

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

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14 **Abstract**

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

28

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

31

32 1. Introduction

33 One of the most complex polysaccharides that exist in the cell wall of all higher
34 plants is pectin (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). Pectin
35 is not a single structure and comprises of a family of plant cell wall polysaccharides that
36 contain galacturonic acid (GalA) linked at α -1,4 positions. It mainly consists of a GalA-
37 rich backbone, known as homogalacturonan (HG \approx 65%) which is partially methyl-
38 esterified in C-6 and O-acethyl-esterified in positions 2 and 3 (Mohnen, 2008).
39 Rhamnose residues interrupt the HG structure to form rhamnogalacturonan I (RG-I \approx
40 20-35%) which is based on a backbone consisting of a repeating disaccharide of GalA
41 and rhamnose residues. In addition, some rhamnose residues may contain sidechains
42 consisting of α -L-arabinose and/or β -D-galactose (arabinans, galactans and
43 arabinogalactans). RG-II constitutes \approx 2-10% of pectin and is the most complex, but is
44 also believed to be the most conserved part of pectin molecules. RG-II has a HG
45 backbone and is branched with rhamnose and other minor sugars such as fucose,
46 glucuronic acid and methyl-esterified glucuronic acid among other rare carbohydrates
47 such as apiose, 2-O-methylxylose, and 2-O-methylfucose (Holck, Hotchkiss, Meyer,
48 Mikkelsen, & Rastall, 2014; Noreen et al., 2017).

49 The biological effects of pectins have been mainly studied on *in vitro* assays and
50 they are highly fermentable dietary fibres. Furthermore, pectic-oligosaccharides (POS)
51 have been proposed as a new class of prebiotics capable of exerting a number of health-
52 promoting effects (Olano- Martin, Gibson, & Rastall, 2002). These benefits include a
53 desirable fermentation profile in the gut (Gómez, Gullón, Yáñez, Schols, & Alonso,
54 2016), potential *in vitro* anti-cancer properties (Maxwell et al., 2015), potential for
55 cardiovascular protection (Samuelsson et al., 2016), as well as antibacterial, anti-
56 inflammatory and antioxidant properties, among others (Míguez, Gómez, Gullón,

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

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

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

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

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

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

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

115

116 **2. Materials and methods**

117 *2.1 Raw material*

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

124 *2.2 Pectin extraction and modification*

125 Sunflower pectin was extracted from 1 kg of dried substrate by suspending in 20
126 L of sodium citrate (0.7 %) at 52 °C, pH 3.2 for 184 min under agitation and the residue
127 was precipitated with ethanol and then freeze-dried (Muñoz-Almagro et al. 2018b).
128 Artichoke pectin was extracted using a cellulase from *Trichoderma reesei* (Celluclast[®]
129 1.5 L, Novozymes, Bagsvaerd, Denmark) in an orbital shaker at 50 °C, pH 5 with
130 constant shaking (200 rpm) following the method described by Sabater et al. (2018).

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

137 The extracted sunflower and artichoke pectins, as well as the commercial citrus
138 pectin were then subjected to an enzymatic treatment using a commercial cellulase from
139 *Aspergillus niger* (Sigma Aldrich, Steinheim, Germany) with pectinolytic activity to
140 reduce their Mw. Then, the resulting material was transferred to a continuous membrane
141 reactor to separate the modified pectin from oligosaccharides and free sugars formed
142 (Olano-Martin, Mountzouris, Gibson & Rastall, 2001). The reactor consisted of an
143 ultrafiltration dead-end stirred cell (model 8000, Amicon, Watford, U.K.) where the
144 substrate was added and then pushed from a pressurized feed tank filled with water at a
145 rate matching the permeate flow rate. All filtrations were carried out with an Ultracel®
146 ultrafiltration disk membrane, with a Mw cut-off (MWCO) of 3 kDa and a diameter of
147 76 mm as determined by the manufacturers. Checking of absence of low molecular
148 weight carbohydrates in the ultrafiltered samples was accomplished by the analysis of
149 the resulting retentates and permeates by SEC-ELSD following the method described in
150 subsection 2.3.2. All pectin and MP samples were free from monosaccharides, as well
151 as oligosaccharides below 10 kDa (Figure 1).

152 2.3 Characterisation of pectin and enzymatic-modified pectin samples

153 2.3.1 Monosaccharide analysis

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

156 were analysed by gas chromatography (GC) carried out with an Agilent Technologies
157 gas chromatograph (7890A) equipped with a flame ionisation detector (FID). Prior to
158 GC analysis, trimethylsilyl oximes (TMSO) of monosaccharides were formed
159 (Cardelle-Cobas, Martínez-Villaluenga, Sanz, & Montilla, 2009). 500 μ L of hydrolysed
160 samples were evaporated to remove the acid and then 400 μ L of phenyl- β -glucoside
161 (0.5 mg/mL) used as internal standard (I.S.) were added. Afterward, the mixture was
162 dried at 40 °C in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland).
163 Sugar oximes were formed by adding 250 μ L hydroxylamine chloride (2.5%) in
164 pyridine and heating the mixture at 70 °C for 30 min. Subsequently, the oximes
165 obtained in this step were silylated with hexamethyldisylazane (250 μ L) and TFA (25
166 μ L) at 50 °C for 30 min. Derivatisation mixtures were centrifuged at 6,700 x g for 2 min
167 and supernatants were injected in the GC-FID.

168 Analyses were carried out using a DB-5HT capillary column (15 m x 0.32 mm x
169 0.10 μ m, J&W Scientific, Folsom, California, USA). Nitrogen was used as carrier gas at
170 a flow rate of 1 mL/min. Injector and detector temperatures were 280 and 385 °C,
171 respectively. The oven temperature was programmed from 150 to 380 °C at a heating
172 ratio of 1 °C/min until 165 °C and then up to 300 °C at a heating rate of 10 °C/min.
173 Injections were made in the split mode (1:5).

174 Data acquisition and integration were done using Agilent ChemStations software
175 (Wilmington, DE, USA). Response factors were calculated after duplicate analysis of
176 standard solutions (glucose, mannose, rhamnose, arabinose, galactose, GalA and
177 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).

199 2.4 Determination of *in vitro* fermentation properties and prebiotic activity

200 2.4.1 Faecal Inocula

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

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

208 2.4.2 *In vitro* batch fermentations

209 Sterile stirred batch culture fermentation systems were set up and aseptically filled
210 with a volume of sterile, basal medium: (per litre) 2 g peptone water, 2 g yeast extract,
211 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 $CaCl_2 \cdot 6H_2O$,
212 2 g $NaHCO_3$, 2 mL Tween 80, 0.05 g haemin, 10 μ L vitamin K1, 0.5 g L-cysteine HCl,
213 0.5 g bile salts and 4 mL resazurin (0.25 g/L). Medium was sterilised at 120 °C for 30
214 min before aseptically dispensing into the sterile fermenters. Sterile stirred fermenters
215 were filled with 9 mL of autoclaved basal medium and were gassed overnight by
216 constant sparging oxygen-free nitrogen to maintain anaerobic conditions. 100 mg of
217 substrates were added (final concentration of 1% (w/v)) to the respective fermentation
218 just prior to the addition of the faecal inoculum (1 mL). The temperature was
219 maintained at 37 °C using a water jacket and the pH was maintained between 6.7 and
220 6.9 using an automated pH controller (Fermac 260; Electrolab, Tewkesbury, UK). The
221 batch cultures were run for a period of 48 h and samples were taken from each vessel at
222 0 and 24 h for bacterial enumeration by fluorescent *in situ* hybridisation (FISH) and at
223 0, 10, 24, 36 and 48 h for SCFA by GC-FID. 3 extra vessels with inulin,

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

226 2.4.3 Short-chain fatty acid (SCFA) analysis

227 Before chemical analysis, samples from each fermentation time were centrifuged at
228 13,000 x g for 10 min to obtain the supernatant. The clear solutions were kept at -20 °C
229 until analysis. SCFA analysis was carried out using GC-FID based on the method
230 described by (Richardson, Calder, Stewart, & Smith, 1989). Before analysis, samples
231 were thawed on ice and then vortexed. After that, 400 µL of each sample were taken
232 into a glass tube and 25 µL of 2-ethylbutyric acid (0.1 M) (IS) was added. Following
233 that, 250 µL of concentrated HCl and 1.5 mL of diethyl ether were added and the
234 solution was mixed 1 min and centrifuged 10 min at 2,000 x g. 400 µL of the upper
235 layer (ether layer) was transferred to a GC screw-cap vial and 50 µL of *N*-(*tert*-
236 butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) was added and leave 72 h
237 to produce fully derivatisation.

238 A 5890 Series II Gas Chromatograph (Hewlett Packard) fitted with a Rtx-1 10 m x
239 0.18 mm column with a 0.20 µm coating (Crossbond 100 % dimethyl polysiloxane;
240 Restek) was used for analysis. Helium was used as carrier gas at a flow rate of 0.7
241 mL/min. Injector and detector temperatures were 275 °C. Oven temperature was
242 programmed from 63 °C for 3 min and then heated to 190 °C at a heating ratio of 3
243 °C/min and held at 190 °C for 3 min. Injections were made in the split mode (100:1).
244 SCFA standards analysis was also carried out to quantify concentrations of all
245 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 , ddH₂O 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, ddH₂O 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 N₂ 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).

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 <i>Bacteroidaceae</i> and <i>Prevotellaceae</i> , <i>Porphyromonadaceae</i> and some	CCA ATG TGG GGG ACC TT	46	48	Manz <i>et al.</i> (1996)
Bif164	<i>Bifidobacterium spp.</i>	CAT CCG GCATTA CCA CCC	50	50	Langendijk <i>et al.</i> (1995)
Chis150	Most of the <i>Clostridium histolyticum</i> group (Clostridium cluster I and II)	TTA TGC GGT ATT AAT CT(C/T) CCT TT	50	50	Franks <i>et al.</i> (1998)
Erec482	Most of the <i>Clostridium coccoides-Eubacterium rectale</i> group (Clostridium cluster XIVa and XIVb)	GCTTCT TAGTCA (A/G)GT ACC G	50	50	Franks <i>et al.</i> (1998)
Lab158	<i>Lactobacillus; Enterococcus</i>	GGT ATT AGC A(C/T)C TGT TTC CA	50	50	Harmsen <i>et al.</i> (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)

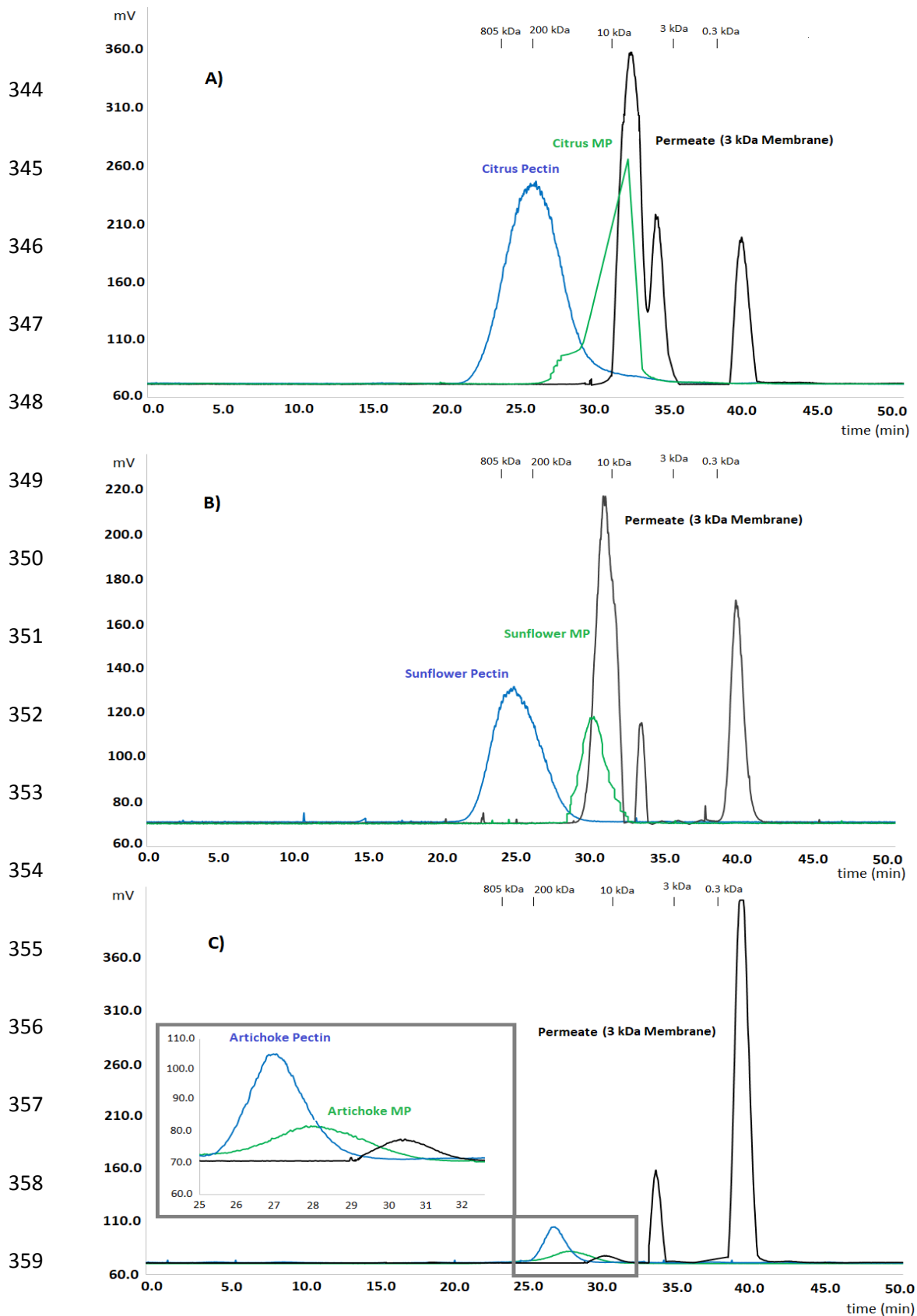
*Monosaccharide content (%) is referred regarding the total carbohydrate measured on each sample.

308 arabinan and arabinogalactan branches to the RG-I chains. The amount of rhamnose and
309 arabinose with respect to GalA may also indicate the substitution of the
310 rhamnogalacturonan branching along the HG with arabinan and arabinogalactan
311 structures (Manderson et al., 2005; Yuliarti, Goh, Matia-Merino, Mawson, & Brennan,
312 2015). The high content of arabinose and GalA determined in artichoke samples support
313 the data obtained in previous studies (Femenia, Robertson, Waldron, & Selvendran,
314 1998; Sabater et al., 2018). Galactose content in all samples was higher than other
315 neutral sugars, with the exception of arabinose in artichoke pectin, which may also
316 indicate the presence of galactose-based oligosaccharides branched to the HG backbone.
317 Xylose that can be present in more complex structural features of pectin, such as RG-II
318 regions or arabinoxylans and xylogalacturonan (Maxwell et al., 2012), ranged from
319 0.9% to 2.3%. Lastly, glucose (from 0.9% to 16.7%) and mannose (from 0.1% to 2.4%)
320 were found in all samples and they could likely derive from non-pectic polysaccharides
321 extracted in minor amounts together the target pectins, such as xyloglucan,
322 hemicellulose, and/or cellulose (Yapo, 2009; Wang et al., 2016; Sabater et al., 2018).

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

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

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



360 **Figure 1.** SEC-ELSD profiles of pectins (blue), enzymatic-modified pectins (MP)
 361 (green), and corresponding ultrafiltrated permeates (black) derived from A) citrus, B)
 362 sunflower, and C) artichoke sources. Elution positions of standard polysaccharide
 363 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 log₁₀ in population were determined. Some authors indicated that increments of 0.5
375 - 1.0 log₁₀ 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 (log ₁₀ 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 coccooides/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 log₁₀) 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

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.

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

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

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

488

489 **4. Conclusions**

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

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

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

513

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519

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