*-acetyl-3,4,6-tri-*O*-methyl-mannitol indicating a β-(2 → 1)-linked backbone. Results indicate an overall linear structure of the *P. glomerata* fructan with D-glucopyranosyl end group, however it was not possible to identify the derivative 1,5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol for glucose. The absence of this derivative in the results may be because it was only present in low amounts, according to Chen et al. [34] and Izydorczyk [10].

The MALDI-TOF mass spectrum (Fig. 2) was similar to inulin standard and both had ions with a mass difference of 162 Da, which corresponds to fructose/glucose residues. In the spectra it was possible to identify the presence of sodium adducts $[M+Na]^+$ agreeing with the data presented for inulin by Oliveira et al. [25]. The optical rotation was $[\alpha]_D^{20} - 56$ (c 0.1, H_2O) which was close to the determined by Wu et al. [23] and Budavari et al. [24] for inulin and fructooligosaccharides.

The GPC refractive index (RI) elution profiles for chicory inulin Orafti® GR (DP < 10) can be seen in Fig. 3(a), this for *P. glomerata* inulin in Fig. 3(b). It is shown in the RI profiles presented that the *P. glomerata* sample tested elute at similar elution time (approximately 60 min) that the standadard chicory inulin sample suggesting that they have approximately the same molar mass.

Evans et al. [37] shown that the RI and M_w elution profiles for the chicory inulin samples with small M_w values become more erratic. This is as a consequence of the fact that the light scattering signal is very weak due to the low molecular mass (and in certain cases low concentration) of the fructan molecules eluting. Increasing the overall sample concentration will increase the light scattering intensity but the refractive index signal can then become saturated. This means that low molecular mass molecules particularly for the chicory inulin Orafti® GR and *P. glomerata* inulin samples, are, therefore, not taken into account when the average
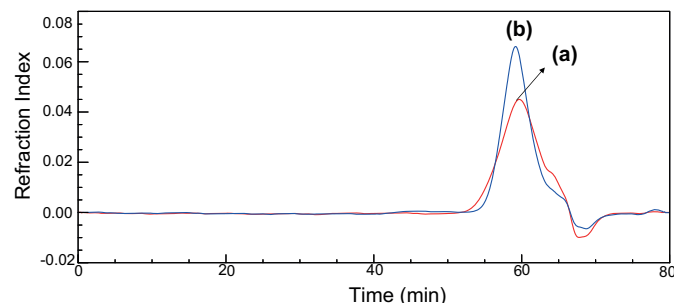

Fig. 3. HPSEC-RI elution profile of: (a) Standard inulin (Orafti® GR) and (b) *P. glomerata* inulin from roots. Analytical conditions: four Waters Ultrahydrogel columns were used in sequence and with different exclusion limit: 7×10^6 (column 2000); 4×10^5 (column 500); 8×10^4 (column 250) and 5×10^3 (column 120). The eluent used was sodium nitrite solution 0.2 mol/L with sodium azide 0.02% (w/v) with a flow of 0.6 ml/min, monitored by the peristaltic pump 515 WATERS.
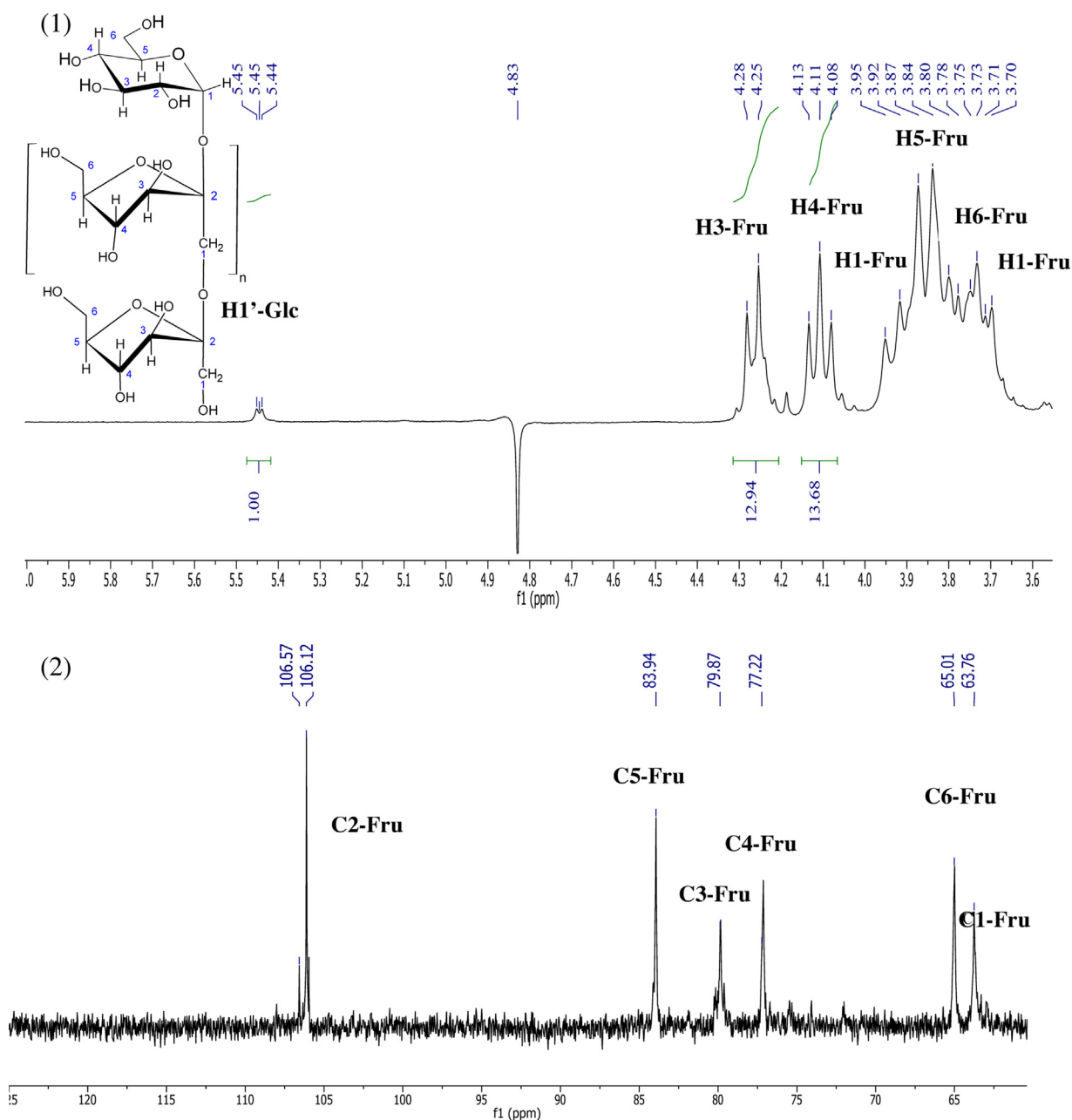
Fig. 4. ^{1}H and ^{13}C NMR (300.05 MHz and 75.45 MHz) spectra of inulin from *P. glomerata* roots in D_2O.

M_w and M_n values are determined using the instrument software. However, the M_w and M_n of *P. glomerata* inulin can be estimated by comparison with Orafti® GR ($M_w = 2500$ and $M_n = 1500$) which was established was obtained using pullulan and dextran Standards [37].

The ^{1}H NMR spectrum (Fig. 4) showed the presence of 1 signal in the anomeric region at δ 5.45 (*J* = 3.8 Hz), which corresponded to H1-Glu and other signals at δ 4.11 (H3-Fru, *J* = 8.0 Hz), δ 4.27 (H4-Fru), and *J* = 8.4 Hz, and between δ 3.70 and 3.95 (H1-Fru, H5-Fru and H6-Fru). The DP calculated by ^{1}H NMR was also 13. The ^{13}C NMR spectrum revealed characteristic peaks of anomeric carbons at δ 106.57 and 106.12, and sugar ring carbons linked to oxygen in the region δ 63.76–83.96. The absence of anomeric carbons peaks in the DEPT-135 ^{13}C NMR spectrum indicates that quaternary carbon atoms are present in the compound. The reversed peaks at δ 63.90 and 65.01 were assigned to methylene group signals. Three other signals (δ 77.22, 79.87, and 83.94) were assigned to C—H

group signals. ^{1}H and ^{13}C NMR chemical shifts were also identified by comparison with data from the published literature, and were found to be similar to the inulin standard spectrum [20,25,38–40].

In COSY, TOCSY, and HMBC, spectrum chemical shifts of D-fructofuranosyl units were fully assigned based on data from the published literature [35,38]. In NOESY, it was possible to determine a β-configuration at the anomeric center of fructosyl residues, which was evident by the correlation between H1/H3-Fru. The sequence of glycosyl residues was determined from an HMBC experiment (Fig. 5). The HMBC spectrum showed a correlation between the H1-Fru/C2-Fru of fructosyl residues and indicate the presence of (2 → 1)-linked β-D-fructofuranosyl [38].

3.2. Prebiotic test

As inhabitants of the gastrointestinal tract, lactobacilli and bifidobacteria use a wide range of carbohydrate sources as a

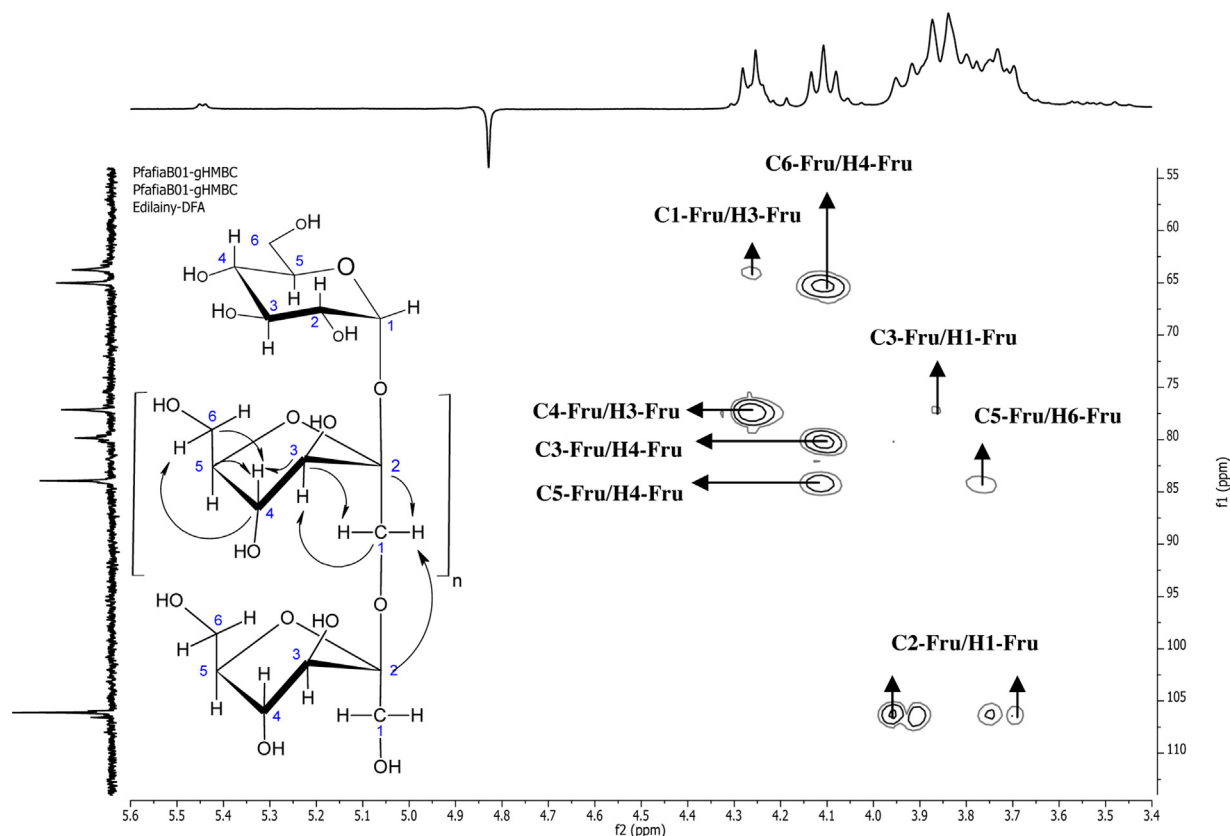**Fig. 5.** HMBC correlation map of inulin from *P. glomerata* roots in D_2O.

growth substrate, including monosaccharides, oligosaccharides, and polysaccharides [41]. As representatives of saccharolytic microflora, they have an enzymatic assembly, such as glycoside hydrolases [42], particularly β-fructofuranosidase which are responsible for the degradation of polysaccharides and oligosaccharides into fermentable sugars. The final metabolic products of this fermentation are short-chain fatty acids (SCFAs), such as butyric, acetic, and propionic acids [43], as well as the gases carbon dioxide and hydrogen [44].

This study demonstrated that *Pfaffia* inulin enhance the growth of both bifidobacterial and lactobacilli populations; although strain specific differences in fermentability were detected (Table 2). Strains from the genus *Bifidobacterium* utilized the medium containing *Pfaffia* inulin to a certain extent (particularly strains JKM and AVNB3-P1).

The lactobacilli strains *Lbc. gasseri* PHM-7E1 and *Lbc. acidophilus* CCDM 151 utilized *Pfaffia* inulin as a carbon source, with particularly high growth rate ($P < 0.05$) recorded for the strain *Lbc. gasseri*

Table 2
Fermentability of inulin from *P. glomerata* roots by lactobacilli and bifidobacteria.

	P	GR	WCH	BM
Lactobacillus fermentum RL25	0.97 ± 0.18[b]	4.40 ± 0.18[e]	3.90 ± 0.22[bc]	0.73 ± 0.20[b]
Lactobacillus casei subsp. *paracasei* PE1TB-P	0.43 ± 0.25[a]	1.15 ± 0.31[a]	3.13 ± 0.25[a]	0.18 ± 0.09[a]
Lactobacillus animalis CCDM 382	1.08 ± 0.23[b]	0.98 ± 0.31[a]	4.00 ± 0.37[bc]	0.88 ± 0.21[b]
Lactobacillus acidophilus CCDM 151	2.60 ± 0.36[d]	4.38 ± 0.17[e]	4.16 ± 0.21[c]	0.95 ± 0.44[b]
Lactobacillus gasseri PHM-7E1	3.57 ± 0.40[e]	4.53 ± 0.22[e]	4.23 ± 0.29[c]	0.90 ± 0.36[b]
Lactobacillus gasseri CCDM 214	1.20 ± 0.40[b]	1.13 ± 0.17[a]	4.18 ± 0.13[c]	0.30 ± 0.14[a]
Average (lactobacilli)	1.64 ± 1.15[β]	2.76 ± 1.73[γ]	3.95 ± 0.44[δ]	0.65 ± 0.39[α]
Bifidobacterium bifidum CCDM 559	0.75 ± 0.13[ab]	3.10 ± 0.26[bc]	3.06 ± 0.38[a]	0.18 ± 0.09[a]
Bifidobacterium animalis subsp. *lactis* CCDM 94	1.02 ± 0.25[b]	1.00 ± 0.26[a]	4.05 ± 0.29[c]	0.23 ± 0.09[a]
Bifidobacterium breve CCDM 562	0.93 ± 0.25[ab]	2.93 ± 0.22[b]	3.08 ± 0.47[a]	0.23 ± 0.13[a]
Bifidobacterium bifidum JOV	1.93 ± 0.35[c]	3.37 ± 0.42[c]	4.18 ± 0.39[c]	0.17 ± 0.06[a]
Bifidobacterium bifidum JKM	1.97 ± 0.30[c]	3.90 ± 0.30[d]	4.13 ± 0.46[c]	0.17 ± 0.12[a]
Bifidobacterium adolescentis AVNB3-P1	1.80 ± 0.46[c]	3.10 ± 0.46[bc]	3.50 ± 0.57[ab]	0.23 ± 0.12[a]
Average (bifidobacteria)	1.40 ± 0.58[¥]	2.82 ± 0.99[£]	3.62 ± 0.63[€]	0.20 ± 0.09[$]

P – medium containing *pfaffia* inulin as a carbon source, GR – Medium containing standard GR Orafti inulin as a carbon source, WCH – Wilkins-Chalgren broth (positive control), BM – basal medium without any sugar (negative control).
[abcde] – data in columns with different superscripts differ ($P < 0.05$).
[αβγδ,$¥£€] – data in lines with different superscripts differ ($P < 0.05$).

Table 3
Production of lactic and acetic acids in the media containing *Pfaffia* FOS after 24 h of incubation (measured using isotachophoresis, from triplicate determination ± sandard deviation).

	Lactic acid (mg/100 ml)	Acetic acid (mg/100 ml)
Lbc. fermentum RL 25	31 ± 7	14 ± 4
Lbc. casei subsp. *paracasei* PE1TB-P	25 ± 5	16 ± 4
Lbc. animalis CCDM 382	18 ± 5	13 ± 3
Lbc. acidophilus CCDM 151	70 ± 3	34 ± 4
Lbc. gasseri PHM-7E1	136 ± 5	39 ± 3
Lbc. gasseri CCDM 214	22 ± 5	19 ± 2
Bif. bifidum CCDM 559	26 ± 6	14 ± 4
Bif. animalis subsp. *lactis* CCDM 94	22 ± 3	33 ± 3
Bif. breve CCDM 562	28 ± 6	24 ± 6
Bif. bifidum JOV	54 ± 5	48 ± 4
Bif. bifidum JKM	54 ± 5	43 ± 4
Bif. adolescentis AVNB3-P1	46 ± 5	32 ± 4

PHM-7E1. The growth was evaluated to be comparable with the growth of this strain in the control medium, additionally the acids' production (Table 3) rose significantly, namely, the production of lactic acid of the strain PHM-7E1 in the *Pfaffia*-containing medium made about 1360 mg/L.

All strains tested in this study were able to utilize *Pfaffia* inulin. Based on the available literature, the DP (as well as the chain length) represents an important factor influencing the oligo- or polysaccharide fermentation and thus utilization by bacteria [45]. Longer chains are fermented more slowly than short chains, with a longer period being required for them to be utilized. In addition, shifting protein fermentation to the more distal parts of the large intestine may have a beneficial function by lowering the production of carcinogenic substances [46]. However, the rate of fermentation may also be dependent on other factors, such as saccharide structure (*e.g.*, glycosidic linkage, degree of molecule branching), the bacteria-substrate relationship, and fermentation products [47]. Metabolic collaboration also seems to play an important role in sugars fermentation in various bacterial species present in the colon [44].

An important criterion that prebiotics must meet is that they must selectively stimulate the growth of intestinal bacteria that contribute to the health and well-being of the host [48]. According to the available published literature, in addition to supporting the growth of desirable bacteria, many prebiotic candidates support the growth of potentially harmful and pathogenic bacteria present in the colon [49,50]. In prebiotics, selective fermentability in favor of beneficial bacteria is required. Thus, further studies are required to characterize the selectivity of *Pfaffia* inulin and to gain sufficient data to evaluate their potential prebiotic effects.

4. Conclusion

In this study, inulin-type polysaccharides were isolated from *P. glomerata* roots. The extracted polysaccharides had 68.86% total sugar content, of which 59.66% was fructose. Based on 2D NMR spectra, the extracted polysaccharides consist of $(2 \rightarrow 1)$-linked β-ᴅ-fructofuranosyl units, with one possible glucose unit on the non-reducing end. GC–MS, NMR spectra, and MALDI-TOF-MS confirmed the structure and presence of inulin as the major component of the roots. Furthermore, it was concluded that representatives of the genera *Lactobacillus* and *Bifidobacterium* utilize *Pfaffia* inulin as a carbon source, although this ability was found to be strain specific. The potential prebiotic effect of *Pfaffia* inulin requires further specification, and confirmation of its selective fermentability by beneficial bacteria.

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