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Influence of the Phenolic Compounds of a Concentrated Tomato Pulp on the Modulation of the Intestinal Microbiota

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ABSTRACT

Tomatoes and their derivatives contain bioactive compounds such as phenolic compounds, carotenoids and vitamins which positively contribute to human health. This study aimed to evaluate the bioaccessibility and biotransformation of phenolic compounds in a tomato pulp using static *in vitro* model of digestion and an *in vitro* colonic fermentation. The *in vitro* digestion of the concentrated tomato pulp (CTP) enhanced total phenolic compounds content, while colonic fermentation decreased the bioaccessibility of phenolic compounds over 72 hours of fermentation. Tomato pulp increased propionic, butyric and valeric acids production compared to control and also ammonium ions production. *Lactobacillus* and *Bifidobacterium* remained present after 48 h and, although a significant reduction ($p < 0.05$) in total coliforms and *E. coli* counts in the presence of tomato pulp was also observed, *Clostridium* was the predominant microorganism during colonic fermentation.

Keywords: Ammonium ions, Bioaccessibility, Colonic fermentation, Short chain fatty acids.
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INTRODUCTION

Tomatoes and derivatives are great sources of bioactive compounds which are known to promote proper body function and health benefits (Vallverdú-Queralt et al., 2011; Salehi et al., 2019). This effect has been attributed, in part, to the antioxidant capacity demonstrated by the phenolic compounds present in the fruit. Regular consumption and adequate amounts of vegetables, fruits and beverages rich in phenolic compounds are associated with the prevention of several chronic non-transmissible diseases (Aboul-Enein et al., 2013; Catalkaya et al., 2020). Foods that have flavonoids can contribute to health through their neuroprotective and cardiovascular effects (Bernatova, 2018). The ingestion of flavonoids as natural compounds present in *natura* foods has the advantage of being rapidly absorbed in the intestine after digestion and avoids side effects for the consumer (Agrawal, 2011).

However, the bioactivity of a compound is directly related to its bioaccessibility, which consists of the amount of a certain component that is released from the food matrix in the gastrointestinal tract, making it available for intestinal absorption (Heaney, 2001). There are many studies evaluating food processing impact on the bioaccessibility of bioactive compounds (Barba et al., 2017; Cilla et al., 2018; Briones-Labarca et al., 2019). Likewise, colonic fermentation has been extensively investigated in order to verify the contribution of these compounds in the modulation of the gut microbiota by application of *in vitro* models that simulate fermentative activity (Mosele et al., 2015; Cueva et al., 2017; Guo et al., 2020). Research has shown that the beneficial health properties of phenolic compounds are partly related to their interaction with the gut microbiota (Queipo-Ortuno et al., 2012). Analysis of short-chain fatty acids and ammonium ions produced by gut microbiota during colonic fermentation are useful indicators to evaluate the beneficial effects of phenolic compounds on colon health.

The main goal of the present study was to simulate an *in vitro* gastrointestinal digestion and colonic fermentation of phenolic compounds of concentrated tomato pulp in order to evaluate its influence on the modulation of the gut microbiota.

MATERIALS AND METHODS

Fruit material and sample preparation

Fresh ripe Italian-type tomatoes were purchased from the local market (Rio de Janeiro, Brazil). Fruits were washed, sanitized by immersion in chlorine solution 200 ppm for 20 min, rinsed with potable water and then manually cut and depulped in a horizontal depulper (Bonina 0.25 dF - Itametal, Itabuna, Brazil) equipped with a 0.8 mm diameter sieve that separated the whole pulp from the peels and seeds. The obtained pulp was concentrated once for 120 min in a jacketed pan (Incapri Máquinas, São Paulo, Brazil) equipped with a steam generation boiler, under constant agitation of 85 rpm and temperature ranging between 80-90 °C, monitored by a thermocouple (Salcasterm 200 - Salcas). The concentrated tomato pulp (CTP) was placed in plastic flasks and stored at -18 ºC until use.

In vitro gastrointestinal digestion assay

The static *in vitro* digestion assay was performed according to the standardized INFOGEST© protocol (Brodkorb et al., 2019). The method comprises the use of simulated digestive fluids, made of electrolytes with concentrations based on physiological conditions to simulate the oral, gastric and intestinal phases of gastrointestinal digestion. The Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were prepared as described by Minekus et al. (2014).

In the oral phase, 5 g of CTP was weighted in a 50 ml centrifuge tube. 4 ml of SSF stock solution (pH 7.0 ± 0.1), 0.5 ml of amylase solution 75 U/ml (Sigma®-Aldrich A3176;), 25 µl of 0.3 M $CaCl_2$ and 475 µl of ultrapure water were added to a final volume of 10 ml. The tube was incubated under stirring (100 rpm) in a water bath (Dubnoff-Novatecnica/Brazil) at 37 ºC for 2 minutes. To proceed to the gastric phase, 8 ml of SGF stock solution (pH 3.0 ± 0.1), 0.5 ml of pepsin solution 2000 U/ml (Sigma®-Aldrich P7000;), 5 µl of 0.3 M $CaCl_2$, 995 µl of ultrapure water were added to a final volume of 20 ml. The pH of the mixture was adjusted to 3.0 ± 0.2 with 1 M HCl and the tube was incubated under stirring (100 rpm) for 2 hours in the water bath at 37 ºC. For the intestinal phase, the pH of the gastric phase was adjusted to 7 with 1 M NaOH to perform intestinal digestion. Then, 8.5 ml of SIF stock solution (pH 7.0 ± 0.1), 5 ml of pancreatin solution 100 U/ml (Sigma®-Aldrich P1750;), 2.5 ml of bile solution (Sigma®-Aldrich B3883 in SIF) and 40 µl of 0.3 M $CaCl_2$ were added to a final volume of 40 ml. The intestinal phase occurred for 2 hours in a water bath under stirring at 37 ºC. All enzyme activities were determined according to Minekus et al. (2014). At the end of each digestion stage of incubation, the tubes were immersed in an ice bath to interrupt the enzyme activity. Each sample was analyzed in triplicate and using a control tube.

In vitro colonic fermentation

This project was approved by the research ethics committee of Clementino Fraga Filho Hospital from the Federal University of Rio de Janeiro, Brazil (approval number 4.502.928).

Fresh fecal samples from a single healthy donor (body mass index between 18.5 and 24.9 kg/m^2, with regular bowel function, no use of antibiotics, prebiotics or probiotics for the previous 3 months and alcoholic beverages for the last 48 h before feces collection) were collected in a sterile container, on-site, at the day of the experiment.

Fecal fermentation experiments were performed according to Inada et al. (2020), with slight modifications. The fecal suspension was prepared by diluting the feces (5 % (w/v)) in a nutrient-rich culture medium. The culture medium and all the materials used were previously autoclaved. The medium was saturated with CO_2 for 48 h inside an anaerobic chamber and the experiment was conducted in a laminar flow to avoid external contaminations.

In glass containers with a screw cap, the fecal suspension was mixed with the digested sample from the final gastrointestinal digestion (1:1 v/v). The assay was conducted using two controls: fecal suspension only (C1) to determine the presence of basal phenolic compounds in the fecal sample and the nutrient-rich culture medium and the digested sample (C2) to evaluate the chemical transformation of phenolic compounds. All flasks were filled with nitrogen to ensure anaerobic conditions. The fermentation was performed by incubating under anaerobiosis at 37 °C for 0, 24, 48 e 72 h.

Analytical methods

Analysis of flavonoids and phenolic acids

The concentrated tomato pulp sample was submitted to phenolic compounds extraction according to the methodology described by Nascimento et al. (2017). The analysis of free phenolic acids was performed by the extraction with 4 ml methanol: water (50:50 v/v, pH 2), followed by mechanical stirring for 1 hour and centrifugation (5000 G) for 10 minutes. The supernatant was collected. Then, 4 ml of acetone: water (70:30; v/v) was added and the mechanical stirring and centrifugation steps were repeated. The supernatant was collected. 3 ml of both supernatants were mixed and transferred to a

1.5 ml vial for the chromatographic injection. The extraction of hydrolyzed phenolics was carried on with a 5 ml solution of NaOH (2 mol/l) containing 1 % of ascorbic acid and 10 mM EDTA that was added to the samples followed by heat at 61-63 °C for 60 minutes. Then, 1.5 ml of HCl (6 mol/l) was added. The solution was vortexed for 10 seconds and centrifuged (2700 rpm) for 10 minutes. The supernatant was collected, and 6.5 ml of ethyl acetate was added. The organic phase was removed and the extraction with ethyl acetate was repeated. The organic fraction was dried under a nitrogen gas (N_2) flow and then diluted in methanol for chromatographic analysis.

The analyses of the two fractions (free and hydrolyzed phenolic acids) of CTP were performed in a High-Performance Liquid Chromatograph Alliance Waters™ model 2690/5, a Waters™ photodiode array detector model 2996 (270, 310 and 370 nm), with a Thermo Hypersil BDS C18 column (100 mm × 4.6 mm × 2.4 µm), a 1.0 ml/min flow, an injection volume of 10 µl, a run time of 28 min, and the elution mode gradient used an aqueous solution of 0.15 % phosphoric acid (95 %) and acetonitrile (5 %). The quantification of flavonoids and phenolic acids was performed by external standardization.

The other samples (fractions from digestion and colonic fermentation) were centrifuged after remove aliquots from each step, then the supernatant was collected and filtered through a 0.22 µm filter until chromatographic analysis. The identification of compounds was performed by comparing retention times and UV/Vis spectra with commercial analytical standards (purity ≥ 95 %) from Sigma-Aldrich™ (USA). External standard method was used for quantifications. The results were expressed in mg of compound per 100 g of tomato pulp.

Microbiological counts

Sample aliquots (1 ml) from *in vitro* colonic fermentation were serially diluted in sterile peptone water (0.1 %) and cultivated on a selective culture medium for each evaluated microorganism. Agar De Man Rogosa and Sharpe (MRS) acidified with glacial acetic acid (pH = 5.4) for *Lactobacillus* spp. and Reinforced Clostridial Agar (RCA) for *Clostridium* spp. RCA was added with Bifidobacterium iodoacetate medium 25 (BIM-25) for *Bifidobacterium* spp. Microbiological counts were performed at initial time (time 0) and after 48 hours of *in vitro* colonic fermentation. The plates were incubated for 5 days in anaerobic conditions at 37 °C for all culture medium. Total coliforms and *Escherichia coli* analysis were performed inoculating samples in Petrifilm® (3M Corporation, St. Paul, MN, USA) at 35 °C for 24 h and 48 h, respectively.

Short Chain Fatty Acids (SCFAs) analysis

The extraction of SCFAs was performed according to Adorno et al. (2014), after *in vitro* colonic fermentation. SCFAs were analyzed by high-resolution gas chromatography system (7890A- Agilent Technologies, U.S.) fitted with a flame ionization detector (FID) and a fused silica capillary column (FFAP- Free Fat Acids Phase) (25 m x 0.2 mm x 0.30 µm). The identification was performed by comparing the retention times of samples with pure patterns of fatty acids injected under the same conditions. Quantification was determined using the internal standard addition method (crotonic acid) and the results were expressed in mmol of fatty acid per liter of digested sample.

Ammonium ion determination

Ammonium ions (NH_4^+) were determined at the initial time (t = 0) and after 24, 48 e 72 h of incubation, using a portable Hanna Checker HI733 colorimetric ammonia analyzer (Hanna Instruments, USA). The results were expressed as mg of NH_4^+ per liter of sample. Ammonium ions (NH_4^+) were determined in triplicate.

Statistical analysis

All results of *in vitro* gastrointestinal digestion phases and colonic fermentation time were reported as mean ± standard deviation of three repetitions. In colonic fermentation, the phenolic content of the control containing only feces (C1) was subtracted from the content of phenolic compounds found on the fermented tomato pulp (with fecal suspension). The differences between digestion phases and colonic fermentation time were determined by One-way ANOVA and Tukey´s test with a significant level of 5 %. All analyses were performed using the software Statsoft Statistica 10 (Tulsa, USA).

RESULTS AND DISCUSSIONS

Bioaccessibility and phenolic compounds profile throughout gastrointestinal digestion

Although it is important to know the phenolic profile in fresh tomato pulp (not concentrated), unfortunately, it was not possible to show these results in this study. It is known that the phenolic composition of tomatoes is influenced by growing conditions, the type and the maturity stage of fruit and also interfered by the time and temperature of processing its derivatives (Kelebek et al., 2017; Asensio et al., 2019; Coyago-Cruz et al., 2022).

Normally, caffeic, chlorogenic and coumaric acids, quercetin and rutin are the main phenolic compounds found in fresh tomatoes and related products (Epriliati and Ginjom, 2012; Cruz-Carrión et al., 2022). Nevertheless, in the present study, only three phenolic compounds were identified and quantified in the non-digested concentrated tomato pulp (two phenolic acids

Table 1. Phenolic compounds content (mg/100 g) of digested fraction after *in vitro* digestion and colonic fermentation.

Compound	Undigested sample	Gastric digestion	Intestinal digestion (% B)	Colonic Fermentation (% B)			
				t = 0	t = 24	t = 48	t = 72
Ferulic acid	0.06 ± 0.00	nd	nd	nd	nd	nd	nd
p-Coumaric acid	0.02 ± 0.00	nd	nd	nd	nd	nd	nd
Gallic acid	nd	1.81 ± 0.12 [a]	23.77 ± 2.02 [b]	0.56 ± 0.05 [c]	0.08 ± 0.01 [d]	0.08 ± 0.01 [d]	0.10 ± 0.01 [d]
Hydroxyphenylacetic acid	nd	nd	nd	nd	4.60 ± 0.22 [a]	4.29 ± 0.26 [a]	4.41 ± 0.31 [a]
Catechin	nd	12.82 ± 0.56 [a]	158.83 ± 16.80 [b]	4.95 ± 0.33 [c]	10.58 ± 0.25 [a]	11.57 ± 0.17 [a]	11.94 ± 0.04 [a]
Rutin	1.21 ± 0.07 [a]	1.12 ± 0.06 [a]	0.82 ± 0.09 [a] (68)	0.01 ± 0.00 [c] (1)	nd	nd	nd
Total phenolic compounds	1.29 ± 0.59 [a]	15.75 ± 6.56 [b]	183.42 ± 85.34 [c] (14219)	5.52 ± 2.71 [d]	15.26 ± 5.27 [b]	15.94 ± 5.81 [b]	16.45 ± 5.99 [b] (1275)

Values reported as the sum of free and hydrolysable fractions and presented as mean (mg/100 g) ± standard deviation (n = 3).(% B): Bioaccessibility (%). nd: not detected. Total phenolic compounds: reported as the sum of the identified compounds. Undigested sample: concentrated tomato pulp before simulated digestion. Different letters on the same row indicate significant difference between the fractions of *in vitro* digestion and colonic fermentation according to Tukey's test ($p < 0.05$).

and one flavonoid), totaling 1.29 mg/100 g among the detected compounds (Table 1).

Other substances with a characteristic spectrum of phenolic compounds were detected, but they could not be fully identified due to a lack of analytical standards. The major compound identified in the samples was the flavonoid rutin (93 %), followed by two hydroxycinnamic acids: ferulic acid (5 %) and p-coumaric acid (2 %). These values are in part in contrast to previously reported for tomato pastes processed by hot break (Vallverdú-Queralt et al., 2012; Kelebek et al., 2017) that observed rutin, naringenin, quercetin and chlorogenic acid as the main phenolic compounds. However, it has to be considered that in both studies, the pulp processing was not similar to the one performed in the present study.

As phenolic compounds seem not to be largely modified at the oral stage, it was chosen not to evaluate the samples of this phase of digestion (Pinacho et al., 2015; Mosele et al., 2016). Regarding total phenolic compounds content, the obtained bioaccessibility higher than 100 % suggests that the release of phenolic compounds from the food matrix could be enhanced by gastrointestinal digestion and/or that phenolics were metabolized from more complex structures (Cueva et al., 2017).

Ferulic acid and p-coumaric acid were not detected in the digestion fractions, indicating that they could have been negatively affected by the digestion or as both substances were present in low concentrations in the undigested sample, their released contents during digestion samples were less than the method detection limit used.

Gallic acid and catechin showed different behavior. They were not identified in the undigested pulp but were released in the gastric phase of digestion with a remarkable increase (13 and 12 times more, respectively) in the intestinal fraction ($p < 0.05$). Nevertheless, the bioaccessibility of these compounds could not be calculated since they were not detected in the undigested tomato pulp. The phenolic compounds could be trapped by dietary fiber or bonded to other components within the food matrix, and thus unable to be released and extracted by the extraction method or maybe these compounds were previously present in the concentrated tomato pulp as one of its derivatives (Cueva et al., 2017 apud Ruíz-García et al., 2022; Alara et al., 2021).

Rutin did not appear susceptible to digestion conditions and no significant difference ($p > 0.05$) was observed in its content throughout the digestive process. It was the only phenolic compound identified in the undigested sample and detected after digestion, showing high release after gastric and intestinal digestions (93 % and 68 %, respectively) becoming accessible to be absorbed by the small intestine or fermented by the gut microbiota in the colon.

Effect of colonic fermentation in phenolic compounds biotransformation

The individual phenolic composition at colonic fermentation stage is shown in Table 1. Only gallic acid (10 %), catechin (89 %) and rutin (0.18 %) were detected and then available for colonic microbiota at the beginning of colonic fermentation (t=0). Regarding the phenolic compound's biotransformation, it was observed that fermentation time did not affect the phenolic composition after 24 h fermentation.

Biotransformation of phenolic compounds occurs

through different enzymatic reactions including hydroxylation, oxidation, decarboxylation, methylation, isomerization, hydration, dehydrogenation and glycosylation catalyzed by the gut microbiota (Cardona et al., 2013; Gowd et al., 2019; Catalkaya et al., 2020). Ferulic acid and p-coumaric acid were not detected throughout the entire digestion and colonic fermentation phases. Gallic acid content decreased to 86% after 24 h fermentation suggesting its biotransformation. A possible metabolic pathway for gallic acid biotransformation is to undergo a reduction reaction into pyrogallol (Wang et al., 2022). On the other hand, catechin content increased 114 % after 24 h fermentation. The results showed catechin as the predominant compound along colonic fermentation, remaining constant after 24 h fermentation. Li et al. (2022) reported the opposite since it was not detected catechin after 24 hours of fermentation, indicating that it was rapidly and completely metabolized by the fecal microbiota. Rutin was detected in very low concentration at 0 h fermentation time and was no longer detected along the process. This small fraction seemed to be rapidly degraded and/or modified by microbial enzymes. It is possible that rutin has been catabolized into hydroxyphenyl acetic acid, as noted by Havilik et al. (2020) when evaluating the catabolism of rutin into phenolic acids by the gut microbiota in the presence of fibers.

Hydroxyphenylacetic acid, which was not detected during digestion steps nor at 0 h fermentation, was identified from 24 hours onwards, remaining constant until the end of fermentation. Hydroxyphenylacetic acid is a phenolic metabolite produced by the intestinal microbiota during colonic fermentation and, along with hydroxyphenylpropionic acid, are the main products of the fermentation of phenolic acids and flavonoids (Catalkaya et al., 2020). Cárdenas-Castro and collaborators (2021) also observed 3-hydroxyphenylacetic acid as the major metabolite after 48 h colonic fermentation of phenolic compounds of tomato.

Gut microbiota profile and metabolism during fermentation of concentrated tomato pulp

To our knowledge, no prior studies have examined the effects of tomato pulp and products on intestinal bacteria. In the present study, counts of different bacterial groups were used to monitor microbial behavior during colonic fermentation of concentrated tomato pulp. So, *Clostridium* spp., total coliforms and *E. coli* were used as representative pathogenic bacteria of the intestinal microbiota, while *Lactobacillus* spp. and *Bifidobacterium* spp. were associated as beneficial bacteria.

Counts of the microbial groups after 48 h colonic fermentation are presented in Figure 1. CTP did not promote the growth of intestinal microbiota. Except for *Clostridium* spp, in which the results indicated that the addition of CTP did not affect its multiplication after 48 h ($p > 0.05$), a significant population decrease in the other microbial groups studied ($p < 0.05$; $\Delta \log \geq 1$) was observed in response to CTP addition to the feed medium compared to the control at 48 h fermentation. The obtained results are in contrast to the expected in relation to the literature available for other matrices since sufficient evidence showed that, regarding the beneficial prebiotic effects, polyphenols exert the result by enhancing the growth of the probiotic bacterial family such as *Bifidobacteriaceae* and *Lactobacillaceae* and by inhibiting pathogenic bacteria such as *E. coli, Clostridium perfringens* and *Helicobacter pylori* (Guergoletto et al., 2016; Morais et al., 2016).

This result can be attributed to the presence of phenolic compounds in the sample since they seem to be associated with antibacterial effects due to the toxicity of the phenol groups (Espín et al., 2017).

In order to evaluate the influence of CTP on the fermentative activity of gut microbiota, the content of volatile short chain fatty acids (SCFA) and the production of ammonium ions was also analyzed during colonic fermentation of CTP. Table 2 shows the SCFA production during colonic fermentation of CTP. Production of acetic acid was not observed for the entire fermentation time compared to the control sample. Production of the other SCFA was increased ($p < 0.05$) at the first 24 h fermentation, reaching a plateau after 48 h incubation ($p > 0.05$). Propionic, butyric and valeric acids were the major SCFA found during fermentation of CTP, being followed by a lower production of hexanoic acid, not detected at 0 h fermentation, and also by the branched-chain fatty acids (BCFA): isobutiric and isovaleric acids. Except for acetic acid, SCFA and BCFA production was significantly higher than the control sample ($p < 0.05$). The obtained results are similar to the study carried out by Wang et al. (2022) in which a significant increase in acetic, propionic, butyric, isobutyric and valeric acids production after 4 h and a plateau after 16 h of tomato fermentation was observed. After gastrointestinal digestion, CTP probably still presented enough content of macronutrients such as dietary fiber, polysaccharides and proteins which served as an energy source for the gut microbiota yielding SCFA and BCFA as end-metabolites of gut microbiota metabolism (Danneskiold-Samsøe et al., 2019; Rios-Covian et al., 2020). Acetate, propionate and butyrate represent about 95 % of the fatty acids present in the gut, being crucial in metabolic functions and intestinal health (Canfora et al., 2015). Despite the major production of SCFA during colonic fermentation, the intestinal microbiota can also produce lower amounts of isobutyric, isovaleric, and 2- methylbutyric acids, known as (BCFA) (Rios-Covian et al., 2020). In the human intestine, the BCFA is produced during the fermentation of branched-chain amino acids (valine, leucine and isoleucine) mainly by genera *Bacteroides* and *Clostridium* (Smith and Macfarlane, 1998; Aguirre et al., 2016). It was demonstrated that the fiber-bacterial community

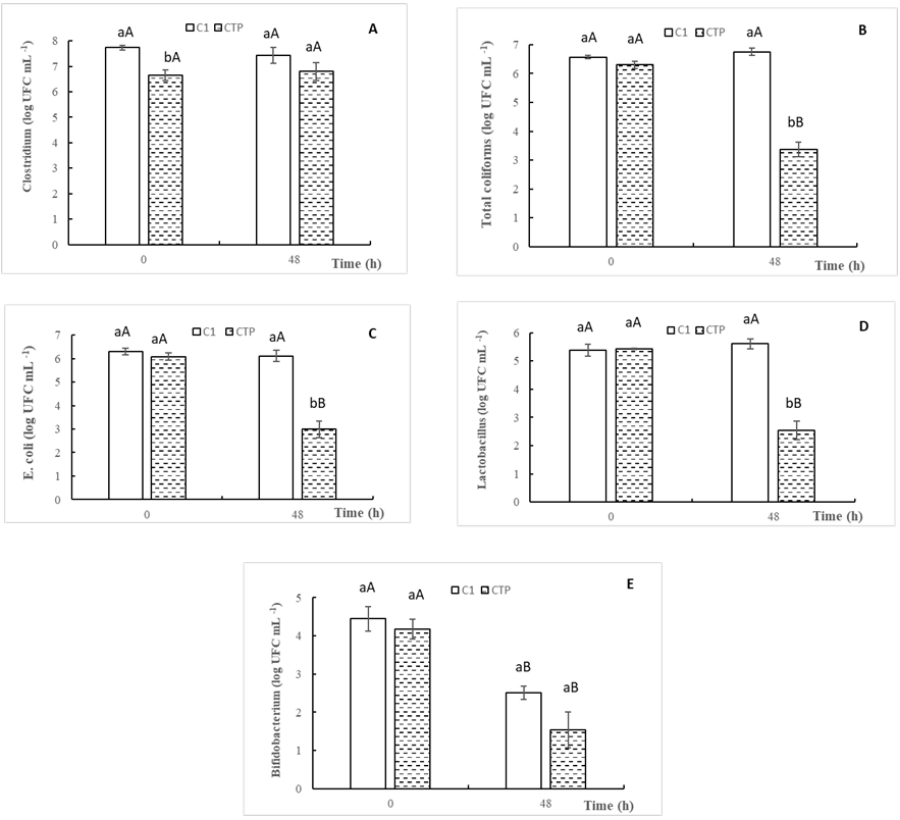

Figure 1: Population of *Clostridium* spp. (A), Total coliforms (B), *E. coli* (C), *Lactobacillus spp.* (D) and *Bifidobacterium* spp. (E) during the fermentation of control with feces (C1) and concentrated tomato pulp with feces (CTP) at the initial time (t = 0 h) and after 48 hours of incubation. Different lower case letters at the same time indicate significant difference between C1 and CTP, according to Tukey´s test ($p < 0.05$). Different uppercase letters in the same column indicate significant differences between the control with feces (C1) or tomato pulp with feces (CTP) at different time, according to Tukey´s test ($p < 0.05$).

dependency for the type of SCFAs produced was not only related to the type of substrates offered to the microbiota, but also to the interactions between the bacteria in the intestinal environment (Reichardt et al., 2018).

Ammonium ions production increased throughout the colonic fermentation both for Control (C1) and for the concentrated tomato pulp (CTP). CTP fermentation produced more ammonium ions than the control C1. At the first 24 h of incubation was observed an increase in the ammonium ions content of C1 (145 to 415 mg NH_4^+/l) and CTP (124 to 569 mg NH_4^+/l) during the fecal reaction, representing an increase of 186 and 358 %, respectively. At 48 h and 72 h fermentation, the fecal reaction generated significantly less ammonia compared to the previous time. Protein catabolism during digestion by colonic bacteria can lead to the production of appreciable levels of ammonia in the gastrointestinal tract which can be hazardous to human health since it is closely related to the pathogenesis of hepatic encephalopathy (Chen et al., 2021). Bacterial species able to grow on individual amino acids as nitrogen and energy source include many of the 'putrefactive' *Clostridium, Peptostreptococcus* and *Fusobacterium* species (Smith and Macfarlane, 1996; Richardson et al., 2013). In the present study, the results showed that *Clostridium* was the major microorganism in tomato pulp after 24 hours of fermentation, indicating a correlation between bacterial growth and ammonium production.

CONCLUSIONS

The most abundant and bioaccessible phenolic compound in concentrated tomato pulp during the simulated gastrointestinal digestion was rutin. However, it was completely catabolized at the beginning of colonic fermentation. Gallic and hydroxyphenilacetic acids, which have not been detected in the undigested sample, were delivered after 24 h of colonic fermentation. Overall, in the present study, the fermentation of CTP seemed to have not promoted a beneficial effect on gut microbiota and gut metabolism. Although it increased BCFA production, it did not promote the multiplication of

Table 2. Short Chain Fatty Acid (SCFA) during 72 h of *in vitro* colonic fermentation.

Analysis	Time (h)	C1	CTP
	0	4.465 ± 0.113^{aA}	5.570 ± 0.512^{bA}
	24	27.052 ± 2.098^{aB}	27.217 ± 1.183^{aBC}
	48	33.306 ± 1.875^{aC}	33.373 ± 3.793^{aCD}
Acetic acid	72	39.084 ± 3.485^{aD}	36.903 ± 4.554^{bD}
	0	1.383 ± 0.001^{aA}	1.002 ± 0.270^{aA}
	24	6.034 ± 0.578^{aB}	37.717 ± 2.296^{bBCD}
	48	7.47 ± 0.948^{aC}	41.142 ± 4.533^{bCD}
Propionic acid	72	8.545 ± 0.748^{aD}	41.430 ± 4.222^{bD}
	0	0.7 ± 0.002^{aA}	0.651 ± 0.361^{aA}
	24	14.784 ± 1.007^{aB}	41.481 ± 1.490^{bBCD}
	48	17.369 ± 1.010^{aC}	45.801 ± 5.607^{bCD}
Butiric acid	72	19.362 ± 0.987^{aD}	47.714 ± 5.146^{bBCD}
	0	0.227 ± 0.001^{aA}	0.364 ± 0.078^{aA}
	24	2.493 ± 0.190^{aB}	39.901 ± 1.186^{bBC}
	48	3.362 ± 0.564^{aC}	49.337 ± 6.659^{bCD}
Valeric acid	72	3.965 ± 0.455^{aD}	55.109 ± 7.487^{bD}
	0	nd^{A}	nd^{A}
	24	0.36 ± 0.001^{aB}	3.873 ± 0.363^{bBC}
	48	0.763 ± 0.010^{aC}	5.824 ± 0.805^{bCD}
Hexanoic acid	72	1.116 ± 0.101^{aD}	6.959 ± 0.951^{bD}
	0	0.055 ± 0.00^{aA}	nd^{bA}
	24	1.228 ± 0.010^{aB}	2.902 ± 0.040^{bB}
	48	1.697 ± 0.035^{aC}	3.915 ± 0.353^{bCD}
Isobutyric acid	72	2.066 ± 0.385^{aD}	4.478 ± 0.366^{bD}
	0	0.158 ± 0.001^{aA}	0.156 ± 0.001^{aA}
	24	2.293 ± 0.245^{aB}	5.241 ± 0.167^{bB}
	48	3.109 ± 0.476^{aC}	6.766 ± 0.623^{bCD}
Isovaleric acid	72	3.633 ± 0.273^{aD}	7.807 ± 0.761^{bD}

Values reported as mean (mmol/l) ± standard deviation (n = 3). Different lower case letters in the same row indicate significant differences between the control with feces (C1) and tomato pulp with feces (CTP) at the same time, according to Tukey's test ($p < 0.05$). Different capital letters in the same column indicate significant differences between the control with feces (C1) or tomato pulp with feces (CTP) at different time, according to Tukey's test ($p < 0.05$).

beneficial bacteria and increased the ammonia content in the culture system, which can exert a toxic effect on the host.

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