In vitro fermentation properties of pectins and enzymatic-modified pectins obtained from different renewable bioresources

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Abstract

The suitability of artichoke and sunflower by-products as renewable sources of pectic compounds with prebiotic potential was evaluated by studying their ability to modulate the human faecal microbiota *in vitro*. Bacterial populations and short-chain fatty acid (SCFA) production were measured. Reduction of the molecular weight of artichoke pectin resulted in greater stimulation of the growth of *Bifidobacterium*, *Lactobacillus* and *Bacteroides/Prevotella*, whilst this effect was observed only in *Bacteroides/Prevotella* for sunflower samples. In contrast, the degree of methylation did not have any impact on fermentability properties or SCFA production, regardless of the origin of pectic compounds. Although further *in vivo* studies should be conducted, either pectin or enzymatically-modified pectin from sunflower and artichoke by-products might be considered as prebiotic candidates for human consumption showing similar ability to promote the *in vitro* growth of beneficial gut bacteria as compared to well-recognized prebiotics such as inulin or fructo-oligosaccharides.

Keywords: pectin, modified pectin, *in vitro* fermentation, prebiotic properties, SCFA, gut microbiota.

1. Introduction

One of the most complex polysaccharides that exist in the cell wall of all higher plants is pectin (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). Pectin is not a single structure and comprises of a family of plant cell wall polysaccharides that contain galacturonic acid (GalA) linked at α-1,4 positions. It mainly consists of a GalA-rich backbone, known as homogalacturonan (HG ≈ 65%) which is partially methyl-esterified in C-6 and O-acethyl-esterified in positions 2 and 3 (Mohnen, 2008). Rhamnose residues interrupt the HG structure to form rhamnogalacturonan I (RG-I ≈ 20-35%) which is based on a backbone consisting of a repeating disaccharide of GalA and rhamnose residues. In addition, some rhamnose residues may contain sidechains consisting of α-L-arabinose and/or β-D-galactose (arabinans, galactans and arabinogalactans). RG-II constitutes ≈ 2-10% of pectin and is the most complex, but is also believed to be the most conserved part of pectin molecules. RG-II has a HG backbone and is branched with rhamnose and other minor sugars such as fucose, glucuronic acid and methyl-esterified glucuronic acid among other rare carbohydrates such as apiose, 2-*O*-methylxylose, and 2-*O*-methylfucose (Holck, Hotchkiss, Meyer, Mikkelsen, & Rastall, 2014; Noreen et al., 2017).

The biological effects of pectins have been mainly studied on *in vitro* assays and they are highly fermentable dietary fibres. Furthermore, pectic-oligosaccharides (POS) have been proposed as a new class of prebiotics capable of exerting a number of health-promoting effects (Olano- Martin, Gibson, & Rastall, 2002). These benefits include a desirable fermentation profile in the gut (Gómez, Gullón, Yáñez, Schols, & Alonso, 2016), potential *in vitro* anti-cancer properties (Maxwell et al., 2015), potential for cardiovascular protection (Samuelsson et al., 2016), as well as antibacterial, anti-inflammatory and antioxidant properties, among others (Míguez, Gómez, Gullón,

Gullón, & Alonso, 2016). Nevertheless, the details of the underlying mechanisms are still largely unknown and additional studies are needed on the structure-function interrelationship, as well as on the claimed effects caused by POS in humans (Gullón et al., 2013).

Apart from POS, whose degree of polymerization range from 3 to 10, during the past few years there has been a flourishing interest towards pectin derivatives, especially the so-called "modified pectins" (MP), a term standing for pectin-derived, water-soluble polysaccharide of lower molecular weight (Mw) than the original pectin and, normally, produced from citrus peel and pulp (Holck et al., 2014). These compounds can be obtained from pectins in their native form using chemical and enzymatic treatments, which produce lower Mw HG and fragments enriched in RG (Morris, Belshaw, Waldron, & Maxwell, 2013). The break-down of pectins not only leads to modification of their physico-chemical and gelling properties (Ngouémazong, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015), but also modulation of their bioactivity (Morris et al., 2013).

There are several *in vitro* and *in vivo* studies on the ability of MP to inhibit tumour growth and metastasis (Morris et al., 2013; Nangia-Makker et al., 2002; Park, Park, Hong, Suh, & Shin, 2017). Citrus MP inhibits *in vitro* and *in vivo* angiogenesis in different types of cancer by blocking the association of galectin-3 to its receptors (Zhang, Xu, & Zhang, 2015). Other beneficial health properties might include the reduction of atherosclerotic lesions (Lu et al., 2017), anti-inflammatory and antioxidant properties (Popov & Ovodov, 2013; Ramachandran, Wilk, Melnick, & Eliaz, 2017) or immunostimulatory properties (Vogt et al., 2016). However, most of these studies were performed using cell cultures or in mice and extrapolation of the results to human or clinical investigations should be considered with caution.

Nonetheless, only a few recent studies have addressed the prebiotic potential of MP in terms of the fermentation properties. A slight or no increase was observed in the faecal lactobacilli count during an *in vivo* study with rats fed with citrus MP (Odun-Ayo, Mellem, & Reddy, 2017). Di et al. (2017) compared five structurally different citrus pectic samples (3 of them were POS and 2 were MP) and found that two POS and one MP exhibited bifidogenic effects with similar fermentabilities in human faecal cultures. These authors concluded that Mw and degree of methylation did not affect their bifidogenic properties; however, structural diversity in pectic compounds is possible as long as significant arabino- and galacto-oligosaccharide content is present. Fanaro et al. (2005) investigated the effect of acidic oligosaccharides from pectin on intestinal flora and stool characteristics in infants, showing that they were well tolerated as ingredient in infant formulae but did not affect intestinal microecology.

To the best of our knowledge, the fermentation and prebiotic properties of pectin derived from artichoke (Sabater, Corzo, Olano, & Montilla, 2018) and sunflower (Muñoz-Almagro, Rico-Rodríguez, Wilde, Montilla, & Villamiel, 2018b) by-products have not been explored. In the case of artichoke, only one previous study showed a selective growth of two specific strains, i.e. *Lactobacillus plantarum* 8114 and *Bifidobacterium bifidum* ATCC 11863 which was ascribed to the combination of its high inulin and low methylated pectin contents (Fissore, Santo Domingo, Gerschenson, & Giannuzzi, 2015). Also, Costabile et al. (2010) reported, in a double-blind, cross-over study carried out in healthy adults, a pronounced prebiotic effect (i.e., increasing of bidifobacteria and lactobacilli) of a very-long-chain inulin derived from artichoke on the human faecal microbiota composition. The lack of knowledge of potential alternative sources of active pectic compounds for human consumption is surprising as previous studies reported that structure and composition can make a significant difference to the

fermentation properties (Onumpai, Kolida, Bonnin, & Rastall, 2011). Thus, bifidogenic properties seem to highly depend on the composition and structure of pectins, with neutral sugar content and GalA:Rha ratio being critical factors (Di et al., 2017).

In this context, considering the structural diversity of pectins dependent on their origin, the aim of this study was to evaluate the effect of a variety of pectins and enzymatic-modified pectins from different sources (in particular, citrus, sunflower and artichoke) on the profile changes in human faecal microbiota population and fermentation metabolites, i.e. short-chain fatty acids.

2. Materials and methods

2.1 Raw material

Sunflower by-products based on heads and leftover stalks and artichoke by-products derived from external bracts, leaves and stems, were supplied by Syngenta AG and Riberebro S.L. (Spain), respectively. Prior to experiments, raw material was ground with a knife mill to particle size < 500 μm. Commercial citrus pectin (trade name Ceampectin®, ESS-4400) was kindly provided by CEAMSA (Porriño, Pontevedra, Spain).

2.2 Pectin extraction and modification

Sunflower pectin was extracted from 1 kg of dried substrate by suspending in 20 L of sodium citrate (0.7 %) at 52 ºC, pH 3.2 for 184 min under agitation and the residue was precipitated with ethanol and then freeze-dried (Muñoz-Almagro et al. 2018b). Artichoke pectin was extracted using a cellulase from *Trichoderma reesei* (Celluclast® 1.5 L, Novozymes, Bagsvaerd, Denmark) in an orbital shaker at 50 °C, pH 5 with constant shaking (200 rpm) following the method described by Sabater et al. (2018).

After hydrolysis, samples were centrifuged (1,300 x *g* for 10 min at 4 °C) and supernatants were filtered through cellulose paper. Residues were washed and precipitated in 70 % ethanol, centrifuged (1,200 x *g*, 20 min) and then freeze-dried. Extraction yield of pectin (expressed as percentage) represents the amount of pectin extracted from 100 g of initial dried raw material, being 10.0% and 22.1% the obtained values for sunflower and artichoke pectin, respectively.

The extracted sunflower and artichoke pectins, as well as the commercial citrus pectin were then subjected to an enzymatic treatment using a commercial cellulase from *Aspergillus niger* (Sigma Aldrich, Steinheim, Germany) with pectinolytic activity to reduce their Mw. Then, the resulting material was transferred to a continuous membrane reactor to separate the modified pectin from oligosaccharides and free sugars formed (Olano-Martin, Mountzouris, Gibson & Rastall, 2001). The reactor consisted of an ultrafiltration dead-end stirred cell (model 8000, Amicon, Watford, U.K.) where the substrate was added and then pushed from a pressurized feed tank filled with water at a rate matching the permeate flow rate. All filtrations were carried out with an Ultracel® ultrafiltration disk membrane, with a Mw cut-off (MWCO) of 3 kDa and a diameter of 76 mm as determined by the manufacturers. Checking of absence of low molecular weight carbohydrates in the ultrafiltered samples was accomplished by the analysis of the resulting retentates and permeates by SEC-ELSD following the method described in subsection 2.3.2. All pectin and MP samples were free from monosaccharides, as well as oligosaccharides below 10 kDa (Figure 1).

2.3 Characterisation of pectin and enzymatic-modified pectin samples

2.3.1 Monosaccharide analysis

Monosaccharide analysis was performed after the acid hydrolysis of samples with 2 M trifluoroacetic acid (TFA) at 110 °C for 4 h. After that, released monosaccharides

were analysed by gas chromatography (GC) carried out with an Agilent Technologies gas chromatograph (7890A) equipped with a flame ionisation detector (FID). Prior to GC analysis, trimethylsilyl oximes (TMSO) of monosaccharides were formed (Cardelle-Cobas, Martínez-Villaluenga, Sanz, & Montilla, 2009). 500 µL of hydrolysed samples were evaporated to remove the acid and then 400 µL of phenyl-β-glucoside (0.5 mg/mL) used as internal standard (I.S.) were added. Afterward, the mixture was dried at 40 °C in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland). Sugar oximes were formed by adding 250 µL hydroxylamine chloride (2.5%) in pyridine and heating the mixture at 70 °C for 30 min. Subsequently, the oximes obtained in this step were silylated with hexamethyldisylazane (250 µL) and TFA (25 µL) at 50 °C for 30 min. Derivatisation mixtures were centrifuged at 6,700 x *g* for 2 min and supernatants were injected in the GC-FID.

Analyses were carried out using a DB-5HT capillary column (15 m x 0.32 mm x 0.10 µm, J&W Scientific, Folson, California, USA). Nitrogen was used as carrier gas at a flow rate of 1 mL/min. Injector and detector temperatures were 280 and 385 °C, respectively. The oven temperature was programmed from 150 to 380 °C at a heating ratio of 1 °C/min until 165 °C and then up to 300 °C at a heating rate of 10 °C/min. Injections were made in the split mode (1:5).

Data acquisition and integration were done using Agilent ChemStations software (Wilmington, DE, USA). Response factors were calculated after duplicate analysis of standard solutions (glucose, mannose, rhamnose, arabinose, galactose, GalA and xylose) over the expected concentration range in samples, (0.01–2 mg) and IS (0.2 mg).

178 2.3.2 Estimation of the molecular weight (Mw)

179 Estimation of Mw was carried out by Size Exclusion Chromatography (SEC)
180 according to the method described by (Muñoz-Almagro, Rico-Rodriguez, Villamiel, &
181 Montilla, 2018a). The analysis was performed on a LC Agilent Technologies 1220
182 Infinity LC System 1260 (Agilent Technologies, Germain), equipped with two
183 consecutive TSK-GEL columns (G5000 PW_{XL}, 7.8 x 300 mm, particle size 10 µm,
184 G2500 PW_{XL}, 7.8 x 300 mm, particle size 6 µm) connected in series with a TSK-Gel
185 guard column (6.0mm×400mm) (Tosoh Bioscience, Stuttgart, Germany). Samples (20
186 µL) were eluted with 0.1 M NaCl at a flow rate of 0.5 mL/min for 50 min at 30 °C. The
187 eluent was monitored with an Evaporative Light Scattering Detector (ELSD)
188 (Boeblingen, Germain) at 30 °C. Pullulans of Mw 805, 200, 10, 3 and 0.3 kDa were
189 used as standards to calibration. All the Mw values specified were weight-average.

190 2.3.3. Estimation of the degree of methylation

191 Degree of methylation of samples was determined by Fourier transform infrared
192 spectroscopy (FTIR). KBr discs were prepared mixing the pectin and enzymatic-
193 modified pectin samples with KBr (1:100) and pressing. FTIR spectra Bruker IFS66v
194 (Bruker, US) were collected in absorbance mode in the frequency range of 400-4000
195 cm^{-1}, at a resolution of 4 cm^{-1} (mid infrared region) with 250 co-added scans. The
196 degree of methylation was determined as the average of the ratio of the peak area at
197 1747 cm^{-1} (COO-R) and 1632 cm^{-1} (COO^-) as previously described (Singthong, Cui,
198 Ningsanond, & Douglas Goff, 2004).

2.4 Determination of *in vitro* fermentation properties and prebiotic activity

2.4.1 Faecal Inocula

Faecal samples from five healthy adults (2 male, 3 female, mean age of 30.6 ± 4.2 years old) who had not consumed prebiotic or probiotic products, nor had received antibiotic treatment within 3 months before study were obtained *in situ*. Samples were kept in an anaerobic cabinet and used within a maximum of 15 min after collection.

Faecal samples were diluted (10% w/w) in anaerobic phosphate-buffered saline (PBS; 0.1 mol/L, pH 7.4, Oxoid, Basingstoke, UK) and homogenised in a stomacher (Stomacher 400, Seward, UK) at normal speed for 2 min.

2.4.2 *In vitro* batch fermentations

Sterile stirred batch culture fermentation systems were set up and aseptically filled with a volume of sterile, basal medium: (per litre) 2 g peptone water, 2 g yeast extract, 0.1 g NaCl, 0.04 g K_2HPO_4, 0.04 g KH_2PO_4, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 6H_2O$, 2 g $NaHCO_3$, 2 mL Tween 80, 0.05 g haemin, 10 μL vitamin K1, 0.5 g L-cysteine HCl, 0.5 g bile salts and 4 mL resazurin (0.25 g/L). Medium was sterilised at 120 °C for 30 min before aseptically dispensing into the sterile fermenters. Sterile stirred fermenters were filled with 9 mL of autoclaved basal medium and were gassed overnight by constant sparging oxygen-free nitrogen to maintain anaerobic conditions. 100 mg of substrates were added (final concentration of 1% (w/v)) to the respective fermentation just prior to the addition of the faecal inoculum (1 mL). The temperature was maintained at 37 °C using a water jacket and the pH was maintained between 6.7 and 6.9 using an automated pH controller (Fermac 260; Electrolab, Tewkesbury, UK). The batch cultures were run for a period of 48 h and samples were taken from each vessel at 0 and 24 h for bacterial enumeration by fluorescent *in situ* hybridisation (FISH) and at 0, 10, 24, 36 and 48 h for SCFA by GC-FID. 3 extra vessels with inulin,

fructooligosaccharides (FOS) and no added carbohydrate source were also included as positive and negative control, respectively.

2.4.3 Short-chain fatty acid (SCFA) analysis

Before chemical analysis, samples from each fermentation time were centrifuged at 13,000 x *g* for 10 min to obtain the supernatant. The clear solutions were kept at -20 °C until analysis. SCFA analysis was carried out using GC-FID based on the method described by (Richardson, Calder, Stewart, & Smith, 1989). Before analysis, samples were thawed on ice and then vortexed. After that, 400 μL of each sample were taken into a glass tube and 25 μL of 2-ethylbutyric acid (0.1 M) (IS) was added. Following that, 250 μL of concentrated HCl and 1.5 mL of diethyl ether were added and the solution was mixed 1 min and centrifuged 10 min at 2,000 x *g*. 400 μL of the upper layer (ether layer) was transferred to a GC screw-cap vial and 50 μL of *N*-(*tert*-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) was added and leave 72 h to produce fully derivatisation.

A 5890 Series II Gas Chromatograph (Hewlett Packard) fitted with a Rtx-1 10 m x 0.18 mm column with a 0.20 μm coating (Crossbond 100 % dimethyl polysiloxane; Restek) was used for analysis. Helium was used as carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were 275 °C. Oven temperature was programmed from 63 °C for 3 min and then heated to 190 °C at a heating ratio of 3 °C/min and held at 190 °C for 3 min. Injections were made in the split mode (100:1). SCFA standards analysis was also carried out to quantify concentrations of all compounds.

246 2.4.4 Enumeration of bacterial populations

247 Enumeration of the target faecal bacteria groups was achieved by FISH with
248 fluorescently labelled 16S rRNA probes according to the method described by (Wagner,
249 Hornt, & Daims, 2003). Briefly, 375 µL aliquots were obtained from each fermenter
250 and were mixed with 1.125 mL 4% (w/v), ice-cold paraformaldehyde and fixed for 4-10
251 h at 4 °C. Fixed cells were then centrifuged at 13,000 x *g* for 5 min and washed twice on
252 1 mL cold filter-sterilised PBS (0.1 M). The washed cells were then resuspended in 150
253 µL PBS and 150 µL of absolute ethanol (99 %) and stored at −20 °C until analysis.

254 To obtain an appropriate number of fluorescent cells in each field of view of the
255 microscope, samples to hybridise were then diluted in a suitable volume of PBS with
256 1% (v/v) of sodium dodecyl sulphate, and 20 µL of the dilution was added to each well
257 of a six-well polytetrafluoroethylene/poly-L-lysine-coated slide (Tekdon Inc., Myakka
258 City, USA). Samples were dried at 48-50 °C for 15 min in a desktop plate incubator and
259 dehydrated in an alcohol series (50, 80 and 96% (v/v) ethanol, 2 min each) and placed
260 again at 48-50 °C to evaporate the excess of ethanol before adding the hybridisation
261 solution. 50 µL of hybridisation solution (per 1 mL; 5 M NaCl 180 µL, 1 M Tris/HCl 20
262 µL, ddH_2O 799 µL, 1 µL SDS 10% (w/v) and 100 µL of probe) was added to each well
263 and left to hybridise for 4 h in a microarray hybridisation incubator (Grant-Boekel, UK)
264 at 46-50 °C depending on the probe. After hybridisation, slides were washed in 50 mL
265 washing buffer (5 M NaCl 9 mL, ddH_2O 40 mL and 1 M Tris/HCl 1 mL) for 15 min
266 and dipped in cold distilled water for 2-3 seconds. Slides were then dried with
267 compressed N_2 and a drop of PVA-DABCO antifade (polyvinyl alcohol mounting
268 medium with 1,4-diazabicyclo (2.2.2) octane) was added onto each well. A coverslip
269 (20 mm, thickness no. 1; VWR) was placed on each slide and cell numbers of
270 microorganisms were determined by direct counting under an epifluorescence

271 microscope (Eclipse 400; Nikon, Surrey, UK) with Fluor 100 lens. A total of 15 random
272 fields of view were counted for each well.

273 The oligonucleotide probes used and conditions for each one are detailed in **Table 1**.
274 These probes were selected to account for major bacterial groups in the Actinobacteria
275 (Bif164), Bacteroidetes (Bac303), and Firmicutes (Lab158, Erec482, Chis150) phyla.

276 *2.5 Statistical Analysis*

277 Statistical analysis was performed using SPSS for Windows, version 23.0. One-way
278 analysis of variance (ANOVA) and Tukey's *post hoc* test was used to determine
279 significant differences among the bacterial group populations and organic acid
280 concentrations among the different substrates. Differences were considered significant
281 at $p < 0.05$ (n=5).

282

Table 1

Oligonucleotide probes used in this study for FISH enumeration of bacteria.

Probe	Specificity	DNA Sequence (5' to 3')	Temperature (°C)		Reference
			HB*	WB*	
Bac303	Most *Bacteroidaceae* and *Prevotellaceae*. some *Porphyromonadaceae*	CCA ATG TGG GGG ACC TT	46	48	Manz *et al.* (1996)
Bif164	*Bifidobacterium spp.*	CAT CCG GCATTA CCA CCC	50	50	Langendijk *et al.* (1995)
Chis150	Most of the *Clostridium histolyticum* group (Clostridium cluster I and II)	TTA TGC GGT ATT AAT CT(C/T) CCT TT	50	50	Franks *et al.* (1998)
Erec482	Most of the *Clostridium coccoides-Eubacterium rectale* group (Clostridium cluster XIVa and XIVb)	GCTTCT TAGTCA (A/G)GT ACC G	50	50	Franks *et al.* (1998)
Lab158	*Lactobacillus; Enterococcus*	GGT ATT AGC A(C/T)C TGT TTC CA	50	50	Harmsen *et al.* (1999)

*HB: hybridisation buffer; WB: washing buffer

3. Results and discussion

The yields of extraction of pectin from artichoke (22.1%) and sunflower by-products (10.0%) were in line with those obtained for other well-established sources of pectin, such as citrus peel (Kurita, Fujiwara, & Yamazaki, 2008), lime peel (Dominiak et al., 2014), apple pomace (Wikiera, Mika, Starzyńska-Janiszewska, & Stodolak, 2015) or passion fruit peels (Liew, Chin, Yusof, & Sowndhararajan, 2016), suggesting their potential use as renewable pectin sources.

3.1 Characterisation of pectin and enzymatic-modified pectin samples

Pectins from different sources (that is, citrus, artichoke and sunflower) and their enzymatic modified polysaccharides (modified pectin (MP)) were evaluated in this study. Neutral sugars and GalA content, average degree of methylation and average estimated Mw are included in **Table 2**. The GalA:Rha ratio displayed in the table shows the number of GalA residues per Rha residue, giving an indication of the RG-I backbone respect to HG content. Thus, a lower value shows a compound richer in RG-I chains. Ara:Rha and Gal:Rha ratios indicate the number of neutral sugar residues attached to the RG-I backbone.

As expected, GalA was the major monosaccharide residue in all pectic samples, ranging from 46.5 % (w/w) to 88.1% (w/w). The lowest values of GalA content were observed in those samples which had the highest values of rhamnose content. In consequence, the GalA:Rha ratio indicated that citrus MP, artichoke pectin, artichoke MP and citrus pectin were the most enriched samples in RG-I as compared to sunflower samples, which were the most enriched in HG structure according to the monomeric composition (27.4 and 24.1 for GalA:Rha ratio for sunflower pectin and sunflower MP, respectively). Instead, artichoke pectin and MP presented high amounts of arabinose, surpassing rhamnose content, which could be indicative of a highly enriched structure in

Table 2

Chemical characterisation of pectins and enzymatic-modified pectins from different renewable bioresources**.**

Sample	Monosaccharide (%*)							Average Mw (kDa)	GalA:Rha	Ara:Rha	Gal:Rha	Average degree of methylation (%)
	Xylose	**Arabinose**	**Rhamnose**	**Galactose**	**Mannose**	**Glucose**	**Galacturonic acid**					
Citrus Pectin	0.9 ± 0.0	3.5 ± 0.0	5.8 ± 0.0	20.2 ± 0.1	1.4 ± 0.0	1.8 ± 0.0	66.5 ± 0.2	800-100	11.52	0.61	3.50	70.7
Citrus MP	1.3 ± 0.2	3.7 ± 0.2	9.8 ± 0.1	14.0 ± 0.3	2.4 ± 0.3	13.3 ± 0.3	55.6 ± 0.6	12.0-10.0	5.70	0.38	1.44	14.2
Sunflower Pectin	2.2 ± 0.1	1.1 ± 0.0	3.2 ± 0.4	4.3 ± 0.0	0.1 ± 0.0	0.9 ± 0.0	88.1 ± 0.9	800-100	27.39	0.35	1.35	45.7
Sunflower MP	0.9 ± 0.0	2.3 ± 0.0	3.2 ± 0.1	12.2 ± 0.0	1.3 ± 0.0	1.8 ± 0.0	78.2 ± 0.5	12.5	24.13	0.71	3.77	17.0
Artichoke Pectin	1.1 ± 0.1	18.9 ± 0.6	7.6 ± 0.1	8.2 ± 0.3	1.0 ± 0.3	16.7 ± 0.7	46.5 ± 0.6	>500	6.13	2.50	1.09	8.9
Artichoke MP	2.3 ± 0.2	10.7 ± 0.1	5.4 ± 0.0	21.1 ± 0.0	1.2 ± 0.0	3.9 ± 0.0	55.5 ± 0.8	300-80	10.34	1.99	3.94	8.5

Analysis were carried out at least in duplicate (n=2)
*Monosaccharide content (%) is referred regarding the total carbohydrate measured on each sample.

arabinan and arabinogalactan branches to the RG-I chains. The amount of rhamnose and arabinose with respect to GalA may also indicate the substitution of the rhamnogalacturonan branching along the HG with arabinan and arabinogalactan structures (Manderson et al., 2005; Yuliarti, Goh, Matia-Merino, Mawson, & Brennan, 2015). The high content of arabinose and GalA determined in artichoke samples support the data obtained in previous studies (Femenia, Robertson, Waldron, & Selvendran, 1998; Sabater et al., 2018). Galactose content in all samples was higher than other neutral sugars, with the exception of arabinose in artichoke pectin, which may also indicate the presence of galactose-based oligosaccharides branched to the HG backbone. Xylose that can be present in more complex structural features of pectin, such as RG-II regions or arabinoxylans and xylogalacturonan (Maxwell et al., 2012), ranged from 0.9% to 2.3%. Lastly, glucose (from 0.9% to 16.7%) and mannose (from 0.1% to 2.4%) were found in all samples and they could likely derive from non-pectic polysaccharides extracted in minor amounts together the target pectins, such as xyloglucan, hemicellulose, and/or cellulose (Yapo, 2009; Wang et al., 2016; Sabater et al., 2018).

In both artichoke samples the degree of methylation was the lowest (8.9 and 8.5 % for pectin and MP, respectively), whereas MP samples from citrus and sunflower had moderately higher values (14.2 and 17.0 %, respectively) and citrus and sunflower pectin had the highest data of all samples with 70.7 % and 45.7 % of degree of methylation, respectively. This behaviour could be ascribed to the pectin methyl esterase activity of the enzyme employed to produce the corresponding MP.

On the other hand, all resulting MP showed a reduction of the Mw as compared to their respective pectin due to the polygalacturonase enzyme activity, which was concomitant with a decrease in GalA and an increase in RG-I to HG. However, modified artichoke pectin showed a decrease in arabinose which led to a higher relative

content of GalA compared to its parent pectin. The initial high content of arabinose observed in artichoke pectin could be related to the resulting high Mw of artichoke MP following enzymatic treatment. It is well known that arabinose is present in pectin as arabinan side chains and, consequently, a high degree of branching may create steric hindrance impairing the efficient cutting of the main chain composed by GalA. The decrease in Mw was correlated to the diminution of degree of methylation observed in citrus and sunflower samples. It is interesting to note that citrus and sunflower MP exhibited a Mw of 10-12.5 kDa which is in line with other modified pectins obtained from citrus (~ 10 kDa) that have shown to be effective supplements in the treatment of cancer and other diseases (Morris et al., 2013). Artichoke MP showed a small decrease in this parameter which is in accordance with its high Mw, as shown in Figure 1.

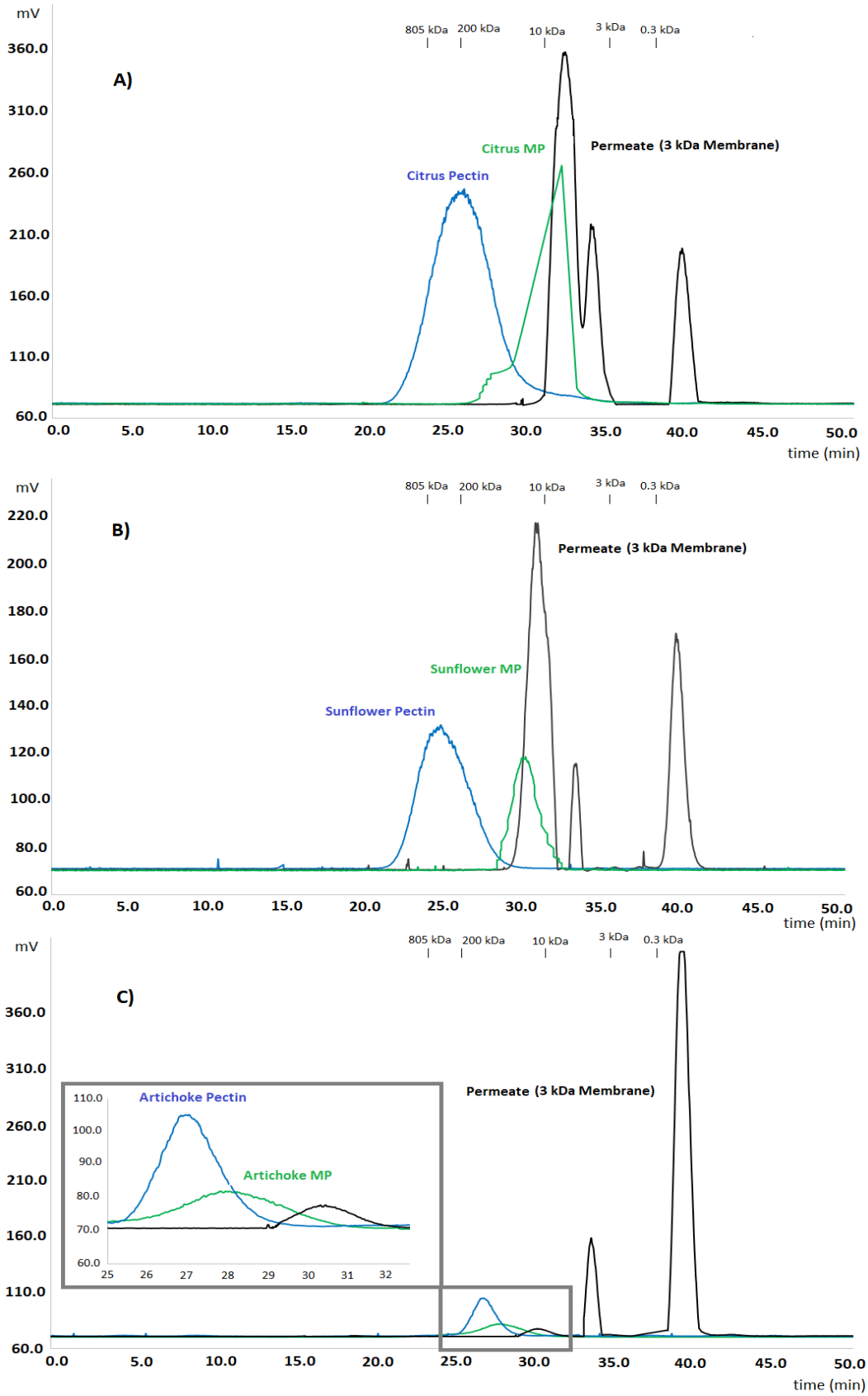

Figure 1. SEC-ELSD profiles of pectins (blue), enzymatic-modified pectins (MP) (green), and corresponding ultrafiltrated permeates (black) derived from A) citrus, B) sunflower, and C) artichoke sources. Elution positions of standard polysaccharide polymers (pullulans) are indicated by arrows.

364 3.2. *In vitro* fermentation

365 3.2.1 Bacterial population changes during *in vitro* fermentation

366 Changes in the human faecal bacterial populations during the *in vitro* fermentation
367 with the different pectins and enzymatic-modified pectins after 24 h are shown in **Table**
368 **3**. A significant increase ($p < 0.05$) of *Bifidobacterium* (Bif164) population for all
369 carbohydrate samples was observed after 24 h of fermentation. It is well known that
370 oligosaccharides deriving from pectins have bifidogenic activities, however there are
371 also studies that have demonstrated a bifidogenic effect in intact pectins suggesting a
372 potential role of this polysaccharide as a prebiotic (Gómez et al., 2016; Yang, Martínez,
373 Walter, Keshavarzian, & Rose, 2013). In our study, numerical increases up to 0.79 –
374 1.19 $\log_{10}$ in population were determined. Some authors indicated that increments of 0.5
375 - 1.0 $\log_{10}$ in bifidobacteria could be considered as a major shift in the gut microbiota

Table 3

Bacterial populations (log10 cells per ml) enumerated by FISH at 0 and 24 h of in vitro fermentation with Inulin, FOS, citrus pectin, citrus modified pectin (MP), sunflower pectin, sunflower MP, artichoke pectin and artichoke MP.

Probe/Strain	Time point (h)	Bacterial concentration (log10 cells/mL)								
		Control	**Inulin**	**FOS**	**Citrus Pectin 800-100 kDa**	**Citrus MP 10.0 – 12.0 kDa**	**Sunflower Pectin 800-100 kDa**	**Sunflower MP 12.50 kDa**	**Artichoke Pectin > 500 kDa**	**Artichoke MP 300-80 kDa**
Bif164	**0**	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)	8.63 (0.08)
	24	8.75 (0.03)^{a}	9.52 (0.15)^{bc,1}	9.48 (0.05)^{bc,1}	9.42 (0.06)^{b,1}	9.63 (0.04)^{cd,1}	9.72 (0.12)^{cd,1}	9.74 (0.06)^{cd,1}	9.50 (0.14)^{bc,1}	9.82 (0.13)^{d,1}
Bac303	**0**	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)	8.46 (0.08)
	24	8.59 (0.08)^{a}	9.36 (0.05)^{ef,1}	9.39 (0.04)^{f,1}	9.05 (0.08)^{bc,1}	9.06 (0.03)^{bc,1}	9.02 (0.09)^{b,1}	9.19 (0.07)^{cd,1}	9.23 (0.11)^{de,1}	9.45 (0.04)^{f,1}
Lab158	**0**	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)	8.35 (0.12)
	24	8.38 (0.07)^{a}	9.05 (0.04)^{de,1}	8.98 (0.03)^{cd,1}	8.65 (0.06)^{b,1}	9.04 (0.03)^{d,1}	9.05 (0.02)^{d,1}	8.98 (0.09)^{cd,1}	8.92 (0.05)^{c,1}	9.17 (0.05)^{e,1}
Erec482	**0**	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)	8.50 (0.07)
	24	8.51 (0.04)^{a}	8.97 (0.11)^{bc,1}	9.08 (0.11)^{c,1}	9.02 (0.05)^{bc,1}	9.06 (0.06)^{c,1}	8.83 (0.11)^{b,1}	8.97 (0.07)^{bc,1}	8.95 (0.06)^{bc,1}	9.01 (0.11)^{bc,1}
Chis150	**0**	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)	8.29 (0.05)
	24	8.35 (0.04)^{a}	8.77 (0.06)^{b,1}	8.72 (0.03)^{b,1}	8.70 (0.09)^{b,1}	8.73 (0.03)^{b,1}	8.77 (0.01)^{b,1}	8.70 (0.02)^{b,1}	8.72 (0.04)^{b,1}	8.70 (0.06)^{b,1}

A control sample without carbohydrate source is also included. Experiments were carried out in batch cultures systems inoculated with faecal inocula from five healthy human donors. Results shown as mean (n = 5) with the corresponding standard deviation in parentheses.

$^{a.\ b.\ c}$ Significant differences ($p < 0.05$) between substrates are indicated with different letters in the same row.

^{1} Significant difference ($p < 0.05$) from the 0 h value for each bacterial group and for the same substrate.

376 towards a potentially healthier composition of intestinal microbiota (Kolida & Gibson,
377 2007). Thus, all pectic samples could be considered bifidogenic under the studied
378 conditions. Remarkably, artichoke MP was the substrate, which promoted the
379 significantly highest growth in bifidobacteria among all assayed samples, including
380 positive controls as inulin and FOS which in turn showed a similar bifidobacterial
381 growth as compared to sunflower and citrus samples. This fact could be attributed to the
382 high combined content of arabinose and galactose found in artichoke MP (**Table 2**)
383 according to previous studies reporting a correlation between arabinose and galactose
384 content with bifidogenic properties (Di et al., 2017; Manderson et al., 2005; Onumpai et
385 al., 2011). Moreover, a positive effect of the decrease of Mw in pectin on its ability to
386 promote bifidobacteria growth was observed for citrus and artichoke sources since their
387 MP derivatives exhibited a significant ($p < 0.05$) increase as compared to unmodified
388 pectin (9.63 vs 9.42 $\log_{10}$ for citrus and 9.82 vs. 9.50 for artichoke), whereas sunflower
389 pectin and MP presented a statistically identical bifidogenic activity. Evidently, there
390 was not any significant increase during fermentation of negative controls, confirming
391 the suitability of these substrates as a carbon source for the metabolism of
392 bifidobacteria. The degree of methylation did not have impact on the bifidogenic
393 properties. More specifically, sunflower samples had different value of this parameter
394 with the same bifidogenic activity and artichoke samples had almost the same one with
395 different bifidogenic activity.

396 The second highest increase (up to 0.56 – 0.93 $\log_{10}$) was observed in
397 *Bacteroides/Prevotella* (Bac303) population. This general increase is explained by the
398 fact that *Bacteroides* species are major carbohydrate-degrading organisms in the gut and
399 have the capacity to degrade diverse plant polysaccharides, including pectins
400 (Dongowski, Lorenz, & Anger, 2000; Flint, Scott, Duncan, Louis, & Forano, 2012;

401 Onumpai et al., 2011). Indeed, many *Bacteroides* strains from human faeces can
402 produce pectinolytic enzymes, including polygalacturonase and pectin methylesterase
403 (Dekker & Palmer, 1981; Jensen & Canale-parola, 1986). Therefore, *Bacteroides* can be
404 involved in cross-feeding with *Bifidobacteria* by releasing breakdown products of
405 pectin or MP which might be utilised by the latter, thus, promoting their growth. Inulin,
406 FOS and artichoke MP samples exhibited the highest increase in *Bacteroides*. With
407 respect to the effect of Mw on *Bacteroides/Prevotella* growth, sunflower and artichoke
408 MP demonstrated a significantly higher increase than their respective pectins. This
409 difference could be attributed to the galactan chains branched to the RG-I since Gal:Rha
410 ratio increased in both sunflower and artichoke MP after the enzymatic hydrolysis.

411 A significant increase in *Lactobacillus/Enterococcus* (Lab158) was also observed
412 for all tested carbohydrate samples, with the most significant increases found in inulin
413 and artichoke MP. Similar to *Bifidobacterium*, *Lactobacillus* is considered one of the
414 major microbial targets for prebiotic action due to their health effects. The high
415 increment in *Lactobacillus/Enterococcus* population following artichoke MP
416 fermentation further established the correlation of arabinose and galactose content with
417 the prebiotic properties. Mw did not affect sunflower samples but it seemed to have an
418 impact on citrus and artichoke sources, in a similar manner to the behaviour observed
419 for *Bifidobacterium* selectivity.

420 *Clostridium coccoides/Eubacterium rectale* (Erec482) showed a significant increase
421 in all tested samples but no significant differences were found among any of the
422 carbohydrate substrates including inulin and FOS. Increase in *Eubacterium rectale* is of
423 particular interest due to its ability to produce butyrate (Manderson et al., 2005). Di et
424 al. (2017) reported an increase of Erec482 numbers when testing a citrus MP of similar
425 Mw (9.2 kDa), although they did not find a positive correlation with the determined

426 butyrate concentrations. In the same way, Chen et al. (2013) reported enhanced
427 *Eubacteria* growth on apple pectin compared to the respective POS, suggesting that the
428 Mw was not a relevant factor. In our work, similar behaviour was observed since all
429 pectic samples resulted in a significant stimulation of the butyrate producing bacteria
430 groups (Erec482) and no differences were found between samples with different Mw or
431 origin.

432 *Clostridium histolyticum* (Chis150) population displayed the lowest changes in all
433 cases, leading to a rather moderate increase (lower than 0.5 $\log_{10}$) after 24 h of
434 fermentation. No significant differences among any substrates were observed after
435 fermentation. In general, *Clostridium* species are considered as potentially harmful
436 bacteria, so in this way, all pectic samples induced a favourable behaviour.

437 3.2.2 Short-chain fatty acids (SCFA) production

438 Acetate, propionate, butyrate and total SCFA formation was analysed throughout
439 the fermentation in batch cultures (**Table 4**). Total SCFA concentration increased
440 strongly during the first 10 or 24 h of fermentation in all tested substrates. In general
441 terms, neither the degree of methylation nor Mw of pectin samples had an influence on
442 the SCFA production, as reflected by the values contained in **Table 4**.

443 Acetate was the most abundant SCFA, followed by propionic and butyric acids in
444 all substrates. Formation of acetate has been related to an enhancement of the ileal
445 motility, a protection against genotoxic agents and pathogens and an increase of colonic
446 blood (Hong et al., 2005). In our study, the only significant differences found between
447 samples after 48 h of analysis were with artichoke and citrus MPs. Results demonstrated
448 a sharp increase of this compound in the first 10 h of fermentation. Although it is

449

Table 4

SCFA concentrations (mM) determined by GC-FID at 0, 10, 24, 36 and 48 h on *in vitro* fermentations with Inulin, FOS, citrus pectin, citrus modified pectin (MP), sunflower pectin, sunflower MP, artichoke pectin and artichoke MP.

SCFA	Time point (h)	Mean SCFA concentration (mM) in substrate								
		Control	**Inulin**	**FOS**	**Citrus Pectin 800-100 kDa**	**Citrus MP 10.0 – 12.0 kDa**	**Sunflower Pectin 800-100 kDa**	**Sunflower MP 12.50 kDa**	**Artichoke Pectin > 500 kDa**	**Artichoke MP 300-80 kDa**
Acetate	**0**	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)	3.80 (1.55)
	10	12.28 (3.30)^{a,1}	36.99 (6.60)^{b,1}	54.64 (15.10)^{bcd,1}	61.65 (11.81)^{cd,1}	68.49 (6.19)^{d,1}	50.64 (9.15)^{bcd,1}	58.47 (4.13)^{cd,1}	49.35 (5.54)^{bcd,1}	42.19 (3.77)^{bc,1}
	24	21.11 (3.31)^{a,2}	62.44 (11.68)^{bcd,2}	57.89 (14.88)^{bc}	78.42 (9.02)^{cd}	78.83 (12.87)^{cd}	82.65 (11.80)^{d,2}	69.21 (10.29)^{bcd}	55.33 (1.62)^{b}	50.86 (7.81)^{b}
	36	26.18 (4.49)^{a}	65.24 (11.98)^{bc}	63.68 (10.80)^{bc}	71.18 (11.38)^{bc}	78.95 (11.71)^{c}	78.95 (11.62)^{c}	67.64 (9.27)^{bc}	55.64 (4.57)^{b}	55.60 (11.09)^{b}
	48	17.62 (3.38)^{a}	64.42 (10.55)^{bc}	63.55 (10.86)^{bc}	77.99 (14.69)^{bc}	85.40 (11.34)^{c}	78.49 (13.31)^{bc}	73.87 (10.49)^{bc}	61.00 (12.27)^{bc}	55.94 (8.95)^{b}
Propionate	**0**	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)	0.54 (0.27)
	10	2.28 (0.92)^{a}	7.58 (1.91)^{ab}	12.20 (6.90)^{b}	8.13 (3.22)^{ab,1}	11.6 (3.76)^{b,1}	6.35 (2.33)^{ab,1}	10.00 (1.10)^{ab,1}	11.35 (4.10)^{b,1}	11.27 (3.17)^{b,1}
	24	4.7 (1.26)^{a,2}	18.17 (4.69)^{b,2}	15.64 (6.76)^{b,1}	12.12 (4.58)^{ab}	16.04 (1.86)^{b}	11.82 (1.26)^{ab,2}	13.84 (0.90)^{b,2}	13.49 (3.98)^{b}	15.69 (1.37)^{b,2}
	36	4.1 (0.60)^{a}	19.51 (5.68)^{cd}	23.77 (2.89)^{d}	11.92 (4.26)^{b}	16.15 (2.26)^{bcd}	11.80 (1.47)^{b}	14.12 (1.61)^{bc}	14.55 (3.51)^{bc}	16.97 (1.62)^{bcd}
	48	2.12 (0.99)^{a}	18.41 (4.95)^{bc}	20.69 (6.22)^{c}	12.71 (3.51)^{b}	17.35 (2.12)^{bc,2}	13.60 (3.01)^{bc}	14.15 (1.82)^{bc}	14.10 (3.61)^{bc}	16.10 (2.03)^{bc}
Butyrate	**0**	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)	0.96 (0.17)
	10	1.29 (0.64)^{a}	6.57 (0.75)^{b}	3.43 (2.17)^{ab}	1.82 (1.94)^{a}	2.51 (1.36)^{a}	2.22 (1.10)^{a}	3.26 (1.52)^{ab}	2.70 (1.88)^{a}	2.74 (1.12)^{a}
	24	1.77 (1.03)^{a}	9.13 (2.22)^{b,1}	5.20 (3.28)^{ab}	4.52 (1.94)^{a,1}	4.94 (1.54)^{ab,1}	4.54 (2.19)^{a,1}	5.42 (1.59)^{ab,1}	5.30 (1.68)^{ab,1}	4.50 (0.78)^{a,1}
	36	2.31 (0.67)^{a,1}	9.66 (3.14)^{c}	7.60 (3.20)^{bc,1}	5.86 (2.26)^{abc,2}	5.35 (1.87)^{abc,2}	5.13 (1.88)^{abc}	6.23 (1.80)^{abc}	5.25 (2.22)^{abc}	4.98 (1.34)^{ab}
	48	1.06 (0.38)^{a}	9.08 (2.87)^{b}	8.40 (3.85)^{b}	4.92 (2.08)^{ab}	5.89 (1.59)^{ab}	4.78 (1.31)^{ab}	6.33 (2.61)^{b}	6.04 (3.04)^{b}	4.98 (1.87)^{ab}
Total	**0**	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)	5.26 (1.90)
	10	15.84 (4.61)^{a,1}	49.14 (13.14)^{b,1}	83.76 (17.01)^{c,1}	71.42 (12.18)^{bc,1}	82.95 (6.94)^{c,1}	59.21 (11.65)^{bc,1}	71.74 (6.46)^{bc,1}	63.89 (10.69)^{bc,1}	56.20 (7.58)^{b,1}
	24	27.83 (3.69)^{a,2}	90.30 (11.28)^{bcd,2}	84.14 (17.36)^{bcd}	94.15 (11.21)^{bcd}	102.36 (14.94)^{d}	99.01 (11.79)^{cd,2}	89.70 (9.42)^{bcd}	74.99 (3.97)^{bc}	72.42 (9.24)^{b}
	36	32.24 (4.55)^{a}	95.90 (13.77)^{b}	90.74 (15.10)^{b}	88.97 (14.71)^{b}	97.03 (18.08)^{b}	95.88 (12.16)^{b}	89.64 (10.97)^{b}	77.27 (8.63)^{b}	79.42 (13.64)^{b,2}
	48	21.21 (3.96)^{a}	90.38 (18.27)^{bc}	91.45 (11.89)^{bc}	95.63 (16.72)^{bc}	109.42 (12.10)^{c}	96.87 (13.57)^{bc}	98.84 (9.49)^{bc,2}	83.19 (17.16)^{bc}	77.02 (11.35)^{b}

A control sample without carbohydrate source was also included. Experiments were carried out in batch cultures systems inoculated with faecal inocula from five healthy human donors. Results shown as mean (n = 5) with the corresponding standard deviation in parentheses.

[a. b. c] Significant differences ($p < 0.05$) between substrates are indicated with different letters in the same row.

[1] Significant difference ($p < 0.05$) from the 0 h value for each SCFA and for the same substrate.

[2] Significant difference ($p < 0.05$) from the 10 h value for each SCFA and for the same substrate.

challenging to attribute a particular fermentation end-product to a specific bacterial group in a mixed culture system, overall the increase in acetate is in agreement with the dynamics of the microbial populations, since all samples promoted the growth of *Bifidobacterium* and *Lactobacillus* (**Table 3**), which are acetate producers. Additionally, these end-products may serve as substrates for other bacteria due to metabolic cross-feeding (Belenguer et al., 2006). Acetate is generated by many bacterial groups that inhabit the colon, with approximately one-third of the product coming from reductive acetogenesis (Miller & Wolin, 1996). In contrast, bacterial groups that form propionate and butyrate are specialised and are of particular interest in terms of their beneficial effects. The main propionate-producing bacteria in the human colon are *Bacteroides* and *Clostridium* whereas butyrate production is related to bacterial groups such as *Clostridium histolyticum* (clusters I, II, IV, XIVa, XV and XVI) and *Eubacterium rectale*.

An increase in propionate concentration was seen in all samples after 48 h of fermentation, whereas fermentation of inulin and FOS resulted in the highest increase among all samples. Similarly to acetate, the high variability found among the five donors meant that propionate differences between all samples were not considered statistically significant ($p > 0.05$) during the first 24 h of fermentation. However, the increase in this end-product is in good agreement with the increase in *Bacteroides* population displayed in **Table 3.** Propionate has also been shown to exert beneficial effects on host health, such as reduction of food intake and enhancement of satiety via augmentation of the satiety hormone leptin (Zeng, 2014), and a protective role against carcinogenesis through the decrease in human colon cancer cell growth via hyperacetylation of histone proteins and stimulation of apoptosis (Hinnebusch, Meng, Wu, Archer, & Hodin, 2002; Jan et al., 2002).

Butyrate production resulted in a significant increase in all samples after 24 h of fermentation. FOS and inulin showed the highest increase after 48 h of fermentation, although non-significant differences were observed among all substrates due to the high inter-individual variability (**Table 4**). The low but significant increase in butyrate levels are in accordance with the increase of Erec482 and Chis150 numbers which also include some of the major butyrate-produces (*Eubacterium rectale* and *Clostridium histolyticum)*. Although acetate, propionate and butyrate are all metabolised to some extent by the epithelium to provide energy, butyrate plays a critical role in maintaining colonic health and moderating cell growth (Zeng, 2014). Compared to acetate and propionate, butyrate exhibits strong anti-inflammatory properties, likely mediated by inhibition of TNF-α production, NF-κB activation, and IL-8, -10, -12 expression in immune and colonic epithelial cells and a protective role against colon cancer (Bailón et al., 2010; Zeng, 2014).

4. Conclusions

Findings in this work highlight the suitability of artichoke and sunflower by-products as renewable sources of bioactive pectic compounds since the reported yields were within the range observed for other well-established pectin sources. To the best of our knowledge, this is the first evidence of prebiotic potential of pectic compounds from sunflower and artichoke and also supports the important role played by the arabinose-rich rhamnogalacturonic acids in stimulating *Bifidobacteria*. A positive effect of decreasing molecular weight on fermentation properties was found in artichoke and citrus sources since their respective enzymatically-modified pectins promoted significantly higher growth in *Bifidobacterium* and *Lactobacillus* than the corresponding unmodified pectin. In the case of sunflower, this behaviour was only

observed in *Bacteroides/Prevotella*, which also grew to significantly higher population levels on artichoke MP as compared to the unmodified pectin. No significant effects of the molecular weight of pectin samples on SCFA production were observed, although this could be due to the high inter-individual variability observed in acetate, propionate and butyrate formation. Likewise, the degree of methylation did not have any significant impact on the fermentability nor SCFA production, regardless the origin of the pectic compounds.

To conclude, although further *in vivo* studies should be conducted, our data reveal that either pectin or enzymatically-modified pectin from sunflower and artichoke by-products might be considered as efficient prebiotic candidates for human consumption showing similar ability to promote the *in vitro* growth of beneficial gut bacteria as *Bifidobacterium* and *Lactobacillus* in comparison to well-recognized prebiotics as inulin and FOS.

Acknowledgements

This work has been funded by MINECO of Spain, Project AGL2014-53445- R. The authors thank Syngenta and Riberebro for supplying the sunflower heads and artichoke by-products, respectively.

Declarations of interest: none

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