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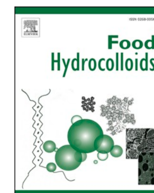

Rhamnogalacturonan–I pectin and derived oligosaccharides obtained from sugar beet pulp and discarded red beetroot: Characterization and comparative study of their antioxidant and prebiotic properties

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A B S T R A C T

Rhamnogalacturonan–I (RG–I) pectin recovered from sugar beet pulp (SBP) and discarded red beetroot (DRB) were subjected to enzymatic hydrolysis to obtain pectooligosaccharides (POS). The hydrolysates with the highest amount of oligomer with a degree of polymerization from 2 to 6 were selected for a detailed characterization of the composition and the structure by MALDI-TOF MS and ^{1}H NMR. The yields of pectin and POS products ranged from 32.7% (g DRB POS/g DRB) to 37.0% (g SBP Pectin/g SBP) with the POS content from 64.4% (DRB products) to 68.7% (SBP POS). A comparative study was conducted between SBP Pectin, SBP POS, DRB Pectin, DRB POS, and commercial prebiotics (inulin and fructooligosaccharides) for their antioxidant capacity and prebiotic potential. The results revealed the much higher antioxidant capacity of DRB products (DPPH: 176.8 μmol Trolox equivalent/g pectin and 162.5 μmol Trolox equivalent/g POS) over the other products. Both bacteria strains evaluated grew with all substrate products. DRB POS and inulin exhibited the highest maximum growth rate for *Lactobacillus rhamnosus* (0.25 and 0.26 h^{-1}, respectively), whereas *Bifidobacteria longum* grew faster on DRB Pectin (0.89 h^{-1}). The short chain fatty acids and lactate production were also measured. Highest concentrations arose using SBP products as substrates (125.3 mM for pectin and 115.7 mM for POS with *Lactobacillus rhamnosus* and 87.8 mM for pectin and 95.0 mM for POS with *Bifidobacterium longum*). The results demonstrated the potential applications of SBP and especially DRB products in the food industry due to their high antioxidant and prebiotic properties.

1. Introduction

Prebiotics are non-digestible food ingredients that selectively promote the growth of beneficial gut bacteria (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). The gut microbiome includes large families of bacteria, including *Lactobacillus* and *Bifidobacterium* genera which are recognized for their potential to exert a positive influence on gut health (Wang, Zhang, & Zhang, 2021). *Lactobacilli* facilitate the digestion and absorption of nutrients in food, metabolize toxic waste products, produce amino acids, and protect against bacterial infections and stress-induced intestinal barrier dysfunction (Dempsey & Corr, 2022; Gao et al., 2022). *Bifidobacteria* have health benefits, including improving gut health, enhancing immune function, and preventing or alleviating gastrointestinal disorders (Shah, 2011). Due to the health benefits of prebiotics, their global market was estimated at US$6 Billion in 2022 and is projected to reach US$13.8 Billion by 2030 (www.researchandmarkets.com). This fact has increased interest in developing new prebiotics to meet the growth in demand.

Non-digestive oligosaccharides are considered as prebiotics since they can resist gastric acidity and gastrointestinal absorption and are

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fermented by intestinal microflora (Gibson et al., 2004). In this way, pectin has been studied as a source of pectin-derived oligosaccharides (POS) (Yeung, Kang, So, Jung, & Chang, 2021). Pectin is a complex heteropolysaccharide found in plant cell walls with different regions, including homogalacturonan (HG), rhamnogalacturonan I (RG–I), and the less common rhamnogalacturonan II (RG–II). The "hairy" region (RG–I) backbone is composed of galacturonic acid linked to rhamnose. The rhamnose molecules often have side chains of neutral oligosaccharides, mainly arabinose and galactose (Waldron & Faulds, 2007). The RG–I region can be a source of POS with prebiotic properties. Previous research (Zhu et al., 2019) demonstrated a positive relationship between the branching degree and the neutral sugar content of pectin with the prebiotic activity.

Nowadays, different kinds of biomass are being explored due to their high pectin content. In this topic, sugar beet pulp (SBP), a byproduct obtained in the sugar industry with an approximate annual production of 13 million tons in Europe (www.pulp2value.eu) has been studied as a POS source due to its high content in RG–I pectin (del Amo-Mateos, López-Linares, García-Cubero, Lucas, & Coca, 2022; Gómez et al., 2019; Mao et al., 2019; Prandi et al., 2018). On the other hand, global food waste production amounts to 1.3 billion tons annually, with fruit and vegetables contributing 42% to the total (del Amo-Mateos et al., 2023). Among fruit and vegetables, discarded red beetroot (DRB) is well known for its large amounts of bioactive compounds, such as antioxidants and natural pigments, among others (Dhiman et al., 2021). Moreover, pectin extraction from red beetroot has previously been reported by some authors (del Amo-Mateos et al., 2023; Hotchkiss et al., 2022).

To enhance the prebiotic activity of pectin, a need arises to obtain molecules with smaller molecular weights, as they are preferred by gut bacteria (Al-Tamim, Palframan, Cooper, Gibson & Rastall, 2006). Cano et al. (2021) studied different methods to obtain oligogalacturonic acids (OGalA), including H_2O_2, trifluoroacetic acid, and HCl hydrolysis. However, only HCl hydrolysis showed precise and repeatable results. Enzymatic hydrolysis (EH) is another method previously applied in POS production (Babbar et al., 2016; Prandi et al., 2018). EH offers several advantages over acid hydrolysis, such as low toxicity, low corrosion, and no environmental damage. Moreover, EH operates under mild conditions, which helps to preserve the nutritional and functional properties of the product (de Moura, Macagnan, & da Silva, 2015).

Bacterial growth and the production of organic acids have been used to evaluate bacterial activity (Gibson et al., 2004). The modeling of bacterial growth can reduce measured data to valuable parameters, such as maximum specific growth rate (μ_{max}), lag period or generation time (Zwietering, Jongenburger, Rombouts, & Riet, 1990). The short chain fatty acids (SCFA), acetate, propionate and butyrate, and lactate are other metabolites produced during prebiotic fermentation and contribute to the overall health and balance of the host (Gullón, Gullón, Sanz, Alonso, & Parajó, 2011).

Although the antioxidant and prebiotic properties of SBP POS have been previously reported, there is a lack of literature on the prebiotic properties of RG–I enriched pectin, as its branch structure could also promote gut bacteria growth, and SCFA and lactate production. Moreover, pectin and POS obtained from DRB have not been evaluated for their prebiotic potential.

The present research aims to study the antioxidant and prebiotic properties of RG-I pectin and POS recovered by hydrothermal microwave-assisted extraction (MAE) and purified by diafiltration and ultrafiltration using SBP and DRB as raw materials. The production of POS from RG-I pectin extracts was assessed by EH using Viscozyme L. The enzyme dilution and EH time effects were evaluated on the oligosaccharide production. Pectin and POS products were characterized by their composition, molecular weight distribution, and structural features. The products obtained were compared to commercial prebiotics for their antioxidant capacity, bacterial growth, SCFA, and lactate production using two bacteria (*Lactobacillus rhamnosus* and *Bifidobacteria longum*). Overall, the present study can contribute to achieving the 3rd and 12th Sustainable Development Goals of the United Nations: good health and well-being and responsible consumption and production, respectively, through the sustainable development of food products with antioxidant and prebiotic capacities from waste or agro-industrial byproducts.

2. Materials and methods

2.1. Raw material

SBP and DRB were kindly supplied by AB Azucarera Iberia (Toro, Spain) and Huercasa (Sanchonuño, Spain), respectively. The SBP was washed, dried at 60 °C, and ground before use. The DRB underwent a cleansing process with water to eliminate residual dirt, and a juice extraction procedure was subsequently carried out (Kenwood PureJuice One JMP400WH) to separate the pomace. The pomace was washed, dried at 60 °C, and ground before use.

2.2. Extraction and purification of pectin

The scheme of the process for pectin production can be found in Fig. 1.

RG-I pectin was recovered from SBP and DRB by hydrothermal MAE under optimal conditions determined in previous studies (del Amo-Mateos et al., 2022; 2023): 165 °C and 11.9 min for SBP, and 160 °C and 5.3 min for DRB. The extraction was carried out in a Multiwave PRO SOLV reactor 50 Hz with a Rotor type 16HF100 (Anton Paar GmbH, Austria).

The MAE extracts rich in POS were subjected to a purification process involving two membrane steps: continuous diafiltration and ultrafiltration. The purification process was performed per the previously reported procedure (del Amo-Mateos, Cáceres, Coca, García-Cubero & Lucas, 2024). The purification was done using a Minimate TFF system (Pall Corporation, USA) equipped with a polyethersulfone membrane with a molecular weight cut-off of 3 kDa (Pall Corporation, USA).

Part of the concentrated extracts was subjected to freeze-drying (Telstar LyoQuest 55, Spain), obtaining two pectin products denoted as 'SBP Pectin' and 'DRB Pectin' based on the respective raw material used. Another fraction of the concentrated extracts was stored at 4 °C until used in EH experiments (Fig. 1).

2.3. POS production by enzymatic hydrolysis

The concentrated extracts were subjected to EH with Viscozyme L supplied by Novozymes A/S (Denmark). Viscozyme is known for its pectinase activity (Babbar et al., 2016; Combo, Aguedo, Goffin, Wathelet, & Paquot, 2012). The concentrated extract was adjusted to pH 4.5 with NaOH and heated until 45 °C. Once the set temperature was reached, the enzyme solution was added in a ratio enzyme solution to extract of 10% (v/v) and stirred at 150 rpm.

The effects of the EH time and enzyme dilution on the production of oligomers were studied. The EH time was set at 5, 15, and 30 min, and three enzyme dilutions were analyzed: 1/25 (165.4 EPGU/mL), 1/50 (82.7 EPGU/mL), and 1/100 (41.4 EPGU/mL). Enzyme blanks of each experiment were carried out as a control.

The hydrolysates with the highest concentration of degree of polymerization (DP) 2–6 oligomers were subjected to freeze-drying (Telstar LyoQuest 55, Spain) (Fig. 1). The resulting solid products were named 'SBP POS' and 'DRB POS.'

2.4. Evaluation of potential prebiotic properties

2.4.1. Microorganisms and culture media

Two bacterial strains, *Lacticaseibacillus rhamnosus* DSM 20021 (*L. rhamnosus*) and *Bifidobacterium longum* subsp. *longum* DSM 20219

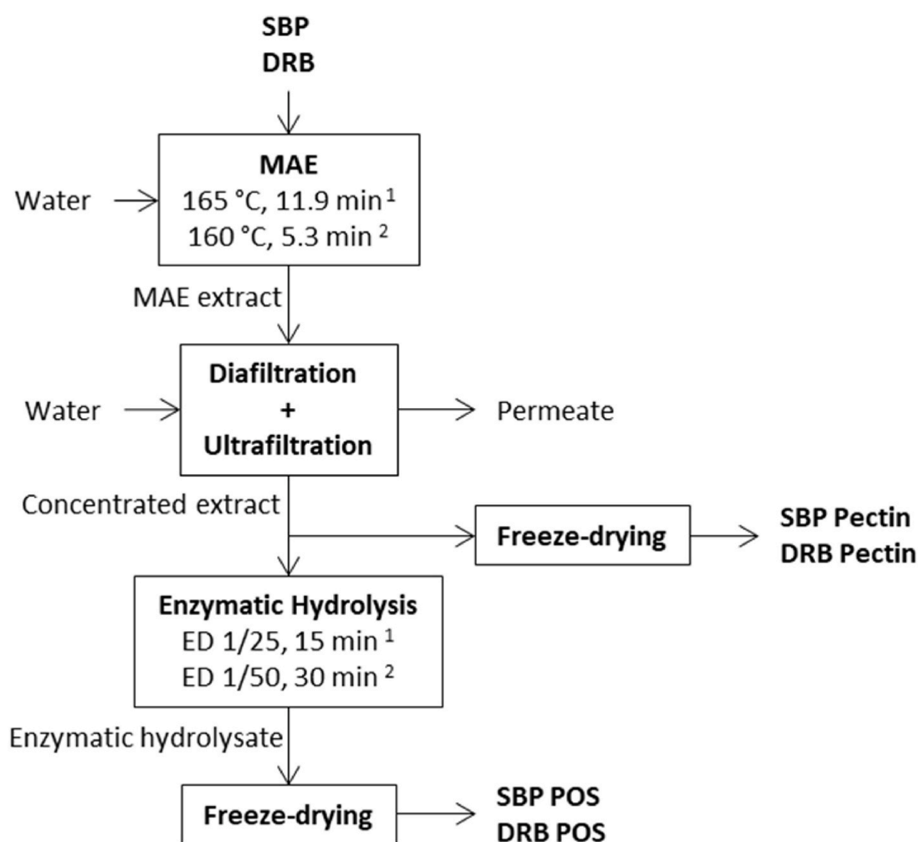

Fig. 1. Schematic diagram for obtaining the pectin and POS products from SBP and DRB used in the comparative study. [1] Operating conditions for SBP stream. [2] Operating conditions for DRB stream.

(*B. longum*) were obtained from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Leibniz, Germany) and maintained in 20% (v/v) glycerol at −80 °C until used. *L. rhamnosus* and *B. longum* were activated in sterilized de Man, Rogosa, and Sharp (MRS, Merck) broth and Reinforced Clostridial Medium (RCM, Sigma-Aldrich) broth, respectively, at 37 °C, 100 rpm, for 24 h under anaerobic conditions achieved by flushing with free O_2 nitrogen (Carburos Metálicos, Spain).

2.4.2. Fermentation assays and bacterial growth

Six different broths were prepared for each bacterium to evaluate the prebiotic activities. The carbon source of the MRS and RCM was replaced by the freeze-dried products (SBP Pectin, DRB Pectin, SBP POS or DRB POS), inulin (Tentorium Energy S.L., Spain) or FOS (Sigma-Aldrich) in a concentration of 1% (w/v). Inulin and FOS, commercially available as prebiotics, were included as control substances. The broths were sterilized at 121 °C for 15 min.

2.5 mL of bacterial cells were inoculated into 22.5 mL of each broth. The fermentation took place in a 100 mL clear crimp cap sample vial with a rubber septum at 37 °C at 100 rpm under anaerobic conditions. Sampling was done at 0, 2, 4, 6, 8, 10, 24, and 48 h. The experiments were done in triplicate. To assess the progress of the fermentation, absorbance measurements were taken in triplicate at 595 nm using a UV–Vis spectrophotometer (Uvmini-1240, Shimadzu Suzhou Instruments Wfg. Shimadzu Corporation, Kyoto, Japan). The bacterial cell concentration was calculated through a calibration curve relating absorbance to the cell dry weight.

The modified Logistic model (eq. (1)) and Gompertz model (eq. (2)) (Gibson, Bratchell, & Roberts, 1987) were used to fit the bacterial growth data:

$$\log(N_t) = A + \frac{C}{1 + \exp(-B \cdot (t - M))} \quad (1)$$

$$\log(N_t) = A + C \cdot \exp[-\exp(-B \cdot (t - M))] \quad (2)$$

where N_t is the concentration of bacteria (mg/L) at time t (h), and A, B, C and M are the parameters of the models. The maximum growth rate (μ_{max}, h^{-1}), lag (h) and generation time (G. time, h) were calculated from each model as described below.

For the Logistic model (eqs. (3)–(5)):

$$\mu_{max} = B \cdot C/4 \quad (3)$$

$$lag = M - 2/B \quad (4)$$

$$G.\ time = 4 \cdot \log(2)/(B \cdot C) \quad (5)$$

For the Gompertz model (eqs. (6)–(8)):

$$\mu_{max} = B \cdot C/e \quad (6)$$

$$lag = M - 1/B \quad (7)$$

$$G.\ time = e \cdot \log(2)/(B \cdot C) \quad (8)$$

Moreover, the maximum growth rate was calculated from the experimental data plotting the bacterial concentration logarithm against time. The maximum growth rate corresponded to the slope of the linear part of the curve.

2.4.3. Determination of the fermentation products

The SCFAs (acetic, propionic, and butyric acids) and lactate produced during fermentation were determined. The culture medium was collected at 0, 4, 8, 24, and 48 h, centrifuged at 13000 rpm for 5 min in an accuSpin Micro 17R (Fisher Scientific, Germany), and the supernatant was filtered through 0.22 μm nylon filters. The filtered sample (1000 μL) was added to 0.3 μL 96% H_2SO_4 and analyzed in a gas

chromatograph Agilent 7820A (Agilent, USA) equipped with a flame ionization detector and a packed column (10% SP-100 + 1% H_3PO_4 on Chromosorb® W acid washed 100/120 mesh size, 2 m × 3.175 mm; Teknokroma, Spain) to determine acetic, propionic, and butyric acids. The temperature of the injection port and FID was set at 350 °C. The oven temperature was initially maintained at 160 °C for 13 min, then increased to 180 °C at a heating rate of 3 °C/min, and held for 6.5 min. The carrier gas was nitrogen at a 45 mL/min flow rate. Hydrogen and air flow rates were 45 and 350 mL/min, respectively. The lactic acid content in the filtered sample was determined by High-performance liquid chromatography (HPLC) in a refractive index detector (Waters 2414, USA) equipped with an Aminex HPX-87 H analytical column (Waters, USA) at 60 °C. The mobile phase was 0.01 N H_2SO_4 at a 0.6 mL/min flow rate.

2.5. Statistical analysis

Python 3.11.4 was used to obtain the bacterial growth model parameters. The dual annealing global optimization algorithm was used to minimize the mean squared error (MSE) between the experimental and calculated data ($\log(N_t)$). A statistical software Statgraphics Centurion XVIII was used for data analysis. An ANOVA test was conducted to assess the relationships between operational parameters and to determine significant differences. A statistical significance of $p < 0.05$ was established.

2.6. Analytical methods

2.6.1. Monomer, oligomer, and degradation compounds analysis

Freeze-dried products were first redissolved in deionized water. The monomer and oligomer content of galacturonic acid, glucose, galactose, rhamnose, and arabinose and the concentration of degradation compounds (formic and acetic acids, HMF, and furfural) were analyzed by HPLC. The monomer content was also determined in the concentrated extracts and enzymatic hydrolysates.

To determine the oligomer content, the samples were subjected to acid hydrolysis, and the oligomers were calculated as the total content minus the monomer content (del Amo-Mateos et al., 2023). The HPLC analysis was performed in a refractive index detector (Waters 2414, USA) using an Aminex HPX-87 H analytical column (Waters, USA) at 60 °C. The mobile phase was 0.01 N H_2SO_4 at a 0.6 mL/min flow rate. Before each analysis, the samples were filtered through a 0.22 μm nylon filter.

2.6.2. Molecular weight distribution

The molecular weight distribution of the concentrated extracts and the enzymatic hydrolysates was determined by High-pressure size exclusion chromatography (HPSEC) with a refractive index detector (Waters 2414, USA) and an Ultrahydrogel 250 column (Waters, Japan). The analysis was carried out with ultrapure water as a mobile phase at a flow rate of 0.7 mL/min at 35 °C. Dextran standards were used as molecular weight standards from 1 to 670 kDa.

2.6.3. Analysis of the degree of polymerization distribution of the enzymatic hydrolysates

Determining the DP (DP 2–6) content in the enzymatic hydrolysates was conducted according to the method described by (López-Linares, Lucas, García-Cubero, Jiménez, & Coca, 2020). HPLC analysis was used with a refractive index detector (Waters 2414) and an Aminex HPX-42A column (Bio-Rad, Richmond, USA). The analysis was performed at an operating temperature of 80 °C with ultrapure water as the mobile phase at a flow rate of 0.6 mL/min. To calibrate the results, standards of xylooligosaccharides, including xylobiose (DP2), xylotriose (DP3), xylotetrose (DP4), xylopentose (DP5), and xylohexose (DP6) (Megazyme International, Ireland) were used.

2.6.4. Antioxidant capacity

The antioxidant capacity of the commercial prebiotics (inulin and FOS), SBP Pectin, DRB Pectin, SBP POS and DRB POS was analyzed using three methods (del Amo-Mateos et al., 2023): DPPH radical scavenging, FRAP, and ABTS radical scavenging. A Trolox solution in methanol was used as standard. The absorbance was measured in a UV–Vis spectrophotometer (Shimadzu, Japan). The results were expressed as Trolox equivalent (TE) in μmol/g.

2.6.5. Structural characteristics

The structural features of the pectin and POS products were analyzed by the Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and the ^1H proton nuclear magnetic resonance (NMR). Both analyses were carried out in the Laboratory of Instrumental Techniques (LTI) of the University of Valladolid.

The MALDI-TOF MS analysis was carried following the method previously reported (del Amo-Mateos et al., 2023). Briefly, the samples (SBP Pectin, DRB Pectin, SPB POS, and DRB POS) were firstly redissolved in distilled water and treated with an ion-exchange resin (Lewatit MonoPlus S 108 H), provided by Lanxess AG (Germany), at room temperature for 30 min. After centrifugation, 1 μL of the sample was mixed with 1 μL of the matrix solution (25 mg/mL of 2,5-dihydroxybenzoic acid in acetonitrile 50% and NaCl 2 mM) on a MALDI plate. The MALDI-TOF MS analysis was performed using an Autoflex speed workstation (Bruker Daltonics, Germany).

For the ^1H NMR, approximately 7 mg of the sample (SBP Pectin, DRB Pectin, SPB POS, and DRB POS) were placed in a vial, and 0.7 mL of D_2O were added to create the deuterium "lock" condition. Subsequently, the sample was sonicated and heated until complete dissolution. The resulting solution was then transferred to a 5 mm NMR tube for analysis. The NMR spectrometer used for the experiments was a Bruker Avance Neo (500 MHz, 2 channels) equipped with an i-Probe. The experimental conditions for ^1H NMR, suppressing the residual signal of HDO (4.70 ppm) using Bruker's zgcppr sequence: spectral width of 7812.5 Hz, acquisition time of 2.09 s, a relaxation time between pulses of 2 s, and a total number of 64 pulses. All measurements were conducted at a constant temperature of 25 °C.

The spectra were processed using exponential apodization with a value of 0.3 Hz. These spectra were processed and analyzed using MestreNova 12.0 and TopSpin4.30 software. The chemical shifts of the ^1H nuclei are reported in parts per million (ppm) and referenced to TMS.

3. Results and discussion

3.1. Enzymatic hydrolysis of pectic extracts

The oligosaccharides derived from the RG-I pectin are being studied for their potential prebiotic properties (Gómez, Gullón, Yáñez, Schols, & Alonso, 2016; Prandi et al., 2018; Yeung et al., 2021). After the hydrothermal MAE and purification process, a concentrated extract rich in RG-I pectin with high molecular weight was obtained (Fig. 2). However, low molecular weight oligosaccharides are preferred by gut bacteria (Al-Tamimi, Palframan, Cooper, Gibson, & Rastall, 2006). To break the high-molecular-weight molecules present in the concentrated extracts and produce oligosaccharides, EH was carried out with Viscozyme L. The effect of the EH on the depolymerization of pectin was evaluated by the molecular weight distribution (Fig. 2) and the concentration of oligosaccharides with a DP 2–6 (Fig. 3).

According to Fig. 2, even though there were differences in the molecular weight distribution of the concentrated extracts, the chromatographic profiles obtained for the enzymatic hydrolysates of SBP and DRB were similar. The EH effectively fragmented the high molecular weight compounds, substantially reducing the broadband signal within the 20–1000 kDa range. This reduction was observed in the three enzymatic dilutions and at the three EH times studied. Moreover, new peaks appeared in the 2–10 kDa range, showing lower molecular weight at

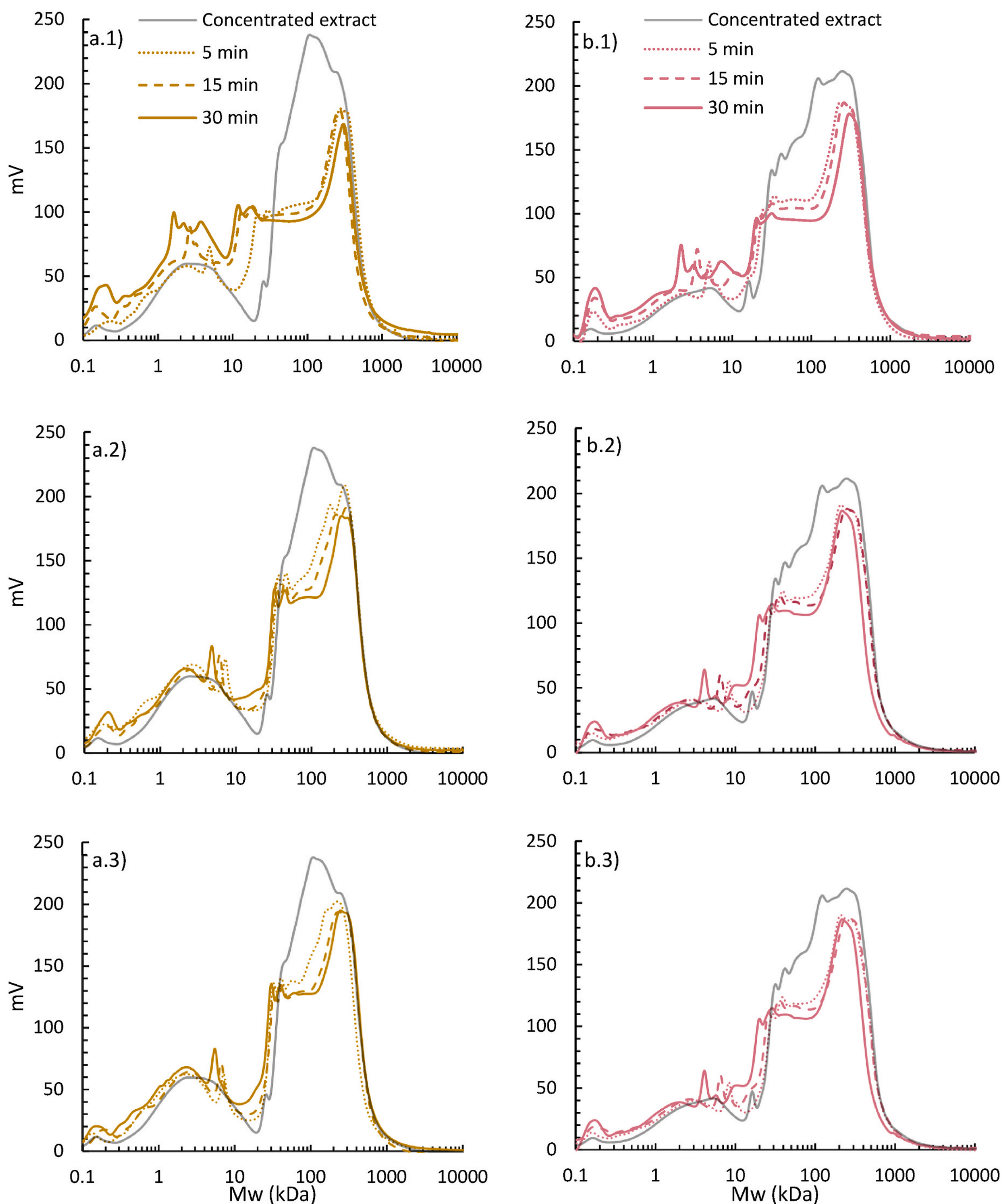

Fig. 2. Molecular weight distribution of the concentrated extracts and enzymatic hydrolysates from SBP (a) and DRB (b) at different enzyme dilutions: 1/25 (1), 1/50 (2) and 1/100 (3) and enzymatic hydrolysis times.

longer EH times, mainly when the 1/25 enzyme dilution was used (Fig. 2a.1 and 2b.1). Finally, the signal of the peak corresponding to 0.1–0.2 kDa increased after EH, especially at longer EH times and low enzymatic dilution (Fig. 2a.1 and 2b.1), which may correspond to monomeric compounds. Sabater, Ferreira-Lazarte, Montilla, and Corzo (2019) found similar trends in the molecular weight distribution of POS samples obtained from different raw materials (citrus or apple pectin) by EH with Viscozyme (4 and 16 U/mL) for 30 and 60 min.

The quantitative analysis of monomers and DP 2–6 oligomers was assessed to obtain more information about the influence of the EH on the molecular weight of POS. The results can be found in Fig. 3. The monomer concentrations revealed the same trends as those observed in molecular weight distribution, and their concentrations increased with longer EH times and a lower enzymatic dilution. In contrast, the concentration of low DP oligomers did not exhibit a clear trend. This might be explained by the ongoing formation of oligomers through the breakdown of the polysaccharides and their further degradation into monomers.

The EH results for the SBP concentrated extract (Fig. 3a) showed that the maximum oligomer concentrations were reached at low enzymatic dilutions (1/25 and 1/50) after 15 min. In contrast, using a more diluted enzyme (1/100), 30 min was needed to reach the highest oligomer

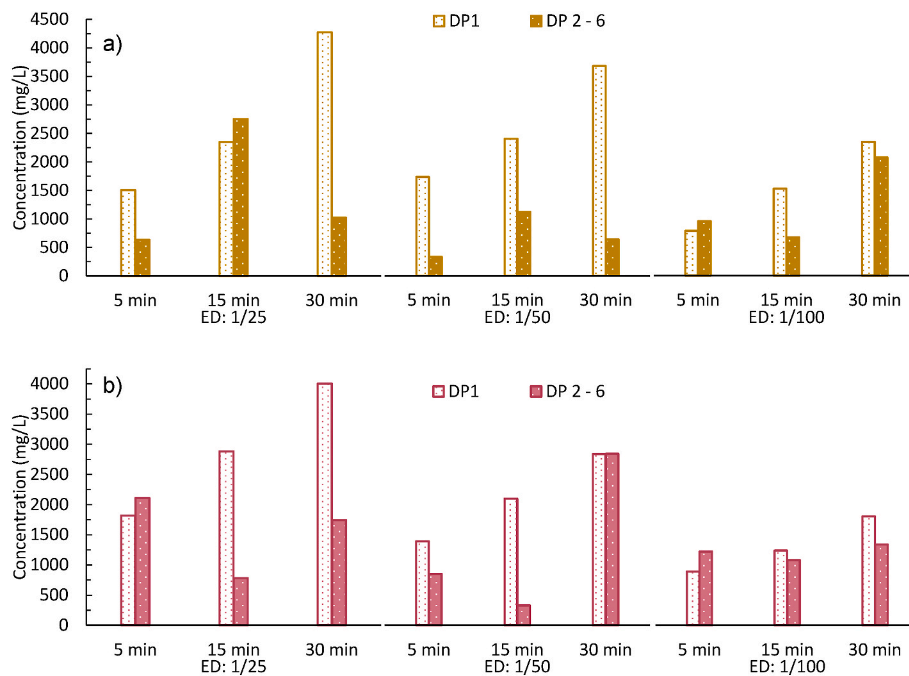

Fig. 3. Monomer (DP 1) and oligomer content (DP 2–6) in enzymatic hydrolysates from SBP (a) and DRB (b) at different enzymatic dilutions and enzymatic hydrolysis times. ED: enzyme dilution.

concentration. Regarding DRB (Fig. 3b), the three enzymatic dilutions showed similar trends. The more diluted enzymes (1/50 and 1/100) yielded the highest oligomer content over a longer time (30 min); whereas the 1/25 enzyme dilution achieved the highest oligomer concentration after 5 min of EH. The enzymatic hydrolysates with the highest concentrations of DP 2–6 (2750 mg/L for SBP and 2843 mg/L for DRB) were obtained using enzymatic dilutions of 1/25 and 1/50 for 15 and 30 min in the concentrated extracts of SBP and DRB, respectively. These selected hydrolysates were then evaluated for their antioxidant and prebiotic properties after freeze drying (Fig. 1).

The results confirmed the efficiency of Viscozyme L in obtaining oligomers from pectin-rich extracts. Other studies have also obtained POS from different raw materials using Viscozyme. For instance, Gómez et al. (2019) produced POS from lemon peel using 3 U/g for 7.5 or 24 h. Prandi et al. (2018) loaded SBP pectin in an enzyme membrane reactor using an enzyme dose of 413.6 and 82.7 EPGU/mL.

3.2. Pectin and POS products: yield, composition, and structural characteristics

Table 1 summarizes the global yield and composition for both pectin and POS products. The four products exhibited similar yields, ranging from 32.7 to 37.0% (g product/g raw material). These yields surpassed the values reported in previous research based on alcohol precipitation for pectin recovery. For instance, Yeung et al. (2021) recovered pectin from okra pods by alkaline extraction followed by ethanol precipitation, achieving a yield of 17.08% (g pectin/g okra pods). Similarly, Mao et al. (2019) reported a yield of 23.7% (g pectin/g SBP) after alkaline extraction and isopropanol precipitation. Moreover, the authors' previous research using the same MAE conditions for recovering POS from SBP (165 °C, 11.9 min) reported a yield of 20.0% after ethanol precipitation (del Amo-Mateos, Cáceres, Coca, García-Cubero & Lucas, 2024). The higher yields obtained in this study might be attributed to the higher efficiency of the membrane process for RG-I pectin purification compared to ethanol precipitation. While ethanol precipitation has been widely used for HG pectin recovery, its efficacy decreases when targeting RG-I pectin.

The concentration in OGalA, the main pectin component, remained similar in all the fractions, ranging from 14.8% (DRB POS) to 15.7% (DRB Pectin), with no significant differences observed between the products. However, some differences were observed in the AraOS content, with SBP products displaying a higher content compared to DRB products. This difference can be attributed to the composition of the raw materials (del Amo-Mateos et al., 2022; 2023). The presence of rhamnan

Table 1
Yield and characterization of pectin and POS products (%, dry basis).

	SBP Pectin	DRB Pectin	SBP POS	DRB POS
Yield (%, g product/g raw material)	37.0 ± 0.3 ^{a}	34.4 ± 0.0 ^{a,b}	33.4 ± 1.6 ^{b}	32.7 ± 1.6 ^{b}
Monomers	n.d.	n.d.	2.9 ± 0.1 ^{a}	2.2 ± 0.1 ^{b}
Degradation compounds	n.d.	n.d.	n.d.	n.d.
OGalA	15.2 ± 0.3 ^{a}	15.7 ± 0.6 ^{a}	15.0 ± 0.4 ^{a}	14.8 ± 0.3 ^{a}
GlcOS	n.d.	n.d.	n.d.	n.d.
GalOS	10.6 ± 0.2 ^{a}	10.7 ± 0.2 ^{a}	10.3 ± 0.1 ^{a}	10.7 ± 0.3 ^{a}
RhaOS	3.9 ± 0.1 ^{b}	4.6 ± 0.1 ^{a}	3.4 ± 0.0 ^{c}	3.9 ± 0.1 ^{b}
AraOS	37.0 ± 0.9 ^{b}	33.5 ± 0.3 ^{d}	39.4 ± 0.1 ^{a}	34.9 ± 0.4 ^{c}
POS	66.8 ± 1.6 ^{a,b}	64.4 ± 0.6 ^{b}	68.7 ± 0.5 ^{a}	64.4 ± 1.0 ^{b}

n.d.: not detected; OGalA: Oligogalacturonoides; GlcOS: glucooligosaccharides; GalOS: galactooligosaccharides; RhaOS: rhamnooligosaccharides; AraOS: arabinooligosaccharides; POS: pectooligosaccharides.
[a-d] Means in the same row not followed by a common superscript letter are significantly different ($p < 0.05$).

moieties in all fractions, along with their high concentration in arabinose and galactose, suggests that the products were rich in RG-I pectin. The POS content ranged from 64.4 to 68.7%, with the highest values corresponding to the SBP products. All the products exhibited purity levels within the range of commercial prebiotics, which exhibit a diverse degree of purity. Examples include galactooligosaccharides (GalOS) (55% purity), isomalto-oligosaccharides (50% purity), or inulin (90% purity) (Sugkhaphan & Kijroongrojana, 2009). The monomer content accounted for less than 3%.

For a better understanding of the oligosaccharide structures present in the products, a MALDI-TOF MS analysis was conducted. The corresponding spectra can be found in Fig. S1, while the mass signals and suggested structures are detailed in Table 2. This analysis confirmed the presence of a wide variety of oligosaccharide structures. Repeating units of 132 m/z, indicative of pentoses, were found in all the products. Based on the product compositions (Table 1), these might be attributed to arabinose molecules. Furthermore, a predominant presence of oligomers was observed. These oligomers were primarily composed of hexuronic acid (galacturonic acid according to the composition), linked to hexoses (likely galactose or rhamnose) and pentoses (arabinose). These findings aligned with the structure of RG-I pectin. In addition, oligomer structure formed only by hexoses was also detected (428 m/z). Methyl and acetyl groups were identified in some structures. Some differences between the products obtained from the raw materials were found. SBP products displayed larger oligomer structures (1885, 2018, 2149 m/z), while the largest one found in DRB products had a mass of 1752 m/z. Lastly, products resulting from EH (POS) exhibited signals at lower m/z values, particularly in SBP POS (421, 488, 518, 660, 680 m/z). The complex oligomer structures found in this study were consistent with data reported for POS obtained from melon by-products (Rico, Gullón, & Yáñez, 2020) or pectin recovered through acid extraction from red beetroot (Hotchkiss et al., 2022).

The structural characteristics of pectin and POS products were further examined by ^{1}H NMR spectroscopy. The spectra (Fig. 4) of the four products were very similar. The presence of signals over 4.4–4.8, especially in the POS products (Fig. 4b and d) indicated the existence of

Table 2
MALDI-TOF MS results and suggested structures of pectin and POS products.

m/z [a]	Structure [b]	SBP Pectin	DRB Pectin	SBP POS	DRB POS
421	HexA2Me2	–	–	+	–
428	Hex2Ac2	+	+	+	+
436	HexHexAAc	+	–	–	–
478	HexA2Ac2	+	+	–	–
488	Pent2HexAc	–	–	+	+
518	PentHexAAc4	–	–	+	+
560	HexA3Me	+	+	+	+
612	PentHexA2Me2Ac	+	+	+	+
650	Pent2HexAAc4	+	+	+	+
660	HexA3Ac2	–	–	+	–
668	HexHexA2Ac2	+	+	+	+
680	PentHexA2Ac3	–	–	+	–
692	PentHexA3Me	+	+	+	+
825	Pent2HexA3Me	+	+	+	+
957	Pent3HexA3Me	+	+	+	+
1090	Pent4HexA3Me	+	+	+	+
1222	Pent5HexA3Me	+	+	+	+
1355	Pent6HexA3Me	+	+	+	+
1487	Pent7HexA3Me	+	+	+	+
1620	Pent8HexA3Me	+	+	+	+
1752	Pent9HexA3Me	+	+	+	+
1885	Pent10HexA3Me	+	–	+	–
2018	Pent11HexA3Me	+	–	+	–
2149	Pent6Hex5GalA2	+	–	+	–

"+" indicates m/z value was found in the spectra of the product; "-" indicates m/z value was not found in the spectra of the product.

[a] m/z: nominal mass/charge corresponding to the [M], $[M+Na]^{+}$ or $[M+K]^{+}$ adducts.

[b] Pent: Pentose; Hex: hexose; HexA: hexuronic acid; Me: methyl; Ac: acetyl.

β-anomers (Zhang et al., 2023). The intense signal at 3.83 ppm corresponded to the esterified galacturonic acid ($-OCH_3$) and the signal at 2.17 and 2.08 ppm might be attributed to the acetyl groups of galacturonic acid ($-COCH_3$). Moreover, the chemical shifts of the →4)-α-Gal*p*A-(1→ (H-1: 5.10 ppm; H-2: 3.72 ppm; H-3: 3.97 ppm; H-4: 4.32 ppm; H-5: 4.51 ppm) were found in all the products, indicating the presence of the galacturonan backbone (Zhang et al., 2023). The H-1 of α-Ara*f*-(1→, →3)-α-Ara*f*-(1→ and →5)-α-Ara*f*-(1→ were found at 5.19, 5.16 and 5.13 ppm respectively (Lemaire et al., 2020). Additionally, the H-2, H-3, H-4, and H-5 of arabinose could be attributed to the signals found at 4.23, 4.14, 4.11 and 4.05 ppm, respectively (Zhang et al., 2023). Moreover, the proton signals at 1.36 and 1.26 ppm could be attributed to the rhamnose structures →2,4)-α-Rha*p*-(1→ and →2)-α-Rha*p*-(1→, respectively (Shi et al., 2017). These results confirmed that all the products were consistent with the RG-I pectin structure.

3.3. Determining the antioxidant capacity

Assessing the antioxidant capacity of products for food applications is of great importance. Antioxidants play a crucial role in preserving food quality, extending shelf life, and potentially offering health benefits to consumers. These benefits include protection against oxidative stress, which is associated with various chronic diseases.

Performing at least two assays is recommended to characterize the antioxidant capacity of a food product, since different assays can measure different pools of antioxidants (Sadowska-Bartosz & Bartosz, 2022). Based on this, the antioxidant capacity of commercial prebiotics (inulin and FOS), pectin and POS products was evaluated by three assays: DPPH, FRAP and ABTS. The results are displayed in Table 3. The inulin exhibited no detectable (DPPH and FRAP) or marginal levels (ABTS) of antioxidant activity. The FOS sample only showed an antioxidant capacity similar to SBP Pectin in the ABTS analysis, the results obtained in the other two assays (DPPH and FRAP) being negligible in comparison to the pectin and POS products.

The antioxidant activity was found to be significantly dependent ($p < 0.05$) on the raw material. DRB products exhibited much higher antioxidant activities than SBP, independently of the analytical method used. Red beetroot is well known for its antioxidant capacity. Although its antioxidant components are mainly found in the juice (del Amo-Mateos et al., 2023), some of them remained in the pomace and were present in the pectin and POS products. DRB Pectin and DRB POS exhibited the most active radical scavenger capacity against DPPH (176.8 μmol TE/g) and ABTS (251.2 μmol TE/g) radicals, respectively. In addition, the highest FRAP was achieved in DRB POS (62.0 μmol TE/g). In contrast, the differences between the pectin and POS products were much lower in comparison to the differences between SBP and DRB. Mengíbar, Mateos-Aparicio, Miralles, and Heras (2013) found an indirect relationship between the molecular weight and antioxidant capacity. Molecular weight distribution profiles (Fig. 2a1 and 2 b2) revealed that after EH, high molecular weight molecules persisted in the POS products in addition to oligomers. The presence of these polysaccharides with higher molecular weights may account for the similar antioxidant activity observed in the POS and pectin products.

3.4. Prebiotic activity

3.4.1. Bacterial growth using different carbohydrate sources

To evaluate the prebiotic potential of the pectin and POS products, a comparative fermentation study was conducted. FOS and inulin, which are known for their prebiotic properties and are commercially available, and the pectin and POS products were used as fermentation substrates. *Lactobacilli* and *Bifidobacteria* play a crucial role in maintaining gut health, safeguarding against harmful pathogens. Due to the importance of these bacteria, the fermentation growth of *L. rhamnosus* and *B. longum* were studied. Both bacteria grew in pectin and POS products. It is worth noting that the products were rich in RG-I pectin with a limited amount

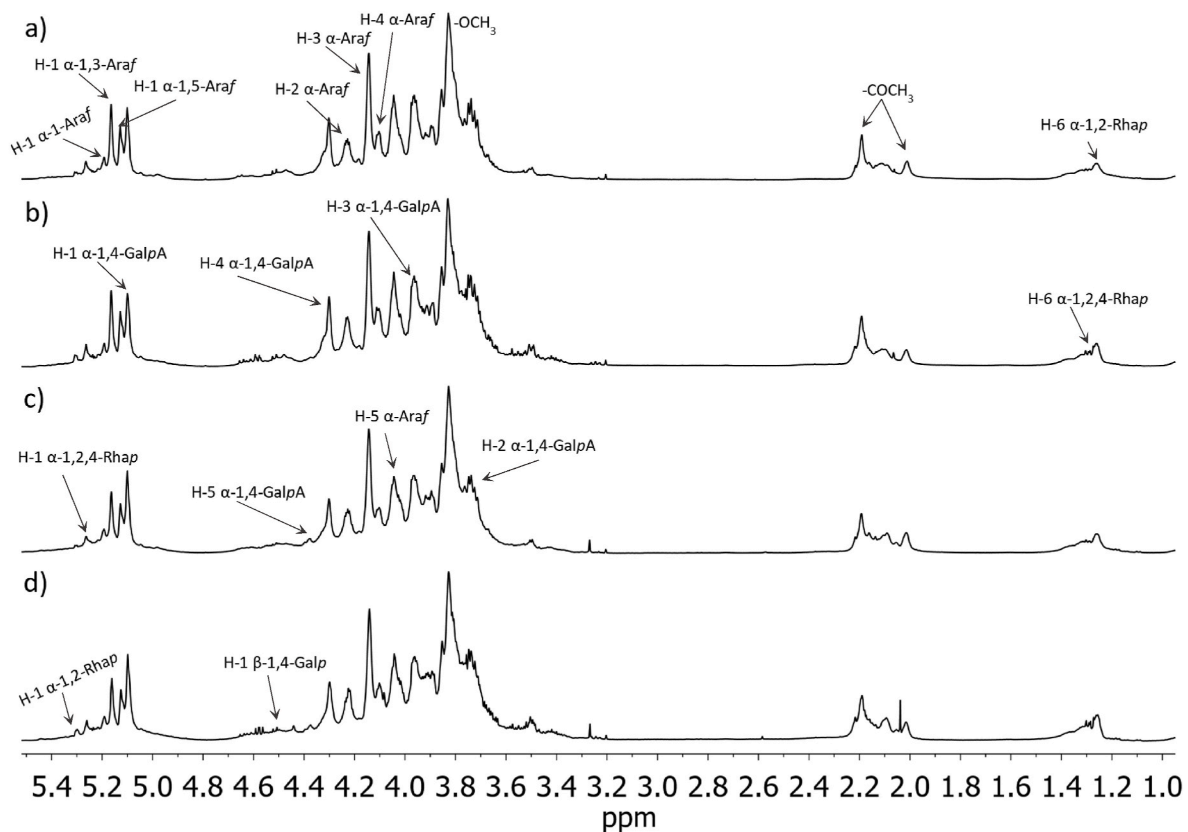

Fig. 4. ^{1}H NMR spectrum of SBP Pectin (a), SBP POS (b), DRB Pectin (c), and DRB POS (d).

Table 3
Antioxidant capacity (μmol TE/g) of commercial prebiotics (inulin and FOS) and pectin and POS products.

	DPPH	FRAP	ABTS
Inulin	BLLD	BLLD	5.0 ± 0.2 $^{d,-}$
FOS	5.4 ± 0.3 ^{e}	0.0 ± 0.0 ^{c}	192.9 ± 0.7 ^{c}
SBP Pectin	56.5 ± 1.4 ^{d}	44.1 ± 1.9 ^{b}	200.1 ± 6.5 ^{b,c}
DRB Pectin	176.8 ± 0.3 ^{a}	61.0 ± 1.3 ^{a}	247.9 ± 6.9 ^{a}
SBP POS	66.5 ± 2.9 ^{c}	43.9 ± 0.2 ^{b}	206.6 ± 3.2 ^{b}
DRB POS	162.5 ± 1.5 ^{b}	62.0 ± 1.3 ^{a}	251.2 ± 5.3 ^{a}

Data are presented as mean ± SD (n = 3). BLLD: below the lower limit of detection.
$^{a\text{-}d}$ Means in the same column not followed by a common superscript letter are significantly different ($p < 0.05$).

of galacturonic acid (Table 1). Previous research has shown significant growth of *Bifidobacteria* (Manderson et al., 2005; Zhu et al., 2019) and *Lactobacillus* (Yeung et al., 2021) in RG-I rich POS, and growth inhibition of *Lactobacillus* in the presence of free GalA (Prandi et al., 2018). The bacterial growth parameters are summarized in Table 4.

The experimental maximum growth rate (Table 4) was determined from the logarithmic bacteria concentration curve (Fig. S2) to compare the effect of carbohydrate sources on bacterial growth. *L. rhamnosus* exhibited different growth rates, depending on the carbohydrate source (Inulin ≈ DRB POS > SBP POS ≈ FOS > SBP Pectin = DRB Pectin). Inulin (0.26 h^{-1}) and DRB POS (0.25 h^{-1}) showed the highest μ_{max} without significant differences between them. The growth rate in SBP POS (0.21 h^{-1}) was similar to that obtained using FOS (0.20 h^{-1}). Thus, *L. rhamnosus* growth in POS products was comparable to that observed in commercial prebiotics. In contrast, pectin products exhibited the lowest μ_{max} values (0.18 h^{-1}). These results revealed that molecular weight may affect *L. rhamnosus* growth, as shorter molecules were better fermented. The study of Yeung et al. (2021) also found faster growth of *L. rhamnosus* using POS as substrate than using its native pectin. The results in Gómez et al. (2014) showed lower *Lactobacillus/Enterococcus* growth using pectin from orange peel than with inulin or FOS.

In contrast, the *B. longum* growth rate exhibited a higher dependence on the raw material source (DRB Pectin ≫ DRB POS ≫ Inulin > FOS > SBP Pectin > SBP POS). DRB Pectin (0.89 h^{-1}) and DRB POS (0.53 h^{-1}) showed a notably much higher μ_{max} compared to commercial prebiotics and SBP products. The growth rate for SBP Pectin (0.17 h^{-1}) and SBP POS (0.15 h^{-1}) exhibited the lowest values. Contrary to the *L. rhamnosus* results, *B. longum* did not exhibit a preference for lower molecular weight molecules, since the μ_{max} values observed in pectin were higher than in POS products. These results were contrary to those reported by Yeung et al. (2021), who observed a higher bacterial growth using POS products as carbohydrate source than pectin recovered from okra pods. Zhu et al. (2019) concluded that sugar content contributes significantly to prebiotic activity. The higher prebiotic activity observed with the pectin in our study, as compared to the results obtained by Yeung et al. (2021), may be attributed to the prevalence of the RG–I pectin region recovered in the present study (Table 1) in contrast to the HG pectin obtained in this previous research. Previous research indicated that an increase of 0.5–1.0 log in *Bifidobacteria* concentrations might represent a significant change for a more favorable composition of the intestinal microbiota (Zhu et al., 2019). Therefore, as can be seen in Fig. S2, inulin, FOS, pectin, and POS products could all be considered bifidogenic.

The prebiotic properties of POS derived from SBP has already been studied (Gómez et al., 2016, 2019; Leijdekkers, Bink, Geutjes, Schols, & Gruppen, 2013; Prandi et al., 2018). Gómez et al. (2019) reported μ_{max} values for different bacteria using SBP POS as a carbohydrate source. The value for *L. rhamnosus* GG was 0.05 h^{-1}. The difference with the value obtained in this study (0.18 h^{-1}) could be due to the extraction method used for POS recovery from SBP. Gomez et al. (2019) used a hydrothermal treatment at 160–163 °C with an operation time of 287–357 min. The exposure of bioactive molecules to high temperatures

Table 4
Parameters of bacteria growth using different carbohydrate sources: maximum growth rates (μ_{max}, h^{-1}) and generation time (G. time, h).

Species	Carbohydrate source	Experimental	Logistic model		Gompertz model	
		μ_{max}	μ_{max}	G. time	μ_{max}	G. time
L. rhamnosus DSM20021	Inulin	0.26 ± 0.00 ^{a,3}	0.29 ± 0.00 ^{a,2}	1.03 ± 0.01 ^{c,1}	0.31 ± 0.00 ^{a,1}	0.98 ± 0.01 ^{d,2}
	FOS	0.20 ± 0.01 ^{b,1}	0.21 ± 0.01 ^{c,1}	1.45 ± 0.06 ^{b,1}	0.22 ± 0.01 ^{c,1}	1.37 ± 0.06 ^{c,1}
	SBP Pectin	0.18 ± 0.01 ^{c,1}	0.17 ± 0.01 ^{d,2}	1.82 ± 0.09 ^{a,1}	0.18 ± 0.01 ^{e,1,2}	1.72 ± 0.08 ^{b,1}
	DRB Pectin	0.18 ± 0.01 ^{c,1}	0.15 ± 0.01 ^{d,2}	1.98 ± 0.07 ^{a,1}	0.16 ± 0.01 ^{f,1,2}	1.88 ± 0.07 ^{a,1}
	SBP POS	0.21 ± 0.01 ^{b,1}	0.21 ± 0.01 ^{c,1}	1.45 ± 0.06 ^{b,1}	0.20 ± 0.01 ^{d,1}	1.44 ± 0.08 ^{c,1}
	DRB POS	0.25 ± 0.00 ^{a,2}	0.26 ± 0.00 ^{b,1,2}	1.14 ± 0.02 ^{b,c,1}	0.27 ± 0.01 ^{b,1}	1.11 ± 0.02 ^{d,1}
B. longum DSM20219	Inulin	0.29 ± 0.01 ^{c,1}	0.28 ± 0.00 ^{c,2}	1.09 ± 0.01 ^{d,1}	0.29 ± 0.00 ^{c,1}	1.04 ± 0.01 ^{c,2}
	FOS	0.23 ± 0.00 ^{d,1}	0.21 ± 0.00 ^{d,2}	1.45 ± 0.02 ^{c,1}	0.22 ± 0.00 ^{d,1}	1.37 ± 0.01 ^{b,2}
	SBP Pectin	0.17 ± 0.00 ^{e,1}	0.15 ± 0.00 ^{e,2}	2.01 ± 0.04 ^{b,1}	0.15 ± 0.00 ^{e,2}	2.02 ± 0.03 ^{a,1}
	DRB Pectin	0.89 ± 0.01 ^{a,1}	0.83 ± 0.01 ^{a,2}	0.36 ± 0.01 ^{f,1}	0.87 ± 0.01 ^{a,1,2}	0.35 ± 0.00 ^{e,2}
	SBP POS	0.15 ± 0.00 ^{f,1}	0.14 ± 0.00 ^{e,2}	2.14 ± 0.04 ^{a,1}	0.15 ± 0.00 ^{e,1}	2.00 ± 0.04 ^{a,2}
	DRB POS	0.53 ± 0.02 ^{b,1}	0.59 ± 0.00 ^{b,1}	0.51 ± 0.00 ^{e,1}	0.58 ± 0.02 ^{b,1}	0.52 ± 0.02 ^{d,1}

Data are presented as mean ± SD of three different experiments.
a-d Means in the same column not followed by a common superscript letter are significantly different ($p < 0.05$) within each bacterial species.
1-3 Means in the same row not followed by a common superscript number are significantly different ($p < 0.05$) within each parameter.

during long operation times could lead to its degradation (García-Cubero et al., 2023). The short operating time required by MAE (11.9 min) could have avoided POS degradation.

To the best of our knowledge, the prebiotic potential of pectin and POS derived from DRB has not been evaluated. The comparative study carried out in this work revealed that DRB POS promoted significantly higher bacterial growth than the SBP products and FOS for both bacteria tested. Moreover, DRB POS showed similar bacterial growth to inulin for *L. rhamnosus* and higher growth for *B. longum*.

The modeling of bacterial growth is a valuable tool in microbiological research and allows insights to be gained into the dynamics of bacterial populations under different conditions. The Logistic and Gompertz models were used to analyze and understand the growth of *L. rhamnosus* and *B. longum* across different carbohydrate sources. The R^2 and MSE measures were considered to assess the model fitting (Tables S1 and S2). Overall, both the Logistic (R^2 = 0.96–0.99; MSE = $9.84 \cdot 10^{-3}$ – $1.35 \cdot 10^{-1}$) and Gompertz (R^2 = 0.97–1.00; MSE = $9.90 \cdot 10^{-3}$ – $1.14 \cdot 10^{-1}$) models achieved high R^2 and relatively low MSE values. However, when examining the differences between the experimental and modeled μ_{max} values (Table 4), there were some variations. The lag time observed in bacterial growth for SBP POS with *B. longum* (Fig. S2d) aligned with the Gompertz model's prediction of a lag time of 1.13 ± 0.06 h. The lag time for the other products, as calculated by the models, was 0.00 ± 0.00 h (data not shown).

The generation time represents the time required for a bacterial population to double during the exponential growth phase. This parameter is helpful to understand the rate at which bacteria can multiply under specific conditions. The experimental data showed that *L. rhamnosus* doubled its size in the first 2 h of fermentation, independently of the carbohydrate source. The values calculated by the Logistic (1.03–1.98 h) and Gompertz (0.98–1.88 h) model were in accordance with experimental data. On the other hand, *B. longum* doubled its size within the first 2 h of fermentation, except when using SBP substrates. It took 2–4 h of fermentation using SBP substrates to double its size. The values calculated by the Logistic (0.36–2.14 h) and Gompertz (0.35–2.02 h) models agreed with the experimental data, confirming that both models fit the bacterial growth.

3.4.2. Short chain fatty acids and lactate production

The metabolism of carbohydrates by the gut bacteria results in the production of SCFAs and lactate. These play a crucial role in the treatment of the metabolic syndrome, bowel disorders and some kinds of cancer (Den Besten et al., 2013). The results of SCFA and lactate production during the fermentation of *L. rhamnosus* and *B. longum* can be found in Fig. 5. The total concentration of SCFA and lactate after 48 h of *L. rhamnosus* fermentation ranged from 100.9 mM (DRB POS) to 125.3 mM (SBP Pectin) (Fig. 5a.1). The results revealed that SCFA and lactate concentrations in the presence of SBP or DRB products were comparable to, or even significantly higher (SBP Pectin) than, those of the commercial prebiotics. In the case of *B. longum*, the concentrations after 48 h were lower and ranged from 52.4 mM (inulin) to 95.0 mM (SBP POS) (Fig. 5a.2). The SCFA and lactate concentrations, using DRB and SBP products as substrates, were higher than that obtained with inulin and comparable to that obtained by FOS. Overall, the highest SCFA concentrations with both bacteria were achieved with SBP products. SBP Pectin and SBP POS showed the highest content in arabinose (Table 1) and, according to previous research, arabinose-rich hairy regions produced more SCFA (Zhu et al., 2019).

As can be seen (Fig. 5b.1 and b.2), acetate was the most abundant product during the fermentation of both bacteria, followed by lactate. Both acetate and lactate accounted for 95% or more of the total fermentation products at any fermentation time (data not shown). After 48 h of fermentation, butyrate and propionate accounted for less than 1% of the total products (Fig. 5b.1 and b.2). As in previous research, the acetate concentration was much higher than those of butyrate and propionate (Yeung et al., 2021). This is because butyrate and propionate are not the major metabolites of *Lactobacilli* or *Bifidobacteria*. On the other hand, lactate is a major product of *Lactobacilli* and *Bifidobacteria* (Louis, Duncan, Sheridan, Walker, & Flint, 2022). Lactate was produced during the fermentation and, after 8 h (*L. rhamnosus*) and 24 h (*B. longum*), its concentration started to decrease in all media (data not shown). This trend was also observed in the study of Gullón et al. (2011), where lactate was produced within the first 7 h of fermentation of fecal inoculum in the presence of oligosaccharides. According to Yeung et al. (2021), lactate is an intermediate metabolite that is converted into other SCFAs by gut bacteria. This fact explained the decreasing concentration during fermentation time.

4. Conclusions

This work demonstrated the prebiotic properties of RG–I pectin and derived POS recovered from SBP and DRB by hydrothermal MAE and purified by a diafiltration and ultrafiltration process. The POS were produced from pectin extracts by EH under the conditions found to obtain the highest amount of oligomer with a DP between 2 and 6. DRB products exhibited a much higher antioxidant capacity than SBP products or commercial prebiotics. Both bacteria studied, *L. rhamnosus* and *B. longum*, grew in the presence of all the substrates. Inulin and DRB POS were found to promote the highest maximum growth rate for *L. rhamnosus*, and DRB Pectin for *B. longum*. The highest SCFA and lactate production was found when using SBP products for both bacteria, while inulin produced the lowest amount. Overall, this research

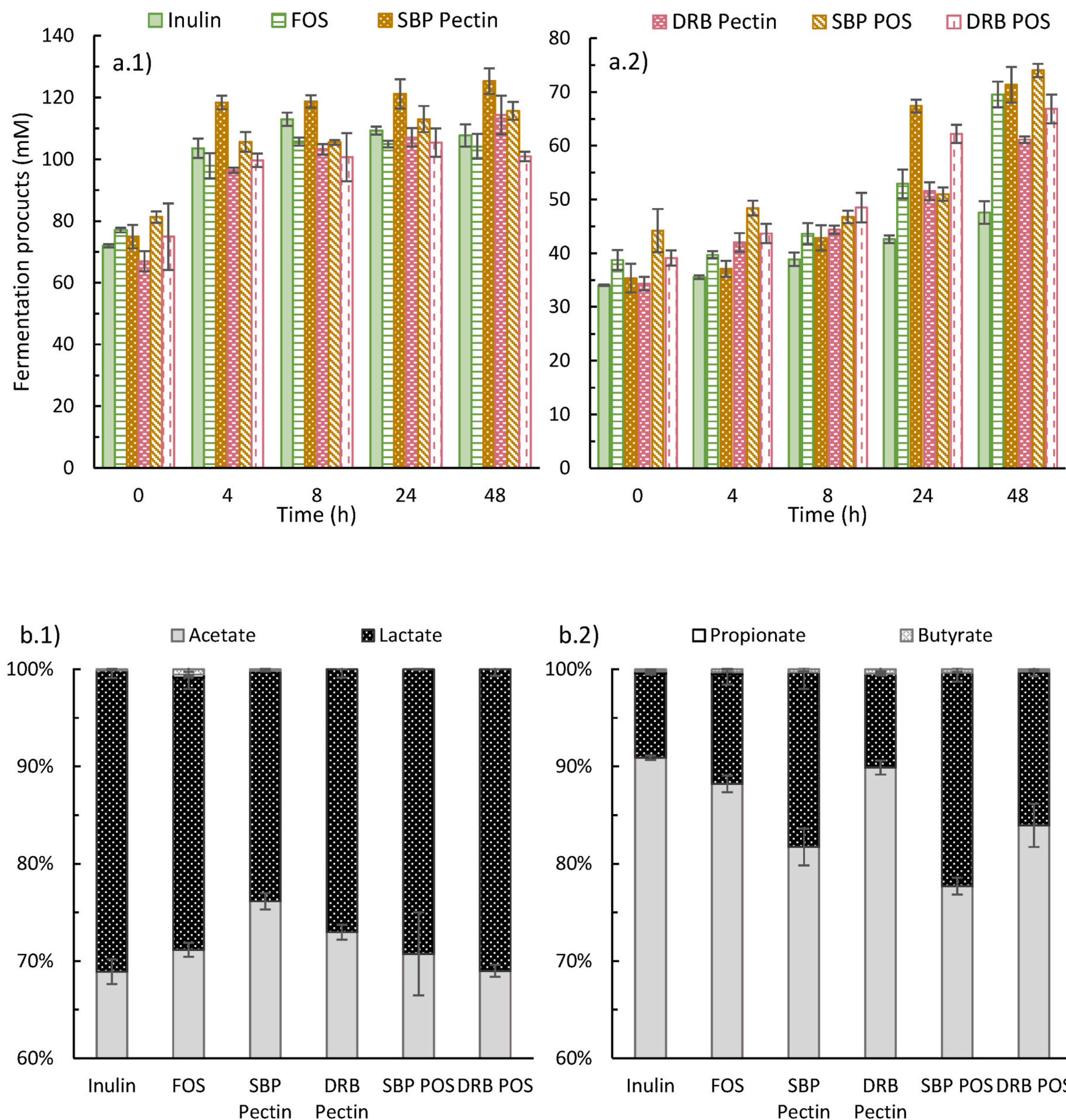

Fig. 5. Short chain fatty acids (SCFA) and lactate production during *L. rhamnosus* (1) and *B. longum* (2) fermentation: time courses of fermentation products (acetate, lactate, propionate and butyrate) (a) and profile after 48 h of fermentation (b).

demonstrated the high potential of RG–I pectin and POS from SBP, but especially from DRB, in food applications for their content in antioxidants and potential prebiotic properties.

CRediT authorship contribution statement

Esther del Amo-Mateos: Writing – original draft, Methodology, Investigation. **Rebeca Pérez:** Methodology, Investigation. **Alejandro Merino:** Software, Methodology. **Susana Lucas:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **M. Teresa García-Cubero:** Project administration, Methodology, Funding acquisition. **Mónica Coca:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.109955.

References

Al-Tamimi, M. A. H. M., Palframan, R. J., Cooper, J. M., Gibson, G. R., & Rastall, R. A. (2006). In vitro fermentation of sugar beet arabinan and arabino-oligosaccharides by the human gut microflora. *Journal of Applied Microbiology, 100*(2), 407–414. https://doi.org/10.1111/j.1365-2672.2005.02780.x

Babbar, N., Baldassarre, S., Maesen, M., Prandi, B., Dejonghe, W., Sforza, S., et al. (2016). Enzymatic production of pectic oligosaccharides from onion skins. *Carbohydrate Polymers, 146*, 245–252. https://doi.org/10.1016/j.carbpol.2016.03.011

Cano, M. E., García-Martín, A., Ladero, M., Lesur, D., Pilard, S., & Kovensky, J. (2021). A simple procedure to obtain a medium-size oligogalacturonic acids fraction from orange peel and apple pomace wastes. *Food Chemistry, 346*. https://doi.org/10.1016/j.foodchem.2020.128909

Combo, A. M. M., Aguedo, M., Goffin, D., Wathelet, B., & Paquot, M. (2012). Enzymatic production of pectic oligosaccharides from polygalacturonic acid with commercial pectinase preparations. *Food and Bioproducts Processing, 90*(3), 588–596. https://doi.org/10.1016/j.fbp.2011.09.003

de Moura, F. A., Macagnan, F. T., & da Silva, L. P. (2015). Oligosaccharide production by hydrolysis of polysaccharides: A review. *International Journal of Food Science and Technology, 50*(Issue 2), 275–281. https://doi.org/10.1111/ijfs.12681

del Amo-Mateos, E., Cáceres, B., Coca, M., Teresa García-Cubero, M., & Lucas, S. (2024). Recovering rhamnogalacturonan-I pectin from sugar beet pulp using a sequential ultrasound and microwave-assisted extraction: Study on extraction optimization and membrane purification. *Bioresource Technology, 394*, Article 130263. https://doi.org/10.1016/j.biortech.2023.130263

del Amo-Mateos, E., Fernández-Delgado, M., Lucas, S., López-Linares, J. C., García-Cubero, M. T., & Coca, M. (2023). Valorization of discarded red beetroot through the recovery of bioactive compounds and the production of pectin by surfactant-assisted microwave extraction. *Journal of Cleaner Production, 389*. https://doi.org/10.1016/j.jclepro.2023.135995

del Amo-Mateos, E., López-Linares, J. C., García-Cubero, M. T., Lucas, S., & Coca, M. (2022). Green biorefinery for sugar beet pulp valorisation: Microwave hydrothermal processing for pectooligosaccharides recovery and biobutanol production. *Industrial Crops & Products, 184*. https://doi.org/10.1016/j.indcrop.2022.115060

Dempsey, E., & Corr, S. C. (2022). Lactobacillus spp. for gastrointestinal health: Current and Future Perspectives. *Frontiers in Immunology, 13*. https://doi.org/10.3389/fimmu.2022.840245. Frontiers Media S.A.

Den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *The Journal of Lipid Research, 54*(9), 2325–2340. https://doi.org/10.1194/jlr.R036012

Dhiman, A., Suhag, R., Chauhan, D. S., Thakur, D., Chhikara, S., & Prabhakar, P. K. (2021). Status of beetroot processing and processed products: Thermal and emerging technologies intervention. *Trends in Food Science & Technology, 114*, 443–458. https://doi.org/10.1016/j.tifs.2021.05.042. Elsevier Ltd.

Gao, H., Li, X., Chen, X., Hai, D., Wei, C., Zhang, L., et al. (2022). The functional roles of Lactobacillus acidophilus in different Physiological and Pathological Processes. *Journal of Microbiology and Biotechnology, 32*(Issue 10), 1226–1233. https://doi.org/10.4014/jmb.2205.05041. Korean Society for Microbiolog and Biotechnology.

García-Cubero, M. T., del Amo-Mateos, E., Fernández-Delgado, M., López-Linares, J. C., Coca, M., & Lucas, S. (2023). Microwave-assisted pretreatment of Lignocellulosic Residues. In *Handbook of biorefinery research and Technology* (pp. 1–24). Springer Netherlands. https://doi.org/10.1007/978-94-007-6724-9_67-1.

Gibson, A. M., Bratchell, N., & Roberts, T. A. (1987). The effect of sodium chloride and temperature on the rate and extent of growth of Clostvidiurn botulinurn type A in pasteurized pork slurry. *Journal of Applied Bacteriology, 62*, 479–490.

Gibson, G. R., Probert, H. M., Loo, J. Van, Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews, 17*(2), 259–275. https://doi.org/10.1079/nrr200479

Gómez, B., Gullón, B., Remoroza, C., Schols, H. A., Parajó, J. C., & Alonso, J. L. (2014). Purification, characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes. *Journal of Agricultural and Food Chemistry, 62*(40), 9769–9782. https://doi.org/10.1021/jf503475b

Gómez, B., Gullón, B., Yáñez, R., Schols, H., & Alonso, J. L. (2016). Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation. *Journal of Functional Foods, 20*, 108–121. https://doi.org/10.1016/j.jff.2015.10.029

Gómez, B., Peláez, C., Martínez-Cuesta, M. C., Parajó, J. C., Alonso, J. L., & Requena, T. (2019). Emerging prebiotics obtained from lemon and sugar beet byproducts: Evaluation of their in vitro fermentability by probiotic bacteria. *Lebensmittel-Wissenschaft & Technologie, 109*, 17–25. https://doi.org/10.1016/j.lwt.2019.04.008

Gullón, B., Gullón, P., Sanz, Y., Alonso, J. L., & Parajó, J. C. (2011). Prebiotic potential of a refined product containing pectic oligosaccharides. *Lebensmittel-Wissenschaft & Technologie, 44*(8), 1687–1696. https://doi.org/10.1016/j.lwt.2011.03.006

Hotchkiss, A. T., Chau, H. K., Strahan, G. D., Nuñez, A., Simon, S., White, A. K., et al. (2022). Structural characterization of red beet fiber and pectin. *Food Hydrocolloids, 129*. https://doi.org/10.1016/j.foodhyd.2022.107549

Leijdekkers, A. G. M., Bink, J. P. M., Geutjes, S., Schols, H. A., & Gruppen, H. (2013). Enzymatic saccharification of sugar beet pulp for the production of galacturonic acid and arabinose; a study on the impact of the formation of recalcitrant oligosaccharides. *Bioresource Technology, 128*, 518–525. https://doi.org/10.1016/j.biortech.2012.10.126

Lemaire, A., Duran Garzon, C., Perrin, A., Habrylo, O., Trezel, P., Bassard, S., et al. (2020). Three novel rhamnogalacturonan I- pectins degrading enzymes from Aspergillus aculeatinus: Biochemical characterization and application potential. *Carbohydrate Polymers, 248*. https://doi.org/10.1016/j.carbpol.2020.116752

López-Linares, J. C., Lucas, S., García-Cubero, M. T., Jiménez, J. J., & Coca, M. (2020). A biorefinery based on brewer's spent grains: Arabinoxylans recovery by microwave assisted pretreatment integrated with butanol production. *Industrial Crops & Products, 158*. https://doi.org/10.1016/j.indcrop.2020.113044

Louis, P., Duncan, S. H., Sheridan, P. O., Walker, A. W., & Flint, H. J. (2022). Microbial lactate utilisation and the stability of the gut microbiome. *Gut Microbes, 3*. https://doi.org/10.1017/gmb.2022.3

Manderson, K., Pinart, M., Tuohy, K. M., Grace, W. E., Hotchkiss, A. T., Widmer, W., et al. (2005). In vitro determination of prebiotic properties of oligosaccharides derived from an orange juice manufacturing by-product stream. *Applied and Environmental Microbiology, 71*(12), 8383–8389. https://doi.org/10.1128/AEM.71.12.8383-8389.2005

Mao, Y., Lei, R., Ryan, J., Arrutia Rodriguez, F., Rastall, B., Chatzifragkou, A., et al. (2019). Understanding the influence of processing conditions on the extraction of rhamnogalacturonan-I "hairy" pectin from sugar beet pulp. *Food Chemistry, X*, 2. https://doi.org/10.1016/j.fochx.2019.100026

Mengíbar, M., Mateos-Aparicio, I., Miralles, B., & Heras, Á. (2013). Influence of the physico-chemical characteristics of chito-oligosaccharides (COS) on antioxidant activity. *Carbohydrate Polymers, 97*(2), 776–782. https://doi.org/10.1016/j.carbpol.2013.05.035

Prandi, B., Baldassarre, S., Babbar, N., Bancalari, E., Vandezande, P., Hermans, D., et al. (2018). Pectin oligosaccharides from sugar beet pulp: Molecular characterization and potential prebiotic activity. *Food & Function, 9*(3), 1557–1569. https://doi.org/10.1039/c7fo01182b

Rico, X., Gullón, B., & Yáñez, R. (2020). Environmentally friendly hydrothermal processing of melon by-products for the recovery of bioactive pectic-oligosaccharides. *Foods, 9*(11). https://doi.org/10.3390/foods9111702

Sabater, C., Ferreira-Lazarte, A., Montilla, A., & Corzo, N. (2019). Enzymatic production and characterization of pectic oligosaccharides derived from citrus and apple pectins: A GC-MS study using random forests and association rule learning. *Journal of Agricultural and Food Chemistry, 67*(26), 7435–7447. https://doi.org/10.1021/acs.jafc.9b00930

Sadowska-Bartosz, I., & Bartosz, G. (2022). Evaluation of the antioxidant capacity of food products: Methods, applications and limitations. *Processes, 10*(10). https://doi.org/10.3390/pr10102031. MDPI.

Shah, N. P. (2011). BACTERIA, BENEFICIAL | Bifidobacterium spp.: Morphology and physiology. In J. W. Fuquay (Ed.), *Encyzlopedia of dairy sciences* (2nd ed., pp. 381–387). https://doi.org/10.1016/B978-0-12-374407-4.00043-1

Shi, H., Yu, L., Shi, Y., Lu, J., Teng, H., Zhou, Y., et al. (2017). Structural characterization of a rhamnogalacturonan I domain from ginseng and its inhibitory effect on galectin-3. *Molecules, 22*(6). https://doi.org/10.3390/molecules22061016

Sugkhaphan, P., & Kijroongrojana, K. (2009). Optimization of prebiotics in soybean milk using mixture experiments. *Songklanakarin Journal of Science and Technology, 31*(5), 481–490.

Waldron, K. W., & Faulds, C. B. (2007). 1.05 - cell wall polysaccharides: Composition and structure. In H. Kamerling (Ed.), *Comprehensive glycoscience* (pp. 181–201). https://doi.org/10.1016/B978-044451967-2/00005-2

Wang, X., Zhang, P., & Zhang, X. (2021). Probiotics regulate gut microbiota: An effective method to improve immunity. *Molecules, 26*(19), 6076. https://doi.org/10.3390/molecules26196076

Yeung, Y. K., Kang, Y. R., So, B. R., Jung, S. K., & Chang, Y. H. (2021). Structural, antioxidant, prebiotic and anti-inflammatory properties of pectic oligosaccharides hydrolyzed from okra pectin by Fenton reaction. *Food Hydrocolloids, 118*. https://doi.org/10.1016/j.foodhyd.2021.106779

Zhang, S., Waterhouse, G. I. N., Du, Y., Fu, Q., Sun, Y., Wu, P., et al. (2023). Structural, rheological and emulsifying properties of RG-I enriched pectins from sweet and sour cherry pomaces. *Food Hydrocolloids, 139*. https://doi.org/10.1016/j.foodhyd.2022.108442

Zhu, R., Wang, C., Zhang, L., Wang, Y., Chen, G., Fan, J., et al. (2019). Pectin oligosaccharides from fruit of Actinidia arguta: Structure-activity relationship of prebiotic and antiglycation potentials. *Carbohydrate Polymers, 217*, 90–97. https://doi.org/10.1016/j.carbpol.2019.04.032

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Riet, K. V. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*, 1875–1881.

Web references

Home - PULP2VALUE Accessed November 3, 2023.

Prebiotics - Global strategic business report (researchandmarkets.com) Accessed November 2, 2023.