Food Research International 129 (2020) 108848

Contents lists available at ScienceDirect

Food Research International

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

Effects of gastrointestinal digested polyphenolic enriched extracts of Chilean currants (*Ribes magellanicum* and *Ribes punctatum*) on *in vitro* fecal microbiota

Alberto Burgos-Edwards[a], Arantxa Fernández-Romero[b], Manuel Carmona[c], Israel Thuissard-Vasallo[b], Guillermo Schmeda-Hirschmann[a,*], Mar Larrosa[b,*]

[a] Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca 3460000, Chile
[b] Nutrition, Microbiota and Health Group, Faculty of Biomedical Sciences, Universidad Europea de Madrid, Villaviciosa de Odón, Madrid 28670, Spain
[c] Food Technology Lab, School of Architecture, Engineering and Design, Universidad Europea de Madrid, Villaviciosa de Odón, Madrid 28670, Spain

ARTICLE INFO

Keywords:
Bifidobacterium spp.
Faecalibacterium prausnitzii
Akkermansia muciniphila
Lactobacillus spp.
Clostridium cluster XIVa
Ribes spp.
In vitro fermentation

ABSTRACT

Chilean currants (*Ribes magellanicum* and *Ribes punctatum*) are wild polyphenol-rich berries with interesting bioactivities in several *in vitro* models. The aim of this study was to investigate the effects of the pre-digested PEE (polyphenol-enriched extract) in a simulated colon model. Fruits were extracted, submitted to simulated gastrointestinal digestion and further colonic fermentation with feces from healthy human donors. Samples were taken at 1, 4, 8 and 24 h of incubation, monitoring pH, ammonia, branched-chain fatty acids (BCFA), short-chain fatty acids (SCFA) and bacterial growth. FOS (fructooligosaccharides) and fecal slurry without treatments were positive and negative control, respectively. Both *Ribes* species reduced ($p < 0.05$) both BCFA and SCFA at 24 h. *R. punctatum* promoted the growth ($p < 0.05$) of beneficial bacteria such as *Clostridium* cluster XIVa, and *Faecalibacterium prausnitzii*; while a trend to increase *Akkermansia muciniphila* was observed. *R. magellanicum* increased ($p < 0.05$) *Clostridium* cluster XIVa population. *Escherichia coli*, *Lactobacillus* spp. and *Bifidobacterium* spp. remained unaffected. Our results suggest that polyphenols from *R. punctatum* and *R. magellanicum* may modulate both bacterial metabolism and some selected gut beneficial bacteria under simulated conditions. Therefore, Chilean currants might be useful as supplements to maintain a healthy colon; however, further *in vivo* studies are needed to confirm their effect and their mechanisms.

1. Introduction

The human gut microbiota is a group of commensal microorganisms, comprising bacteria, fungi, viruses, and protozoa that inhabit the intestinal tract. It contributes to maintain normal physiology through defense against pathogens, synthesis of vitamins, amino acids, and metabolites (Singh et al., 2017). Diet influences gut microbiota (De Filippo et al., 2010). A high intake of digestible saccharides, animal-derived fats and proteins, together with a low intake of diet fiber (so-called "Western diet") decrease the presence of health-promoting species in gut microbiota such as: *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Lactobacillus spp.*, *Bifidobacterium spp.*, *Clostridium* cluster XIVa; as well as bacterial diversity (Healey, Murphy, Brough, Butts, & Coad, 2017; Tuohy, Conterno, Gasperotti, & Viola, 2012). Western diet also decreases the generation of beneficial metabolites, for instance short-chain fatty acids (SCFA) (Tan et al., 2014; Verbeke et al., 2015), increase toxic fermentation products and produces a gut microbiota enrichment of potentially detrimental bacteria (Blachier et al., 2017; Windey, De Preter, & Verbeke, 2011). The tight diet-microbiota relation and its implication in health, encourage the investigation of bioactive components in food as a novel therapeutic approach.

Polyphenols are naturally occurring compounds with a wide range of health-promoting properties. These compounds may modulate the bacterial communities in the gut (Duda-Chodak, Tarko, Satora, & Sroka, 2015; Tuohy et al., 2012). Individual polyphenols such as 3-caffeoylquinic acid (3-CQA), 5-caffeoylquinic acid (5-CQA), quercetin-rutinoside and genistin display bifidogenic effects (Mayta-Apaza et al., 2018). Anthocyanins and flavan-3-ol monomers promote *Bifidobacterium* and

Abbreviations: BCFA, branched-chain fatty acids; 3-CQA, 3-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid; FOS, fructooligosaccharides; GID, gastrointestinal digestion; HCA, hydroxycinnamic acids; IBD, inflammatory bowel diseases; ID-PEE, intestinal digested polyphenol-enriched extract; PEE, polyphenol-enriched extract; SCFA, short-chain fatty acids

* Corresponding authors.
E-mail addresses: schmeda@utalca.cl (G. Schmeda-Hirschmann), mar.larrosa@universidadeuropea.es (M. Larrosa).

https://doi.org/10.1016/j.foodres.2019.108848
Received 28 May 2019; Received in revised form 18 October 2019; Accepted 19 November 2019
Available online 03 December 2019
0963-9969/

Lactobacillus abundance (Hidalgo et al., 2012; Tzounis et al., 2008); while flavan-3-ol and 5-CQA increase the butyrate-producer group *Clostridium coccoides–Eubacterium rectale* (Mills et al., 2015; Tzounis et al., 2008). As expected, polyphenol-rich extracts seem to modulate the microbiota. The extent of the effect and the strains involved depends on the polyphenolic profile of the source. Among berries, blueberry (Vendrame et al., 2011) and blackcurrants (Molan, Liu, & Plimmer, 2014) promote the growth of *Lactobacillus* and *Bifidobacterium* species in healthy humans. The last one also inhibits potential *Clostridium pathogenic* species. Cranberry supplementation to obese mice led to higher abundance of *A. muciniphila* population, which is associated with beneficial metabolic effects (Anhê et al., 2014). In addition, polyphenols are also capable of influencing microbiota metabolism by inhibiting microbial activities, causing changes in microbiota metabolites like short chain fatty acids (SCFA), branched chain fatty acids (BCFA), ammonia, hydrogen sulfide, etc) (Bazzocco, Mattila, Guyot, Renard, & Aura, 2008).

The Chilean currants *Ribes magellanicum* and *Ribes punctatum* are a rich source of hydroxycinnamic acids (HCA), flavonols, dihydroflavonols, and anthocyanins (Jiménez-Aspee et al., 2016). Therefore, these berries may be a good candidate for prebiotic supplementation.

In addition, these fruits exhibit interesting bioactivities. Their polyphenolic-enriched extracts (PEEs) show antioxidant activity through several *in vitro* methods, maintaining or increasing them throughout gastrointestinal digestion (Burgos-Edwards, Jiménez-Aspee, Thomas-Valdés, Schmeda-Hirschmann, & Theoduloz, 2017) or under simulated colonic conditions (Burgos-Edwards, Jiménez-Aspee, Theoduloz, & Schmeda-Hirschmann, 2018), respectively. The PEEs are cytoprotective against stress induced by oxygen peroxide and methylglyoxal (2015; Jiménez-Aspee et al., 2016), by increasing the detoxifying enzymes such as catalase, superoxide dismutase, glutathione peroxidase and reductase (Theoduloz, Burgos-Edwards, Schmeda-Hirschmann, & Jiménez-Aspee, 2018).

The polyphenol profile undergoes considerable modifications during the gastrointestinal tract (Burgos-Edwards et al., 2017). Therefore, it's recommended to take into account its influence. In this sense, the *in vitro* digestion model is a valid tool, allowing the consideration of such impact on polyphenols before the assessment of their bioactivity (Brown et al., 2014).

To date, the effect of *in vitro* colonic fermentation on the polyphenols from Chilean currants and its impact on its bioactivity have been described (Burgos-Edwards et al., 2018). However, no information could be found about the influence of their polyphenols on gut bacteria. Therefore, this study aimed to assess the potential impact of gastrointestinal digested PEEs from *R. magellanicum* and *R. punctatum*, on the growth of bacterial groups as well as the pH, ammonia content SCFA and BCFA formation, by means of batch-culture fermentation system with feces from healthy human donors.

2. Materials and methods

2.1. Chemicals and standards

Methanol, sodium bicarbonate, calcium chloride dihydrate, magnesium sulphate heptahydrate and formic acid were purchased from Merck (Darmstadt, Germany). Ethyl acetate HPLC grade and acetic acid were from VWR chemicals (Fontenay-sous-Bois, France). Sodium chloride, potassium phosphate monobasic, potassium phosphate dibasic, sodium hydroxide, Tween 80 and yeast extract were purchased from Scharlau (Barcelona, Spain). Sodium sulfate anhydrous was purchased from Labkem (Barcelona, Spain). Amberlite XAD-7 HP resin, porcine pancreatin, lipase from porcine pancreas, porcine bile extract, amyloglucosidase from *Aspergillus niger*, α-amylase from porcine pancreas, α-glucosidase from *Saccharomyces cerevisiae*, hemin, L-cysteine hydrochloride hydrate, vitamin K1, resazurin sodium salt, bile salts for medium, fructooligosaccharide (FOS), Trizma maleate buffer, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, 2-ethylbutiric acid, and 4-methylvaleric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Pepsin A was purchased from Biomol GmbH (Hamburg, Germany). Ultrapure water was obtained from a Barnsted EasyPure water system (Thermo Scientific, Marietta, OH, USA). BD BBL™ Trypticase™ Peptone was purchased from BD Biosciences (Sparks, MD, USA). GENbox anaerobic sachets were purchased from BioMérieux (Marcy L'Etoile, France).

2.2. Sample collection and processing

Ripe fruits of *Ribes* spp. were collected during the summer months (January - February) in 2015 and 2016. *Ribes magellanicum* Poir. was collected from Laguna Verde, Parque Nacional Conguillío, Región de la Araucanía, Chile. *Ribes punctatum* Ruiz & Pav. was collected from Las Trancas, Región del Ñuble, Chile. The phenolic-enriched extracts (PEEs) were prepared following the protocol described by Jiménez-Aspee et al. (2015). Briefly, the fruits were washed, homogenized with a blender (Thomas TH-501V, Thomas Elektrogeräte, Germany) and lyophilized. Homogenates were extracted with MeOH/formic acid (99:1, v/v) under sonication (15 min), then filtered and concentrated. Acidified methanol was employed as a solvent system due to the higher stability of anthocyanins at low pH; minimizing the possible losses occasioned by exposure to light and temperature during the extraction procedure (Naczk & Shahidi, 2004). Each extract was dissolved in water, filtered and mixed with Amberlite XAD-7-HP® resin, under constant stirring for 40 min. Then, it was washed with water, re-extracted with MeOH/formic acid (99:1, v/v), concentrated, and subsequently freeze-dried to obtain the phenolic-enriched extracts (PEEs).

2.3. In vitro gastrointestinal digestion (GID)

The PEEs were subjected to a two-step *in vitro* GID procedure as previously described (Burgos-Edwards et al., 2017). Briefly, each PEE (250 mg) was incubated with 14.4 mL of simulated gastric fluid (SGF; pH 1.2) for 30 min, in the dark under constant shaking (180 rpm, 37 °C). The pH was adjusted (4.5 ± 0.2) and 75 μL of amyloglucosidase (120 mg/mL) was added, with 30 min of incubation. After pH adjustment (6.9 ± 0.2), 830 μL of α-amylase (120 mg/mL) was added, incubating for 45 min. Intestinal phase started with the addition of 14.4 mL of simulated intestinal fluid (SIF; pH 7.5) to the previous mixture, incubating 30 min. Finally, 1.8 mL of lipase (0.63% w/v) and bile extract (1.60% w/v) solution was added and further incubated (30 min) to obtain the GID samples. Samples were centrifuged (10 min, 3000 rpm, 4 °C) and supernatants were frozen at −80 °C until analyses. Polyphenols recovery was performed with cartridges HF Bond Elut C18 (Agilent Technologies, Santa Clara, CA, USA), following a previous described procedure (Burgos-Edwards et al., 2017); yielding the intestinal digested polyphenol-enriched extracts (ID-PEE).

2.4. Pre-reduced sterile bacterial growth medium

The fermentation medium containing inorganic salts and peptone and yeast extract as protein sources was prepared according to Tzounis et al. (2008). The pH of the medium was adjusted to 7.0 ± 0.1 and it was then sterilized at 121 °C for 15 min, at 1 atm. Finally, the oxygen was displaced from the medium by overnight incubation (16–18 h) within an anaerobic chamber (Baker Ruskinn Concept 4000, Bridgend, UK), full-filled with a gaseous mixture of N_2 and H_2 (90:10). Similarly, the PBS (0.1 M, pH 7.2) for fecal slurry preparations was supplemented with resazurin (1 mg/L) and L-cysteine (0.5 g/L) and was incubated overnight to ensure pre-reduced environment.

2.5. In vitro batch-culture fermentation of GID-extracts

Fresh fecal materials were obtained from three healthy male donors

(30–39 years old). The number of subjects was chosen based on previous studies similar to ours (Hidalgo et al., 2012; Mills et al., 2015; Rodríguez-Costa et al., 2018; Sáyago-Ayerdi, Zamora-Gasga, & Venema, 2019). The procedure was approved by the Ethics Committee on Drug Research of Comunidad de Madrid (Reference number 07/694487.9/17). The volunteers declared the absence of intestinal diseases or antibiotics treatment for the previous 6 months. Samples were collected in sterile containers and transported to the lab in anaerobic jars provided with gas generation sachets. The fecal material was processed within 2 h from the collection. Fecal suspensions (1:10, w/v) were prepared separately, by mixing 5 g of feces with 50 mL of pre-reduced PBS. The suspensions were homogenized manually within stomacher® bags (Seward Limited, West Sussex, UK) for 3 min and then filtered, resulting in fecal slurries from each donor. The ID-PEEs were separately dissolved in pre-reduced PBS and sterilized through 0.22 μm syringe filters. FOS was dissolved in pre-reduced PBS and sterilized by autoclave.

The fermentation experiments were conducted in sterile Falcon tubes containing the ID-PEE, the pre-reduced medium (pH 7.0) and the fecal slurry. The ID-PEE of each berry was incubated at 40, 80 and 160 μg/mL. Doses were chosen assuming 60–240 mg of polyphenols arriving within chyme at the colon, in a volume of 1.5 L, according to Bazzocco et al. (2008). These concentrations are easily reached *in vivo* at the terminal ileum (Brown et al., 2015). FOS (1%, w/v) and fecal slurry without sample were employed as a positive and negative control, respectively. The mixtures were incubated at 37 °C under anaerobic conditions and aliquots of 3 mL were collected at 1, 4, 8 and 24 h for analyses. Each aliquot was further centrifuged (15 min, 5000 rpm, 4 °C), taking the supernatant for pH, ammonium and SCFA determination while the pellet was used for bacterial DNA extractions. Supernatants as well as pellets were stored at −80 °C until analysis. Three independent experiments were conducted and incubations for each donor were carried out in duplicate.

2.6. Determination of pH, and ammonia content

The pH of the obtained supernatants at each time-point was measured using a basic 20+ Crison pH meter (Hach Lange, Barcelona, Spain) following the methodology described by Dai & Karring (2014). The ammonia content was determined employing a high-performance ammonia selective ion electrode (Orion™, ThermoFisher Scientific, Waltham, MA, USA), as previously described (Moreno-Pérez et al., 2018). Briefly, 300 μL of each supernatant was diluted in MilliQ water (1:10, v/v), alkalized with 30 μL of NaOH (1 M) and immediately measured. The ammonia content was calculated by means of a standard curve, built with serial dilutions of ammonium chloride (0.1 M) following the electrode manufacturer's instructions. The results were expressed as parts per million (ppm).

2.7. Short-chain fatty acids and branched-chain fatty acids

SCFA and BCFA were extracted following the procedure described by García-Villalba et al. (2012), with some modifications. First, an aliquot of each supernatant was mixed with an equal volume of phosphoric acid (1%, v/v). Second, 300 μL of the sample was spiked with 20 μL of 4-methylvaleric acid (100 mM) employed as a recovery standard. Third, the mixture was extracted with 300 μL of ethyl acetate, homogenized with vortex for 1 min and centrifuged (3 min, 10000 rpm), transferring the upper phases to a new Eppendorf tube. This procedure was repeated three times. Finally, anhydrous sodium sulfate was added to remove water traces. The samples were vortexed, centrifuged and transferred to GC-vials for analyses. Analyses were carried out with an Agilent GC System 7820A chromatograph equipped with a DBWax 121-7037LT column and an Agilent Series MSD 5975 detector (Agilent Technologies, Inc. Santa Clara, CA, USA). Injection volume was 1 μL of sample or standard for each analysis. The data was acquired by selective ion monitoring (SIM). The target and qualifier ions are detailed in Table 1. SCFA and BCFA quantification was performed by means of an eight-point external calibration curve (0.015 to 2 mM) with reference standards. 2-ethylbutiric acid was used as the internal standard.

Table 1
Mass of the target and qualifier ions employed for the identification and quantification of short-chain fatty acids (SCFA).

SCFA	Target ion (*m/z*)	Qualifier ions (*m/z*)
Acetic acid	43	45; 60
Propionic acid	74	73; 57
Butyric acid	73	60
Isobutyric acid	73	88
Valeric acid	73	60
Isovaleric acid	87	60
Caproic acid	87	60; 73
2-Ethylbutiric acid	88	73; 87
4-Methylvaleric acid	73	74; 60

2.8. Bacterial DNA extraction

The bacterial DNA was extracted from the pellets obtained after centrifugation of 3 mL of each fermented sample. The E.Z.N.A.® Stool DNA Kit (Omega Biotek, Norcross, GA, USA) and a bead-beating homogenizer (Bullet Blender Storm, Next Advance, Troy, NY, USA) were employed for the extractions. The concentration and purity of bacterial DNA were determined with a NanoDrop equipment (ThermoFisher Scientific, Waltham, MA, USA).

2.9. Quantitative PCR analysis

Quantitative PCR (qPCR) analysis was carried out for monitoring the abundance of the following bacteria: *Clostridium cluster* XIVa (Matsuki et al., 2002), *Bifidobacterium* spp. (Delroisse et al., 2008), *Lactobacillus spp*. (Vigsnæs, Holck, Meyer, & Licht, 2011), *F. prausnitzii* (Son et al., 2015), *A. muciniphila* (Collado, Derrien, Isolauri, de Vos, & Salminen, 2007), *E. coli* and total bacteria (Ott, Musfeldt, Ullmann, Hampe, & Schreiber, 2004). Primers sequences, concentrations and annealing temperature are detailed in Table 2.

A CFX Connect™ Real-Time PCR Detection System (BioRad, Barcelona, Spain) was employed for bacterial quantification, using SYBR Green I chemistry (BioRad, Barcelona, Spain) as a fluorescence probe. The analyses were performed in 20 μL reactions including one microliter (10 ng) of DNA template and 200–500 nM of primers. Cycling parameters were as follows: 95 °C during 10 min, then 40 cycles at 95 °C for 15 s, 1 min at the established annealing temperature and 72 °C for 45 s. Afterwards, melting curve analysis were performed, measuring fluorescence while the temperature increased from 50 °C to 95 °C. Bacterial populations were quantified through standard curves built with serial dilutions of DNA from a known number of bacteria, corresponding to each species cultivated under anaerobic conditions. Absolute quantification of each bacterial taxa was performed by the standard-curve (SC) method using the formula DNA (copy) = 6.02×10^{23}(copy/mol) × DNA amount (g)/ DNA length (dp) × 660 (g/mol/dp). The genome sizes used were 5.44, 3.09, 2.70, 2.43, 5.13, 1.99, and 5.44 Mbp (base pairs) for *E. coli*, *F. prausnitzii, A. muciniphila, Bifidobacterium* spp., *Clostridium* spp., and *Lactobacillus* spp., and total bacteria, respectively. Results are represented as Log_{10} of absolute bacterial number.

2.10. Statistical analysis

The statistical analyses were performed with the Statistical Package for Social Sciences for Windows software, version 22.0 (SPSS Inc., Chicago, IL, USA). Significant differences among non-treated control

Table 2
Sequence, annealing temperature, and concentration of primers.

Target	Primers sequence		Annealing temperature (°C)	Concentration (nM)	Reference
	Reverse	Forward			
Clostridium cluster XIVa	5′-AGT TTY ATT CTT GCG AAC G-3′	5′-CGG TAC CTG ACT AAG AAG C-3′	61	500	Matsuki et al. (2002)
Bifidobacterium spp.	5′-CCC CAC ATC CAG CAT CCA-3′	5′-CGC GTC YGG TGT GAA A-3′	58	300	Delroisse et al. (2008)
Lactobacillus spp.	5′-CAC CGC TAC ACA TGG AG-3′	5′-AGC AGT AGG GAA TCT TCC A-3′	61	200	Vigsnæs et al. (2011)
Faecalibacterium prausnitzii	5′-GTC GCA GGA TGT CAA GAC-3′	5′-CCC TTC AGT GCC GCA GT-3′	48	500	Son et al. (2015)
Akkermansia muciniphila	5′-CCT TGC GGT TGG CTT CAG AT-3′	5′-CAG CAC GTG AAG GTG GGG AC-3′	49	500	Collado et al. (2007)
Escherichia coli	5′-CTT TGG TCT TGC GAC GTT AT-3′	5′-AGA AGC TTG CTC TTT GCT GA-3′	55	250	Bressa et al. (2017)
Total bacteria 27F/518R	5′-ATT ACC GCG GCT GCT GG-3′	5′-GAG TTT GAT CMT GGC TCA G-3′	61	200	Ott et al. (2004)

and treatments were assessed employing the Student's *t* test. Statistical significance was established at $p \leq 0.05$. Correlation analyses were performed using the Pearson correlation coefficient. The Benjamini-Hochberg false discovery rate was used as a p-value adjustment method to minimize type I error. Data were expressed as the mean ± SEM. Graphics were constructed with GraphPad Prism® version 7.0 (San Diego, CA).

3. Results and discussion

The polyphenolic-enriched extracts (PEEs) from two Chilean currants (*R. magellanicum* and *R. punctatum*) were assessed for prebiotic activity through the measurement of the pH, ammonia, branched chain fatty acids (BCFA), short chain fatty acids (SCFA), and six colonic bacterial taxa during a simulated colonic fermentation for 24 h.

3.1. pH and ammonia variations throughout in vitro fermentation.

Fecal pH and ammonia content are parameters associated with colon health. Both are considered indirect indicators of bacterial fermentation and metabolites production (Yao, Muir, & Gibson, 2015). Therefore, we monitored both parameters at four time-points (1, 4, 8, and 24 h) along the *in vitro* fermentation experiments. FOS treatments significantly decreased ($p < 0.05$) the pH of the medium at 4, 8 and 24 h after exposure respect to the non-treated control (Fig. 1). Other authors reported similar results (Cantu-Jungles et al., 2018) in which the pH drop was attributed to the SCFA generated from FOS fermentation, leading to a more acidic environment. Although a decrease in the pH is desirable from a health point of view, the pH of the samples treated with Chilean currants ID-PEEs and the non-treated control showed no significant differences (Fig. 1). In the same way, fecal pH remained unaffected after cranberry supplementation to healthy adults (Rodríguez-Morató, Matthan, Liu, de la Torre, & Chen, 2018). However, a reduced fecal pH was observed after dietary intervention with black currant (*Ribes nigrum*) commercial extracts in healthy subjects (Molan et al., 2014). These results indicate that changes in fecal pH after consumption of berries are probably due to their insoluble fiber content rather than their polyphenols (Jakobsdottir, Nilsson, Blanco, Sterner, & Nyman, 2014).

Regarding the ammonia content, samples with the ID-PEEs and the non-treated control showed no significant differences (Fig. 1). Other polyphenol sources influenced this parameter with divergent results. The increase in ammonia produced by a high-protein diet was not counteracted by the administration of proanthocyanidin-rich polyphenol extract from avocado peel (Cires et al., 2019). Ammonia production remained unchanged after 24 h of fermentation with mango peel extracts in a dynamic *in vitro* model of the colon, increasing it at longer times than in our study (48 and 72 h) (Sáyago-Ayerdi et al., 2019). Cranberry and grape seed polyphenols also increased ammonium production in a dynamic gastrointestinal simulator, the so-called SHIME (Sánchez-Patán et al., 2015). On the other hand, orange juice (Duque, Monteiro, Adorno, Sakamoto, & Sivieri, 2016), black tea and red wine grape extracts (Kemperman et al., 2013) reduced its formation, also in a SHIME model. FOS treatment attenuated significantly ($p < 0.05$) the ammonia increment among 4 and 24 h of incubation (Fig. 1). In our study, FOS as non-digestible carbohydrate may act as carbon source, delaying ammonia accumulation from protein fermentation (Verbeke et al., 2015; Windey et al., 2011). The catabolism of proteins by bacteria leads to potentially harmful metabolites, including ammonium, amines, cresols, indoles, and phenols (Blachier et al., 2017; Yao et al., 2015). In fact, the excessive protein fermentation is associated with the pathogenesis of IBD and colorectal carcinogenesis (Windey et al., 2011). Therefore, the modulation of protein fermentation through dietary supplementation might be useful to prevent the excessive accumulation of its metabolites.*3.2. Branched-chain fatty acids (BCFA) production*

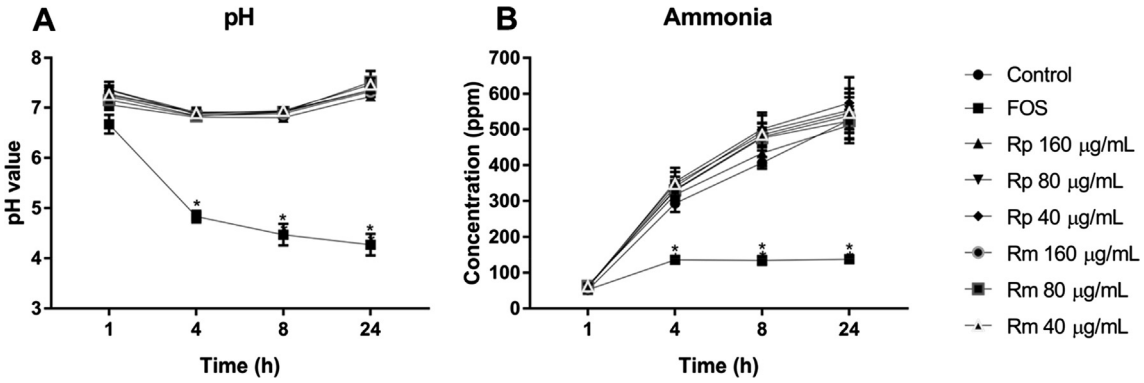

Fig. 1. pH and ammonia variations during *in vitro* colonic fermentation of *Ribes punctatum* (Rp) and *R. magellanicum* (Rm) at 40, 80 and 160 μg/mL. The results are expressed as mean ± SEM (n = 3). The symbol (*) point out significant differences ($p < 0.05$) among control and samples, by the Student's *t*-test.

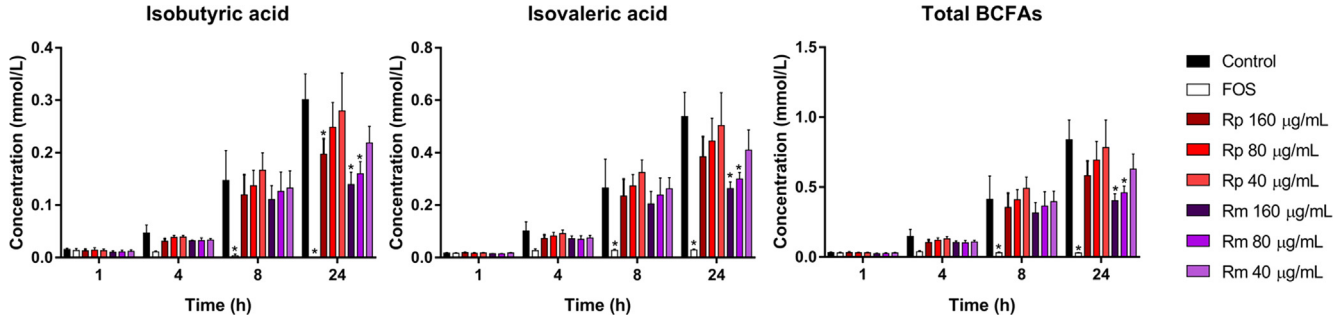

Fig. 2. Branched-chain fatty acids (BCFA) formation during simulated colonic fermentation with the intestinal digested polyphenols from *R. punctatum* (Rp) and *R. magellanicum* (Rm) at 40, 80 and 160 μg/mL. The results are shown as mean ± SEM (n = 3). Significant differences ($p < 0.05$) in Student's *t*-test between treatments and control are indicated (*) in the graphics.

BCFA are considered specific markers of protein fermentation as they are produced exclusively from amino acids (Windey et al., 2011; Yao et al., 2015). Therefore, we monitored variations in isobutyric and isovaleric acids and the total sum (Total BCFA) throughout the fermentation experiment (Fig. 2). FOS decreased total BCFA by 75–96 % compared to the negative control. These reductions were significant ($p < 0.05$) after 8 and 24 h; supporting the potential displacement of amino acids, as a carbon source by FOS, as we observed for ammonia (Verbeke et al., 2015; Windey et al., 2011). Among currants, *R. magellanicum* dose-dependently decreased total BCFA ($p < 0.05$) at 24 h respect to the non-treated control (Fig. 2); with reductions by 45% and 52% at 80 and 160 μg/mL, respectively. *R. punctatum* treatments showed the same trend in total BCFA, but only the diminution by 37% of isobutyric acid was significant ($p < 0.05$), after 24 h of incubation at 160 μg/mL. Extracts-induced changes in bacterial BCFA production have been previously informed. Predigested mango peel extract reduced isobutyric acid after 24 h of incubation within an *in vitro* model, in agreement with our results (Sáyago-Ayerdi et al., 2019). Cranberry and grape seed polyphenolic extracts did not elicit any changes in BCFA within SHIME (Sánchez-Patán et al., 2015). Additionally, in a study with rats fed with fiber enriched with polyphenol-rich extracts, it was informed that neither strawberry, black currant or chokeberry supplementations were able to alter isobutyric and isovaleric acids in caecum (Kosmala, Zduńczyk, Karlińska, & Juśkiewicz, 2014). Similar results were found with proanthocyanidin-rich polyphenol extract from avocado peel that did not modify BCFA in rats fed with a high-protein diet (Cires et al., 2019).

Although BCFA and ammonia are produced by protein fermentation (Yao et al., 2015), only the first ones were reduced by ID-PEEs under our experimental conditions. These data suggest a potential influence of Chilean currants polyphenols on protein fermentation by human colonic bacteria. The fact that ammonia production was not altered could be due to the alternative formation of ammonia by urea hydrolysis; meanwhile, the measured BCFA derive exclusively from valine and leucine (Blachier et al., 2017; Windey et al., 2011).

3.2. Short chain fatty acids (SCFA) analyses

SCFA are major end products of gut microbiota fermentation activity that have a positive impact in the host health, serving as energy source for colonocytes, having anti-inflammatory and anticarcinogenic effects and regulating the lipid metabolism (Koh, De Vadder, Kovatcheva-Datchary, & Backhed, 2016). Polyphenols from natural sources may regulate the gut microbiota fermentative process (Tuohy, Conterno, Gasperotti, Viola, & Pvt, 2014). We assessed the potential impact of *R. punctatum* and *R. magellanicum* polyphenols on SCFA production, monitoring SCFA for 24 h at four time-points (Fig. 3). In fecal samples, acetic, propionic and butyric acids account for 95% of the measured SCFAs, in a proportion of approximately 60:20:20, respectively (Verbeke et al., 2015). Under our experimental conditions, the main SCFA reached 94%, in a molar ratio of approximately 57:19:18 (acetic, propionic and butyric acid) in the control, in line with the previously observed.

Among treatments, FOS increased significantly ($p < 0.05$) the acetic acid content between 4 and 8 h relative to the control (Fig. 3). Propionic acid remained unaffected for eight hours, decreasing ($p < 0.05$) after 24 h, whereas butyric acid was higher ($p < 0.05$) in FOS samples at all time-points. However, valeric acid decreased ($p < 0.05$) in FOS treatments between 4 and 24 h. Although FOS increased ($p < 0.05$) caproic acid during the first hour, it was reduced ($p < 0.05$) after 8 and 24 h. The total SCFA significantly increased ($p < 0.05$) with FOS. These results are in agreement with the reported beneficial effects for FOS, yielding acetate and butyrate predominantly over other SCFA (Cantu-Jungles et al., 2018). The ID-PEEs did not produce significant changes in total SCFA. However, the extracts decreased individual SCFA (except for acetic acid) mostly at the final

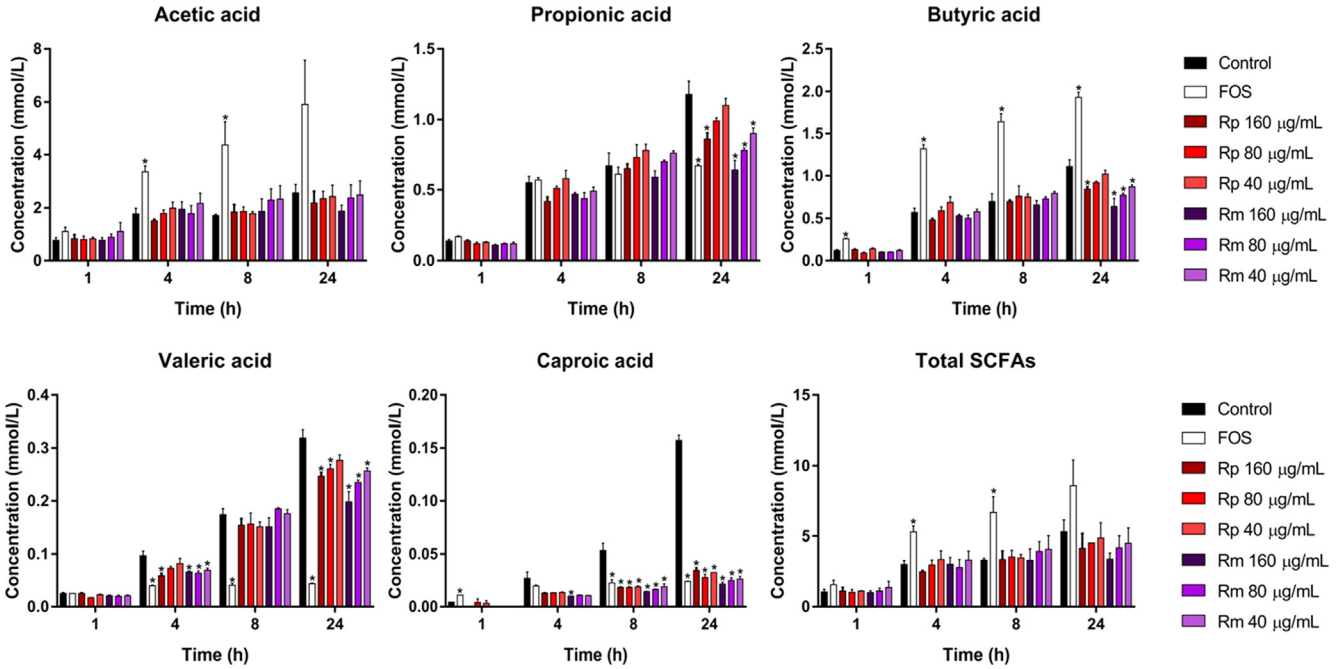

Fig. 3. Production of short chain fatty acids (SCFA), after simulated colonic fermentation with the intestinal digested polyphenols from *R. punctatum* (Rp) and *R. magellanicum* (Rm) at 40, 80 and 160 µg/mL. Results are depicted as mean ± SEM (n = 3). Significant differences ($p < 0.05$) in Student's *t*-test between treatments and control are indicated (*) in the graphics.

stage of the incubation. ID-PEEs did not induce significant changes in acetic acid content compared with the control (Fig. 3). Propionic and butyric acid decreased ($p < 0.05$) after ID-PEE treatments at 24 h. Reductions account for 22% in both acids with *R. punctatum* (160 µg/mL); while *R. magellanicum* (160 and 80 µg/mL) reduced propionic acid by 34–46 % and butyric acid by 22–42 %. The valeric acid content decreased ($p < 0.05$) by 40% and 19–25% at 4 and 24 h, respectively, with *R. punctatum*. All treatments with *R. magellanicum* affected the valeric acid content, decreasing it by 40% and 19–38% after 4 and 24 h of incubation, respectively. Caproic acid was also significantly affected ($p < 0.05$) by ID-PEEs, decreasing by 67% after eight hours. Past 24 h, *R. punctatum* inhibited its production ($p < 0.05$) by 75–88 % and *R. magellanicum* by 88%.

Similar effects were reported for red wine grape extract, which reduced butyric acid (Kemperman et al., 2013); pre-digested mango peel, decreasing caproic and butyric acids (Sáyago-Ayerdi et al., 2019); and apple extracts, reducing the sum of acetic, propionic and butyric acids (Bazzocco et al., 2008), employing *in vitro* fermentation methods. On the other hand, cranberry and grape seed polyphenols did not influence SCFA in SHIME or mice (Sánchez-Patán et al., 2015; Singh et al., 2018); while black tea raised acetic acid production (Kemperman et al., 2013) and orange juices increased acetic, propionic and butyric acids (Duque et al., 2016). Kosmala et al. (2014) measured SCFA production in rat caecum after the administration of fibers/polyphenol-rich extracts combinations. They reported a decrease in samples with proanthocyanidin-rich extracts, including black currant and chokeberry. In addition, isolated proanthocyanidins reduced SCFA under *in vitro* conditions (Bazzocco et al., 2008). We described the polyphenolic profile of *R. punctatum* and *R. magellanicum* ID-PEEs in a previous work, reporting proanthocyanidin monomers and oligomers in both extracts (Burgos-Edwards et al., 2017). Proanthocyanidins might contribute to the observed decrease in SCFAs, probably through the inhibition of saccharolytic enzymes from microorganisms (Scalbert, 1991), the inhibition of polysaccharides degradation by microbial cell-wall degrading enzymes (Bazzocco et al., 2008), or direct inhibition of bacteria using heme through their iron chelation activity (Marín, Miguélez, Villar, & Lombó, 2015). The breakdown of polysaccharides by these enzymes is a necessary step in the synthesis of SCFAs (Bazzocco et al., 2008).

SCFA may act as an energy source, anti-inflammatory, anti-tumorigenic, gut integrity promoters, and antimicrobial against pathogens (Tan et al., 2014; Verbeke et al., 2015; Windey et al., 2011). Thus, high SCFA generation may be involved in the beneficial effects of prebiotics (Tan et al., 2014). On the other hand, Tuohy et al. (2012) suggested that the *in vitro* inhibition of SCFA by polyphenols might be beneficial under *in vivo* conditions. Such inhibition may prolong SCFA production until the distal colon, preventing the increase of potentially harmful proteolysis-derived metabolites.

3.3. Changes in bacterial populations by Chilean currants digested extracts.

The influence of *Ribes* ID-PEEs on health-related bacteria was investigated by qPCR analyses, addressing total bacteria, three phylogenetic groups, and three specific bacteria. The *Lactobacillus* and *Bifidobacterium* genera were selected because they are well-known health-promoting bacteria, and *Clostridium* cluster XIVa as it is a predominant group in intestinal microbiota, which is involved in the global maintenance of gut function and is a producer of SCFA (Lopetuso, Scaldaferri, Petito, & Gasbarrini, 2013). As bacteria health-promoting species *A. muciniphila* was selected for its important role in the prevention of obesity and diabetes (Dao et al., 2016) and *F. prausnitzii* for its anti-inflammatory properties (Sokol et al., 2008). In contrast, *E. coli* was selected for its potential pathogenic activity (Mirsepasi-Lauridsen, Vallance, Krogfelt, & Petersen, 2019). Changes in bacterial abundance elicited by *R. punctatum* and *R. magellanicum* are shown in Figs. 4 and 5, respectively. Low counts of *C.* cluster XIVa bacteria have been associated with a higher risk of IBD and colorectal cancer. Thus, their selective regulation through diet is considered as a novel strategy to achieve immunomodulation, maintaining colonic health (Cantu-Jungles et al., 2018; Healey et al., 2017). The ID-PEE of *R. punctatum* promoted a significant ($p < 0.05$) growth in this bacterial group at 160 µg/mL, reaching 0.2 log units over the control (Fig. 4). The mentioned occurred at 1 h, without significant differences ($p < 0.05$) at other time-points. *R. magellanicum* ID-PEE augmented ($p < 0.05$) *C.* XIVa cluster population by 0.2–0.26 log units during the first hour of

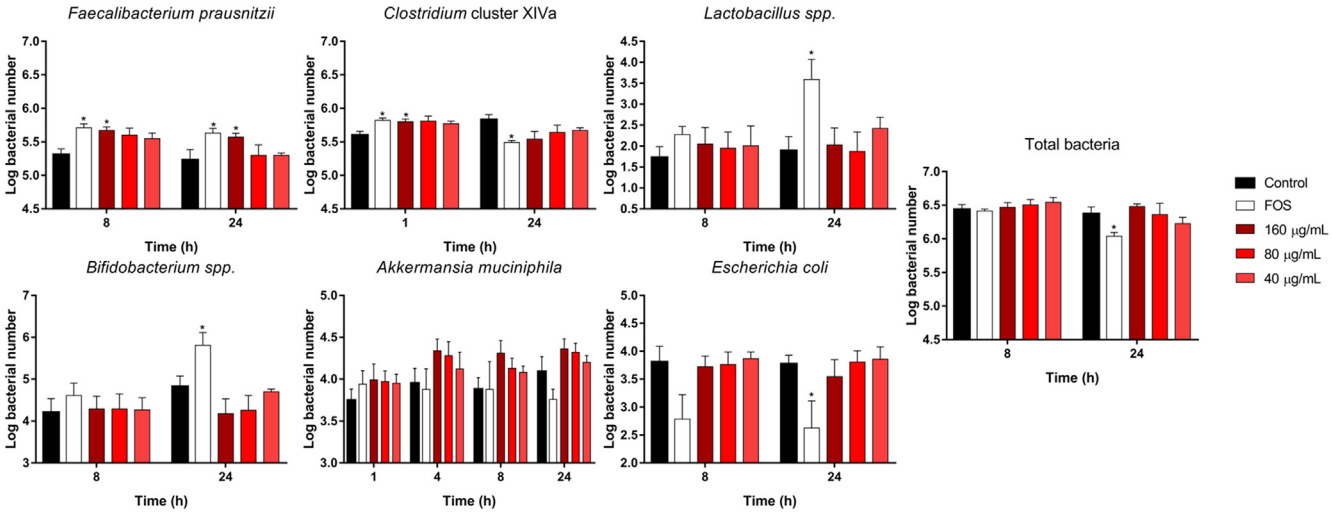

Fig. 4. Influence of polyphenols from *R. punctatum* on the human bacterial composition during the simulated colonic fermentation. Results are expressed as Log_{10} bacterial number ± SEM. Statistical significance (Student's *t*-test, $p < 0.05$) between control and treatments are indicated (*) in the graphics.

incubation (Fig. 5). However, these increments were not maintained. Similarly to Chilean currants, tart cherries polyphenolic-rich extract increased *C.* XIVa cluster growth during *in vitro* fermentation with a SHIME model. The authors attributed the activity to the main components 3-CQA and 5-CQA (Mayta-Apaza et al., 2018). In addition, pure 5-CQA promoted the growth of *Clostridium coccoides-Eubacterium rectale* group (Mills et al., 2015). We detected both mentioned HCA esters in *R. punctatum* and *R. magellanicum* (Burgos-Edwards et al., 2017). Thus, the possible contribution of these HCA to the growth of the *C.* cluster XIVa bacteria should be considered. The duration of the effect is also in agreement with the rapid degradation of these HCA under fermentation conditions (Burgos-Edwards et al., 2018). The flavan-3-ols from both ID-PEE (Burgos-Edwards et al., 2017), might also participate in the growth-promotion of this bacteria, considering that stimulation of *C. coccoides-E. rectale* group by flavan-3-ols was previously observed *in vitro* (Tzounis et al., 2008). FOS showed an initial increment ($p < 0.05$) of 0.22 log units on this bacterial group; no changes were observed at the middle time-points, decreasing ($p < 0.05$) 0.34 log units after 24 h. Other authors have also observed a decrease in the members of this cluster during *in vitro* incubation with FOS, supporting our results (Cantu-Jungles et al., 2018; Mills et al., 2015).

Among other important members of a healthy gut microbial community are *Lactobacillus* and *Bifidobacterium* genera. These bacteria are able to reduce the incidence and severity of intestinal inflammation diseases, to improve gut barrier, to maintain low LPS levels and to prevent colorectal cancer (Duda-Chodak et al., 2015; Healey et al., 2017; Singh et al., 2017). It is relevant to discover growth-promoters of these species as an alternative therapy for the above mentioned properties. FOS significantly increased ($p < 0.05$) *Bifidobacterium* spp. and *Lactobacillus* spp. populations by 0.99 and 1.70 log units, respectively, at 24 h after exposure (Fig. 4). The potential effect of ID-PEE from *R. punctatum* on both bacteria was assessed, without significant changes ($p < 0.05$) during 24 h (Fig. 4). Samples treated with *R. magellanicum* did not show a growth-promoter effect ($p < 0.05$) of the mentioned species either (Fig. 5). In agreement with our results, wine polyphenols extract, rich in quercetin, flavan-3-ols and anthocyanins, did not affect these bacterial groups under *in vitro* conditions (Sánchez-Patán et al., 2012). Likewise, oat bran polyphenols did not show promoting-effects on *Bifidobacterium* group in an *in vitro* fermentation model of the gut microbiota (Kristek et al., 2019). In fecal samples from healthy volunteers, cranberry powder did not alter the abundance of *Bifidobacterium* or *Lactobacillus* species either (Rodríguez-Morató et al., 2018). Moreover, *Aronia melanocarpa* juice decreased the abundance of *Bifidobacterium* species in SHIME (Wu et al., 2018). On the other hand,

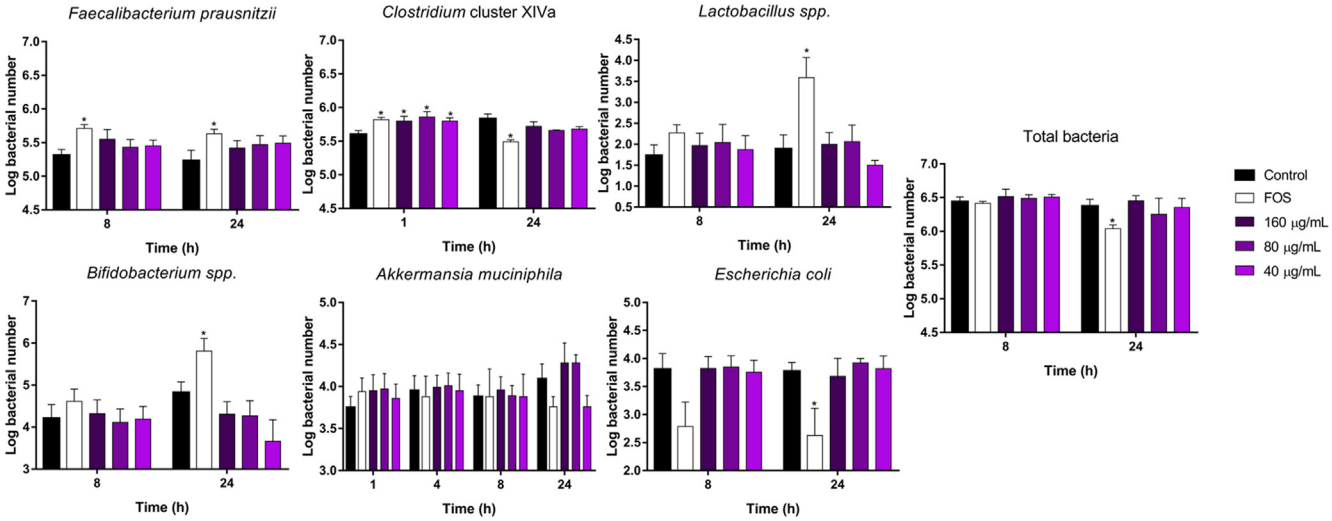

Fig. 5. Changes in abundance of human colonic bacteria elicited by *R. magellanicum* polyphenols along time, during the *in vitro* fermentation. Results are expressed as Log_{10} bacterial number ± SEM. Statistical differences ($p < 0.05$) by Student's *t*-test respect to the control are pointed out (*).

polyphenol rich Sea buckthorn berries juice promoted the growth of *Lactobacillus* and *Bifidobacterium* in a simulated human digestion model (Attri & Goel, 2018), blueberry polyphenol extract increased *Bifidobacterium* in mice after 12 weeks of supplementation (Jiao et al., 2019) and blueberry (*Vaccinium angustifolium*) drink promoted the growth of both mentioned bacteria after six-week consumption in human (Vendrame et al., 2011). These growth-promoter effects were also observed after administration of black currants commercial powders to healthy humans (Molan et al., 2014). Polyphenol-rich *Pandanus tectorius* fruit extract administered to mice for 6 weeks increased *Lactobacillus* population, that correlated with a decrease in serum lipids (Wu et al., 2019). Both *Bifidobacterium* and *Lactobacillus* species are increased by anthocyanins *in vitro* (Hidalgo et al., 2012), supporting their possible involvement in berries prebiotic-like activity. The loss of anthocyanins during simulated GID might explain the absence of growth-promoter effect in ID-PEEs (Burgos-Edwards et al., 2017). Further, polyphenols from *R. punctatum* and *R. magellanicum* are catabolized by the microbiota during *in vitro* fermentation, generating quercetin and luteolin aglycons as metabolites (Burgos-Edwards et al., 2018). It has been reported that flavonoid aglycons, such as quercetin may inhibit the growth of gut bacteria, including *Lactobacillus* and *Bifidobacterium* species (Duda-Chodak et al., 2015). One important consideration is also the employed dose of polyphenols. In our study the concentrations of polyphenols employed are very low (micrograms), trying to simulate the biological conditions, whereas most *in vitro* studies are carried out with doses of milligrams and even grams. Recently, the effect of anthocyanins in microbiota has been reviewed by Igwe, Charlton, Probst, Kent, and Netzel (2019), observing that only a proliferative effect on *Bifidobacterium* was observed in those studies carried out with high doses of anthocyanins (Igwe et al., 2019).

The butyrate-producing bacterium *F. prausnitzii* is considered an important member of the health colonic community (Flint, Scott, Louis, & Duncan, 2012). Reduced abundance was reported in IBD and obese patients (Healey et al., 2017; Singh et al., 2017). We monitored the effect of the ID-PEEs on *F. prausnitzii* abundance during 24 h, contrasting to the non-treated control. FOS and *R. punctatum* did not produce significant variations ($p < 0.05$) in *F. prausnitzii* population during the first four hours of incubation (Fig. 4). After 8 h, *R. punctatum* (160 μg/mL) and FOS increased *F. prausnitzii* population by 0.36 and 0.40 log units, respectively. This growth-promoter effect was maintained ($p < 0.05$) until 24 h, increasing 0.34 log units over control for *R. punctatum* (160 μg/mL) and 0.4 log units for FOS. On the other hand, *R. magellanicum* did not induce significant variations ($p < 0.05$) within 24 h of incubation (Fig. 5). Similar results were informed for high-fat diet fed mice, whose gut microbiota was enriched in *Faecalibacterium* species after the administration of Lingonberry (*Vaccinium vitis-idaea* L.) extract; which was linked to reduced inflammation markers (Heyman-Lindén et al., 2016) (Heyman-Lindén et al., 2016). On the other hand, Mayta-Apaza et al. (2018) informed a decrease in the relative abundance of *Faecalibacterium* spp. after administration of polyphenol-rich tart cherry (*Prunus cerasus*) juice to healthy humans. Our results suggest that *R. punctatum* might promote the growth of the health-promotor bacterium *F. prausnitzii*. This is the first study addressing the effect of polyphenols from *Ribes* species on the mentioned bacteria.

The mucin-degrader bacterium *A. muciniphila* is also considered a member of the healthy microbiota since its low abundance is associated with obesity, psoriatic arthritis, and IBD (Flint et al., 2012; Singh et al., 2017); therefore, it was addressed in this study. FOS did not significantly change ($p < 0.05$) bacterial abundance; in fact, the population decreased after 24 h compared to the control. Neither *R. punctatum* nor *R. magellanicum* produced a significant increase ($p < 0.05$) in the population of *A. muciniphila*, although there was a tendency to increase with *R. punctatum* treatment. Increments showed dose-dependency, and the highest dose increased *A. muciniphila* by 0.2, 0.39, 0.43, and 0.27 log units at 1, 4, 8, and 24 h, respectively (Fig. 4). This trend was not observed for *R. magellanicum* samples. This bacterium increased after treatments with polyphenols from black tea, red wine grape extracts and Aronia juice within SHIME, and also in microbiota of mice supplemented with green tea polyphenols (Kemperman et al., 2013; Ushiroda et al., 2019; Wu et al., 2018). Lingonberries also promoted its growth in high-fat diet mice after 11 weeks of administration (Heyman-Lindén et al., 2016). Cranberry consumption did not affect *A. muciniphila* populations in healthy subjects (Rodríguez-Morató et al., 2018); however, its polyphenol-rich extract administrated to mice not only increased *A. muciniphila* populations, it was associated with improved metabolism of glucose and lipids as well as anti-inflammatory effects (Anhê et al., 2014). We observed a dose-dependent trend to increase *A. muciniphila* abundance after *R. punctatum* treatments. The differences among individuals' initial microbiota might have influenced the global data making it difficult to find significant differences. This effect was observed before by other authors. They proposed to classify volunteers based on basal microbiota to avoid wrong interpretations of results (Healey et al., 2017; Mayta-Apaza et al., 2018).

The last monitored bacterium in this study was *E. coli*, whose proliferation inside the colon is associated with higher risk of IBD, unlike the other assessed bacteria (Duda-Chodak et al., 2015; Singh et al., 2017). FOS decreased significantly ($p < 0.05$) its abundance by 1.15 log units at 24 h (Fig. 4); whereas *R. punctatum* and *R. magellanicum* did not produce significant changes ($p < 0.05$) compared to the control (Figs. 4 and 5). Cranberry and grape seed extracts reduced members of the *Enterobacteriaceae* family within SHIME (Sánchez-Patán et al., 2015). Chilean currants polyphenols did not affect the growth of *E. coli* possibly due to the low content of flavonoid aglycones in the digested extracts (Burgos-Edwards et al., 2017). It has been suggested that flavonoid aglycones such as quercetin may inhibit the growth and motility of intestinal bacteria, including *E. coli*; while their glycosides are less active or devoid of this effect (Duda-Chodak et al., 2015; Marín et al., 2015). We did not observe that effect in Chilean currants, probably due to low proportion of flavonoid aglycones within the extracts (Burgos-Edwards et al., 2017). Anthocyanins can also inhibit the growth of *E. coli* (Puupponen-Pimia et al., 2001), however, anthocyanins initially present in the *Ribes* extracts were extensively degraded after the *in vitro* digestion process (Burgos-Edwards et al., 2017). Additional studies are needed to obtain a clearer picture of the role of polyphenol bacterial metabolites, while the antimicrobial effect of polyphenols is demonstrated (Daglia, 2012) there is a lack of studies addressing the antimicrobial effect of polyphenol metabolites derived from digestion and fermentation processes.

3.4. Correlation analyses.

Correlation analyses were carried out in order to detect associations among bacteria and the measured metabolites. In control samples, a strongly positive association was observed between the presence of ammonia and all the measured SCFA and BCFA (Fig. 6). These results were expected since both SCFA, BCFA and ammonia are end products of protein metabolism, the main food source in control samples (Macfarlane, Gibson, Beatty, & Cummings, 1992). SCFA production was also strongly correlated with BCFA production and no relationship was found between pH levels and ammonia (Fig. 6). On contrary, the correlation between ammonia levels and pH was positive with FOS treatment (Fig. 6), indicating that the decrease in pH was accompanied by a decrease in ammonia production, as shown by other studies carried out with fermentable carbohydrates (Terpend, Possemiers, Daguet, & Marzorati, 2013). The addition of FOS to the fermentation medium resulted in the loss of correlation between ammonia and BCFA and between SCFA and BCFA (Fig. 6), probably by the interference of carbohydrates with the protein metabolism (Macfarlane et al., 1992; Swanson et al., 2002). In contrast, pH correlated with most fatty acids indicating that these fatty acids are a determining factor of fecal pH when FOS are added to the fermentation medium (Fig. 6), whereas in control samples, pH is independent of fatty acids (Fig. 6). Treatments

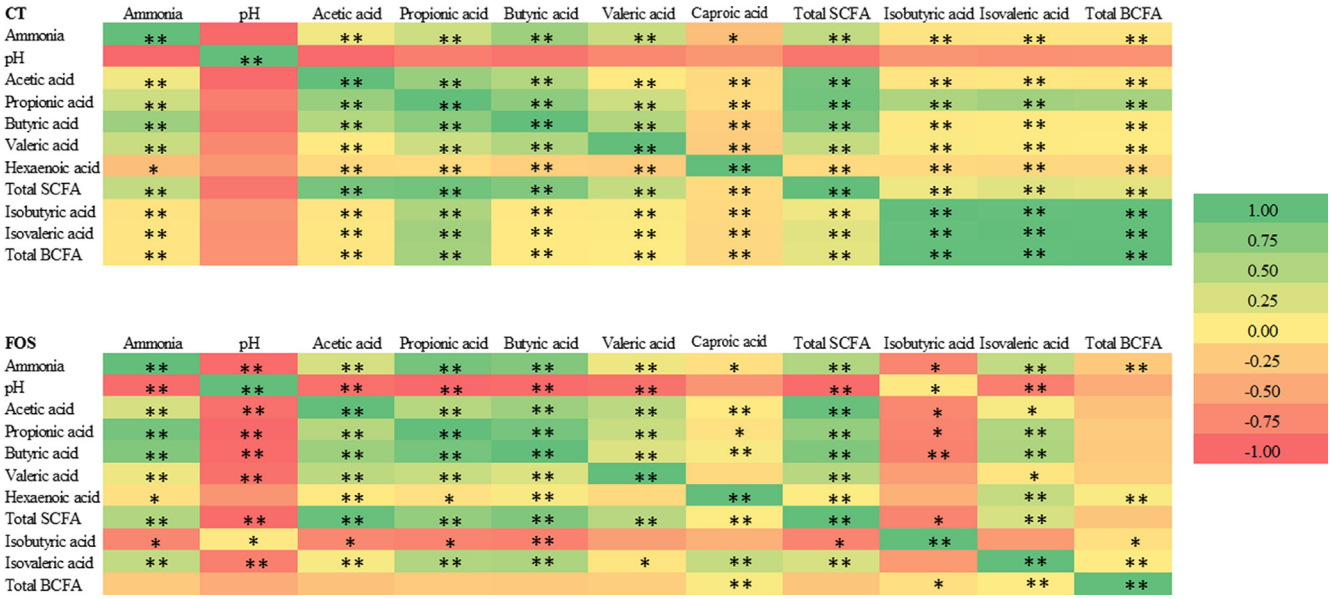

Fig. 6. Correlation heatmap between SCFA, BCFA, ammonia and pH for control and FOS treatment. * ($p < 0.05$) ** ($p < 0.001$).

with both *R. magellanicum* and *R. punctatum* at all concentrations tested showed very similar correlation profiles to the control samples showing a high correlation between ammonia, SCFA and BCFA and not with pH (data not shown).

Correlation analyses between the different bacterial taxa showed bidirectional interactions between them in the presence of different treatments. In control samples it was observed a positive interaction between *C.* cluster XIVa and *E. coli,* between *Lactobacillus* and *Bifidobacterium* and also between *Bifidobacterium* and *E. coli* (Fig. 7). Negative significant correlations where detected between *F. prausnitzii* and *A. muciniphila* and also between *A. muciniphila*, *Lactobacillus* and *Bifidobacterium*. These relationships were modified by treatment with FOS, which made significant the negative relationship between *C.* cluster XIVa and *Lactobacillus* and *Bifidobacterium* genera (Fig. 7). As expected, the differential significant correlations caused by the ID-PEE treatments in comparison with control occurred in *F. prausnitzii* and *C.* cluster XIVa. In general, ID-PEE modified the relationship of *F. prausnitzii* with *C.* cluster XIVa and *Bifidobacterium* that became significantly positive (Fig. 7C), probably due to cross-feeding that exists between these bacterial groups (Riviere, Selak, Lantin, Leroy, & De Vuyst, 2016).

The correlation study between bacteria and the rest of the parameters determined under the influence of the different treatments was performed for *F. prausnitzii* since it was the bacterium that was significantly modified by the *R. puntactum* treatment. In this analysis, no significant correlations were detected between *F. prausnitzii* with any of the parameters measured for both control and FOS treatments. In

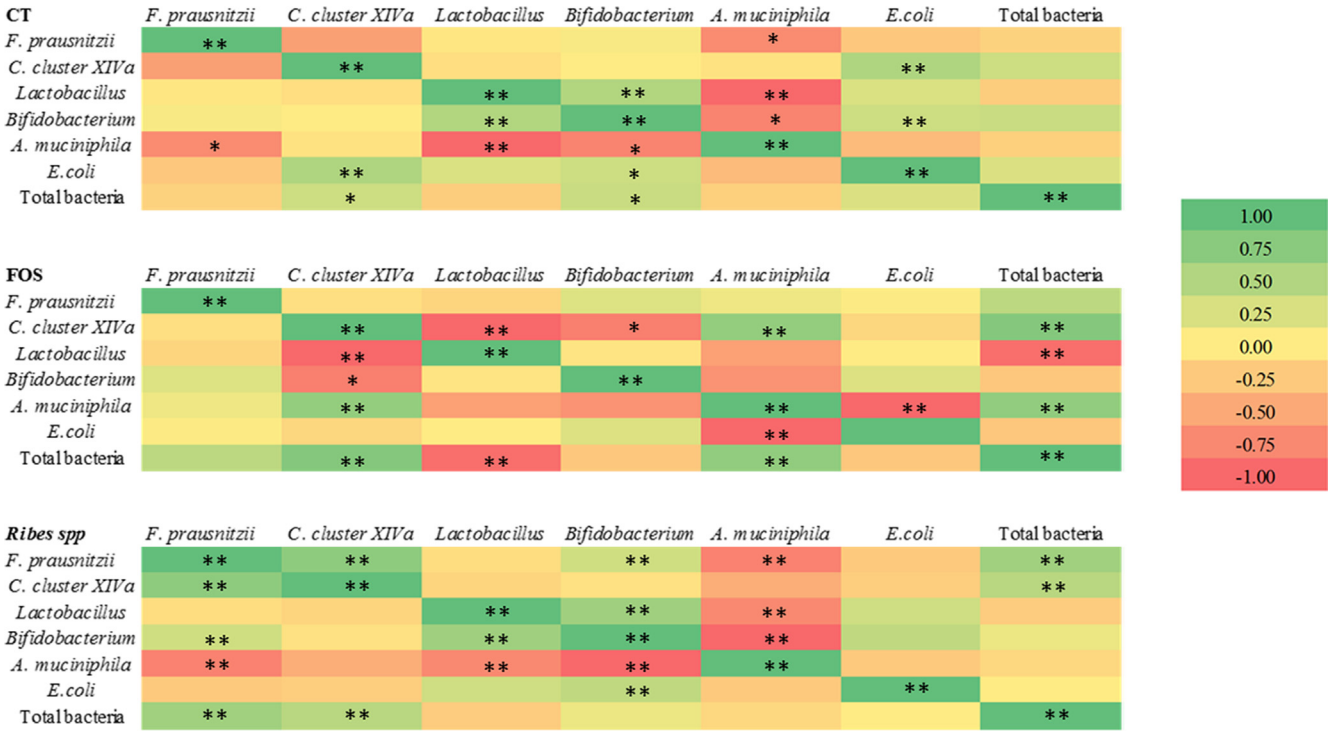

Fig. 7. Heatmap showing correlations of bacterial taxa under control, FOS and ID-PEE treatments. * ($p < 0.05$) ** ($p < 0.001$).

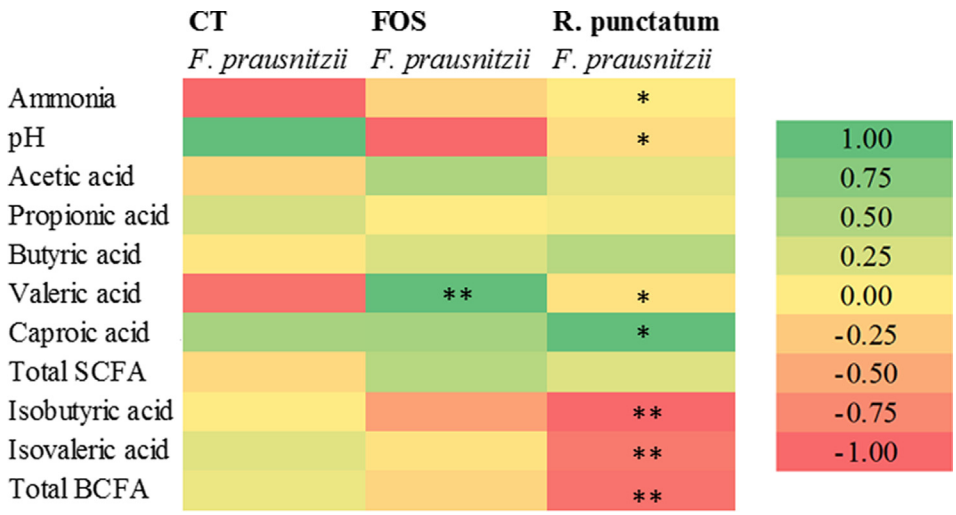

Fig. 8. Heatmap depicting associations between *F. prausnitzii* and pH, ammonia, SCFA and BCFA. * ($p < 0.05$) ** ($p < 0.001$).

contrast, the treatments with *R. punctatum* established negative significant correlations between the presence of *F. prausnitzii* and the BCFA (Fig. 8). Thus, the increase in *F. prausnitzii* could be partly responsible for the decrease in the observed BCFA concentration. These results would be in agreement with those observed in rats with colitis, in which the concentration of BCFA acids decreased after the administration of *F. prausnitzii* by gavage (Zhang et al., 2014). Although *F. prausnitzii* is a butyrate a producer, *R. punctatum* treatment decreased butyrate, this could be due to the fact that *F. prausnitzii* is a lactate-utilizing bacteria (Duncan, Louis, & Flint, 2004), and the culture medium employed in this study lacked it.

4. Conclusions

In summary, the ID-PEE did not modify parameters such as pH or ammonia and did not increase taxonomic groups classically considered as prebiotics (*Lactobacillus* and *Bifidobacterium*). Although the total levels of SCFA were not modified, the ID-PEE mainly decreased butyrate and propionate SCFA. The concentration of BCFA, which production is related to protein fermentation, was reduced by *Ribes punctatum* and *Ribes magellanicum* treatments. Both extracts increased the presence of *Clostridium* cluster XIVa in the first hours of incubation and a tended to increase the presence of *A. muciniphila*. *R. punctatum* increased the presence of *F. prausnitzii,* a bacterial specie with anti-inflammatory properties, what suggest that Chilean currants intake may be beneficial for colonic health. To the best of our knowledge, this is the first study describing the modulation of gut microbiota and its metabolites by Chilean berries. Although we performed a complete simulation of the gastrointestinal tract, using low concentrations of ID-PEE it should be taken into account the implicit limitations of the batch-culture fermentation method (closed system with limiting substrate and only one dose of treatment). Therefore, further *in vivo* experiments are needed to confirm these findings, considering this work as a first step to validate the potential prebiotic-like effect of Chilean currants in the human colon.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgements

This work was supported by the Ministry of Science, Innovation and Universities, Spain: Ramón y Cajal program (grants 2012_11910 and 2014_16307) and the AGL2016-77288-R project; The National Commission for Scientific and Technological Research of Chile CONICYT-PCHA/Doctorado Nacional/2015-21151561 (A. Burgos Edwards) and Universidad de Talca (PIEI-QUIM-BIO). The authors acknowledge the study participants.

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