Food Research International 160 (2022) 111702

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Roasting and frying modulate the phenolic profile of dark purple eggplant and differently change the colon microbiota and phenolic metabolites after *in vitro* digestion and fermentation in a gut model

Lorenzo Nissen [a,b,c,1], Alice Cattivelli [d,1], Flavia Casciano [a], Andrea Gianotti [a,b,c], Davide Tagliazucchi [d,*]

[a] Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum - University of Bologna, V.le Fanin 44, 40127 Bologna, Italy
[b] Interdepartmental Centre of Agri-Food Industrial Research (CIRI), Alma Mater Studiorum - University of Bologna, P.za G. Goidanich 60, 47521 Cesena (FC), Italy
[c] Centre for Applied Biomedical Research – CRBA, Alma Mater Studiorum - Università di Bologna, Policlinico di Sant'Orsola, Bologna, Italy
[d] Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2 - Pad. Besta, 42100 Reggio Emilia, Italy

ARTICLE INFO

Keywords:
Thermal treatments
Hydroxycinnamic acids
Prebiotic index
Food processing
Mass spectrometry
Microbiomics

ABSTRACT

The way of cooking vegetables could differently affect the phenolic profiles of foods and their impact on human colon microbiota. In this work, we investigated the stability and bioaccessibility as well as the impact and fate of dark purple eggplant (DPE) phenolic compounds in the gut microbiota after grilling or frying in comparison to the raw one. After cooking, DPE underwent a gastro-intestinal digestion along with a proximal colon fermentation using the short-term batch model MICODE (multi-unit *in vitro* colon gut model). During the process, the phenolic compounds profiles (through high-resolution mass spectrometry) and microbiomics (qPCR of 14 core taxa) analyses were performed. Results showed that thermal treatments increased the amount of extractable phenolic compounds as well as their bioaccessibility. The highest gastro-intestinal release was observed in fried DPE (2468.46 ± 13.64 μmol/100 g), followed by grilled DPE (1007. 96 ± 12.84 μmol/100 g) and raw DPE (900.93 ± 10.56 μmol/100 g). Mass spectrometry analysis confirmed that colonic bacteria were able to metabolize DPE phenolic compounds mainly to 3-(3′-hydroxyphenyl)propanoic acid. Furthermore, results indicated that frying was better than grilling in terms of fostering more the growth of beneficial bacterial taxa and limiting that of opportunistic taxa. For example, fried DPE determined an increase in abundance of *Bifidobacteriaceae Lactobacillales* of 2.66 and 3.80 times. This work is one of the first exploring how cooking methods can affect the phenolic composition of DPE and differently impact on the colon microbiota tuning and modifying the food functionalities.

1. Introduction

Eggplant is a typical component of the Mediterranean Diet, which is considered a notable source of phenolic compounds, especially hydroxycinnamic acid-derivatives mainly conjugates with quinic acid or amides (Martini, Conte, Cattivelli, & Tagliazucchi, 2021). The world-wide eggplant production in 2019 was >50 million of tons per year making this vegetable one of the most important crop of the solanaceous family together with tomato, potato and pepper. In the USA the consumption per capita of eggplant was estimated about 0.5 kg in 2020 (Karimi, Kazemi, Samani, & Simal-Gandara, 2021). Several health benefits have been linked to eggplant intake, especially related to the prevention of chronic pathologies such as cardiovascular diseases, cancer and inflammatory conditions (Gürbüz et al., 2018; Nishimura et al., 2019). These supposed beneficial effects of eggplant consumption have been correlated to the presence of phenolic compounds and in particular of hydroxycinnamic acids (Gürbüz et al., 2018). Various *in vivo* and *in vitro* studies demonstrated the potential cardio-protective effects as well as the anti-diabetic, anti-carcinogenic and anti-inflammatory properties of hydroxycinnamic acids and derived metabolites (Clifford, 2000; Sova, & Saso, 2020).

Besides composition, technological treatments may alter the food

* Corresponding author.
E-mail address: davide.tagliazucchi@unimore.it (D. Tagliazucchi).
[1] Both authors equally contributed to this work.

https://doi.org/10.1016/j.foodres.2022.111702
Received 26 April 2022; Received in revised form 9 July 2022; Accepted 15 July 2022
Available online 18 July 2022

composition including the quali/quantitative profile of beneficial compounds and their possibility to be bioaccessible and thereafter bioavailable. Indeed, differently from many Mediterranean Diet vegetable foods, eggplant is consumed exclusively after cooking, largely after frying and grilling that are the two most common thermal treatments to which eggplant are subjected. Various studies pointed out that cooking procedure may modify the phenolic profile of vegetables foods also resulting in a differential release of individual phenolic compounds after *in vitro* gastro-intestinal digestion (Valli, et al., 2016; Saa, Di Silvestro, Dinelli, & Gianotti, 2017; Cattivelli, Conte, Martini, & Tagliazucchi, 2021; Martini et al., 2021). Furthermore, variations in the phenolic composition after cooking and *in vitro* digestion resulted in a different pattern of colonic metabolites after *in vitro* fermentation of raw and cooked cardoon, green pepper and cactus cladodes (De Santiago, Gill, Carafa, Tuohy, De Peña, & Cid, 2019; Juániz et al., 2016; Juániz et al., 2017). In addition, it has been shown that both food composition and thermal treatments altered the composition of the gut microbiota (Pérez-Burillo et al., 2018).

Specifically, increasing evidence suggests that the supposed health effects of phenolic compounds are probably related to the interaction with gut microbiota including the bio-activation of phenolic compounds by gut bacterial metabolism and the preservation of a healthy microbial population (Marhuenda-Muñoz et al., 2019). For example, phenolic compounds may enhance the relative abundance of *Lactobacillus* and *Bifidobacterium* species that are involved in the protection of the intestinal epithelium, as well as of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, which exhibited anti-inflammatory effects (Corrêa et al., 2019; Kasprzak-Drozd, Oniszczuk, Stasiak, & Oniszczuk, 2021; Mena, & Bresciani, 2020). On the other hand, gut bacteria can modify the structure of phenolic compounds generating metabolites that are more easily absorbed than the parent compounds and presented important biological activities (Koudoufio et al., 2020; Marhuenda-Muñoz et al., 2019).

In order to shed light on the network involving food, processes, bioaccessibility, and bioavailability for humans, more recently *in vitro* gut models were used to study some mechanisms involved in gut microbiota interactions with food components including plant phytochemicals. *In vitro* gut models are considered a proper solution because they can explain the impact of prebiotics on human gut microbiota, focusing on the shift in the core microbial groups and that of selected species and their metabolites, assaying composition and abundance in the community over time (Nissen, Casciano & Gianotti, 2020a). In particular, we performed this study employing the gut model MICODE (Multi-Unit *in vitro* Colon Model), previously used with success to study food matrices rich in phenolic compounds (Nissen et al., 2021a).

Therefore, the objective of this study was to assess the effect of the cooking methods (grilling and frying) on the dark purple eggplant phenolic compounds, their release during *in vitro* gastro-intestinal digestion and the main metabolites produced after their *in vitro* colonic fermentation. Moreover, the shift of fecal microbiota groups associated to those changes were also evaluated.

2. Materials and methods

2.1. Dark purple eggplant (DPE) cooking and in vitro gastro-intestinal digestion

DPE (Black Beauty variety) samples underwent to two different cooking treatments, i.e. frying and grilling, as reported in Martini et al. (2021). Briefly, for each treatment, a whole DPE fruit was weighed, washed with distilled water, dried with paper towels and cut in slices of approximatively 1.0 cm. One portion (corresponding to about half of the eggplant weight) was immediately frozen at −80 °C and used as raw sample, whereas the other portion was subjected to the cooking treatment. For the grilling procedure, the DPE slices were cooked at 120 °C for 10 min on a grilling plate. For the frying procedure, the DPE slices were fried at 170 °C for 10 min in sunflower oil by using a domestic deep fryer. After frying, DPE slices were drained of and dabbed with paper towels to absorb the oil in excess, and stored at −80 °C until analysis. The *in vitro* gastro-intestinal digestion was carried out on raw and cooked DPE samples by applying the INFOGEST protocol (Minekus et al., 2014). The full description of the digestion protocol is reported in Martini et al (2021). At the end of the intestinal phase, an aliquot of sample (1 mL) was withdrawn, centrifuged (10000*g*, 10 min, 4 °C) and the supernatant stored at −80 °C for mass spectrometry analysis. Another portion of 1 g of the sample was subjected to the *in vitro* colonic fermentation trials as described below. The remaining material (about 30 mL of digested sample) was stored at −80 °C. Cooking treatments and digestions were carried out in triplicate.

2.2. Colonic fermentation

2.2.1. Fecal donors

The fecal samples used for this study were obtained from three lean healthy individuals that respected the inclusion criteria as previously reported (Connolly et al., 2012; Koutsos et al., 2017; Oba et al., 2020; Nissen et al., 2021a; Nissen et al., 2021b; Arnal et al., 2021). Briefly, the three healthy subjects, two females and one male aged between 30 and 45 y did not undergo antibiotic treatment for at least 3 months prior to stool collection, did not intentionally consume pre- or probiotic supplements before the experiment, and had no history of bowel disorders. Additionally, the donors were normal weight, not smokers, not chronically consuming any drug, and not alcoholic drink consumers. Fecal samples were donated two times (with an interval of seven days) for the two biological replicas. Fecal samples were collected, and processed as previously described (Nissen et al., 2021a; Nissen et al., 2021b). Briefly, to collect fecal samples, donors were provided and instructed to use a collection kit, which includes a stool collector (Sarstedt AG & Co. KG, Nümbrecht, Germany) and an anaerobic jar with a O_2 catalyst (Oxoid, Thermo Fisher Scientific, Waltham, MA, USA). Fecal samples were then maintained at 4 °C and processed within 2 h. The fecal slurry was prepared by homogenizing 6 g of feces (2 g of each donation) in 54 mL of O_2 reduced phosphate-buffered saline (PBS). The three healthy donors were told of the study's aims and procedures and gave their verbal consent for their fecal matter to be used for the experiments, in agreement with the ethics procedures required at the University of Bologna.

2.2.2. Fecal batch-culture fermentation and sampling

Colonic fermentations were conducted for 24 h in independent vessels using an *in vitro* gut model, MICODE (Nissen et al., 2021a; Nissen et al., 2021b), obtained by the assembly of Minibio Reactors (Applikon Biotechnology BV, Delft, NL) and controlled by Lucullus PIMS software (Applikon Biotechnology BV, NL). The preparation of the experiments was made according to published procedures (Connolly et al., 2012; Koutsos et al., 2017; Wang et al., 2020) and described in detail in Nissen et al. (2021a). Briefly, fermentation vessels were filled aseptically with 90 mL of basal medium (Connolly et al., 2012; Koutsos et al., 2017). Once the proximal colon ecological condition was reached, a first vessel was aseptically loaded with 10 mL of a mixtures including fecal slurry (FS) (10 % w/v of human feces in O_2 reduced PBS) and 1 g of the solid portion of *in vitro* digested fried DPE (FDPE), a second vessel was loaded with 10 mL of FS including 1 g of the solid portion of *in vitro* digested grilled DPE (GDPE), a third vessel was loaded with 10 mL of FS including 1 g of the solid portion of *in vitro* digested raw DPE (RDPE), and a fourth vessel was set as the blank control (BC) and was loaded with 10 mL of FS including 1 mL of digestive enzymes. Batch cultures were run under controlled conditions for a period of 26.55 h including the baseline (BL) (2.55 ± 0.11 h) as described in Nissen et al. (2021b). Sampling was performed as reported in Nissen et al. (2021b). Samples of the different time points were used for UHPL/HR-MS and qPCR. Technical replicas of analyses were conducted in triplicates from two independent biological experiments.

2.3. Identification and quantification of the phenolic compounds by ultra high performance liquid chromatography/high resolution mass spectrometry (UHPLC/HR-MS)

2.3.1. Phenolic compounds and faecal metabolites extraction

The extraction of phenolic compounds from raw and cooked non-digested DPE was performed according to Martini et al. (2021). Raw and cooked samples were mixed with a water/methanol/formic acid (28:70:2, v/v/v) solution in a ratio of 1:2 (w/v). After homogenization, the samples were incubated at 37 °C for 30 min, centrifuged (6000*g*, 20 min, 4 °C) and the collected supernatant analysed by UHPLC/HR-MS.

Samples collected at the end of the *in vitro* gastro-intestinal digestion were analysed by UHPLC/HR-MS without any further extraction procedure.

Faecal metabolites were extracted following the protocol of Juániz et al. (2017). Briefly, fermented samples were mixed with the extraction solution (water/methanol/formic acid; 19.9:80:0.1, v/v/v) in a ratio of 1:1 (v/v). Then the mixtures were vortexed for 1 min and centrifuged at 12000*g* for 10 min at 4 °C. The supernatants were then collected and analysed by UHPLC/HR-MS.

2.3.2. Phenolic profile of raw and cooked non-digested, in vitro digested and in vitro fermented dark purple eggplant

Qualitative and quantitative profiles of phenolic compounds were performed using an UHPLC Ultimate 3000 separation module equipped with a C18 column (Acquity UPLC HSS C18 Reversed phase, 2.1 × 100 mm, 1.8 μm particle size, Waters, Milan, Italy) and coupled with a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Data were acquired by both negative and positive ionization modes. The composition of the mobile phases, the elution gradient and the mass spectrometry parameters are fully described in Martini, Tagliazucchi, Minelli, & Lo Fiego (2020).

Quantification was carried out by building external calibration curves with the available standard compounds as depicted in Table S1.

The name of the phenolic metabolites generated during colonic bacterial fermentation is reported by following the recommendation previously reported (Kay et al., 2020).

2.4. Microbiota analyses

2.4.1. Enumeration of bacterial groups and qPCR prebiotic index (qPI)

Bacterial DNA was extracted just after sampling (Purelink Microbiome DNA Purification Kit by Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) and quantified (Biodrop, Biochrom ltd., Cambridge, UK). Enumeration of bacterial groups was made by qPCR to evidence changes in the microbiota after fermentation (Tanner et al., 2014; Westfall, Lomis & Prakash, 2018; Tsitko et al., 2019; Tamargo et al., 2022) following previous protocols (Modesto et al., 2011; Nissen et al., 2020b; Nissen et al., 2021b). 14 different bacterial taxa (*Eubacteria*, *Firmicutes*, *Bacteroidetes*, *Lactobacillales*, *Bifidobacteriaceae, Enterobacteriaceae*, *Clostridium* group I, *Clostridium* group IV, *Bacteroides-Prevotella-Porphyromonas* (BPP) group, *Atopobium-Collinsella-Eggerthella* (ATOP) group, *Bifidobacterium longum*, *Escherichia coli*, *Faecalibacterium prausnitzii*, and *Akkermansia muciniphila*) (Table S2) were assessed by qPCR on a QuantStudio 5 System (Applied Biosystem, Thermo Fisher, USA). The qPCR Prebiotic Index (qPI) was calculated as reported in Nissen et al. (2021b).

2.5. Data processing and statistical analysis

The shifts in abundance of bacterial groups were calculated as Log_2(F/C) on raw data (Love, Huber & Anders, 2014) obtained from sextuplicates. Also, from the raw data, the qPI was obtained after normalization with the mean centering method. ANOVA followed by a Tukey post-hoc test was applied using Statistica v 8.0 (Tibco, Palo Alto, CA, USA). The differences were considered significant with $p < 0.05$. To address specific correlations among bacteria and metabolites, two independent datasets were merged and computed by Spearman Rank analysis and visualized with a two-way joining heatmap, including Pearson dendrograms with complete linkage. The dataset of correlations was performed with Statistica v. 8.0 (Tibco, USA) and the heatmap was produced with the Expression tool on https://www.heatmapper.ca (Babicki et al., 2016).

3. Results and discussion

3.1. Phenolic compound profiles of raw and cooked DPE

The phenolic compound profiles of raw (RDPE) fried (FDPE) and grilled (GDPE) eggplant was investigated through high-resolution mass spectrometry. The mass spectral data of the identified phenolic compounds are reported in supplementary Table S3 whereas the quantitative data are displayed in Table 1. A total of 48 phenolic compounds were detected and quantified in DPE samples, being the *trans* isomer of 5-*O*-caffeolyquinic acid the principal compound in both raw and cooked DPE samples, where it accounted for >65 % of the total amount of phenolic compounds. In general, caffeoylquinic acids was the most representative class of phenolic compound accounting for the 78.0 %, 73.0 % and 79.8 % of total phenolic compounds in RDPE, FDPE and GDPE, respectively. In addition, feruloylquinic acids made up the 15.9 %, 14.3 % and 17.3 % of total phenolic compounds in RDPE, FDPE and GDPE. Small amounts of flavonols and anthocyanins were also identified in eggplant samples, especially in RDPE (Table 1). These data are in agreement with previous studies showing that hydroxycinnamic acids and, in particular, caffeoylquinic acids dominated the phenolic profiles of eggplant (Gürbüz, Uluişik, Frary, Frary, & Doğanlar, 2018; Martini et al., 2021; Niño-Medina, Urías-Orona, Muy-Rangel, & Heredia, 2017).

RDPE displayed a total amount of 900.9 ± 14.8 μmol/100 g of eggplant of phenolic compounds (Table 1). After submitting eggplant to frying and grilling, total phenolic compounds increased by 174 % and 12 %, respectively. After grilling, a small increase in the amount of caffeoylquinic and feruloylquinic acids of 14.5 % and 21.8 %, respectively, was detected. At the same time, the amount of dicaffeoylquinic acids and hydroxycinnamic acids-spermidine derivatives decreased by about 29 % and 64 %. Grilling also caused strong degradation of flavonols (~63 %) and especially of anthocyanins (~81 %). On the contrary, frying treatment brought about an increase in all of the classes of hydroxycinnamic acids. The highest increase in terms of concentration was found for caffeoylquinic acids and feruloylquinic acids. However, the utmost percentage increase was detected for hydroxycinnamic acids-spermidine derivatives (~932 %) and dicaffeoylquinic acids (~272 %).

3.2. Phenolic compound profiles of raw and cooked DPE after in vitro gastro-intestinal digestion

As reported in Table 1, the bioaccessibility of phenolic compounds in DPE following *in vitro* gastro-intestinal digestion was greatly influenced by the applied cooking method. In RDPE, only trace amounts of some hydroxycinnamic acids were detected after the digestion procedure. On the contrary, the two thermal treatments caused an important increase in the amount of phenolic compounds released after *in vitro* digestion, with a bioaccessibility index of 47.9 % and 24.7 % in FDPE and GDPE, respectively. In absolute terms, frying was the thermal treatment that caused the highest release of phenolic compounds after digestion (1182.2 ± 24.4 μmol/100 g of eggplant) whereas after grilling the amount of released phenolic compounds was 4.8 times lower (248.7 ± 6.9 μmol/100 g of eggplant). A similar cooking effect was already described for other food matrices such as cardoon, green pepper and onion as well as for eggplant (Cattivelli et al., 2021; Juániz et al., 2016; Juániz et al., 2017; Martini et al., 2021). In all of these samples, frying and grilling resulted in a higher bioaccessibility of phenolic compounds respect to the raw food. These results may be a consequence of the

Table 1
Concentration of individual phenolic compounds in raw and cooked dark purple eggplant. Results are expressed in μmol of phenolic compound/100 g of eggplant. Bioaccessibility index (BI) is defined as the ratio between the amount of a specific phenolic compound after *in vitro* digestion and the amount in the solvent extract expressed as percentage value.

Compound	*RDPE*			*FDPE*			*GDPE*		
	Before digestion	After digestion	BI (%)	Before digestion	After digestion	BI (%)	Before digestion	After digestion	BI (%)
Hydroxycinnamic acids									
3-*O*-Caffeoylquinic acid *cis*	1.54 ± 0.03	n.d.	0	31.42 ± 1.09	76.56 ± 0.73	243.7	2.36 ± 0.01	80.9 ± 0.54	3427
4-*O*-Caffeoylquinic acid *cis*	1.15 ± 0.02	n.d.	0	4.04 ± 0.02	2.37 ± 0.01	58.7	2.34 ± 0.01	1.51 ± 0.01	64.5
5-*O*-Caffeoylquinic acid *trans*	609.33 ± 8.73	0.11 ± 0.01	0.02	1618.40 ± 9.27	792.92 ± 17.18	49.0	697.16 ± 11.41	118.95 ± 5.70	17.1
4-*O*-Caffeoylquinic acid *trans*	20.02 ± 0.12	n.d.	0	140.26 ± 0.50	175.67 ± 4.26	125.2	30.28 ± 0.20	13.19 ± 0.20	43.6
5-*O*-Caffeoylquinic acid *cis*	70.66 ± 0.84	n.d.	0	8.29 ± 0.10	25.61 ± 0.06	308.9	72.12 ± 0.98	17.78 ± 0.25	24.7
Di-hydro-caffeoylquinic acid	0.03 ± 0.00	n.d.	0	0.41 ± 0.01	0.10 ± 0.01	24.4	0.04 ± 0.00	n.d.	0
3-*O*-Feruloylquinic acid *cis*	0.31 ± 0.01	0.34 ± 0.01	109.7	1.81 ± 0.03	0.63 ± 0.01	34.8	n.d.	n.d.	n.d.
4-*O*-Feruloylquinic acid *cis*	0.13 ± 0.01	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5-*O*-Feruloylquinic acid *trans*	24.85 ± 0.26	0.46 ± 0.01	1.9	44.30 ± 0.33	34.80 ± 0.95	78.6	27.73 ± 0.96	11.06 ± 0.14	39.9
O-Feruloylquinic acid	2.13 ± 0.04	n.d.	0	14.10 ± 0.23	n.d.	0	3.88 ± 0.01	n.d.	0
4-*O*-Feruloylquinic acid *trans*	97.50 ± 2.95	n.d.	0	274.44 ± 5.77	0.59 ± 0.01	0.2	120.77 ± 1.11	n.d.	0
O-Feruloylquinic acid	9.13 ± 0.63	n.d.	0	8.56 ± 0.58	5.16 ± 0.03	60.3	13.55 ± 0.33	1.07 ± 0.01	7.9
5-*O*-Feruloylquinic acid *cis*	9.20 ± 0.15	n.d.	0	9.91 ± 0.07	n.d.	0	8.60 ± 0.07	n.d.	0
1,4-Di-*O*-caffeoylquinic acid	0.60 ± 0.03	n.d.	0	9.15 ± 0.12	14.85 ± 0.53	162.3	0.48 ± 0.02	0.36 ± 0.01	75.0
1,5-Di-*O*-caffeoylquinic acid	19.45 ± 0.07	n.d.	0	55.77 ± 0.37	15.83 ± 0.21	28.4	6.31 ± 0.04	0.13 ± 0.01	2.1
3,5-Di-*O*-caffeoylquinic acid	3.06 ± 0.10	n.d.	0	6.47 ± 0.07	n.d.	0	1.05 ± 0.02	n.d.	0
4,5-Di-*O*-caffeoylquinic acid	n.d.	n.d.	n.d.	14.23 ± 0.53	13.69 ± 0.10	96.2	0.50 ± 0.01	0. 46 ± 0.02	92.0
3,4-Di-*O*-caffeoylquinic acid	n.d.	n.d.	n.d.	0.36 ± 0.01	0.20 ± 0.00	55.6	n.d.	n.d.	n.d.
N^1,N^5-Caffeoyl-caffeoyl-spermidine	0.67 ± 0.01	n.d.	0	2.94 ± 0.08	0.38 ± 0.01	12.9	0.49 ± 0.01	0.14 ± 0.01	28.6
N^1,N^{10}-caffeoyl-caffeoyl-spermidine	3.65 ± 0.03	n.d.	0	33.99 ± 0.39	1.77 ± 0.01	5.2	2.61 ± 0.05	n.d.	0
N^1,N^5-Di-hydro-caffeoyl-caffeoyl-spermidine	6.88 ± 0.15	n.d.	0	54.18 ± 0.60	2.30 ± 0.01	4.2	3.54 ± 0.09	n.d.	0
N^1,N^{10}- Di-hydro-caffeoyl-caffeoyl-spermidine	1.63 ± 0.01	n.d.	0	14.34 ± 0.17	0.63 ± 0.01	4.4	0.90 ± 0.01	n.d.	0
N^1,N^5-Di-hydro-caffeoyl-di-hydro-caffeoyl-spermidine	7.92 ± 0.52	n.d.	0	108.79 ± 1.43	4.30 ± 0.02	4.0	7.11 ± 0.08	n.d.	0
Hydroxycinnamic acid	n.d.	n.d.	n.d.	0.31 ± 0.01	0.14 ± 0.01	45.2	0.14 ± 0.01	n.d.	0
Coumaric acid	n.d.	0.52 ± 0.01	n.f.	0.53 ± 0.01	0.52 ± 0.01	98.1	n.d.	0.40 ± 0.01	n.f.
Di-hydro-coumaric acid isomer	n.d.	n.d.	n.d.	n.d.	0.31 ± 0.01	n.f.	0.17 ± 0.01	0.28 ± 0.01	164.7
Di-hydro-coumaric acid isomer	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.17 ± 0.01	0.24 ± 0.01	141.2
Caffeic acid	0.16 ± 0.01	n.d.	0	1.02 ± 0.02	9.68 ± 0.16	949.0	0.25 ± 0.01	0.16 ± 0.01	64.0
Di-hydroxycinnamic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	0.02 ± 0.01	0.03 ± 0.00	150.0
Di-hydro-caffeic acid	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.00	n.f.	n.d.	n.d.	n.d.
Caffeoylshikimic acid isomer	0.42 ± 0.01	n.d.	0	0.05 ± 0.01	0.04 ± 0.00	80.0	0.14 ± 0.01	n.d.	0
Caffeoylshikimic acid isomer	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.00	n.f.	n.d.	n.d.	n.d.
Caffeoyl-tryptophan-hexoside isomer	0.52 ± 0.01	n.d.	0	0.42 ± 0.01	n.d.	0	0.07 ± 0.01	n.d.	0
Caffeoyl-tryptophan-hexoside isomer	0.76 ± 0.01	n.d.	0	3.50 ± 0.10	n.d.	0	0.18 ± 0.01	n.d.	0
Caffeoyl-hexoside isomer	0.09 ± 0.01	n.d.	0	0.01 ± 0.00	n.d.	0	0.05 ± 0.01	n.d.	0
Caffeoyl-hexoside isomer	0.04 ± 0.01	n.d.	0	0.44 ± 0.01	0.11 ± 0.00	25.0	0.08 ± 0.01	0.03 ± 0.00	37.5
Caffeoyl-hexoside isomer	0.01 ± 0.00	0.02 ± 0.00	200.0	0.08 ± 0.00	0.05 ± 0.00	62.5	0.04 ± 0.01	0.04 ± 0.00	100.0
Caffeoyl-hexoside isomer	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01 ± 0.01	n.d.	0
3-*O*-Coumaroylquinic acid	n.d.	0.42 ± 0.01	n.f.	n.d.	n.d.	n.d.	n.d.	1.25 ± 0.01	n.f.
5-*O*-Coumaroylquinic acid *trans*	2.52 ± 0.04	0.15 ± 0.01	6.0	2.63 ± 0.04	1.90 ± 0.04	72.2	2.63 ± 0.02	0.58 ± 0.01	22.1
4-*O*-Coumaroylquinic acid	n.d.	0.17 ± 0.01	n.f.	n.d.	0.37 ± 0.01	n.f.	n.d.	0.28 ± 0.01	n.f.
5-*O*-Coumaroylquinic acid *cis*	0.83 ± 0.02	0.12 ± 0.01	14.5	0.29 ± 0.01	0.28 ± 0.01	96.6	0.63 ± 0.01	0.32 ± 0.01	50.8
Total hydroxycinnamic acids	**895.22 ± 10.56**	**2.31 ± 0.03**	**0.3**	**2465.44 ± 13.34**	**1181.88 ± 17.75**	**47.9**	**1006.43 ± 12.84**	**248. 44 ± 5.74**	**24.7**
Hydroxybenzoic acids									
Hydroxybenzoic acid isomer	0.03 ± 0.00	n.d.	0	0.14 ± 0.00	0.06 ± 0.00	42.9	0.06 ± 0.00	0.06 ± 0.00	100.0
Hydroxybenzoic acid isomer	0.04 ± 0.00	0.06 ± 0.00	150.0	0.07 ± 0.00	0.08 ± 0.00	114.3	0.07 ± 0.00	0.09 ± 0.00	112.5
Protocatechuic acid	0.01 ± 0.00	n.d.	0	0.07 ± 0.00	0.10 ± 0.00	14.3	0.08 ± 0.00	0.08 ± 0.00	100.0
Total hydroxybenzoic acids	**0.08 ± 0.00**	**0.06 ± 0.00**	**75.0**	**0.28 ± 0.00**	**0.24 ± 0.00**	**85.7**	**0.21 ± 0.00**	**0.23 ± 0.00**	**109.5**
Flavonols									
Quercetin-3-*O*-rutinoside	1.10 ± 0.01	n.d.	0	0.58 ± 0.01	n.d.	0	0.26 ± 0.01	n.d.	0
Quercetin-3-*O*-hexoside-rhamnoside	0.03 ± 0.00	n.d.	0	0.02 ± 0.00	0.01 ± 0.00	50.0	0.02 ± 0.00	n.d.	0
Kaempferol-3-*O*-hexoside	0.02 ± 0.00	n.d.	0	n.d.	n.d.	n.d.	0.01 ± 0.00	n.d.	0
Kaempferol-3-*O*-rutinoside	0.09 ± 0.00	n.d.	0	0.03 ± 0.00	0.01 ± 0.00	33.3	0.07 ± 0.00	0.01 ± 0.00	14.3
Kaempferol-3-*O*-hexoside-rhamnoside	0.01 ± 0.00	n.d.	0	0.01 ± 0.00	n.d.	0	0.01 ± 0.00	n.d.	0

(*continued on next page*)

Table 1 (*continued*)

Compound	*RDPE*			*FDPE*			*GDPE*		
	Before digestion	After digestion	BI (%)	Before digestion	After digestion	BI (%)	Before digestion	After digestion	BI (%)
Kaempferol-3-*O*-hexoside-hexoside	0.02 ± 0.00	n.d.	0	0.02 ± 0.00	0.01 ± 0.00	50.0	0.02 ± 0.00	n.d.	0
Myricetin-3-*O*-rutinoside	0.12 ± 0.01	n.d.	0	0.80 ± 0.01	0.01 ± 0.00	1.25	0.14 ± 0.01	n.d.	0
Total flavonols	**1.39 ± 0.01**	**n.d.**	**0**	**1.46 ± 0.01**	**0.04 ± 0.00**	**2.7**	**0.52 ± 0.01**	**0.01 ± 0.00**	**1.9**
Anthocyanins									
Delphinidin-3-*O*-rutinoside isomer	3.83 ± 0.03	n.d.	0	1.12 ± 0.02	n.d.	0	0.64 ± 0.02	n.d.	0
Delphinidin-3-*O*-rutinoside isomer	0.05 ± 0.00	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Delphinidin-3-*O*-rutinoside-5-*O*-hexoside isomer	0.23 ± 0.01	n.d.	0	0.15 ± 0.00	n.d.	n.d.	0.13 ± 0.00	n.d.	0
Delphinidin-3-*O*-rutinoside-5-*O*-hexoside isomer	0.06 ± 0.01	n.d.	0	n.d.	n.d.	0	n.d.	n.d.	n.d.
Cyanidin-3-*O*-rutinoside isomer	0.09 ± 0.00	n.d.	0	0.01 ± 0.00	n.d.	n.d.	0.04 ± 0.00	n.d.	0
Total anthocyanins	**4.25 ± 0.03**	**n.d.**	**0**	**1.28 ± 0.02**	**n.d.**	**0**	**0.81 ± 0.02**	**n.d.**	**0**
Total	**900.93 ± 10.56**	**2.37 ± 0.03**	**0.3**	**2468.46 ± 13.64**	**1182. 16 ± 17.75**	**47.9**	**1007. 96 ± 12.84**	**248. 67 ± 5.74**	**24.7**

Different letters within the same row mean significant different ($P < 0.05$) values.
n.d. means that the compound was not detected in the sample.
n.f. means newly formed compound.
Abbreviations are: RDPE, raw dark purple eggplant; FDPE, fried dark purple eggplant; GDPE, grilled dark purple eggplant.

matrix softening effect due to the thermal treatments, which enhance the release of phenolic compounds from the food matrices (Palermo, Pellegrini, & Fogliano, 2014).

The classes of compounds with the lowest bioaccessibility in both FDPE and GDPE were feruloylquinic acids and hydroxycinnamic acids-spermidine derivatives. Caffeoylquinic and dicaffeoylquinic acids showed higher bioaccessibility than the other hydroxycinnamic acids in both the cooked samples (Table 1). Some compounds in both FDPE and GDPE showed a bioaccessibility index higher than 100 %. The increase in concentration of these compounds may be the consequence of both isomerization and hydrolysis reactions. Acyl migration from 5-*O*-caffeoylquinic acid to the 4- and 3-*O*-isomers was observed after *in vitro* digestion of both GDPE and FDPE. Former works revealed isomerization phenomena during *in vitro* digestion of several food matrices from 5-caffoylquinic acid to the 4- and 3-isomers (Juániz et al., 2017; Martini et al., 2019; Martini et al., 2021; Monente, Ludwig, Stalmach, De Pena, Cid, & Crozier, 2015). Similarly, the increase in 1,4-di-*O*-caffeoylquinic acid concentration observed in digested FDPE may be due to the isomerization of other di-caffeoylquinic acids isomers as already observed (Juániz et al., 2017; Martini et al., 2021). Likewise, isomerization of 5-*O*-coumaroylquinic acid to the 4- and 3-acyl structure may account for the changes in the concentration of coumaroylquinic acid isomers observed after digestion (Kahle et al., 2011; Martini et al., 2019). Furthermore, the increase in the concentration of 5-*O*-caffeoylquinic acid and caffeic acid may result from the hydrolysis of di-caffeoylquinic acids and caffeoylquinic acids, respectively. Previous studies pointed out the formation of hydrolysis products of di- and mono-caffeoylquinic acids after incubation under alkaline conditions (as found in the intestinal fluid) or with pancreatic juice (Da Encarnação, Farrel, Ryder, Kraut, & Williamson, 2015; Deshpande, Jaiswal, Matei, & Kuhnert, 2014; Kahle et al., 2011).

3.3. Identification of phenolic compound metabolites after in vitro faecal fermentation

Previous studies involving healthy humans and ileostomists demonstrated that, after consumption of coffee, only about the 30 % of hydroxycinnamic acids were absorbed in the small intestine and appeared in plasma as parent compounds and/or sulphate and glucuronide conjugates (Stalmach et al., 2009; Stalmach, Steiling, Williamson, & Crozier, 2010). On the contrary, the 70 % of ingested hydroxycinnamic acids reached the colon where they underwent to the action of the colonic microbiota (Stalmach et al., 2009; Stalmach et al., 2010). Similar results were obtained after consumption of artichoke or yerba mate where >80 % of absorbed hydroxycinnamic acids appeared in the plasma or urine as colonic metabolites (Domínguez-Fernández, Young Tie Yang, Ludwig, Clifford, Cid, & Rodríguez-Mateos, 2022; Gómez-Juaristi, Martínez-López, Sarria, Bravo, & Mateos, 2018). Therefore, following *in vitro* gastro-intestinal digestion, raw and cooked DPE were subjected to faecal fermentation and the phenolic compounds and metabolites were analysed at different time points (6, 18 and 24 h) by high-resolution mass spectrometry. As reported in Tables 2-4, most of the native phenolic compounds were degraded after 6 h of *in vitro* fermentation. Independently on the treatment, isomers of caffeoylquinic acids, dicaffeoylquinic acids, coumaroylquinic acids and caffeic acid-hexosides were totally degraded after 6 h of incubation. Contemporaneously, an increase of 3′,4′-dihydroxycinnamic acid (caffeic acid) concentration as well as the appearance of several isomers of two related metabolites, namely di-hydro-caffeoylquinic acid and 3-(3′,4′-dihydroxyphenyl)propanoic acid (di-hydro-caffeic acid), was observed. Otherwise, some residual amounts of two feruloylquinic acid isomers were recorded together with the appearance of 4′-hydroxy-3′-methoxycinnamic acid (ferulic acid) and 3-(4′-hydroxy-3′-methoxyphenyl) propanoic acid (di-hydro-ferulic acid). Additional newly formed metabolites detected after 6 h of fermentation were 3-(3′-hydroxyphenyl) propanoic acid (di-hydro-coumaric acid), 3-(4′-hydroxyphenyl)propanoic acid (phloretic acid), 3,4-dihydroxybenzoic acid (protocatechuic acid), 4-hydroxy-3-methoxybenzoic acid (vanillic acid) and 4′-hydroxy-3′-methoxyphenylacetic acid (homovanillic acid) (Tables 2-4). After 18 h of fermentation, the majority of the previously identified phenolic compounds and metabolites disappeared in all the DPE samples. The metabolite present at the highest concentration after 18 and 24 h in each sample was 3-(3′-hydroxyphenyl)propanoic acid although some others metabolites such as 3-(3′,4′-dihydroxyphenyl)propanoic acid, 3-(4′-hydroxyphenyl)propanoic acid, 3,4-dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid and 4′-hydroxy-3′-methoxyphenylacetic acid were still present.

Metabolic pathways of eggplant hydroxycinnamic acids degradation by colonic microbiota are described in Fig. 1. The first step in the metabolic pathway of caffeoylquinic acids (Fig. 1) was the reduction of the C2-C3 double bond leading to the appearance of several isomers of di-hydro-caffeoylquinic acid (Juániz et al., 2017; Tomas-Barberan et al., 2014). Caffeoylquinic acids may also be a substrate of bacterial esterase, which are able to cleave the bond between quinic acid and 3′,4′-dihydroxycinnamic acid releasing this last compound (Andreasen, Kroon, Williamson, & Garcia-Conesa, 2001; Juániz et al., 2017; Ludwig, De Peña, Cid, & Crozier, 2013). Several cinnamoyl esterase-producing bacteria have been isolated from human microbiota belonging to

Table 2
Amount of phenolic metabolites produced during *in vitro* fecal fermentation of raw dark purple eggplant. Results are expressed in μmol of phenolic compound/100 of eggplant.

Compound	Synonim	6 h	18 h	24 h
Di-hydro-caffeoylquinic acid isomer 1	/	n.d.	n.d.	n.d.
Di-hydro-caffeoylquinic acid isomer 2	/	32.72 ± 1.63	8.00 ± 0.11	n.d.
Di-hydro-caffeoylquinic acid isomer 3	/	1.60 ± 0.02	0.38 ± 0.01	n.d.
Di-hydro-caffeoylquinic acid isomer 4	/	2.55 ± 0.05	0.54 ± 0.01	n.d.
5-*O*-Feruloylquinic acid *trans*	/	15.11 ± 0.64	2.31 ± 0.02	n.d.
4-*O*-Feruloylquinic acid	/	14.96 ± 0.21	2.96 ± 0.04	n.d.
3′,4′-Dihydroxycinnamic acid	Caffeic acid	0.97 ± 0.03	0.64 ± 0.03	n.d.
4′-Hydroxy-3′-methoxycinnamic acid	Ferulic acid	26.11 ± 0.59	n.d.	n.d.
3-(3′-Hydroxyphenyl) propanoic acid	Di-hydro-coumaric acid	20.61 ± 0.69	37.67 ± 0.47	27.18 ± 0.22
3-(4′-Hydroxyphenyl) propanoic acid	Phloretic acid	2.48 ± 0.02	3.14 ± 0.05	2.68 ± 0.04
3-(3′,4′-Dihydroxyphenyl) propanoic acid	Di-hydro-caffeic acid	0.69 ± 0.02	0.52 ± 0.01	0.47 ± 0.01
3-(4′-Hydroxy-3′-methoxyphenyl) propanoic acid	Di-hydro-ferulic acid	n.d.	n.d.	n.d.
Hydroxybenzoic acid	/	n.d.	n.d.	n.d.
3,4-Dihydroxybenzoic acid	Protocatechuic acid	4.09 ± 0.02	0.15 ± 0.06	0.08 ± 0.02
4-Hydroxy-3-methoxybenzoic acid	Vanillic acid	2.11 ± 0.03	0.02 ± 0.00	0.20 ± 0.01
4′-Hydroxy-3′-methoxyphenylacetic acid	Homovanillic acid	0.16 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
N^1,N^5-Di-hydro-caffeoyl-di-hydro-caffeoyl-spermidine	/	n.d.	n.d.	n.d.
Total		**124.16 ± 1.99**	**56.34 ± 0.57**	**30.63 ± 0.23**

Different letters within the same row mean significant different ($P < 0.05$) values.
n.d. means that the compound was not detected in the sample.

Table 3
Amount of phenolic metabolites produced during *in vitro* fecal fermentation of fried dark purple eggplant. Results are expressed in μmol of phenolic compound/100 of eggplant.

Compound	Synonim	6 h	18 h	24 h
Di-hydro-caffeoylquinic acid isomer 1	/	n.d.	n.d.	n.d.
Di-hydro-caffeoylquinic acid isomer 2	/	27.36 ± 0.02	7.63 ± 0.04	7.51 ± 0.02
Di-hydro-caffeoylquinic acid isomer 3	/	1.60 ± 0.01	n.d.	n.d.
Di-hydro-caffeoylquinic acid isomer 4	/	2.30 ± 0.02	n.d.	n.d.
5-*O*-Feruloylquinic acid *trans*	/	8.68 ± 0.13	n.d.	n.d.
4-*O*-Feruloylquinic acid	/	11.28 ± 0.26	n.d.	n.d.
3′,4′-Dihydroxycinnamic acid	Caffeic acid	0.76 ± 0.01	n.d.	n.d.
4′-Hydroxy-3′-methoxycinnamic acid	Ferulic acid	21.19 ± 0.85	2.90 ± 0.11	2.06 ± 0.16
3-(3′-Hydroxyphenyl) propanoic acid	Di-hydro-coumaric acid	92.03 ± 3.09	215.00 ± 3.50	216.01 ± 4.76
3-(4′-Hydroxyphenyl) propanoic acid	Phloretic acid	3.63 ± 0.22	5.38 ± 0.09	4.81 ± 0.14
3-(3′,4′-Dihydroxyphenyl) propanoic acid	Di-hydro-caffeic acid	0.28 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
3-(4′-Hydroxy-3′-methoxyphenyl) propanoic acid	Di-hydro-ferulic acid	4.21 ± 0.02	n.d.	n.d.
Hydroxybenzoic acid	/	n.d.	0.16 ± 0.01	0.36 ± 0.01
3,4-Dihydroxybenzoic acid	Protocatechuic acid	5.58 ± 0.02	0.61 ± 0.07	0.54 ± 0.02
4-Hydroxy-3-methoxybenzoic acid	Vanillic acid	0.98 ± 0.01	0.01 ± 0.00	0.17 ± 0.01
4′-Hydroxy-3′-methoxyphenylacetic acid	Homovanillic acid	0.16 ± 0.00	0.14 ± 0.01	0.06 ± 0.00
N^1,N^5-Di-hydro-caffeoyl-di-hydro-caffeoyl-spermidine	/	0.09 ± 0.00	0.48 ± 0.02	0.36 ± 0.01
Total		**180.34 ± 3.23**	**232.64 ± 3.50**	**232.20 ± 4.76**

Different letters within the same row mean significant different ($P < 0.05$) values.
n.d. means that the compound was not detected in the sample.

species of *Lactobacillus gasseri*, *Bifidobacterium lactis* and *Escherichia coli* (Couteau, McCartney, Gibson, Williamson, & Faulds, 2001). However, as already suggested, caffeoylquinic acids reduction proceeded faster than ester hydrolysis being di-hydro-caffeoylquinic acids the major detected metabolic products after 6 h of fermentation (Juániz et al., 2017; Tomas-Barberan et al., 2014). The next metabolic step was represented by the hydrolysis of the ester bond of di-hydro-caffeoylquinic acid and the reduction of 3′,4′-dihydroxycinnamic acid, which converged on the formation of 3-(3′,4′-dihydroxyphenyl)propanoic acid (Juániz et al., 2017; Ludwig et al., 2013; Tomás-Barberán et al., 2014). This last intermediate may have different fates as reported in Fig. 1. The propionic side chain of 3-(3′,4′-dihydroxyphenyl)propanoic acid may underwent to β-oxidation producing 3,4-dihydroxybenzoic acid, which can be further dehydroxylated to hydroxybenzoic acid (Cortés-Martín, Selma, Tomás-Barberán, González-Sarrías, & Espín, 2020; Williamson, & Clifford, 2017). Alternatively, 3,4-dihydroxybenzoic acid may be formed from 3-(3′,4′-dihydroxyphenyl)propanoic acid by two consecutive α-oxidation steps that proceeded through the formation of 3′,4′-dihydroxyphenylacetic acid (homoprotocatechuic acid) (Juániz et al., 2017). However, this last intermediate was not detected at any time during fermentation, suggesting that this pathway could be of minor importance respect to direct β-oxidation. However, the major catabolic pathway for 3-(3′,4′-dihydroxyphenyl)propanoic acid degradation was the dehydroxylation to 3-(3′-hydroxyphenyl)propanoic acid.

Another important class of hydroxycinnamic acids found in *in vitro* digested eggplant samples was represented by the different isomers of feruloylquinic acids, whose metabolic pathway during fermentation is depicted in Fig. 1. Feruloylquinic acids were metabolized slowly respect to caffeoylquinic acids by gut microbiota since some residual amounts of these compounds were detected after 6 h of fermentation. Furthermore, the most important degradative pathway for feruloylquinic acids was the ester hydrolysis that resulted in the fast appearance of 4′hydroxy-3′-methoxycinnamic acid that derived from the cleavage of the quinic acid moiety from the parent compound (Ludwig et al., 2013). Indeed, 4′hydroxy-3′-methoxycinnamic acid was reduced to the 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid intermediate that was further degraded to 4-hydroxy-3-methoxybenzoic acid (directly through β-oxidation or through two steps of α-oxidation via the intermediate 4′-hydroxy-3′-methoxyphenylacetic acid) and 3-(4′-hydroxyphenyl)propanoic acid (Fig. 1). The low measured amounts of these last compounds in the fermented eggplant samples may suggest the presence of alternative metabolic pathways. Previous studies reported that the most important metabolites identified after colonic fermentation of ferulic acid (4′hydroxy-3′-methoxycinnamic acid) were 3-(4′-hydroxy-3′-methoxyphenyl)propanoic acid, 3-(3′,4′-dihydroxyphenyl)propanoic acid and 3-(3′-hydroxyphenyl)propanoic acid (Duncan et al., 2016; Russel et al., 2008). In this case, the metabolic pathway involved the reduction of 4′-hydroxy-3′-methoxycinnamic acid to 3-(4′-hydroxy-3′-

Table 4
Amount of phenolic metabolites produced during *in vitro* fecal fermentation of grilled dark purple eggplant. Results are expressed in μmol of phenolic compound/100 of eggplant.

Compound	Synonim	6 h	18 h	24 h
Di-hydro-caffeoylquinic acid isomer 1	/	9.47 ± 0.02	n.d.	n.d.
Di-hydro-caffeoylquinic acid isomer 2	/	36.21 ± 0.28	n.d.	n.d.
Di-hydro-caffeoylquinic acid isomer 3	/	1.80 ± 0.08	n.d.	n.d.
Di-hydro-caffeoylquinic acid isomer 4	/	2.95 ± 0.13	n.d.	n.d.
5-*O*-Feruloylquinic acid *trans*	/	10.23 ± 0.31	n.d.	n.d.
4-*O*-Feruloylquinic acid	/	17.05 ± 0.24	n.d.	n.d.
3′,4′-Dihydroxycinnamic acid	Caffeic acid	1.20 ± 0.06	n.d.	n.d.
4′-Hydroxy-3′-methoxycinnamic acid	Ferulic acid	31.68 ± 0.19	0.14 ± 0.01	n.d.
3-(3′-Hydroxyphenyl) propanoic acid	Di-hydro-coumaric acid	26.33 ± 0.14	41.28 ± 0.79	49.13 ± 1.26
3-(4′-Hydroxyphenyl) propanoic acid	Phloretic acid	3.58 ± 0.09	2.86 ± 0.07	2.10 ± 0.05
3-(3′,4′-Dihydroxyphenyl) propanoic acid	Di-hydro-caffeic acid	0.42 ± 0.01	0.32 ± 0.01	0.42 ± 0.02
3-(4′-Hydroxy-3′-methoxyphenyl) propanoic acid	Di-hydro-ferulic acid	2.70 ± 0.26	n.d.	n.d.
Hydroxybenzoic acid	/	n.d.	n.d.	n.d.
3,4-Dihydroxybenzoic acid	Protocatechuic acid	4.80 ± 0.03	1.46 ± 0.01	0.01 ± 0.00
4-Hydroxy-3-methoxybenzoic acid	Vanillic acid	2.27 ± 0.05	n.d.	n.d.
4′-Hydroxy-3′-methoxyphenylacetic acid	Homovanillic acid	0.18 ± 0.00	0.07 ± 0.01	n.d.
N^1,N^5-Di-hydro-caffeoyl-di-hydro-caffeoyl-spermidine	/	n.d.	n.d.	n.d.
Total		**124.16 ± 0.63**	**46.12 ± 0.79**	**51.66 ± 1.26**

Different letters within the same row mean significant different ($P < 0.05$) values.
n.d. means that the compound was not detected in the sample.

methoxyphenyl)propanoic acid followed by demethylation of the methoxy group at C3′ and further dehydroxylation at C4′ (Duncan et al., 2016; Russel et al., 2008). It is possible to speculate that this catabolic pathway also occurred during *in vitro* fermentation of raw and cooked DPE such that the metabolism of feruloylquinic acids converged to the formation of 3-(3′-hydroxyphenyl)propanoic acid.

Concerning the effect of cooking, DPE heat treatment appeared to influence only the amount of produced metabolites, without any effect on the catabolic pathway. These results are in accordance with previous studies that found that cooking of cardoon, green pepper and artichoke had only a quantitative impact on the metabolites produced during *in vitro* colonic fermentation (Domínguez-Fernández, Ludwig, De Peña, & Cid, 2021; Juániz et al., 2016; Juániz et al., 2017). FDPE, which showed the highest amount of phenolic compounds after *in vitro* digestion, also displayed the highest amount of metabolites after 24 h of *in vitro* fermentation (232.2 ± 5.1 μmol/100 g of eggplant) followed by GDPE (51.7 ± 1.3 μmol/100 g of eggplant) and RDPE (30.6 ± 0.3 μmol/100 g of eggplant) eggplant. In all the samples, 3-(3′-hydroxyphenyl)propanoic acid was the major produced metabolite accounting for the 89 %, 93 % and 95 % of total phenolic compounds after 24 h of *in vitro* fermentation in RDPE, FDPE and GDPE, respectively. *In vitro* studies showed that 3-(3′-hydroxyphenyl)propanoic acid exhibited anti-inflammatory properties by reducing prostanoid production in human fibroblast stimulated with cytokines and anti-diabetic activity by promoting survival of pancreatic β-cells and insulin secretion (Fernández-Millán et al., 2014; Larrosa et al., 2009; Russell et al., 2008). Moreover, 3-(3′-hydroxyphenyl)propanoic acid was able to relax *in vitro* vascular smooth cells and to decrease *in vivo* blood pressure in healthy and spontaneously hypertensive rats (Najmanová et al., 2016).

3.4. Shift of total bacteria and microbiota eubiosis

qPCR absolute quantifications were targeted to 14 different bacterial taxa (Table S2) related to the core microbiota, including total Eubacteria and the two main phyla of the human healthy colon, i.e. *Bacteroidetes* and *Firmicutes*. Considering the total Eubacteria, in respect to the abundances at the baseline (BL) and apart from the values of the blank control (BC), just GDPE at 24 h scored a significant reduction in abundance of 1.66 folds less ($p < 0.05$) (Table 5). The two other samples, independently from the time points, had slight not significant increases ($p > 0.05$). It is noteworthy that at the 24 h the value of GDPE was 2.91 and 2.72 folds significantly lower than RDPE and FDPE, respectively ($p < 0.05$). These evident results indicated that GDPE, alike the BC, limited the growth of total colon Eubacteria.

Quantifications of *Bacteroidetes* phylum showed significant changes principally at the 24 h, where all values were significant in respect to the BL ($p < 0.05$) (Table 5). In particular, RDPE had the top increase of 1.75 folds and GDPE had the top reduction of 1.56 folds, in comparison to the BL ($p < 0.05$). Additionally, RDPE was the sole that significantly increased *Bacteroidetes* content at 18 h ($p < 0.05$). The higher loads of *Bacteroidetes* after fermentation of RDPE could indicate a preferable substrate described by longer fiber chains, and thus more specific sites for *Bacteroidetes*, which is rich in fiber degrading enzymes.

Considering *Firmicutes* (Table 5), significant changes were seen for each sample at the 24 h and for GDPE at 18 h ($p < 0.05$). At 24 h, increases were found for each eggplant sample, with no difference among each other ($p > 0.05$). These outputs could indicate opposite scenarios depending on which group of *Firmicutes* was the most fostered; a positive outlook could be due to increase of *Lactobacillales*, while a negative could be due to increase of *Clostridiaceae*.

As a general parameter for microbiota eubiosis, we chose the *Firmicutes/Bacteroidetes* (F/B) ratio (Table 6). Within this ratio, a value over two is usually linked to microbiota dysbiosis (Blaut et al., 2002; Koliada et al., 2017; Zhou et al., 2017; Martinez et al., 2021). The fecal samples at the baseline had a F/B of 0.81 and this eubiosis condition was maintained by RDPE (0.51) and by FDPE (0.88) ($p > 0.05$), but not by GDPE (2.64) ($p < 0.05$). These results indicate that during the 24 h of fermentation RDPE and FDPE did not perturb the colon core microbiota of healthy donors and were able to provide a substrate nutritionally adapted. Oppositely, fermentations on GDPE triggered a dysbiosis condition that mirrored the higher *Firmicutes* content. A potential explanation could be related to the occurrence of the Maillard reaction between DPE fiber and amino compounds during grilling, making DPE fiber no longer available for gut microbiota fermentation (Pérez-Burillo et al., 2018). Previous studies suggested that during grilling the degree of the Maillard reaction was stronger than during frying (Pérez-Burillo, Rufián-Henares, & Pastoriza, 2019).

3.5. Shift in selected bacterial taxa

To give evidence to the shifts of colon core microbiota, we quantify the abundance of some bacterial taxa selected as renown to be active responders to fiber substrate fermentation and modulators of host wellbeing (Table 5). A first set was related to beneficial microbes, while a second set was related to opportunistic and dysbiontic microbes.

Lactobacillales and *Bifidobacteriaceae* with the exceptions of few species are related to the host's wellbeing and eubiosis of the colon microbiota (Sanders et al., 2019). *Lactobacillales* includes also *Lactobacillaceae* and *Enterococcaceae*. These three families are implicated in the biotransformation of phenolic compounds; for example *Lactobacillaceae* are able to increase epicatechin as well as gallic, caffeic, and ferulic acids

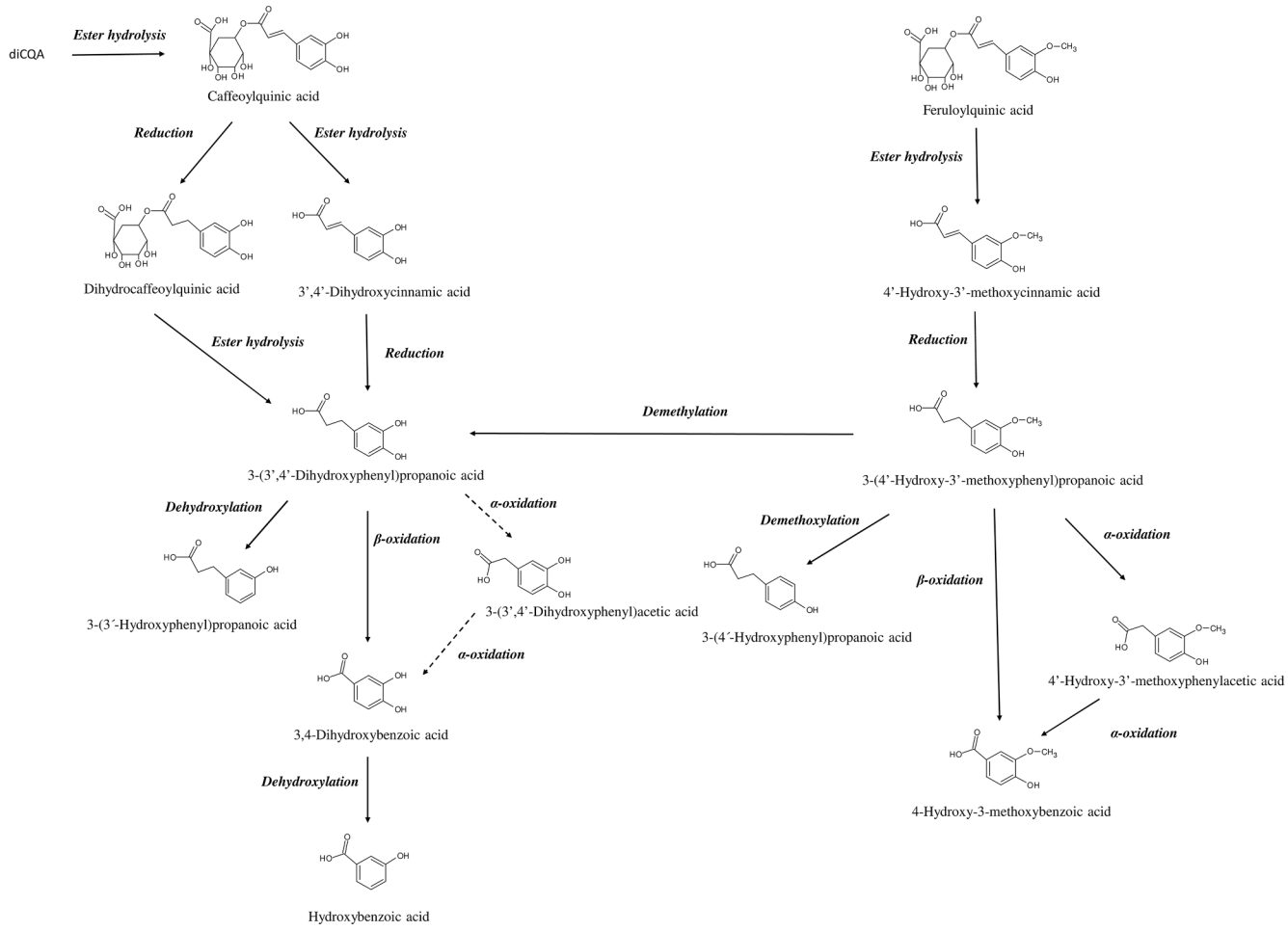

Fig. 1. Proposed metabolic pathways for phenolic compounds microbial metabolism after *in vitro* gastro-intestinal digestion and faecal fermentation of dark purple eggplant.

content (Liu et al., 2021); *Enterococcaceae* are able to promote *O*-deglycosylation and *C*-ring fission of several flavonols (Corrêa et al., 2019), whereas *Bifidobacteriaceae* are able to metabolize homovanillic acids (Boto-Ordonez et al., 2014) and hydrolyze chlorogenic acids (Iqbal et al., 2020). In our experiments, *Lactobacillales* and *Bifidobacteriaceae* had a different trend during fermentation (Table 5). The first taxon at 18 h scored a significant increase in abundance just for FDPE. At the 24 h, all DPE substrates were able to increase the abundance of *Lactobacillales*. Similarly, on a long-term fermentation of phenolic compounds on gut models, other authors found that *Enterococcaceae* was the most fostered family (Kemperman et al., 2013; Tamargo et al., 2022).

From our results at 18 h, *Bifidobacteriaceae* taxa were significantly fostered ($p < 0.05$) by any DPE substrates, while at 24 h a significant increase was seen just for FDPE ($p < 0.05$) (Table 5). A similar fashion was observed even at the species level (Table 5). In fact, targeting *B. longum*, a significant increase was seen just after FDPE fermentation ($p < 0.05$). This feature could mean that *Bifidobacteriaceae* are more specific in metabolizing phenolic compounds than *Lactobacillales*, as well as are more sensible to some detrimental compounds generated by pyrolysis of grilled foods. It is reported that *Bifidobacteriaceae* are somehow inhibited by a certain class of polyphenol, *e.g.* that of black tea (Kemperman et al., 2013) rather than other, *e.g.* that of olive (Nissen et al., 2021a). Additionally, it is known that *Lactobacillales* can resist to high content of phenolic compound (Khubber et al., 2022), because owns an enzymatic arsenal with a wide target range, able to de-esterification, hydrolysis, or conversion of phenolics to individual acids (gallic, quinic, caffeic, *p*-coumaric, ferulic, dihydrocaffeic, dihydroferulic acid, vinylcatechol, vinylguaiacol) (Filannino, Di Cagno & Gobbetti, 2018; Khubber et al., 2022).

Among other beneficial taxa, *Clostridium* group IV and *Faecalibacterium prausnitzii* are reported to be involved in biotransformation of phenolic compounds (Braune, & Blaut, 2016). From our results (Table 5), *Clostridium* group IV increased significantly at the 24 h just after FDPE fermentation that had even the highest significant increase in *F. prausnitzii* too. *F. prausnitzii* belongs to *Clostridium* group IV related to important SCFA producers and its presence is associated to a healthy gut. A decrease in the number of these bacteria is observed in patients with inflammatory bowel disease (Zhang et al., 2019). Like our results, these taxa are reported to increase after dietary clinical trial with phenolic compounds from red wine (Moreno-Indias et al., 2016; Wiciński et al., 2020). Lastly, *Akkermansia muciniphila* has been reported to be exalted from phenolic compounds presence, alike that some phenolic compounds are acting as prebiotics (Rodríguez-Daza et al., 2021). From our results (Table 5) this taxon was significantly fostered both at 18 h and at 24 h by any eggplant substrate, but was not by the BC ($p < 0.05$). In particular, RDPE and FDPE behaved similarly, and their mean increment, 3.5 ± 0.28 Log_2(F/C), was 1.8-fold higher than that after GDPE fermentation.

Another commensal group that is involved mainly in fiber degradation is the *Bacteroides-Prevotella* group, covered by the BPP (*Bacteroides–Prevotella–Porphyromonas*) target. Generally, these bacteria are sensible to both fibers (Patrascu et al., 2017) and phenolic compounds. For example, Tamargo et al., (2022) found a significant increase of this group after phenolic compounds fermentation on a gut model. From our

Table 5
Shift in bacterial abundances of selected targets by enumeration with qPCR.

qPCR Target	Cells/mL	Log_2(F/C)		ANOVA*
Eubacteria	**Baseline**	**18 h**	**24 h**	
RDPE	9.55E + 08 ± 2.27E + 07	0.02^{A}	0.57^{A}	*0.139711*
GDPE	9.55E + 08 ± 2.27E + 07^{a}	-0.06^{aA}	-1.66^{bB}	*0.001852*
FDPE	9.55E + 08 ± 2.27E + 07	0.04^{A}	0.61^{A}	*0.063910*
BC	9.55E + 08 ± 2.27E + 07^{a}	-1.88^{bB}	-3.34^{cC}	*0.000001*
		0.002320	*0.000001*	**P value**
Bacteroidetes	**Baseline**	**18 h**	**24 h**	
RDPE	2.73E + 08 ± 3.24E + 07^{b}	1.84^{aA}	1.75^{aA}	*0.015355*
GDPE	2.73E + 08 ± 3.24E + 07^{a}	0.88^{aA}	-1.56^{bB}	*0.006992*
FDPE	2.73E + 08 ± 3.24E + 07^{b}	-0.08^{bB}	1.30^{aA}	*0.002790*
BC	2.73E + 08 ± 3.24E + 07^{a}	-0.79^{aB}	-4.15^{bC}	*0.025874*
		0.015736	*0.000542*	**P value**
Firmicutes	**Baseline**	**18 h**	**24 h**	
RDPE	2.20E + 08 ± 6.43E + 07	0.22^{bAB}	1.09^{A}	*0.087025*
GDPE	2.20E + 08 ± 6.43E + 07^{b}	1.04^{abA}	1.35^{aA}	*0.035807*
FDPE	2.20E + 08 ± 6.43E + 07^{b}	-0.16^{bB}	1.43^{aA}	*0.000006*
BC	2.20E + 08 ± 6.43E + 07^{a}	0.07^{abAB}	-1.51^{bB}	*0.000262*
		0.000091	*0.010083*	**P value**
Lactobacillales	**Baseline**	**18 h**	**24 h**	
RDPE	5.99E + 06 ± 7.00E + 05^{b}	-0.03^{bA}	3.82^{aA}	*0.000974*
GDPE	5.99E + 06 ± 7.00E + 05^{b}	0.51^{bA}	3.07^{aA}	*0.000023*
FDPE	5.99E + 06 ± 7.00E + 05^{b}	1.23^{bA}	3.80^{aA}	*0.000001*
BC	5.99E + 06 ± 7.00E + 05^{a}	-1.24^{bB}	-0.86^{bB}	*0.001798*
		0.006568	*0.000322*	**P value**
Bifidobacteriaceae	**Baseline**	**18 h**	**24 h**	
RDPE	9.67E + 07 ± 9.60E + 06^{b}	2.13^{aA}	-4.02^{cBC}	*0.045509*
GDPE	9.67E + 07 ± 9.60E + 06^{b}	1.31^{aA}	-2.32^{cB}	*0.012910*
FDPE	9.67E + 07 ± 9.60E + 06^{b}	3.13^{aA}	2.66^{aA}	*0.014113*
BC	9.67E + 07 ± 9.60E + 06^{a}	-1.66^{bB}	-6.41^{cC}	*0.004711*
		0.007112	*0.002270*	**P value**
Enterobacteriaceae	**Baseline**	**18 h**	**24 h**	
RDPE	5.94E + 06 ± 1.17E + 06^{b}	0.53^{bAB}	2.31^{aA}	*0.040556*
GDPE	5.94E + 06 ± 1.17E + 06^{b}	-0.09^{bB}	1.57^{aB}	*0.000202*
FDPE	5.94E + 06 ± 1.17E + 06	0.30^{AB}	0.56^{C}	*0.054133*
BC	5.94E + 06 ± 1.17E + 06^{c}	0.86^{bA}	2.75^{aA}	*0.000006*
		0.000001	*0.000171*	**P value**
BPP group	**Baseline**	**18 h**	**24 h**	
RDPE	1.38E + 07 ± 1.99E + 06^{b}	1.52^{aA}	2.42^{aAB}	*0.040071*
GDPE	1.38E + 07 ± 1.99E + 06^{b}	0.55^{bAB}	1.30^{aB}	*0.000353*
FDPE	1.38E + 07 ± 1.99E + 06^{b}	-0.59^{bB}	2.83^{aA}	*0.017136*
BC	1.38E + 07 ± 1.99E + 06	-0.97^{B}	-0.77^{C}	*0.391845*
		0.049358	*0.025514*	**P value**
ATOP group	**Baseline**	**18 h**	**24 h**	
RDPE	3.10E + 04 ± 1.37E + 03	0.27^{B}	0.87^{B}	*0.328140*
GDPE		3.76^{aA}	5.32^{aA}	*0.011461*
	3.10E + 04 ± 1.37E + 03^{b}			
FDPE	3.10E + 04 ± 1.37E + 03^{b}	0.62^{abB}	1.26^{aB}	*0.014034*
BC	3.10E + 04 ± 1.37E + 03^{b}	0.62^{bB}	8.54^{aA}	*0.001073*
		0.004022	*0.000656*	**P value**
Clostridium Group I	**Baseline**	**18 h**	**24 h**	
RDPE	1.34E + 03 ± 2.58E + 02	0.88^{B}	0.72^{C}	*0.040768*
GDPE	1.34E + 03 ± 2.58E + 02^{b}	3.60^{aA}	2.58^{aB}	*0.000002*
FDPE	1.34E + 03 ± 2.58E + 02	0.90^{B}	0.64^{C}	*0.059008*
BC	1.34E + 03 ± 2.58E + 02^{c}	4.78^{bA}	8.74^{aA}	*0.007743*
		0.000002	*0.000044*	**P value**
Clostridium Group IV	**Baseline**	**18 h**	**24 h**	
RDPE	1.28E + 07 ± 1.40E + 06^{a}	-0.94^{bB}	-1.74^{bB}	*0.002343*
GDPE	1.28E + 07 ± 1.40E + 06^{a}	-0.88^{bB}	-1.40^{bB}	*0.001558*
FDPE	1.28E + 07 ± 1.40E + 06^{b}	1.52^{aA}	1.97^{aA}	*0.002567*
BC	1.28E + 07 ± 1.40E + 06^{a}	-3.36^{bC}	-4.17^{bC}	*0.043406*
		0.000001	*0.001605*	**P value**
Faecalibacterium prausnitzii	**Baseline**	**18 h**	**24 h**	
RDPE	1.46E + 03 ± 5.20E + 02^{b}	4.58^{a}	8.56^{a}	*0.001407*
GDPE	1.46E + 03 ± 5.20E + 02^{b}	3.59^{a}	5.69^{a}	*0.001564*
FDPE	1.46E + 03 ± 5.20E + 02^{b}	3.78^{a}	8.81^{a}	*0.001359*
BC	1.46E + 03 ± 5.20E + 02^{a}	-0.88^{ab}	-3.62^{b}	*0.001090*
		0.010691	*0.023811*	**P value**
Bifidobacterium longum	**Baseline**	**18 h**	**24 h**	
RDPE	6.58E + 07 ± 1.35E + 06	−0.28	-0.57^{AB}	*0.053207*
GDPE	6.58E + 07 ± 1.35E + 06	−0.37	0.41^{AB}	*0.060214*
FDPE	6.58E + 07 ± 1.35E + 06^{b}	-0.12^{b}	1.86^{aA}	*0.000033*
BC	6.58E + 07 ± 1.35E + 06^{a}	-1.09^{b}	-3.40^{cB}	*0.003734*
		0.051701	*0.006037*	**P value**
Akkermansia muciniphila	**Baseline**	**18 h**	**24 h**	
RDPE	4.24E + 03 ± 6.04E + 02^{b}	3.05^{aA}	3.30^{aAB}	*0.000001*
GDPE	4.24E + 03 ± 6.04E + 02^{b}	2.05^{aA}	1.88^{aB}	*0.000002*
FDPE	4.24E + 03 ± 6.04E + 02^{b}	2.24^{aA}	3.70^{aA}	*0.000024*
BC	4.24E + 03 ± 6.04E + 02^{a}	-2.42^{bB}	-5.16^{bC}	*0.000303*
		0.000012	*0.000096*	**P value**
Escherichia coli	**Baseline**	**18 h**	**24 h**	
RDPE	9.10E + 02 ± 2.28E + 02	0.40^{B}	0.82^{B}	*0.131025*
GDPE	9.10E + 02 ± 2.28E + 02^{b}	0.12^{bB}	3.43^{aB}	*0.033926*
FDPE	9.10E + 02 ± 2.28E + 02^{b}	0.06^{bB}	2.42^{aB}	*0.000017*
BC	9.10E + 02 ± 2.28E + 02^{c}	4.54^{bA}	9.50^{aA}	*0.000019*
		0.000109	*0.043282*	**P value**

* ANOVA and post hoc Tukey ($P < 0.05$). For each bacterial target, values with different letters are significantly different among a sample and among a time point, with lower- or upper-case letter, respectively. RDPE = raw dark purple

eggplant; GDPE = grilled dark purple eggplant; FDPE = fried dark purple eggplant; BC = blank control.

Table 6
Shift in *Firmicutes* to *Bacteroidetes* ratio.

Sample	*Firmicutes/Bacteroidetes*			ANOVA*
	Baseline	**18 h**	**24 h**	
RDPE	0.81 ± 0.50^{a}	0.26 ± 0.16^{cB}	0.51 ± 0.20^{bC}	*0.001769*
GDPE	0.81 ± 0.50^{b}	0.90 ± 0.40^{bA}	2.64 ± 0.48^{aB}	*0.000161*
FDPE	0.81 ± 0.50^{b}	1.24 ± 0.54^{aA}	0.88 ± 0.55^{bC}	*0.000558*
BC	0.81 ± 0.50^{b}	1.46 ± 0.67^{bA}	5.04 ± 0.67^{aA}	*0.002569*
		0.000353	*0.000545*	**P value**

* ANOVA and post hoc Tukey ($P < 0.05$). For each bacterial target, values with different letters are significantly different among a sample and among a time point, with lower- or upper-case letter, respectively. RDPE = raw dark purple eggplant; GDPE = grilled dark purple eggplant; FDPE = fried dark purple eggplant; BC = blank control.

results, the BPP group increased significantly at 18 h ($p < 0.05$) just after RDPE fermentation, while at 24 h for all the DPE ($p < 0.05$) (Table 5).

Considering the opportunistic bacterial taxa, we have targeted the *Enterobacteriaceae*, *Clostridium* group I, the *Atopobium–Collinsella-Eggerthella* (ATOP) group, and *Escherichia coli* (Table 5).

Enterobacteriaceae at 24 h increased after fermentation of all sample, including the BC. Although this increment was not significant for FDPE ($p < 0.05$). In particular, the increment scored by FDPE was 2.8, 4.1, and 4.9-folds lower than RDPE, GDPE and BC, respectively.

Likely was the fashion of fermentation for *E. coli* that at 24 h increased in any fermentation condition, including the BC, and significantly in three out of four samples. Even in this case, the increase scored by the BC was the top one, more than four time higher than the mean increment observed by the eggplant samples, 2.2 ± 1.32 Log_2(F/C). In comparison to each other, this time was GDPE that made *E. coli* grow more; 1.4 and 4.2-folds more than FDPE and RDPE, respectively.

Clostridium group I surged in any case at any time point, but significantly just for GDPE and BC ($p < 0.05$). In particular, at 24 h, RDPE and FDPE had a similar increase that was 3.8 and 12.9-folds lower than GDPE and BC, respectively.

Lastly, considering the ATOP group, increases were observed in any substrate at any time. In detail, ATOP raised at 24 h significantly for GDPE, FDPE and BC ($p < 0.05$), but GDPE and BC had respectively 4.2 and 6.7-fold more abundance in comparison to FDPE. This group engages in the biotransformation of phenolic compounds, because *Atopobium parvulum* for example possess tannases (Jiménez, et al., 2014), while *Atopobium* spp. is correlated to protocatechuic acid production after colonic fermentation of berry phenolic compounds (Williamson & Clifford, 2017). In similar works of fermentation, the abundance of *Eggerthella* was enhanced by berry phenolic compounds (Rodríguez-Daza et al., 2021). From this scenario, seems that GDPE was the substrates that made the opportunistic group grow mostly, whereas FDPE brought about a considerable increase in many beneficial taxa Previous *in vitro* and *in vivo* studies showed that vegetable cooking reshapes the gut microbiota with effects attributable to an altered physical access to dietary fibers, to changes in the amount and/or type of plant-derived compounds or to the generation of new compounds, such as Maillard reaction products, after thermal treatments (Carmody et al., 2019; Pérez-Burillo et al., 2018).

3.6. qPCR prebiotic index

Considering the results (Table 7), we found out that the fermented substrate with the best prebiotic activity was FDPE after 24 h, followed by RDPE and GDPE after 24 h. Thus, GDPE had the lowest prebiotic index that was 2.48 folds lower than that of FDPE at 24 h. In comparison to FDPE, RDPE scored 1.35-fold lower values at 24 h. The blank control

Table 7
Scores of qPCR Prebiotic Index (qPI).

Sample	qPCR Prebiotic Index			ANOVA*
	Baseline	**18 h**	**24 h**	
RDPE	0.30 ± 0.05^{c}	0.51 ± 0.01^{bB}	0.72 ± 0.07^{aC}	*0.000032*
GDPE	0.30 ± 0.05^{c}	0.39 ± 0.01^{aA}	0.33 ± 0.01^{bB}	*0.000012*
FDPE	0.30 ± 0.05^{c}	0.97 ± 0.03^{aA}	0.53 ± 0.03^{bC}	*0.000001*
BC	0.30 ± 0.05^{b}	0.29 ± 0.01^{abA}	0.01 ± 0.01^{aA}	*0.000001*
		0.000022	*0.000001*	**P value**

* ANOVA and post hoc Tukey ($P < 0.05$). For each bacterial target, values with different letters are significantly different among a sample and among a time point, with lower- or upper-case letter, respectively. RDPE = raw dark purple eggplant; GDPE = grilled dark purple eggplant; FDPE = fried dark purple eggplant; BC = blank control.

scored for any time points lower values than any FDPE, GDPE and RDPE cases (all significant, but one) and reached the lowest value of the dataset at the endpoint (7.47-fold lower than FDPE 24 h).

Anyhow, at 18 h, results showed that the qPI values were similar among the three eggplant samples, but FDPE and RDPE were still higher than GDPE ($p > 0.05$). Lastly, the BC sample scored significant lower values than each eggplants sample ($p < 0.05$). Thus, the different prebiotic index of FDPE, RDPE and GDPE could be mostly due to a different structural composition of the eggplant fibers with different portion of soluble fibers generated after the different cooking conditions, as well as by different transformation of eggplant phenolics with prebiotic capacity. Similarly to most know prebiotics (*e.g.* fructooligosaccharides), it is recognized that soluble fibers are excellent substrates for production of postbiotics (*e.g.* short chain fatty acids) in the large intestine (Oba et al, 2020). Additionally, in line with the latest definition of prebiotics (Gibson et al., 2017), there is evidence that some phenolics could foster the growth of beneficial bacteria, thus owning a prebiotic nature (Rodríguez-Daza et al., 2021).

3.7. Correlations among phenolics and microbial taxa

Spearman Rank Correlations ($p < 0.05$), two-joining-way Heatmaps, and Pearson cluster analysis were performed by comparison of two different normalized datasets, each derived from values of relative quantification (microbial taxa and phenolics) of the whole experimental dataset (Fig. 2). The significance of correlations is reported in Fig. S1. From the left-side dendrograms, two main clusters and a smaller one were identified. The two main clusters were then distributed in two different subgroups defined by the topside dendrograms related to phenolics variables. In cluster 1A the most significant positive correlations were held by taxa *Bifidobacteriaceae*, *B. longum* and *Clostridium* group IV towards 3,4-dihydroxybenzoic, 4′-hydroxy-3′-methoxyphenylacetic, hydroxybenzoic, 3-(3′-hydroxyphenyl)propanoic and 4′-hydroxy-3′-methoxycinnamic acids. In cluster 1B, the same bacterial variables were negatively correlated mainly to 3′,4′-dihydroxycinnamic, di-hydrocaffeoylquinic isomer 3, 5-*O*-feruloylquinic and 3-(3′,4′-dihydroxyphenyl)propanoic acids. The most significant negative correlations were seen for *B. longum* to each of the four compounds aforementioned. *Bifidobacteriaceae* are able of both *O*-deglycosylation and *C*-ring fission of kaempferol, quercetin, and myricetin releasing protocatechuic acid (Murota, Nakamura & Uehara, 2018). *Bifidobacteriaceae* and species from *Clostridium* group IV are able also to hydrolyze ester bonds, *C*-ring cleavage, and dihydroxylation of anthocyanins and proanthocyanidins releasing hydroxybenzoic acids (Braune, & Blaut 2016).

In cluster 3 the trend was opposite. The bacterial variables that resulted to have more significant correlations were *Enterobacteriaceae* and *Bacteroidetes* (and minorly *Bacteroides-Prevotella* of BPP target) principally towards 3′,4′-dihydroxycinnamic acid, di-hydrocaffeoylquinic isomer 3, 5-*O*-feruloylquinic and 3-(3′,4′-dihydroxyphenyl)propanoic acids (cluster 3B). While on cluster 3A the

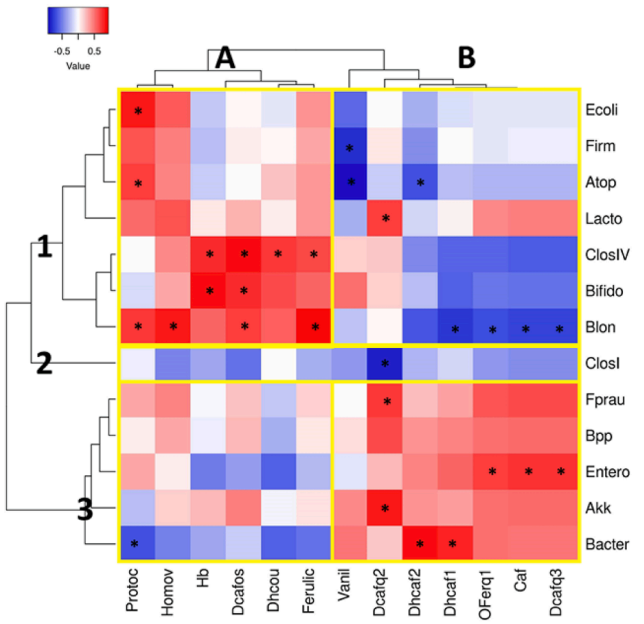

Fig. 2. Interomics, Spearman Rank Correlations among the microbiota dataset and the phenolic compounds dataset. Left side dendrogram identifies by Pearson analysis three major clusters among bacterial groups. Top dendrogram identifies by Pearson analysis two major clusters among phenolic compounds. Significance of correlations are provided as supplementary material (Fig. S1). X axis labels: Protoc = protocatechuic acid (3,4-dihydroxybenzoic acid); Homov = homovanillic acid (4′-hydroxy-3′-methoxyphenylacetic acid); HB = hydroxybenzoic acid; Dcafos = N^1,N^5-di-dihydrocaffeoylsperimidine; Dhcou = Dihydro-coumaric acid (3-(3′-hydroxyphenyl)propanoic acid); Ferulic = ferulic acid (4′-hydroxy-3′-methoxycinnamic acid); Vanil = vanillic acid (4-hydroxy-3-methoxybenzoic acid); Dcafq2 = di-hydro-caffeoylquinic acid isomer 2; Dhcaf2 = di-hydro-caffeic acid isomer 2 (3-(3′,4′-dihydroxyphenyl)propanoic acid); Dhcaf1 = di-hydro-caffeic acid isomer 1 (3-(3′,4′-dihydroxyphenyl)propanoic acid); Oferq1 = 5-*O*-feruloylquinic acid *trans*; Caf = caffeic acid (3′,4′-dihydroxycinnamic acid); Dcafq3 = di-hydro-caffeoylquinic acid isomer 3. Y axis labels: Ecoli = *Escherichia coli*; Firm = *Firmicutes*; Atop = *Atopobium-Collinsella-Eggerthella* group; Lscto = *Lactobacillales*; Clos IV = *Clostridium* group IV; Bifido = *Bifidobacteriaceae*; Blon = *Bifidobacterium longum*; Clos I = *Clostridium* group I; Fprau = *Faecalibacterium prausnitzii*; Bpp = *Bacteroides-Prevotella-Porphyromonas* group; Entero = *Enterobacteriaceae*; Akk = *Akkermansia muciniphila*; Bacter = *Bacteroidetes*.

negative correlations were significant just for 3,4-dihydroxybenzoic acid and *Bacteroidetes*.

4. Conclusion

Heat treatments increased the extractability of eggplant phenolic compounds and were essential to promote the release of DPE phenolic compounds during *in vitro* gastro-intestinal digestion. Fried DPE displayed the highest amount of phenolic compounds following *in vitro* gastro-intestinal digestion. Additionally, gut microbiota exhibited a considerable metabolic activity resulting in a thorough modification in the chemical structure of DPE phenolic compounds. The colon metabolic pathways of the different phenolic compounds in DPE converged towards the production of 3-(3′-hydroxyphenyl)propanoic acid, independently from the samples. Fried DPE displayed the highest amount of metabolites.

Colonic fermentation of fried and raw DPE resulted in a considerable increase in many beneficial taxa. A positive correlation among the most important phenolic metabolites and *Bifidobacteriaceae*, *B. longum* and *Clostridium group IV* suggesting their involvement in the colonic metabolism of hydroxycinnamic acids.

Therefore, eggplant frying can be considered as the most appropriate cooking treatment to achieve the highest amount of bioaccessible phenolic compounds and metabolites also favoring the growth of beneficial colonic bacteria resulting in a shift in the microbiota composition that more efficaciously metabolize phenolic compounds. Since the amount and type of phenolic metabolites and the colonic microbiota composition influence human health, presented results suggest that the application of different cooking methods may have distinctive effects on human health. Meanwhile the gut microbiota composition and its metabolic activity can be modulate not only by the cooking treatments but also by the type of food and phenolic compounds profile. Therefore, more studies are needed in order to unravel the effect of different foods in combination with diverse cooking techniques on gut microbiota diversity and phenolic compounds metabolism.

CRediT authorship contribution statement

Lorenzo Nissen: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Alice Cattivelli:** Conceptualization, Software, Formal analysis, Investigation, Data curation, Writing – review & editing. **Flavia Casciano:** Software, Formal analysis, Investigation, Writing – review & editing. **Andrea Gianotti:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Davide Tagliazucchi:** Conceptualization, Methodology, Software, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Acknowledgements

The authors acknowledge the Fondazione Cassa di Risparmio di Modena for funding the HPLC-ESI-Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer system at the Centro Interdipartimentale Grandi Strumenti (CIGS). The authors also acknowledge the personnel of Centre for Applied Biomedical Research – CRBA, *Alma Mater Studiorum* - Università di Bologna, Policlinico di Sant'Orsola, Bologna, Italy.

Funding: This research did not receive any external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the ethics procedures required at the University of Bologna.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111702.

References

Andreasen, M. F., Kroon, P. A., Williamson, G., & Garcia-Conesa, M. T. (2001). Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *Journal of Agricultural and Food Chemistry, 49*, 5679–5684.

Arnal, M. E., Denis, S., Uriot, O., Lambert, C., Holowacz, S., Paul, F., et al. (2021). Impact of oral galenic formulations of Lactobacillus salivarius on probiotic survival and interactions with microbiota in human in vitro gut models. *Beneficial microbes, 12*, 381–396.

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J. R., Maciejewski, A., et al. (2016). Heatmapper: Web-enabled heat mapping for all. *Nucleic Acids Research, 44*, W147–W153.

Blaut, M., Collins, M. D., Welling, G. W., Doré, J., van Loo, J., & de Vos, W. (2002). Molecular biological methods for studying the gut microbiota: The EU human gut flora project. *British Journal of Nutrition, 87*, S203–S211.

Boto-Ordóñez, M., Urpi-Sarda, M., Queipo-Ortuño, M. I., Tulipani, S., Tinahones, F. J., & Andres-Lacueva, C. (2014). High levels of Bifidobacteria are associated with increased levels of anthocyanin microbial metabolites: A randomized clinical trial. *Food & Function, 5*, 1932–1938.

Braune, A., & Blaut, M. (2016). Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes, 7*, 216–234.

Carmody, R. N., Bisanz, J. E., Bowen, B. P., Maurice, C. F., Lyalina, S., Louie, K. B., et al. (2019). Cooking shapes the structure and function of the gut microbiome. *Nature Microbiology, 4*, 2052–2063.

Cattivelli, A., Conte, A., Martini, S., & Tagliazucchi, D. (2021). Influence of cooking methods on onion phenolic compounds bioaccessibility. *Foods, 10*, 1023.

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture, 80*, 1033–1043.

Connolly, M. L., Tuohy, K. M., & Lovegrove, J. A. (2012). Wholegrain oat-based cereals have prebiotic potential and low glycaemic index. *British Journal of Nutrition, 108*, 2198–2206.

Corrêa, T., Rogero, M. M., Hassimotto, N., & Lajolo, F. M. (2019). The two-way polyphenols-microbiota interactions and their effects on obesity and related metabolic diseases. *Frontiers in Nutrition, 6*, 188.

Cortés-Martín, A., Selma, M. V., Tomás-Barberán, F., González-Sarrías, A., & Espín, J. C. (2020). Where to look into the puzzle of polyphenols and health? The postbiotics and gut microbiota associated with human metabotypes. *Molecular Nutrition and Food Research, 64*, Article e1900952.

Couteau, D., McCartney, A. L., Gibson, G. R., Williamson, G., & Faulds, C. B. (2001). Isolation and characterization of human colonic bacteria able to hydrolyse chlorogenic acid. *Journal of Applied Microbiology, 90*, 873–881.

Da Encarnação, J. A., Farrel, T. L., Ryder, A., Kraut, N. U., & Williamson, G. (2015). In vitro enzymic hydrolysis of chlorogenic acids in coffee. *Molecular Nutrition and Food Research, 59*, 231–239.

De Santiago, E., Gill, C. I. R., Carafa, I., Tuohy, K. M., De Peña, M. P., & Cid, C. (2019). Digestion and colonic fermentation of raw and cooked *Opuntia ficus-indica* cladodes impacts bioaccessibility and bioactivity. *Journal of Agricultural and Food Chemistry, 67*, 2490–2499.

Deshpande, S., Jaiswal, R., Matei, M. F., & Kuhnert, N. (2014). Investigation of acyl migration in mono- and dicaffeoylquinic acids under aqueous basic, aqueous acidic, and dry roasting conditions. *Journal of Agricultural and Food Chemistry, 62*, 9160–9170.

Domínguez-Fernández, M., Ludwig, I. A., De Peña, M. P., & Cid, C. (2021). 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 & Function, 12*, 1996–2011.

Domínguez-Fernández, M., Young Tie Yang, P., Ludwig, I. A., Clifford, M. N., Cid, C., & Rodriguez-Mateos, A. (2022). In vivo study of the bioavailability and metabolic profile of (poly)phenols after sous-vide artichoke consumption. *Food Chemistry*, *367*, 130620.

Duncan, S. H., Russel, W. R., Quartieri, A., Rossi, M., Parkhill, J., Walker, A. W., et al. (2016). Wheat bran promotes enrichment within the human colonic microbiota of butyrate-producing bacteria that release ferulic acid. *Environmental Microbiology, 18*, 2214–2225.

Fernández-Millán, E., Ramos, S., Alvarez, C., Bravo, L., Goya, L., & Martín, M.Á. (2014). Microbial phenolic metabolites improve glucose-stimulated insulin secretion and protect pancreatic beta cells against tert-butyl hydroperoxide-induced toxicity via ERKs and PKC pathways. *Food and Chemical Toxicology, 66*, 245–253.

Filannino, P., Di Cagno, R., & Gobbetti, M. (2018). Metabolic and functional paths of lactic acid bacteria in plant foods: Get out of the labyrinth. *Current Opinion in Biotechnology, 49*, 64–72.

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology, 14*, 491–502.

Gómez-Juaristi, M., Martínez-López, S., Sarria, B., Bravo, L., & Mateos, R. (2018). Absorption and metabolism of yerba mate phenolic compounds in humans. *Food Chemistry, 240*, 1028–1038.

Gürbüz, N., Uluişik, S., Frary, A., Frary, A., & Doğanlar, S. (2018). Health benefits and bioactive compounds of eggplant. *Food Chemistry, 268*, 602–610.

Iqbal, Y., Cottrell, J. J., Suleria, H. A., & Dunshea, F. R. (2020). Gut microbiota-polyphenol interactions in chicken: A review. *Animals, 10*, 1391.

Jiménez, N., Santamaría, L., Esteban-Torres, M., de las Rivas, B., & Muñoz, R. (2014). Contribution of a tannase from Atopobium parvulum DSM 20469T in the oral processing of food tannins. *Food Research International*, *62*, 397-402.

Juániz, I., Ludwig, I. A., Bresciani, L., Dall'Asta, M., Mena, P., Del Rio, D., Cid, C., & De Peña, M. P. (2016). Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and faecal fermentation. *Journal of Functional Foods*, *27*, 201–213.

Juániz, I., Ludwig, I. A., Bresciani, L., Dall'Asta, M., Mena, P., Del Rio, D., Cid, C., & De Peña, M. P. (2017). Bioaccessibility of (poly)phenolic compounds of raw and cooked cardoon (*Cynara cardunculus* L.) after simulated gastrointestinal digestion and fermentation by human colonic microbiota. *Journal of Functional Foods*, *32*, 195–207.

Kahle, K., Kempf, M., Schreier, P., Scheppach, W., Schrenk, D., Kautenburger, T., et al. (2011). Intestinal transit and systemic metabolism of apple polyphenols. *European Journal of Nutrition, 50*, 507–522.

Karimi, A., Kazemi, M., Samani, S. A., & Simal-Gandara, J. (2021). Bioactive compounds from by-products of eggplant: Functional properties, potential applications and advances in valorization methods. *Trends in Food Science and Technology, 112*, 518–531.

Kasprzak-Drozd, K., Oniszczuk, T., Stasiak, M., & Oniszczuk, A. (2021). Beneficial effects of phenolic compounds on gut microbiota and metabolic syndrome. *International Journal of Molecular Sciences, 22*, 3715.

Kay, C. D., Clifford, M. N., Mena, P., McDougall, G. J., Andres-Lacueva, C., Cassidy, A., et al. (2020). Recommendations for standardizing nomenclature for dietary (poly) phenol catabolites. *The American Journal of Clinical Nutrition, 112*, 1051–1068.

Kemperman, R. A., Gross, G., Mondot, S., Possemiers, S., Marzorati, M., Van de Wiele, T., et al. (2013). Impact of polyphenols from black tea and red wine/grape juice on a gut model microbiome. *Food Research International, 53*, 659–669.

Khubber, S., Marti-Quijal, F. J., Tomasevic, I., Remize, F., & Barba, F. J. (2022). Lactic acid fermentation as a useful strategy to recover antimicrobial and antioxidant compounds from food and by-products. *Current Opinion in Food Science, 43*, 189–198.

Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., et al. (2017). Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiology, 17*, 1–6.

Koudoufio, M., Desjardins, Y., Feldam, F., Spahis, S., Delvin, E., & Levy, E. (2020). Insight into polyphenol and gut microbiota crosstalk: Are their metabolites the key to understand protective effects against metabolic disorders? *Antioxidants, 9*, 982.

Koutsos, A., Lima, M., Conterno, L., Gasperotti, M., Bianchi, M., Fava, F., et al. (2017). Effects of commercial apple varieties on human gut microbiota composition and metabolic output using an in vitro colonic model. *Nutrients, 9*, 533.

Larrosa, M., Luceri, C., Vivoli, E., Pagliuca, C., Lodovici, M., Moneti, G., et al. (2009). Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. *Molecular Nutrition and Food Research, 53*, 1044–1054.

Liu, L., Zhang, C., Zhang, H., Qu, G., Li, C., & Liu, L. (2021). Biotransformation of polyphenols in apple pomace fermented by β-glucosidase-producing *Lactobacillus rhamnosus* L08. *Foods, 10*, 1343.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology, 15*, 1–21.

Ludwig, I. A., De Peña, M. P., Cid, C., & Crozier, A. (2013). Catabolism of coffee chlorogenic acids by human colonic microbiota. *Biofactors, 39*, 623–632.

Marhuenda-Muñoz, M., Laveriano-Santos, E. P., Tresserra-Rimbau, A., Lamuela-Raventós, R. M., Martínez-Huélamo, M., & Vallverdú-Queralt, A. (2019). Microbial phenolic metabolites: Which molecules actually have an effect on human health? *Nutrients, 11*, 2725.

Martini, S., Conte, A., & Tagliazucchi, D. (2019). Bioactivity and cell metabolism of *in vitro* digested sweet cherry (*Prunus avium*) phenolic compounds. *International Journal of Food Sciences and Nutrition, 70*, 335–348.

Martini, S., Tagliazucchi, D., Minelli, G., & Lo Fiego, D. P. (2020). Influence of linseed and antioxidant-rich diets in pig nutrition on lipid oxidation during cooking and in vitro digestion of pork. *Food Research International, 137*, Article 109528.

Martini, S., Conte, A., Cattivelli, A., & Tagliazucchi, D. (2021). Domestic cooking methods affect the stability and bioaccessibility of dark purple eggplant (*Solanum melongena*) phenolic compounds. *Food Chemistry, 341*, Article 128298.

Martinez, J. E., Kahana, D. D., Ghuman, S., Wilson, H. P., Wilson, J., Kim, S. C., et al. (2021). Unhealthy lifestyle and gut dysbiosis: A better understanding of the effects of poor diet and nicotine on the intestinal microbiome. *Frontiers in Endocrinology, 12*, 649.

Mena, P., & Bresciani, L. (2020). Dietary fibre modifies gut microbiota: What's the role of (poly)phenols? *International Journal of Food Sciences and Nutrition, 71*, 783–784.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static *in vitro* digestion method suitable for food – An international consensus. *Food & Function, 5*, 1113–1124.

Modesto, M., Stefanini, I., D'Aimmo, M. R., Nissen, L., Tabanelli, D., Mazzoni, M., et al. (2011). Strategies to augment non-immune system based defence mechanisms against gastrointestinal diseases in pigs. *NJAS - Wagening Journal of Life Science, 58*, 149–156.

Monente, C., Ludwig, I. A., Stalmach, A., De Pena, M. P., Cid, C., & Crozier, A. (2015). In vitro studies on the stability in the proximal gastrointestinal tract and bioaccessibility in Caco-2 cells of chlorogenic acids from spent coffee grounds. *International Journal of Food Sciences and Nutrition, 66*, 657–664.

Moreno-Indias, I., Sanchez-Alcoholado, L., Pérez-Martínez, P., Andres-Lacueva, C., Cardona, F., Tinahones, F. J., et al. (2016). Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients. *Food & Function, 7*, 1775–1787.

Murota, K., Nakamura, Y., & Uehara, M. (2018). Flavonoid metabolism: The interaction of metabolites and gut microbiota. *Bioscience, Biotechnology, and Biochemistry, 82*, 600–610.

Najmanová, I., Pourová, J., Vopršalová, M., Pilařová, V., Semecký, V., Nováková, L., et al. (2016). Flavonoid metabolite 3-(3-hydroxyphenyl)propionic acid formed by human microflora decreases arterial blood pressure in rats. *Molecular Nutrition and Food Research, 60*, 981–991.

Niño-Medina, C., Urías-Orona, V., Muy-Rangel, M. D., & Heredia, J. B. (2017). Structure and content of phenolics in eggplant (*Solanum melongena*) – a review. *South African Journal of Botany, 111*, 161–169.

Nishimura, M., Suzuki, M., Takahashi, R., Yamaguchi, S., Tsubaki, K., Fujita, T., et al. (2019). Daily ingestion of eggplant powder improves blood pressure and

psychological state in stressed individuals: A randomized placebo-controlled study. *Nutrients, 11*, 2797.

Nissen, L., Casciano, F., & Gianotti, A. (2020a). Intestinal fermentation in vitro models to study food-induced gut microbiota shift: An updated review. *FEMS Microbiology Letters, 367*, fnaa097.

Nissen, L., Bordoni, A., & Gianotti, A. (2020b). Shift of volatile organic compounds (VOCs) in gluten-free hemp-enriched sourdough bread: A metabolomic approach. *Nutrients, 12*, 1050.

Nissen, L., Casciano, F., Chiarello, E., Di Nunzio, M., Bordoni, A., & Gianotti, A. (2021a). Colonic in vitro model assessment of the prebiotic potential of bread fortified with polyphenols rich olive fiber. *Nutrients, 13*, 787.

Nissen, L., Valerii, M. C., Spisni, E., Casciano, F., & Gianotti, A. (2021b). Multiunit in vitro colon model for the evaluation of prebiotic potential of a fiber plus D-limonene food supplement. *Foods, 10*, 2371.

Oba, S., Sunagawa, T., Tanihiro, R., Awashima, K., Sugiyama, H., Odani, T., et al. (2020). Prebiotic effects of yeast mannan, which selectively promotes Bacteroides thetaiotaomicron and Bacteroides ovatus in a human colonic microbiota model. *Scientific reports, 10*(1), 1–11.

Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of Food and Agriculture, 94*, 1057–1070.

Patrascu, O., Béguet-Crespel, F., Marinelli, L., Le Chatelier, E., Abraham, A. L., Leclerc, M., et al. (2017). A fibrolytic potential in the human ileum mucosal microbiota revealed by functional metagenomic. *Scientific Reports, 7*, 40248.

Pérez-Burillo, S., Pastoriza, S., Jiménez-Hernández, N., D'Auria, G., Pilar Francino, M., & Rufián-Henares, J. A. (2018). Effect of food thermal processing on the composition of the gut microbiota. *Journal of Agricultural and Food Chemistry, 66*, 11500–11509.

Pérez-Burillo, S., Rufián-Henares, J. A., & Pastoriza, S. (2019). Effect of home cooking on the antioxidant capacity of vegetables: Relationship with Maillard reaction indicators. *Food Research International, 121*, 514–523.

Rodríguez-Daza, M. C., Pulido-Mateos, E. C., Lupien-Meilleur, J., Guyonnet, D., Desjardins, Y., & Roy, D. (2021). Polyphenol-mediated gut microbiota modulation: Toward prebiotics and further. *Frontiers in Nutrition, 8*, Article 689456.

Russell, W. R., Scobbie, L., Chesson, A., Richardson, A. J., Stewart, C. S., Duncan, S. H., et al. (2008). Anti-inflammatory implications of the microbial transformation of dietary phenolic compounds. *Nutrition and Cancer, 60*, 636–642.

Saa, D. T., Di Silvestro, R., Dinelli, G., & Gianotti, A. (2017). Effect of sourdough fermentation and baking process severity on dietary fibre and phenolic compounds of immature wheat flour bread. *LWT-Food Science and Technology, 83*, 26–32.

Sanders, M. E., Merenstein, D. J., Reid, G., et al. (2019). Probiotics and prebiotics in intestinal health and disease: From biology to the clinic. *Nature Review Gastroenterology Hepatology, 16*, 605–616.

Sova, M., & Saso, L. (2020). Natural sources, pharmacokinetics, biological activities and health benefits of hydroxycinnamic acids and their metabolites. *Nutrients, 12*, 2190.

Stalmach, A., Mullen, W., Barron, D., Uchida, K., Yokota, T., Cavin, C., et al. (2009). Metabolite profiling of hydroxycinnamate derivatives in plasma and urine following the ingestion of coffee by humans: Identification of biomarkers of coffee consumption. *Drug Metabolism and Disposition, 37*, 1759–1768.

Stalmach, A., Steiling, H., Williamson, G., & Crozier, A. (2010). Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Archives of Biochemistry and Biophysics, 501*, 98–105.

Tamargo, A., Cueva, C., Silva, M., Molinero, N., Miralles, B., Bartolomé, B., et al. (2022). Gastrointestinal co-digestion of wine polyphenols with glucose/whey proteins affects their bioaccessibility and impact on colonic microbiota. *Food Research International, 111010*.

Tanner, S. A., Zihler Berner, A., Rigozzi, E., Grattepanche, F., Chassard, C., & Lacroix, C. (2014). In vitro continuous fermentation model (PolyFermS) of the swine proximal colon for simultaneous testing on the same gut microbiota. *PloS One, 9*(4), Article e94123.

Tomas-Barberan, F., García-Villalba, R., Quartieri, A., Raimondi, S., Amaretti, A., Leonardi, A., et al. (2014). In vitro transformation of chlorogenic acid by human gut microbiota. *Molecular Nutrition and Food Research, 58*, 1122–1131.

Tsitko, I., Wiik-Miettinen, F., Mattila, O., Rosa-Sibakov, N., Seppänen-Laakso, T., Maukonen, J., et al. (2019). A small in vitro fermentation model for screening the gut microbiota effects of different fiber preparations. *International Journal of Molecular Sciences, 20*, 1925.

Valli, V., Danesi, F., Gianotti, A., Di Nunzio, M., Saa, D. L. T., & Bordoni, A. (2016). Antioxidative and anti-inflammatory effect of in vitro digested cookies baked using different types of flours and fermentation methods. *Food Research International, 88*, 256–262.

Wang, X., Gibson, G. R., Sailer, M., Theis, S., & Rastall, R. A. (2020). Prebiotics inhibit proteolysis by gut bacteria in a host diet-dependent manner: A three-stage continuous in vitro gut model experiment. *Applied and Environmental Microbiology, 86*, e02730-19.

Westfall, S., Lomis, N., & Prakash, S. (2018). A polyphenol-rich prebiotic in combination with a novel probiotic formulation alleviates markers of obesity and diabetes in Drosophila. *Journal of Functional Foods, 48*, 374–386.

Williamson, G., & Clifford, M. N. (2017). Role of the small intestine, colon and microbiota in determining the metabolic fate of polyphenols. *Biochemical Pharmacology, 139*, 24–39.

Wiciński, M., Gębalski, J., Mazurek, E., Podhorecka, M., Śniegocki, M., Szychta, P., et al. (2020). The influence of polyphenol compounds on human gastrointestinal tract microbiota. *Nutrients, 12*, 350.

Zhang, M., Zhou, L., Wang, Y., Dorfman, R. G., Tang, D., Xu, L., et al. (2019). *Faecalibacterium prausnitzii* produces butyrate to decrease c-Myc-related metabolism and Th17 differentiation by inhibiting histone deacetylase 3. *International Immunology, 31*, 499–514.

Zhou, S., Wang, Y., Jacoby, J. J., Jiang, Y., Zhang, Y., & Yu, L. L. (2017). Effects of medium-and long-chain triacylglycerols on lipid metabolism and gut microbiota composition in C57BL/6J mice. *Journal of Agricultural and Food Chemistry, 65*, 6599–6607.