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## Prebiotic effect of predigested mango peel on gut microbiota assessed in a dynamic *in vitro* model of the human colon (TIM-2)

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### ABSTRACT

Mango (*Mangifera indica* L.) peel (MP), is a by-product from the industrial processing to obtain juices and concentrates, and is rich in polyphenols and dietary fiber (DF). DF content of dried MP is about 40%. The aim of this study was to determine the prebiotic potential of this by-product submitting predigested mango ('Ataulfo') peel to a dynamic *in vitro* model of the human colon. Dried MPs were predigested following an enzymatic treatment and separating digestion products and undigested material by diafiltration. The predigested samples were fermented in a validated *in vitro* model of the colon (TIM-2) using human fecal microbiota and sampled after 0, 24, 48 and 72 h. A carbohydrate mixture of standard ileal effluent medium (SIEM) was used as control. Production of short chain fatty acids (SCFA), branched chain fatty acids (BCFA) and ammonia profiles were determined in both lumen and dialysates. Microbiota composition was determined by sequencing 16S rRNA gene V3–V4 region. Principal component (PC) analysis of fermentation metabolites and relative abundance of genera was carried out. Fermentation of MP resulted in SCFA concentrations resembling those found in the SIEM experiments, with a 56:19:24 molar ratio for acetic, propionic and butyric acids, respectively. BCFA and ammonia were produced in similar concentrations in both samples. About 80 bacterial genera were identified after fermentation of MP, with an 83% relative abundance of *Bifidobacterium* at 24 h. Three PC were identified; PC1 was influenced by a high *Bifidobacterium* abundance and low metabolites production. PC2 resulted in a decrease of other genera and an increase of metabolites studied. The relative abundance at 72 h in MP was distributed over 4 genera *Bifidobacterium*, *Lactobacillus*, *Dorea*, and *Lactococcus*. Our results suggest MP as a potential prebiotic ingredient.

### 1. Introduction

Mango (*Mangifera indica* L.) is known by its bright color, sweet taste, and delicious flavor (Singh, Singh, Sane, & Nath, 2013). Mango production is located as the fifth place of fruit crops grown worldwide, led by India with 18 million tonnes, followed by Indonesia, Thailand, China and Mexico (FAOSTAT, 2016). 'Ataulfo' mango is a Mexican variety that is highly accepted because it has unique sensory features, such as; firm consistency, sweet drupe, and intense aroma (Instituto Mexicano de la Propiedad Industrial, 2003). The most common industrial processing for mango is to obtain juices and concentrates, and about 50–55% of the fruit is discarded as a by-product, represented by seeds, peel, and paste (Santos, 2002). Unfortunately, this untreated waste most of the time is discarded directly in the environment and can be the cause of soil contamination. Yet, they are rich in polyphenols (PP) and dietary fiber (DF). Therefore, the possibility of using these compounds as additives or active ingredients in the food industry is of great interest

(Gorinstein et al., 2011).

In recent years, research on mango by-products (peel and paste) has demonstrated it to be a good source of DF and bioactive compounds, as PP (Blancas-Benitez et al., 2015). DF is known as non-starch polysaccharide, which reaches the human colon, and can be degraded by enzymes of the gut microbiota (Aura, 2008). Some DF show a fermentative property producing short chain fatty acids (SCFA; mainly acetic, propionic, and butyric acid) (Blaut & Klaus, 2012). This could also lead to specific changes in the composition and activity in the microbiota conferring health benefits to the host, in which case the DF is considered to be a prebiotic (Gibson et al., 2017; Roberfroid et al., 2010). However, to consider if a fiber has potential prebiotic effect it is necessary to evaluate its influence on the gut microbiota composition and production of SCFA. *In vivo* experiments (in animals or humans) have several limitations, such as high costs, ethical constraints and difficulty in sampling from the gut. Therefore, *in vitro* systems, despite their incomplete physiological environment such as absence of host

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cells, which mimic the microbial composition and activity, are good alternatives (Venema & Van den Abbeele, 2013). A dynamic system that simulates the kinetic conditions in the gastrointestinal tract, the TNO *in vitro* model of the colon (TIM-2) has been validated (Koen Venema & Van den Abbele, 2013) and used for almost two decades to study the effect of (potential) prebiotics on the gut microbiota. This model allows a rapid first screening of compounds while at the same time the mode of action can be studied mechanistically (Koenen, Rubio, Mueller, & Venema, 2016). This study aimed to determine the amount of DF in mango peel (MP), and to evaluate the prebiotic effect of predigested MP on the gut microbiota in this validated, dynamic *in vitro* model of the human colon (TIM-2).

## 2. Materials and methods

### 2.1. Sample preparation

Agro-industrial by-product (peel) of mango (*Mangifera indica L.* 'Ataulfo') from concentrate processing was provided by MexiFrutas®, S.A. de C.V. in Nayarit, Mexico. The origin of the sample and their preparation are described in an earlier publication (Blancas-Benitez et al., 2015). Indigestible fraction (IF) was evaluated by the method of Saura-Calixto, Garcia-Alonso, Goni, and Bravo (2000). Briefly, 300 mg of sample were incubated with pepsin (300 mg/mL in 0.2 M HCl-KCl buffer, pH 1.5, 37 °C, 1 h, P-7000, Sigma-Aldrich), pancreatin (5 mg/mL in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, P-1750, Sigma-Aldrich), and α-amylase (1 mL of a 120 mg/mL solution in Tris maleate buffer 0.1 M; pH 6.9, 37 °C, 16 h, A-3176, Sigma-Aldrich). After the enzymatic hydrolysis the samples were centrifuged (15 min, 25 °C, 3000 × g) and supernatants were removed. The residues are regarded as the insoluble indigestible fraction (IIF) and quantified by gravimetric methods. Supernatants were incubated (45 min, 60 °C) with 100 µL amyloglucosidase (A-9913, Sigma-Aldrich), transferred into dialysis tubes (D9652-30.48 m avg., 12,400 Da, Sigma Aldrich) and dialyzed against water for 48 h. The dialyzed supernatants (solution inside the dialysis tubes) constitute the soluble indigestible fraction (SIF). A SIF aliquot was hydrolyzed with 1 M sulfuric acid (100 °C for 90 min) for measuring sugars in SIF after reaction with dinitrosalicylic acid. TIF was quantified as the sum of IIF + SIF. The extractable and non-extractable polyphenols (hydrolyzable polyphenols) were determined as previously described (Hartzfeld, Forkner, Hunter, & Hagerman, 2002; Pérez-Jiménez et al., 2008) and antioxidant capacity (ABTS and FRAP) (Benzie & Strain, 1996; Re et al., 1999). All results are expressed as g/100 g DW.

### 2.2. Microbiota collection and standardization

Fecal samples were collected from donors at Maastricht University at Campus Venlo, The Netherlands, as follows: fresh fecal samples were collected from three healthy individuals (1 male and 2 female, average age: 43 years), without dietary restrictions, apparently free from gastrointestinal diseases and who did not receive antibiotic treatment during the previous 3 months, samples were placed in a gastight bag and immediately into a plastic jar containing an anaerocult strip (AnaeroGen™, Cambridge, UK). Samples were transported in anaerobic conditions at 15 °C in a period not longer than 3 h to the laboratory, mixed and standardized (500 g) in an anaerobic cabinet (Sheldon Lab – Bactron IV, Cornelius, OR, USA) according to Venema, Nuenen, Smeets-Peeters, Minekus, and Havenaar (2000). In brief, 450 mL of 10 × concentrated dialysis liquid was added, 2490 mL of demi-water, and 560 g of glycerol. The fecal material was aliquoted, frozen in liquid nitrogen, and stored at –80 °C until inoculation in TIM-2.

### 2.3. Mango peel predigestion

The equivalent to 70 g of indigestible carbohydrates were used. To a

gastric electrolytic solution (adjusted to, pH 3.0 with 1 mol/L HCl) containing the MP, 350 mg pepsin was added (P-7012, Sigma-Aldrich) and the solution was incubated (30 min, 37 °C). Then the pH was adjusted to 7.0 and 52.5 g of pancreatin (Pfizer, city, Germany) was added and incubation was continued during 3 h, after which the sample was centrifuged (20 min, 8000g, Centurion Scientific, K243R, Germany). Supernatants were separated and the pellet was washed, and the washing fluid was combined with the supernatant, and diafiltered (Sureflux, Nipro Europe NV, Zaventum, Belgium) using a peristaltic pump, to remove small digestion products and water, leading to a reduction of almost 6 times the volume. This fraction without digestion products was then mixed back with the pellet, and this was considered as the indigestible fraction.

### 2.4. TIM-2: dynamic *in vitro* model of large intestine

The TIM-2 system is a validated, dynamic, computer-controlled model that simulates the human proximal colon, mimicking body temperature, lumen pH, absorption of water and microbial metabolites through a semipermeable membrane inside the model, mixing and transporting the intestinal contents with peristaltic movements, using an anaerobic microbiota from human origin (Maathuis, Hoffman, Evans, Sanders, & Venema, 2009; Reimer et al., 2014). The fermentation was carried out by inoculating with 70 mL of the standardized microbiota (described above) plus 50 mL of dialysis liquid, containing (per liter): 2.5 g K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 4.5 g NaCl, 0.005 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.45 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.05 g bile and 0.4 g cysteine·HCl, plus 1 mL of a vitamin mixture containing (per liter): 1 mg menadione, 2 mg D-biotin, 0.5 mg vitamin B12, 10 mg pantothenate, 5 mg nicotinamide, 5 mg p-aminobenzoic acid and 4 mg thiamine. The total volume of the system was 120 mL. The microbiota was adapted to the model conditions with the simulated ileal effluent medium (SIEM), which simulates the indigestible fraction of a high fiber diet that can reach the colon and is fermented. SIEM contained the following components (g/L): 9.0 pectin, 9.0 xylan, 9.0 arabinogalactan, 9.0 amylopectin, 43.7 casein, 74.6 starch, 31.5 Tween 80, 43.7 bactopeptone, 0.7 ox-bile, 4.7 K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 8.4 NaCl, 0.009 FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.7 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.8 CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.05 bile, 0.02 haemin, and 0.3 cysteine·HCl, plus 1.5 mL of the vitamin mixture. The pH was adjusted to 5.8. All medium components were purchased at Tritium Microbiology (Eindhoven, the Netherlands). The microbiota was adapted to the model conditions with the SIEM for 20 h and after that, a 4 h starvation period allowed the bacteria to ferment all available carbohydrates in the system prior to the addition of the test compound, predigested MP. After the starvation period, samples were collected at time-point zero (t<sub>0</sub>), and SIEM medium was fed as control sample in one unit of the system, while the predigest MP (7.5 g/day, 2.5 mL/h), mixed with SIEM without the indigestible carbohydrates, was fed in three different units of the system, and a 72 h experimental period started. Samples of the lumen and dialysate were collected at time 0 h, 24 h, 48 h and 72 h, as is shown in Fig. 1.

### 2.5. Analytical methods

#### 2.5.1. Short chain fatty acids (SCFA), branched chain fatty acids (BCFA), lactate, and ammonia analysis

To evaluate SCFA, samples (lumen and dialysates) were centrifuged (20 min, 14,000 rpm, Centurion Scientific, K243R, Germany) and supernatant was then diluted using 1.5 mM sulfuric acid, mixed and filtered (0.45 µm PTFE). The samples were injected using an 883 Basic IC plus system with suppressed conductivity detection (Metrohm, Herisau, Switzerland). Chromatograms were recorded using the Metrohm MagIC net 3.1 Software. Separation of the acids was achieved isocratically on a 300 mm × 7.8 mm, 7 µm particle size ICsep ION300 Ion exclusion column (Transgenomic, New Haven, CT, USA), using 1.5 mM sulfuric acid as mobile phase. A 3.5mmx1mm, 0.2 µm particle size Metrosep

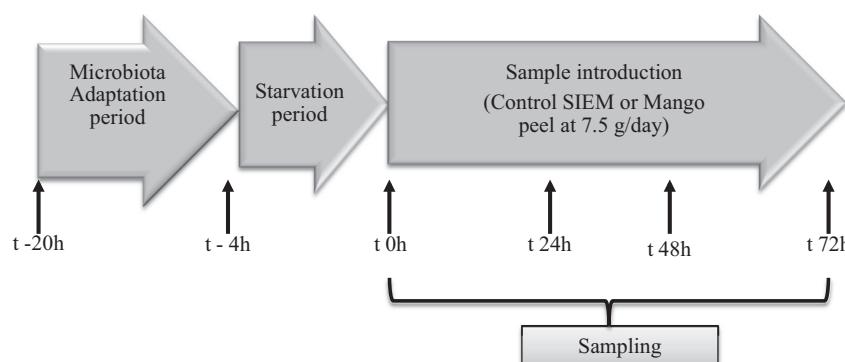


Fig. 1. Experimental setup of TIM-2 fermentation experiment.

RP2 Guard column (Metrohm, Herisau, Switzerland) was installed as pre-column. Samples were injected through a 10  $\mu\text{L}$  loop, using a 730  $\mu\text{l}$  sample changer (Metrohm, Herisau, Switzerland) and eluted at a flow rate of 0.4 ml/min and a temperature of 65 °C, with a pressure of 5.5 MPa. The suppressor system was regenerated using a 100 mM LiCl solution. Conductivity detector was used in positive mode. The eluent and suppressor fluid were degassed using an ultrasonic bath. Standards for succinic, lactic, acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, and caproic acids were purchased from Sigma Aldrich (Sigma Chemical, St. Louis, MO). Lactic acid standard was prepared from the sodium salt. Formic acid was purchased from Actuall chemicals (Oss, The Netherlands). A 25 mM mixed stock standard solution was prepared and diluted to produce calibration standards.

Ammonia was quantified enzymatically using the Berthelot reaction, briefly; samples were reacted with an alkaline phenol and sodium hypochlorite to produce an indophenol (660 nm). Samples were evaluated using an auto analyzer (Coba Mira plus, Roche, Almere, Netherlands). The analysis was performed by BioANalyTiX (Mook, Netherlands). The concentrations were calculated based on a calibration curve of known concentrations.

### 2.5.2. Microbiota composition in SIEM control and mango peel

The change in abundance of bacterial genera was determined using next generation sequencing. The V3–V4 region of the 16S rRNA gene was amplified and sequenced using an Illumina MiSeq. DNA extraction and sequencing was performed by Baseclear (Leiden, the Netherlands). QIIME 1.9.1 was used to convert the raw-data into abundances using an established bioinformatic pipeline as described by Aguirre, Ramiro-García, Koenen, and Venema (2014).

### 2.6. Statistical analysis

Data were subjected to Student's *t*-test. Principal component analysis (PCA) of fermentation metabolites was performed based on the mean values of triplicates. Components were calculated without rotation and the number of extracted factors were based on eigenvalues > 1.0 and explained variance (%) > 70. All analyses were performed using STATISTICA software, version 10.0 (StatSoft, Inc. 1984–2007, Tulsa, OK, USA).

## 3. Results and discussion

### 3.1. Indigestible fraction (IF) and polyphenols (PP) content in mango peel

IF in mango peels was about 60%, corresponding to all non-digestible compounds and comprising dietary fiber (both soluble and insoluble), extractable and non-extractable PP, resistant protein and other indigestible compounds (Saura-Calixto et al., 2000). Table 1 shows the content of soluble, insoluble, and total (IF) for MP, which is generally recognized as an excellent source of pectin with low protein content (Kermani, Shpigelman, Pham, Van Loey, & Hendrickx, 2015).

Table 1

Total indigestible fraction, total soluble polyphenols, hydrolyzable polyphenols content and antioxidant capacity in mango peel.<sup>a</sup>

	Mango peel (g/100 g)
Total indigestible fraction	52.61 ± 0.16
Soluble indigestible fraction	27.63 ± 0.94
Insoluble digestible fraction	24.98 ± 1.10
Total soluble polyphenols (mg EAG/g)	102.81 ± 0.02
Antioxidant capacity	
ABTS (mmol TE/g)	1293.65 ± 9.05
FRAP (mmol TE/g)	735.68 ± 6.99
Hydrolyzable polyphenols (mg EAG/g)	11.65 ± 0.31

<sup>a</sup> Data are the mean ± SEM (n = 3).

Depending on the variety and ripeness the polysaccharide fraction is rich in arabinose, galactose, galactan, and arabinogalactan, which are side chains of pectic substances (Yashoda, Prabha, & Tharanathan, 2005). The soluble IF/insoluble IF ratio was about 1:1. A balanced soluble dietary fiber: insoluble dietary fiber ratio of 1:1 has been recommended for human diet (Geerkens et al., 2015). The total soluble polyphenols (TSP) and hydrolyzable polyphenols (HP) represented about 11.46% of the total IF. The antioxidant capacity (Table 1) was higher than for other plant-foods, such as, cereals or other fruits. Bioaccessibility of TSP for mango peel has been reported to be around 44% (Blancas-Benitez et al., 2015), which means that both non-bioaccessible TSP and HP can reach the colon and potentially be used as substrate by the colonic microbiota. This is one way of how the composition of the IF can influence changes of the microbiota composition during the fermentation process.

### 3.2. Short chain fatty acids (SCFA) production in SIEM control and mango peels samples

SCFA are produced in the proximal colon through the fermentation of (amongst others) indigestible carbohydrates. The cumulative amount of SCFA produced during the fermentation time (0, 24, 48, and 72 h) of SIEM and MP, are shown in Table 2. Cumulative SCFA production during the fermentation did not show statistical differences ( $p > 0.05$ ) between SIEM and MP, only at 72 h the cumulative amount of butyric acid in SIEM experiments was higher than in experiments with MP. The most abundant SCFA were acetic, propionic, and butyric acid, comprising 95% of the total SCFA, while other acids such as, valeric, capric, succinic, lactic, and formic acid are formed as intermediate metabolites. Total cumulative SCFA produced during the fermentation of SIEM and MP were 121.3–152 mmol respectively. The concentration of SCFA produced in the colon can reach ~150 mM. The molar ratio of the most common SCFA in the studied samples was for SIEM 54: 19: 27, and MP 56: 19: 24 acetic, propionic, and butyric acid respectively. The molar ratio of these three SCFA is commonly found to be between 60% acetic, 25% propionic, and 15% butyric acid (Cook & Sellin, 1998) and

**Table 2**

Mean ( $\pm$  SEM) cumulative amount of short chain fatty acids (mmol) in simulated ileal effluent medium (SIEM) and mango peel (MP).<sup>1</sup>

	SIEM	Mango peel
Acetic acid		
24 h	14.42 $\pm$ 0.79a	19.01 $\pm$ 0.66a
48 h	42.15 $\pm$ 1.81a	38.98 $\pm$ 3.49a
72 h	69.40 $\pm$ 2.09a	64.33 $\pm$ 4.12a
Propionic acid		
24 h	4.73 $\pm$ 1.48a	5.02 $\pm$ 0.43a
48 h	15.25 $\pm$ 1.43a	12.32 $\pm$ 1.27a
72 h	24.56 $\pm$ 2.05a	21.78 $\pm$ 0.74a
Butyric acid		
24 h	3.76 $\pm$ 2.01a	5.82 $\pm$ 0.42a
48 h	19.34 $\pm$ 3.47a	15.29 $\pm$ 1.04a
72 h	34.87 $\pm$ 4.95b	27.84 $\pm$ 0.11a
Valeric acid		
24 h	0.46 $\pm$ 0.10a	0.65 $\pm$ 0.38a
48 h	2.28 $\pm$ 0.86a	1.86 $\pm$ 1.38a
72 h	5.14 $\pm$ 1.79a	4.63 $\pm$ 2.47a
Caprylic acid		
24 h	0.36 $\pm$ 0.20a	0.17 $\pm$ 0.09a
48 h	0.93 $\pm$ 0.35a	0.25 $\pm$ 0.05a
72 h	1.76 $\pm$ 0.40b	0.45 $\pm$ 0.16a
Succinic acid		
24 h	0.16 $\pm$ 0.11a	0.013 $\pm$ 0.01a
48 h	0.55 $\pm$ 0.36a	0.04 $\pm$ 0.03a
72 h	0.55 $\pm$ 0.37a	0.20 $\pm$ 0.15a
Lactic acid		
24 h	0.13 $\pm$ 0.13a	0.0005 $\pm$ 0.0003a
48 h	0.03 $\pm$ 0.03a	0.004 $\pm$ 0.0036a
72 h	0.02 $\pm$ 0.02a	0.004 $\pm$ 0.0034a
Formic		
24 h	0.43 $\pm$ 0.19a	0.68 $\pm$ 0.07a
48 h	1.15 $\pm$ 0.32a	1.53 $\pm$ 0.16a
72 h	1.43 $\pm$ 0.50a	2.02 $\pm$ 0.41a
Total		
24 h	25.84 $\pm$ 2.66a	31.38 $\pm$ 1.95a
48 h	90.15 $\pm$ 7.22b	70.28 $\pm$ 7.06a
72 h	152.00 $\pm$ 8.20a	121.27 $\pm$ 6.70a

<sup>1</sup> Significant differences evaluated using Student's *t*-test (sample  $\times$  interaction time,  $p < 0.05$ ). Different letters (a or b) between columns indicate significant differences.

60:20:20 acetic, propionic and butyric acid (McNeil, 1984). In our experiments the molar ratio of butyric acid was slightly higher than reported before.

SCFA promote the production of mucin and gastrointestinal peptide, they can be utilized by colonocytes, and those that are not used by the gut epithelium are absorbed via portal vein to reach other organs, such as liver and muscle (Verbeke et al., 2015). Acetate stimulates cholesterol and long chain fatty acid synthesis in the liver (Delzenne, Neyrinck, Bäckhed, & Cani, 2011). A study in mice showed that acetate from colonic fermentation can cross the blood-brain barrier and suppress appetite in the hypothalamus (Frost et al., 2014). Propionic acid reduces liponeogenesis, cholesterol synthesis and recently has been demonstrated to be involved in the activation of G-protein-coupled receptors (GPR-41, and GPR-43) releasing satiety hormones (Kimura et al., 2011). At the end of the experimental period (time 72 h) butyric acid was statistically different ( $p < 0.05$ ) between samples, where SIEM was higher than MP. Butyric acid production for MP was similar to cassava by-product (Bussolo de Souza et al., 2014) and similar to a mixture of linear short-chain FOS and long chain inulin from chicory (Koenen et al., 2016) fermented in the same system (TIM-2). Butyric acid is used as an energy source by epithelial cells and it has restorative ability in colitis. In colon cancer it regulates proliferation, differentiation, and cell apoptosis by inhibiting histone-D-acetylase (Fung, Cosgrove, Lockett, Head, & Topping, 2012). Succinic and lactic acid did

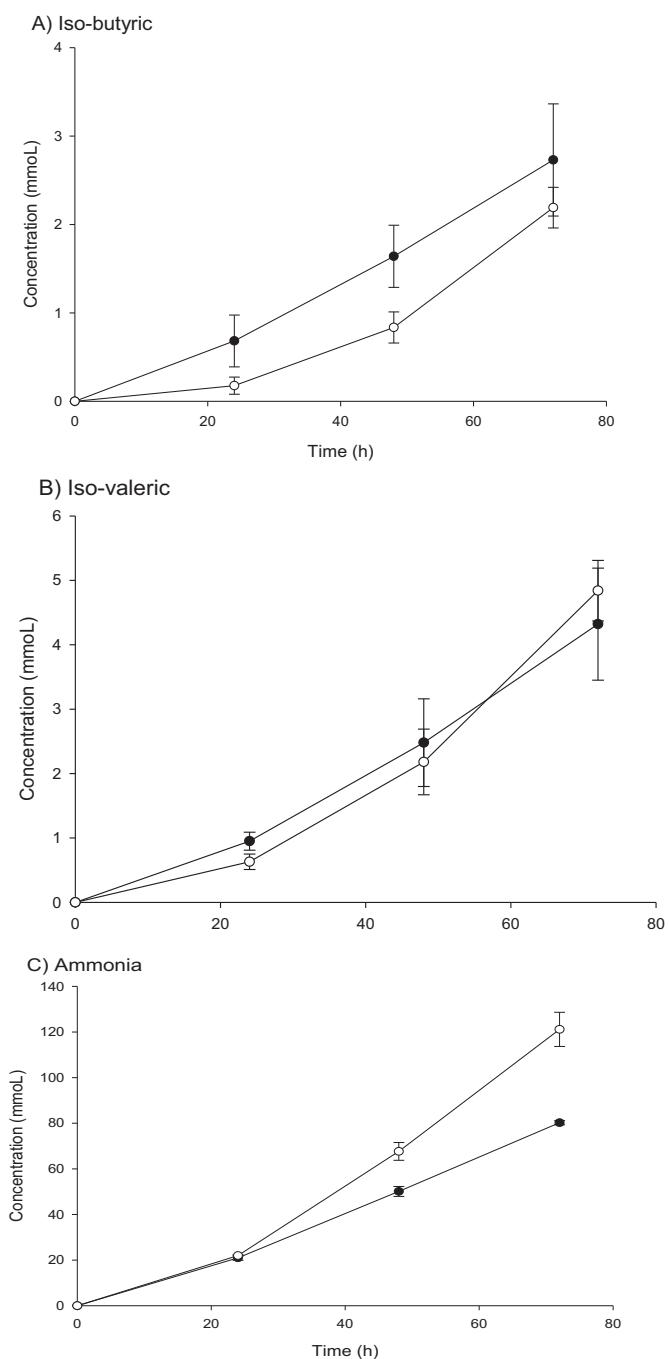


Fig. 2. Mean ( $\pm$  SEM) cumulative concentrations ( $n = 3$ ) of iso-butyric acid, iso-valeric acid and ammonia (mmol) (-●-) SIEM and mango peel (-○-).

not show statistical differences ( $p > 0.05$ ) (Table 2). These acids serve as intermediates in the metabolism of SCFA and generally they do not accumulate in the colon (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). Lactic acid only accumulates when the substrate is fermented fast (Maathuis et al., 2009). Low concentrations of valeric acid were observed (Table 1). SCFA are the main agonist for free fatty acid receptors (FFAR2) (Alvarez-Curto & Milligan, 2016). Moreover, middle chain fatty acids and long chain fatty acids receptor (GPR48) had been found on cells of the immune system (Murray, Rathmell, & Pearce, 2015). Small concentrations of caprylic acid were quantified in the fermented samples. Formic acid was also not statistically different ( $p > 0.05$ ) between samples. It generally serves as substrate for acetogenic bacteria, which are able to convert it into acetic acid (Louis,

Hold, & Flint, 2014).

### 3.3. Branched chain fatty acids (BCFA) and ammonia cumulative concentration

The BCFA, iso-butyric acid was lower for MP, but was no longer statistically different between samples at 72 h (Fig. 2), while iso-valeric acid did not show statistical differences ( $p > 0.05$ ) during the fermentation. The production of BCFA is related to microbiota fermentation of amino acids valine, leucine and isoleucine (Le Roy et al., 2013). Iso-butyrate, 2-methyl-butyrate and iso-valerate demonstrated anti-oxidant properties and contributed generally about 5% to total acid production (Davila et al., 2013). Ammonia amounts were higher at 48 and 72 h in MP. Ammonia can affect the exchange of ions in the colon and may act as a regulator of colonic sodium-ion absorption (Wutzke, Lotz, & Zipprich, 2010). The toxicity attributed to ammonia could be to its ability to protonate and deprotonate different compounds in host cells (Kleiner, Traglauer, & Domm, 1998).

### 3.4. Changes in microbiota composition in SIEM and mango peel

An average of 500 OTUs were identified in each sample (data not shown) with about 80 genera. About 90% of the relative abundance in the fermented samples belonged to the genera *Bifidobacterium*, *Bacteroides*, *Blautia*, *Collinsella*, *Dorea*, *Lactobacillales*, *Lactococcus*, *Ruminococcaceae*, *Streptococcus*, and *Lachnospiraceae*. Fig. 3 shows the relative abundance of the genera during the fermentation process. *Bifidobacterium* was the most abundant genus in the studied samples, however; changes in its abundance were from 51 to 16% in SIEM, and 62 to 83% at 24 h in MP, and 47% at 72 h of fermentation. *Bacteroides* in SIEM was relatively constant between 14 and 20%, however with MP it did not present abundance higher than 1%. Although the SCFA production in the samples studied were similar, although specifically butyric acid was higher in SIEM than MP at the end of the fermentation (Table 2), the fermentability is highly influenced by the type of non-starch polysaccharides present in the samples and influences the abundance of certain bacterial members. In our experiments, in MP fermentations *Bifidobacterium* species were higher than *Bacteroides*, which showed a decreased in abundance over time. Intake of apples in eight humans similarly resulted in increased fecal *Bifidobacterium* and decreased *Bacteroides* (Fåk et al., 2015; Shinohara, Ohashi, Kawasumi, Terada, & Fujisawa, 2010).

*Blautia* increased during the fermentation from 1.97 to 9.32% at 72 h in SIEM but in MP it was only 3.66% at 72 h of fermentation. *Blautia* helps to digest complex carbohydrates and abundance of these bacteria is a strong indication of a healthy gut. *Blautia* levels are higher in healthy people when compared to patients with liver disease or

colorectal cancer, or children with diabetes (Alou, Lagier, & Raoult, 2016). *Collinsella* was more abundant in SIEM than MP. This genus is known for its ability to ferment a wide range of carbohydrates, including starch, forming products such as hydrogen gas and ethanol. Goldman et al. (2009) describe *Collinsella aerofaciens* as “the most predominant and ubiquitous microorganism isolated from the human gut.” *Dorea* genus was present in MP fermentations at 6.69% at the end of the fermentation time, while in SIEM it was lower (3.29%). *Dorea* is a carbohydrate utilizing bacteria. However, it has been reported that it could be increased when a high protein diet is fed (Aguirre et al., 2016). An unknown genus of the order *Lactobacillales* was highly abundant in MP with 19.85% of abundance at 72 h, compared to 3.53% in SIEM. *Bifidobacterium* and *Lactobacillus* are two genera commonly determined to evaluate the prebiotic effect of dietary carbohydrates (Rycroft, Jones, Gibson, & Rastall, 2001). However, a novel definition from the International Scientific Association from Probiotics and Prebiotics (ISAPP) concluded: “a prebiotic is consider as a substrate that is selectively utilized by host microorganism conferring health benefit”. Considering carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids (Gibson et al., 2017). The abundance of *Lactococcus* in SIEM decreased during the fermentation time but this behavior was not observed for MP. An unknown genus of the family *Ruminococcaceae* showed abundance of 11.93% at 72 h in SIEM, but in MP abundance was only 1.59% at the same time. *Ruminococcus* is a common gut microbe that breaks down complex carbohydrates, and they are most common in the digestive tracts of people with carb-heavy diets, where in particular they are a keystone species in degradation (Ze, Duncan, Louis, & Flint, 2012). *Streptococcus* abundance was lower than 1% in SIEM at 72 h but in MP was only 0.18% at the same fermentation time. The unknown genus of the family *Lachnospiraceae* was relatively abundant 20.09% in SIEM, but not for the MP where it reached only 1.34%. Particularly *Lachnospiraceae* family includes the *Roseburia* and *Blautia* genera, which are related with the production of SCFA and they significantly reduced the severity of the colitis by selectively blocking the recruitment of monocytes/macrophages, but not of other cells (Duncan, Louis, & Flint, 2007).

### 3.5. PCA analysis

PCA was performed to determine correlations with microbial metabolic patterns in the samples at different fermentation times. Three principal components (PC) were obtained (eigenvalues  $> 1$ ) that explained 74.2% of the total variance, with PC 1, 2, and 3 explaining 29.88, 26.85, and 17.51% respectively of all variance in the microbiota genera and microbial metabolites production. Loading scatter plots for the three PC are shown in Fig. 4. PC1 in the positive axis (Fig. 4a) was

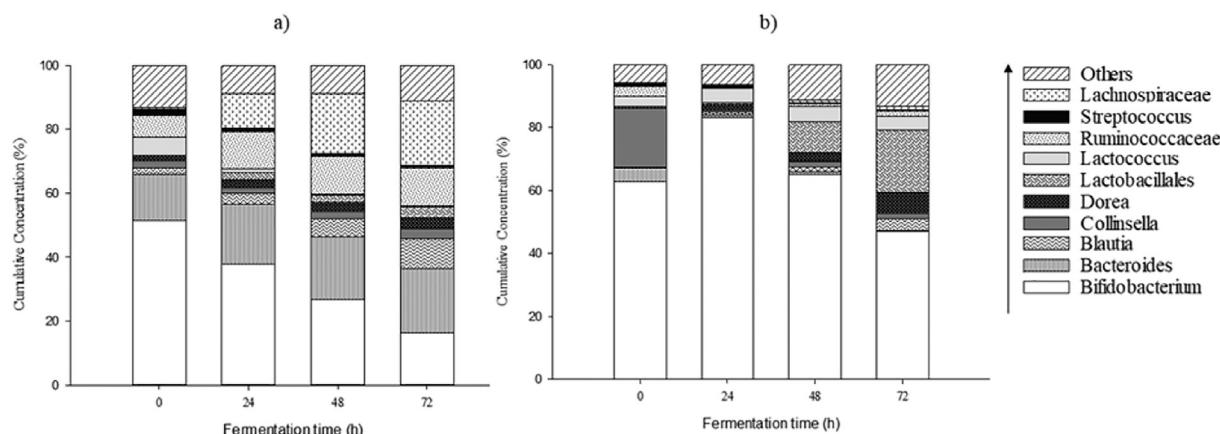
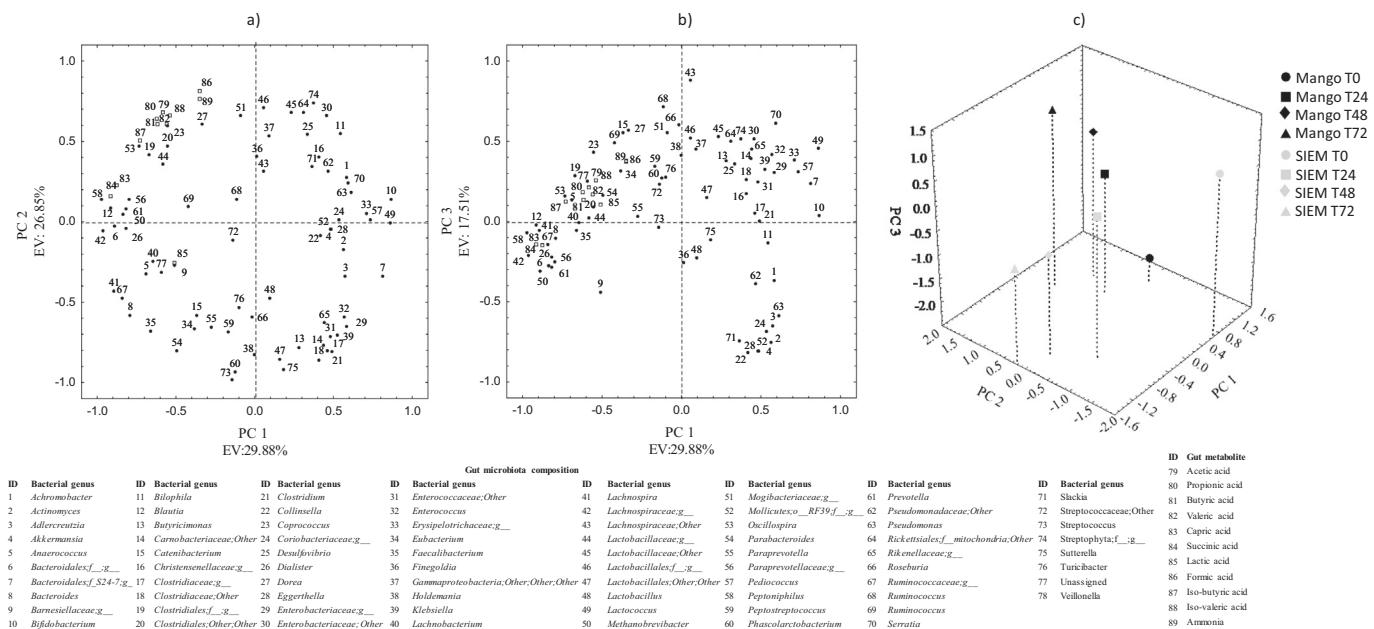


Fig. 3. Changes in bacterial genera composition (relative abundance) of during a) SIEM and b) Mango peel extracts during *in vitro* colonic fermentation at different times.



**Fig. 4.** Correlation of gut microbiota (genus level) and microbial metabolites in the *in vitro* colonic fermentation of SIEM and Mango peel extracts using principal component analysis (PCA): loading scatter plot for a) PC1 vs. PC2; b) PC1 vs. PC3 and c) PCA scores plot of the samples.

highly influenced by the genera *Bifidobacterium* (ID: 10), unknown genus of the order *Bacteroidales* (ID: 7), and *Lactococcus* (ID: 49), present in high abundance. The PC1 negative axis comprises compounds that were produced at relatively low levels during the fermentation process, where metabolites such as acetic acid (ID: 79), propionic acid (ID: 80), butyric acid (ID: 81) and valeric acid (ID: 82) apparently were consumed and the presence of other species in lower relative abundances. PC2 in the positive axis was influence by the production of different metabolites such as formic acid (ID: 86), iso-valeric acid (ID: 89) and ammonia (ID: 90) and genera that appear during the fermentation as more abundant, such as another unknown genus of the order *Lactobacillales* (ID: 46), while genera in the PC2 negative axis are present at lower relative abundances. PC3 in the positive axis (Fig. 4b) is influence by *Lachnospiraceae* (ID: 43), *Ruminococcus* (ID: 68), *Serratia* (ID: 70) and in the negative axis by other genera in lower relative abundances. Fig. 4c, shows the changes in the microbiota composition during the fermentation, where SIEM Control changes from PC1 to PC2, and Mango peel from PC1 to PC3 and finally PC2.

#### **4. Conclusions**

Mango peel has a high IF that can be fermented, it showed a production of SCFA similar to SIEM Control and other dietary fiber material such as cassava by-product. Mango peel can promote the growth of *Bifidobacterium* with a maximum at 24 h of fermentation, while at 72 h mango peel favoured the growth of *Bifidobacterium* and *Lactobacillus*. The production of SCFA are not always related to the growth of a particular genus but can also influence the abundance of other bacterial species with specific health properties through cross-feeding or other mechanisms. The indigestible fraction of MP can potentially be used as a prebiotic ingredient.

## **Conflict of interest**

Authors declare any conflict of interest.

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