Food Chemistry 362 (2021) 130167

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Prebiotic potential and chemical characterization of the poly and oligosaccharides present in the mucilage of *Opuntia ficus-indica* and *Opuntia joconostle*

José M. Cruz-Rubio *, Monika Mueller, Helmut Viernstein, Renate Loeppert, Werner Praznik *

Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

ARTICLE INFO

Keywords:
Nopal
Heteropolysaccharides
Pectic substances
Dietary fiber
Gastrointestinal microbiome
Prebiotics

ABSTRACT

The mucilage extracted from the convection-dried cladodes of *O. ficus-indica* and *O. joconostle,* two species of economic importance, delivered three fractions after methanol precipitation. Two were composed of high molar mass polysaccharides, and one included water-soluble mono-, di-, and oligosaccharides. The large polysaccharides have a molar mass range of 4.0×10^3 to 8.0×10^5 g·mol^{-1} and are consistently composed of galactose, arabinose, xylose, and rhamnose; however, the content of galacturonic acid was different between both fractions and species. Their fermentability by selected probiotics was relatively low, 11–27 % compared to glucose, and decreased with increasing levels of galacturonic acid in the molecules. In the third fraction, previously unreported oligosaccharides were found. These include simple- and complex-structured galactooligosaccharides with arabinosyl-, xylosyl- and galacturonosyl acid residues. Their fermentability by prebiotic species can be ascribed more to their structural characteristics and monosaccharide composition than their molecular dimensions.

1. Introduction

The cladodes and fruit of nopal (*Opuntia* spp.) have been a food staple for centuries in North and Central America (Piperno, 2011), and have been recently touted as a functional ingredient (Patel, 2014; Stintzing & Carle, 2005); they are often used in weight-loss diets, particularly in Mexico (Onakpoya, O'Sullivan, & Heneghan, 2015). Cladodes for human consumption (*nopales*) are sourced from *Opuntia ficus-indica* and its different cultivars (Sáenz et al., 2013). *O. joconostle* has been widely cultivated as well, but it is favored for the production of its acidic, savory fruits instead for the cladodes (Morales, Ramírez-Moreno, Sanchez-Mata, Carvalho, & Ferreira, 2012).

The cladodes of *Opuntia* spp. have a high content of both soluble and insoluble fiber on a dry matter basis (Hernandez-Urbiola, Perez-Torrero, & Rodriguez-Garcia, 2011; Peña-Valdivia, Trejo, Arroyo-Pena, Sanchez Urdaneta, & Balois Morales, 2012); soluble fiber is present in the form of a highly viscous solution named mucilage while insoluble fiber is present mostly as lignin and hemicelluloses. The proportion between soluble and insoluble fiber is quite variable and related to the cultivar and age of the cladode, with lignin content increasing with cladode age. The content and composition of the mucilage is dependent on the *Opuntia* species and developmental stage of the cladodes (López-Palacios, Peña-Valdivia, Reyes-Agüero, & Rodríguez-Hernández, 2011). Authors of recent studies have discussed the potential of mucilage as a functional food, as there is evidence of hypoglycemic effect on diabetic-induced rats of different ages after nopal intake (Nuñez-Lopez, Paredes-Lopez, & Reynoso-Camacho, 2013). Additionally, the ability of the mucilage to modify the microbiota in rats was shown, including a decreasing metabolic endotoxemia, glucose insulinotropic peptide, glucose intolerance, lipogenesis, and metabolic inflexibility after a diet high in fat and sugar (Sanchez-Tapia et al., 2017). Furthermore, the mucilage has shown a prebiotic potential with an increase in lactobacilli and bifidobacteria and a slight decrease in pathogenic bacteria (Guevara-Arauza et al., 2012).

The monosaccharide composition of the mucilage of *O. ficus-indica* includes xylose, rhamnose, galactose, arabinose and galacturonic acid, with a proposed structure of a highly branched polysaccharide with a main backbone composed of rhamnosyl-, galactosyl- and galacturonic acid residues and branching side chains of xylosyl- and arabinosyl-residues (Trachtenberg & Mayer, 1981). On the contrary, a more recent study (Di Lorenzo et al., 2017) has found two major carbohydrate populations: an unbranched β-linked galactan polymer and a highly

* Corresponding author.
E-mail addresses: jmcruz1982@gmail.com (J.M. Cruz-Rubio), werner.praznik@univie.ac.at (W. Praznik).

https://doi.org/10.1016/j.foodchem.2021.130167
Received 6 January 2021; Received in revised form 19 April 2021; Accepted 18 May 2021
Available online 21 May 2021

branched arabinan-rich polysaccharide. However, this last work does not attempt to place the galacturonic acid residues in their proposed structures.

To the best of our knowledge, no publication has discussed the carbohydrate profile and molecular dimensions of the mucilage and their relation to the fermentability by probiotic bacteria. Moreover, in all the studies mentioned before, the mucilage was extracted from the fresh cladodes, then dried and used. As the fresh cladodes of *O. ficus-indica* have a limited shelf-life they are often dried and milled to be sold as a flour, either as an ingredient in prepared foods to increase the fiber content, or ingested directly as a dietary supplement, touting the same functional properties of the fresh plant. Previous work in our group (Cruz-Rubio, Mueller, Loeppert, Viernstein, & Praznik, 2020) has shown that different drying treatments, from lyophilization to convection drying, while delivering some modifications to the molecular dimensions, have no significant effect on the fermentation of the polysaccharides present in the mucilage by selected probiotic bacteria. Moreover, an extensive literature search did not yield any results on the presence (or characteristics) of naturally occurring oligosaccharides, mainly as previous work has focused on the high molar mass components of the mucilage. Therefore, the aim of this work was to fractionate the poly- and oligosaccharides that form the mucilage isolated from convection-dried cladodes of two *Opuntia* species -*Opuntia ficus-indica* and *Opuntia joconostle*- and subsequently characterize their molecular dimensions, monosaccharide composition and structural composition, as well to evaluate their prebiotic potential by four widely used probiotic bacteria.

2. Material and methods

2.1. Mucilage extraction

Cladodes from *O. ficus-indica* (L.) Mill and *O. joconostle* F.A.C. Weber, grown in the same region (Cuquio, Jalisco, Mexico) and harvested at the same age, were collected and the thorns and glochids removed. The cladodes were convection-dried (succinctly, at 50 °C for 36 h and then milled to a fine flour) as described in detail before (Cruz-Rubio et al., 2020). The dry flour was then extracted overnight three times with acetone at a ratio of 20:1 acetone:dry cladode (*v/w*) under vigorous stirring. Acetone was then removed by centrifugation (9 000 × *g*, 15 min). The same procedure was applied with 95 $\%_{v/v}$ methanol, and finally the flour was left to dry at room temperature.

To extract the mucilage, the washed and dried flour was extracted thrice with deionized water at 80 °C for 1 h. The water:solids ratio (*v/w*) was 35:1, 25:1 and 20:1 consecutively. After each extraction, the supernatant was separated by centrifugation (24 000 × *g*, 30 min). The three supernatants (mucilage) were pooled together and further centrifugated for 90 min at 24 000 × *g* to remove fines, with the solids-free supernatant separated and lyophilized until further use. Supplementary Fig. S1 shows the purification and subsequent purification steps of the samples created for this investigation.

2.2. Fractional methanol precipitation

4 g of dry mucilage were dissolved in 40 ml of water and vigorously stirred at 60 °C overnight. After this step, the solution was centrifuged at 12 000 × *g* for 20 min and the precipitate discarded. To this solution, an equal volume of pure methanol (i.e. 40 ml) was slowly added while being stirred. The solution was left in agitation for 30 min, and centrifuged at 9000 × *g* for 20 min. The supernatant was collected, and the separated precipitate was washed with 20 ml of 50 $\%_{v/v}$ methanol, centrifugated at the same conditions, and this last washing step was repeated four times. The precipitate was then lyophilized and named fraction 0–50 (F0-50). The supernatants from all the previous steps were pooled together and 150 ml of 100 % methanol were added to reach a methanol concentration of ca. 80 %. The suspension was centrifuged, the supernatant collected, and the pellet was washed four times with 80 % methanol as described before. The pellet was lyophilized and became fraction 50–80 (F50-80); the supernatants were collected, pooled together and the methanol removed by evaporation under reduced pressure. This soluble material was lyophilized and designated fraction 80–100 (F80-100).

2.3. Preparative size exclusion chromatography (prepSEC)

2.5 ml of 8 $\%_{w/v}$ solutions of F80-100 were injected into a preparative SEC system which consisted of one precolumn (20 × 1.5 cm) packed with Toyopearl HW 40-S (Tosoh Bioscience GmbH), followed by a 110 × 1.5 cm column packed with Sephacryl 200-HR (GE Healthcare Life Sciences) and a 125 × 1.5 cm column packed with Bio-Gel P-2 (Bio-Rad Laboratories, Inc.). The separation eluent was distilled water at a flow rate of 0.6 ml·min^{-1}. A differential refractive index detector (WellChrom K-2301, Knauer GmbH) and an analog chart recorder (REC-102, GE Healthcare Life Sciences) were used for monitoring and recording the separation profile. The plots were digitalized using WebPlotDigitizer (Rohatgi, 2020). The collected fractions' size was 6 ml and were pooled in adequate fractions corresponding to the separation profile (Fig. 1 B). For *Opuntia ficus-indica* fractions F1 to F7 were collected. For *O. joconostle* only fractions F1 to F5 were found. In order to obtain enough material, 6 replicate injections were performed, and equal fractions were pooled together and freeze dried until further use.

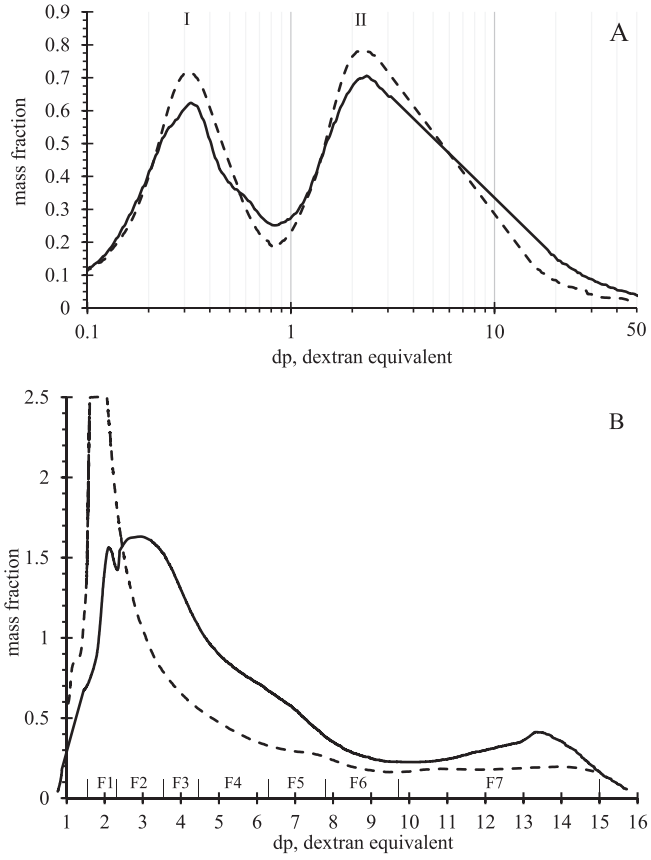

Fig. 1. A: Degree of polymerization distribution of F80-100 from analytical SEC system. Molecular dimensions of peaks I and II are given in Table 3. B: Degree of polymerization distribution of F80-100 from the preparative SEC system. *O. ficus-indica* (solid) collected F1 to F7 and *O. joconostle* (dotted), collected F1 to F5.

2.4. Monosaccharide composition and content

2.4.1. Total carbohydrate content

A phenol–sulfuric semi-micro method was used for the quantification of carbohydrate content of the samples (Cruz-Rubio et al., 2020). Briefly, the samples were dissolved in water to a final concentration of 22.5 μg/ml. To 150 μl of this solution (after cooling in ice water) 600 μl of concentrated H_2SO_4 at 4 °C were added, the mixture thoroughly agitated, and incubated at 80 °C for 30 min. After the reaction cooled to room temperature, 10 μl of 90 % phenol were added followed by vigorous mixing and left to proceed for 30 min. 200 μl of the mixture were transferred to a well of a 96-well microplate, and the absorbance was read at 480 nm. Standards closely matching the average monosaccharide composition of the samples were used to prepare the calibration curve.

2.4.2. Hydrolysis and thin layer chromatography (TLC)

Shortly, the monosaccharide composition was determined after hydrolysis of the polysaccharides by incubating a 10 mg/ml saccharide solution with 2 M trifluoroacetic acid for three hours at 110 °C, as described in more detail in our previous report (Cruz-Rubio et al., 2020).

The presence and type of monosaccharides were evaluated by thin layer chromatography (TLC). HPTLC silica gel 60 (Merck KGaA) plates were used, and after injection of 3 μl of hydrolyzed sample or 1 μl of standard per lane, the plates were developed thrice with acetonitrile: 0.3 % ammonium hydroxide (17:3) as mobile phase. Detection was achieved by immersion in thymol (0.2 $\%_{w/v}$) and sulfuric acid (5 $\%_{v/v}$) in methanol reagent. Color was developed by placing the plate in an oven for 2 min at 95 °C. Standards were prepared at 1 mg/ml of each monosaccharide (rhamnose, xylose, arabinose, mannose, glucose, galactose, sucrose, glucuronic acid, and galacturonic acid) in 50 $\%_{v/v}$ methanol.

2.4.3. High performance anion exchange chromatography – Pulsed amperometric detection (HPAEC-PAD)

For the quantification of monosaccharides an ICS-3000 system (Thermo Fisher Scientific Inc.) with a Carbopac PA1 precolumn (4 × 50 mm) plus column (4 × 250 mm) and pulsed amperometric detection was used. Briefly, 10 μl of hydrolysate were diluted in water 1:50 and were injected into the system, with an elution gradient as follows: −20 to −2.5 min, 200 mM NaOH. −2.5 to 22 min, NaOH 10 mM + 2 mM NaOAc. 22 to 40 min, ramp up to 200 mM NaOH and 200 mM NaOAc. 40 to 50 min, 200 mM NaOH at a flow 1 $ml{\cdot}min^{-1}$. The standard carbohydrate waveform was used for detection. A more detailed description can be found in (Cruz-Rubio et al., 2020).

2.4.4. 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization of the monosaccharides

The derivatization method proposed by Wu, Jiang, Lu, Yu, and Wu (2014) was followed with slight modifications. 20 μl of hydrolyzed polysaccharide were placed in the bottom of 16 × 100 mm screw-cap test tubes and evaporated to dryness at room temperature under reduced pressure. Once dry, 200 μl of 32 % ammonia solution were added and the contents vortexed until all the solids were dissolved. 200 μl of methanolic 0.3 M PMP were added, the tube tightly capped and incubated at 70 °C for 30 min under vigorous stirring. After the reaction was completed, the contents were evaporated to dryness under reduced pressure. To the dry contents of the tube, 200 μl of 50 % methanol were added, vortexed thoroughly, and then evaporated to dryness. This step was performed thrice, in order to remove the ammonia and other volatile salts. Once finished, 400 μl of water were added to the tube and the contents thoroughly mixed. To this solution 500 μl of $CHCl_3$ were added, mixed vigorously, centrifuged at 3 000 × *g* and the organic phase was carefully discarded. This step was repeated three times after which the aqueous phase was transferred to an Eppendorf tube and centrifuged at 22 000 × *g* for 15 min. 250 μl of supernatant were taken from the tubes and placed in the chromatography vials for injection.

2.4.5. C18 reverse phase HPLC of the PMP-derivatized monosaccharides

An Ultimate 3000 UHPLC system (Thermo Fisher Scientific Inc.) with a diode array detector was used. The mobile phase consisted of acetonitrile:phosphate buffer 0.1 M pH 6.7 in a proportion 84.3:15.7 (*v/v*) at a flow rate of 1.8 $ml{\cdot}min^{-1}$. The phosphate buffer was prepared gravimetrically by dissolving 6.707 g of sodium phosphate monobasic and 6.246 g of sodium phosphate dibasic in 1000 ml of type I water. The buffer solution was filtered (0.22 μm) under vacuum and ultrasound, and further treated with ultrasound under reduced pressure for 1 h to completely remove gases from the eluent. After the thorough degassing, the buffer was volumetrically mixed with the acetonitrile. The column system used consisted of a Poroshell 120 EC-C18 precolumn (4.6 × 5 mm, 2.7 μm) followed by a Poroshell 120 EC-C18 (4.6 × 100 mm, 2.7 μm) column (Agilent Technologies, Inc.). The column was kept at 30 °C, the injection volume was 5 μl, and a detection wavelength of 245 nm was used; the run time was 22 min.

2.5. TLC characterization of the oligosaccharides

O. ficus-indica fractions F1 to F7 and *O. joconostle* fractions F1 to F5 were characterized by HPTLC. 5 μl of sample at 4 mg/ml, or 1 μl of standard at 1 mg/ml per monosaccharide in 60 $\%_{v/v}$ methanol per lane were injected on 10 × 20 cm HPTLC silica gel 60 (Merck KGaA) plates using a Linomat 5 (Camag AG). Next, the plates were developed thrice with 1-propanol:water 7:3.5 *v/v* as the mobile phase (Dongowski, 1996). Detection was achieved by immersion in thymol (0.2 $\%_{v/v}$) and sulfuric acid (5 $\%_{v/v}$) in methanol reagent. Color was developed by placing the plate in an oven for 2 min at 95 °C. The plates were digitalized using a flatbed scanner, and the brightness and contrast modified to try and match the live appearance of the plate; however, the colours were not represented faithfully in the digital version.

2.6. Determination of molar mass distributions by analytical size exclusion chromatography (anSEC)

The molar mass distribution was evaluated using a SEC column system as described in Cruz-Rubio et al. (2020). Shortly, 1 mg of saccharide (dissolved in 300 μl of eluent) were injected into a system consisting of a pre column (10 × 100 mm) packed with Toyopearl HW 40S plus three 10 × 300 mm columns filled with Superose 6, Superose 12, and Toyopearl HW 40S respectively. The eluent was 50 mM NaCl + 0.01 $\%_{w/v}$ NaN_3 at a flow rate of 0.6 $ml{\cdot}min^{-1}$. Detection was achieved by using a differential refractive index detector, and the system was calibrated using narrow standard dextrans (Pharmacosmos A/S) from a M_w of 667 800 (dextran 670) to 180 (glucose). CPCwin (a.h. group, Graz, Austria) was used for calculation of the molecular dimensions. The M_w results are reported as dextran equivalent. The calculated molar mass was converted to degree of polymerization (dp) by dividing by a factor of 162 $g{\cdot}mol^{-1}$ per dp unit. This factor corresponds to an anhydrohexose and was chosen as a representative value between the included anhydropentoses and anhydrouronic acids in the saccharides.

2.7. Determination of the fermentability by probiotic strains

The fermentability, and by extension prebiotic potential, of the isolated carbohydrates was evaluated by measuring the growth of the probiotic strains *L. rhamnosus* GG (ATCC 53103), *L. acidophilus* (DSM 13241), *B. longum* ssp. *infantis* (ATCC 15697) and *B. animalis* ssp. *lactis* Bb-12 (DSM 15954) in the presence of the tested saccharide using a previously described method (Mueller, Reiner, Fleischhacker, Viernstein, Loeppert, & Praznik, 2016). In brief, the bacterial strains were incubated in Man-Rogosa-Sharpe (MRS) medium (10 g of peptone from casein, 8 g of meat extract, 4 g of yeast extract, 1 g di-potassium-hydrogen phosphate, 2 g Tween 80, 2 g of di-ammonium-hydrogen

citrate, 5 g of sodium acetate, 0.2 g of magnesium sulfate, 0.5 g cysteine hydrochloride, and 0.04 g manganese sulfate per liter) overnight. Yeast extract was obtained from Oxoid (Hampshire, UK). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). The OD_{600} of the fresh overnight culture was measured and the required amount of cells was calculated. After washing the bacteria using phosphate buffered saline (PBS) supplemented with 0.5 g/L cysteine hydrochloride three times, the cells were resuspended in MRS-medium without carbohydrate source. Bacteria were incubated without sugar (negative control) or with the respective samples (1 $\%_{w/w}$ sample) in honeycomb plates with a final volume of 200 μl and a starting OD_{600} of 0.1 at 37 °C for 48 h. The OD_{600} was measured using a Bioscreen C microbiological growth monitoring system (Oy Growth Curves Ab Ltd) every hour after mixing by shaking for 15 sec. All samples were tested in duplicated or triplicates at various concentrations. The average was calculated and compared to the OD_{600} increase in the presence of the positive control glucose. To calculate the relative growth rate (RGR), the OD_{600} of the negative control was subtracted from the OD_{600} of the sample plus probiotic, and this value divided by the negative-corrected OD_{600} value of glucose. The raw growth data was normalized by dividing the relative grow rate by the carbohydrate content in order to be able to compare samples with differing saccharide content.

3. Results and discussion

Before the extraction of mucilage, the dry, powdered cladodes were extracted with acetone and methanol in order to remove rests of water, apolar components such as chlorophyll, free available monosaccharides, and salts. This procedure prevents interferences in the following analytical treatments and reduced non-fermentable material for the determination of prebiotic potential determination. Moreover, methanol can be easily removed and does not form an azeotrope in comparison to ethanol, allowing for a better handling during the subsequent carbohydrate characterization. For food use, methanol can be easily substituted for ethanol.

The hydrothermally (at 80 °C) extracted mucilage spontaneously builds up supramolecular clusters with the available polysaccharides held together by ionic and hydrophilic interactions; additionally, minor components such as salts and protein can support this building process. Low concentrations and high temperatures help reduce these interactions between the polymer molecules and allow a better insight into the real molecular dimensions of the mucilage polysaccharides.

We had previously found that the mucilage extracted from convection-dried *O. ficus-indica* and *O. joconostle* cladodes have a molar mass distribution in a range of 10^4 to 10^6 g·mol^{-1} for both species (Cruz-Rubio et al., 2020). The average molar mass for *O. ficus-indica* was 3.7 × 10^5 g·mol^{-1} and 4.7 × 10^5 g·mol^{-1} for *O. joconostle*, with a dispersity value of 3.4 for the former and 2.6 for the latter. Moreover, we found that the average molar mass ($\bar{M}_w$) decreased and the dispersity increased after the long-time convection drying process; e.g. for *O. ficus-indica* the $\bar{M}_w$ decreased to 2.6 × 10^5 g·mol^{-1}, while the dispersity increased to 3.7. These results indicate that the mucilages include lower molar mass components. Furthermore, this decrease in $\bar{M}_w$ allows for the assumption of a partial breakdown of the mucilage matrix after the extended heating time.

3.1. Fractional methanol precipitation of the mucilage: yields, composition and molecular dimensions of the polysaccharide fractions

To obtain more information about the actual molecular dimensions of the polysaccharides a relatively simple fractionation technique by means of methanol precipitation was applied. In this case the increasing concentration of organic solvent reduce the ionic and hydrophilic interactions between the molecules and allows for an easy separation of the high molar mass polysaccharides from the low molar mass components. Using this procedure, naturally occurring oligosaccharides, which are non-covalently bonded to the mucilage polysaccharides could be separated and identified.

This methanol precipitation procedure yielded three fractions: two precipitates (F0-50 and F50-80) and one supernatant (F80-100). Precipitation occurred after increasing the solutions' methanol concentration up to 50 $\%_{v/v}$. The precipitate, F0-50, is a polysaccharide-enriched fraction including polysaccharide populations with an average molar mass of 2.0 × 10^5 g·mol^{-1} for *O. ficus-indica* and 1.9 × 10^5 g·mol^{-1} for *O. joconostle* (Table 1). After increasing the concentration of methanol to 80 $\%_{v/v}$ two more fractions were obtained. The precipitate (F50-80) is a fraction composed mostly of the more water-soluble polysaccharides, with an average molar mass of 2.6 × 10^5 g·mol^{-1} for *O. ficus-indica* and 1.5 × 10^5 g·mol^{-1} for *O. joconostle*, with a lower amount of high molar mass polysaccharides. The supernatant became F80-100; a fraction composed mostly of salts, di- and oligosaccharides along with non-specified water-soluble components. The population of oligomers in F80-100 delivers an average molar mass of 4.8 × 10^2 g·mol^{-1} for *O. joconostle* and 6.3 × 10^2 g·mol^{-1} for *O. ficus-indica*. After lyophilizing all the fractions, the proportion of F0-50 : F50-80 : F 80–100 is approximately 60 : 20 : 20 $\%_{w/w}$. The broad molar mass distribution induced by the methanol precipitation steps in the mucilage indicates a partial breakdown of the supramolecular assembly into predominantly covalently structured molecules in each fraction.

Table 2 shows the monosaccharide composition of the fractions obtained after the fractional precipitation. For the quantitative analysis of the monosaccharides and uronic acids two chromatographic techniques were utilized: high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and reverse phase chromatography (rp HPLC) with pre-derivatization of the monosaccharides with PMP. Both methods delivered similar results and were applied together as the mean ± the limit of agreement. The main reason for using both methods was to be certain of the monosaccharide composition. While HPAEC-PAD is the method of choice in our group given its relative simplicity and sensitivity, certain monosaccharide pairs, such as xylose and mannose cannot be fully resolved, and therefore, quantified. The use of an orthogonal separation method (in addition to the qualitative TLC) allowed to resolve this issue satisfactorily and helped assure that no artifacts were described in the results. A more detailed discussion and comparison of these methods can be seen in Fig. S3 in the Supplementary Material.

The separation and yield of these three fractions are similar for both species; however, there are considerable differences in the monosaccharide composition between species. Table 2 shows the monosaccharide composition of all the produced fractions. Fractions F0-50 and F50-80 deliver similar values compared to the original convection-dried mucilage as reported in (Cruz-Rubio et al., 2020); the main monosaccharide present is galactose (ca. 32 $\%_{mol}$), followed by arabinose; a higher level of galacturonic acid is in *O. ficus-indica* than in

Table 1
Molecular dimensions of the methanol fractionated poly- and oligosaccharides from *Opuntia* spp. mucilage.

	Yield $\%_{w/w}$	$\bar{M}_w$[1] (g·mol^{-1})	$\bar{M}_n$[2] (g·mol^{-1})	$Đ$[3]	Distribution (g·mol^{-1})
	O. ficus-indica				
F0-50	60.2	2.0 × 10^5	5.0 × 10^4	4.0	5.0 × 10^3 – 8.0 × 10^5
F50-80	20.4	2.6 × 10^5	0.5 × 10^4	5.2	4.0 × 10^3 – 8.0 × 10^5
F80-100	19.4	6.3 × 10^2	4.4 × 10^2	1.4	2.0 × 10^2 – 2.3 × 10^3
	O. joconostle				
F0-50	59.9	1.9 × 10^5	2.5 × 10^4	7.6	5.0 × 10^3 – 8.0 × 10^5
F50-80	21.3	1.5 × 10^5	1.5 × 10^4	5.2	4.0 × 10^3 – 8.0 × 10^5
F80-100	18.8	4.8 × 10^2	3.5 × 10^2	1.4	2.0 × 10^2 – 2.1 × 10^3

[1] average mass molar mass.
[2] average number molar mass.
[3] dispersity, $Đ = \bar{M}_w/\bar{M}_n$.

Table 2
Monosaccharide composition of the purified polysaccharides after fractional methanol precipitation and oligosaccharides obtained by prepSEC from F80-100.

	dp[a]	rhamnose[b] (%mol)	arabinose[b] (%mol)	xylose[b] (%mol)	galactose[b] (%mol)	glucose[b] (%mol)	gal. acid[b] (%mol)
O. ficus-indica							
F0-50		8.8 ± 0.9	17.8 ± 1.5	13.3 ± 0.4	29.1 ± 0.9	1.8 ± 1.9	29.3 ± 0.9
F50-80		9.9 ± 2.1	23.6 ± 1.9	18.0 ± 1.5	35.3 ± 3.1	2.3 ± 0.1	11.0 ± 2.5
F80-100		15.7 ± 0.9	19.4 ± 1.6	9.0 ± 0.3	31.4 ± 3.4	21.4 ± 1.0	3.1 ± 2.2
prepSEC fractions from F80-100							
F1	suc	6.9 ± 0.8	7.2 ± 0.5	6.6 ± 0.9	23.2 ± 2.7	55.6 ± 6.2	n.d.
F2	2–4	11.1 ± 1.9	12.2 ± 2.1	7.8 ± 1.2	51.4 ± 3.7	17.4 ± 1.6	n.d.
F3	3–5	14.1 ± 1.0	17.1 ± 5.3	11.4 ± 1.8	48.5 ± 4.7	10.0 ± 0.6	n.d.
F4	4–6	15.2 ± 0.6	10.7 ± 1.3	7.2 ± 3.1	47.4 ± 3.3	19.4 ± 0.9	n.d.
F5	3–7	9.7 ± 0.0	11.3 ± 1.2	7.0 ± 0.5	46.8 ± 2.5	15.9 ± 0.1	9.3 ± 0.9
F6	8–9	10.4 ± 1.6	42.4 ± 4.6	12.3 ± 3.1	19.6 ± 0.2	4.9 ± 0.2	10.4 ± 0.0
F7	9–14	11.9 ± 2.0	44.4 ± 1.7	11.1 ± 1.1	17.7 ± 0.5	3.1 ± 0.4	11.8 ± 0.5
O. joconostle							
F0-50		3.4 ± 1.1	28.4 ± 1.3	8.1 ± 0.3	39.8 ± 0.7	0.6 ± 0.1	19.7 ± 0.2
F50-80		6.1 ± 0.4	29.3 ± 3.3	15.5 ± 0.8	35.0 ± 1.6	0.8 ± 0.6	13.2 ± 0.7
F80-100		12.2 ± 0.4	11.4 ± 1.0	6.1 ± 0.7	40.0 ± 0.3	28.9 ± 0.7	1.3 ± 0.4
prepSEC fractions from F80-100							
F1	glc, suc	9.0 ± 1.4	13.3 ± 0.1	n.d.	19.6 ± 2.8	58.1 ± 4.3	n.d.
F2	2–4	11.6 ± 0.8	12.5 ± 2.0	n.d.	45.0 ± 0.7	30.9 ± 1.8	n.d.
F3	3–5	10.4 ± 1.6	5.9 ± 0.5	2.7 ± 0.4	39.8 ± 0.3	41.2 ± 2.8	n.d.
F4	4–6	5.3 ± 0.9	9.7 ± 1.4	7.5 ± 0.9	45.9 ± 0.7	30.5 ± 2.1	1.1 ± 0.1
F5	3–8	8.0 ± 0.7	19.5 ± 2.4	10.2 ± 0.9	31.1 ± 3.5	25.4 ± 3.1	6.3 ± 0.3

n.d.; under limit of detection.
[a] range of main saccharides estimated from TLC analysis.
[b] ± limit of agreement, calculated as 1.96 × S_d, (n = 3).

O. joconostle. Their correspondence to the monosaccharide composition of the total mucilage is clear as these two fractions form the bulk of the mucilage, being 81 % for *O. ficus-indica* and 87 % for *O. joconostle*. The level of glucose in these fractions was reduced to a minimal concentration due to the high solubility of the low molar mass starch components such as maltooligosaccharides.

The level of galacturonic acid in fraction F0-50 was higher by approx. 30 % in *O. ficus-indica* and 20 % in *O. joconostle* than in the original mucilage; however, in fraction F50-80 the level of galacturonic acid was around 13 % relatively lower and documents the better solubility of its mainly neutral polysaccharide components.

The soluble fraction F80-F100 (19 and 18 $\%_{w/w}$ yields for *O. ficus-indica* and *O. joconostle* respectively) shows a quite different profile, both in molar mass distribution and monosaccharide composition. Besides the high level of glucose, it shows a high content of galactose, 31.4 $\%_{mol}$ for *O. ficus-indica*, 40.0 $\%_{mol}$ for *O. joconostle,* and a low part of galacturonic acid with 3.1 $\%_{mol}$ and 1.3 $\%_{mol}$ respectively. Furthermore, TLC analysis documents different kinds of mono-, di- and oligosaccharides. Therefore, we decided to investigate the low molar mass carbohydrate populations in more detail by further separating this fraction by means of preparative SEC.

3.2. Monosaccharide composition, molecular dimensions and structural features of the oligosaccharides from F80-100

Table 2 shows a difference in the monosaccharide composition of fraction F80-100 between the *Opuntia* species, particularly in the content of glucose. However, after analysis of the prepSEC fractions it becomes evident that this glucose derives from existing sucrose and maltooligosaccharides (MOS) in the fractions.

Fig. 1 A (anSEC) shows that fraction F80-100 is separated into two major peaks in both species. One is mostly composed of salts and other low molar mass components (peak I), and a second one with a dp distribution between 1 and 20. Table 3 shows that the dispersity of peak II for both species is over 2. Therefore, a prepSEC step was performed on F80-100 to purify and fractionate the low molar mass carbohydrates.

Table 3
Molecular dimensions and yield of the purified oligosaccharides after fractional methanol precipitation and prepSEC fractionation.

	F80-100, analytical SEC							
	O. ficus-indica				*O. joconostle*			
	Yield ($\%_{w/w}$)	$\bar{dp}_w$[1]	$\bar{dp}_n$[2]	$Đ$[3]	Yield ($\%_{w/w}$)	$\bar{dp}_w$[1]	$\bar{dp}_n$[2]	$Đ$[3]
I[a]	32.9	salts	35.8	salts				
II	67.1	9	4	2.25	64.2	8	4	2.00
	F80-100, preparative SEC fractions							
	Yield ($\%_{w/w}$)	CHO[4] (%)	dp range[5]		Yield ($\%_{w/w}$)	CHO[4] (%)	dp range[5]	
F1	8.0	33.5	2		5.1	39.2	1–2	
F2	26.3	43.5	2–4		35.0	37.1	2–3	
F3	21.1	49.5	3–5		30.0	50.0	3–4	
F4	15.0	60.2	4–6		17.1	77.2	4–6	
F5	9.0	60.0	3–7		15.9	89.2	5–8	
F6	8.1	82.5	8–9					
F7	12.5	100.0	9–15					

[1] Mass average degree of polymerization, calculated as dextran equivalent. 162 used as monomer M_w for dp calculation.
[2] Number average degree of polymerization.
[3] Dispersity, $Đ = \bar{M}_w/\bar{M}_n$.
[4] Total carbohydrate content.
[5] degree of polymerization estimated from the TLC analysis.
[a] see Fig. 1 A for fraction assignment.

Fig. 1 B shows the fractionated profiles for both species. The fractions with components dp ≤ 1, which include minor parts of monosaccharides, salts and other non-identified low molar mass components were excluded for further analysis. *O. ficus-indica* was divided into seven fractions, with a dp distribution from 1 to 15, while *O. joconostle* was divided only into five fractions, 1 < dp < 8.

Two TLC analyses were carried out on the prepSEC fractions. Fig. 2 shows the samples separated using an eluent optimized for the

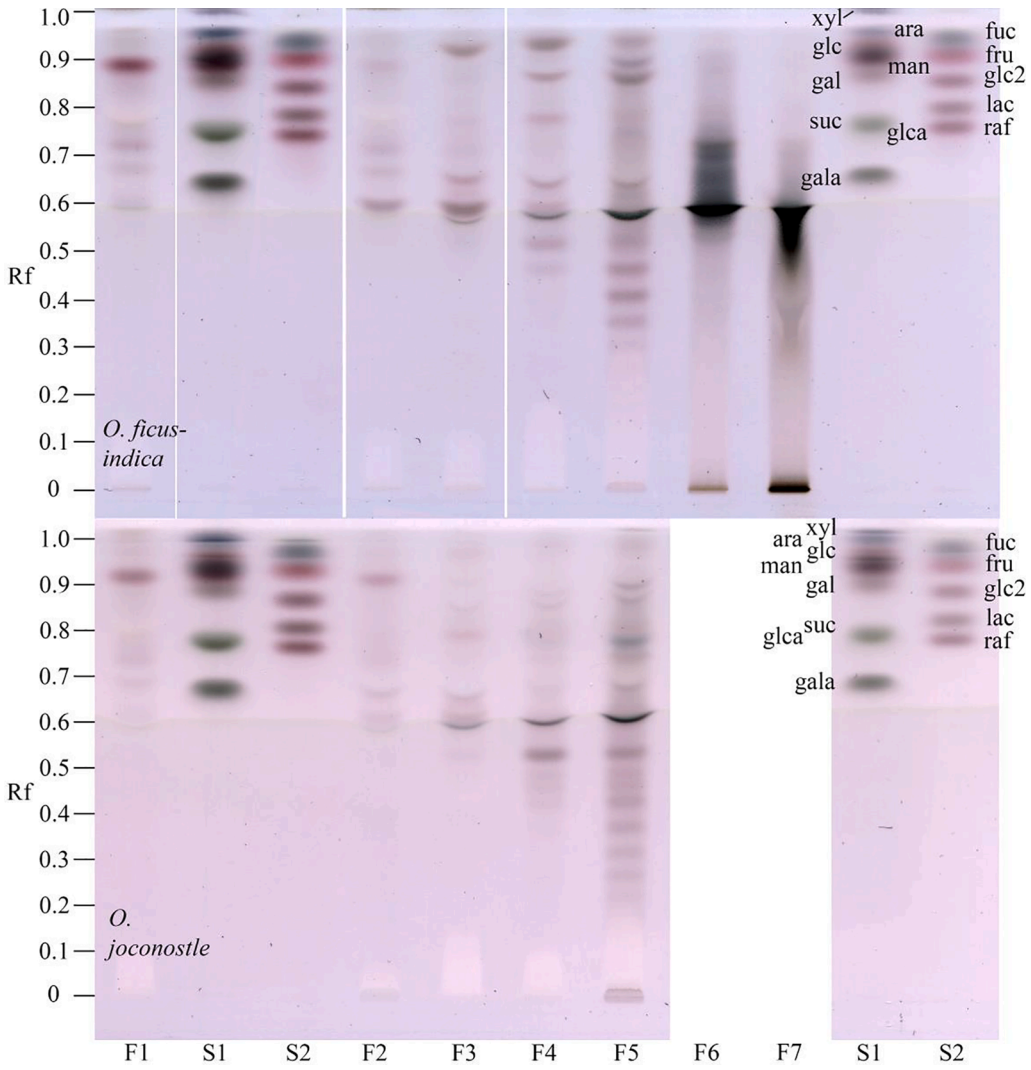

Fig. 2. TLC pattern of the oligosaccharide fractions from F80-100 from preparative SEC separation. *O. ficus-indica* (top), *O. joconostle* (bottom). Standard S1: xyl, xylose; ara, arabinose; glc, glucose; gal, galactose; suc, sucrose; glca, glucuronic acid; gala, galacturonic acid. Standard S2: fuc, fucose; fru, fructose; glc2, maltose; lac, lactose; raf, raffinose.

separation of oligosaccharides, while Fig. S2 (Supplementary Material) utilizes an eluent optimized for mono and disaccharide separation. The prepSEC fractions containing only salts or monosaccharides were removed from both figures in order to be able to align the oligosaccharide-containing samples between the two species into similar retention times.

The TLC pattern in Fig. 2 and Fig. S2 (Supplementary Material) delivers additional information about the nature of the separated mono-, di- and oligosaccharides by using specific visualizing reagents. Moreover, it is easy to see the position and nature of neutral and acidic oligosaccharides; the acidic polysaccharides derivatize into very deep, greenish color compounds, while the neutral saccharides become pink for the hexoses, and yellow or blue for the pentoses or methyl-pentoses. However, the similarities or differences in monosaccharide composition of the fractions only hint at the nature of the oligosaccharides present in F80-100.

F1 and F2 (for both species) contain a very high proportion of sucrose, which is hardly detectable from F3 onwards. Thus, the high levels of glucose in F1 and F2 (Table 2) derives primarily from the hydrolysis of sucrose. If we multiply the glucose content in F80-100 by the F80-100 proportion of the mucilage, we get a glucose content of 4.8 $\%_{mol}$ for *O. ficus-indica* and 5.4 $\%_{mol}$, for *O. joconostle*, values like those reported before (Cruz-Rubio et al., 2020).

Giving further clues of the structure of the separated oligosaccharides, at the eluent conditions used (pH close to neutral), differently dissociated species of the acidic polysaccharide exist, potentially broadening the bands. This effect is quite apparent in Fig. 2 where F7, the fraction with the higher galacturonic acid content shows a diffused pattern due to the number of dissociated species present for the same acidic carbohydrate molecule.

These methods, in combination with the content of carbohydrate in the fractions (phenol–sulfuric method), allow to make inferences as to the identity and composition of the oligosaccharides, and further along, its influence on their fermentability by the probiotic strains.

3.2.1. *Oligosaccharides from O. ficus-indica*

As stated before, F1 contains a high amount of sucrose. If we remove the glucose coming from its hydrolysis, the composition of F1 gets closer to that of the neutral oligosaccharides (F2 to F4), namely oligosaccharides composed mostly of galactose (ca. 50 $\%_{mol}$) and ca. 15 $\%_{mol}$ each for rhamnose, arabinose and xylose. The dp is around 2–3 and includes dimers of galactose as well as some of not identified carbohydrate construction.

F2 shows a similar pattern as F1, with a reduced sucrose band and a slight increase in the dp 3–4 bands. The high level of glucose hints to maltotri- and maltotetraoligomers; the content of galactose, rhamnose,

arabinose and xylose indicates a mixture of differently structured neutral oligomers.

The TLC pattern of F3 delivers bands of oligosaccharides with surprising intensity at 0.6 Rf. This value indicates relatively complex-structured oligosaccharides with dp of 3–5. Additionally, a homologous maltooligomer series was found in the fraction. It is worth noting that F1 to F3 have a relatively low carbohydrate content (50–62 $\%_{w/w}$), which will in turn also affect the results of the fermentability of these fractions.

F4 still has a relatively low content of carbohydrate at 77 %, but with an apparent increase in dp to 4–6, as there are three well defined bands between Rf 0.55 and 0.40, showing several complex-structured oligosaccharides of galactosyl-, arabinosyl- and xylosyl- residues with a dominance of pentosyl groups due to the more purplish color, as opposed to the pink color of hexose-dominated oligosaccharides.

Fraction 5, dp 3–8, includes a minor amount of galacturonic acid reflected in the TLC bands of the oligosaccharides between Rf 0.6 and 0.65 due to the deep, greenish color. Additionally, the TLC bands between Rf 0.35 and 0.55 come from the neutral complex-structured oligosaccharides with galactosyl-, arabinosyl- and xylosyl- residues in the molecules.

The TLC bands of F6 between Rf 0.75 and 0.50 show an intense dark color. The Rf is quite high, even partly higher than galacturonic acid, giving evidence that this fraction is a pectic oligosaccharide with a backbone of galacturonic acid residues (therefore being less available for interaction with the silica). The monosaccharide composition further indicates a branching characteristic with galactosyl-, rhamnosyl-, arabinosyl- and xylosyl residues.

F7 has an almost equal monosaccharide composition to F6 but with a higher content of galacturonic acid. However, the TLC pattern and the SEC separation behavior are quite different between these two fractions. The charged galacturonic acid residues in this oligosaccharide molecules may be arranged peripherally and allow an intense interaction with the stationary phases of SEC and TLC. These results support the assumption that this fraction is composed mostly of acidic arabinogalactan oligomers, with a main chain composed of galactosyl residues interposed with arabinosyl- and galacturonic acid residues in the side chains with predominantly terminal residues of galacturonic acid (Chintalwar et al., 1999).

Furthermore, the high content of complex-structured branched oligosaccharides in F3 to F7 supports the evidence of breakdown of mucilage polymers to smaller molecular units by the convection drying (particularly the long-time treatment) of cladodes from *O. ficus-indica*.

3.2.2. Oligosaccharides from O. joconostle

Similarly to F1 from *O. ficus-indica*, *O. joconostle*s' F1 is composed mostly of glucose, sucrose and probably other dimers of galactose. The monosaccharide composition (Table 2) shows a high level of glucose and relatively low amount of galactose, arabinose and rhamnose. The total absence of xylose is notable compared to the results of *O. ficus-indica*. The total content of carbohydrate is around 40 % and confirms the relatively high level of salt and other non-identified components in this fraction.

In general, the monosaccharide composition of F2 to F5 shows a high amount of galactose and glucose and supports the hypothesis that a population of maltooligosaccharides as well as galactooligosaccharides are present in these fractions. To proof this hypothesis, the fractions were treated separately with *endo*-1,4-β-galactanase and α-amylase and qualitatively evaluated by TLC (results not shown). The treatment with *endo*-1,4-β-galactanase delivered a significant amount of galactose and di-galactose and confirms the splitting of β-1,4- linkages in the oligomers. The treatment with α-amylase released a significant amount of glucose, evidence for the splitting of the maltooligomers and confirms their presence in the fractions.

F2 and F3 are similarly composed, with a high level of maltooligomers and β-1,4- linked galactooligosaccharides of dp 2–5. Minor parts of rhamnosyl-, arabinosyl- and xylosyl residues indicate possible branching structure in the galactooligomers. Similarly to F1, the total content of carbohydrate in F2 and F3 is around 40 % and confirms the presence of salt and other components in the fractions.

F4 has a relatively high level of total carbohydrate (72 %) and shows well defined bands with dp 4–6 in the TLC pattern. The content of glucose is approx. 30 $\%_{mol}$ for the existing maltooligosaccharides in the fraction. The other oligosaccharides are composed mostly of galactose (approx. 46 $\%_{mol}$), with increasing parts of rhamnose, arabinose and xylose compared to F3. These results support the increasing branched characteristics of the existing galactooligomers in this fraction as well. A minor part of galacturonic acid residues in the structure composition was found as well.

F5, with a content of carbohydrate of 89 %, shows a lot of defined bands in the TLC pattern with a dp between 3 and 8. As before, some of these bands derive from maltooligomers. Further bands reflect the complex branched oligomers with galactosyl-, rhamnosyl- and galacturonic acid residues proposed in the main chain and interposed with arabinosyl- and xylosyl residues predominantly terminal in the side chains.

In contrast to the results of *O. ficus-indica*, a thermal breakdown of mucilage polymers from the convection drying process could not be found, the only degradation products derive from starch polymers, which are present in CAM (Crassulacean Acid Metabolism) plants as a transitory carbohydrate reserve (Weise, van Wijk, & Sharkey, 2011). This starch can be split into a series of maltooligosaccharides and are found in the prepSEC fractions from mucilage fraction F80-100. The results of the prepSEC fractionation document the developing of complex-structures built up from the relatively simple structures found in F2 and F3 to complex branched molecules in F4 and F5, which can be further reflected in the mucilage fraction F0-50 and F50-80.

3.3. Fermentability of the different poly- and oligosaccharides extracted from O. ficus-indica and O. joconostle by probiotic strains

3.3.1. Monosaccharides as carbon source for the selected probiotic strains

In this investigation, four widely considered probiotic strains (*L. acidophilus*, *L. rhamnosus*, *B. longum* ssp. *infantis* and *B. animalis* ssp. *lactis*) were tested for their ability to ferment the monosaccharides present in the mucilage polysaccharides under our testing conditions. To facilitate the comparison, all results are reported as growth relative to glucose, since this was (unsurprisingly) the sugar that prompted the greatest growth response. The results are listed in Table S4 in the Supplementary Material.

In order to grow, the selected microorganism must be able to metabolize the monosaccharide as a carbon source as well as to contain the necessary enzymes to cleave the glycosidic linkages forming the polysaccharide into monosaccharides or saccharides small enough to be transported into the cell.

Glucose and galactose were metabolized quickly by all four probiotic strains. In contrast, xylose and arabinose led only to a significant growth for three out of the four strains (*L. acidophilus, B. animalis* ssp. *lactis* and *B. longum* ssp. *infantis*). Rhamnose induced a medium RGR of 38 to 51 % and is the only monosaccharide (in addition to glucose and galactose) to elicit a growth response in *L. rhamnosus*. However, rhamnose cannot be metabolized by *B. longum* ssp. *infantis*. Moreover, in our test system, none of the microorganisms showed any growth with galacturonic acid as the carbon source.

3.3.2. Fermentability of the methanol fractionated poly-and oligosaccharides (F0-50, F50-80 and F80-100)

Table 4 shows the fermentability of fractions F0-50, F50-80 and F80-100 from both *Opuntia* species. The dependency of the growth rates on the molecular dimension, monosaccharide composition, and found structural features of all fractions was investigated in detail.

Table 4
Prebiotic potential of poly- and oligosaccharides extracted from the mucilage of two *Opuntia* species.

	L. acidophilus RGR[a] (%)	*L. rhamnosus* RGR[a] (%)	*B. longum* ssp. *infantis* RGR[a] (%)	*B. animalis* ssp. *lactis* RGR[a] (%)
	O. ficus-indica			
F0-50	14	11	10	18
F50-80	18	10	14	21
F80-100	20	11	26	27
	prepSEC fractions from F80-100			
F1	45	48	84	57
F2	34	18	41	25
F3	18	26	28	20
F4	20	33	17	7
F5	17	23	22	17
F6	7	–	11	–
F7	17	–	12	–
	O. joconostle			
F0-50	11	9	17	2
F50-80	20	13	22	14
F80-100	15	19	28	36
	prepSEC fractions from F80-100			
F1	43	64	41	56
F2	54	73	57	65
F3	22	38	28	30
F4	8	19	16	26
F5	9	19	3	0

[a] Relative Growth Rate to glucose. Value corrected for carbohydrate content by normalizing against carbohydrate content.

3.3.2.1. Fermentability as a function of molecular dimensions. The fractions from *O. ficus-indica* were fermentable by all four probiotic strains and led to a relative growth increase ranging between 11 and 27 % (Table 4). *B. longum* ssp. *infantis* shows a notable dp dependency, as it grew more with the fraction of the lowest dp (F80-100), namely 26 % compared to F50-80 (14 % RGR increase) and F0-50 (10 % RGR increase). *B. animalis* and *L. acidophilus* show a similar trend of higher fermentability as the dp decreases, but it is not as pronounced as with *B. longum*.

All but one of the included fractions of *O. joconostle* are significantly fermentable as indicated by a RGR increase ranging from 9 to 36 % (Table 4). For 3 out of the 4 strains (*L. rhamnosus* GG, *B. animalis* ssp. *lactis* and *B. longum* ssp. *infantis*), a dependency in fermentability to the molecular dimension was found. In fact, F80-100 was fermented faster than F0-50. F0-50 was hardly metabolized by *B. animalis* ssp. *lactis* leading to a growth increase of only 2 %.

3.3.2.2. Influence of monosaccharide composition and structural features on fermentability. As stated before, the carbohydrate composition and type of linkages in the polysaccharides have a major impact on the growth behavior of the tested probiotic strains. A good example of this is F0-50. *B. animalis* grows quite well in *O. ficus-indica* compared to *O. joconostle*, despite the higher content of non-fermentable galacturonic acid (Supplementary Material Table S4). Since the molar mass distributions and monosaccharide composition are relatively similar, the branching characteristics is left as the controlling factor in this difference. A query into the genome of *B. animalis* in the Carbohydrate Active Enzymes (http://www.cazy.org/) database (Lombard, Golaconda Ramulu, Drula, Coutinho, & Henrissat, 2014) shows that this microorganism does not encode for polysaccharide lyases, one of the main classes of enzymes able to degrade complex polysaccharides. This leaves only with glycoside hydrolases. The results for the query correlate well with the results of Table 3, as there are listed several α- and β-galactosidases, α-L-arabinofuranosidases, *endo*-(1,4)-β- and β-xylosidases, α- and β- amylases and glucosidases, and α-fucosidases, while there is no mention of rhamnosidases or galacturonidases.

In contrast to the differences in F0-50, F50-80 from both species have an almost equal monosaccharide composition (galactose ~35 $\%_{mol}$, arabinose ~25 $\%_{mol}$, xylose ~16 $\%_{mol}$ and rhamnose ~8 $\%_{mol}$) and carbohydrate content (~90 %). Yet, for both F0-50 and F50-80, a trend can be seen: *L. acidophilus* and *L. rhamnosus* have similar growth values, while *B. longum* and *B. animalis* differ between species, with *B. longum* being more active in *O. joconostle* and *B. animalis* is more active in *O. ficus-indica*. Moreover, both F50-80 fractions have the highest proportion of the most fermentable sugars (galactose, arabinose, and xylose), and the lowest content of galacturonic acid. This indicates that the more pectic-like material, needing lyases for depolymerization that these microorganisms do not produce, was precipitated in the F0-50 step; this could be an explanation for the fact that this fraction shows the highest growth values.

F80-100, as the results show, is metabolized by the bacteria, but since there is a large content of sucrose and maltooligosaccharides, the results are difficult to interpret as they belong to the mixture. Therefore, the analysis of the results will be performed on the individual constituents of F80-100 as separated by prepSEC.

3.3.3. The fermentability F80-100 and its preparative SEC fractionated oligosaccharides

Table 4 shows the relative growth rates of the four applied bacterial strains for both F80-100 as a whole as well as the preparative *SEC* fractions. The prebiotic effectivity as tested is related to the total carbohydrate content and to the presence of fermentable mono-, di and oligosaccharides such as glucose, sucrose and maltooligomers, all non-prebiotics since they are fermented before reaching the gut. To correct for the differing carbohydrate amounts, the raw relative growth rates were normalized by dividing by the total carbohydrate content as found by the phenol–sulfuric method, as the samples were prepared all to the same mass-concentration. The term RGR will be used for the discussion in this section to reflect the use of the calculated, corrected relative growth rates.

In the prepSEC fractions, however, prebiotic oligosaccharides with adequate monosaccharide composition and structure were present, allowing for a suitable interpretation of their prebiotic potential related to the mucilage components, as well as the chance to analyze the influence of the monosaccharide composition and structure of the oligosaccharides in the ability of the microorganisms to utilize them as a carbon source.

3.3.3.1. Fermentability of oligosaccharides from O. ficus-indica. In general, all fractions were well fermented, and show a decreasing RGR value trend as the molecular dimensions increase. Because of minor availability of samples F6 and F7 only *L. acidophilus* and *B. longum* were tested in these fractions. The high RGR values (45 to 84 %) for F1 are justified by the high fermentability of glucose, fructose and sucrose (Mueller et al., 2016), all coming from the plant energy metabolism pool. F2 has a rest of sucrose, and already includes the maltooligomers from transitory starch. This is reflected in the lower RGR values for all strains. Fractions F3 to F5 include more complex-structured mucilage oligomers besides the present maltooligomers, and thus the RGR values are reduced to the range of 17 – 33 %, except for *B. animalis*, which delivered a very low RGR value of 7 % when incubated with F4.

F6 and F7, composed primarily of mucilage oligomers and having a relatively high level of galacturonic acid, show similar values to those of the high molar mass mucilage polysaccharides. It is worth noting that for *L. acidophilus*, there was an RGR increase from F6 to F7, signaling a better availability of fermentable sugar residues in the latter, despite the larger molecular dimensions.

3.3.3.2. Fermentability of oligosaccharides from O. joconostle. The pre-pSEC fractions of *O. joconostle* likewise show a decreasing RGR trend with increasing molecular dimensions. Only F5 induces no growth of *B. longum* and *B. animalis*. All fractions include mono-, disaccharides or plant metabolism oligosaccharides such as maltooligosaccharides. F1 and F2 share the same high content of sucrose as *O. ficus-indica*, similarly delivering high RGRs. Moreover, the fractions contain 20–40 % maltooligosaccharides, corresponding (by definition) to an equal contribution to the RGR. The included galactooligosaccharides (with an increasingly complex structure) are responsible for the rest of the growth. However, F5 induces almost no growth for *B. longum* and *B. animalis*. Given the presence of the easily hydrolysable malto- and galactooligosaccharides (via the corresponding present gluco- and galacto- hydrolases) we can only assume the presence of a low-molar mass compound that inhibits the growth of these microorganisms.

4. Conclusions

The mucilage extracted from the convection-dried cladodes of *O. ficus-indica* and *O. joconostle* is partly composed of supramolecular assemblies built of conjugated, non-covalent bonded complex-structured neutral and acidic polysaccharides. Moreover, it has been shown for the first time that these assemblies allow for the inclusion of low molecular weight components, particularly mono-, di- and oligosaccharides. The oligosaccharides found in this work have been so far overlooked in the literature regarding the analysis and characterization of the mucilage of *Opuntia* spp.

Two high molar mass heteropolysaccharides fractions with partly pectic characteristics along a fraction of low molar mass poly- and oligosaccharides with minor amounts of galacturonic acid residues were found. The reported results represent the molar mass distribution of the covalently structured molecules, and likewise document the disruption of the non-covalent bonds in the mucilage matrix.

The low molar mass fraction includes carbohydrates from the plant primary metabolism and are present in both species.

O. ficus-indica oligosaccharides include oligomers with a potential galactosyl-linked main chain and potentially interposed by arabinosyl-, xylosyl- and increasing galacturonopyranosyl acid residues in the side chains. The presence of such complex oligosaccharides in these fractions supports their possible origin as products of heteropolysaccharide degradation after the convection drying of the cladodes.

The oligosaccharides found in *O. joconostle* show a series of maltooligomers along an increasing amount of galactooligomers with increasing rhamnosyl-, arabinosyl-, xylosyl- and galacturonic acid residues in the molecules. These results document the development of heteropolysaccharide structure building, from simple oligomer units to the complex molecules present in the high-molar mass polysaccharides. The possible hydrothermal breakdown of mucilage into smaller units in a manner analogous to *O. ficus-indica* was not found.

The prebiotic potential of the high molar mass heteropolysaccharides for both *Opuntia* species was relatively low and can be explained by the lack of complex- and pectic polysaccharide degrading enzymes in the four bacterial species tested. The slightly higher fermentability of the low molar mass poly- and oligosaccharides can be justified by the lower level of galacturonic acid residues in the fraction.

In general, the different relative growth rates of the oligosaccharides by the tested probiotic strains can be ascribed more to their structural characteristics and monosaccharide composition than their molecular dimensions.

Finally, our results indicate that it does not make economic sense to isolate the natural occurring oligosaccharides (or produced from processing-related degradation of the polysaccharides) present in *Opuntia* spp. given their low concentration. However, their production via a deliberate partial hydrothermal or enzymatic degradation of the heteropolysaccharides into low molar mass oligosaccharides can support the developing of novel products for the stimulation of specific microorganisms (or genera of microorganisms) in the gut microbiota.

CRediT authorship contribution statement

José M. Cruz-Rubio: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Monika Mueller:** Methodology, Formal analysis, Resources, Writing - review & editing. **Helmut Viernstein:** Funding acquisition, Writing - review & editing, Supervision. **Renate Loeppert:** Writing - review & editing. **Werner Praznik:** Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Many thanks to Anita Csorba and Viktoria Horvath for conducting experimental work. Jose M. Cruz-Rubio thanks CONACYT for scholarship 440539 and Biotrendy SA de CV for providing the *Opuntia* spp. samples. Monika Mueller thanks Stadt Wien for Hochschuljubiläumstiftung grant H-275198/2016.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130167.

References

Chintalwar, G., Jain, A., Sipahimalani, A., Banerji, A., Sumariwalla, P., Ramakrishnan, R., & Sainis, K. (1999). An immunologically active arabinogalactan from *Tinospora cordifolia*. *Phytochemistry, 52*(6), 1089–1093. https://doi.org/10.1016/s0031-9422(99)00386-6.

Cruz-Rubio, J. M., Mueller, M., Loeppert, R., Viernstein, H., & Praznik, W. (2020). The Effect of cladode drying techniques on the prebiotic potential and molecular characteristics of the mucilage extracted from *Opuntia ficus-indica* and *Opuntia joconostle*. *Scientia Pharmaceutica, 88*(4), 43. https://doi.org/10.3390/scipharm88040043.

Di Lorenzo, F., Silipo, A., Molinaro, A., Parrilli, M., Schiraldi, C., D'Agostino, A., … Lanzetta, R. (2017). The polysaccharide and low molecular weight components of *Opuntia ficus indica* cladodes: Structure and skin repairing properties. *Carbohydrate Polymers, 157*, 128–136. https://doi.org/10.1016/j.carbpol.2016.09.073.

Dongowski, G. (1996). Determination of saturated and unsaturated oligogalacturonic acids by means of thin-later chromatography. *Journal of Chromatography A, 756* (1–2), 211–217. https://doi.org/10.1016/s0021-9673(96)00637-1.

Guevara-Arauza, J. C., de Jesús Ornelas-Paz, J., Pimentel-González, D. J., Rosales Mendoza, S., Soria Guerra, R. E., & Paz Maldonado, L. M. T. (2012). Prebiotic effect of mucilage and pectic-derived oligosaccharides from nopal *(Opuntia ficus-indica)*. *Food Science and Biotechnology, 21*(4), 997–1003. https://doi.org/10.1007/s10068-012-0130-1.

Hernandez-Urbiola, M. I., Perez-Torrero, E., & Rodriguez-Garcia, M. E. (2011). Chemical analysis of nutritional content of prickly pads (*Opuntia ficus indica*) at varied ages in an organic harvest. *International Journal of Environmental Research and Public Health, 8*(5), 1287–1295. https://doi.org/10.3390/ijerph8051287.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., & Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research, 42*(Database issue), D490–495. https://doi.org/10.1093/nar/gkt1178.

López-Palacios, C., Peña-Valdivia, C. B., Reyes-Agüero, J. A., & Rodríguez-Hernández, A. I. (2011). Effects of domestication on structural polysaccharides and dietary fiber in nopalitos (*Opuntia* spp.). *Genetic Resources and Crop Evolution, 59*(6), 1015–1026. https://doi.org/10.1007/s10722-011-9740-3.

Morales, P., Ramírez-Moreno, E., Sanchez-Mata, M.d. C., Carvalho, A. M., & Ferreira, I. C. F. R. (2012). Nutritional and antioxidant properties of pulp and seeds of two xoconostle cultivars (Opuntia joconostle F.A.C. Weber ex Diguet and Opuntia matudae Scheinvar) of high consumption in Mexico. *Food Research International, 46* (1), 279–285. https://doi.org/10.1016/j.foodres.2011.12.031.

Mueller, M., Reiner, J., Fleischhacker, L., Viernstein, H., Loeppert, R., & Praznik, W. (2016). Growth of selected probiotic strains with fructans from different sources relating to degree of polymerization and structure. *Journal of Functional Foods, 24*, 264–275. https://doi.org/10.1016/j.jff.2016.04.010.

Nuñez-Lopez, M. A., Paredes-Lopez, O., & Reynoso-Camacho, R. (2013). Functional and hypoglycemic properties of nopal cladodes *(O. ficus-indica)* at different maturity

stages using in vitro and in vivo tests. *Journal of Agriculture and Food Chemistry, 61* (46), 10981–10986. https://doi.org/10.1021/jf403834x.

Onakpoya, I. J., O'Sullivan, J., & Heneghan, C. J. (2015). The effect of cactus pear (*Opuntia ficus-indica*) on body weight and cardiovascular risk factors: A systematic review and meta-analysis of randomized clinical trials. *Nutrition, 31*(5), 640–646. https://doi.org/10.1016/j.nut.2014.11.015.

Patel, S. (2014). Opuntia cladodes (nopal): Emerging functional food and dietary supplement. *Mediterranean Journal of Nutrition and Metabolism, 7*(1), 11–19. https://doi.org/10.3233/mnm-140003.

Peña-Valdivia, C. B., Trejo, C., Arroyo-Peña, V. B., Sánchez Urdaneta, A. B., & Balois Morales, R. (2012). Diversity of unavailable polysaccharides and dietary fiber in domesticated nopalito and cactus pear fruit (*Opuntia* spp.). *Chemistry & Biodiversity, 9*(8), 1599–1610. https://doi.org/10.1002/cbdv.v9.810.1002/cbdv.201200047.

Piperno, D. R. (2011). The origins of plant cultivation and domestication in the new world tropics. *Current Anthropology, 52*(S4), S453–S470. https://doi.org/10.1086/659998.

Rohatgi, A. (2020). WebPlotDigitizer. Pacifica, California, USA, https://automeris.io/WebPlotDigitizer.

Sáenz, C., Berger, H., Rodríguez-Félix, A., Galleti, L., Corrales García, J., & Sepúlveda, E. (2013). *Agro-industrial utilization of cactus pear*. Rome: Food and Agriculture Organization.

Sanchez-Tapia, M., Aguilar-Lopez, M., Perez-Cruz, C., Pichardo-Ontiveros, E., Wang, M., Donovan, S. M., Tovar, A. R., & Torres, N. (2017). Nopal (*Opuntia ficus indica*) protects from metabolic endotoxemia by modifying gut microbiota in obese rats fed high fat/sucrose diet. *Scientific Reports, 7*(1), 4716. https://doi.org/10.1038/s41598-017-05096-4.

Stintzing, F. C., & Carle, R. (2005). Cactus stems (*Opuntia* spp.): A review on their chemistry, technology, and uses. *Molecular Nutrition & Food Research, 49*(2), 175–194. https://doi.org/10.1002/mnfr.200400071.

Trachtenberg, S., & Mayer, A. M. (1981). Composition and properties of *Opuntia ficus-indica* mucilage. *Phytochemistry, 20*(12), 2665–2668. https://doi.org/10.1016/0031-9422(81)85263-6.

Weise, S. E., van Wijk, K. J., & Sharkey, T. D. (2011). The role of transitory starch in C(3), CAM, and C(4) metabolism and opportunities for engineering leaf starch accumulation. *Journal of Experimental Botany, 62*(9), 3109–3118. https://doi.org/10.1093/jxb/err035.

Wu, X., Jiang, W., Lu, J., Yu, Y., & Wu, B. (2014). Analysis of the monosaccharide composition of water-soluble polysaccharides from *Sargassum fusiforme* by high performance liquid chromatography/electrospray ionisation mass spectrometry. *Food Chemistry, 145*, 976–983. https://doi.org/10.1016/j.foodchem.2013.09.019.