TITLE:

Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and fecal fermentation.

SHORT TITLE: Catabolism of pepper (poly)phenols after digestion and fecal fermentation

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ABSTRACT

A total of 21 (poly)phenolic compounds (free and bound) were quantified in raw, olive oil fried, sunflower oil fried and griddled green pepper before and after a simulated gastrointestinal digestion. Flavonoids, particularly quercetin rhamnoside, were the main compounds. The bioaccessibility of (poly)phenolic compounds after gastrointestinal digestion was higher in cooked (>82%) than in raw (48%) samples, showing a positive effect of heat treatment on the release of (poly)phenols from the vegetal matrix. Additionally, a fecal fermentation was carried out for 24h. A time-dependent microbial metabolic activity was observed, which resulted firstly (<5h) in the hydrolysis of flavonoid glycosides and then in the formation of 3 catabolites, namely 3,4-dihydroxybenzoic acid, dihydrocaffeic acid and 3-(3'-hydroxyphenyl)propionic acid, this being by far the most abundant. Catabolic pathways for colonic microbial degradation of flavonoids and hydroxycinnamic acids have been proposed. Griddled pepper showed the highest amount of (poly)phenols both after gastrointestinal digestion and colonic fermentation.

KEYWORDS

Polyphenols; *In vitro* bioaccessibility; *In vitro* gastrointestinal digestion; Colonic catabolism; Heat treatment; Pepper

1. Introduction

Europe produces millions of tonnes of a broad range of fruits and vegetables thanks to its varied climatic and topographic conditions. Mediterranean countries such as Turkey, Spain, Italy, Greece or France are the largest producers of vegetables (Eurostat, 2015), and are also the countries characterized by the highest intake of fruits and vegetables. Plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenol rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably, but definitely not solely due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013).

Phenolics are compounds with at least one aromatic ring with one or more hydroxyl groups attached and could be divided into several classes. Flavonoids are one of the main polyphenolic compounds found in vegetables. The majority of flavonoids occur naturally as glycosides rather than aglycones, like in green peppers, one of the most consumed vegetables in the Mediterranean countries such as Spain (MAGRAMA, 2015). Quercetin rhamnoside and luteolin 7-*O*-(2-apiosyl-6-malonyl) glucoside are the most abundant phenolic compounds in green peppers, accounting for around 80% of total phenolics (Juániz et al., 2016; Marin, Ferreres, Tomás-Barberán, & Gil, 2004). However, it must be taken into account that many dietary vegetables are usually eaten after different cooking methods and that (poly)phenolic compounds can be either degraded or released from plant tissue structures by thermal processes, depending on the cooking methods and their time and temperature conditions, as well as the type of vegetable (Juániz et al., 2016; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Palermo, Pellegrini, & Fogliano, 2014; Pellegrini et al., 2009; Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015).

67 After ingestion, (poly)phenols can also be modified in the gastrointestinal tract by digestive
68 enzymes and, consequently, their bioaccessibility might be affected. The stomach reduces the
69 particle size of food, in turn potentially enhancing the release of phenolic compounds
70 (Scalbert, Morand, Manach, & Remesy, 2002). Additionally, glycosylation influences
71 absorption at intestinal level, since glycosidic flavonoids are more bioaccesible than their
72 aglyconic forms (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Deconjugation
73 can take place in the lumen by the action of membrane-bound lactase phlorizin-hydrolase
74 (LPH) and aglycones may then be absorbed passively through the epithelium. The epithelial
75 cells can also hydrolyze the glycosides by the action of cytosolic β-glucosidase and
76 consequently aglycones may be formed after absorption by the active sodium-dependent
77 glucose transporter, SGLT-1 (Day et al., 2000; Nemeth et al., 2003). Nevertheless, the levels
78 of flavonoids in plasma after dietary intake are low (Aura, 2008), which is probably related to
79 their limited absorption. Absorbed compounds undergo phase II enzymatic metabolism and
80 they can be conjugated with glucuronic acid, sulphate and methyl groups in the liver and
81 enterohepatic recirculation may result in some recycling back to the small intestine through
82 bile excretion, so parent compounds could not be detected in plasma (Aura, 2008; Del Rio et
83 al., 2013). A large part of the ingested (poly)phenols could then reach the colon where they
84 could be transformed by the local microbiota to smaller and more absorbable molecules. Gut
85 microbiota metabolism can also modulate the health effects of dietary (poly)phenolic
86 compounds by altering absorption, bioavailability, and biological activity, so biological
87 effects should not be only attributed to the native compounds present in foods but also to their
88 metabolites (Duda-Chodak, Tarko, Satora, & Sroka, 2015).
89 Therefore, this work aimed at (1) investigating the effect of a simulated gastrointestinal
90 digestion on the (poly)phenolic fraction of both, raw and cooked green peppers, a vegetable

commonly consumed as crude in salads and cooked in several ways in the Mediterranean Diet, and (2) identifying and quantifying the main metabolites derived from an *in vitro* microbial colonic fermentation.

2. Material and methods

2.1 Chemical and reagents

Sweet Italian green pepper (*Capsicum annuum*), olive oil (refined and virgin olive oil blend) and sunflower oil were obtained from local stores. Selection of oils was based on their high consumption by Spanish consumers for frying.

Human saliva α-amylase (852 U/mg protein), pepsin (674 U/mg), pancreatin (4xUPS), and bile salts (for digestion) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous dipotassium hydrogen phosphate and soluble starch were from Carlo Erba Reagents (Milan, Italy). Methanol, 99% formic acid, acetonitrile, bile salts (for fermentation), calcium chloride, (+)-arabinogalactan, tryptone, yeast extract, buffered peptone water, Dulbecco's phosphate buffer saline, casein sodium salt from bovine milk, pectin from citrus fruits, mucin from porcine stomach-type III, sodium hydrogen carbonate, potassium phosphate monobasic, magnesium sulfate monohydrate, guar gum, Tween 80, xylan from Birchwood, L-cysteine hydrochloride monohydrate and iron(II)-sulfate heptahydrate, resazurin redox indicator sodium hydroxide, ethyl acetate, and citric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potassium chloride and sodium chloride were obtained from Merk (Darmstadt, Germany). Pure phenolic standards for high-performance liquid chromatographic (HPLC) and tandem mass spectrometric (MS/MS) analyses of rutin, luteolin-4-glucoside, quercetin, luteolin, 5-caffeoylquinic acid (5-CQA), caffeic acid, *p*-coumaric acid, dihydrocaffeic acid, 3-(3'-hydroxyphenyl)propionic acid, and protocatechuic acid were also purchased from Sigma-Aldrich (St. Louis, MO, USA).

115 2.2 Samples preparation

116 Chopped green pepper (300 g) was fried with olive or sunflower oils (30 mL) at 115 ºC for 10
117 minutes in a non-stick frying pan. Then, temperature was decreased to 108 ºC for 5 minutes.
118 Chopped green pepper was also submitted to a heating process at 150 ºC for 10 minutes and
119 then at 110 ºC for 5 minutes in a non-stick griddle without oil addition. Then, raw and cooked
120 green peppers were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain), and
121 stored at -18ºC until further analysis.

122 2.3 Simulated gastrointestinal digestion

123 A three step *in vitro* digestion model was carried out in a bioreactor according to Minekus et
124 al. (2014) and Monente et al. (2015 b) adapted to our laboratory. Briefly, 2 g of each sample
125 was weighted in a 100 mL vessel placed and heated in a water bath at 37 °C. The vessel was
126 magnetically stirred and connected to a pH sensor. The three steps were carried out in absence
127 of light. Simulated salivary, gastric and intestinal fluids (SSF, SGF and SIF) (Table 1
128 supplementary information) were employed for each step. First, oral digestion was performed
129 by adding 14 mL of the stock SSF solution, 250 μL of an α-amylase solution (1.3 mg mL^{-1}),
130 0.10 mL of 0.3M $CaCl_2$, and water up to 20 mL. The sample was shaken for 30 min at 37 °C.
131 Second, the gastric digestion step was carried out at pH 3 with 1M HCl It was started by
132 adding 15 mL of SGF, 1.19 mL of a pepsin solution (1 g of pepsin in 10 mL of 0.1 M HCl),
133 0.01 mL of 0.3M $CaCl_2$ and water up to 20 mL. After 2 h incubation, the final intestinal step
134 was carried out by adding 22 mL of SIF, 10 mL of a pancreatin solution (0.008 g mL^{-1}), 5 mL
135 of bile salts (0.025 g mL^{-1}), 0.08 mL of 0.3M $CaCl_2$ and water up to 40 mL. The pH was then
136 adjusted to 7 with 1M NaOH and the samples were incubated for 2 h. All samples were frozen
137 and lyophilized in a freeze dryer Cryodos-80 (Telstar), and stored at -18ºC until further

138 analysis. Each green pepper sample was digested in duplicate and then the two repetitions
139 were mixed and homogenized.
140 2.4 *In vitro* fecal fermentation
141 The *in vitro* fecal fermentation was carried out according to Dall'Asta et al. (2012) adapted to
142 the samples under study.
143 2.4.1 *In vitro* fermentation growth medium preparation
144 The composition for 1 L of growth medium was 2.5 g of soluble starch, 2.5 g of peptone, 2.5
145 g of tryptone, 2.25 g of yeast extract, 2.25 g of NaCl, 2.25 g of KCl, 1 g of pectin, 2 g of
146 mucin, 1.5 g of casein, 1 g of arabinogalactan, 0.75 g of $NaHCO_3$, 0.35 g of $MgSO_4H_2O$, 0.5 g
147 of guar, 0.5 g of xylan, 0.4 g of L-cysteine $HCl \cdot H_2O$, 0.25 g of KH_2PO_4, 0.25 g of K_2HPO_4,
148 0.2 g of bile salt, 0.04 g of $CaCl_2$, 0.0025 g of $FeSO_4 \cdot 7H_2O$, 0.5 mL of Tween 80, and 2 mL
149 of resazurin solution (0.025%, w/v) as an anaerobic indicator. The growth medium was
150 sterilized at 121°C for 15 min in glass vessels (12 mL) before sample preparation.
151 2.4.2 Fecal slurry
152 Fresh fecal samples were collected from four healthy donors who did not have previous
153 intestinal disease, were not treated with antibiotics for the previous 3 months, and followed a
154 polyphenol-free diet (avoiding fruits and vegetables, nuts, legumes, high-fiber products, and
155 beverages such as tea, coffee and fruit juices, as well as alcohol) for 2 days before fecal
156 collection. Samples were immediately stored in an anaerobic jar and then diluted with
157 Dulbecco's phosphate buffer saline at 1% (w/v) and homogenized to obtain a 10% (w/w)
158 slurry to be used as the fermentation starter.
159 2.4.3 Fermentation conditions
160 The final fermentation volume was 4 mL, made of 45% growth medium, 45% of fecal slurry,
161 and 10% of food sample extract (prepared dissolving 0.5 g of dried sample in 4 mL of PBS).

The fermentation starter and samples were introduced in the vessel containing sterilized growth medium, sealed with a rubber seal, and flushed through a double needle with nitrogen to create an anaerobic condition. Vessels were then incubated for 24 h at 37°C at 200 strokes/min in a Dubnoff bath (ISCO, Milan, Italy) and collected after 15 min, 5 and 24 h for further analysis. Following incubation, fecal metabolism was stopped by adding 0.4 mL of acetonitrile to the 4 mL of fermented sample and samples were frozen (-18°C) until metabolite extraction and subsequent analysis. All experiments were carried out in triplicate.

2.5 Phenolic compound and metabolite extraction.

The extraction of free (poly)phenolic compounds from raw and cooked both non-digested and digested green pepper was performed according to Sánchez-Salcedo, Mena, Garcia-Viguera, Martinez & Hernandez (2015) with some modifications. Briefly, 50 mg of each sample was extracted with 1 mL of methanol/acidified water (0.1% formic acid) (80:20 v/v), sonicated for 90 min, followed immediately by 1 min of vortex mixing, and centrifuged for 10 min at 14000 rpm (HERMLE Labortechnik GmbH, Wehingen, Germany). The supernatant was collected and the residue was re-extracted using 0.5 mL of methanol/acidified water (80:20 v/v), sonicated for 25 min in a sonic bath, vortex for 1 min and centrifuged for 10 min at 14000 rpm. Both supernatants were combined and stored in the freezer at -18ºC until LC/MS^n analyses.

The extraction of the bound (poly)phenolic compounds was performed following the method reported by Zaupa et al. (2014). The residue obtained from previous extractions was further hydrolyzed with 1.5 mL of 2 M sodium hydroxide and kept at room temperature for 1 h. After alkaline hydrolysis, the pH of the mixture was adjusted to pH 3 by adding 1.35 mL of 3 M citric acid. The bound phenolic compounds were then extracted with 4 mL of ethyl acetate. After 10 min at 14000 rpm centrifugation, 1 mL of the ethyl acetate supernatant was dried

under vacuum by rotary evaporation (Savant SPD121P, SpeedVac Concentrator, Thermo Scientific, Inc., San Jose, CA, U.S.A.) and the residue was dissolved in 0.25 mL of methanol/acidified water (0.1% formic acid) (80:20 v/v) for the LC/MS^n analyses.

For the extraction of fecal metabolites, 0.1 mL aliquots of fermented samples were transferred to a clean microfuge tube. (Poly)phenolic compounds and possible metabolites derived from the fecal fermentation were extracted by adding 0.1 mL of methanol/acidified water (0.1% formic acid) (80:20 v/v), vortexed and centrifuged at 14000 rpm for 10 min. Aliquots of 100 µL were then transferred to vials and subjected to LC/MS^n analysis.

2.6 Identification and quantification of (poly)phenolic compounds and metabolites

Qualitative and quantitative analysis of (poly)phenolic compounds were carried out using an Accela UHPLC 1250 equipped with a linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, U.S.A.) fitted with a heated ESI probe (H-ESI-II, Thermo Fisher Scientific Inc., San Jose, CA, U.S.A.).

To determine (poly)phenols of green pepper, a preliminary analysis was carried out in a full scan, data-dependent MS^3 mode, scanning from m/z of 100 to 1500 to identify the compounds. Consequently, a selective full scan MS^2 mode analysis, monitoring specific *m/z*, was performed to quantify the previous identified (poly)phenolic compounds. For UHPLC separation, mobile phase A was 0.1% (v/v) formic acid in acetonitrile and mobile phase B was 0.1% (v/v) formic acid in water. Separations were carried out by means of a C18 BlueOrchid column (50 × 2 mm; 1.8 µm particle size; Knauer, Berlin, Germany), with an injection volume of 5 µL, column oven temperature of 30 °C and elution flow rate of 0.3 mL/min. The mobile phases comprised a program of 0-3 min, 5% A; 3-12 min, 5-40% A; 12-13 min, 40-80% A; 13-16 min, 80% A and then return to 5% A in one min and maintained the gradient until the end of the analysis (21 min) to re-equilibrate the column. The MS functioned in

210 negative ionization mode, with capillary temperature set at 275 °C, while the source was
211 maintained at 300 °C. The sheath gas flow was 50 units, while auxiliary and sweep gases
212 were set both to 5. The source voltage was 3 kV and the capillary voltage and tube lens were -
213 2 and -58 V, respectively. All compounds were fragmented with pure helium gas (99.9999%)
214 using a CID of 35.
215 The analysis of potential phenolic metabolites resulted from the *in vitro* human fecal
216 microbiota degradation, was also performed firstly in full scan, data-dependent MS^3 mode,
217 scanning from m/z of 100 to 1500, for a comprehensive compound identification, and, later,
218 in a full scan data-dependent MS^2 mode, monitoring the specific identified ions for the
219 quantification. Chromatographic separation was performed using a XSELECTED HSS T3 (50
220 x 2.1 mm, 2.5 μm particle size, Waters, Milford, MA, USA) and employing the conditions
221 and the same solvents previously described, except for the elution gradient. The mobile phase
222 was made of 0-0.5 min, 2% A; 0.5-9 min, 2−45% A; 9-9.5 min, 45-80% A; 9.5-12.5 min,
223 80% A and then return to 2% A in 0.5 min. The MS worked in negative ionization mode, with
224 a capillary temperature of 275 °C, while the source was maintained at 250 °C. The sheath gas
225 flow was 40 units, while auxiliary and sweep gases were set to 5 units. The source voltage
226 was 3 kV, and the capillary voltage and tube lens were -9 and -53 V, respectively. All
227 metabolites were fragmented using a CID of 30.
228 Quantification was performed with calibration curves built with the available standard
229 compounds. Quercetin derivatives were quantified in rutin equivalents. Luteolin derivatives
230 were quantified as luteolin 4-glucoside equivalents, all caffeoylquinic acids were quantified
231 using 5-CQA, while caffeic acid derivatives and coumaric acid derivatives were quantified
232 respectively as caffeic acid and coumaric acid equivalents.

Chromatograms and spectral data were acquired using XCalibur software 2.1 (Thermo Fisher Scientific Inc., San Jose, CA, U.S.A.). Each sample was analysed in triplicate.

2.7 Bioaccessibility of (poly)phenolic compounds

The percentage of bioaccessibility of (poly)phenolic compounds after simulated gastrointestinal digestion or fecal fermentation was calculated as following:

$$\text{Bioaccessibility (\%)} = \frac{\text{PCA}}{\text{PCB}} * 100$$

Where PCA is the total (Poly)phenolic Compounds content in samples (nmol/g dm) After *in vitro* digestion or fecal fermentation and PCB is the total (Poly)phenolic Compounds content in samples (nmol/g dm) Before *in vitro* digestion or fecal fermentation.

2.8 Statistical analysis

Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter. Bonferroni test was applied as *a posteriori* test for detecting significantly different means ($p < 0.05$). All statistical analyses were performed using the STATA v.12.0 software package.

3. Results and discussion

A total of 21 (poly)phenolic compounds were identified and quantified (Table 1) in green pepper samples. Specifically, 6 quercetin derivatives, 9 luteolin derivatives and 6 hydroxycinnamic acids were detected and quantified. Flavonoids, particularly quercetin rhamnoside, were the main compounds found both in raw and cooked samples. All flavonoids were mainly detected in the analyzed free fraction, although some of them, as rutin, quercetin glucoside, quercetin rhamnoside, luteolin 8-*C*-hexoside and luteolin 7-*O*-(2-apiosyl) glucoside, were also found in the bound fraction. In contrast, some hydroxycinnamic

256 acids, such as caffeic and coumaric acids, were found only in the bound fraction. These
257 compounds could probably be linked to pepper fiber fraction and released from the food
258 matrix only after the hydrolysis process. Additionally, the applied alkaline hydrolysis process
259 could have degraded some phenolic compounds, such as caffeic acids derivatives, CQAs or
260 coumaroylquinic acid, so they could be detected after hydrolysis into their corresponding
261 caffeic and coumaric acids (Monente, Ludwig, Irigoyen, De Pena, & Cid, 2015 a).
262 Figure 1 and Table 1 show free and bound (poly)phenolic compounds of green pepper which
263 have been quantified by UHPLC both in raw and in cooked vegetables treated with different
264 cooking methods, comparing also how the *in vitro* gastrointestinal digestion affected the final
265 amount of (poly)phenolic compounds. Before digestion, raw pepper presented a total of
266 12.664 μmol (poly)phenolic compounds/g dm, of which 66% were detected as free
267 compounds and 34% as bound compounds. After submitting green pepper to a frying process
268 both with olive oil and sunflower oil, total (poly)phenolic compounds decreased by more than
269 a half, resulting reduced to 4.715 and 5.113 μmol/g dm in olive oil and sunflower oil fried
270 green pepper, respectively. Although free (poly)phenolic compounds decreased significantly
271 after frying process, the overall loss of (poly)phenolic compounds was mainly due to the
272 decrease of bound compounds. Furthermore, phenolic compounds exclusively found in olive
273 oil, such as oleuropein, pinoresinol, tyrosol and hydroxytyrosol were not detected in olive oil
274 fried green pepper, probably because olive oil used for frying was a blend mainly constituted
275 by refined olive oil with a little amount of virgin olive oil, where phenolic compounds were
276 hardly present (Boskou, 2009). Similarly, sunflower oil was also refined, and (poly)phenols
277 identified in oil fried green pepper samples were the same detected in raw ones. Therefore, the
278 contribution of the oils to the (poly)phenols of cooked samples was scarce.

279 The amount of (poly)phenolic compounds of griddled green pepper (11.475 μmol/g dm) was
280 also lower than in raw green pepper, but the reduction was much lower than after frying
281 process. Free (poly)phenolic compounds were strongly affected, but bound compounds
282 decreased only 5%, representing the 29% of the total (poly)phenolic compounds of griddled
283 green pepper. The decrease of bound compounds observed in all cooked samples with respect
284 to raw green pepper, could be due to the thermal destruction of cell walls and sub-cellular
285 compartments during the cooking process that increases the release of these compounds. The
286 lower release of bound compounds in the griddled samples was probably due to the effect of
287 the higher temperature applied during this cooking process compared to frying, which could
288 increase the formation of high molecular weight end products typical for the Maillard
289 reaction, such as melanoidins, that could retain phenolic compounds into their structures.
290 Some studies confirmed that higher roasting temperature induced higher formation of
291 melanoidins and that melanoidins content also depends on the extent of roasting (Bekedam,
292 Loots, Schols, Van Boekel, & Smit, 2008 a; Sacchetti et al., 2016). Additionally, some studies
293 about coffee reported the incorporation of chlorogenic acids and other phenolic compounds
294 into melanoidins, which may reach an astounding 54% mainly by non-covalent interactions
295 (Bekedam, Schols, Van Boekel, & Smit, 2008 b; Monente et al., 2015 a; Morales, Somoza, &
296 Fogliano, 2012; Nunes & Coimbra, 2010).

297 **Simulated gastrointestinal digestion**

298 The (poly)phenolic compound composition was also influenced by gastrointestinal digestion
299 (Figure 1). In raw green pepper, an evident decrease (more than 50%) of total (poly)phenolic
300 compounds was observed after digestion. The loss was mainly due to the decrease of the
301 bound fraction, which represented the 34% of total (poly)phenolic compounds in raw green
302 pepper before digestion and was reduced to 11% after digestion. This change may be due to

303 the release of the bound compounds from the food matrix as a consequence of the enzymatic
304 action. In griddled pepper, a decrease in total (poly)phenolic compounds (around 20%) was
305 also observed after gastrointestinal digestion, however the loss was lower than what occurred
306 in raw samples. The high amount of bound compounds, probably attached to complex
307 structures derived from the Maillard reaction which took place during the griddled process,
308 appeared to be more stable during the *in vitro* digestion process, resulting in their release from
309 the food matrix without any subsequent degradation. In fried samples, both with olive oil and
310 sunflower oil, the fat content of the samples could have exerted a protective effect against
311 enzymatic action and the final amount of (poly)phenolic compounds was not affected by
312 gastrointestinal digestion. In olive oil fried green pepper only 5% of total (poly)phenolic
313 compounds were degraded by enzymatic action and no significant changes were observed in
314 the amount of total (poly)phenolic compounds of sunflower oil fried green pepper.
315 Considering the individual (poly)phenolic compounds (Table 1), no new compounds were
316 found after the digestion process although aglyconic forms could be expected. Actually, the
317 loss of the glycosidic moieties is due to membrane-bound glycosylases found on the brush
318 border of the mammalian small intestine (Day et al., 2000; Nemeth et al., 2003), which are
319 clearly not present under the adopted conditions.
320 Finally, it can be concluded that the bioaccessibility of phenolic compounds after
321 gastrointestinal digestion was higher in cooked samples than in raw ones. The 82%, 96% and
322 100% of the total amount of (poly)phenolic compounds of undigested griddled green pepper,
323 olive oil fried green pepper and sunflower oil fried green pepper, respectively, were
324 bioaccessible after gastrointestinal digestion, compared to 48% in raw pepper. These results
325 are in accordance with the data reported by others, who demonstrated a higher bioaccessibility
326 of (poly)phenolic compounds after food thermal treatment (Girgin & El Nehir, 2015).

Furthermore, griddled pepper showed the largest amount of phenolic compounds still present in the matrix after the digestion process (9.447 μmol (poly)phenolic compounds/g dm).

(Poly)phenolic compounds degradation during fecal fermentation

After the intake of flavonoids and non-flavonoids, the circulation levels of these compounds in plasma are low (Aura, 2008), probably related to their limited absorption (Dupas, Baglieri, Ordonaud, Tome, & Maillard, 2006; Jaganath, Mullen, Edwards, & Crozier, 2006; Manach et al., 2005; Monente et al., 2015 b; Walle, 2004). Moreover, polyphenol bioavailability in the first gastrointestinal tract has been estimated less than 20% (Hu, 2007). In the present study, the (poly)phenolic compounds detected in digested samples have been considered as the compounds which potentially reach the colon and could be metabolized by the microbiota.

(Poly)phenolic profiles of green pepper at the beginning of the experiment and during the fecal fermentation (15 min, 5 h and 24 h of incubation) are shown in Figure 2. An important microbial metabolic activity was observed, which resulted in the hydrolysis of flavonoid glycosides and the consequent formation of aglycones, principally quercetin and luteolin, as a first step of the fecal fermentation (Figure 2 A). Quercetin derivatives were quickly metabolized and during the first few minutes of colonic biotransformation, the amount of quercetin-based compounds was halved, while quercetin aglycone increased simultaneously. The highest amount of quercetin was detected after 5 hours of fecal incubation. However, the recovered amount of quercetin after 5 h did not correspond to the total of the native quercetin derivatives, indicating that also the aglycone, once released, could be rapidly degraded, in agreement with previously reported results (Serra et al., 2012). Similarly, luteolin aglycone was rapidly released at the beginning of the fecal incubation. However, luteolin derivatives were not degraded so fast, letting hypothesize that luteolin could have also derived from quercetin dehydroxylation, as illustrated in Figure 3. In general, *O*-glucosides of both

351 quercetin and luteolin were almost completely metabolized by the intestinal microbiota while
352 *C*-glycosides were much more slowly degraded, and some of them, as for example luteolin 8-
353 *C*-hexoside, the main flavonoid derivative found in all samples after fecal fermentation
354 (Tables 2, 3, 4 and 5), were still present after 24 h of fecal incubation. This result is in
355 agreement with those reported by Hein, Rose, Van't Slot, Friedrich & Humpf (2008), who
356 observed a complete metabolism of *O*-glycoside compounds between 20 min and 4 hours,
357 whereas *C*-glycoside compounds which only partially reduced. The same study showed that
358 the released aglycones were completely metabolized within 8 hours. Nevertheless, in the
359 present study, luteolin aglycone was substantially metabolized within 5 hours, whereas low
360 amounts of quercetin still remained after 24 h of fecal incubation. The highlighted difference
361 between the compared studies could be linked to the source of native compounds used in the
362 model, as Hein and colleagues (2008) employed only standard molecules, not a food matrix.
363 Therefore, it could be hypothesized that food matrices used in the present study could have
364 influenced the metabolism of quercetin derivatives, preventing their complete degradation.
365 The catabolic pathways proposed for quercetin and luteolin microbial degradation in the colon
366 are shown in Figure 3. In accordance with Serra et al. (2012), quercetin is subjected to ring
367 fission, resulting in the formation of dihydrocaffeic acid, which could be then further
368 degraded to new catabolites. Ring fission could also result in the formation of protocatechuic
369 acid (Rechner et al., 2004). In the case of luteolin, according to Serra et al. (2012), only
370 dihydrocaffeic acid could be generated.
371 Concerning phenolic acids, the compounds detected on the digested fraction were quickly
372 metabolized by gut microbiota and no native compounds were detected after 5 h of fecal
373 incubation (Figure 2 B). An increase in caffeic acid amount was detected at the beginning of
374 the fecal fermentation, probably due to the cleavage of quinic acids (Ludwig, de Peña, Cid, &

375 Crozier, 2013; Rechner et al., 2004; Tomás-Barberán et al., 2014) and to the deglycosilation
376 of caffeic acid glucosides, as illustrated in Figure 3. In agreement with Breynaert et al. (2015),
377 caffeoylquinic acids and their main metabolite, i.e. caffeic acid, were not detectable anymore
378 after 5 h of colonic incubation, indicating a complete degradation of these compounds. In
379 accordance with the literature (Ludwig et al., 2013; Rechner et al., 2004; Tomás-Barberán et
380 al., 2014), caffeic acid was further metabolized into dihydrocaffeic acid by reduction of the
381 double bond.
382 Additionally to caffeic acid, quercetin and luteolin aglycones, a total of 3 catabolites, namely
383 3,4-dihydroxybenzoic acid (protocatechuic acid), dihydrocaffeic acid and 3-(3'-
384 hydroxyphenyl)propionic acid were generated during fecal fermentation as products of
385 degradation of (poly)phenolic compounds (Figure 2 C). As previously discussed,
386 dihydrocaffeic acid could be considered an intermediate catabolite of quercetin and caffeic
387 acid catabolism, which undergoes further dihydroxylation and results in the production of 3-
388 (3'-hydroxyphenyl)propionic acid, by far the most abundant catabolite found after fecal
389 incubation of green pepper samples. Moreover, protocatechuic acid was detected in
390 substantial amounts in 5 h fermented samples, and still remained in considerable amounts
391 after 24 h fecal incubation in cooked green pepper (Tables 2, 3, 4 and 5). As previously
392 discussed, it could be generated through the ring fission of quercetin (Rechner et al., 2004), or
393 through the α-oxidation of dihydrocaffeic acid passing via 3',4'-dihydroxyphenylacetic acid
394 (homoprotocatechuic acid) as intermediate (Ludwig et al., 2013). However,
395 dihydroxyphenylacetic acid was not detected in the present study, probably because of its
396 rapid rate of conversion to 3,4-dihydroxybenzoic acid (protocatechuic acid) (Ludwig et al.,
397 2013). Actually, some authors (Aura et al., 2002) detected a higher concentration of
398 dihydroxyphenylacetic acid within 2 h of incubation, a time point not considered in the

399 present study. Like dihydroxyphenylacetic acid, other minor catabolites previously reported
400 as being generated after (poly)phenol compound fecal biotransformation, such as phenylacetic
401 acid, hydroxyphenylacetic acid, hydroxybenzoic acid or benzoic acid (Aura et al., 2002;
402 Ludwig et al., 2013; Serra et al., 2012) were not detected in the present study. This could be
403 due to the different fermentation times applied, as 3-hydroxyphenylacetic acid was formed by
404 dehydroxylation of 3,4-dihydroxyphenylacetic acid after 8 h incubation (Aura et al., 2002),
405 while other catabolites presented the maximum amount after 48 h of fecal incubation and only
406 low quantities were detected after 24 h (Serra et al., 2012).
407 Finally, regarding the influence of heat treatment on fecal metabolism, only differences in the
408 total amount of catabolites were observed between raw and cooked green pepper samples
409 (Tables 2, 3, 4 and 5), whereas no differences were detectable in the number of catabolites
410 and in the suggested metabolic pathways for (poly)phenolic microbial degradation in the
411 colon. The total amount of (poly)phenolic compounds and catabolites in green pepper samples
412 remained around 47-59 % bioaccesible after 24 h of fecal incubation. Griddled green pepper,
413 which was the sample with the highest amount of (poly)phenolic compounds after the *in vitro*
414 gastrointestinal digestion, presented the highest amount of compounds after colonic
415 fermentation (4198 μmol/ g dm), following by sunflower oil fried pepper, raw pepper and
416 olive oil fried pepper (2.719, 2.480 and 2.210 μmol/g dm, respectively). No significant
417 differences were found between raw and fried pepper, both with sunflower oil and olive oil.
418 **4. Conclusions**
419 In summary, despite the consistent degradation of (poly)phenolic compounds after cooking
420 processes, the different heat treatments applied in this study seemed to exert a positive effect
421 on the release of phenolic compounds from green pepper during gastrointestinal digestion. In
422 griddled green pepper, the higher temperature applied during this thermal process compared

to frying could increase the formation of Maillard reaction compounds, such as melanoidins, which could form complex structures with (poly)phenolic compounds attached, resulting less accessible to digestive enzyme activities and improving their stability during digestion steps. On the other hand, the fat content of the fried samples could exert a protective effect against enzymatic action. Thus, the bioaccessibility of phenolic compounds after gastrointestinal digestion was higher in cooked samples than in raw one, especially in griddled green pepper, which showed the highest amount of phenolic compounds after the digestion process. Additionally, gut microbiota showed a high metabolic activity resulting in a large modification of (poly)phenolic compounds into new metabolites. Griddled green pepper was still the sample with the highest amount of bioaccesible (poly)phenolic compounds, even after the fecal fermentation step.

The metabolites formed during fecal fermentation may have an influence on the intestinal microflora (Blaut, Schoefer, & Braune, 2003) and their bioactive properties can be different from the activity of their parent compounds. Some studies demonstrated beneficial effects of phenolic catabolites, such as antioxidant, anti-inflammatory, anti-hyperglycemic, neuroprotective activities and positive effects on oxidative stress (Duda-Chodak et al., 2015; Masella et al., 2012; Verzelloni et al., 2011). However, the positive effects of these metabolites on health are still not clearly defined, and more studies are necessary in order to better understand the real actions of these compounds within the human organism. Further studies are also clearly needed to investigate the absorption of (poly)phenolic compounds and their subsequent transformation during phase II enzymatic metabolism resulting in glucuronidated, sulphated and methylated derivatives (Aura, 2008; Del Rio et al., 2013).

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581

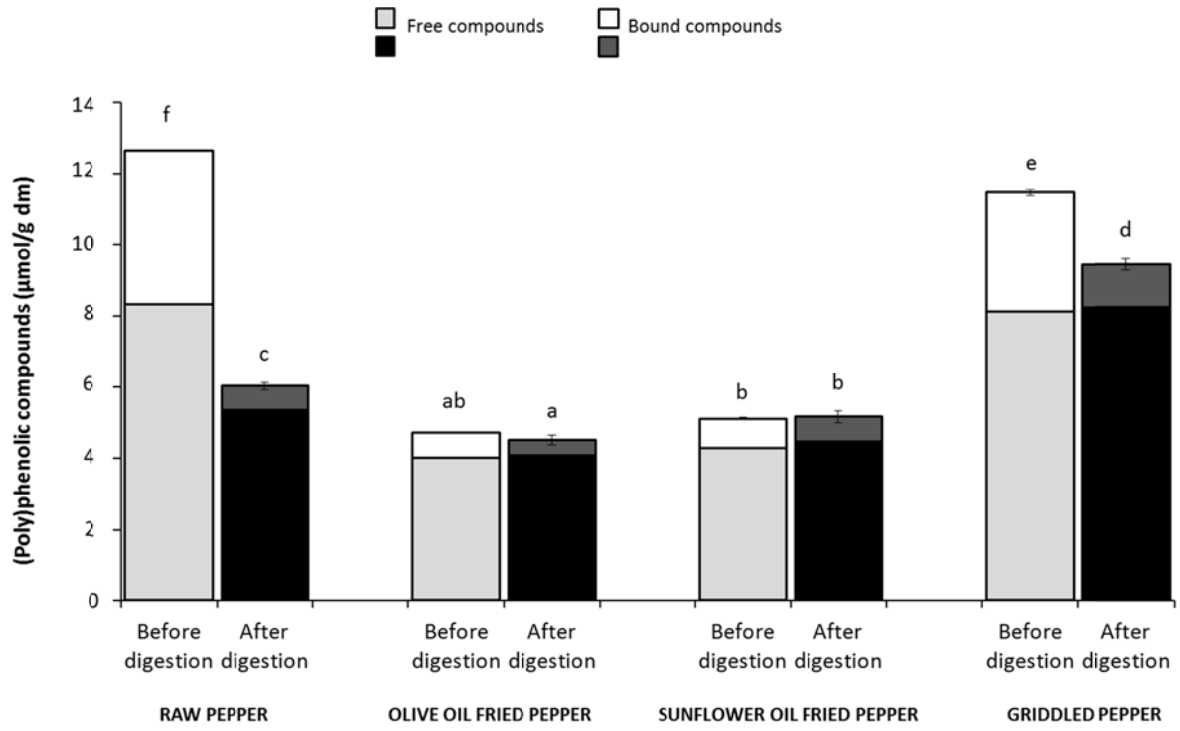
Figure caption

Figure 1. Free, bound and total (poly)phenolic compounds of green pepper both raw and cooked with different heat treatments, and after an *in vitro* gastrointestinal digestion. Results are expressed as mean ± standard deviation (n=3).

Figure 2. (Poly)phenolic compounds profiles of griddled green pepper during 24 h fecal fermentation. A) Main (poly)phenolic compounds (flavonoids) degradation profiles and production of their corresponding aglycones. B) Minor (poly)phenolic compounds (hydroxycinnamic acids) degradation profiles. C) Main (poly)phenolic catabolites production profiles after *in vitro* fecal fermentation. C is the control sample before fecal fermentation.

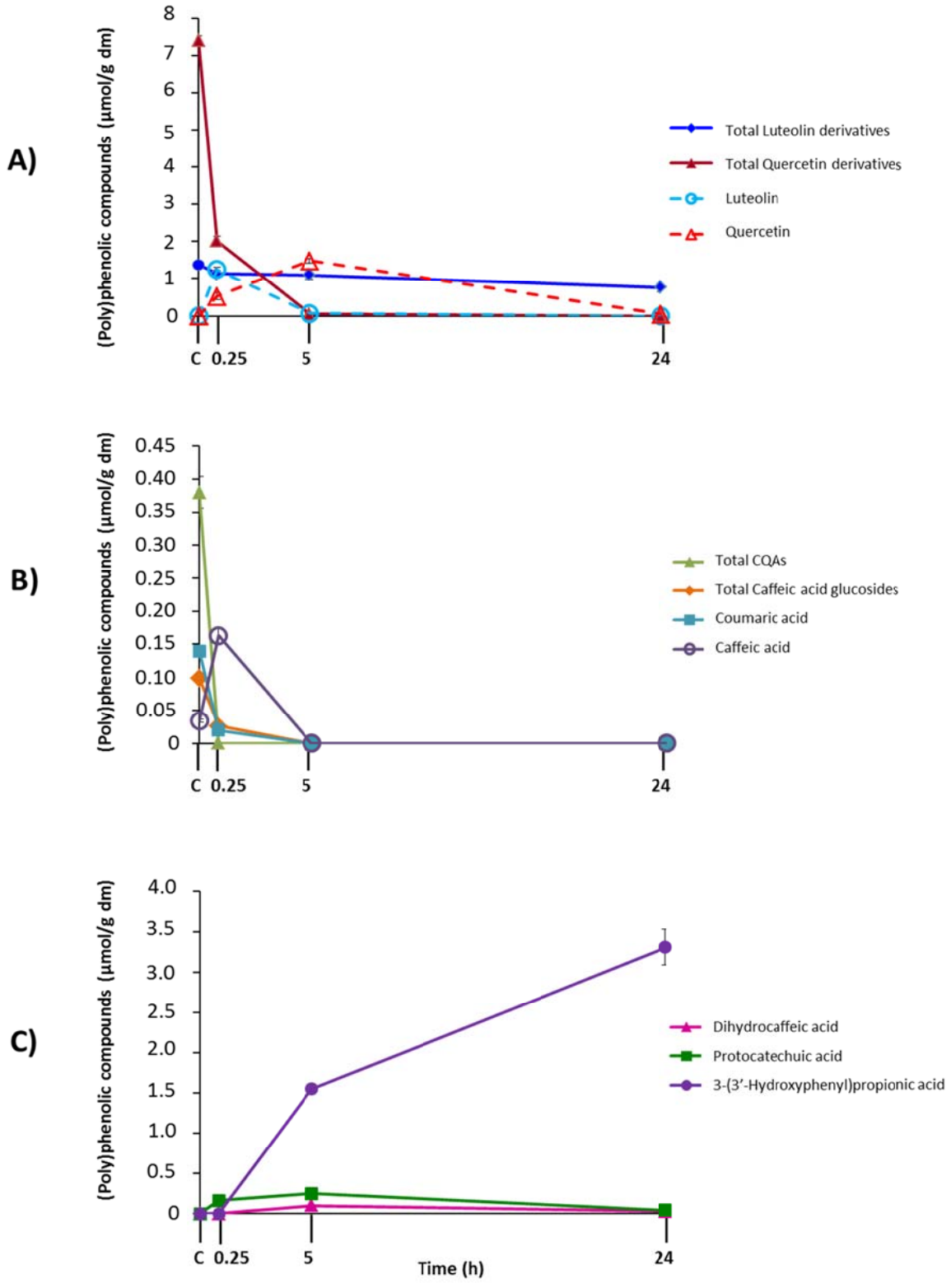
Figure 3. Proposed catabolic pathways for microbial degradation of (poly)phenolics in the colon after digestion of green pepper.

Figure 1.



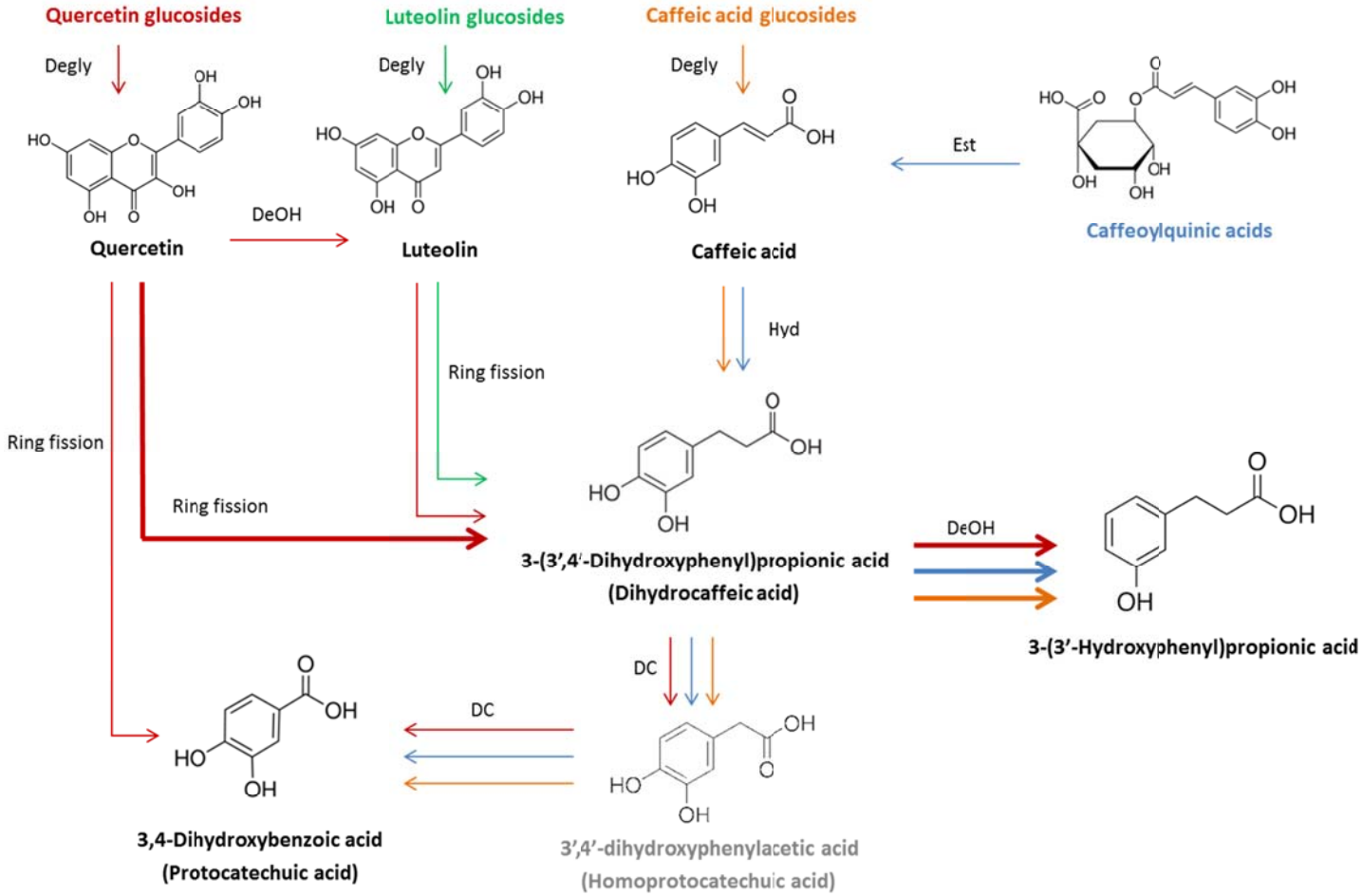
Different letters mean significant differences (p<0.05) between total (poly)phenolic compounds.

Figure 2.


A)
(Poly)phenolic compounds (µmol/g dm)
8
7
6
5
4
3
2
1
0
C
0.25
5
24
Total Luteolin derivatives
Total Quercetin derivatives
Luteolin
Quercetin
B)
(Poly)phenolic compounds (µmol/g dm)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
C
0.25
5
24
Total CQAs
Total Caffeic acid glucosides
Coumaric acid
Caffeic acid
C)
(Poly)phenolic compounds (µmol/g dm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
C
0.25
5
24
Time (h)
Dihydrocaffeic acid
Protocatechuic acid
3-(3'-Hydroxyphenyl)propionic acid

.

Figure 3.



Degly, Deglycosilation; DeOH, Dehydroxylation; Est, Ester hydrolysis; Hyd, Hydrogenation; DC, Decarboxylation. Detected metabolites are in black, non-detected metabolites are in grey. Red arrows show quercetin glucoside catabolic pathway, green arrows correspond to luteolin derivatives colonic pathways, orange arrows evidence caffeic acid derivatives pathways and blue arrows indicate chlorogenic acid metabolic pathways. Bold arrows indicate major pathways.

Table 1. Free and bound (poly)phenolic compounds in raw and cooked green pepper (fried in olive oil, fried in sunflower oil and griddled) before and after an *in vitro* gastrointestinal digestion. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Raw pepper		Olive oil fried pepper		Sunflower oil fried pepper		Griddled pepper	
	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion
Quercetin 3-glucoside-7-rhamnoside								
Free compounds	0.043 ± 0.002^{c}	0.021 ± 0.003^{a}	0.020 ± 0.002^{a}	0.019 ± 0.001^{a}	0.026 ± 0.003^{ab}	0.026 ± 0.004^{ab}	0.039 ± 0.004^{bc}	0.044 ± 0.005^{c}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin 3-sambubioside-7-rhamnoside								
Free compounds	0.096 ± 0.013^{c}	0.057 ± 0.000^{ab}	0.044 ± 0.003^{a}	0.038 ± 0.004^{a}	0.049 ± 0.004^{a}	0.041 ± 0.001^{a}	0.084 ± 0.011^{bc}	0.086 ± 0.003^{bc}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Rutin								
Free compounds	0.073 ± 0.026^{ab}	0.050 ± 0.007^{a}	0.063 ± 0.003^{ab}	0.062 ± 0.002^{ab}	0.081 ± 0.010^{b}	0.066 ± 0.001^{ab}	0.178 ± 0.011^{d}	0.142 ± 0.001^{c}
Bound compounds	0.003 ± 0.001^{b}	nd^{a}	nd^{a}	nd^{a}	0.002 ± 0.000^{b}	0.003 ± 0.000^{b}	0.007 ± 0.001^{c}	0.002 ± 0.000^{b}
Quercetin glucoside								
Free compounds	0.501 ± 0.116^{c}	0.276 ± 0.004^{ab}	0.238 ± 0.001^{ab}	0.224 ± 0.008^{a}	0.381 ± 0.003^{bc}	0.331 ± 0.028^{ab}	0.761 ± 0.085^{d}	0.502 ± 0.005^{c}
Bound compounds	0.180 ± 0.003^{c}	0.042 ± 0.000^{a}	0.031 ± 0.015^{a}	0.032 ± 0.003^{a}	0.049 ± 0.009^{ab}	0.060 ± 0.002^{ab}	0.244 ± 0.013^{d}	0.089 ± 0.016^{b}
Rutin isomer								
Free compounds	nd^{a}	nd^{a}	0.010 ± 0.002^{b}	0.011 ± 0.002^{b}	0.013 ± 0.001^{b}	0.011 ± 0.002^{b}	0.024 ± 0.001^{c}	0.026 ± 0.003^{c}
Bound compounds	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.002 ± 0.000^{b}	nd^{a}
Quercetin rhamnoside								
Free compounds	5.001 ± 0.116^{d}	4.061 ± 0.032^{c}	2.362 ± 0.032^{a}	2.820 ± 0.137^{ab}	2.489 ± 0.022^{a}	3.051 ± 0.127^{b}	4.797 ± 0.109^{d}	5.777 ± 0.214^{e}
Bound compounds	2.911 ± 0.267^{d}	0.458 ± 0.008^{ab}	0.378 ± 0.003^{ab}	0.243 ± 0.036^{a}	0.487 ± 0.016^{ab}	0.404 ± 0.040^{ab}	2.020 ± 0.011^{c}	0.753 ± 0.081^{b}
Luteolin 6,8.di-C-glucoside								
Free compounds	0.040 ± 0.000^{ab}	0.026 ± 0.001^{ab}	0.035 ± 0.001^{ab}	0.026 ± 0.000^{ab}	0.043 ± 0.004^{b}	0.024 ± 0.000^{a}	0.061 ± 0.001^{c}	0.063 ± 0.010^{c}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 6-C-hexoside-8-C-pentoside								
Free compounds	0.084 ± 0.003^{c}	0.048 ± 0.004^{a}	0.066 ± 0.004^{b}	0.048 ± 0.006^{a}	0.083 ± 0.002^{c}	0.044 ± 0.002^{a}	0.114 ± 0.001^{d}	0.115 ± 0.003^{d}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 6-C-pentoside-8-C-hexoside								
Free compounds	0.045 ± 0.001^{b}	0.026 ± 0.001^{a}	0.042 ± 0.000^{b}	0.030 ± 0.001^{a}	0.044 ± 0.006^{b}	0.021 ± 0.002^{a}	0.052 ± 0.000^{b}	0.066 ± 0.004^{c}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 8-C-hexoside								
Free compounds	0.281 ± 0.020^{d}	0.069 ± 0.001^{a}	0.223 ± 0.004^{cd}	0.151 ± 0.028^{b}	0.237 ± 0.006^{cd}	0.171 ± 0.023^{bc}	0.363 ± 0.011^{e}	0.292 ± 0.003^{de}
Bound compounds	0.060 ± 0.002^{c}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.040 ± 0.000^{b}	nd^{a}
Luteolin 7-O-(2-apiosyl)glucoside								
Free compounds	0.388 ± 0.025^{ab}	0.573 ± 0.060^{c}	0.252 ± 0.025^{a}	0.291 ± 0.052^{ab}	0.306 ± 0.009^{ab}	0.367 ± 0.008^{ab}	0.350 ± 0.004^{ab}	0.435 ± 0.003^{bc}
Bound compounds	0.514 ± 0.011^{d}	0.099 ± 0.004^{a}	0.099 ± 0.017^{a}	0.082 ± 0.001^{a}	0.099 ± 0.001^{a}	0.125 ± 0.014^{a}	0.351 ± 0.015^{c}	0.181 ± 0.007^{b}
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside I								
Free compounds	0.358 ± 0.020^{c}	0.070 ± 0.016^{a}	0.124 ± 0.015^{b}	0.044 ± 0.011^{a}	0.123 ± 0.020^{b}	0.059 ± 0.006^{a}	0.289 ± 0.012^{c}	0.160 ± 0.036^{b}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd

Luteolin acetylglucoside I								
Free compounds	0.030 ± 0.000^{c}	0.009 ± 0.002^{a}	0.013 ± 0.001^{a}	0.008 ± 0.000^{a}	0.014 ± 0.001^{a}	0.007 ± 0.000^{a}	0.028 ± 0.002^{bc}	0.022 ± 0.002^{b}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II								
Free compounds	nd^{a}	nd^{a}	0.028 ± 0.005^{c}	0.012 ± 0.000^{ab}	0.019 ± 0.007^{bc}	0.005 ± 0.001^{ab}	0.084 ± 0.002^{d}	0.033 ± 0.002^{c}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin acetylglucoside II								
Free compounds	nd^{a}	nd^{a}	0.002 ± 0.000^{b}	nd^{a}	0.003 ± 0.001^{b}	nd^{a}	0.020 ± 0.000^{d}	0.010 ± 0.001^{c}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid glucoside I								
Free compounds	0.183 ± 0.010^{d}	0.068 ± 0.004^{b}	0.142 ± 0.002^{c}	0.072 ± 0.007^{b}	0.124 ± 0.003^{c}	0.032 ± 0.003^{a}	0.243 ± 0.003^{e}	0.062 ± 0.001^{b}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid glucoside II								
Free compounds	0.918 ± 0.004^{d}	nd^{a}	0.026 ± 0.001^{b}	nd^{a}	0.026 ± 0.002^{b}	0.026 ± 0.001^{b}	0.043 ± 0.001^{c}	0.037 ± 0.005 ^{bc}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid								
Free compounds	nd	nd	nd	nd	nd	nd	nd	nd
Bound compounds	0.272 ± 0.010^{d}	nd^{a}	0.043 ± 0.000^{b}	nd^{a}	0.034 ± 0.007^{b}	0.018 ± 0.003^{ab}	0.221 ± 0.013^{c}	0.034 ± 0.002^{b}
5-CQA								
Free compounds	0.967 ± 0.036^{f}	nd^{a}	0.280 ± 0.005^{cd}	0.209 ± 0.007^{bc}	0.201 ± 0.011^{bc}	0.156 ± 0.009^{b}	0.517 ± 0.013^{e}	0.335 ± 0.029^{d}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
4-CQA								
Free compounds	0.155 ± 0.0020^{e}	nd^{a}	0.046 ± 0.003^{c}	0.023 ± 0.001^{b}	0.024 ± 0.006^{b}	0.028 ± 0.004^{b}	0.085 ± 0.002^{d}	0.045 ± 0.005^{c}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Coumaric acid								
Free compounds	nd	nd	nd	nd	nd	nd	nd	nd
Bound compounds	0.388 ± 0.008^{c}	0.079 ± 0.009^{a}	0.149 ± 0.002^{b}	0.069 ± 0.006^{a}	0.157 ± 0.005^{b}	0.087 ± 0.005^{a}	0.460 ± 0.022^{d}	0.139 ± 0.002^{b}
Total (poly)phenolic compounds								
Free compounds	8.337^{c}	5.354^{b}	4.016^{a}	4.089^{a}	4.285^{a}	4.464^{a}	8.130^{c}	8.250^{c}
Bound compounds	4.328^{d}	0.679^{a}	0.700^{a}	0.426^{a}	0.828^{ab}	0.696^{a}	3.345^{c}	1.198^{b}
Total compounds	**12.664^{f}**	**6.033^{c}**	**4.715^{ab}**	**4.514^{a}**	**5.113^{b}**	**5.150^{b}**	**11.475^{e}**	**9.447^{d}**

Different letters for each row indicate significant differences ($p \leq 0.05$) among samples.

Table 2. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of raw green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.021 ± 0.003	0.016 ± 0.001	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.057 ± 0.000	0.012 ± 0.003	nd	nd
Rutin	0.050 ± 0.007	0.007 ± 0.002	nd	nd
Quercetin glucoside	0.318 ± 0.003	nd	nd	nd
Rutin isomer	nd	nd	nd	nd
Quercetin rhamnoside	4.519 ± 0.024	2.314 ± 0.128	0.029 ± 0.002	nd
Total quercetin derivatives	4.965 ± 0.025	2.348 ± 0.136	0.029 ± 0.002	nd
Quercetin	nd	0.245 ± 0.023	0.619 ± 0.064	0.029 ± 0.008
Luteolin derivatives				
Luteolin 6,8-di-*C*-glucoside	0.026 ± 0.001	0.046 ± 0.008	0.019 ± 0.001	0.025 ± 0.002
Luteolin 6-*C*-hexoside-8-*C*-pentoside	0.048 ± 0.004	0.090 ± 0.003	0.059 ± 0.027	0049 ± 0.006
Luteolin 6-*C*-pentoside-8-*C*-hexoside	0.026 ± 0.001	0.051 ± 0.001	0.037 ± 0.003	0.027 ± 0.005
Luteolin 8-*C*-hexoside	0.069 ± 0.001	0.150 ± 0.025	0.099 ± 0.02	0.117 ± 0.006
Luteolin 7-*O*-(2-apiosyl)glucoside	0.672 ± 0.057	0.415 ± 0.024	0.005 ± 0.002	0.001 ± 0.000
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside I	0.070 ± 0.016	nd	nd	nd
Luteolin acetylglucoside I	0.009 ± 0.002	nd	nd	nd
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside II	nd	nd	nd	nd
Luteolin acetylglucoside II	nd	nd	nd	nd
Total luteolin derivatives	0.921 ± 0.072	0.753 ± 0.053	0.220 ± 0.035	0.219 ± 0.009
Luteolin	nd	1.220 ± 0.102	0.043 ± 0.010	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.068 ± 0.004	0.049 ± 0.00	nd	nd
Caffeic acid glucoside II	nd	nd	nd	nd
Caffeic acid	nd	0.015 ± 0.002	nd	nd
5-CQA	nd	nd	nd	nd
4-CQA	nd	nd	nd	nd
Coumaric acid	0.079 ± 0.009	0.057 ± 0.014	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.047 ± 0.002	nd
Protocatechuic acid	nd	0.235 ± 0.009	0.299 ± 0.006	nd
3-(3′-Hydroxyphenyl)propionic acid	nd	nd	1.213 ± 0.108	2.232 ± 0.204
Total (poly)phenolic compounds	**6.033**	**4.922**	**2.469**	**2.480**

Table 3. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of olive oil fried green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.019 ± 0.001	0.012 ± 0.002	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.038 ± 0.004	0.003 ± 0.000	nd	nd
Rutin	0.062 ± 0.002	nd	nd	nd
Quercetin glucoside	0.256 ± 0.006	nd	nd	nd
Rutin isomer	0.011 ± 0.002	0.012 ± 0.001	nd	nd
Quercetin rhamnoside	3.063 ± 0.101	0.841 ± 0.063	nd	nd
Total quercetin derivatives	3.449 ± 0.107	0.868 ± 0.062	nd	nd
Quercetin	nd	0.608 ± 0.070	0.643 ± 0.036	0.020 ± 0.001
Luteolin derivatives				
Luteolin 6,8-di-*C*-glucoside	0.026 ± 0.000	0.039 ± 0.006	0.035 ± 0.008	0.025 ± 0.003
Luteolin 6-*C*-hexoside-8-*C*-pentoside	0.048 ± 0.006	0.082 ± 0.023	0.058 ± 0.002	0.050 ± 0.001
Luteolin 6-*C*-pentoside-8-*C*-hexoside	0.030 ± 0.001	0.050 ± 0.012	0.042 ± 0.001	0.028 ± 0.002
Luteolin 8-*C*-hexoside	0.151 ± 0.028	0.304 ± 0.030	0.363 ± 0.022	0.361 ± 0.043
Luteolin 7-*O*-(2-apiosyl)glucoside	0.373 ± 0.054	0.141 ± 0.018	0.004 ± 0.001	nd
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside I	0.044 ± 0.011	nd	nd	nd
Luteolin acetylglucoside I	0.008 ± 0.000	nd	nd	nd
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside II	0.012 ± 0.000	nd	nd	nd
Luteolin acetylglucoside II	nd	nd	nd	nd
Total luteolin derivatives	0.693 ± 0.033	0.616 ± 0.088	0.502 ± 0.034	0.464 ± 0.045
Luteolin	nd	0.735 ± 0.088	0.057 ± 0.002	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.072 ± 0.007	0.014 ± 0.002	nd	nd
Caffeic acid glucoside II	nd	nd	nd	nd
Caffeic acid	nd	0.134 ± 0.002	nd	nd
5-CQA	0.209 ± 0.007	nd	nd	nd
4-CQA	0.023 ± 0.001	nd	nd	nd
Coumaric acid	0.069 ± 0.006	0.003 ± 0.000	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.057 ± 0.005	nd
Protocatechuic acid	nd	0.131 ± 0.00	0.212 ± 0.000	0.073 ± 0.009
3-(3′-Hydroxyphenyl)propionic acid	nd	nd	0.810 ± 0.102	1.652 ± 0.111
Total (poly)phenolic compounds	**4.514**	**3.109**	**2.280**	**2.210**

Table 4. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of sunflower oil fried green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.026 ± 0.004	0.015 ± 0.002	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.041 ± 0.001	0.006 ± 0.000	nd	nd
Rutin	0.069 ± 0.002	nd	nd	nd
Quercetin glucoside	0.391 ± 0.026	nd	nd	nd
Rutin isomer	0.011 ± 0.002	0.019 ± 0.00	nd	nd
Quercetin rhamnoside	3.455 ± 0.167	1.278 ± 0.192	nd	nd
Total quercetin derivatives	3.991 ± 0.138	1.318 ± 0.197	nd	nd
Quercetin	nd	0.384 ± 0.098	0.435 ± 0.004	0.005 ± 0.000
Luteolin derivatives				
Luteolin 6,8-di-*C*-glucoside	0.024 ± 0.000	0.056 ± 0.007	0.029 ± 0.007	0.026 ± 0.002
Luteolin 6-*C*-hexoside-8-*C*-pentoside	0.044 ± 0.002	0.085 ± 0.003	0.058 ± 0.012	0.064 ± 0.002
Luteolin 6-*C*-pentoside-8-*C*-hexoside	0.021 ± 0.002	0.088 ± 0.005	0.049 ± 0.009	0.038 ± 0.005
Luteolin 8-*C*-hexoside	0.171 ± 0.023	0.424 ± 0.074	0.365 ± 0.054	0.317 ± 0.063
Luteolin 7-*O*-(2-apiosyl)glucoside	0.492 ± 0.023	0.180 ± 0.021	0.004 ± 0.001	nd
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside I	0.059 ± 0.005	nd	nd	nd
Luteolin acetylglucoside I	0.007 ± 0.000	nd	nd	nd
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside II	0.005 ± 0.001	nd	nd	nd
Luteolin acetylglucoside II	nd	nd	nd	nd
Total luteolin derivatives	0.824 ± 0.009	0.833 ± 0.109	0.505 ± 0.080	0.444 ± 0.068
Luteolin	nd	0.913 ± 0.070	0.034 ± 0.008	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.032 ± 0.003	0.019 ± 0.001	nd	nd
Caffeic acid glucoside II	0.026 ± 0.001	nd	nd	nd
Caffeic acid	0.018 ± 0.003	0.135 ± 0.009	nd	nd
5-CQA	0.156 ± 0.009	nd	nd	nd
4-CQA	0.028 ± 0.004	nd	nd	nd
Coumaric acid	0.087 ± 0.005	0.006 ± 0.000	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.055 ± 0.002	nd
Protocatechuic acid	nd	0.140 ± 0.003	0.146 ± 0.006	0.034 ± 0.004
3-(3′-Hydroxyphenyl)propionic acid	nd	nd	1.281 ± 0.042	2.236 ± 0.070
Total (poly)phenolic compounds	**5.161**	**3.749**	**2.457**	**2.719**

Table 5. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of griddled green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.044 ± 0.005	0.019 ± 0.001	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.086 ± 0.003	0.005 ± 0.000	nd	nd
Rutin	0.144 ± 0.001	nd	nd	nd
Quercetin glucoside	0.591 ± 0.011	nd	nd	nd
Rutin isomer	0.026 ± 0.003	0.024 ± 0.001	nd	nd
Quercetin rhamnoside	6.530 ± 0.133	1.969 ± 0.126	0.045 ± 0.004	nd
Total quercetin derivatives	7.419 ± 0.116	2.017 ± 0.129	0.045 ± 0.004	nd
Quercetin	nd	0.528 ± 0.094	1.482 ± 0.061	0.052 ± 0.007
Luteolin derivatives				
Luteolin 6,8-di-*C*-glucoside	0.063 ± 0.010	0.094 ± 0.019	0.073 ± 0.009	0.050 ± 0.006
Luteolin 6-*C*-hexoside-8-*C*-pentoside	0.115 ± 0.003	0.159 ± 0.006	0.149 ± 0.012	0.125 ± 0.010
Luteolin 6-*C*-pentoside-8-*C*-hexoside	0.066 ± 0.004	0.110 ± 0.021	0.132 ± 0.012	0.060 ± 0.002
Luteolin 8-*C*-hexoside	0.292 ± 0.003	0.571 ± 0.103	0.733 ± 0.091	0.529 ± 0.098
Luteolin 7-*O*-(2-apiosyl)glucoside	0.616 ± 0.023	0.197 ± 0.021	0.008 ± 0.001	0.002 ± 0.000
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside I	0.160 ± 0.036	nd	nd	nd
Luteolin acetylglucoside I	0.022 ± 0.002	nd	nd	nd
Luteolin 7-*O*-(2-apiosyl-6-malonyl)glucoside II	0.033 ± 0.002	nd	nd	nd
Luteolin acetylglucoside II	0.010 ± 0.001	nd	nd	nd
Total luteolin derivatives	1.377 ± 0.028	1.130 ± 0.078	1.095 ± 0.124	0.767 ± 0.116
Luteolin	nd	1.243 ± 0.063	0.072 ± 0.012	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.062 ± 0.001	0.026 ± 0.001	nd	nd
Caffeic acid glucoside II	0.037 ± 0.005	nd	nd	nd
Caffeic acid	0.034 ± 0.002	0.163 ± 0.011	nd	nd
5-CQA	0.334 ± 0.029	nd	nd	nd
4-CQA	0.045 ± 0.005	nd	nd	nd
Coumaric acid	0.139 ± 0.002	0.020 ± 0.004	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.100 ± 0.004	0.027 ± 0.005
Protocatechuic acid	nd	0.162 ± 0.000	0.251 ± 0.001	0.041 ± 0.004
3-(3′-Hydroxyphenyl)propionic acid	nd	nd	1.551 ± 0.055	3.311 ± 0.221
Total (poly)phenolic compounds	**9.447**	**5.289**	**4.597**	**4.198**