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In vitro colonic fermentation of Mexican "taco" from corn-tortilla and black beans in a Simulator of Human Microbial Ecosystem (SHIME®) system

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ABSTRACT

A Mexican staple food prepared with corn "tortilla" (*Zea mays* L.) and common beans (*Phaseolus vulgaris* L.) is named as "taco". It was fermented in an *in vitro* colonic Simulator of Human Microbial Ecosystem (SHIME®) to evaluate the effect in short chain fatty acids (SCFA), ammonia production, and the growth of total presumptive counts for anaerobic bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium* spp., and total coliforms in the three simulated reactors of the human colon. After two weeks of stabilization, the simulator was fed during 9 days with the mixture of 50 g of beans and 50 g of tortilla mixed with 100 mL of carbohydrate based medium. Every third day, samples were collected from the three simulated colon vessels for the corresponding analysis. The production of the SCFA was higher during the treatment period than the basal period in the three colon sections. The acetate was produced in higher concentration (191.9 mmol/L) than propionate and butyrate (29.1 and 55.0 mmol). During the treatment period, the higher molar ratio (%) for acetate, propionate, and butyrate were 84: 14: 24, respectively. The ammonia ions as well as the growth of presumptive coliforms were reduced ($p < 0.05$) in the three simulated colon vessels during the treatment. Finally, *in vitro* fermentation of Mexican "taco" showed a possible potential functional profile of an ancestral staple food due to the production of SCFA that may exert beneficial effects.

1. Introduction

Mexican foods are worldwide known because of the nutritional importance contribution of its ingredients being the first cuisine of a country accepted in 2010, for the Intergovernmental Committee by the Safeguarding of the Intangible Cultural Heritage of UNESCO (Gálvez & Salinas, 2015). Recently, studies reported drastic changes in food consumption in Mexico (Flores et al., 2010). In that sense, the intake of native foods has decreased and the intake of high fat diets has increased (Rivera et al., 2002). However, two of the most native foods in the Mexican diet are corn and beans. Even with the changes in Mexican dietary habits the consumption of beans is about 10 kg per person/per year (*Phaseolus vulgaris* L.) (Gálvez & Salinas, 2015) and approximately 95% of Mexicans consume corn (*Zea mays L.*) cooked as "tortilla" (flat discs with a diameter from 12 to 18 cm and from 1 to 4 mm thick). The consumption of tortilla in Mexico per person/per day is 325 g (Rojas-Molina et al., 2007), and supplies 70% and 50% of the daily-consumed calories and the daily-consumed protein, respectively (Sáyago-Ayerdi, Tovar, Osorio-Díaz, Paredes-López, & Bello-Pérez, 2005). Besides, the content of protein in beans is about 20–25%, where 40–50% is represented by the protein phaseolin and 10–27% is represented by lectins (Carrasco-Castilla et al., 2012). Its high relative resistance to proteolysis but the cooked processes improves digestibility of phaseolin (Yin, Tang, Wen, Yang, & Yuan, 2010). Even α-amylase inhibitor (α-AL), arcelin, and phytohemagglutinin (PHA) reduce protein digestibility, these members of the protein profile in beans could also exert anticarcinogenic effects (González de Mejía, Valadez-Vega, Reynoso-Camacho, & Loarca-Piña, 2005). Also, common beans contain micronutrients as well as, bioactive compounds distributed in the different anatomic parts of the bean seed. In this sense, the pericarp contents dietary fiber (DF), minerals, and phenolic compounds (PC), specifically anthocyanins in black beans: delphinidin, petunidin, malvidin and pelargonidin (Chávez-Mendoza & Sánchez, 2017). Starch, resistant starch (RS), and DF are the major constituents of carbohydrates present in corn tortilla and beans. It has been reported that the content of soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber

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(TDF) in black beans was 5.6, 25.5 and 31.2% (Silva-Cristobal, Osorio-Díaz, Tovar, & Bello-Pérez, 2010), respectively, and in tortilla was 2.4, 10.5 and 12.9% (Bello-Pérez et al., 2014), respectively. Besides, the indigestible fraction (IF) is a physiological concept that comprises most components of vegetable foods that escape digestion and absorption in the small intestine, reaching the colon where they are susceptible to bacterial fermentation, *e.g.* TDF (Saura-Calixto, García-Alonso, Goni, & Bravo, 2000).

The mixture of bean and corn tortilla is called "taco" and it is traditionally consumed in Mexico. The "Taco" has important quantities of DF. The content of SDF, IDF, TDF in bean-corn flour was 2.3%, 10.4% and 12.76% (Treviño-Mejía, Luna-Vital, Gaytán-Martínez, Mendoza, & Loarca-Piña, 2016), respectively, and it has been reported a content of RS of 3.9% (Sáyago-Ayerdi, Tovar, Osorio-Díaz, Paredes-López, & Bello-Pérez, 2005) in "taco", which resist human gastrointestinal digestion conditions and reach the colon (Silva-Cristobal, Osorio-Díaz, Tovar, & Bello-Pérez, 2010). In that sense, several studies reported that the main products of the fermentation of RS are the short chain fatty acids (SCFA) (Ahmed, Segal, & Hassan, 2000; Henningsson, Nyman, & Bjork, 2002; Nugent, 2005; Topping, 2007) as butyrate, propionate and acetate whose may exert benefits to the colon health (Hamer et al., 2008; Haralampu, 2000; Huda-Faujan et al., 2010; Scheppach, Bartram, & Richter, 1995).

The gastrointestinal (GI) human digestion has been a scientific approach, hence, several GI *in vitro* systems has been designed due to *in vivo* studies have limitations (ethical policies, high experimental costs and difficulty in sampling from the gut) (Sáyago-Ayerdi, Zamora-Gasga, & Venema, 2017). Thus, the static models are the most representative digestive systems, but these models do not reproduce the multiple changes along the process in pH and secretion flow rated and even dynamic models do, *i.e.*, the Dynamic Gastric Model (DGM) only simulates a partial process of GI digestion (the stomach) (Guerra et al., 2012). A dynamic *in vitro* system that integrates the whole GI tract from stomach to colon is the Simulator of Human Microbial Ecosystem, SHIME®, that has a proven system to study aspects of human intestinal microbiota during food fermentation (De Boever, Deplancke, & Verstraete, 2000; Van De Wiele, Boon, Possemiers, & Verstraete, 2004). It consists of five double-jacketed vessels that simulate the environmental conditions of pH, residence time, inoculum, and temperature of the stomach, the small intestine and the ascending, transverse, and descending colon with a total retention time of 72 h (Molly, Woestyne, & Verstraete, 1993; Sivieri et al., 2014). The focus in the use of the SHIME® system has been to evaluate the prebiotic effect of the DF, *i.e.*, fructooligosaccharides (FOS) (Daguet, Pinheiro, Verhelst, Possemiers, & Marzorati, 2016) and to evaluate the probiotic effect of Lactobacillus (Barroso et al., 2014) but, as far as we know, the SHIME® system has not been used to evaluate the effects of the fermentation of a more complex food matrix. Besides, the profile of microbial metabolites produced is strongly determined by the compounds of the food matrix that would reach the colon (Flint, Duncan, Scott, & Louis, 2015). Thus, the aim of this study was to evaluate the effect in short chain fatty acids (SCFA) production, ammonia production and the growth of certain presumptive bacterial groups during the *in vitro* colonic fermentation of Mexican "taco" in the SHIME® reactor system.

2. Materials and methods

2.1. Preparation of the Mexican "taco"

Corn "tortillas" were bought in a local supermarket in Tepic, Nayarit, Mexico. 'Negro Jamapa' variety of common beans was donated (Cadena Agroalimentaria de Frijol de Nayarit). They were washed with tap water and cooked to controlled pressure until the same degree of tenderness of each bean was obtained. Mexican "taco" was made under the 5:5 bean-tortilla proportions because it was the properly proportion according to the general experimental protocol (Molly, Woestyne, Smet, & Verstraete, 1994). The content of RS was measured as the non-hydrolyzed enzymatic starch fraction in the small intestine (Goñi, Garcia-Diz, Mañas, & Saura-Calixto, 1996). The total indigestible fraction (TIF) (Saura-Calixto, García-Alonso, Goni, & Bravo, 2000) was evaluated this protocol simulates the physiological conditions of human digestion. The PC were evaluated in supernatants from a previous organic aqueous extraction (Pérez-Jiménez et al., 2008) using a microplate reader (Biotek® Synergy HT, USA). The absorbance was read at 750 nm (Montreau, 1972) modified from Alvarez-Parrilla, de la Rosa, Amarowicz, and Sahidi (2010). The composition in RS, TIF and PC of the studied "taco" is shown in the Table 1.

Table 1
Phenolic compounds (PC), resistant starch (RS) and total indigestible fraction (TIF) content of the Mexican "taco", 5:5 bean-tortilla proportion (g/100 g DW)[a].

Parameter	Mexican "taco"
PC[b]	3.70 ± 0.37
RS	4.65 ± 0.04
Indigestible fraction (IF) content	
Soluble IF	2.98 ± 0.33
Insoluble IF	28.98 ± 1.80
Total IF[c]	31.11 ± 2.28

[a] Data are means of three repetitions ± standard deviation (n = 3).; Dry weight (DW).
[b] mg GAE/g DW. Gallic acid equivalentes (GAE).
[c] Total IF = Sum of soluble IF + insoluble IF.

2.2. Dynamic SHIME® model

Briefly, the SHIME system, developed at Ghent University, is a dynamic model of the human gastrointestinal tract. The vessels of the SHIME® system operated at 37 °C and were stirred continuously using a magnetic stirrer. The inside of each vessel was kept in anaerobiosis through the daily injection of N_2 for 30 min, and the pH of each portion of the tract was automatically adjusted with the addition of 0.5 N NaOH or 0.5 N HCl (Molly, Woestyne, Smet, & Verstraete, 1994; Possemiers, Verthé, Uyttendaele, & Verstraete, 2004). Stomach conditions were simulated in reactor one, at pH 2 with pepsin from porcine gastric mucosa (Sigma, P6887) at a concentration of 1 g/L, under micro-aerophilic conditions for 2 h, at 37 °C (Possemiers, Marzorati, Verstraete, & Van de Wiele, 2010). The passage of food in the small intestine was simulated in reactor two by the addition of 60 mL artificial pancreatic and bile liquid [6 g/L oxgall (Sigma, Brazil), 1.9 g/L pancreatin from porcine pancreas (Sigma, P1750) and 12.5 g/L $NaHCO_3$], pH 6.5–7.5 (Molly, Woestyne, Smet, & Verstraete, 1994; Possemiers, Verthé, Uyttendaele, & Verstraete, 2004). Colonic fermentation were simulated in reactor three (ascendant colon-AC), four (descendent colon-DC) and five (transverse colon-TC), at the beginning of the experiments, the colonic reactors were inoculated with non-gas producing (methane < 3 ppm) stool samples from three adult volunteers who declared did not taken antibiotics in the two years prior. As the production of methane gas is directly related to an increase of acetic acid and consequently, the increase in total SCFA (Wolever, Robb, Ter Wal, & Spadafora, 1993), the volunteers classified as methane producers were excluded. For this classification, the breath methane dosage of four lean volunteers was performed through a Quintron digital Breathtracker Microlyzer at the Pedriatric Gastroenterology Department of the São Paulo Federal School. The stool inoculum was prepared using the method described by Possemiers, Marzorati, Verstraete, and Van de Wiele (2010)). The microbial inoculum was stabilized for two weeks (basal period) on a carbohydrate-based medium [(3 g/L of starch (Unilever, Brazil), 2 g/L of pectin (Sigma, USA), 4 g/L of type III mucin from porcine stomach (Sigma, USA), 1 g/L of xylan (Sigma, USA), 1 g/L of peptone (Acumedia, USA), 1 g/Lof arabinogalactan (Sigma, USA), 0.4 g/L of glucose

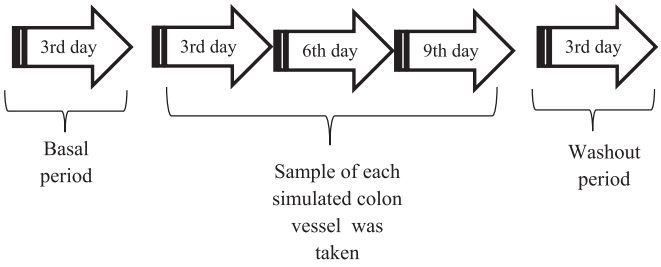

Fig. 1. Experimental setup of SHIME® during "taco" fermentation. Basal period after two weeks of stabilization (system fed only the carbohydrate-based medium). Sample of each simulated colon vessel in the SHIME® system during 9 days (every 3rd day was taken sample for the corresponding analysis). The washout period last 3 days and the system was submitted under the same conditions of the basal period.

(Synth,Brazil), 3 g/L of yeast extract (Acumedia, USA), and 0.5 g/L of Lcysteine (Sigma, USA). Three times *per* day, the liquid feed was entered to the system (Payne et al., 2003; Possemiers, Verthé, Uyttendaele, & Verstraete, 2004) to adapt the microorganisms to the specific environmental conditions (pH range, retention time and available carbon sources) of the three sections of human colon (Possemiers, Verthé, Uyttendaele, & Verstraete, 2004). After two weeks of stabilization (basal period), the reactor was fed during 9 days (every 3rd day was taken sample for the corresponding analysis) with the mixture of 50 g of cooked beans and 50 g of tortilla mixed with 100 mL of carbohydrate-based medium and ground in a blender to obtain a homogeneous particle. The mixture of the "taco" with the carbohydrate-based medium produced a semisolid mixture that simulated the physicochemical characteristics of the "chemo". During washout period, that lasted 3 days, only the carbohydrate-based medium was added to the system (100 mL three times a day). The experimental setup of SHIME® during "taco" fermentation is shown in Fig. 1.

2.3. Microbiological analysis

2.3.1. Analysis of microbiota composition

The intestinal microbiota composition analysis was based on the enumeration of total presumptive counts for the anaerobic bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium* spp., and total coliforms. Total presumptive anaerobic bacteria amounts were determined by plating on Standard Methods Agar (Himedia, Brazil) and anaerobic incubation (Anaerogen Anaerobic System, Probac do Brazil) at 37 °C/48 h. Agar MRS (Himedia, Brazil) was used to determine the population of presumptive lactobacilli. BIM-25 agar with anaerobic incubation at 37 °C/72 h was used to determine the population of presumptive *Bifidobacterium* spp. (Sanguinetti, Dias, & Simpson, 1994). Presumptive *Clostridium* spp. (Munoa & Pares, 1988) was enumerated anaerobically by using Reinforced Clostridial Agar at 37 °C/48 h. Petrifilm™ EC (3 M) with anaerobic incubation at 37 °C/48 h was used to determine the population of total presumptive coliforms. For sampling purposes, 1 mL of a sample taken from each reactor was suspended in 9 mL of peptone water. Serial dilutions were prepared and inoculated into the selective culture media. The plate counts analysis was performed in triplicate.

2.4. Ammonium analysis in digested sample

The ammonia content was determined at 25 °C using a selective ion meter (710A model, Orion) coupled to an ammonia selective-ion electrode (Orion 95–12). This equipment was calibrated using 0.1 M standard ammonium chloride solutions. It was added to every 25 mL of sample took once a week from the simulated colon vessels, throughout the three experimental periods (basal, treatment and washout), 0.5 mL ionic strength adjuster (ISA, Orion) solution, a pH-adjusting and ionic force solution (Bedani, 2008). The analysis was performed in triplicate.

2.5. Analysis of short-chain fatty acids (SCFA')

The SCFA were extracted from the samples with diethyl ether, after the addition of 2-methyl hexanoic acid as an internal standard. The SCFA were quantified using a gas chromatograph equipped with a flame-ionization gas detector, a capillary split/spitless injector and an HP-INNOWAX column with a 30 m × 0.25 mm × 0.25 µm inlet (Shidmadzu GC2010) with a flow rate of 1.56 mL/min of hydrogen as the carrier. The temperatures of the column, injector and detector were 170, 150 and 280 °C, respectively (Van De Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2007). The analyses were made in triplicate and the samples were collected according to the experimental set up (Fig. 1) from the vessels were frozen at −20 °C.

2.6. Statistical analysis

Data of all the analysis are means of three replicates. Significance of all results was evaluated using the statistical software STASTICA, version 10 (Statsoft. 1984–2011 Inc., Tulsa, OK, U.S.A.) with one-way ANOVA, and individual means were compared using the Tukey's test ($p < 0.05$).

3. Results and discussion

3.1. Microbial qualitative changes during "taco" passage through the SHIME® reactor

The changes in intestinal presumptive microbiota during the fermentation of "taco" are shown in Fig. 2. Presumptive counts of total coliforms growth decreased in all the three colon vessels (AC, TC and DC) in the first 3 days of treatment; almost 3 log CFU decrease was observed in the AC. Indeed, in the same period time as presumptive counts of total coliforms, presumptive counts of total anaerobes decreased 2 log CFU in the TC, 1 log CFU in DC and almost 1 log CFU in the AC. In the 6th of treatment, 2 log CFU decreased in presumptive counts of *Clostridium* spp. was found in the simulated TC but in the 9th day of treatment, until 1 log CFU increase was observed in all the three colon vessels. Non-significant differences ($p > 0.05$) were observed during the washout period in the AC and TC.

Presumptive counts of *Lactobacillus* spp. during the treatment was decreased until 2 log CFU in all the colon three vessels. Nevertheless, during the washout period 1 log CFU increase was observed in the AC. In the other hand, after 3 days of treatment, almost 1 log CFU increase in presumptive counts of *Bifidobacterium* spp. population was observed in the simulated AC. But, at 6th day of treatment, 2 log CFU decrease was observed in all the three colon sections. In the simulated DC was observed 1 log CFU decrease ($p < 0.05$) during the washout period.

The main behavior of the growth of the presumptive microorganisms during the fermentation of this staple food was the decreased in the different colon sections, and this behavior was particularly observed for the presumptive counts of *Clostridium* spp. and coliforms. Nevertheless, it is important to take into account that during the fermentation of an IF of a complex food matrix could exist different interactions that limit the availability or bioaccessibility of substrates. Chemical interactions between the food complex matrix (*e.g.* covalent bonds) or the formation of complex among protein-carbohydrates fermented could limited the bioaccessibility of certain nutrients (substrates) to the gut microbiota that may influenced the variations in the microbial groups growth (Jakobek, 2015). The selective effects of any substrate may suggest that species that become more dominant during treatment express metabolic routes for its degradation or they are more resistant to these compounds (Jin & Hattori, 2012). Hence, it would be more difficult for the gut microbiota to access or metabolize the fermentable components of a complex food matrix that reach the colon. This could explain the reliability growth of the presumptive microbial groups measured in this study. The presumptive *Lactobacilli* population

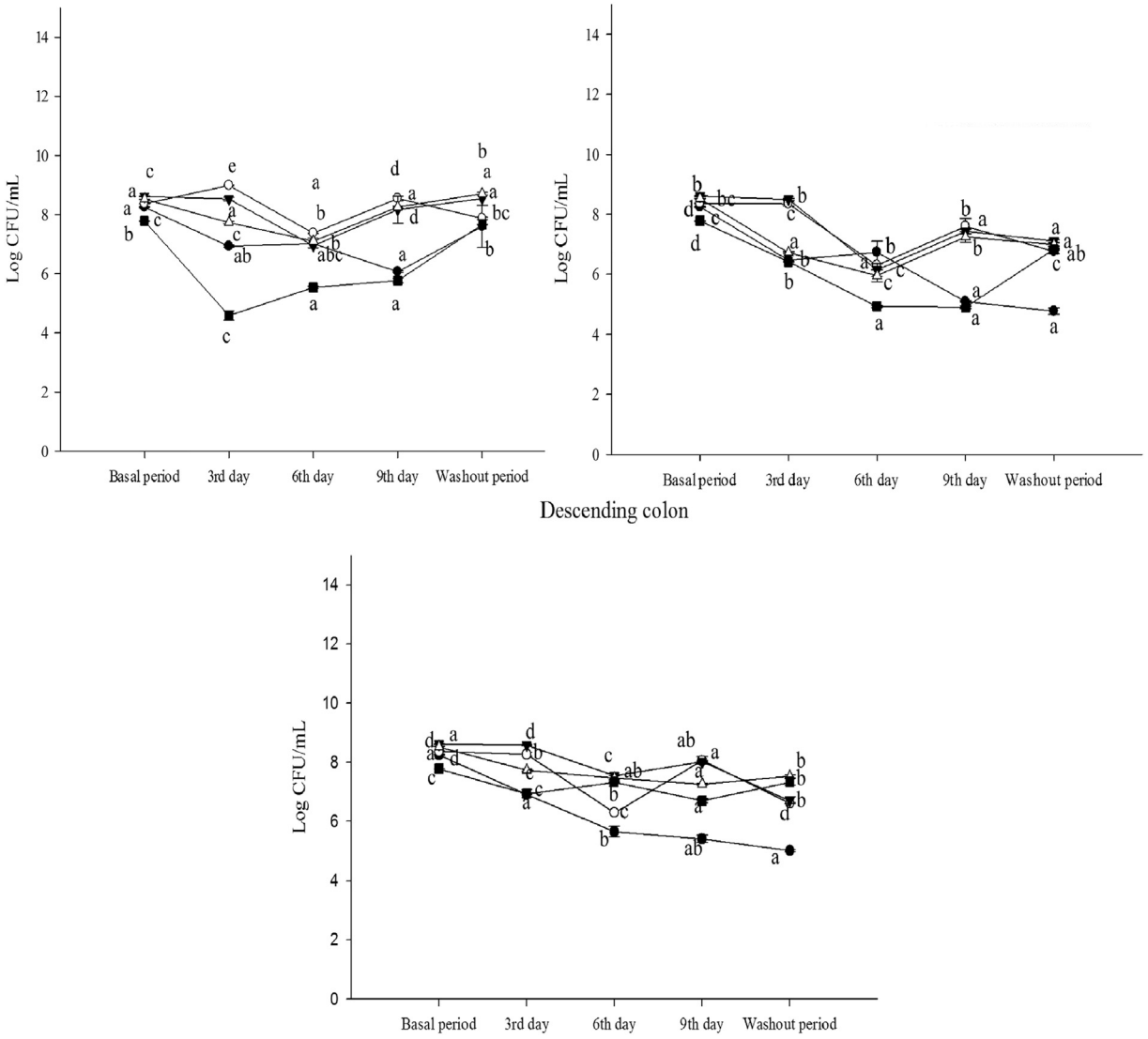

Fig. 2. Average of presumptive counts measurements, expressed in log CFU/mL, for the total plate counts on specific growth media in the corresponding periods in the ascending, transverse and descending colon vessels. —●— *Lactobacillus spp.* —○— *Bifidobacterium spp.* —▼— *Clostridium spp.* —△— Total anerobes. —■— Total coliforms. Data are means of three replicates ± standard deviation. Averages with different letters represent statistical difference the Tukey's test ($p < 0.05$) in the same microbial group.

Table 2
Ammonium ion production (ppm) in SHIME® run during the treatment[1].

	Ascending colon	Transverse colon	Descending colon
Basal period	431.66 ± 1.52^{d}	512.33 ± 12.01^{d}	577.33 ± 21.93^{d}
3rd day	53.16 ± 3.78^{a}	121.00 ± 3.60^{b}	200.66 ± 12.74^{a}
6th day	36.76 ± 2.10^{b}	149.00 ± 1.00^{a}	212.00 ± 3.60^{a}
9th day	49.56 ± 3.82^{a}	155.00 ± 2.00^{a}	267.00 ± 14.73^{b}
Washout period	411.00 ± 8.54^{c}	490.00 ± 5.00^{c}	525.00 ± 4.32^{c}

Different uppercase letters in the same column represent statistical difference to the Tukey's test ($p < 0.05$).

[1] Data are means of three replicates ± standard deviation ($n = 3$).

as well as, certain species of presumptive *Bifidobacteria* population or presumptive *Clostridium* spp. could not express metabolic routes for degradation of PC and other substrates available during the treatment. The effect of dietary polyphenols on the microbiota community due to the microbial breakdown products whose may increase or decrease the growth of bacterial populations in the human intestine (Lee, Jenner, Low, & Lee, 2006). For example, black tea polyphenols and red wine/grape juice (RWGE) polyphenols were fermented in the SHIME® system showing that both polyphenols sources evaluated stimulated *Klebsiella*, and *Akkermansia* and reduced *Bifidobacteria*, *B. coccoides* and *Anaeroglobus* but particularly, black tea polyphenols reduced *Victivallis* and RWGE promoted growth of *Alistipes*, *Cloacibacillus*, *Victivalli* but *Subdoligranulum* and *Bacteroides* were decreased (Kemperman et al., 2013). Then, PC could exert antibacterial properties (Kemperman, Bolca, Roger, & Vaughan, 2010), which could be attributed to the decrease of presumptive anaerobes and total coliforms.

In addition, the gut microbiota in the intestinal metabolism can modulate the effects that PC and other fermentable compound may exert in human health due to the modification in absorption, bioavailability and biologic activity of those, outstanding, the biological effects could not be just attribute to the native compound in food, also to their metabolites (Duda-Chodak, Tarko, Satora, & Sroka, 2015).

3.2. Ammonium ion production

A significant reduction ($p < 0.05$) in ammonium ions production in AC, TC and DC (Table 2) colon sections was observed during the treatment. At the first 3 days, an 86.8, 76.2 and 64.3% reduction ($p < 0.05$) was observed in AC, TC, and DC vessels, respectively. In the

6th day of treatment, a 91% reduction ($p < 0.05$) was observed in the simulated AC, being the higher inhibition percentage in the three vessels. During the washout period an increase ($p < 0.05$) was observed in all the simulated colon vessels. Ammonia is a key product of metabolic pathways in many organisms, encountered frequently at the crossroads of catabolism and anabolism and their production may be the result of total digestion of tissues proteins by less specific microbial proteinases (Kleiner, Traglauer, & Domm, 1998). Frequently, the reason for this toxicity may be a consequence of the molecules ability to protonate and deprotonate rapidly, forming molecular species with very different lipophilic charges (Kleiner, Traglauer, & Domm, 1998). Hence, the decrease during the treatment in ammonium ion production was a positive result because high concentrations of this metabolic product could act as a tumor cancer promotor in colon (Hughes, Magee, & Bingham, 2000; Scott, Gratz, Sheridan, Flint, & Duncan, 2013). Since 1942, it has been reported that decrease in ammonium ion production is a consequence of the fermentation of carbohydrate in cultures because of glucose ability to inhibit synthesis of alanine and glutamate deaminases and aspartase attributed to low pH because of the production of certain acids (nowadays, SCFA's) (Epps & Gale, 1942). It has been reported that the ammonium concentration in the intestinal lumen increases progressively from the ascending colon to the descending colon due to the higher rate of protein fermentation in the descending colon relative to the ascending colon (Macfarlane, Gibson, & Cummings, 1992). Then, the lower ammonium production in the ascending colon is attributed to low pH and high availability of carbohydrates in this region (Smith & Macfarlane, 1998).

3.3. Analysis of short chain fatty acids (SCFA)

The concentration (mmol/L) of SCFA produced is shown in the Table 3. The production of acetate was among 120.83 to 191.99 mmol/L during the fermentation, non-significant differences ($p > 0.05$) in propionate and butyrate production were observed. Acetate is the most abundant SCFA in the colon and it represents more than the 50% of the total SCFA detected in human feces (Louis, Scott, Duncan, & Flint, 2007). The production of acetate can be due to the content RS and DF of the "taco". In the other hand, the producers species of propionate and butyrate could not grew selectively during the treatment in this study because SCFA production may be influence by the number and species of microorganisms present in the colon, as well as, the substrate available (Macfarlane & Macfarlane, 2003), *e.g.*, *Coprococcus* species such as *Faecalibacterium prausnitzii* within the *Ruminococcaceae* (clostridial cluster IV) and *Eubacterium rectale* within the *Lachnospiraceae* (clostridial cluster XIVa) are producers of butyrate (Geirnaert et al., 2014). Polysaccharides extracted from cooked black bean seeds have been fermented in a static system and the concentration of acetate (39–51 mmol/L), propionate (8–12 mmol/L), and butyrate (8–15 mmol/L) were evaluated (Campos-Vega et al., 2009). The maximum amount of acetate, propionate and butyrate production during the fermentation of Mexican "taco" in this study was 191.9, 31.2, and 74.1 mmol/L, respectively. Zamora-Gasga et al., 2018 reported the production of SCFA in traditional Mexican corn "tortilla" fermented in a static *in vitro* digestion system. After 48 h of fermentation, the production of acetate, propionate and butyrate was 498, 293.75 and 331.96 mmol/L per 100 mg of traditional Mexican corn "tortilla" fermented. Nevertheless, the calculation of the molar ratio for acetate, propionate and butyrate (A:P:B molar ratio) would let us to analyze if the abundance expressed in percentage (%), of SCFA during the treatment was higher or lower than the basal period and washout period and if it was higher or lower compare with other fermented foods. The molar ratio for acetate, propionate and butyrate (A:P:B ratio) calculated with the average production of SCFA during the basal period with Mexican "taco" and the washout period in each simulated colon vessel is shown in the Fig. 3.

In order to compare the molar ratio proportions for SCFA during the fermentation of the Mexican "taco" with another important sources of polysaccharides fermented in the SHIME® system, it was analyzed the molar ratio for SFCA during FOS and orange juice fermentation. In the simulated TC the molar ratio for butyrate in the 6th day of fermentation of Mexican "taco" was 24.0% while the molar ratio during FOS fermentation was 12.3%. In the AC and DC, the molar ratio for propionate in the 9th day of the treatment with Mexican "taco" was 8.75 and 14.78%, respectively, and compared with the molar ratio of FOS was 2.9 and 13.4%, respectively (Sivieri et al., 2014) using the same system. Besides, in the same simulated regions mentioned, AC and DC, the molar ratio for acetate during the treatment with Mexican "taco" was 84.3 and 75.3% during the 6th and 3rd day of fermentation, respectively, compared with the 66.4 and 64.5% molar ratio for acetate during FOS fermentation (Sivieri et al., 2014). However, the molar ratio for butyrate in the 3rd day of the treatment with Mexican "taco" was 21.4% in the simulated AC, while during the fermentation of fresh

Table 3
Short-chain fatty acid (SFCA) concentration (mmol/L) from the ascending colon (AC), transverse colon (TC), and descending colon (DC) vessels as a result of the treatment with the Mexican "taco"[1].

SCFA	Experimental period	AC	TC	DC
Acetate	Basal period	38.72 ± 14.89^{b}	43.25 ± 4.92^{a}	35.15 ± 3.01^{c}
	3rd day	191.99 ± 23.00^{a}	168.23 ± 3.67^{c}	164.59 ± 5.75^{a}
	6th day	142.13 ± 9.15^{a}	150.25 ± 14.46^{bc}	160.95 ± 1.48^{a}
	9th day	149.16 ± 15.19^{a}	120.83 ± 3.29^{b}	129.10 ± 18.44^{ab}
	Washout period	49.77 ± 16.37^{b}	61.69 ± 9.51^{a}	106.44 ± 10.87^{b}
Propionate	Basal period	7.35 ± 0.19^{a}	9.99 ± 2.70^{a}	9.56 ± 0.05^{a}
	3rd day	5.27 ± 0.73^{a}	31.24 ± 12.01^{a}	21.88 ± 2.28^{a}
	6th day	4.92 ± 2.27^{a}	23.73 ± 3.02^{a}	24.06 ± 0.40^{a}
	9th day	17.92 ± 8.27^{a}	18.49 ± 0.41^{a}	29.14 ± 9.76^{a}
	Washout period	10.44 ± 3.55^{a}	16.60 ± 6.48^{a}	29.29 ± 10.91^{a}
Butyrate	Basal period	10.27 ± 1.60^{a}	15.21 ± 6.14^{a}	17.04 ± 2.78^{a}
	3rd day	53.83 ± 9.57^{b}	74.19 ± 44.88^{a}	32.03 ± 6.05^{a}
	6th day	21.36 ± 9.72^{a}	55.03 ± 4.74^{a}	40.81 ± 2.00^{a}
	9th day	37.62 ± 6.52^{ab}	37.08 ± 2.49^{a}	69.42 ± 41.89^{a}
	Washout period	16.44 ± 8.52^{a}	34.21 ± 21.35^{a}	60.05 ± 34.23^{a}
Total SCFA	Basal period	56.35 ± 13.09^{a}	68.45 ± 13.78^{a}	61.75 ± 0.16^{b}
	3rd day	251.11 ± 12.68^{c}	273.67 ± 53.21^{b}	218.49 ± 2.58^{a}
	6th day	168.42 ± 21.16^{b}	229.01 ± 22.23^{b}	225.83 ± 0.11^{a}
	9th day	204.71 ± 29.98^{bc}	176.41 ± 0.39^{ab}	227.67 ± 13.68^{a}
	Washout period	76.66 ± 4.30^{a}	112.51 ± 18.32^{a}	195.79 ± 34.27^{a}

Different uppercase letters in the same column represent statistical difference to the Tukey's test ($p < 0.05$).
[1] Data are means of three replicates ± standard deviation (n = 3).

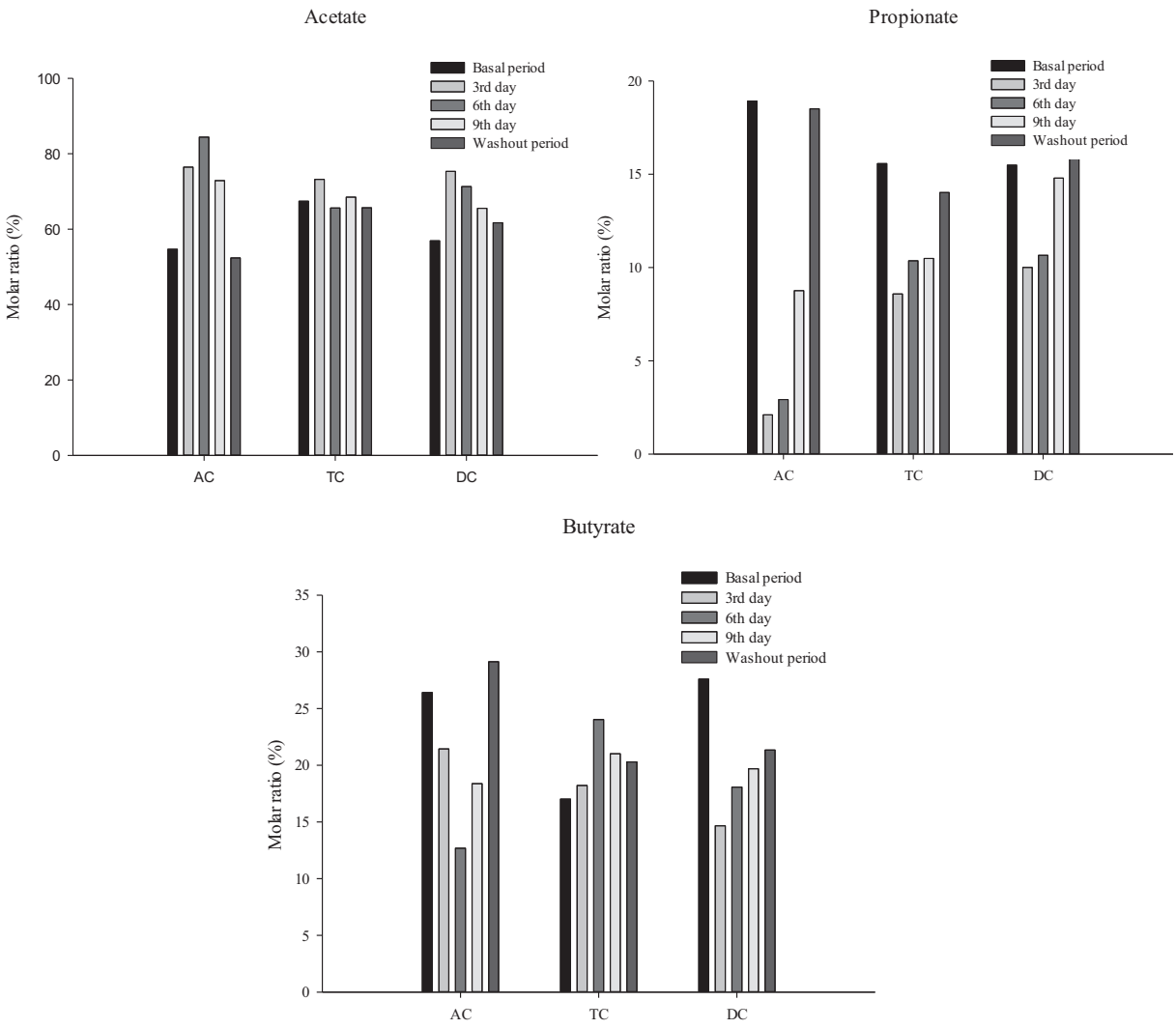

Fig. 3. Molar ratio for acetate, propionate and butyrate (A:P:B ratio) of the average production in the ascending colon (AC), the transverse colon (TC), and the descending colon (DC) vessel of the SHIME® system as a result of the treatment with the Mexican "taco".

orange juice was 11.8% in the same simulated colon vessel (Duque, Monteiro, Adorno, Sakamoto, & Sivieri, 2016). Moreover, the maximum molar ratio of acetate during the fermentation of chitin-glucan in a TWINSHIME® reactor system was 53% and among 14–24% for butyrate (Marzorati, Maquet, & Possemiers, 2017). The molar ratio for acetate was higher during the treatment with Mexican "taco" (84.3%) than the molar ratio of acetate during the fermentation of chitin-glucan while the molar ratio for butyrate during the fermentation of Mexican "taco" was the same value reported during the chitin-glucan fermentation. The molar ratio for the production of propionate and butyrate by the microbiota after 48 h of traditional Mexican corn "tortilla" fermentation was 26.14% and 29.54%, respectively (Zamora-Gasga et al., 2018). This molar ratio is higher than the molar ratio for the production of propionate and butyrate by the microbiota (14.78% in the DC and 24% in the TC, respectively) during the fermentation of Mexican "taco" in the SHIME® system but the molar ratio for acetate during the treatment with Mexican "taco" was higher (84.3% in the AC) than the molar ratio for the production of acetate by the microbiota was higher during the treatment in traditional Mexican corn "tortilla" (51.23%). Even an increase in acetate, propionate and butyrate production was not observed during the "taco" fermentation, the molar ratio for acetate was higher compared to the molar ratio for acetate during other polysaccharides fermentation. Thus, the Mexican "taco" could be an enriched substrate for the microbiota for the production of acetate.

4. Conclusions

Mexican "taco" showed a possible potential functional profile of an ancestral staple food due to the production of SCFA and the decrease in ammonium ion concentrations during its fermentation. In order to improve our knowledge of the effects in gut microbiota metabolism of the colonic fermentation of cooked food (even the preparation method could influence during the fermentation) as eaten (mixturing protein, carbohydrates, fiber, bioactive compounds) and the possible effects in host health of gut metabolites, combination of *in vitro* and *in vivo* studies are needed.

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