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In vitro gastrointestinal digestion and colonic fermentation of tomato (*Solanum lycopersicum* L.) and husk tomato (*Physalis ixocarpa* Brot.): Phenolic compounds released and bioconverted by gut microbiota

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ABSTRACT

Two of the most important Mexican plant-foods are tomato (*Solanum lycopersicum* L.) and husk tomato (*Physalis ixocarpa* Brot.). In this study three objectives were followed: *i)* to evaluate the bioaccessible phenolic compounds (PC) in T and HT during upper gastrointestinal digestion, *ii)* to *in vitro* ferment the indigestible fractions of the samples to evaluate the short-chain fatty acids (SCFA) production, *iii)* the microbial metabolites, bioconverted PC and volatile organic compounds (VOCs) generated during the fermentation. Vanillic acid was the most bioaccessible PC and after 48 h, 3-hydroxyphenylacetic acid was the most abundant microbial metabolite identified in both samples. The identification of VOCs belonging to terpenes (and derivatives) group in T and HT can be product of the microbial metabolism of carotenoids. The study shows new knowledge of the *in vitro* intestinal digestion and fermentation of T and HT final compounds with biological potential which should be evaluated in further studies.

1. Introduction

Plant-foods contain a heterogeneous and broad profile of bioactive compounds (BC) such as phenolic compounds (PC). A tremendous amount of research has associated the consumption of PC with the prevention on the development of different diseases (Catalkaya et al., 2020; Williamson, Kay, & Crozier, 2018). One of the countries in the world with the most biodiverse plant-foods is Mexico. From the estimated 23,400 plants in Mexico, 3000 have shown health effects (Mercado-Mercado, Blancas-Benítez, Zamora-Gasga, & Sáyago-Ayerdi, 2019). Of note, two of the most important members of Mexican plant-foods and members of the Solanaceae family are tomato (*Solanum lycopersicum* L.), which is a component of the daily diet in most of the world (Gürbüz Çolak, Eken, Ülger, Frary, & Doğanlar, 2020) and husk tomato (*Physalis ixocarpa* Brot.).

The bioactive compounds content in tomato has been extensively characterized (Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010) and the health benefits that these may exert have been evaluated (Navarro-González, García-Alonso, & Periago, 2018). In contrast, the studies on the husk tomato BC profile are scarce despite its wide used in Mexican sauces (Cárdenas-Castro et al., 2019) and its important pectin content which makes husk tomato a soluble dietary fiber source (Morales-Contreras, Contreras-Esquivel, Wicker, Ochoa-Martínez, & Morales-Castro, 2017). Nevertheless, the health effects of the bioactive compounds or PC depend on their release from the food matrix during the gastrointestinal process (bioaccessibility) (Tulipani et al., 2012). Several factors affect the bioaccessibility of phenolic compounds, such as processing and food matrix interactions (Martini, Conte, Cattivelli, & Tagliazucchi, 2021; Tomas et al., 2018). Since, it is known that just a percentage of the total ingested PC are bioaccessible in

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the small intestine, a considerable portion is associated with the indigestible fraction (IF) escapes the intestinal digestion, and reaches the colon where the gut microbiota will bioconvert the PC and produced microbial metabolites, some of which have been shown to be more bioactive than their precursors (Aravind, Wichienchot, Tsao, Ramakrishnan, & Chakkaravarthi, 2021; Williamson et al., 2018). Furthermore, the analysis of the volatile organic compounds (VOCs) also produced by gut microbiota during colonic fermentation could be an important indicator and non-invasive method to correlate the effects of IF with colon health promotion (de Lacy Costello et al., 2014; Zamora-Gasga et al., 2018ab).

Knowledge of the *in vitro* gastrointestinal digestion of tomato is currently limited, considering that studies have only evaluated the effect of processing tomato on the content of PC, or food matrix composition on the bioaccessibility of carotenoids, particularly lycopene (Lu et al., 2020). Additionally, a lack of knowledge exists on the bioaccessibility of PC from husk tomato and on the analysis of VOCs produced during *in vitro* colonic fermentation of tomato and husk tomato. Thus, the aim of this work was to evaluate the bioaccessible phenolic compounds (PC) in tomato and husk tomato during upper gastrointestinal digestion and to *in vitro* ferment the indigestible fractions of the samples to evaluate the short-chain fatty acids (SCFA) production as well as the microbial metabolites, bioconverted PC and volatile organic compounds (VOCs) generated during the fermentation.

2. Materials and methods

2.1. Sample preparation

Raw tomato and husk tomato were purchased in a local supermarket in Tepic, Nayarit, México. Tomato and husk tomato were selected based on mature condition according to visual color, soft finger texture, size and shape. Samples were frozen (-80 °C), freeze-dried (FreeZone 6, Labconco, Kansas, City, MO), grounded (Nutribullet, NBR-0804B, USA), sieved (0.5 μm) and stored in sealed bags at −20 °C until analysis.

2.2. In vitro gastrointestinal digestion

Freeze-dried samples were subjected to an *in vitro* gastrointestinal digestion model following the Blancas-Benitez, Pérez-Jiménez, Montalvo-González, González-Aguilar, and Sáyago-Ayerdi (2018) protocol. First, 300 mg of freeze-dried tomato or husk tomato were submitted to oral digestion with α-amylase from human saliva (A0521, Sigma-Aldrich, St. Louis, MO, USA, 1000–3000 units/mg protein; 30 μL of a 0.049 mg/mL solution in 0.05 M phosphate buffer, pH 6.9, 37 °C, 2 min). In order to simulate gastric digestion, the samples were incubated in a shaking water bath with pepsin (P-7000, Sigma-Aldrich, ≥ 250 units/mg solid; 0.2 mL of a 300 mg/mL solution in 0.2 M HCl-KCl buffer, pH 1.5, 40 °C, 2 h). The intestinal digestion was simulated by adding pancreatin (P-1750, Sigma-Aldrich, 4 × USP specifications; 3 mL of a 5 mg/mL solution in 0.1 M phosphate buffer, pH 7.5, 37 °C, 6 h), and α-amylase (A-6255, Sigma-Aldrich, ≥ 1000 units/mg protein; 2 mL of a 120 mg/mL solution in 0.1 M Tris-Maleate buffer, pH 6.9, 37 °C, 16 h). After the intestinal digestion, the PC released were considered as "bioaccessible". The samples from this stage were centrifuged (Hermle Z 323 K; Wehingen, Germany) (3500×*g*, 15 min, 4 °C) and the supernatant was used to identified the PC by HPLC-DAD-ESI-MS described in below sections.

2.3. Isolation and quantification of indigestible fraction (IF), and in vitro colonic fermentation of tomato and husk tomato

The IF was evaluated as was quantified by Zamora-Gasga et al. (2018a,2018b). The gastrointestinal digestion pellets were considered as the insoluble indigestible fraction (IIF) and the supernatant was submitted to a dialysis (D9527-30.48 m avg. flat width 43 mm, 14000 Da, Sigma-Aldrich), this was considered as the soluble indigestible fraction (SIF). Total indigestible fraction (TIF) was considered as the sum of both fractions (IIF and SIF). On the other hand, IF was isolated according to the amendments proposed by Tabernero, Venema, Maathuis, and Saura-Calixto (2011). The isolated TIF was fermented according to Zamora-Gasga et al. (2018a,b). A pool of fresh fecal samples collected was prepared from four healthy volunteers (25–28 years), two male and two female (Nutritional status based on body mass index; normal-weight for all donators). The volunteers declared no gastrointestinal diseases, no intake of antibiotics at least 3 months before the beginning of the study and no consumption of tomato and husk tomato at least one month before the donation. The fecal samples were collected by the volunteers in plastic containers in which anaerobic conditions were simulated using Anaerocult A (Merck, Darmstadt, Germany). Then, samples were diluted with 0.1 mol/L, pH 7 phosphate buffer and homogenized to obtain a 10 g/100 mL final solution as a pool faecal suspension. During the *in vitro* colonic fermentation, anaerobic conditions of human colon were simulated using a gas mixture (10:10:80 H_2:CO_2:N_2). Sterile basal nutrient medium containing peptone water (2 g/L), yeast extract (2 g/L), NaCl (0.1 g/L), K_2HPO_4 (0.04 g/L), KH_2PO_4 (0.04 g/L), $MgSO_4{\cdot}7H_2O$ (0.01 g/L), $CaCl_2{\cdot}2H_2O$ (0.01 g/L), $NaHCO_3$ (0.01 g/L), cysteine HCl (0.5 g/L), bile salts (0.5 g/L), tween 80 (2 mL/L) and 0.2 g haematin (diluted in 5 mL of NaOH) was adjusted to pH 7. Tubes (Falcon™ 352196, length 120 mm, 15 mL, Fisher Scientific) containing 9 mL of basal culture medium were inoculated with 1 mL of fecal suspension and 100 mg of indigestible fraction (considered as substrate) isolated from tomato or husk tomato. All incubations were collocated in triplicate into a shaking (60 rpm) water bath at 37 °C. In parallel, two different controls were used: a) raffinose (100 mg, R0514, Sigma-Aldrich), used as a fermentable carbohydrate that leads to the production of short chain fatty acids (SCFA) by the gut microbiota, which represented the positive control and b) the fecal suspension in the culture media without addition of substrate, used as negative control. Samples were collected and centrifuged at 6, 12, 24 and 48 h and centrifuged (Hermle Z 323 K; Wehingen, Germany) (6000 rpm, 15 min, 4 °C). Supernatants were divided for the analytical assays described below and were immediately stored at −80 °C until analysis.

2.4. TSP and antioxidant capacity (AOX) in fermented samples and PC extraction from the isolated indigestible fractions

In order to quantify the total soluble polyphenols (TSP), supernatants from fermentation samples (6, 12, 24 and 48 h) were mixed with sodium carbonate (7.5% w/v) and Folin-Ciocalteús reagent following the protocol modified by Alvarez-Parrilla, de la Rosa, Amarowicz, and Shahidi (2011) using a microplate reader (Biotek® Synergy HT, USA) with Gen5 software. The absorbance was read at 750 nm and gallic acid was used as standard. The results from *in vitro* colonic fermentation were expressed as mg gallic acid equivalents GAE/g substrate dry weight (DW). The aliquots from the *in vitro* colonic fermentation were used to assay the reduction of the DPPH (2,2-Diphenyl-1-picrylhidrazyl) radical. The assays were conducted with the modifications described by Alvarez-Parrilla et al. (2011) using an aqueous solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) as standard. The results were expressed as mM Trolox equivalent TE/g substrate DW.

2.5. Identification of PC by HPLC-DAD-ESI-MS

An aqueous-organic extraction was performed on the isolated IF (250 mg) with 50% methanol/50% water: 70% acetone/30% water (50:50 v/v) according to Pérez-Jiménez et al. (2008). The supernatant of this extraction was used to identify the PC "available for the gut microbiota" by HPLC-DAD-ESI-MS. Then, the supernatant identified as "available for gut microbiota" and samples from intestinal digestion considered as "bioaccessible" and from *in vitro* colonic fermentation were dispensed in microtubes (Eppendorf, Hamburg, Germany) (2 mL),

centrifuged (Vacufuge plus, Eppendorf) (14000 rpm, 20 min) and filtered through a 0.45 μm nylon membrane filter (Merck Millipore Ltd., Cork, Ireland) and dispensed in chromatographic vials.

The identification of PC by HPLC-DAD-ESI-MS was determined according to Blancas-Benitez et al. (2018) with slight modifications. A HPLC Agilent 1260 series system (Agilent Technologies, Santa Clara, CA, USA) was used equipped with an Agilent G4212-60008 UV–Vis diode array detector (DAD) and coupled with a 6120 Agilent simple Quadrupole LC/MS, equipped with an electrospray ionization interface in negative ionization mode (N_2 as drying gas flow, 13.0 L/min; nebulizer pressure, 40 psi; gas drying temperature. 350 °C; capillary voltage, 3500 V) and 10 μL was automatically injected (flow rate 0.4 mL/min) onto a Poroshell 120 EC-C18 column (4.6 mm × 150 mm, particle size 2.7 m) (Agilent Technologies). The elution gradient was carried out using water containing 0.1% formic acid (A) and acetonitrile (B) (Sigma Aldrich). The data analysis was performed using OpenLab CDS, ChemStation Edition software (Agilent Technologies). Characterization of the PC was based on retention time (Rt) in DAD and mass spectrometric signal (single MS scan in the 100–1000 *m/z* range) directly compared with the Rt of analytical standards (Supplementary material). External calibration curves were prepared to quantify the compounds identified. When the standard was not available, the calibration curve corresponding to its phenolic precursor was used to tentatively quantify the compound. The chemicals from Sigma-Aldrich used for calibration curves were: 3,4-dihydroxybenzoic acid (37580), 4-hydroxybenzoic acid (240141), luteolin (L9283), 4-hydroxyphenylacetic acid (H50004), *trans*-ferulic acid (128708), vanillic acid (94770), quercetin (Q4951), sinapic acid (D7927), chlorogenic acid (C3878), caffeic acid (C0625), catechin (C1788), kaempferol (K0133), naringenin (N5893), myricetin (70050) and 3-(4-hydroxyphenyl)-propionic acid (H52406).

2.6. Volatile organic compounds (VOCs) quantification by headspace solid-phase micro-extraction (HS-SPME-GC/MS) analysis

The evaluation of VOCs concentrations was performed according to Zamora-Gasga et al. (2018a,b). The volatile constituents were analyzed with an Agilent 5977A mass selective detector coupled to an Agilent 7890B gas chromatograph (Agilent Technologies), equipped with a HP-5MS capillary column (30 m × 0.250 mm × 0.25 μm; Agilent Technologies). Supernatant from fermentation samples were weighed (500 mg) and were placed into a 20 mL vial sealed with a magnetic cap with polytetra-fluor-ethylene/silicon septum. Thereafter, the sample was submitted to an incubation with polydimethylsiloxane-divinylbenzene-carboxen fiber, under gentle shaking (250 rpm at 45 °C). After 120 min, the fiber was inserted into the injection port of the GC system for thermal desorption (240 °C for 10 min) for a GC/MS analysis. Quantification of SCFA was obtained by means of acetic, propionic and butyric acid standard curves (Sigma-Aldrich) and comparing the mass spectra of the samples with the data system library MSD ChemStation software (F.01.03.2357, The NIST Mass Spectral Search Program, Version 2.2). The results were expressed in mmol/L produced per 100 mg DW of substrate. Tentative identification of the other volatile components was done comparing the mass spectra of the samples with the data system library MSD ChemStation software (F.01.03.2357, The NIST Mass Spectral Search Program, Version 2.2). Acetic acid was used as internal standard to calculate the relative concentration of all volatile organic compounds. The results were expressed in mmol/L.

2.7. Statistical analysis

All analyses were performed in triplicate (n = 3); mean values and standard deviations from each determination were calculated. Data were processed by ANOVA/Fisheŕs least significant difference test for all samples (with significance set at $p < 0.05$), according to normality test (Shapiro-Wilk test) and homogeneity variance (Levenés test). VOCs profiles were evaluated between substrates and fermentation times using principal components analysis (PCA). All analyzes were performed using STATISTICA software, version 10.0 (StatSoft Inc. 1984–2007, Tulsa, OK, USA).

3. Results and discussion

3.1. PC released after the intestinal digestion (bioaccessible) and associated to the indigestible fraction (available for the gut microbiota), in tomato and husk tomato

The PC released in the intestinal digestion stage (bioaccessible) is more relevant to evaluate than the initial content of PC in the starting (husk) tomato material. In support of this notion, the bioaccessible PC may exert physiological functions while the non-bioaccessible phenolics, i.e. the PC associated with the IF, can reach the colon and be used as substrates by the gut microbiota and bioconverted in new compounds with different biological properties (Mercado-Mercado et al., 2019). Seventeen and twelve phenolic compounds were identified as “bioaccessible” and “available for the gut microbiota”, respectively (Supplementary material). However, Cárdenas-Castro et al. (2021) reported forty-five and thirty-eight compounds in starting Mexican sauces and predigested sauces (available for the gut microbiota) made with mainly husk tomato and mixed with onion, garlic, coriander and hot pepper. The differences in the higher number and heterogeneous PC profile identified in the sauces than in raw husk tomato suggested the significant contribution of the addition of other vegetables. On the other hand, total PC bioaccessible in tomato and husk tomato was 132.18 and 158.13 mg/100 g DW, respectively (Table 1). Notably, the total PC available for the gut microbiota significantly decreased to 13% of the total PC bioaccessible (17.59 and 20 0.61 mg/100 g DW in tomato and husk tomato, respectively). The bioaccessible PC could not be totally absorbed, then, just a percentage will be “bioavailable” (absorbed and reach the bloodstream, organs or cells). In our study was simulated the absorption of PC during the intestinal digestion through passive diffusion using cellulose membranes (dialysis). Then, the decrease of the total PC available for the gut microbiota compared to those bioaccessible in tomato and husk tomato indicated that 87% of the bioaccessible PC could be potentially bioavailable.

The bioaccessible PC and the non-bioaccessible PC (available for the gut microbiota) are shown in Table 1 and they were classified into three PC groups: hydroxycinnamic acids and derivatives, hydroxybenzoic acids and derivatives and flavonoids. The most bioaccessible PC in tomato and husk tomato was vanillic acid (124.48 and 140.16 mg/100 g DW, respectively), a hydroxybenzoic acid. As far as we know, there are no studies on PC profiles in raw husk tomato (*Physalis ixocarpa*) but Medina-Medrano et al. (2015) reported PC composition of other species of the genus *Physalis*. Phenolic acids were found mainly, in the fruit (1.24 to 50.57 mg GAE/100 g bs). Several studies have investigated the PC content and profile in tomatoes (Gómez-Romero et al., 2010; Martínez-Huélamo et al., 2015; Vallverdú-Queralt, Medina-Remón, Andres-Lacueva, & Lamuela-Raventos, 2011). The differences on the PC profile found in tomato may be explained considering the type or variety of the tomatoes evaluated, agricultural treatments, ripeness, processing, environmental conditions or sub-optimal extraction of the compounds analyzed (Gómez-Romero et al., 2010). Elbadrawy and Sello (2016) reported 3.31 mg/100 g DW of vanillic acid in Egyptian tomatoes but it was not reported to be present in Spanish tomatoes. Homovanillic acid hexose was the second most abundant PC found in Spanish tomatoes (25 mg/100 g DW) (Gómez-Romero et al., 2010).

In general, hydroxycinnamic acids and their derivatives have been reported to be the most abundant family in tomatoes but differences in the compounds quantified belonging this PC group in tomatoes are remarkable. Zanfini, Franchi, Massarelli, Corbini, and Dreassi (2017) and Jeż, Wiczkowski, Zielińska, Białobrzewski, and Błaszczak (2018) reported a content of chlorogenic acid for Italian and Polish tomatoes of 10.27 and 3.09 mg/100 g DW, respectively but their bioaccessibility was

Table 1
Phenolic compounds (PC) released during *in vitro* upper intestinal digestion (bioaccessible) and associated with the indigestible fraction (available for the gut microbiota) of tomato and husk tomato[1].

	Bioaccessible		Available for the gut microbiota	
Tentative compound	Tomato	Husk tomato	Tomato	Husk tomato
Hydroxybenzoic acids and derivatives				
Dihydroxybenzoic acid	0.34 ± 0.06^{a}	0.45 ± 0.01^{a}	nd	nd
Dihydroxybenzoic acid pentose	nd	nd	0.19 ± 0.01	nd
Vanillic acid	124.48 ± 0.95^{a}	140.16 ± 2.66^{b}	5.27 ± 0.62^{B}	2.44 ± 0.01^{A}
Isovanillic acid	nd	nd	4.01 ± 0.35^{A}	8.05 ± 0.56^{B}
TOTAL (mg/100 g DW)	124.82 ± 1.02^{a} (94.43%)*	140.65 ± 2.65^{b} (89%)*	9.47 ± 0.25^{A} (54%)*	10.50 ± 0.57^{A} (51%)*
Hydroxycinnamic acids and derivatives				
1-Caffeoylquinic acid	0.07 ± 0.01^{a}	0.11 ± 0.02^{a}	nd	nd
5-Caffeoylquinic acid	0.15 ± 0.01	nd	0.14 ± 0.01^{A}	0.05 ± 0.03^{B}
3-Caffeoylquinic acid (Chlorogenic acid)	0.04 ± 0.01	nd	nd	nd
Caffeic acid	nd	nd	0.23 ± 0.01^{B}	0.02 ± 0.01^{A}
Sinapic acid deoxyhexosidehexoside I	2.72 ± 0.04	Traces	nd	nd
Sinapic acid deoxyhexosidehexoside II	nd	Traces	nd	nd
Feruloylquinic acid	nd	13.44 ± 1.38	5.93 ± 0.10^{A}	8.11 ± 0.12^{B}
TOTAL (mg/100 g DW)	3.00 ± 0.03^{a}(2.27%)*	13.55 ± 1.41^{b}(9%)*	6.31 ± 0.10^{A}(36%)*	8.18 ± 0.08^{B}(40%)*
Flavonoids				
Isorhamnetin hexoside I	0.35 ± 0.05	nd	nd	nd
Naringenin dihexoside	0.10 ± 0.02	nd	nd	nd
Kaempferol-O-sambubioside	nd	0.09 ± 0.01	nd	0.07 ± 0.01
Epicatechin gallate	1.85 ± 0.24^{a}	1.20 ± 0.05^{a}	nd	nd
Isorhamnetin hexoside II	0.18 ± 0.02^{a}	0.19 ± 0.05^{a}	nd	nd
Apigenin acetyl hexoside	nd	0.48 ± 0.09	nd	nd
Myricetin-3-O-rutinoside	0.42 ± 0.05^{a}	0.50 ± 0.01^{a}	nd	nd
Kaempferol rutinoside	nd	0.11 ± 0.01	nd	0.07 ± 0.01
Kaempferol-O-glucuronide	nd	Traces	nd	nd
Kaempferol-3-O-(6″-O-acetyl)glucoside	1.08 ± 0.01^{a}	1.00 ± 0.03^{a}	1.39 ± 0.08^{A}	1.34 ± 0.06^{A}
Rutin hexoside	0.32 ± 0.01^{a}	0.36 ± 0.02^{a}	0.41 ± 0.06^{A}	0.41 ± 0.03^{A}
TOTAL (mg/100 g DW)	4.35 ± 0.26^{a} (3.30%)*	3.93 ± 0.06^{a} (2%)*	1.80 ± 0.02^{A} (10%)*	1.92 ± 0.05^{A} (9%)*
TOTAL PHENOLIC COMPOUNDS (mg/100 g DW)	132.18 ± 1.32^{a}	158.13 ± 1.18^{b}	17.59 ± 0.38^{A}	20.61 ± 0.54^{B}

[1] Values represent mean ± SD (n = 3). Different lowercase letters in the same row indicate significant difference ($p < 0.05$) between T and HT for the bioaccessible fraction. Different uppercase letters in the same row indicate significant differences between T and HT for the available for gut microbiota fraction. *Percentage of phenolic compounds groups quantified per sample. nd = not detected.

not investigated. However, in our study, the content of bioaccessible chlorogenic acid was 0.04 mg/100 g DW (Table 1). Then, as previously mentioned, the bioaccessibility of PC could be different than its initial content.

Regarding bioaccessible flavonoids, 11 glycosylated derivatives were quantified in tomato and husk tomato. Derivatives of narigenin have been found as one of the most abundant flavonoids in raw tomatoes (Gómez-Romero et al., 2010) but only naringenin dihexoside was identified as bioaccessible in raw tomato samples in our study which is in line with the report by Martínez-Huélamo et al. (2015) whom identified glycosylates of naringenin in plasma when the bioaccessibility of raw tomatoes and processed tomatoes was evaluated in vivo. Indeed, higher bioaccessibility of naringenin derivatives was found in mechanical/thermal processed tomatoes or when oil (fat rich matrix) was added to tomatoes (Tulipani et al., 2012).

Several bioaccessible kaempferol glycosides were identified in husk tomato such as kaempferol-O-glucuronide, kaempferol-3-O-sambubioside and kaempferol rutinoside (Table 1). These results are in concordance with Medina-Medrano et al. (2015) whom reported glycosylated derivatives of kaempferol as the most abundant compounds in different species of the genus *Physalis*, such as *Physalis philadelphica*. It is noteworthy that Campos-Vidal et al. (2021) associated gastroprotective effects with glycosylated kaempferol derivatives, such as kaempferol-3-O-sambubioside that protected against induced gastric ulcer in rats.

The percentage of the PC groups available for gut microbiota were more balanced compared to the bioaccessible onés PC groups (Table 1). Hydroxybenzoic and hydroxycinnamic acids derivatives and flavonoids were found in percentages ranging from 51 to 54%, 36 to 40%, 10 to 9%, respectively. Particularly, vanillic, isovanillic and feruloylquinic acid were identified in the IF of tomato and husk tomato. Caffeic acid was only identified in the IF of tomato and was not bioaccessible in the small intestine, despite being reported as one of the most representative PC of tomato. This may be explained since that caffeic acid could be associated to cellulose and xylan by hydrogen bonds and this may decrease its bioaccessibility (Jakobek & Matić, 2019). Regarding flavonoids, kaempferol-3-O-(6″-O-acetyl)glucoside and rutin hexoside were the most abundant compounds associated with the IF of tomato and husk tomato.

3.2. Quantification of the IF of tomato and husk tomato

The indigestible fraction comprises mainly dietary fiber and those compounds that resists the digestion such as indigestible proteins, lipids, lignin and bioactive compounds bound to the IF components (Tabernero et al., 2011). The content of the IF of tomato and husk tomato is shown in Fig. 1(a). No significant differences ($p > 0.05$) were observed in the content of soluble indigestible fraction (SIF) in both samples, as opposed to the content of insoluble indigestible fraction (IIF) and total indigestible fraction (TIF) ($p < 0.05$). Gu et al. (2020) and Morales-Contreras et al. (2017) have reported tomato peel and husk tomato as good sources of soluble dietary fiber. However, IIF was significantly higher for tomato than for husk tomato (47.57 and 38.71 g/100 g DW, respectively). Li, Feng, Niu, and Yu (2018) reported dietary fiber in tomato peels contained 48.5% of insoluble dietary fiber, similar with the value obtained. Also, TIF was significantly higher for tomato than for husk tomato (52.86 and 41.84 g/100 g DW, respectively). Previously mentioned, it is suggested that the major component of IF is dietary fiber and their beneficial effects have been studied such as lowering the risk of cardiovascular disease and obesity, increasing satiety, reducing blood sugar and preventing colon cancer associated with the action of the gut microbiota by producing short-chain fatty acids (SCFA) during colonic fermentation (Li et al., 2018).

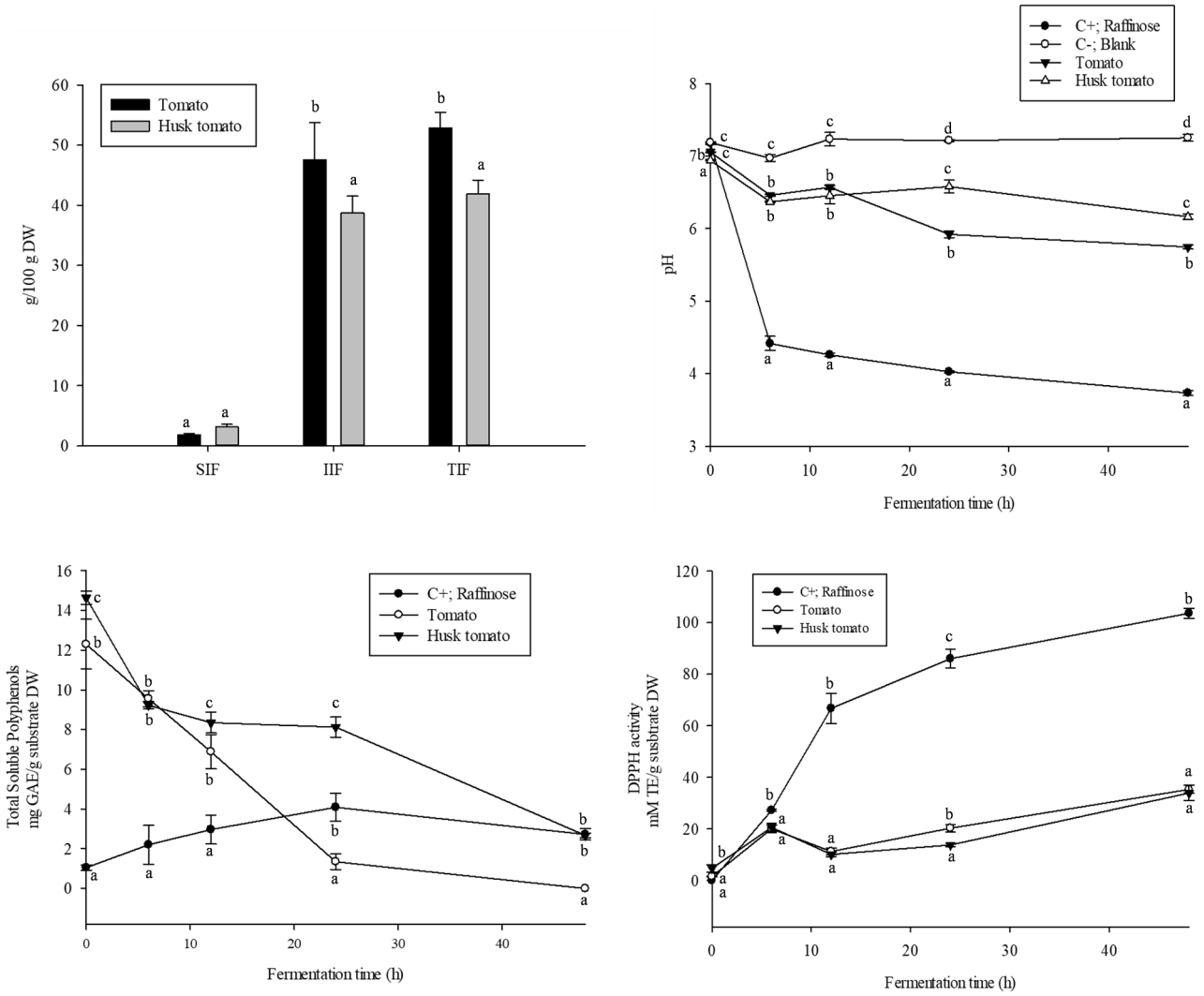


Fig. 1. Changes in (a) soluble indigestible fraction (SIF), insoluble indigestible fraction (IIF) total indigestible fraction (TIF) content in tomato and husk tomato; (b) pH kinetic plot in extracts during *in vitro* colonic fermentation; (c) total soluble polyphenols in extracts during *in vitro* colonic fermentation; (d) DPPH activity plot in extracts during *in vitro* colonic fermentation. Values represent mean ± SD (n = 3). Different lowercase letters at the same fermentation time indicate significant differences between substrates ($p < 0.05$). For (c) and (d) plots, values of blank sample (C-) were removed from total values.

3.3. In vitro colonic fermentation of IF isolated from tomato and husk tomato: pH values, AOX and production of SCFA

Fig. 1 (b) shows the pH values of over time. Measuring pH is a first screening to give an overview of the fermentation process. Initial pH values ranged from 6.9 to 7.2 but after 6 h fermentation the positive control (C+, raffinose substrate) decreased 3 pH units and continued decreasing as the fermentation proceeded. The pH values in the samples in tomato and husk tomato were significantly higher compared to C^+. Nevertheless, the pH of tomato and husk tomato samples declined approximately 1 pH unit after 48 h fermentation. Overall, the acidification of the colonic lumen could influence the prevention of the overgrowth of pathogens in the gut. Furthermore, the lowering of pH values could be associated with the production of SCFA (Peredo-Lovillo, Romero-Luna, & Jiménez-Fernández, 2020). Regarding the TSP content analyzed by folin ciocalteaús reagent and antioxidant capacity (AOX) analyzed by DPPH assay during the fermentation (Fig. 1(c) and (d)), the TSP was significantly higher ($p < 0.05$) in the tomato and husk tomato samples at the start of fermentation than those present in C^+ samples. Notably, the AOX values in C^+ were significantly higher ($p < 0.05$) than tomato and husk tomato samples but as fermentation proceed the response of AOX also increased in tofmato and husk tomato. This observation is in line with data reported by Zamora-Gasga et al. (2018a,2018b) who explained that the production and co-presence of all together microbial metabolites such as butyric acid in *in vivo* evaluation (Yuan, Wang, Bhat, Lohner, & Li, 2018) may increase the AOX values in C^+ samples.

Regarding the SCFA production, the concentrations of these dietary fiber fermentation end-metabolites are shown in Table 2. No production of acetic, propionic and butyric acid was found in samples of tomato and husk tomato after 6 and 12 h fermentation which may suggested slow fermentation of the type of dietary fiber of both fruits. Since fecal samples were collected from individuals that did not habitually ingest (husk) tomato, it could also mean that the gut microbiota needed to adapt to the substrates and induce genes that were capable of degrading the (husk) tomato dietary fiber. After 24 and 48 h fermentation, acetic, propionic and butyric acid production on tomato and husk tomato was significantly lower ($p < 0.05$) than on the C^+. The highest concentration of SCFA is mainly found in the proximal colon, where most carbohydrates are fermented. This leaves the distal colon usually devoid of fermentable carbohydrates and here the fermentation of proteins prevails, with concomitant production of toxic metabolites (Peredo-Lovillo et al., 2020). Thus, if dietary fiber is slowly fermented, it would have a positive effect because it will reach the distal part of the colon to be

Table 2
Short-chain fatty acids (SCFAs, mmol/L) production at 24 and 48 h of *in vitro* fermentation of blank, raffinose and indigestible fraction (IF) isolated from tomato and husk tomato.

SCFA/Fermentation time	Blank	Raffinose	Tomato	Husk tomato
Acetic acid				
24 h	nd	7.7 ± 3.5^{Ac}	16.7 ± 1.2^{Aa}	20.8 ± 1.4^{Ab}
48 h	5.83 ± 1.26^{a}	229.9 ± 18.7^{Bc}	155.5 ± 23.2^{Bb}	154.2 ± 10.4^{Bb}
Propionic acid				
24 h	nd	8.63 ± 1.3^{Ab}	1.8 ± 0.3^{Aa}	nd
48 h	1.3 ± 0.4^{a}	33.7 ± 5.2^{Bd}	21.8 ± 6.0^{Bc}	11.9 ± 3^{b}
Butyric acid				
24 h	0.8 ± 0.1^{Aa}	6.6 ± 1.4^{Ac}	0.51 ± 0.06^{Ab}	nd
48 h	0.7 ± 0.1^{Aa}	30.8 ± 1.0^{Bd}	3.35 ± 0.97^{Bc}	1.3 ± 0.1^{b}
Molar ratio (acetic:propionic:butyric)				
24 h	0:0:100	84:9:7	88:9:3	100:0:0
48 h	75:16:9	78:11:11	77:11:12	92:7:1

[1]Values are reported in mmol/L produced per 100 mg substrate as mean ± SD (n = 3). Different uppercase letters in the same column indicate significant differences between fermentation times for a same substrate, different lowercase letters in the same row indicate significant difference between substrates for a particular time point ($p < 0.05$); nd = not detected.

fermented there, leading to a decrease in protein fermentation and toxic metabolite production (de Lacy Costello et al., 2014). In general, the molar ratio of tomato and husk tomato samples changed after 48 h fermentation (Table 2). Furthermore, the physiological effects that have been reported for acetic acid were that this compound may stimulate the synthesis of lipids whilst propionic acid reduces lipogenesis and cholesterol synthesis and activates receptors that release hormones that exerts anti-inflammatory properties (De Souza, Jonathan, Saad, Schols, & Venema, 2019). Regarding butyric acid, this SCFA serves as a source of energy for the colonocytes and has shown effects on regulation of gene expression (Peredo-Lovillo et al., 2020).

3.4. Microbial metabolites produced during in vitro colonic fermentation: Biotransformation of PC

Growing and new information about the biotransformation of phenolic compounds (PC) from different foods that reach the colon by the gut microbiota is necessary because the possible biological activities for human health may be mainly exerted by the microbial metabolites produced, rather than their initial PC precursors. The biotransformation occurs through different enzymatic reactions such as de-esterification, oxidation, hydroxylation, dehydrogenation, decarboxylation, methylation, hydration, and deglycosylation catalyzed by the gut microbiota (Catalkaya et al., 2020). Metabolites from PC produced during the *in vitro* colonic fermentation of tomato and husk tomato are shown in Table 3. Regarding hydroxybenzoic acids and hydroxycinnamic acids, vanillic acid and feruloylquinic acid were identified at 6 and 12 h, respectively, but after 24 h fermentation they were no longer detected in both fermentations suggesting their biotransformation. The observation for vanillic acid is in line with the identification of vanillin at 24 and 48 h fermentation, which agrees with the data reported by Álvarez-Rodríguez et al. (2003). The presence of hydroxyphenylpropionic acid derivatives at 24 and 48 h in both fermentations indicate the degradation of feruloylquinic acid. Indeed, hydroxyphenylpropionic and hydroxyphenylacetic acids were the major metabolites formed during the *in vitro* colonic fermentation of tomato and husk tomato. In this regard, hydroxyphenylpropionic and hydroxyphenylacetic acids have been described as the major fermentation products of phenolic acids and flavonoids (Catalkaya et al., 2020).

As previously noted, phenolic acids (Table 1) were the main PC substrate for the gut microbiota followed by glycosylated flavonoids. Both these PC groups were proposed to be metabolized to hydroxyphenylpropionic and hydroxyphenylacetic acids according to the

Table 3
Phenolic metabolites (μM/L) produced by gut microbiota during the *in vitro* colonic fermentation of isolated indigestible fraction of tomato (T) and husk tomato (HT) at different time points[1].

Time/Tentative compound	6 h		12 h		24 h		48 h	
	T	HT	T	HT	T	HT	T	HT
4-hydroxybenzoic acid	nd	nd	nd	nd	nd	15.65 ± 3.27	nd	nd
Dihydroxybenzoic acid pentose	nd	nd	nd	nd	11.96 ± 1.49^{a}	13.12 ± 0.80^{a}	7.95 ± 0.83^{b}	nd
Vanillic acid	63.66 ± 0.32^{a}	64.31 ± 0.71^{a}	34.40 ± 1.10^{b}	17.66 ± 0.03^{c}	nd	nd	nd	nd
Feruloylquinic acid	13.73 ± 1.87^{a}	30.36 ± 4.00^{b}	10.32 ± 0.44^{a}	26.93 ± 2.56^{b}	nd	nd	nd	nd
1-Caffeoylquinic acid	nd	nd	nd	nd	Traces	1.76 ± 0.18	nd	nd
Sinapic acid deoxyhexosidehexoside I	2.92 ± 0.56^{ab}	4.87 ± 0.89^{b}	nd	1.55 ± 0.90^{a}	nd	nd	nd	nd
Sinapic acid deoxyhexosidehexoside II	nd	4.93 ± 0.22^{a}	nd	4.83 ± 0.12^{a}	nd	nd	nd	nd
Quercetin dihexoside	nd	nd	nd	nd	2.27 ± 0.34^{a}	1.99 ± 0.85^{a}	Traces	Traces
Kaempferol-3-O-(6″-O-acetyl-glucoside)	nd	nd	nd	nd	nd	nd	nd	4.99 ± 0.12
Theaflavonoside I	nd	nd	nd	nd	nd	nd	nd	6.79 ± 0.76
Kaempferol rutinoside pentoside	nd	nd	nd	nd	nd	nd	nd	0.26 ± 0.10
Quercetin 3,7-di-O-α-L-rhamnopyranoside	nd	nd	nd	nd	2.07 ± 0.34^{a}	2.86 ± 0.38^{a}	nd	11.62 ± 0.24^{b}
Myricetin	nd	nd	nd	nd	nd	1.64 ± 0.32	nd	nd
3-hydroxyphenylacetic acid	nd	nd	nd	nd	43.51 ± 3.35^{a}	nd	44.47 ± 11.55^{a}	43.22 ± 12.47^{a}
3-(4-hydroxyphenyl)propionic acid	5.05 ± 0.12^{a}	5.52 ± 0.03^{a}	11.58 ± 0.74^{b}	11.20 ± 0.36^{b}	nd	33.72 ± 8.03^{c}	nd	nd
3-(3-hydroxyphenyl)propionic acid	nd	nd	nd	nd	36.68 ± 3.85^{d}	27.74 ± 0.88^{c}	2.20 ± 1.23^{b}	7.11 ± 0.36^{a}
Vanillin	nd	nd	nd	nd	7.71 ± 1.36^{a}	8.59 ± 0.07^{a}	6.65 ± 1.80^{a}	7.81 ± 2.82^{a}
TOTAL (μmoles)	85.38 ± 2.24^{d}	110.01 ± 4.17^{b}	56.31 ± 0.79^{a}	62.20 ± 2.11^{a}	104.97 ± 1.86^{b}	107.12 ± 7.26^{b}	68.40 ± 9.79^{ac}	81.82 ± 9.72^{cd}

[1] Values are reported in μmol/L produced per 100 mg substrate as mean ± SD (n = 3). Different lowercase letters in the same row indicate significant difference ($p < 0.05$). nd = not detected.

biotransformation pathways proposed in Fig. 2 (Serra et al., 2012; Williamson et al., 2018). Colonic fermentation of feruloylquinic acid is related to the formation of dihydroferulic acid. Nonetheless, this metabolite was not detected probably because it is considered an (unstable) intermediate metabolite accounting for the increase of 3-(3-hydroxyphenyl)propionic acid and 3-(4-hydroxyphenyl)propionic acid via dehydroxylation. Particularly, after 6 h fermentation, 3-(4-hydroxyphenyl)propionic acid was identified in tomato and husk tomato samples (5.05 and 5.52 μM, respectively) which continued to increase after 12 h fermentation (11.58 and 11.20 μM, in tomato and husk tomato respectively) and after 24 h fermentation only in husk tomato sample (33.72 μM). This metabolite may also come from kaempferol, via C-ring cleavage (Fig. 2). More glycosylated kaempferol compounds were identified in the IF of husk tomato than tomato (Table 1).

Flavonoids were not detected during the first 12 h fermentation but glycosylated quercetin compounds were observed after 24 h in samples obtained from tomato and husk tomato fermentation, and glycosylated kaempferol compounds were identified after 48 h fermentation for husk tomato. It is suggested that, as the fermentation continued, the microbiota could be hydrolyzing and metabolizing the components which that could be interacting with PC. Namely, once they release the bound PC, the microbiota would degrade them. In light of this, 3-hydroxyphenylacetic acid was the major metabolite identified after 48 h fermentation in tomato and husk tomato samples. As is shown in Fig. 2, it may come from the biotransformation of glycosylated quercetin compounds, through C-ring cleavage and dehydroxylation. It is worth mentioning that Verzelloni et al. (2011) evaluated the combination of some catabolites from colonic fermentation including 3-hydroxyphenylacetic acid. The co-presence of different metabolites from PC biotransformation showed neuroprotective effects explained since the synergistic effect of the mixture. The synergistic effect of several microbial metabolites in the increase of physiological functions has been also hypothesized by Williamson and Clifford (2017).

3.5. Volatile organic compounds: PCA of fecal metabolic fingerprint during the in vitro colonic fermentation of tomato and husk tomato

In order to explore the differences among the volatile organic compounds (VOCs) produced during each sample time period (0–6, 6–12, 12–24 and 24–48 h) of the *in vitro* colonic fermentation of C^+ (raffinose), C^- (blank), tomato and husk tomato, a PCA was elaborated based on the VOCs concentrations (Fig. 3). A total of 107 different metabolites classified into the families of alkyl halides, esters, organic sulfides, organic trisulfides, benzothiazoles, naphthalenes, pyridine and derivatives, amides, SCFA, indoles and derivatives, fatty acid derivatives, organic acids, olefins, terpenes and derivatives, alcohols and polyols, carbonyl compounds, alkanes and benzene and substituted derivatives were identified. In general, the two principal components (PCs) of each fermentation period explained>73% of the total variance among the samples. PCA analysis showed a clear-cut separation of the samples into four major clusters. All the samples followed different PCs. The compounds located in the axis of the PCs at the fermentation times evaluated were based on factors coordinated value < -0.75 to for less production (located in the negative axis) and > 0.75 to for higher production (located in the positive axis). Regarding 6 h fermentation, carbonyl compounds (ID: 22), caryophyllene (ID: 49), decanal (ID: 54), alkanes (ID: 55, 69) and benzene and substituted derivatives (ID: 64) were located in the PC1 positive axis. In contrast, SCFA as acetic (ID: 30), butyric (ID:47) and propionic acid (ID:97) and carbonyl compounds as heptanal (ID: 73) and dodecanal (ID: 68) were located in the PC1 negative axis. This metabolic pattern was observed in husk tomato. VOCs (ID: 23, 49, 55, 69) have been reported in faeces of healthy humans (de Lacy Costello et al., 2014). In addition, it was observed that C^+ sample shows a metabolic pattern inverse to that of husk tomato. Regarding PC2, the VOCs located in the positive axis were butylated hydroxytoluene (ID: 48), 3-mehtyl-indole (ID: 77), p-cresol (ID: 92), p-xylene (ID: 99) whilst the VOCs located in the negative axis olefins (ID: 19), benzene and substituted derivatives (ID: 38, 42, 103) and carbonyl compounds (ID: 84). This metabolic pattern was observed in C^-. The VOC profile shown for C- continued to be produced in C^- samples as the fermentation proceeded. This agreed with the reported data of VOCs

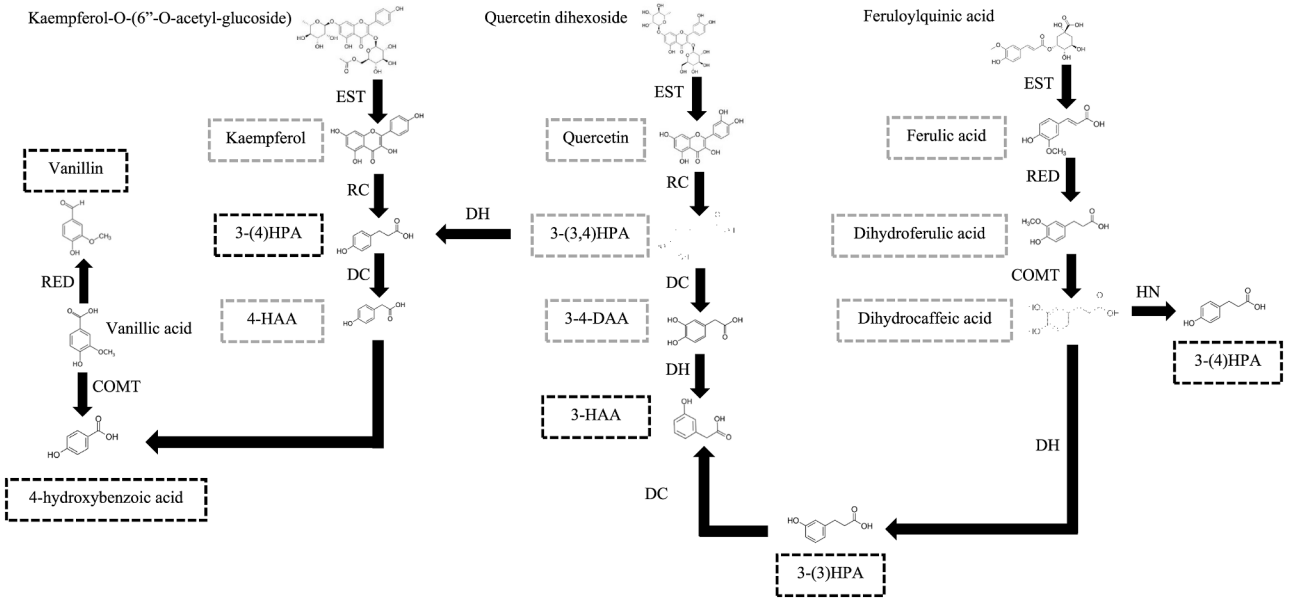
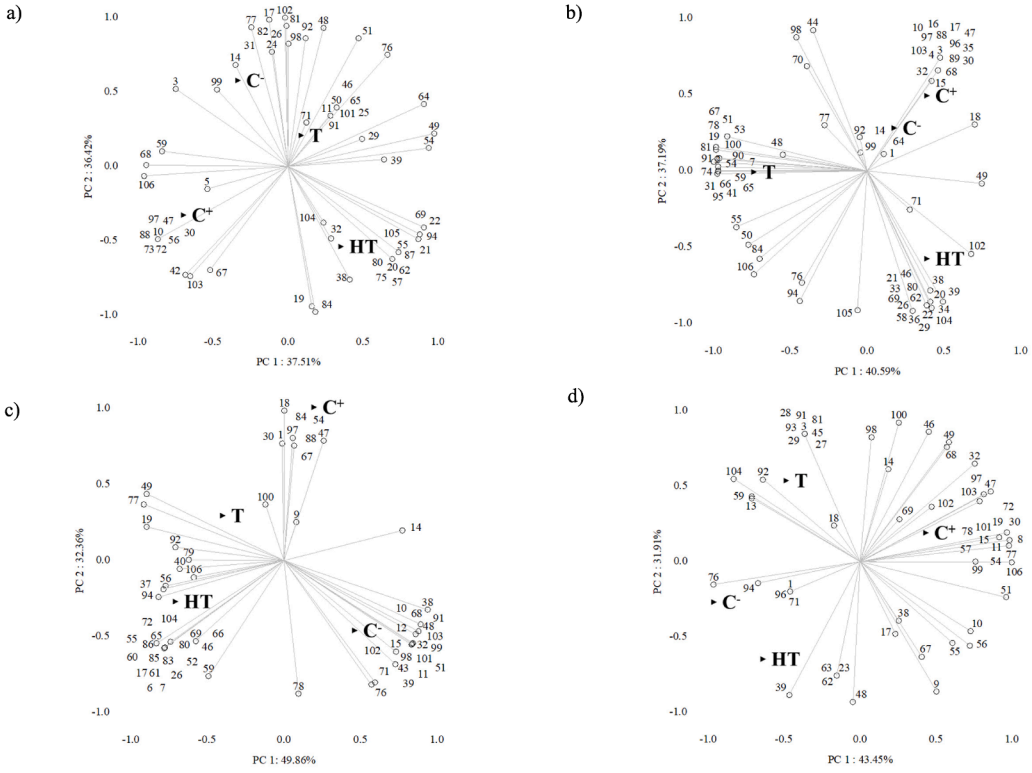


Fig. 2. Proposed biotransformation pathways of the main phenolic compounds in tomato and husk tomato available for the gut microbiota proposed based on Williamson et al. (2018) and Serra et al. (2012). 4-HAA: 4-hydroxyphenylacetic acid; 3-(3,4)HPA: 3-(3,4-Dihydroxyphenyl)propionic acid; 3–4-DAA: 3–4-dihydroxyphenylacetic acid; 3-HAA: 3-hydroxyphenylacetic acid; 3-(3)HPA: 3-(3)-hydroxyphenylpropionic acid; 3-(4)HPA: 3-(4)hydroxyphenylpropionic acid. RC: ring cleavage. DC: decarboxylases. DH: dehydrogenases. EST: esterases. RED: reductases. COMT: Catechol-O-methyltransferases. HN: hydrogenases. Potential intermediates metabolites. Metabolites identified.



ID Volatile Organic Compound.

1 (S)-(+)-6-Methyl-1-octanol
2 α-dehydro-ar-himachalene
3 α-terpineol
4 1,2,5,6-tetrahydropyridine, 1-methylphenyl
5 1,2-Benzenedicarboxylic acid, bis ester
6 1-Butanol, 2-methyl-
7 1-Butanol, 3-methyl
8 1-Decanol
9 1-Decene
10 1-Dodecene
11 1-Hexanol, 2-ethyl
12 1 H-Indole, 4-methyl-
13 1 H-Indole, 5-methyl-
14 1-Nonanol **15** 1-Tetradecene
16 2-(2-Furyl)pyridine
17 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
18 2,4-Di-tert,butylphenol
19 2,5-Cyclohexadiene-1,4-dione, 2,6-diethyl
20 2-Decenal, (E)-
21 2-Heptanal, (Z)-
22 2-Nonenal, (E)-
23 2-Nonenal, (Z)-
24 2-Propenoic acid, pentadecyl ester
25 2-Propenoic acid, tridecyl ester
26 2-Tetradecene, (E)-
27 3-Nonen-1,ol, (Z)-
28 3-Phenylpropanol
29 5,9-Undecadien-2-one, 6, 10-dimethyl-(E)
30 Acetic acid
31 Benzaldehyde
32 Benzaldehyde, 2,4-dimethyl
33 Benzaldehyde, 2-chloro-3-hydroxy-4
34 Benzaldehyde, 3,5-dimethyl
35 Benzaldehyde, 4-methyl
36 Benzenamine, 2-chloro-4,6-dinitro
37 Benzene, 1,2,3,4-tetramethyl
38 Benzene, 1,3-bis(1,1-dimethylethyl)-
39 Benzene, 1,3-dimethyl
40 Benzene, 1-methyl-3-propyl-
41 Benzene, 1-methyl-4-(1-methylethenyl)-
42 Benzeneacetaldehyde
43 Benzenemethanol, 3,5-dimethyl-
44 Benzenemethanol, 4-methyl-
45 Benzenepropanoic acid, methyl ester
46 Benzothiazole
47 Butyric acid
48 Butylated Hydroxytoluene
49 Caryophyllene
50 Cyclodecane
51 Cyclododecane
52 Cyclopropane, nonyl-
53 Cyclotetradecane
54 Decanal
55 Decane
56 Decane, 2-methyl-
57 Decane, 3,7-dimethyl-
58 Decane, 3,8-dimethyl-
59 Decane, 3-methyl-
60 Decane, 4-methyl-
61 Decane, 5-methyl-
62 Decanoic acid, methyl ester
63 Decanoic acid, propyl ester
64 Dibutyl phthalate
65 Dimethyl trisulfide
66 Disulfide, dimethyl
67 D-Limonene
68 Dodecanal
69 Dodecane
70 Dodecane, 4,6-dimethyl-
71 Dodecanoic acid, methyl ester
72 Ethylbenzene
73 Heptanal
74 Hexadecane
75 Hexanal
76 Indole
77 Indole, 3-methyl-
78 Longifolene
79 Mesitylene
80 Methyl salicylate
81 Methyl tetradecan
82 Naphthalene
83 Naphthalene, deca
84 Nonanal
85 Nonane
86 Nonane, 3-methyl
87 Octanal
88 Octanoic acid
89 o-Veratramide
90 Oxime-, methoxy-phenyl-
91 o-Xylene
92 p-Cresol
93 Phenol, 4-ethyl-
94 Phenyl-ethyl Alcohol
95 Phosphorothioic acid, O,O,S-triethyl ester
96 Phthalic acid, 7-bromoheptyl isobutyl ester
97 Propionic acid
98 Propionic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester
99 p-Xylene
100 Silanediol, dimethyl-
101 Terpineol
102 Tetradecane
103 Toluene
104 Trichloromethane
105 Tridecane
106 Undecane
107 Undecanoic acid, 10-methyl-, methyl ester

Fig. 3. Biplots obtained by principal component analysis (PCA) from volatile organic compounds identified by GC/MS during *in vitro* colonic fermentation of isolated indigestible fraction from tomato (T) and husk tomato (HT): a) 6 h fermentation; b) 12 h fermentation; c) 24 h fermentation; d) 48 h fermentation. C^+: positive control (raffinose substrate). C^-: negative control (blank).

profile of blank (substrate absence) during *in vitro* colonic fermentation (Zamora-Gasga et al., 2018a, 2018b). It is shown that tomato sample followed the metabolic pattern inverse to that of C^- (Fig. 3).

For the next time period, after 12 h fermentation, the VOCs located in the PC1 positive axis were carbonyl compounds (ID; 21, 22, 29), benzene and substituted derivatives (ID: 18, 33 34, 36, 38, 39), terpenes and derivatives (ID: 49), alkanes (ID: 58, 69, 102), fatty acid derivatives (ID: 62), esters (ID: 80) and alkyl halides (ID: 104). The PC1 negative axis comprised terpenes and derivatives (ID: 2, 67, 78), alcohols and polyols (ID: 7), olefins (ID: 19), benzene and substituted derivatives (ID: 31, 41, 90, 91), alkanes (ID: 51, 53, 55, 59), carbonyl compounds (ID: 54, 84), organic trisulfides (ID: 65), organic disulfides (ID: 66), fatty acid derivatives (ID: 81) and organic acids (ID: 95). This metabolic pattern was observed in husk tomato whereas in contrast tomato sample shows a metabolic pattern inverse to that of husk tomato. 2-Heptenal, (Z)- (ID: 21) could be generated from the metabolism of polyunsaturated fatty acids (de Lacy Costello et al., 2014). In addition, organic trisulfides and organic disulfides have been commonly reported in faeces (de Lacy Costello et al., 2014).

After 24 h fermentation, the PC1 positive axis comprised olefins (ID: 10, 15), alcohols and polyols (ID: 11, 14), indoles and derivatives (ID: 12), benzene and substituted derivatives (ID: 32, 34, 38, 39, 91, 99, 103), terpenes and derivatives (ID:101), alkanes (ID: 51, 102), carbonyl compounds (ID: 68), organic acids (ID: 98) and the VOCs located in the PC1 negative axis were alcohols and polyols (ID: 6, 7, 94), organic acids (ID: 17), olefins (ID: 19, 26), benzothiazoles (ID: 46), terpenes and derivatives (ID: 49), alkanes (ID: 52, 56, 60, 61, 85, 86), benzene and substituted derivatives (ID: 72, 92), organic trisulfides (ID: 65), organic disulfides (ID: 66), indoles and derivatives (ID: 77), esters (ID: 80), naphthalenes (ID; 82, 83), and alkyl halides (ID: 104). This metabolic pattern was observed in C-. In contrast, it was observed that husk tomato shows a metabolic pattern inverse of that of C^-.

Regarding 48 h fermentation, alcohols and polyols (ID: 8, 11), olefins (ID: 10, 15, 19), SCFA (ID: 30, 47, 97), alkanes (ID: 51, 56, 57, 106), carbonyl compounds (ID: 54), benzene and substituted derivatives (ID: 72, 103), indoles and derivatives (ID: 77), terpenes and derivatives (ID: 78, 101) were located in the PC1 positive axis and indoles and derivatives (ID: 13, 76), alkanes (ID: 59, 104), alcohols and polyols (ID: 94) and benzene and substituted derivatives (ID: 96) were located in the PC1 negative axis. This metabolic pattern was observed in C^+ and it was observed that C^- sample shows a metabolic pattern inverse to that of C^+. On the other hand, after 48 h, in the PC2 positive axis were located terpenes and derivatives (ID: 3, 49), alcohols and polyols (ID: 27, 28), carbonyl compounds (ID: 29) benzene and derivatives (ID: 45, 91, 93), benzothiazoles (ID: 46), fatty acid derivatives (ID: 81) and organic acids (ID: 98). In contrast, the VOCs located in the PC2 negative axis were carbonyl compounds (ID: 23), benzene and substituted derivatives (ID: 39, 48) and fatty acid derivatives (ID: 62, 63). This metabolic pattern was observed in tomato. The inverse metabolic pattern was observed in husk tomato sample. It is worth mentioning that 2,4-Di-*tert*-butylphenol (ID: 18) which seemed to be located in the PC2 positive axis after 48 h, induced apoptosis in gastric adenocarcinoma AGS cells through mitotic catastrophe by increasing acetylation of α-tubulin (Song, Lim, & Cho, 2018). Regarding carbonyl compounds, 5, 9-Undecadien-2-one, 6, 10-dimethyl-(E) (ID: 29) which was found in tomato and husk tomato VOCs profile is closely related to the high oxidation of fatty acids. Ketones can be produced by many species of bacteria as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* or by fungi from the respective alkanoic acid (de Lacy Costello et al., 2014).

Diverse VOCs comprised in the terpenes and derivatives group (ID: 2, 3, 67) were found in the axis were tomato or husk tomato were located at different fermentationś time. It is worth mentioning that α-terpineol (ID: 3) could be an important candidate for the development of analgesic drugs effective for cancer pain control (Gouveia et al., 2018). Studies have reported the content of carotenoids in tomato and husk tomato (Cárdenas-Castro et al., 2019) and it is suggested that terpenes and their derivatives may be produced from the metabolism of carotenoids. Although the knowledge of the VOCs produced during *in vitro* colonic fermentation is increasing, the information is still limited about the health effects of the VOCs found in the gut.

4. Conclusions

The most bioaccessible phenolic compound during the *in vitro* upper gastrointestinal digestion of tomato and husk tomato was vanillic acid which was surprising since the hydroxycinnamates have been reported as the most abundant PC group in some varieties of tomato. In addition, kaempferol glycosides were highly bioaccessible in husk tomato. Previously, kaempferol derivatives were found in different *Physalis* species, but until now there was no information reported on the PC profile of *Physalis ixocarpa*. Moreover, hydroxycinnamates, hydroxybenzoic acids and derivatives and flavonoids were associated with the indigestible fraction of both samples and were available for the gut microbiota. They were fermented by the gut microbiota in more simple phenolic structures, of which some have been shown to have their own biological activity.

Thus, this study showed the biotransformation of phenolic compounds and production of VOCs (including SCFA) during the *in vitro* colonic fermentation of the indigestible fraction of tomato and husk tomato. The major metabolite found after 48 h fermentation in both samples was 3-hydroxyphenylacetic acid, a compound promising neuroprotective potential. Tomato and husk tomato (and the controls used) were located in different PCs as the fermentation proceed according to PCA analysis. The findings of different terpenes and derivatives in tomato and husk tomato samples suggested the metabolism of carotenoids. The compilation of the bioaccessible PC and their biotransformation, as well as the identification of VOCs, could be used for a better understanding of implications of gastrointestinal biotransformation of PC. This should culminate in a complete overview of the compounds that may be responsible for the final biological effects of the consumption of foods that may be widely explored for the initial content of different bioactive compounds, such as for (husk) tomato. However, further work is required to evaluate the biological health properties that the microbial metabolites may exert, alone but better still in combination, as they are produced from the complex BC content of (husk) tomato.

CRediT authorship contribution statement

Alicia P. Cárdenas-Castro: Formal analysis, Investigation, Writing - original draft. **Víctor M. Zamora-Gasga:** Methodology, Software, Validation, Writing - review & editing. **Emilio Alvarez-Parrilla:** Supervision, Writing - review & editing. **Víctor M. Ruíz-Valdiviezo:** Visualization, Writing - review & editing. **Koen Venema:** Resources, Funding acquisition, Writing - review & editing. **Sonia G. Sáyago-Ayerdi:** Conceptualization, Methodology, Resources, Funding acquisition, Validation, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130051.

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