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# Industrial and culinary treatments applied to Piquillo pepper (*Capsicum annuum* cv. *Piquillo*) impact positively on (poly)phenols' bioaccessibility and gut microbiota catabolism†

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Thermal treatments applied to plant-based foods prior to consumption might influence (poly)phenols' bioaccessibility and the metabolization of these compounds by the gut microbiota. In the present research, the impact of industrial (grilling and canning) and culinary (microwaving and frying) treatments on the bioaccessibility and colonic biotransformations of (poly)phenols from Piquillo pepper (*Capsicum annuum* cv. *Piquillo*) were evaluated by *in vitro* gastrointestinal digestion and colonic fermentation models and HPLC-ESI-MS/MS. The application of industrial treatments impacted positively on (poly)phenols' bioaccessibility compared to raw pepper. Microwaving also exerted a positive effect on (poly)phenols' bioaccessibility compared to canning whereas the addition of oil for frying seemed to negatively affect (poly)phenols' release from the food matrix. Throughout the 48 hours of the colonic fermentation process (poly)phenolic compounds were catabolized into different (poly)phenol derivatives whose formation was also positively affected by industrial and culinary treatments. Based on the concentration and time of appearance of these derivatives, catabolic pathways of (poly)phenols from Piquillo pepper were proposed. The major (poly)phenol derivatives identified (3-(3'-hydroxyphenyl)propanoic acid, 4-hydroxy-3-methoxyphenylacetic acid and benzene-1,2-diol) are considered of great interest for the study of their bioactivity and the potential effect on human health.

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## Introduction

*Capsicum annuum* belonging to the *Solanaceae* family is considered one of the most produced and commercialized pepper species worldwide.<sup>1,2</sup> The variety Piquillo (*C. annuum* cv. *Piquillo*), cultivated in the south of Navarra (Spain), exhibits a particular organoleptic attractiveness that makes it a highly appreciated gastronomical product in the context of the Mediterranean diet. Moreover, Piquillo pepper, recognised with the European Protected Designation of Origin (PDO), undergoes a distinctive industrial thermal process for its commercialization: first a grilling treatment by direct flame and a subsequent peeling, followed by a canning technique.

Similar to other *Capsicum annuum* varieties, flavonoid conjugates (luteolin and quercetin derivatives) are the most abundant (poly)phenolic compounds in raw Piquillo pepper.<sup>3-6</sup> However, after industrial grilling due to the high temperatures applied, non-flavonoids become the predominant (poly)phenols.<sup>3</sup>

(Poly)phenols are secondary metabolites highly present in fruits and vegetables with a growing appeal to researchers in recent years due to their known health properties, including the action on gut microbiota.<sup>7,8</sup> Several epidemiological and clinical research studies have been conducted revealing the promising effect that (poly)phenols might exert on human health by lowering the incidence of certain non-communicable diseases including a protective effect against neurocognitive decline, certain types of cancer and cardiovascular diseases.<sup>9-12</sup> These health benefits have been attributed to the reported antioxidant and anti-inflammatory properties which might additionally improve glucose response and insulin regulation, lipid metabolism, blood pressure *etc.* and the co-morbidities associated with these dysregulations.<sup>13-15</sup>

Nevertheless, it has been widely recognized that (poly)phenols' health promoting properties do not depend exclu-

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sively on the concentration present in plants, but on the amount of these compounds that are absorbed and get into systemic blood circulation to reach target cells *i.e.*, their bioavailability.<sup>16</sup> Moreover, their absorption largely depends on the chemical structure of (poly)phenols and their bioaccessibility, which is defined as the amount of a compound that is released from the food matrix and is available to be potentially absorbed.<sup>7,16,17</sup>

Although (poly)phenols might be released from the food matrix, and/or biotransformed during gastrointestinal digestion, they are known to be poorly absorbed in the upper gastrointestinal tract (GIT). Moreover, (poly)phenols are often attached to sugars (*e.g.* glucose, rhamnose...) presenting complex structures that cannot be absorbed by passive diffusion and reach the colon where human microbiota plays an important role in their biotransformation into more absorbable low molecular weight phenolic compounds.<sup>7,8,18,19</sup> The catabolites produced at the colon level have been proposed as those mainly responsible for the potential bioactivity on human health and on gut microbiota composition.<sup>20–25</sup> For this reason (poly)phenols have been recently included in the emerged concept of the 3 'Ps' for gut health (prebiotics, probiotics and (poly)phenols).<sup>23</sup>

Despite the great interest in studying the bioaccessibility and human colonic metabolism of plant (poly)phenols for a better understanding of their potential bioavailability and bioactivity, the available evidence is still limited. The bioaccessibility and colonic biotransformation of (poly)phenols have been previously assessed by Cárdenas-Castro *et al.*,<sup>26,27</sup> in raw *Capsicum annuum* varieties. Nevertheless, vegetables are usually consumed after being submitted to thermal treatments such as canning during industrial processing or culinary techniques (frying, microwaving, grilling, boiling...). As previously reported,<sup>25,28–31</sup> thermal processing, despite provoking losses in (poly)phenolic content, might also have an influence on plant-based foods' matrix. These changes include cell-wall disruption and cleavage of complexes which in turn might impact on (poly)phenols bioaccessibility, and consequently, their bioavailability could be potentially enhanced. In particular, (poly)phenols' bioaccessibility and colonic catabolism of (poly)phenols from Italian green pepper<sup>32</sup> were reported to be positively affected by the application of culinary treatments compared to raw pepper.

Considering that Piquillo pepper is commercialized after the application of industrial treatments (grilling and canning) which impact their (poly)phenol profile and contents,<sup>3</sup> it might be hypothesized that (poly)phenols' bioaccessibility and colonic transformations would also be influenced. Therefore, the aim of the present study was to further evaluate the effect of industrial grilling and canning, and an additional culinary treatment (microwaving and frying) on the bioaccessibility after *in vitro* gastrointestinal digestion of (poly)phenols from Piquillo pepper (*Capsicum annuum* cv. *Piquillo*) by HPLC-ESI-MS/MS. Moreover, the present work aimed to assess the metabolization and the formation of (poly)phenol colonic derivatives after *in vitro* colonic fermentation by HPLC-ESI-MS/MS.

## Material and methods

### Chemical and reagents

For *in vitro* gastrointestinal digestion the enzymes human saliva  $\alpha$ -amylase (97.8 units per mg of solid), pepsin from porcine gastric mucosa (706 units per mg of solid), pancreatin from porcine pancreas (8xUPS) and bile extract porcine were purchased from Sigma-Aldrich (Darmstadt, Germany). Hydrochloric acid, sodium hydroxide, potassium phosphate 2-H<sub>2</sub>O, sodium bicarbonate, sodium chloride, magnesium chloride 6-H<sub>2</sub>O and ammonium carbonate were purchased from Panreac Química SLU (Barcelona, Spain). Potassium chloride was acquired from VWR chemicals (Pennsylvania, USA).

For *in vitro* colonic fermentation, cobalt chloride 6-H<sub>2</sub>O was purchased from Sigma-Aldrich (Darmstadt, Germany). Disodium phosphate 2-H<sub>2</sub>O, potassium chloride, ammonium molybdate, magnesium sulphate H<sub>2</sub>O, zinc sulphate 7-H<sub>2</sub>O and copper sulphate 5-H<sub>2</sub>O were obtained from Panreac Química SLU (Barcelona, Spain). Sodium sulphate 10-H<sub>2</sub>O, urea, calcium chloride, ferrous sulphate 7-H<sub>2</sub>O were acquired from Merck (Darmstadt, Germany).

All chemicals and reagents used for chromatographic analyses were LC-MS grade. Acetonitrile and 99% formic acid were acquired from Scharlau (Barcelona, Spain) and methanol was purchased from Panreac AppliChem (Darmstadt, Germany). Reference standards of phenolic compounds were supplied from different manufacturers and named following the standardized nomenclature proposed by Kay *et al.*<sup>33</sup> Benzene-1,2-diol, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3',4'-dihydroxycinnamic acid, 4'-hydroxycinnamic acid, 4'-hydroxy-3'-methoxycinnamic acid, 3'-hydroxy-4'-methoxycinnamic acid, 4'-hydroxy-3',5'-dimethoxycinnamic acid, 3-phenylpropanoic acid, 3-(3'-hydroxyphenyl)propanoic acid, phenylacetic acid, 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, quercetin, quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, isorhamnetin, kaempferol, apigenin 8-*C*-glucoside, apigenin 6,8-*C*-diglucoside, luteolin, luteolin 7-*O*-glucoside, luteolin 7-*O*-glucuronide, luteolin 8-*C*-glucoside, and naringin, were obtained from Sigma-Aldrich (Darmstadt, Germany). Standards of 3-(3',4'-dihydroxyphenyl)propanoic acid and 3-(4'-hydroxy-3'-methoxyphenyl)propanoic acid were acquired from Alfa Aesar (Kandel, Germany). Apigenin, isorhamnetin 3-*O*-glucoside, kaempferol-7-*O*-glucoside, 2-(3'-Hydroxyphenyl)ethanol and naringenin-7-*O*-glucoside were purchased from Extrasynthese (Lyon, France).

### Sample preparation

Raw Piquillo peppers, as well as grilled by direct flame (aprox. 700 °C for 15 s) and subsequently canned (102 °C for 30 min) following the statements of the Protected Designation of Origin (PDO), were acquired from a local food industry in Lodosa (Navarra, Spain). Then, canned Piquillo pepper was subjected to two different common cooking techniques as pre-



viously described by Del Burgo-Gutiérrez *et al.*<sup>3</sup>: microwaving (750 W for 1 min) and frying with the addition of olive oil (15 mL) in a non-sticky pan (90 °C for 6 min). Immediately after cooking, all samples were cooled. Then, raw and heat-treated (industrial and culinary) peppers were lyophilized in a freeze dryer (Cryodos-80, Telstar, Terrasa, Spain). Finally, each lyophilized sample was ground into powder with a kitchen blender (La Moulinette 700W, Moulinex, Alençon, France) and stored at −18 °C until further analysis.

### Simulated gastrointestinal digestion

For simulated gastrointestinal digestion *in vitro* standardized procedure as described by Brodkorb *et al.*,<sup>34</sup> was followed. The three step (oral, gastric and intestinal) digestion process was performed in absence of light, under magnetic stirring and at controlled temperature (37 °C). Moreover, pH was also regulated and adjusted on each digestion step with 1 M HCl and/or 2 M NaOH (pH = 7 for oral and intestinal steps and pH = 3 for gastric phase). Previously, simulated salivary, gastric and intestinal fluids (SSF, SGF and SIF) were prepared as described in ESI Table S1.† For oral digestion  $\alpha$ -amylase and SSF were added as described in the *in vitro* simulation protocol.<sup>34</sup> Then, pepsin and SGF were used and finally for the intestinal step, pancreatin, bile salts and SIF were added. All digested samples were immediately frozen for enzymatic inhibition, then whole digested content (residue and supernatant) was lyophilized in a freeze dryer (Cryodos-80, Telstar, Terrasa, Spain) and stored at −18 °C until further analysis. *In vitro* gastrointestinal procedure was performed in duplicate for each pepper sample and both replications were mixed and homogenized.

### *In vitro* colonic fermentation

Afterwards both, raw and heat-treated digested pepper samples were subjected to an *in vitro* fermentation process using human faecal inoculum to mimic large intestine conditions following the method described by Domínguez-Fernández *et al.*<sup>30</sup>

Prior to the fermentation experiment, the culture medium consisting of carbonate-phosphate buffer was prepared as described by Mosele *et al.*<sup>35</sup> The medium was reduced in the absence of light and in an anaerobic container for 48 h prior to *in vitro* fermentation experiment. Fresh faecal samples were collected in sterile containers under anaerobic conditions from three healthy volunteers (Aged: 24–38; BMI: 18.5–24.9) who reported not having gastrointestinal diseases, nor antibiotic treatment for the previous 4 months, and having followed a (poly)phenol-free diet for 48 h prior to sample collection. Sample collection protocol was conducted following the guidelines of the Declaration of Helsinki (Ethical approval from the Research Ethics Committee of University of Navarra No. 2021.80). First, faecal samples were homogenized with culture media to obtain 5% (w/v) of faecal slurry by shaking in a stomacher for 1 minute. Then, 10 mL of faecal slurry were mixed in disposable tubes with 125 mg of each lyophilized sample of digested Piquillo pepper, and then flushed with nitrogen to create an anaerobic atmosphere. All tubes were

incubated in anaerobic containers (Becton Dickinson, Sparks, MD, USA), in an orbital shaker (60 rpm) for 48 hours under constant temperature (37 °C). Samples were collected at different times of incubation (2, 6, 24 and 48 h) and faecal metabolism was stopped by adding 60  $\mu$ L of HCl. Samples were immediately frozen and stored at −80 °C. This procedure was performed in triplicate for each pepper sample under study. Parallel to pepper colonic fermentation, two controls were also performed for each incubation time. Control 1 consisted of 10 mL of faecal slurry without digested sample and control 2 contained 10 mL of culture media (without faeces) and 125 mg of each digested pepper sample under study. All fermented pepper samples were lyophilized in a freeze dryer (Cryodos-80, Telstar, Terrasa, Spain) and stored at −18 °C until further analysis.

### (Poly)phenols extraction

(Poly)phenolic compounds and their metabolites were extracted from all pepper samples under study according to the method described by Sánchez-Salcedo *et al.*,<sup>36</sup> with some modifications. Briefly, 25 mg of each sample was mixed with 0.5 mL methanol/acidify water (0.1% formic acid) (50 : 50 v/v) and sonicated for 90 minutes. Afterwards, samples were centrifuged 10 minutes at 18 625g and the supernatant was collected. The residue was re-extracted with 0.25 mL of methanol/acidified water (50 : 50 v/v), sonicated for 25 minutes and centrifuged for 10 minutes at 18 625g. Both supernatants were mixed, filtered with a 0.22  $\mu$ m PVDF syringe filter, and stored at −18 °C until LC-MS/MS analysis. Each sample was extracted in triplicates.

### Identification and quantification of (poly)phenolic compounds

Polyphenols present in the samples after *in vitro* gastrointestinal digestion and colonic fermentation were characterized (identified and quantified) using an HPLC unit model 1200 (Agilent Technologies, Palo 201 Alto, CA, USA) equipped with a triple quadrupole linear ion trap mass spectrometer (3200 Q-TRAP LC-MS/MS) (AB SCIEX, Madrid, Spain), as described previously by Domínguez-Fernández *et al.*,<sup>30</sup> with modifications. Chromatographic separation was carried out on a CORTECS C18 column (3 × 75 mm, 2.7  $\mu$ m) from Waters (Barcelona, Spain) at 30 °C. Mobile phases consisted of 0.1% (v/v) formic acid in water as solvent A, and 100% acetonitrile as solvent B. Elution flow rate was set at 0.6 mL min<sup>−1</sup> and injection volume was 5  $\mu$ L. The 35 minutes gradient elution program started at 5% B (0–1 min), 5–10% B (1–5 min), 10–20% B (5–8 min), 20–14% B (8–8.5 min), 14–20% B (8.5–10.5 min), 20–30% B (10.5–16 min), 30–100% B (16–17.6 min), 100% B (17.6–25.6 min) and then linearly return to the starting conditions (5% B) over 4.8 min and maintained until the end of the analysis (35 minutes) to re-equilibrate the column. The mass spectrometric analysis run in negative ionization mode with the turbo heater operated at 600 °C and Ion Spray voltage was set at −3500 V. Nitrogen was used as nebulizing, turbo heater and curtain gas, and the pressure was set at −60, −65 and −35 psi, respectively.



Decustering potential and entrance potential were set at  $-20$  V and  $-10$  V and collision energy (CE) for each compound was optimized using the same standards as for (poly)phenolic compound identification (Table S2 ESI†). Targeted mass spectrometric analysis was performed based on the previous identification of (poly)phenols in Piquillo pepper<sup>3</sup> and included other possible phenolic derivatives and metabolites commonly described after colonic fermentation of other matrices rich in (poly)phenols.<sup>32,37,38</sup> Identification parameters including  $m/z$ , fragmentation patterns and retention times, were obtained by comparing with pure phenolic standards if available or tentatively identified based on their chemical structures and comparing with databases (Human Metabolome Database, PubChem and MassBank of North America). When pure phenols standards were unavailable, semiquantification was performed with the calibration curves of structurally similar compounds as previously described by Del Burgo-Gutiérrez *et al.*<sup>3</sup>

Analyst software 1.6.3 (AB SCIEX) was used to obtain Chromatograms and spectral data. Results were expressed in micromol ( $\mu\text{mol}$ ) of (poly)phenol per gram (g) of pepper dry matter (dm). To determine the differences between raw and heat-treated peppers after *in vitro* gastrointestinal digestion and colonic fermentation different statistical analysis were applied using the STATA v.15.0 software package. First, for each (poly)phenolic subgroup the normal distribution of the data was assessed with the skewness and kurtosis test. For those normally distributed (poly)phenolic subgroups, one-way analysis of variance (ANOVA) was applied followed by a Levene test to verify the homogeneity of variances. Then, the Tukey test, for homoscedastic subgroups or Tamhane test for heteroscedastic data, were applied as *posteriori* tests, both with significance accepted at  $p < 0.05$ . For those (poly)phenolic subgroups that did not follow a normal distribution of the data, the Kruskal–Wallis test was applied followed by the multiple comparison test *U* Mann–Whitney adjusted by Sidak with significance accepted at  $p < 0.05$ . The corresponding data test applied for each (poly)phenolic subgroup is specified in the respective tables.

### Bioaccessibility

Bioaccessibility (BA) of (poly)phenolic compounds after *in vitro* gastrointestinal digestion was expressed as the percentage of the concentration of each (poly)phenol ( $\mu\text{mol}$  per g per dm) that remained after *in vitro* gastrointestinal digestion in relation to the initial concentration of each (poly)phenol ( $\mu\text{mol}$  per g per dm) in non-digested pepper samples.<sup>39</sup>

## Results and discussion

### Bioaccessibility of Piquillo pepper (poly)phenols after *in vitro* gastrointestinal digestion

In accordance with the regulations established by the Protected Designation of Origin (PDO), Piquillo pepper is submitted to industrial grilling at high temperatures (approx.

$700$  °C for 15 s) followed by peeling and a subsequent canning process (approx.  $120$  °C for 30 min) before commercialization. Moreover, canned pepper is traditionally submitted to an additional culinary process (microwaving or frying) prior to consumption. As previously observed in other plant-based foods, heat treatments might impact on (poly)phenols' bioaccessibility after *in vitro* gastrointestinal digestion.<sup>30–32,39,40</sup>

Therefore, Piquillo peppers – raw and after both, industrial and culinary heat treatments– were submitted to a three-step *in vitro* gastrointestinal digestion (oral, gastric and duodenal) and (poly)phenolic compounds were analysed by HPLC-ESI-MS/MS. Mass spectrometric characteristics of (poly)phenols identified after *in vitro* gastrointestinal digestion are detailed in ESI Table S2.† Bioaccessibility of (poly)phenolic compounds was calculated by comparing the (poly)phenolic content of each compound after *in vitro* gastrointestinal digestion (ESI Table S3†) with the respective content of raw or heat-treated Piquillo peppers prior digestion.<sup>3</sup> Bioaccessibility (%) of total (poly)phenols compounds and (poly)phenols grouped by families in raw and thermally treated Piquillo peppers are reported in Table 1.

**In raw Piquillo pepper** a total of 50 (poly)phenolic compounds from several subclasses were identified after being submitted to an *in vitro* gastrointestinal digestion (Table S3†). These compounds accounted for  $6.22$   $\mu\text{mol}$  per g per dm and presented a total bioaccessibility of 102%, being non-flavonoids the most abundant compounds ( $3.64$   $\mu\text{mol}$  per g per dm) with a total bioaccessibility of 118% (Table 1). The abundance of non-flavonoids in raw digested Piquillo pepper and thus, the high bioaccessibility observed is mainly attributed to the high amounts of phenylacetic and 3-phenylpropanoic acids found after *in vitro* digestion. These compounds were probably generated from the degradation of other (poly)phenols during digestion,<sup>32,41</sup> but most probably were compounds present in Piquillo pepper covalently bound to food matrix and released as a result of gastrointestinal conditions (pH changes and enzymatic activity).<sup>27</sup> Additionally, 3-(3'-hydroxyphenyl)propanoic, 3-(4'-hydroxy-3'-methoxyphenyl)propanoic acids and 2-(3'-hydroxyphenyl)ethanol, were also generated during *in vitro* gastrointestinal digestion in raw digested peppers. On the other hand, flavonoids ( $2.58$   $\mu\text{mol}$  per g per dm) seemed neither to increase due to a release from food matrix nor to be highly degraded during *in vitro* gastrointestinal digestion and presented an overall high bioaccessibility of 84% (Table 1).

**In industrially treated (grilled and canned) peppers**, a total of 54 (poly)phenolic compounds were detected after *in vitro* gastrointestinal digestion (Table S3†). High amounts of 4'-hydroxy-3'-methoxyphenylacetic acid were found after digestion, which also contributed to the even higher bioaccessibility observed for non-flavonoids (120% and 170% respectively) compared to raw digested samples (Table 1). This compound, along with benzene-1,2-diol, benzene-1,2,3-triol and 4-hydroxy-1,2-benzopyrone were not detected in raw digested samples indicating that their appearance might be exclusively attributed to the application of high temperatures during industrial





**Table 1** Percentage of bioaccessibility (BA) of total (poly)phenols and (poly)phenols grouped by families in raw and thermally treated Piquillo pepper. (Poly)phenols concentrations are expressed as  $\mu\text{mol}$  of (poly)phenolic compound per g of pepper (dry matter) (mean  $\pm$  standard deviation,  $n = 3$ ). Content of before *in vitro* gastrointestinal digestion correspond to the results previously reported by Del Burgo-Gutiérrez *et al.*<sup>3</sup>

Compound	Raw			Grilled			Canned			Microwaved			Fried		
	Before digestion	After digestion	BA (%)	Before digestion	After digestion	BA (%)	Before digestion	After digestion	BA (%)	Before digestion	After digestion	BA (%)	Before digestion	After digestion	BA (%)
Benzenediols- trials <sup>b</sup>	nd	Tr	—	0.36 $\pm$ 0.01	0.19 $\pm$ 0.01c	52	0.29 $\pm$ 0.01	0.14 $\pm$ 0.00c	50	0.29 $\pm$ 0.00	0.15 $\pm$ 0.00c	51	0.27 $\pm$ 0.01	0.12 $\pm$ 0.00b	45
Benzotic acids	0.67 $\pm$ 0.02	0.34 $\pm$ 0.01b	51	0.65 $\pm$ 0.01	0.50 $\pm$ 0.01c	78	0.32 $\pm$ 0.02	0.32 $\pm$ 0.01b	100	0.34 $\pm$ 0.01b	0.33 $\pm$ 0.01a	97	0.37 $\pm$ 0.02	0.27 $\pm$ 0.01a	72
Cinnamic acids	2.38 $\pm$ 0.06	1.39 $\pm$ 0.05c	58	1.30 $\pm$ 0.03	0.64 $\pm$ 0.00b	49	0.76 $\pm$ 0.02	0.44 $\pm$ 0.02a	59	0.78 $\pm$ 0.02	0.47 $\pm$ 0.01a	60	0.87 $\pm$ 0.05	0.40 $\pm$ 0.03a	45
Phenylpropanoic acids	nd	0.82 $\pm$ 0.01b	—	0.01 $\pm$ 0.01	0.84 $\pm$ 0.01b	9663	0.01 $\pm$ 0.00	0.94 $\pm$ 0.02c	13 558	0.01 $\pm$ 0.00	0.99 $\pm$ 0.01d	14 133	0.01 $\pm$ 0.00	0.74 $\pm$ 0.01a	11 802
Phenylacetic acids	nd	1.08 $\pm$ 0.05a	—	1.38 $\pm$ 0.03	2.47 $\pm$ 0.07d	179	0.90 $\pm$ 0.03	2.20 $\pm$ 0.06c	244	0.86 $\pm$ 0.03	2.49 $\pm$ 0.09d	289	1.02 $\pm$ 0.04	1.86 $\pm$ 0.01b	183
Others	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	73	0.01 $\pm$ 0.00	Tr	—	0.01 $\pm$ 0.00	Tr	—	0.01 $\pm$ 0.00	Tr	—	0.01 $\pm$ 0.00	Tr	—
Acylquinic acids	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	99	0.01 $\pm$ 0.00	Tr	—	0.01 $\pm$ 0.00	Tr	—	0.01 $\pm$ 0.00	Tr	—	Tr	Tr	—
<b>Non-flavonoids</b>	<b>3.06 <math>\pm</math> 0.08</b>	<b>3.64 <math>\pm</math> 0.06a</b>	<b>118</b>	<b>3.72 <math>\pm</math> 0.06</b>	<b>4.64 <math>\pm</math> 0.12c</b>	<b>120</b>	<b>2.29 <math>\pm</math> 0.07</b>	<b>4.05 <math>\pm</math> 0.01b</b>	<b>170</b>	<b>2.30 <math>\pm</math> 0.07</b>	<b>4.43 <math>\pm</math> 0.07c</b>	<b>186</b>	<b>2.54 <math>\pm</math> 0.08</b>	<b>3.38 <math>\pm</math> 0.05a</b>	<b>128</b>
Flavonols <sup>a</sup>	1.23 $\pm$ 0.00	1.27 $\pm$ 0.00d	103	0.14 $\pm$ 0.00	0.10 $\pm$ 0.01c	77	0.10 $\pm$ 0.00	0.07 $\pm$ 0.00b	72	0.10 $\pm$ 0.00	0.07 $\pm$ 0.00b	77	0.09 $\pm$ 0.00	0.06 $\pm$ 0.00a	66
Flavones <sup>a</sup>	1.48 $\pm$ 0.02	1.22 $\pm$ 0.02c	83	0.25 $\pm$ 0.01	0.22 $\pm$ 0.01ab	86	0.21 $\pm$ 0.01	0.22 $\pm$ 0.01b	104	0.23 $\pm$ 0.00	0.25 $\pm$ 0.00b	112	0.23 $\pm$ 0.01	0.20 $\pm$ 0.00a	91
Flavanones	0.35 $\pm$ 0.01	0.10 $\pm$ 0.00	28	0.06 $\pm$ 0.00	Tr	—	0.02 $\pm$ 0.00	Tr	—	0.01 $\pm$ 0.00	Tr	—	0.02 $\pm$ 0.00	Tr	—
<b>Total flavonoids<sup>a</sup></b>	<b>3.06 <math>\pm</math> 0.03</b>	<b>2.58 <math>\pm</math> 0.02d</b>	<b>84</b>	<b>0.40 <math>\pm</math> 0.01</b>	<b>0.32 <math>\pm</math> 0.01c</b>	<b>81</b>	<b>0.33 <math>\pm</math> 0.01</b>	<b>0.29 <math>\pm</math> 0.01b</b>	<b>90</b>	<b>0.33 <math>\pm</math> 0.00</b>	<b>0.32 <math>\pm</math> 0.00bc</b>	<b>99</b>	<b>0.33 <math>\pm</math> 0.00</b>	<b>0.26 <math>\pm</math> 0.00a</b>	<b>79</b>
<b>Total (poly) phenols</b>	<b>6.12 <math>\pm</math> 0.06</b>	<b>6.22 <math>\pm</math> 0.08d</b>	<b>102</b>	<b>4.12 <math>\pm</math> 0.06</b>	<b>4.99 <math>\pm</math> 0.09c</b>	<b>116</b>	<b>2.62 <math>\pm</math> 0.01</b>	<b>4.34 <math>\pm</math> 0.10b</b>	<b>160</b>	<b>2.63 <math>\pm</math> 0.01</b>	<b>4.75 <math>\pm</math> 0.09c</b>	<b>175</b>	<b>2.88 <math>\pm</math> 0.01</b>	<b>3.64 <math>\pm</math> 0.05a</b>	<b>123</b>

nd = not detected; Tr = traces. Different letters for each row indicate significant differences ( $p \leq 0.05$ ) among digested samples. <sup>a</sup> Data not normally distributed. <sup>b</sup> Normal distribution of the data but no homogeneity of variances.

grilling. Moreover, the greater (poly)phenols bioaccessibility observed in canned peppers compared to raw and grilled, suggests that the cell-wall softening enhancing the release of these low molecular phenolic acids during gastrointestinal digestion was favoured by this industrial processing technique (Table 1).

Similar to the values observed for raw Piquillo pepper, bioaccessibility of total flavonoids seemed not to be highly affected by the application of industrial treatments, being 81% in grilled and 90% in canned samples (Table 1), indicating that gastrointestinal conditions (pH and enzymes) seemed not to degrade these compounds as previously observed in other vegetables.<sup>7,30,32</sup>

Regarding **culinary treatments** applied to canned Piquillo pepper, (poly)phenols bioaccessibility after *in vitro* gastrointestinal digestion was enhanced after microwaving (175%) compared to canned peppers (160% BA), whereas lower bioaccessibility was observed in fried peppers (123%). This suggests that the release of (poly)phenols from food matrix during gastrointestinal digestion is favoured by microwaving.<sup>29</sup> On the contrary, the addition of oil during cooking might modify the food matrix or even trap (poly)phenols interfering in their release during gastrointestinal digestion.<sup>42</sup>

The overall great bioaccessibility (>100% BA) observed for total (poly)phenolic compounds and specially for total non flavonoids (phenylpropanoic acids and phenylacetic acids) in all Piquillo pepper samples was probably associated to the release of these compounds from food matrix as result of gastrointestinal digestion which seemed to be favoured by the application of industrial and culinary heat treatments.

Interestingly, after the application of industrial and culinary heat treatments (poly)phenols of Piquillo pepper seemed to be more bioaccessible (116–175%) than (poly)phenols of other culinary-treated plant based foods such as artichoke (38–59%),<sup>30</sup> cardoon (60–67%),<sup>31,39</sup> cactus cladodes (55–64%)<sup>40</sup> and of other *Capsicum annuum* varieties as Italian green pepper (82–100%)<sup>32</sup> and dried Chiltepin peppers (3.14%).<sup>42</sup> These differences might be explained by the variability in processing conditions (time and temperature) applied to each.

Moreover, food matrix composition and (poly)phenolic profile might also impact on their total bioaccessibility. In particular, bioaccessibility of raw Piquillo pepper (102%) was notably higher than bioaccessibility of raw artichoke (1.6%)<sup>30</sup> and cardoon (2%)<sup>39</sup> probably due to differences in the major (poly)phenols found in these vegetables (CQQ and diCQA) compared to Piquillo pepper. Moreover, despite the similarity in their (poly)phenolic profile, raw Piquillo pepper also presented higher bioaccessibility than raw cactus cladodes (44%)<sup>40</sup> and Italian green pepper (48%).<sup>32,39</sup> This might be explained by the higher amounts of non-flavonoids, especially phenylacetics and phenylpropanoic acids found in Piquillo pepper that were not reported to be present neither in cactus cladodes nor in Italian green pepper. Similar to the present results, other authors have also observed a heightened bioaccessibility (>100%) of total (poly)phenolic compounds in

mung bean coat (350% BA),<sup>8</sup> mango pulp (206% BA)<sup>41</sup> and in free (poly)phenols fraction of carob (389% BA)<sup>43</sup> mainly attributed to the non-flavonoid fraction.

### Impact of the gut microbiota on Piquillo pepper (poly)phenols

It is widely known that only small quantities of (poly)phenols might be absorbed intact in the upper GIT and high amounts reach the colon to be metabolized into low molecular microbial derivatives. These metabolites might exert different health effects and present higher bioactivity than their parent compounds, either at colon level or, once absorbed, at systemic level.<sup>8,20,22,43</sup>

In the present study, pepper samples resulting from the *in vitro* gastrointestinal digestion were subjected to an *in vitro* colonic fermentation. Digested raw and thermally treated Piquillo peppers were incubated under anaerobic conditions with faecal samples for 48 hours. (Poly)phenolic compounds and microbial derivatives were identified and quantified at each incubation point (2, 6, 24 and 48 h) by HPLC-ESI-MS/MS.

A total of 57 (poly)phenolic compounds were identified in Piquillo pepper samples from *in vitro* colonic fermentation. Mass spectrometric characteristics of identified (poly)phenols are detailed in Table S2.† Total (poly)phenolic content and (poly)phenols grouped by families at the different incubation times are reported in Table 2. In Table 3, concentrations of 21 compounds were reported as the most relevant (poly)phenols representing each at least 2% of total content. Content of the other 26 (poly)phenols found in minor quantities (< 2% of total (poly)phenolic content) are reported in Table S4.†

As reported by several authors, some phenolic compounds found after colonic fermentation of plant-based foods might not be exclusively the result of the breakdown of (poly)phenols mediated by the colonic microbiota but might also result from the breakdown of surplus dietary proteins or amino acids (tyrosine, phenylalanine and/or tryptophan) that reach unabsorbed the large intestine.<sup>24,44</sup> In the present research, high concentrations of phenylacetic, 4-hydroxyphenylacetic and 3-phenylpropanoic acids were detected in control 1 samples (faecal samples homogenised with culture medium but without the addition of pepper samples). Therefore, these compounds were classified as “phenolic compounds also derived from other sources” and excluded for the total (poly)phenols calculation. Despite these compounds will not be further discussed, their contents can be consulted in Table S5.†

Overall, an important biotransformation of (poly)phenols was observed throughout the colonic fermentation process, evidencing a great microbial activity on the remaining (poly)phenols of digested Piquillo peppers. Although concentration and time of appearance were different among samples, similar phenolic derivatives were detected in raw and heat treated samples resulting from the colonic fermentation process. Thus, Fig. 1 illustrates the catabolic pathway proposed for the (poly)phenolic compounds of Piquillo pepper (*Capsicum annuum* cv. *Piquillo*).

First, (poly)phenols attached to sugars such as flavonoids glycosides and cinnamic acid glycosides were deglycosylated at



**Table 2** Total (poly)phenolic compounds and (poly)phenols grouped by families from 48 h of colonic fermentation of raw and thermally treated Piquillo pepper. Results are expressed as  $\mu\text{mol}$  of (poly)phenolic compound per g of pepper (dry matter) (mean  $\pm$  standard deviation,  $n = 3$ )

Compound	Raw	Grilled	Canned	Microwaved	Fried
<b>Non-flavonoids</b>					
<b>Benzene diols and triols</b>					
T 0 h <sup>a,c</sup>	Tra	0.191 $\pm$ 0.009c	0.144 $\pm$ 0.003c	0.150 $\pm$ 0.002c	0.119 $\pm$ 0.004b
T 2 h	nda	0.093 $\pm$ 0.006c	0.061 $\pm$ 0.004b	0.103 $\pm$ 0.005c	0.072 $\pm$ 0.005b
T 6 h	0.038 $\pm$ 0.000a	0.267 $\pm$ 0.005c	0.245 $\pm$ 0.023c	0.186 $\pm$ 0.020b	0.179 $\pm$ 0.006b
T 24 h <sup>b</sup>	0.286 $\pm$ 0.013ad	0.631 $\pm$ 0.029bc	0.478 $\pm$ 0.024ab	0.544 $\pm$ 0.026ab	0.307 $\pm$ 0.025cd
T 48 h	0.295 $\pm$ 0.006a	0.661 $\pm$ 0.046c	0.587 $\pm$ 0.032c	0.548 $\pm$ 0.045bc	0.389 $\pm$ 0.002ab
<b>Benzoic acids</b>					
T 0 h <sup>a</sup>	0.341 $\pm$ 0.008b	0.504 $\pm$ 0.010c	0.319 $\pm$ 0.007b	0.331 $\pm$ 0.013b	0.266 $\pm$ 0.003a
T 2 h	0.523 $\pm$ 0.002c	0.398 $\pm$ 0.003b	0.730 $\pm$ 0.026d	0.343 $\pm$ 0.016a	0.395 $\pm$ 0.007ab
T 6 h	0.055 $\pm$ 0.004a	0.092 $\pm$ 0.002a	0.583 $\pm$ 0.020d	0.306 $\pm$ 0.032b	0.421 $\pm$ 0.024c
T 24 h <sup>c</sup>	Tra	Tra	0.167 $\pm$ 0.009ab	0.139 $\pm$ 0.003b	0.614 $\pm$ 0.020c
T 48 h	0.044 $\pm$ 0.005a	0.079 $\pm$ 0.003b	0.065 $\pm$ 0.004ab	0.138 $\pm$ 0.009c	0.246 $\pm$ 0.008d
<b>Cinnamic acids</b>					
T 0 h <sup>a</sup>	1.387 $\pm$ 0.050c	0.641 $\pm$ 0.002b	0.443 $\pm$ 0.019a	0.465 $\pm$ 0.007a	0.396 $\pm$ 0.026a
T 2 h <sup>c</sup>	3.005 $\pm$ 0.017d	0.452 $\pm$ 0.019a	1.062 $\pm$ 0.056bc	0.647 $\pm$ 0.012b	0.784 $\pm$ 0.004c
T 6 h	0.579 $\pm$ 0.021d	0.158 $\pm$ 0.009a	0.281 $\pm$ 0.004c	0.127 $\pm$ 0.011a	0.214 $\pm$ 0.011b
T 24 h	0.004 $\pm$ 0.000b	0.002 $\pm$ 0.000a	0.012 $\pm$ 0.001b	0.011 $\pm$ 0.001b	0.072 $\pm$ 0.002c
T 48 h <sup>b</sup>	0.014 $\pm$ 0.001b	0.005 $\pm$ 0.000a	0.009 $\pm$ 0.001a	0.040 $\pm$ 0.003ab	0.035 $\pm$ 0.002ab
<b>Phenylpropanoic acids</b>					
T 0 h <sup>a</sup>	0.817 $\pm$ 0.012b	0.835 $\pm$ 0.011b	0.943 $\pm$ 0.024c	0.994 $\pm$ 0.005d	0.736 $\pm$ 0.010a
T 2 h	0.669 $\pm$ 0.035d	0.174 $\pm$ 0.005ab	0.303 $\pm$ 0.007c	0.188 $\pm$ 0.016b	0.129 $\pm$ 0.006a
T 6 h <sup>b</sup>	0.419 $\pm$ 0.026bc	0.207 $\pm$ 0.016abc	0.918 $\pm$ 0.069c	0.754 $\pm$ 0.010b	0.240 $\pm$ 0.000a
T 24 h	1.069 $\pm$ 0.119a	2.365 $\pm$ 0.161b	2.382 $\pm$ 0.049a	3.902 $\pm$ 0.246c	5.056 $\pm$ 0.514d
T 48 h	0.651 $\pm$ 0.013c	0.061 $\pm$ 0.003a	0.240 $\pm$ 0.004b	0.057 $\pm$ 0.003a	1.312 $\pm$ 0.018d
<b>Phenylacetic acids</b>					
T 0 h <sup>a</sup>	1.075 $\pm$ 0.049a	2.472 $\pm$ 0.065d	2.197 $\pm$ 0.055c	2.490 $\pm$ 0.060d	1.860 $\pm$ 0.013b
T 2 h <sup>c</sup>	nda	0.322 $\pm$ 0.008bc	0.463 $\pm$ 0.038c	0.500 $\pm$ 0.003c	0.239 $\pm$ 0.024b
T 6 h <sup>b</sup>	nda	0.973 $\pm$ 0.086d	0.628 $\pm$ 0.069c	0.507 $\pm$ 0.036bc	0.500 $\pm$ 0.024b
T 24 h	0.743 $\pm$ 0.028b	1.328 $\pm$ 0.060c	0.447 $\pm$ 0.031a	0.735 $\pm$ 0.040b	0.643 $\pm$ 0.028a
T 48 h <sup>c</sup>	0.274 $\pm$ 0.014a	0.656 $\pm$ 0.051ab	0.774 $\pm$ 0.113ab	0.544 $\pm$ 0.029b	0.353 $\pm$ 0.007ab
<b>Other phenolics</b>					
T 0 h <sup>a</sup>	0.006 $\pm$ 0.000	Tr	Tr	Tr	Tr
T 2 h	0.005 $\pm$ 0.001b	0.008 $\pm$ 0.000c	Tra	0.007 $\pm$ 0.001c	0.008 $\pm$ 0.000c
T 6 h	0.012 $\pm$ 0.001a	0.033 $\pm$ 0.002c	0.021 $\pm$ 0.001b	0.037 $\pm$ 0.004c	0.030 $\pm$ 0.003c
T 24 h <sup>b</sup>	0.047 $\pm$ 0.003cd	0.066 $\pm$ 0.001d	0.027 $\pm$ 0.002ab	0.058 $\pm$ 0.004bc	0.029 $\pm$ 0.003a
T 48 h <sup>c</sup>	0.049 $\pm$ 0.002a	0.070 $\pm$ 0.005a	0.069 $\pm$ 0.004a	0.063 $\pm$ 0.006a	0.102 $\pm$ 0.080a
<b>Total non-flavonoids</b>					
T 0 h <sup>a</sup>	3.635 $\pm$ 0.060a	4.643 $\pm$ 0.116c	4.046 $\pm$ 0.096b	4.430 $\pm$ 0.070c	3.377 $\pm$ 0.047a
T 2 h	4.206 $\pm$ 0.022e	1.438 $\pm$ 0.041b	2.156 $\pm$ 0.041d	1.785 $\pm$ 0.068c	1.389 $\pm$ 0.010a
T 6 h	1.102 $\pm$ 0.049a	1.729 $\pm$ 0.084bc	2.682 $\pm$ 0.035d	1.918 $\pm$ 0.097c	1.583 $\pm$ 0.024b
T 24 h	2.149 $\pm$ 0.083a	4.392 $\pm$ 0.228c	3.516 $\pm$ 0.025b	5.389 $\pm$ 0.205d	6.721 $\pm$ 0.467e
T 48 h	1.329 $\pm$ 0.019a	1.534 $\pm$ 0.061ab	1.755 $\pm$ 0.123b	1.390 $\pm$ 0.078a	2.476 $\pm$ 0.052c
<b>Flavonoids</b>					
<b>Flavonols</b>					
T 0 h <sup>a,b</sup>	1.267 $\pm$ 0.060d	0.104 $\pm$ 0.006c	0.070 $\pm$ 0.001b	0.071 $\pm$ 0.001b	0.057 $\pm$ 0.001a
T 2 h <sup>a,b</sup>	2.791 $\pm$ 0.021c	0.124 $\pm$ 0.009b	0.085 $\pm$ 0.007a	0.094 $\pm$ 0.003a	0.074 $\pm$ 0.004a
T 6 h <sup>b</sup>	0.490 $\pm$ 0.016c	0.067 $\pm$ 0.008b	0.054 $\pm$ 0.004b	0.031 $\pm$ 0.003a	0.028 $\pm$ 0.003ab
T 24 h <sup>b</sup>	0.073 $\pm$ 0.004c	0.052 $\pm$ 0.009c	0.007 $\pm$ 0.001ab	0.023 $\pm$ 0.000b	Tra
T 48 h	0.015 $\pm$ 0.001d	0.008 $\pm$ 0.000b	0.012 $\pm$ 0.001c	Tra	0.020 $\pm$ 0.000cd
<b>Flavones</b>					
T 0 h <sup>a,b</sup>	1.218 $\pm$ 0.017c	0.216 $\pm$ 0.008ab	0.221 $\pm$ 0.010b	0.254 $\pm$ 0.003b	0.204 $\pm$ 0.004a
T 2 h <sup>b</sup>	2.632 $\pm$ 0.024d	0.212 $\pm$ 0.008bc	0.239 $\pm$ 0.010c	0.207 $\pm$ 0.013b	0.163 $\pm$ 0.005a
T 6 h <sup>b</sup>	0.994 $\pm$ 0.012c	0.168 $\pm$ 0.014ab	0.165 $\pm$ 0.005ab	0.215 $\pm$ 0.006a	0.177 $\pm$ 0.001b
T 24 h	0.128 $\pm$ 0.012d	0.015 $\pm$ 0.001a	0.022 $\pm$ 0.001ab	0.040 $\pm$ 0.001b	0.054 $\pm$ 0.003c
T 48 h	0.103 $\pm$ 0.012	nd	nd	Tr	nd
<b>Flavanones</b>					
T 0 h <sup>a</sup>	0.098 $\pm$ 0.001	Tr	Tr	Tr	Tr
T 2 h <sup>b</sup>	0.276 $\pm$ 0.019b	0.004 $\pm$ 0.000a	0.009 $\pm$ 0.001a	0.034 $\pm$ 0.002a	0.032 $\pm$ 0.001a
T 6 h	0.064 $\pm$ 0.006c	0.004 $\pm$ 0.000a	0.006 $\pm$ 0.001a	0.033 $\pm$ 0.002b	0.037 $\pm$ 0.003b
T 24 h <sup>b</sup>	0.008 $\pm$ 0.000b	Tra	Tra	0.030 $\pm$ 0.002b	0.034 $\pm$ 0.005b
T 48 h	nd	nd	nd	nd	nd
<b>Total flavonoids</b>					
T 0 h <sup>a</sup>	2.584 $\pm$ 0.021d	0.320 $\pm$ 0.010c	0.291 $\pm$ 0.010b	0.324 $\pm$ 0.002bc	0.261 $\pm$ 0.004a
T 2 h <sup>b</sup>	5.700 $\pm$ 0.024c	0.340 $\pm$ 0.016b	0.334 $\pm$ 0.015b	0.335 $\pm$ 0.016b	0.270 $\pm$ 0.003a
T 6 h <sup>b</sup>	1.549 $\pm$ 0.031b	0.238 $\pm$ 0.022a	0.225 $\pm$ 0.009a	0.279 $\pm$ 0.010a	0.242 $\pm$ 0.008a
T 24 h <sup>c</sup>	0.209 $\pm$ 0.016c	0.067 $\pm$ 0.009c	0.029 $\pm$ 0.000ab	0.093 $\pm$ 0.002b	0.089 $\pm$ 0.006a



Table 2 (Contd.)

Compound	Raw	Grilled	Canned	Microwaved	Fried
T 48 h <sup>b</sup>	0.118 ± 0.011c	0.008 ± 0.000b	0.012 ± 0.001b	Tr	0.020 ± 0.000ab
<b>Total (poly)phenolic compounds</b>					
T 0 h <sup>a</sup>	6.219 ± 0.075d	4.962 ± 0.092c	4.337 ± 0.104b	4.754 ± 0.068c	3.638 ± 0.050a
T 2 h <sup>b</sup>	9.906 ± 0.032d	1.776 ± 0.056ab	2.945 ± 0.029c	2.115 ± 0.080b	1.902 ± 0.028a
T 6 h <sup>c</sup>	2.651 ± 0.026b	1.967 ± 0.063a	2.907 ± 0.035c	2.197 ± 0.103bc	1.835 ± 0.028a
T 24 h	2.358 ± 0.097a	4.460 ± 0.236c	3.544 ± 0.025b	5.483 ± 0.206d	6.824 ± 0.470e
T 48 h	1.446 ± 0.030a	1.542 ± 0.061ab	1.767 ± 0.123b	1.390 ± 0.078a	2.490 ± 0.051c

nd = not detected; Tr = traces. <sup>a</sup> T 0 h was assumed to correspond to (poly)phenolic content of pepper samples after *in vitro* gastrointestinal digestion. Different letters for each row indicate significant differences ( $p \leq 0.05$ ) among digested samples. <sup>b</sup> Data not normally distributed. <sup>c</sup> Normal distribution of the data but no homogeneity of variances.

the initial steps of colonic fermentation resulting in the release of their respective aglycones. Then, flavonoids might be subjected to a C-ring fission into 3-(3'-4'-dihydroxyphenyl)propanoic acid, followed by a dehydroxylation process into 3-(3'-hydroxyphenyl)propanoic acid.<sup>8,32,37,44</sup> Moreover, 3-(3'-hydroxyphenyl)propanoic acid might also arise from other non-flavonoids such as cinnamic acids (3,4-dihydroxycinnamic and 3-hydroxy-4-methoxycinnamic acid derivatives) *via* reduction of the double bond and dehydroxylation or demethoxylation reactions as proposed by other authors.<sup>45,46</sup> Then, 3-(3'-hydroxyphenyl)propanoic acid might be converted into 3-hydroxyphenylacetic acid by an  $\alpha$ -oxidation of the acyl-chain although this compound might also derive from non-detected intermediates such as 3,4-dihydroxyphenylacetic acid resulting from the  $\alpha$ -oxidation of 3-(3',4'-dihydroxyphenyl)propanoic acid.<sup>45</sup> Otherwise, 3,4-dihydroxyphenylacetic acid might also be  $\alpha$ -oxidated into 3,4-dihydroxybenzoic acid<sup>31,45</sup> and further dehydroxylated into 3- and 4-hydroxybenzoic acid or catabolized into benzene-1,2-diol *via*  $\beta$ -oxidation.<sup>45</sup> Other compounds of relevance such as 4'-hydroxy-3'-methoxyphenylacetic acid might be originated from the  $\alpha$ -oxidation of 3-(4'-hydroxy-3'-methoxyphenyl)propanoic acid derived from the hydrogenation of 4'-hydroxy-3'-methoxycinnamic acid by microbial reductase activity.<sup>45,46</sup>

As can be observed in Table 2, although (poly)phenolic compounds identified during the colonic fermentation process were similar among pepper samples under study, the application of heat treatments, especially industrial processing, impacts on the total and individual (poly)phenols concentrations at the beginning of the colonic fermentation process (T0 h). Therefore, differences in the degradation kinetics and time of appearance of some pepper (poly)phenolic derivatives, as well as in their content, were found throughout the 48 h *in vitro* colonic fermentation.

Fig. 2A illustrates the degradation kinetics and biotransformation of total (poly)phenols and the main subclasses (non-flavonoids and flavonoids) of raw, grilled, and canned peppers through the fermentation process (48 h). In raw Piquillo samples, total (poly)phenolic content showed a notably increase after 2 hours of incubation (from 6.219 to 9.906  $\mu\text{mol g}^{-1}$ ), from which non-flavonoids increased from 3.635 to 4.206  $\mu\text{mol g}^{-1}$ , mainly due to the increase of cin-

amic acids (from 1.387 to 3.005  $\mu\text{mol g}^{-1}$ ), while total flavonoids increased from 2.584 to 5.700  $\mu\text{mol g}^{-1}$  with the increment of all flavonoid subgroups (Table 2). On the contrary, total (poly)phenols decreased in grilled (from 4.987 to 1.776  $\mu\text{mol g}^{-1}$ ) and canned peppers (from 4.337 to 2.945  $\mu\text{mol g}^{-1}$ ) after the same 2 hours of incubation (Table 2 and Fig. 2A). This might suggest that in raw peppers, relevant amounts of (poly)phenols covalently bound to food matrix (e.g., polysaccharides or dietary fibre), were released at the initial steps of colonic fermentation. In grilled and canned peppers, these covalently bound (poly)phenols were probably released from the food matrix due to the high temperatures applied during industrial processing, and therefore, already catabolized at the initial steps of colonic fermentation.

The main (poly)phenols found in raw peppers before colonic fermentation (T0 h) were quercetin, luteolin, cinnamic acids and their respective glycosides. Fig. 2B represents the degradation kinetics of these compounds in raw and in industrially treated peppers (grilled and canned). In raw peppers glycosides are first hydrolysed resulting in an increase of their respective aglycones, which peaked after 2–6 hours of colonic fermentation. Nevertheless, in the case of flavonoid deglycosylation, only luteolin increased after 2 hours of incubation whereas quercetin was not detected at this point. This could be explained by the rapid dehydroxylation of quercetin into luteolin as previously reported in other colonic fermentation studies of (poly)phenols rich plant-based foods.<sup>25,32</sup> In industrially treated peppers flavonoid and cinnamic acid glycosides followed a similar degradation pattern (Fig. 2B). However, since little amounts of flavonoids were initially present (approx. 0.3  $\mu\text{mol g}^{-1}$ ) compared to raw peppers (2.6  $\mu\text{mol g}^{-1}$ ), lower amounts of luteolin were found in grilled and canned peppers (Table 3). As illustrated in Fig. 1B, after 6 hours of colonic fermentation, content of flavonoids and cinnamic acids derivatives was markedly reduced and then, after 24 hours of incubation, these compounds were almost entirely degraded. Similarly, other authors<sup>25,30</sup> reported in other plant-based foods that only low amounts of native (poly)phenols remained intact after *in vitro* colonic fermentation highlighting the importance of studying colonic biotransformation of (poly)phenols for understanding their potential bioactivity. As a result of the colonic degradation of native (poly)phenols



**Table 3** Major (poly)phenolic compounds and derivatives from 48 h of colonic fermentation of raw and thermally treated Piquillo pepper. Results are expressed as  $\mu\text{mol}$  of (poly)phenolic compound per g of pepper (dry matter) (mean  $\pm$  standard deviation,  $n = 3$ )

Compound <sup>a</sup>	Raw	Grilled	Canned	Microwaved	Fried
<b>Non-flavonoids</b>					
<b>Benzene diols and triols</b>					
<b>Benz-1,2-diol</b>					
T 0 h <sup>d,j</sup>	nda	0.177 $\pm$ 0.007d	0.131 $\pm$ 0.003c	0.139 $\pm$ 0.001cd	0.109 $\pm$ 0.003b
T 2 h	nda	0.093 $\pm$ 0.006c	0.061 $\pm$ 0.004b	0.103 $\pm$ 0.006c	0.072 $\pm$ 0.005b
T 6 h	0.038 $\pm$ 0.000a	0.267 $\pm$ 0.005c	0.245 $\pm$ 0.023c	0.186 $\pm$ 0.020b	0.179 $\pm$ 0.006b
T 24 h <sup>e</sup>	0.286 $\pm$ 0.016ab	0.631 $\pm$ 0.029c	0.478 $\pm$ 0.024bc	0.544 $\pm$ 0.026bc	0.307 $\pm$ 0.025ac
T 48 h	0.295 $\pm$ 0.008a	0.661 $\pm$ 0.046c	0.587 $\pm$ 0.032c	0.548 $\pm$ 0.045bc	0.389 $\pm$ 0.002ab
<b>Benzoic acids</b>					
<b>3-OH-BA</b>					
T 0 h <sup>d</sup>	0.004 $\pm$ 0.000	0.028 $\pm$ 0.001	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.010 $\pm$ 0.000
T 2 h	nd	0.042 $\pm$ 0.002	0.136 $\pm$ 0.008	0.127 $\pm$ 0.001	0.088 $\pm$ 0.002
T 6 h	0.055 $\pm$ 0.004	0.053 $\pm$ 0.002	0.117 $\pm$ 0.008	0.112 $\pm$ 0.010	0.098 $\pm$ 0.008
T 24 h	Tr	Tr	Tr	0.011 $\pm$ 0.001	0.066 $\pm$ 0.003
T 48 h	0.044 $\pm$ 0.005	0.079 $\pm$ 0.004	0.065 $\pm$ 0.004	0.138 $\pm$ 0.009	0.091 $\pm$ 0.004
<b>4-OH-BA</b>					
T 0 h	0.023 $\pm$ 0.002	0.030 $\pm$ 0.001	0.038 $\pm$ 0.001	0.039 $\pm$ 0.001	0.024 $\pm$ 0.001
T 2 h	0.091 $\pm$ 0.002	Tr	0.224 $\pm$ 0.013	0.024 $\pm$ 0.000	0.074 $\pm$ 0.003
T 6 h	Tr	nd	0.208 $\pm$ 0.003	0.129 $\pm$ 0.010	0.121 $\pm$ 0.013
T 24 h	Tr	nd	0.123 $\pm$ 0.005	0.042 $\pm$ 0.001	0.244 $\pm$ 0.004
T 48 h	nd	nd	Tr	Tr	0.056 $\pm$ 0.004
<b>3,4-diOH-BA</b>					
T 0 h <sup>d</sup>	0.003 $\pm$ 0.000	0.016 $\pm$ 0.001	0.027 $\pm$ 0.002	0.028 $\pm$ 0.000	0.023 $\pm$ 0.001
T 2 h	Tr	nd	0.153 $\pm$ 0.008	0.007 $\pm$ 0.000	0.072 $\pm$ 0.004
T 6 h	Tr	nd	0.038 $\pm$ 0.000	0.079 $\pm$ 0.007	0.188 $\pm$ 0.013
T 24 h	nd	nd	0.286 $\pm$ 0.016	0.087 $\pm$ 0.005	0.303 $\pm$ 0.023
T 48 h	nd	nd	Tr	Tr	0.099 $\pm$ 0.002
<b>3-MetOH-BA-4-O-GlucSD<sup>b</sup></b>					
T 0 h <sup>d</sup>	0.305 $\pm$ 0.008	0.424 $\pm$ 0.012	0.233 $\pm$ 0.005	0.244 $\pm$ 0.012	0.206 $\pm$ 0.012
T 2 h	0.432 $\pm$ 0.005	0.356 $\pm$ 0.007	0.217 $\pm$ 0.004	0.186 $\pm$ 0.018	0.160 $\pm$ 0.001
T 6 h	Tr	0.040 $\pm$ 0.003	Tr	Tr	Tr
T 24 h	nd	nd	nd	nd	nd
T 48 h	nd	nd	nd	nd	nd
<b>Cinnamic acids</b>					
<b>CA-4'-O-GlucSD<sup>b</sup></b>					
T 0 h <sup>d</sup>	1.025 $\pm$ 0.046	0.395 $\pm$ 0.003	0.262 $\pm$ 0.012	0.282 $\pm$ 0.003	0.257 $\pm$ 0.024
T 2 h	2.117 $\pm$ 0.056	0.354 $\pm$ 0.020	0.784 $\pm$ 0.045	0.461 $\pm$ 0.010	0.551 $\pm$ 0.010
T 6 h	0.556 $\pm$ 0.026	0.143 $\pm$ 0.011	0.242 $\pm$ 0.002	0.079 $\pm$ 0.014	0.203 $\pm$ 0.019
T 24 h	nd	Tr	Tr	Tr	Tr
T 48 h	nd	nd	nd	nd	nd
<b>3',4'-diOH-CA</b>					
T 0 h <sup>d</sup>	0.020 $\pm$ 0.000	0.012 $\pm$ 0.001	0.009 $\pm$ 0.001	0.009 $\pm$ 0.000	0.009 $\pm$ 0.000
T 2 h	Tr	0.012 $\pm$ 0.001	0.054 $\pm$ 0.005	0.053 $\pm$ 0.006	0.039 $\pm$ 0.003
T 6 h	nd	Tr	Tr	Tr	Tr
T 24 h	nd	nd	nd	nd	0.021 $\pm$ 0.002
T 48 h	nd	nd	nd	0.027 $\pm$ 0.002	0.027 $\pm$ 0.002
<b>4'-OH-3'-MetOH-CA</b>					
T 0 h <sup>d</sup>	0.126 $\pm$ 0.003	0.055 $\pm$ 0.001	0.041 $\pm$ 0.001	0.045 $\pm$ 0.000	0.044 $\pm$ 0.001
T 2 h	0.366 $\pm$ 0.038	0.051 $\pm$ 0.002	0.064 $\pm$ 0.003	0.024 $\pm$ 0.001	0.047 $\pm$ 0.002
T 6 h	Tr	Tr	Tr	Tr	Tr
T 24 h	nd	nd	nd	nd	0.014 $\pm$ 0.001
T 48 h	nd	nd	nd	nd	nd
<b>3'-OH-4'-MetOH-CA</b>					
T 0 h <sup>d</sup>	0.017 $\pm$ 0.000	0.005 $\pm$ 0.000	0.006 $\pm$ 0.000	0.007 $\pm$ 0.001	0.006 $\pm$ 0.001
T 2 h	0.354 $\pm$ 0.002	nd	0.081 $\pm$ 0.007	0.048 $\pm$ 0.002	0.084 $\pm$ 0.007
T 6 h	Tr	nd	0.016 $\pm$ 0.001	0.025 $\pm$ 0.002	Tr
T 24 h	nd	nd	nd	nd	nd
T 48 h	nd	nd	nd	nd	nd
<b>Phenylpropanoic acids</b>					
<b>3-(3'-OH-ph)PrA</b>					
T 0 h <sup>d,f</sup>	0.007 $\pm$ 0.000a	0.007 $\pm$ 0.001a	0.007 $\pm$ 0.000a	0.009 $\pm$ 0.001a	0.006 $\pm$ 0.000a
T 2 h <sup>e</sup>	0.064 $\pm$ 0.000	nd	nd	nd	nd
T 6 h <sup>e</sup>	0.263 $\pm$ 0.037bc	Tra	0.676 $\pm$ 0.065c	0.549 $\pm$ 0.012b	Tra
T 24 h	0.997 $\pm$ 0.146a	2.298 $\pm$ 0.158b	2.347 $\pm$ 0.061b	3.838 $\pm$ 0.248c	5.007 $\pm$ 0.629d
T 48 h	0.602 $\pm$ 0.010c	Tra	0.162 $\pm$ 0.002b	Tra	1.257 $\pm$ 0.021d
<b>3-(3',4'-diOH-ph)PrA</b>					
T 0 h <sup>d</sup>	0.017 $\pm$ 0.001	0.017 $\pm$ 0.001	0.017 $\pm$ 0.001	0.017 $\pm$ 0.000	0.013 $\pm$ 0.000

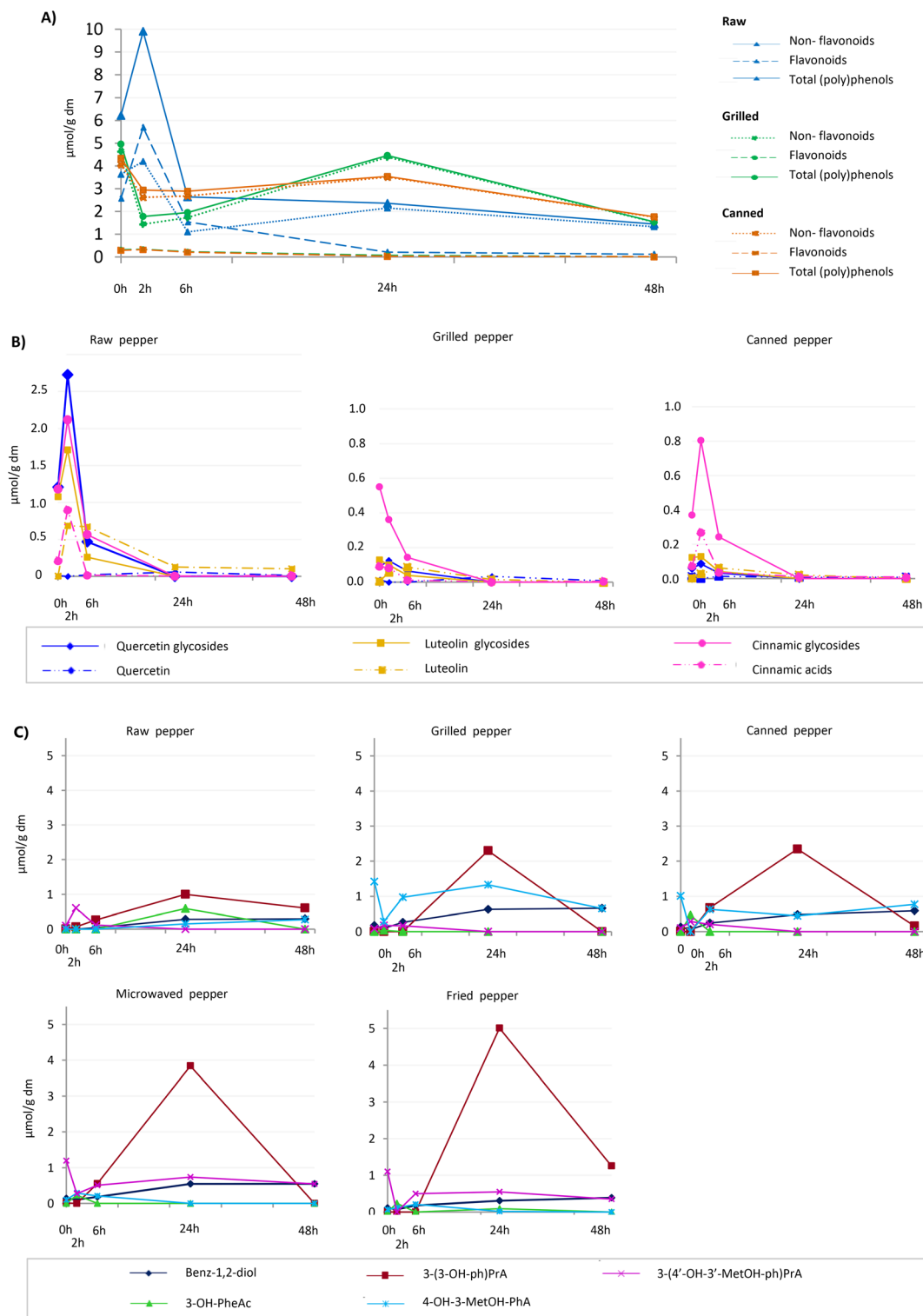


Table 3 (Contd.)

Compound <sup>a</sup>	Raw	Grilled	Canned	Microwaved	Fried
T 2 h	Tr	Tr	0.015 ± 0.000	0.018 ± 0.001	0.011 ± 0.001
T 6 h	0.035 ± 0.004	0.038 ± 0.011	0.036 ± 0.002	0.039 ± 0.001	0.029 ± 0.002
T 24 h	0.072 ± 0.001	0.068 ± 0.005	0.035 ± 0.003	0.063 ± 0.005	0.032 ± 0.000
T 48 h	0.049 ± 0.004	0.061 ± 0.004	0.079 ± 0.003	0.057 ± 0.004	0.055 ± 0.003
<b>3-(4'-OH-3'-MetOH-ph)PrA</b>					
T 0 h <sup>d</sup>	0.101 ± 0.003c	0.082 ± 0.004b	0.098 ± 0.001c	0.098 ± 0.004c	0.069 ± 0.002a
T 2 h <sup>f</sup>	0.605 ± 0.043c	0.174 ± 0.006ab	0.288 ± 0.008b	0.170 ± 0.019	0.118 ± 0.006a
T 6 h	0.121 ± 0.015a	0.169 ± 0.011b	0.206 ± 0.007c	0.167 ± 0.012b	0.211 ± 0.002c
T 24 h	Tr	Tr	Tr	Tr	0.017 ± 0.000
T 48 h	nd	nd	nd	nd	Tr
<b>Phenylacetic acids</b>					
<b>3'-OH-PhA<sup>c</sup></b>					
T 0 h <sup>d</sup>	nd	nd	nd	nd	nd
T 2 h <sup>e</sup>	nda	0.036 ± 0.004b	0.463 ± 0.038c	0.230 ± 0.024bc	0.239 ± 0.024bc
T 6 h	nd	Tr	Tr	Tr	Tr
T 24 h <sup>e</sup>	0.589 ± 0.028c	nda	nda	nda	0.091 ± 0.004b
T 48 h	nd	nd	nd	nd	nd
<b>4'-OH-3'-MetOH-PhA<sup>b</sup></b>					
T 0 h <sup>d</sup>	nda	1.419 ± 0.021d	1.010 ± 0.027b	1.192 ± 0.085c	1.103 ± 0.016bc
T 2 h <sup>f</sup>	nda	0.286 ± 0.006b	nda	0.269 ± 0.004b	nda
T 6 h <sup>e</sup>	nda	0.973 ± 0.086d	0.628 ± 0.069c	0.507 ± 0.036bc	0.500 ± 0.029b
T 24 h	0.155 ± 0.013a	1.328 ± 0.073d	0.447 ± 0.038b	0.735 ± 0.040c	0.551 ± 0.032b
T 48 h <sup>f</sup>	0.274 ± 0.017ab	0.656 ± 0.051a	0.774 ± 0.113a	0.544 ± 0.029b	0.353 ± 0.008a
<b>Other phenolics</b>					
<b>2-(3'-OH-Ph)etOH</b>					
T 0 h <sup>d</sup>	Tr	Tr	Tr	Tr	0.006 ± 0.000
T 2 h	0.005 ± 0.001	Tr	Tr	Tr	Tr
T 6 h	0.012 ± 0.002	0.025 ± 0.002	0.014 ± 0.001	0.030 ± 0.004	0.023 ± 0.002
T 24 h	0.047 ± 0.003	0.059 ± 0.002	0.020 ± 0.003	0.048 ± 0.004	0.016 ± 0.004
T 48 h	0.049 ± 0.002	0.063 ± 0.005	0.065 ± 0.005	0.054 ± 0.005	0.034 ± 0.002
<b>Flavonoids</b>					
<b>Flavonols</b>					
<b>Querc</b>					
T 0 h <sup>d</sup>	0.010 ± 0.000	0.013 ± 0.001	0.011 ± 0.000	0.011 ± 0.000	0.009 ± 0.000
T 2 h	nd	nd	nd	nd	nd
T 6 h	0.020 ± 0.002	Tr	0.013 ± 0.000	Tr	Tr
T 24 h	0.059 ± 0.005	0.034 ± 0.006	0.006 ± 0.000	0.015 ± 0.000	nd
T 48 h	0.012 ± 0.001	0.008 ± 0.001	0.008 ± 0.001	Tr	nd
<b>Querc 3-O-Rha</b>					
T 0 h <sup>d</sup>	0.978 ± 0.003	0.067 ± 0.006	0.043 ± 0.000	0.044 ± 0.001	0.036 ± 0.001
T 2 h	2.390 ± 0.005	0.103 ± 0.010	0.072 ± 0.007	0.077 ± 0.002	0.062 ± 0.003
T 6 h	0.470 ± 0.021	0.064 ± 0.008	0.031 ± 0.003	0.031 ± 0.003	0.028 ± 0.003
T 24 h	Tr	Tr	tr	Tr	Tr
T 48 h	nd	nd	nd	nd	nd
<b>Flavones</b>					
<b>Lut</b>					
T 0 h <sup>d</sup>	0.003 ± 0.000	0.005 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000
T 2 h	0.687 ± 0.043	0.057 ± 0.001	0.027 ± 0.002	0.034 ± 0.001	0.024 ± 0.002
T 6 h	0.672 ± 0.015	0.087 ± 0.010	0.062 ± 0.002	0.124 ± 0.003	0.089 ± 0.001
T 24 h	0.128 ± 0.015	0.015 ± 0.002	0.022 ± 0.001	0.037 ± 0.002	0.036 ± 0.002
T 48 h	0.103 ± 0.014	Tr	Tr	Tr	Tr
<b>Lut-7-O-(2-O-Ap)GlucSD<sup>b</sup></b>					
T 0 h <sup>d</sup>	0.062 ± 0.005	0.016 ± 0.001	0.034 ± 0.001	0.037 ± 0.002	0.032 ± 0.003
T 2 h	1.030 ± 0.010	0.071 ± 0.005	0.088 ± 0.005	0.065 ± 0.009	0.060 ± 0.001
T 6 h	0.169 ± 0.018	0.027 ± 0.002	0.023 ± 0.002	0.015 ± 0.002	0.017 ± 0.001
T 24 h	nd	nd	nd	nd	nd
T 48 h	nd	nd	nd	nd	nd
<b>Lut 7-O-(2-O-Ap-6-O-MaO)GlucSD<sup>b</sup></b>					
T 0 h <sup>d</sup>	0.852 ± 0.002	0.076 ± 0.006	0.042 ± 0.003	0.052 ± 0.001	0.036
T 2 h	0.146 ± 0.029	0.010 ± 0.002	nd	0.007 ± 0.002	Tr
T 6 h	Tr	Tr	nd	Tr	nd
T 24 h	nd	nd	nd	nd	nd
T 48 h	nd	nd	nd	nd	nd
<b>Flavanones</b>					
<b>NarGE<sup>b</sup></b>					
T 0 h <sup>d</sup>	0.068 ± 0.001	Tr	Tr	Tr	Tr
T 2 h	0.221 ± 0.031	0.004 ± 0.000	0.009 ± 0.001	0.034 ± 0.002	0.032 ± 0.001
T 6 h	0.064 ± 0.008	0.004 ± 0.000	0.006 ± 0.001	0.033 ± 0.002	0.037 ± 0.003
T 24 h	0.008 ± 0.001	nd	Tr	0.030 ± 0.002	0.032 ± 0.003







**Fig. 2** Kinetic profile of the major (poly)phenols of raw heat-treated Piquillo peppers during 48 h of *in vitro* faecal fermentation. (A) Profile of degradation of total (poly)phenols and the two main subfamilies (non-flavonoids and flavonoids) of raw and industrially treated (grilled and canned) Piquillo peppers. (B) Degradation of the main native (poly)phenols of raw, grilled and canned Piquillo peppers. (C) Profile of the main colonic derivatives produced in raw Piquillo pepper and after the application of industrial (grilling and canning) and culinary (microwaving and frying) heat treatments.



Interestingly, in raw peppers these end products were not detected until 6 hours of fermentation, whereas in grilled and canned peppers were already present at the beginning of the colonic fermentation. Therefore, it might be suggested that the formation of benzene-1,2-diol and 4-hydroxy-3-methoxyphenylacetic is also associated to the degradation of (poly)phenols during the application of industrial heat treatments.

Regarding culinary treatments, **microwaved and fried pepper** samples showed a similar (poly)phenolic profile to industrially processed samples before colonic fermentation, and therefore, similar colonic degradation kinetics were observed. However, noteworthy differences in (poly)phenols concentrations were found (Table 2). Although at the beginning of colonic fermentation ( $T$  0 h), microwaved digested pepper showed similar (poly)phenol concentrations to grilled and canned pepper, higher contents of phenolic catabolites were observed after 24 h of colonic fermentation (Table 2 and Fig. 2C) suggesting that food matrix changes due to microwaves might enhance (poly)phenols colonic catabolism. Interestingly, although fried peppers exhibited the lowest (poly)phenolic content after the *in vitro* digestion, these samples presented the highest phenolic concentrations after 24 and 48 hours of the colonic fermentation process (6.719 and 2.478  $\mu\text{mol g}^{-1}$  respectively) (Table 2 and Fig. 2C). Therefore, the addition of olive oil seemed to have a protective effect against gastrointestinal degradation possibly by entrapping (poly)phenols in lipid micellar structures, enhancing afterwards the formation of smaller colonic derivatives.<sup>42</sup>

Overall, although after 2 hours of incubation, greater amounts of (poly)phenols were found in raw compared to heat treated peppers, flavonoids and cinnamic acid glycosides present complex structures that might not be potentially absorbed.<sup>7,16,17</sup> Moreover, the (poly)phenols catabolism of raw pepper resulted in reduced amounts of low molecular weight (poly)phenol derivatives compared to heat treated peppers, especially in culinary treated peppers. Therefore, considering the poor bioavailability and consequently low bioactivity of the parent compounds, the application of industrial treatments is of a great interest due to the higher formation of low molecular weight derivatives with potential health effects. Recent research<sup>47</sup> revealed a promising effect of 3-(3'-hydroxyphenyl)propanoic acid, the major colonic catabolite of Piquillo pepper, on the attenuation of atherosclerosis due to their anti-inflammatory properties. Moreover, benzene 1,2-diol, that is an end-colonic metabolite of Piquillo pepper, especially in heat-treated samples, has been positively correlated with *Oscillospira* spp. which is a probiotic candidate with positive effects in obesity-related metabolic diseases,<sup>48</sup> and negatively correlated with *Paraprevotella* spp., even with not a clear cause effect relationship.<sup>49</sup> Other metabolites as 4-hydroxyphenylacetic and 3-phenylpropanoic acids have also been suggested to be associated with a healthy colonic metabotype.<sup>24</sup> Although these two compounds were detected in the present research, only unknown content might derive from gut microbial catabolism of Piquillo pepper (poly)phenols since they can also derive from other food sources. Nevertheless, despite the

known importance of (poly)phenolic metabolites rather than parent compounds on the potential health effects of (poly)phenolic compounds, there is still limited information on the biological activity of individual microbial-derived metabolites.<sup>20,23</sup>

In summary, the present research investigated how the high temperatures applied during industrial processing (grilling and canning) positively impact the bioaccessibility of (poly)phenolic compounds from Piquillo pepper (*Capsicum annuum* cv. *Piquillo*) compared to raw ones. In addition, among the two culinary treatments commonly applied to canned Piquillo pepper prior to consumption, microwaving seemed to favour (poly)phenols' release from food matrix and therefore (poly)phenols' bioaccessibility. The *in vitro* colonic metabolism of raw and heat-treated peppers also revealed the effect of thermal processing on (poly)phenol's degradation kinetics, and on the content of the low molecular weight derivatives formed. Moreover, the extensive biotransformation of (poly)phenols into smaller derivatives observed throughout the colonic fermentation process indicates the great interest of these compounds as those potentially responsible for the health promoting effects of (poly)phenols from Piquillo pepper either at colonic level or in the human organism after being absorbed and reaching target cells. It should be also considered that metabolites might not have an isolated effect but an additive or even a synergistic (or antagonistic) effect in the presence of other (poly)phenolic derivatives. Therefore, further studies on the *in vivo* bioavailability and bioactivity of (poly)phenols from Piquillo pepper (*Capsicum annuum* cv. *Piquillo*) considering the impact of culinary treatments applied for their consumption should be conducted in the future.

## Author contributions

Conceptualization: C.C. and M.-P.D.P.; formal analysis: C.D. B.-G. and I.A.L.; funding acquisition: C.C. and M.-P.D.P.; investigation: C.D.B.-G.; methodology: C.D.B.-G.; project administration: C.C. and M.-P.D.P.; supervision: I.A.L., C.C. and M.-P. D.P.; visualization: C.D.B.-G. and I.A.L.; writing – original draft preparation: C.D.B.-G.; writing – review and editing: C.D.B.-G., C.C., M.-P.D.P., and I.A.L.

## Data availability statement

The raw data files used to generate the experimental results of this study are available on <https://doi.org/10.5281/zenodo.10529682>.

## Conflicts of interest

There are no conflicts to declare.



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## References

- 1 N. de Sá Mendes, M. C. P. Santos, M. C. B. Santos, L. C. Cameron, M. S. L. Ferreira and É. C. B. A. Gonçalves, Characterization of pepper (*Capsicum baccatum*) – A potential functional ingredient, *LWT – Food Sci. Technol.*, 2019, **112**, 108209, DOI: [10.1016/j.lwt.2019.05.107](https://doi.org/10.1016/j.lwt.2019.05.107).
- 2 N. de Sá Mendes and É. C. B. A. Gonçalves, The role of bioactive components found in peppers, *Trends Food Sci. Technol.*, 2020, **99**, 229–243, DOI: [10.1016/j.tifs.2020.02.032](https://doi.org/10.1016/j.tifs.2020.02.032).
- 3 C. Del Burgo-Gutiérrez, I. A. Ludwig, M. P. de Peña and C. Cid, LC-MS/MS Analysis Elucidates the Different Effects of Industrial and Culinary Processing on Total and Individual (Poly)phenolic Compounds of Piquillo Pepper (*Capsicum annuum cv. Piquillo*), *J Agric. Food Chem.*, 2023, **71**(15), 6050–6060, DOI: [10.1021/acs.jafc.2c07829](https://doi.org/10.1021/acs.jafc.2c07829).
- 4 I. Juárez, I. A. Ludwig, E. Huarte, G. Pereira-Caro, J. M. Moreno-Rojas, C. Cid, *et al.*, Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables, *Food Chem.*, 2016, **197**, 466–473, DOI: [10.1016/j.foodchem.2015.10.139](https://doi.org/10.1016/j.foodchem.2015.10.139).
- 5 J. G. López-Velázquez, F. Delgado-Vargas, G. López-Ángulo, E. García-Armenta, M. E. López-López, L. E. Ayón-Reyna, *et al.*, Phenolic profile associated with chilling tolerance induced by the application of a hot water treatment in bell pepper fruit, *J. Food Sci.*, 2020, **857**, 2080–2089, DOI: [10.1111/1750-3841.15310](https://doi.org/10.1111/1750-3841.15310).
- 6 A. Marín, F. Ferreres, F. A. Tomás-Barberán and M. I. Gil, Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.), *J. Agric. Food Chem.*, 2004, **52**(12), 3861–3869, DOI: [10.1021/jf0497915](https://doi.org/10.1021/jf0497915).
- 7 S. Feng, J. Yi, X. Li, X. Wu, Y. Zhao, Y. Ma, *et al.*, Systematic Review of Phenolic Compounds in Apple Fruits: Compositions, Distribution, Absorption, Metabolism, and Processing Stability, *J. Agric. Food Chem.*, 2021, **69**(1), 7–27, DOI: [10.1021/acs.jafc.0c05481](https://doi.org/10.1021/acs.jafc.0c05481).
- 8 J. Xie, N. Sun, H. Huang, J. Xie, Y. Chen, X. Hu, *et al.*, Catabolism of polyphenols released from mung bean coat and its effects on gut microbiota during *in vitro* simulated digestion and colonic fermentation, *Food Chem.*, 2022, **396**, 1337191, DOI: [10.1016/j.foodchem.2022.133719](https://doi.org/10.1016/j.foodchem.2022.133719).
- 9 J. Bié, B. Sepodes, P. C. B. Fernandes and M. H. L. Ribeiro, Polyphenols in Health and Disease: Gut Microbiota, Bioaccessibility, and Bioavailability, *Compounds*, 2023, **3**(1), 40–72, DOI: [10.3390/compounds3010005](https://doi.org/10.3390/compounds3010005).
- 10 D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges and A. Crozier, Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases, *Antioxid. Redox Signal.*, 2013, **18**(14), 1818–1892, DOI: [10.1089/ars.2012.4581](https://doi.org/10.1089/ars.2012.4581).
- 11 M. Obrenovich, Y. Li, M. Tayahi and V. P. Reddy, Polyphenols and Small Phenolic Acids as Cellular Metabolic Regulators, *Curr. Issues Mol. Biol.*, 2022, **44**(9), 4152–4166, DOI: [10.3390/cimb44090285](https://doi.org/10.3390/cimb44090285).
- 12 A. Rodriguez-Mateos, D. Vauzour, C. G. Krueger, D. Shanmuganayagam, J. Reed, L. Calani, *et al.*, Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update, *Arch. Toxicol.*, 2014, **88**(10), 1803–1853, DOI: [10.1007/s00204-014-1330-7](https://doi.org/10.1007/s00204-014-1330-7).
- 13 I. Dini and L. Grumetto, Recent Advances in Natural Polyphenol Research, *Molecules*, 2022, **27**(24), 8777, DOI: [10.3390/molecules27248777](https://doi.org/10.3390/molecules27248777).
- 14 A. Gasmi, P. K. Mujawdiya, S. Noor, R. Lysiuk, R. Darmohray, S. Piscopo, *et al.*, Polyphenols in Metabolic Diseases, *Molecules*, 2022, **27**(19), 6280, DOI: [10.3390/molecules27196280](https://doi.org/10.3390/molecules27196280).
- 15 P. Sun, L. Zhao, N. Zhang, J. Zhou, L. Zhang, W. Wu, *et al.*, Bioactivity of dietary polyphenols: The role in LDL-C lowering, *Foods*, 2021, **10**(11), 1–29, DOI: [10.3390/foods10112666](https://doi.org/10.3390/foods10112666).
- 16 A. Ribas-Agustí, O. Martín-Belloso, R. Soliva-Fortuny and P. Elez-Martínez, Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**(15), 2531–2548, DOI: [10.1080/10408398.2017.1331200](https://doi.org/10.1080/10408398.2017.1331200).
- 17 K. Wojtunik-Kulesza, A. Oniszczuk, T. Oniszczuk, M. Combrzyński, D. Nowakowska and A. Matwijczuk, Influence of *in vitro* digestion on composition, bioaccessibility and antioxidant activity of food polyphenols—A non-systematic review, *Nutrients*, 2020, **12**(5), 1401, DOI: [10.3390/nu12051401](https://doi.org/10.3390/nu12051401).
- 18 T. A. F. Corrêa, M. M. Rogero, N. M. A. Hassimotto and F. M. Lajolo, The Two-Way Polyphenols-Microbiota Interactions and Their Effects on Obesity and Related Metabolic Diseases, *Front. Nutr.*, 2019, **6**, 1–15, DOI: [10.3389/fnut.2019.00188](https://doi.org/10.3389/fnut.2019.00188).
- 19 R. Dong, S. Liu, J. Xie, Y. Chen, Y. Zheng, X. Zhang, *et al.*, The recovery, catabolism and potential bioactivity of polyphenols from carrot subjected to *in vitro* simulated digestion and colonic fermentation, *Food Res. Int.*, 2021, **143**, 110263, DOI: [10.1016/j.foodres.2021.110263](https://doi.org/10.1016/j.foodres.2021.110263).
- 20 G. Di Pede, L. Bresciani, F. Brighenti, M. N. Clifford, A. Crozier, D. Del Rio, *et al.*, *In Vitro* Faecal Fermentation



- of Monomeric and Oligomeric Flavan-3-ols: Catabolic Pathways and Stoichiometry, *Mol. Nutr. Food Res.*, 2022, **66**(21), 2101090, DOI: [10.1002/mnfr.202101090](https://doi.org/10.1002/mnfr.202101090).
- 21 D. Del Juncal-Guzmán, L. M. Hernández-Maldonado, J. A. Sánchez-Burgos, G. A. González-Aguilar, V. M. Ruiz-Valdiviezo, J. Tovar, *et al.*, In vitro, gastrointestinal digestion and colonic fermentation of phenolic compounds in UV-C irradiated pineapple (*Ananas comosus*) snack-bars, *LWT – Food Sci. Technol.*, 2021, **138**, 110636, DOI: [10.1016/j.lwt.2020.110636](https://doi.org/10.1016/j.lwt.2020.110636).
  - 22 A. Burgos-Edwards, F. Jiménez-Aspee, C. Theoduloz and G. Schmeda-Hirschmann, Colonic fermentation of polyphenols from Chilean currants (*Ribes spp.*), and its effect on antioxidant capacity and metabolic syndrome-associated enzymes, *Food Chem.*, 2018, **258**, 144–155, DOI: [10.1016/j.foodchem.2018.03.053](https://doi.org/10.1016/j.foodchem.2018.03.053).
  - 23 J. C. Espín, A. González-Sarrias and F. A. Tomás-Barberán, The gut microbiota: A key factor in the therapeutic effects of (poly)phenols, *Biochem. Pharmacol.*, 2017, **139**, 82–93, DOI: [10.1016/j.bcp.2017.04.033](https://doi.org/10.1016/j.bcp.2017.04.033).
  - 24 G. Williamson and M. N. Clifford, Role of the small intestine, colon and microbiota in determining the metabolic fate of polyphenols, *Biochem. Pharmacol.*, 2017, **139**, 24–39, DOI: [10.1016/j.bcp.2017.03.012](https://doi.org/10.1016/j.bcp.2017.03.012).
  - 25 E. De Santiago, C. I. R. Gill, I. Carafa, K. M. Tuohy, M. P. De Peña and C. Cid, Digestion and Colonic Fermentation of Raw and Cooked *Opuntia ficus-indica* Cladodes Impacts Bioaccessibility and Bioactivity, *J. Agric. Food Chem.*, 2019, **67**(9), 2490–2499, DOI: [10.1021/acs.jafc.8b06480](https://doi.org/10.1021/acs.jafc.8b06480).
  - 26 A. P. Cárdenas-Castro, E. Alvarez-Parrilla, E. Montalvo-González, J. A. Sánchez-Burgos, K. Venema and S. G. Sáyago-Ayerdi, Stability and anti-topoisomerase activity of phenolic compounds of *Capsicum annuum* “Serrano” after gastrointestinal digestion and in vitro colonic fermentation, *Int. J. Food Sci. Nutr.*, 2020, **71**(7), 826–838, DOI: [10.1080/09637486.2020.1734542](https://doi.org/10.1080/09637486.2020.1734542).
  - 27 A. P. Cárdenas-Castro, J. J. Rochín-Medina, K. Ramírez, J. Tovar and S. G. Sáyago-Ayerdi, In Vitro Intestinal Bioaccessibility and Colonic Biotransformation of Polyphenols from Mini Bell Peppers (*Capsicum annuum* L.), *Plant Foods Hum. Nutr.*, 2022, **77**(1), 77–82, DOI: [10.1007/s1130-022-00948-5](https://doi.org/10.1007/s1130-022-00948-5).
  - 28 L. Arfaoui, Dietary plant polyphenols: Effects of food processing on their content and bioavailability, *Molecules*, 2021, **26**(10), 2959, DOI: [10.3390/molecules26102959](https://doi.org/10.3390/molecules26102959).
  - 29 A. Cilla, L. Bosch, R. Barberá and A. Alegría, Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols, *J. Food Compos. Anal.*, 2018, **68**, 3–15, DOI: [10.1016/j.jff.2017.01.009](https://doi.org/10.1016/j.jff.2017.01.009).
  - 30 M. Domínguez-Fernández, I. A. Ludwig, M. P. De Peña and C. Cid, Bioaccessibility of Tudela artichoke (*Cynara scolymus* cv. Blanca de Tudela), (poly)phenols: The effects of heat treatment, simulated gastrointestinal digestion and human colonic microbiota, *Food Funct.*, 2021, **12**(5), 1996–2011, DOI: [10.1039/d0fo03119d](https://doi.org/10.1039/d0fo03119d).
  - 31 E. Huarte, G. Serra, A. Monteagudo-Mera, J. Spencer, C. Cid and M. P. De Peña, Raw and Sous-Vide-Cooked Red Cardoon Stalks (*Cynara cardunculus* L. var. *altilis* DC): (Poly)phenol Bioaccessibility, Anti-inflammatory Activity in the Gastrointestinal Tract, and Prebiotic Activity, *J. Agric. Food Chem.*, 2021, **69**(32), 9270–9286, DOI: [10.1021/acs.jafc.1c03014](https://doi.org/10.1021/acs.jafc.1c03014).
  - 32 I. Juániz, I. A. Ludwig, L. Bresciani, M. Dall’Asta, P. Mena, D. Del Rio, *et al.*, Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation, *J. Funct. Foods*, 2016, **27**, 201–213, DOI: [10.1016/j.jff.2016.09.006](https://doi.org/10.1016/j.jff.2016.09.006).
  - 33 C. D. Kay, M. N. Clifford, P. Mena, G. J. McDougall, C. Andres-Lacueva, A. Cassidy, *et al.*, Recommendations for standardizing nomenclature for dietary (poly)phenol catabolites, *Am. J. Clin. Nutr.*, 2020, **112**(4), 1051–1068, DOI: [10.1093/ajcn/nqaa204](https://doi.org/10.1093/ajcn/nqaa204).
  - 34 A. Brodkorb, L. Egger, M. Alminger, P. Alvito, R. Assunção, S. Ballance, *et al.*, INFOGEST static in vitro simulation of gastrointestinal food digestion, *Nat. Protoc.*, 2019, **14**(4), 991–1014, DOI: [10.1038/s41596-018-0119-1](https://doi.org/10.1038/s41596-018-0119-1).
  - 35 J. I. Mosele, S. Martín-Peláez, A. Macià, M. Farràs, R. M. Valls, Ú. Catalán, *et al.*, Faecal microbial metabolism of olive oil phenolic compounds: In vitro and in vivo approaches, *Mol. Nutr. Food Res.*, 2014, **58**(9), 1809–1819, DOI: [10.1093/ajcn/nqaa204](https://doi.org/10.1093/ajcn/nqaa204).
  - 36 E. M. Sánchez-Salcedo, P. Mena, C. García-Viguera, J. J. Martínez and F. Hernández, Phytochemical evaluation of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits, a starting point for the assessment of their beneficial properties, *J. Funct. Foods*, 2015, **12**, 399–408, DOI: [10.1016/j.jff.2014.12.010](https://doi.org/10.1016/j.jff.2014.12.010).
  - 37 L. Bresciani, G. Di Pede, C. Favari, L. Calani, V. Francinelli, A. Riva, *et al.*, In vitro (poly)phenol catabolism of unformulated- and phytosome-formulated cranberry (*Vaccinium macrocarpon*) extracts, *Food Res. Int.*, 2021, **141**, 110137, DOI: [10.1016/j.foodres.2021.110137](https://doi.org/10.1016/j.foodres.2021.110137).
  - 38 J. I. Mosele, A. Macià and M. J. Motilva, Metabolic and microbial modulation of the large intestine ecosystem by non-absorbed diet phenolic compounds: A review, *Molecules*, 2015, **20**(9), 17429–17468, DOI: [10.1002/mnfr.201400124](https://doi.org/10.1002/mnfr.201400124).
  - 39 I. Juániz, I. A. Ludwig, L. Bresciani, M. Dall’Asta, P. Mena, D. Del Rio, *et al.*, Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (*Cynara cardunculus* L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota, *J. Funct. Foods*, 2017, **32**, 195–207, DOI: [10.1016/j.jff.2017.02.033](https://doi.org/10.1016/j.jff.2017.02.033).
  - 40 E. De Santiago, G. Pereira-Caro, J. M. Moreno-Rojas, C. Cid and M. P. De Peña, Digestibility of (Poly)phenols and Antioxidant Activity in Raw and Cooked Cactus Cladodes (*Opuntia ficus-indica*), *J. Agric. Food Chem.*, 2018, **66**(23), 5832–5844, DOI: [10.1021/acs.jafc.8b01167](https://doi.org/10.1021/acs.jafc.8b01167).
  - 41 J. L. Ordoñez-Díaz, A. Moreno-Ortega, F. J. Roldán-Guerra, V. Ortíz-Somovilla, J. M. Moreno-Rojas and G. Pereira-Caro,



- In vitro gastrointestinal digestion and colonic catabolism of mango (*Mangifera indica* L.) pulp polyphenols, *Foods*, 2020, 9(12), 1836, DOI: [10.3390/foods9121836](https://doi.org/10.3390/foods9121836).
- 42 M. Ovando-Martínez, N. Gámez-Meza, C. C. Molina-Domínguez, C. Hayano-Kanashiro and L. A. Medina-Juárez, Simulated Gastrointestinal Digestion, Bioaccessibility and Antioxidant Capacity of Polyphenols from Red Chiltepin (*Capsicum annuum* L. Var. *glabriusculum*), Grown in Northwest Mexico, *Plant Foods Hum. Nutr.*, 2018, 73(2), 116–121, DOI: [10.1007/s11130-018-0669-y](https://doi.org/10.1007/s11130-018-0669-y).
- 43 Y. A. Chait, A. Gunenc, F. Bendali and F. Hosseinian, Simulated gastrointestinal digestion and in vitro, colonic fermentation of carob polyphenols: Bioaccessibility and bioactivity, *LWT - Food Sci. Technol.*, 2020, 117, 108623, DOI: [10.1016/j.lwt.2019.108623](https://doi.org/10.1016/j.lwt.2019.108623).
- 44 G. Di Pede, L. Bresciani, L. Calani, G. Petrangolini, A. Riva, P. Allegrini, *et al.*, The human microbial metabolism of quercetin in different formulations: An in vitro evaluation, *Foods*, 2020, 9(8), 1–13, DOI: [10.3390/foods9081121](https://doi.org/10.3390/foods9081121).
- 45 C. D. Kay, G. Pereira-caro, I. A. Ludwig, M. N. Clifford and A. Crozier, Anthocyanins and Flavanones Are More Bioavailable than Previously Perceived : A Review of Recent Evidence, *Annu. Rev. Food Sci. Technol.*, 2017, 8, 155–182, DOI: [10.1146/annurev-food-030216-025636](https://doi.org/10.1146/annurev-food-030216-025636).
- 46 G. Pereira-Caro, G. Borges, I. Ky, A. Ribas, L. Calani, D. Del Rio, *et al.*, In vitro colonic catabolism of orange juice (poly) phenols, *Mol. Nutr. Food Res.*, 2015, 59(3), 465–475, DOI: [10.1002/mnfr.201400779](https://doi.org/10.1002/mnfr.201400779).
- 47 J. Feng, C. Ge, W. Li and R. Li, 3-(3-Hydroxyphenyl)propionic acid, a microbial metabolite of quercetin, inhibits monocyte binding to endothelial cells via modulating E-selectin expression, *Fitoterapia*, 2022, 156, 105071, DOI: [10.1016/j.fitote.2021.105071](https://doi.org/10.1016/j.fitote.2021.105071).
- 48 J. Yang, Y. Li, Z. Wen, W. Liu, L. Meng and H. Huang, Oscillospira - a candidate for the next-generation probiotics, *Gut Microbes*, 2021, 13(1), 1987783, DOI: [10.1080/19490976.2021.1987783](https://doi.org/10.1080/19490976.2021.1987783).
- 49 J. I. Mosele, M. J. Gosalbes, A. Macia, L. Rubio, J. F. Vazquez-Castellanos, H. N. Jimenez, *et al.*, Effect of daily intake of pomegranate juice on fecal microbiota and feces metabolites from healthy volunteers, *Mol. Nutr. Food Res.*, 2015, 59(10), 1942–1953, DOI: [10.1002/mnfr.201500227](https://doi.org/10.1002/mnfr.201500227).

