Raw and Sous-Vide-Cooked Red Cardoon Stalks (Cynara cardunculus L. var. altilis DC): (Poly)phenol Bioaccessibility, Anti-inflammatory Activity in the Gastrointestinal Tract, and Prebiotic Activity

Estibaliz Huarte, Gessica Serra, Andrea Monteagudo-Mera, Jeremy Spencer, Concepción Cid, and María-Paz de Peña*

ABSTRACT: The in vitro anti-inflammatory and prebiotic activity and the content and profile of bioaccessible (poly)phenols and catabolites of raw and sous-vide-cooked red cardoon (Cynara cardunculus L. var. altilis DC) were investigated during gastrointestinal (GI) digestion. Raw cardoon after in vitro GI digestion had 0.7% bioaccessible (poly)phenols, which protected against lipopolysaccharide-induced inflammation by counteracting IL-8, IL-6, TNF-α, and IL-10 secretions in differentiated Caco-2 cells. Contrarily, GI-digested sous vide cardoon showed higher (poly)phenol bioaccessibility (59.8%) and exerted proinflammatory effects in Caco-2 cells. (Poly)phenols were highly metabolized during the first 8 h of in vitro fermentation, and nine catabolites were produced during 48 h of fermentation. Colonic-fermented raw and sous-vide-cooked cardoon did not show anti-inflammatory activity in HT-29 cells but presented potential prebiotic activity, comparable to the commercial prebiotic FOS, by stimulating health-promoting bacteria such as Bifidobacterium spp. and Lactobacillus/Enterococcus spp. and by increasing the production of total SCFAs, especially acetate.

KEYWORDS: Cynara, heat treatment, gastrointestinal digestion, (poly)phenols, cytokines, gut microbiota

INTRODUCTION

Diets consisting of low amounts of fruits, vegetables, and other fiber- and prebiotic-rich foods and high amounts of refined grains, alcohol, and ultra-processed foods are associated with changes in the gut microbiota composition and function (namely, dysbiosis) and lead to systemic chronic inflammation (SCI). In addition, there is evidence that SCI is implicated in disease onset or progression of metabolic syndrome. Therefore, it is of great importance to find nutritional or therapeutic interventions that help prevent or reduce an inflammatory state and maintain healthy microbiota.

Cultivated cardoon (Cynara cardunculus L. var. altilis DC), a Mediterranean plant belonging to the family Asteraceae, is mainly cultivated in Spain, Italy, Greece, France, and south Portugal. Cardoon stalks are usually consumed raw in salads or in a boiled form. Although the white variety is better known, stalks from the red cardoon variety are a richer source of (poly)phenols than those of the white variety. In addition, the application of heat treatments, such as frying and grilling, on cardoon stalks exerts a positive effect on the bioaccessibility of (poly)phenols, resulting in higher (poly)phenol bioaccessibility in digested cooked cardoons than in the raw ones after gastrointestinal (GI) digestion. Furthermore, the innovative culinary technique of sous vide cooking promoted a greater amount of caffeoylquinic acids (CQAs) both before and after GI digestion than traditional boiling in other Cynara vegetable-like globe artichoke.

About 90% of dietary (poly)phenols, along with nondigestible carbohydrates and other plant components (such as lignin, resistant proteins, and carotenoids), are resistant to digestion and absorption in the small intestine. Thus, they reach the colon where they can be completely or partially hydrolyzed into smaller and more absorbable compounds by the gut microbiota. In a previous study on white cardoon stalks, gut microbiota action induced the formation of a higher total amount of the (poly)phenol-derived catabolites [protocatechuic acid, dihydrocaffeoylquinic, dihydrocaffeic, and 3-(3-hydroxyphenyl)propionic acids] in fried and griddled cardoon stalks than in raw ones during an in vitro-simulated colonic fermentation. Thus, different bioaccessibilities of bioactive compounds and their catabolites between raw and cooked vegetables could impact their potential biological activity in the GI tract.

There is growing evidence of the ability of (poly)phenols to modulate inflammation, the gut microbiota composition, and metabolic activity. Then, throughout the upper and lower GI digestion processes, red cardoon bioactive compounds, such as (poly)phenols, and/or their resulting catabolites, might exert anti-inflammatory and/or prebiotic activity on the small intestine and/or the colon, before being absorbed into the blood or excreted. To the best of the authors’ knowledge, there...
are no previous studies that evaluate the biological properties of red cardoon and neither the impact of culinary treatments and the GI digestion on its potential bioactivity. Therefore, this study aimed to investigate raw and sous-vide-cooked red cardoon stalks at different stages of in vitro upper and lower digestions (simulated oral-GI digestion and colonic fermentation) in terms of their (1) (poly)phenol bioaccessibility and metabolism by gut microbiota; (2) anti-inflammatory activity in the small intestine (differentiated Caco-2 cells) or colon (HT-29 cells); and (3) potential prebiotic activity on fecal human microbiota.

**MATERIALS AND METHODS**

**Chemicals and Reagents.** Two plants of red cardoon stalks (C. cardunculus L. var. altiss DC) of approx. 50 cm in height and around 2.5 kg each, and harvested the day before, were purchased in a local retail store during the winter season (January 2019). Peptone water, yeast extract, bile salts, and phosphate buffered saline (PBS) tablets were obtained from Oxoid Ltd (Basingstoke, UK). Methanol, acetonitrile and 98% formic acid were acquired from PanReac AppliChem (Darmstadt, Germany). Pure phenolic standards were purchased from Sigma-Aldrich (Steinheim, Germany), except for dihydrocaffeic acid (Alfa Aesar, Thermo Fisher Scientific, Massachusetts, USA), the dicaffeoylquinic acids (diCQAs) 1,4-diCQA and 4,5-diCQA (MedChem Express, New Jersey, USA), and 3,5-diCQA and apigenin (Extrasynthese, Genay, France). Low glucose (1 g/L) Dulbecco’s modified Eagle’s medium (DMEM) GlutaMAX containing pyruvate with and without phenol red; high glucose (4.5 g/L) DMEM with and without phenol red; Dulbecco’s PBS; 0.25% trypsin—ethylenediaminetetraacetic acid (1X); penicillin—streptomycin; heat-inactivated fetal bovine serum (FBS); and MEM nonessential amino acids (100X) were obtained from Gibco (Paisley, UK). The standard fructooligosaccharide (FOS) OraftiP95 was purchased from BENO-Orafti (Oreye, Belgium). Digestive enzymes, lipopolysaccharide (LPS) from Escherichia coli 026:B6, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), oligonucleotide probes, and other chemicals and reagents used were purchased from Sigma Aldrich (Poole, UK).

**Red Cardoon Sample Preparation.** Cardoon stalks were washed, and the spiny skin was removed manually. Then, they were cut into rectangular homogeneous pieces (1.5 x 6 cm approx.), manually mixed together, and divided into two parts (1.8 kg each). One-half was named raw cardoon and lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain). The other half was packaged sous-vide was controlled with a temperature probe. All the volunteers had not taken any antibiotics for at least 6 months before the study and had no history of bowel or GI disease and followed a polyphenol-free diet (avoiding fruits and vegetables, nuts, legumes, high-fiber products, and beverages such as tea, coffee, and fruit juices, as well as alcohol) for 2 days before fecal collection. Fecal slurry from each individual was prepared by homogenizing human feces (10% w/v) in PBS (0.1 M; pH 7.4) in a stomacher (Stomacher 400, Seward, Norfolk, UK) at 460 paddle beats/min for 2 min.

**Simulated Colonic Fermentation.** GI-digested and dialyzed cardoon samples were submitted to an in vitro-simulated colonic fermentation using fecal batch cultures. In order to mimic conditions located in the distal region of the human large intestine, the experiment was run under anaerobic conditions at 37 °C and pH 6.7–6.9 for a period of 48 h. Briefly, sterilized glass water-jacketed vessels (300 mL) were aseptically filled with a presterilized basal nutrient medium (135 mL) containing the following: peptone water (2 g/L), yeast extract (0.1 g/L), KH2PO4 (0.44 g/L), KH3PO4 (0.4 g/L), MgSO4·7H2O (0.01 g/L), CaCl2·6H2O (0.01 g/L), NaHCO3 (2 g/L), Tween 80 (0.1 mL/L), hemin (0.05 g/L), vitamin K1 (10 mL/L), t-cysteine (0.5 g/L), bile salts (0.5 g/L), resazurin (1 mg/L), and distilled water. The vessels were then gassed overnight with O2-free N2 (15 mL Minverse media) and magnetically stirred. The next day, the temperature of the vessels was set to 37 °C by use of a circulating water bath, and the pH was maintained at 6.7–6.9 using an Electrolab pH controller (Tewksbury, UK). Then, 1.5 g of each freeze-dried digest sample (1% w/v final volume) was added to the stirred vessels just before the addition of 15 mL of the fecal slurry (1% w/v final volume). The prebiotic FOS OraftiP95 at 1% w/v final volume was included as the positive fermentation control, and basal nutrient media with no substrate was added as the negative fermentation control (NFC). Batch cultures were run for 48 h and were performed in triplicate with three different fecal volunteers for each substrate. Aliquots from cardoon sample and control fermentation were collected at various time points for (poly)phenol and catabolite analysis (15 mL at 0, 4, 8, 24, and 48 h), cytotoxicity and impact on cytokine secretion analysis in HT-29 cells (5 mL at 8 and 24 h), and bacterial enumeration and lactic acid and short-chain fatty acid (SCFA) analysis (5 mL at 0, 8, 24 and 48 h). Collected aliquots for the analysis of (poly)phenols and catabolites were immediately inactivated with 50 μL of 33% HCl, then freeze-dried (Cryodos-80, Telstar, Terrasa, Spain), and stored at −80 °C.

**Analysis of (Poly)phenols and Catabolites by High-Performance Liquid Chromatography with Tandem Mass Spectrometry.** (Poly)phenols and catabolites were extracted from the undegested, digested (with and without subsequent dialysis), and
fermented cardoon samples, following the Sánchez-Salcedo et al. procedure with some modifications. Briefly, 25 mg of each freeze-dried sample were extracted with 0.5 mL of 80:20 (v/v) methanol/acidified water (0.1% formic acid) and sonicated for 90 min. Afterward, the mixture was centrifuged for 10 min at 19,956 g. The supernatant was collected and the residue was re-extracted using 0.25 mL of 80:20 v/v methanol/acidified water, sonicated for 25 min in a sonic bath, and centrifuged for 10 min at 19,956 g. Both supernatants were mixed, filtered with a 0.22 μm PVDF syringe filter, and stored at −18 °C until high-performance liquid chromatography with tandem mass spectrometry (HPLC−MS/MS) analysis.

(Poly)phenols and catabolites in the extracts were analyzed using an HPLC unit model 1200 (Agilent Technologies, Palo Alto, CA, USA) equipped with a triple quadrupole linear ion-trap mass spectrometer (3200 Q-TRAP, AB SCIEX). The column used was a CORTECS C18 (3 × 75 mm, 2.7 μm) from Waters. The HPLC separation was carried out using 0.1% v/v formic acid in water (solvent A) and acetonitrile (solvent B) at a constant flow rate of 0.6 mL/min and a column oven temperature of 20 °C. The injection volume was 5 μL. The mobile phase program was established as follows: 0−1 min, 5% B; 1−5 min, 5−10% B; 5−8 min, 10−20% B; 8−10.5 min, 20% B; 10.5−16 min, 20−30% B; 16−17.6 min, 30−100% B; 17.6−25.6 min, 100% B, then returned to 5% B in 4.8 min and maintained an isotropic elution until the end of the analysis (35 min). For the identification and quantification of (poly)phenols and catabolites, the ion multiple reaction monitoring mode was used. The MS worked in the negative ionization mode, with a source temperature of 600 °C and the IonSpray voltage of −3500 V. The declustering and entrance potentials were set at −20 and −10 V, respectively. The collision energy was optimized for each compound using the same standards as those used for identification. Compounds were identified by comparing the MS/MS fragmentation pattern and retention time with pure reference standards when available. When no standard was available, compounds were tentatively identified by comparing the MS/MS fragmentation with the literature fragmentation pathway. The retention time and mass spectrometric characteristics of (poly)phenols and catabolites identified as well as the pure reference standards used are shown in Table S1.

(Poly)phenols and catabolites were quantified by using the calibration curves of their own pure reference standard when available. For those compounds with a pure reference standard, 1-CQA and CQA derivatives I−III were quantified as 5-CQA equivalents; diCQA glucosides I−II and succinyl/tylCQAs I−II as 4.5-diCQA equivalents; caffeoyl-hexoside was quantified as isocaffeic acid; and luteolin acetylglycoside was quantified as luteolin 7-O-glucoside equivalents. The limit of quantification (LOQ) was 0.25 μg/mL for hesperidin and phenylactic acid; 0.05 μg/mL for 1,3-diCQA, isoforeric acid, quercetin, 3-hydroxyphenylactic acid, protocatechuic acid, and 4-hydroxybenzoic acid; and 0.025 μg/mL for the rest of the standards used. Chromatograms and spectral data were acquired using Analyst software 1.6.3 (AB SCIEX). Those polyphenols or catabolites detected and quantified in the NFC samples were subtracted from the colonial fermented cardoon samples. Each sample was analyzed in duplicate. The results are presented as the mean of micrograms of (poly)phenol per gram of the cardoon sample dry matter (μg/g dm) ± standard deviation (SD). The bioaccessibility of (poly)phenols from digested (with and without subsequent dialysis) and fermented cardoon samples was determined as follows:

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\text{bioaccessibility} = \frac{\text{TPC}_{\text{after}}}{\text{TPC}_{\text{before}}} \times 100\% 
\]

where TPC_{before} is the total poly(phenol) content before digestion and TPC_{after} is the total poly(phenol) content after digestion/dialysis/fermentation.

**Cell Culture Conditions and Treatments.** Caco-2 cells (human colon adenocarcinoma cells) were purchased from the European Collection of Cell Cultures (ECACC) (Salisbury, Wiltshire UK) and cultured in low glucose (1 g/L) DMEM containing pyruvate and glutamine and supplemented with 1% (v/v) of nonessential amino acids. HT-29 cells (human colon adenocarcinoma cell line) were obtained from ECACC and cultured in high glucose (4.5 g/L) DMEM containing L-glutamine. Cell media from both cell lines were supplemented with 10% v/v FBS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Cells were incubated in a humidified atmosphere at 5% CO₂ and 37 °C and sub-cultured 2–3 times per week.

For experiments with Caco-2 cells, fractions collected after the digestion of cardoon samples were immediately centrifuged at 1543.5g for 15 min, and the resulting supernatants were collected and 0.45 μM filtered to separate the soluble fraction that might be available for absorption into intestinal cells (bio-accessible fraction). For experiments with HT-29 cells, aliquots collected during the fermentation of cardoon samples or from the NFC of the three volunteers at the same fermentation time (8 or 24 h) were combined and centrifuged (1543.5g, 10 min). Then, supernatants were 0.45 and 0.22 μM filtered to obtain the potentially bio-accessible fraction for colon cells. The filtered supernatants of the digestion and fermentation of cardoon samples and the NFC were divided in small aliquots (1.5 mL) and stored at −20 °C for use.

Cells were seeded in 96-well plates (3.4 × 10⁴ cells/mL for Caco-2 cells and 2.2 × 10⁵ cells/mL for HT-29 cells; 150 μL/well) for the MTT assay and in 24-well plates (3.12 × 10⁵ cells/mL for Caco-2 cells and 1.84 × 10⁵ cells/mL for HT-29 cells; 1 mL/well) for the Luminex assay. Caco-2 cells were allowed to grow to confluence for 3 days. HT-29 cells were incubated for 24 h at 37 °C and 5% CO₂, reaching a sub-confluent state prior to the experiments.

Different LPS concentrations and exposure times (0.1−50 μg/mL, 24−72 h) were preliminarily analyzed in both cell lines, and in undifferentiated Caco-2 cells, for their cytotoxicity (MTT assay) and impact on cytokine secretion (Luminex multiplex assay). Undifferentiated Caco-2 cells were allowed to grow to confluence for 19 days at 37 °C and 5% CO₂ prior to the experiments in order to differentiate and develop an enterocyte-like phenotype. Their cell media were changed every 3 days. HT-29 cells were incubated for 24 h at 37 °C and 5% CO₂, reaching a sub-confluent state prior to the experiments.

LPS at 5 and 0.1 μg/mL in the differentiated Caco-2 and HT-29 cells, respectively, and 48 h of exposure were selected as the treatment to induce an inflammatory state in cells on the basis of its ability to stimulate high cytokine secretion without being cytotoxic. Furthermore, the cytotoxicity of supernatants of cardoon sample digestion (2.5−20% v/v in cell media, which corresponds to 1.21−9.68 mg dm/mL of fermentation (0.1−10% v/v in cell media, which corresponds to 9.53−95.32 μg dm/mL) was previously analyzed by the MTT assay in both cell lines. Then, noncytotoxic concentrations were selected for the assessment of the anti-inflammatory potential, which were 2.42 and 9.68 mg cardoon dm/mL for the digested cardoon samples (≥88% MTT reduction relative to the control, both in the absence and in the presence of 5 μg/mL LPS) (Figure S1a) and 23.8 and 95.3 μg cardoon dm/mL for fermented cardoon samples (>80% MTT reduction relative to the control, both in the absence and in the presence of 5 μg/mL LPS) (Figure S1b).

Once the concentrations and exposure times were optimized, the experiments were carried out under the following conditions: on the day of the experiment, the cell medium was removed, and differentiated Caco-2 cells were washed with phenol red-free DMEM at 2.5% FBS and HT-29 cells with PBS. Differentiated Caco-2 cells were pretreated for 1 h with two selected supernatant concentrations of the GI-digested cardoon samples (2.42 and 9.68 mg cardoon dm/mL which are equivalent to 5 and 20% v/v in cell media, respectively). Besides, HT-29 cells were pretreated for 1 h with two selected supernatant concentrations of 8 and 24 h colon-fermented cardoon samples (23.8 and 95.3 μg cardoon dm/mL, which are equivalent to 0.25 and 1% v/v in cell media, respectively) and two supernatant concentrations of 8 and 24 h NFC (0.25 and 1% v/v in cell media). Treatments continued in both cell lines for a further 48 h in the absence and presence of LPS at 5 and 0.1 μg/mL for...
differentiated Caco-2 and HT-29 cells, respectively. Blank controls were introduced in the experiments with both differentiated Caco-2 cells and HT-29 cells consisting of cells incubated with phenol red-free cell media at 2.5% FBS.

**Cytokine Quantification by the Luminox Multiplex Assay.** After the aforementioned treatments (see section “Cell Culture Conditions and Treatment”), well contents were removed transferred to Eppendorf tubes, and stored at −20 °C. The day of the analysis, the tubes were defrosted and centrifuged at 16,000g for 5 min (Centrifuge Thermo Scientific, Heraeus Fresco 17, Massachusetts, United States). The concentration of IL-8, IL-6, IL-1β, TNF-α, and IL-10 on the diluted supernatants was measured using a bead-based Luminox multiplex assay (Human High Sensitive Cytokine Premix Kit A, Magnetic Luminox High Performance Assay, R&D Systems, United Kingdom) following the manufacturer’s instructions. The analysis was carried out with a Luminox 200 System and Luminox Xponent software (Luminox Corporation, Austin, Texas, USA). Three independent experiments were performed.

**Enumeration of Bacterial Populations by Fluorescence In Situ Hybridization.** Aliquots (750 μL) collected from the fermentation of the cardoon samples were immediately centrifuged for 5 min at 11,338 g. The pellet was resuspended in 375 μL of PBS and fixed in 1,125 μL of the 4% w/v paraformaldehyde solution at 4 °C for 4 h. Fixed samples were centrifuged at 11,338 g for 5 min, and the cell pellet was washed twice in 1 mL of sterile cold PBS (0.1 M, pH 7.0) and centrifuged at 11,338 g for 5 min. Finally, the cell pellet was resuspended in PBS (300 μL) and 99% ethanol (300 μL) and stored at −20 °C until analysis. Then, bacterial populations were enumerated by fluorescence in situ hybridization (FISH) using oligonucleotide probes targeting specific regions of 16S rRNA. Probes were commercially synthesized and labeled with the fluorescent dye Cy3. The following probes were used: Bif164 for *Bifidobacterium* spp.; Lab158 for *Lactobacillus/Enterococcus*; Erec482 for *Enterococcus rectale*—*Clostridium cocoides* group (*Clostridium cluster XIVa*); Bac303 for *Bacteroides/Prevotella* group; and EUB338 mixture consisting of EU3B338, EU3B338III, and EU3B338III for total bacteria. Fixed samples were hybridized as described by Daims, Stoecker, and Wagner. Bacterial cells were counted in 15 random fields of view per sample using a fluorescence microscope (Nikon E400 Eclipse, Tokyo, Japan). Three independent fermentation aliquots of cardoon samples with fecal samples from 3 different donors were analyzed.

**Analysis of Lactic Acid and SCFAs by Gas Chromatography with Flame Ionization Detection.** Aliquots (1 mL) collected from the fermentation of cardoon samples were immediately centrifuged (1 min for 10 min) to remove bacteria and other solids. Supernatants were then transferred into clean tubes and frozen at −20 °C. Then, lactic acid and SCFAs from the fermented samples were extracted and derivatized as previously described. Briefly, 500 μL of each sample or standard solution were added to a 100 mm × 16 mm flat-bottomed glass tube with 25 μL of 0.1 M 2-ethylbutyric acid as the internal standard. Then, 250 μL of the concentrated HCI and 1.5 mL of diethyl ether were added to each tube, vortexed for 1 min, and centrifuged (10 min at 752g). Tubes were left at room temperature overnight and the day after, 500 μL of diethyl ether (the receiver layer) were transferred into GC screw-cap vials. Then, 25 μL of N-tert-butyldimethylsilyl)-N-methyltrifluoroacetamide was added to each vial with the ether extract. Then, the vials were left at room temperature for 72 h to allow the acid in the samples to completely derivatize. Then, a 7890B Gas Chromatograph (Agilent Technologies, Cheshire, UK) equipped with FID was used for the analysis of lactic acid and SCFAs in the fermented samples. The column was used a HP-SPM (30 m × 0.25 mm × 0.25 μm; HP-5 3% diphenyl/95% dimethylpolysiloxane) from Agilent Technologies. Both the injector and detector were held at 275 °C. The sample injection volume was 1 μL, and a split ratio of 100:1 was used. The carrier gas helium was set at 5 psi and a flow rate of 6.5 mL/min. The GC oven was held at 63 °C for 3 min, programed to 190 °C at 10 °C/min, and then held constant at 190 °C for 1 min. Lactic acid and SCFAs (acetic, propionic, butyric, isobutyric, and isovaleric acids) were quantified by using a calibration curve performed with a standard solution at concentrations of 6.25–50 mM. Peak areas were integrated and calculated using ChemStation B.01.04.232 software (Agilent Technologies, Cheshire, UK). Three independent fermentation aliquots of cardoon samples with fecal samples from three different donors were analyzed.

**Statistical Analysis.** One-way analysis of variance (ANOVA) with Tukey’s post hoc test was conducted to determine the differences in cytokine concentrations between each treatment and control (untreated cells and NFC-treated cells; with or without LPS stimulation), as well as differences among the raw and cooked cardoon treatments at the same concentration. ANOVA and Tukey’s post hoc test was also applied to determine the significant differences in bacterial counts, and in lactic acid and SCFA concentrations, among treatments (NFC, FOS, raw, and sous-vide-cooked cardoon) at the same fermentation time points (0, 8, 24, and 48 h). Differences in bacterial counts, and in lactic acid and SCFA concentrations, from 0 h of fermentation value within the same treatment were tested using the paired Student’s t test. Differences were considered significant if p value < 0.05. All the statistical analyses were performed using the STATA v12.0 software package.

### RESULTS

**Polyphenol and Catabolite Bioaccessibility during GI-Digestion and Colonic Fermentation.** A total of 19 and 30 (poly)phenols were identified and quantified in raw and sous-vide-cooked red cardoon (nondigested), respectively (Tables 1 and 2). Phenolic acids, and specifically hydroxycinnamic acids, represented >98% of the total content in both samples, with mono- and di-CQAs as the major (poly)phenolic compounds (43.4–48.7 and 55.5–49.2% of total polyphenols, respectively). The remaining (poly)phenols were flavonoids (apigenin, luteolin, quercetin, and hesperetin derivatives), being luteolin derivatives (luteolin 7-O-glucoside, luteolin 7-O-glucuronide, and luteolin acetylglucoside) the most abundant ones.

Despite raw and sous-vide-cooked red cardoon having a similar total content of (poly)phenols (13.15 and 13.14 mg/g dm respectively), differences in the content or in the individual (poly)phenol profile were found. The most abundant (poly)-phenols were 5-CQA (29.3–41.8% of total polyphenols) and 1,5-diCQA (20.7–39.2% of total polyphenols) in both cardoon samples, along with 3,5-diCQA (13.0% of total polyphenols) in raw cardoon, and 1,3-diCQA (18.2%) in the sous vide one. In addition, undigested raw cardoon showed a higher content of 5-CQA (1.4-fold), 1,5-diCQA (3-fold), 3,5-diCQA (1.9-fold), and succinylidiCQA I (2-fold) and II (1.7-fold) than sous vide cardoon. On the contrary, 11 (poly)-phenols (i.e., CQA derivatives 1–III, 3,4-diCQA, diCQA glucoside I, ferulic and isofericulic acids, apigenin, vicenin-2, luteolin, and hesperedin), which were only found in trace amounts or not detected in the raw cardoon, could be quantified in the sous vide one.

After an in vitro simulated oral-GI digestion, the content of individual (poly)phenols substantially decreased in both samples (Table 1), reaching a bioaccessibility of 0.7% (or 0.5% after dialysis) in digested raw cardoon, whereas 59.8% (or 38.1% after dialysis) of (poly)phenols still remained bioaccessible in digested sous-vide-cooked cardoon (Table 2).

During the 48 h in vitro simulated colonic fermentation, (poly)phenol bioaccessibility of raw and sous vide cardoon gradually decreased, while 9 catabolites were detected at different fermentation time points (Table 1). In addition, flavonoid aglycones such as luteolin and apigenin, as well as low-molecular-weight hydroxycinnamic acids, such as caffeic acid, increased during 4–8 h of fermentation but decreased
Table 1. (Poly)phenolic Profile and Catabolites from Raw and Sous-Vide-Cooked Red Cardoon before and after Simulated Oral GI Digestion \textit{In Vitro}, after Subsequent Dialysis, and during a Simulated Colonic Fermentation \textit{In Vitro} (0, 8, 24, and 48 h), as Analyzed by HPLC−MS/MS

| (poly)phenol                       | red cardoon |
|-----------------------------------|-------------|
| Phenolic compound                 | nondigested | digested | digested and dialyzed | 0 h fermented | 4 h fermented | 8 h fermented | 24 h fermented | 48 h fermented |
| Phenolic Acids                    |             |          |                      |              |              |              |                |                |
| Monocaffeoylquinic Acids (CQAs)   |             |          |                      |              |              |              |                |                |
| 1-CQA raw                         | 91.43 ± 5.91| 2.19 ± 0.28| 2.72 ± 0.11          | 2.16 ± 0.07  | 2.79 ± 0.06  | 3.73 ± 0.32  | <LOQ           | <LOQ           |
| sous vide                         | 146.99 ± 2.03| 159.98 ± 33.48| 218.43 ± 1.05      | 128.61 ± 0.92| 125.39 ± 2.85| 1.70 ± 0.06  | 0.94 ± 0.01   | 0.52 ± 0.08   |
| 3-CQA raw                         | 105.75 ± 2.68| 5.52 ± 0.91 | 4.14 ± 0.15         | 2.39 ± 0.06  | 2.81 ± 0.06  | 3.85 ± 0.41  | <LOQ           | nd             |
| sous vide                         | 1018.54 ± 108.87| 871.63 ± 52.04| 607.60 ± 11.68      | 356.66 ± 39.10| 384.91 ± 23.89| 22.01 ± 0.26| 192 ± 0.04    | <LOQ           |
| 4-CQA raw                         | 14.75 ± 0.78 | 5.12 ± 0.56 | 3.07 ± 0.03         | 2.27 ± 0.18  | 2.39 ± 0.01  | 2.80 ± 0.26  | <LOQ           | nd             |
| sous vide                         | 1379.72 ± 68.27| 1021.63 ± 110.42| 548.55 ± 4.84      | 304.21 ± 6.12| 273.17 ± 0.73| 6.77 ± 0.02  | 1.83 ± 0.07   | <LOQ           |
| 5-CQA raw                         | 5495.39 ± 284.01| 42.44 ± 1.62 | 31.12 ± 1.96        | 22.27 ± 0.13 | 7.61 ± 0.02  | 6.35 ± 0.68  | 0.78 ± 0.04   | 0.19 ± 0.04   |
| sous vide                         | 3845.58 ± 253.15| 2337.01 ± 88.40| 1939.52 ± 10.52    | 124425 ± 2.05| 75.81 ± 8.45 | 7.08 ± 0.18  | 2.13 ± 0.01   | 0.31 ± 0.01   |
| CQA Derivative I                  | <LOQ        | nd        | nd                  | nd            | nd            | nd            | nd             | nd             |
| raw                               | 693 ± 1.10  | <LOQ      | nd                  | nd            | nd            | nd            | nd             | nd             |
| sous vide                         | <LOQ        | nd        | nd                  | nd            | nd            | nd            | nd             | nd             |
| CQA Derivative II                 |             |          |                      |              |              |              |                |                |
| raw                               | 0.71 ± 0.08 | 0.25 ± 0.01| 0.62 ± 0.05         | <LOQ          | <LOQ          | <LOQ          | nd             | nd             |
| sous vide                         |             |          |                      |              |              |              | nd             | nd             |
| CQA Derivative III                |             |          |                      |              |              |              | nd             | nd             |
| raw                               | <LOQ        | nd        | nd                  | nd            | nd            | nd            | nd             | nd             |
| sous vide                         | 397 ± 0.57 | 3.36 ± 0.22| 3.95 ± 0.23         | 1.36 ± 0.29  | 1.34 ± 0.12  | <LOQ          | nd             | nd             |
| diCQAs and Derivatives            |             |          |                      |              |              |              |                |                |
| 1,3-diCQA                         |             |          |                      |              |              |              |                |                |
| raw                               | 34.75 ± 0.39| 0.35 ± 0.89 | 1.32 ± 0.02         | <LOQ          | <LOQ          | <LOQ          | <LOQ           | <LOQ           |
| sous vide                         | 2386.53 ± 210.42| 1520.01 ± 105.62| 687.96 ± 14.26   | 425.18 ± 14.90| 406.11 ± 14.85| 243.78 ± 3.12| 6.43 ± 0.08   | 2.33 ± 0.29   |
| 1,5-diCQA                         |             |          |                      |              |              |              |                |                |
| raw                               | 515.20 ± 479.34| 7.09 ± 1.43 | 2.63 ± 0.48         | 1.01 ± 0.03  | 1.11 ± 0.22  | 1.01 ± 0.00  | <LOQ           | nd             |
| sous vide                         | 2724.58 ± 137.79| 1119.44 ± 107.59| 513.46 ± 20.06    | 196.95 ± 65.83| 175.88 ± 18.81| 2.28 ± 0.15  | <LOQ           | nd             |
| 3,5-diCQA                         |             |          |                      |              |              |              |                |                |
| raw                               | 1696.62 ± 109.72| 3.75 ± 0.51 | 2.69 ± 0.08         | 1.58 ± 0.02  | <LOQ          | <LOQ          | nd             | nd             |
| sous vide                         | 898.67 ± 13.87| 406.43 ± 23.66| 255.19 ± 2.69      | 84.90 ± 19.35| 76.29 ± 11.17| 1.73 ± 0.02  | <LOQ           | nd             |
| 3,4-diCQA                         |             |          |                      |              |              |              |                |                |
| raw                               | nd          | nd        | nd                  | nd            | nd            | nd            | nd             | nd             |
| sous vide                         | 174.22 ± 4.22| 118.67 ± 0.25| 396.66 ± 1.34      | 11.44 ± 1.74 | 14.72 ± 0.03 | 1.92 ± 0.07  | nd             | nd             |
| 4,5-diCQA                         |             |          |                      |              |              |              |                |                |
| raw                               | 136.51 ± 8.56| 1.91 ± 0.20 | 1.37 ± 0.16         | 0.92 ± 0.01  | <LOQ          | nd            | nd             | nd             |
| sous vide                         | 217.17 ± 1493| 108.76 ± 4.33| 85.06 ± 2.93       | 68.85 ± 1.04 | 80.06 ± 3.94 | 1.67 ± 0.04  | <LOQ           | <LOQ           |
Table 1. continued

| (poly)phenol | nondigested | digested | digested and dialyzed | 0 h fermented | 4 h fermented | 8 h fermented | 24 h fermented | 48 h fermented |
|--------------|-------------|----------|-----------------------|---------------|---------------|---------------|----------------|----------------|
| diCQA Glucoside I |             |          |                       |               |               |               |                |                |
| raw          | <LOQ        | nd       | nd                    | Nd            | nd            | nd            | nd             | nd             |
| sous vide    | 1.40 ± 0.05 | 1.36 ± 0.00 | 1.40 ± 0.01           | Nd            | nd            | nd            | nd             | nd             |

| diCQA Glucoside II |             |          |                       |               |               |               |                |                |
| raw          | 1.55 ± 0.03 | nd       | nd                    | Nd            | nd            | nd            | nd             | nd             |
| sous vide    | 1.34 ± 0.05 | 1.34 ± 0.00 | 1.40 ± 0.00           | Nd            | nd            | nd            | nd             | nd             |

| Succinyl diCQA I |             |          |                       |               |               |               |                |                |
| raw          | 139.76 ± 3.42 | <LOQ    | <LOQ                 | nd            | nd            | nd            | nd             | nd             |
| sous vide    | 147.99 ± 2.74 | <LOQ    | nd                    | nd            | nd            | nd            | nd             | nd             |

| Succinyl diCQA II |             |          |                       |               |               |               |                |                |
| raw          | 10.83 ± 1.45 | 3.41 ± 0.15 | 3.47 ± 0.04          | 1.59 ± 0.12   | 1.66 ± 0.10   | nd             | nd             | nd             |

| Other Hydroxycinnamic Acids |             |          |                       |               |               |               |                |                |
| Caffeic Acid |             |          |                       |               |               |               |                |                |
| raw          | 5.43 ± 0.09  | 0.16 ± 0.08  | 0.42 ± 0.01           | 0.49 ± 0.09   | 2.92 ± 0.01   | <LOQ           | <LOQ           | <LOQ           |
| sous vide    | 66.72 ± 11.59 | 6.55 ± 0.12 | 0.84 ± 0.05           | 7.39 ± 1.34   | 49.27 ± 4.81  | 1.30 ± 0.00    | 2.76 ± 0.08    | 2.02 ± 0.35    |

| Caffeoyl-Hexoside |             |          |                       |               |               |               |                |                |
| raw          | 1.97 ± 0.30  | <LOQ      | nd                    | nd            | nd            | nd            | nd             | nd             |
| sous vide    | 2.49 ± 1.93  | 1.64 ± 1.32 | 0.77 ± 0.11           | <LOQ          | <LOQ          | nd             | nd             | nd             |

| Ferulic Acid |             |          |                       |               |               |               |                |                |
| raw          | nd          | <LOQ      | nd                    | nd            | nd            | nd            | nd             | nd             |
| sous vide    | 1.05 ± 0.07  | <LOQ      | nd                    | nd            | nd            | nd            | nd             | nd             |

| Isoferulic Acid |             |          |                       |               |               |               |                |                |
| raw          | nd          | nd        | nd                    | nd            | nd            | nd            | nd             | nd             |
| sous vide    | 1.83 ± 0.57  | nd        | nd                    | nd            | nd            | nd            | nd             | nd             |

| p-Coumaric Acid |             |          |                       |               |               |               |                |                |
| raw          | <LOQ        | 0.85 ± 0.06  | 0.74 ± 0.01           | <LOQ          | <LOQ          | <LOQ          | <LOQ           | <LOQ           |
| sous vide    | <LOQ        | <LOQ      | <LOQ                   | <LOQ          | <LOQ          | <LOQ          | <LOQ           | <LOQ           |

| Flavonoids |             |          |                       |               |               |               |                |                |
| Apigenin Derivatives |             |          |                       |               |               |               |                |                |
| Apigenin |             |          |                       |               |               |               |                |                |
| raw          | <LOQ        | <LOQ      | <LOQ                   | <LOQ          | <LOQ          | 0.98 ± 0.01    | nd             | nd             |
| sous vide    | 0.95 ± 0.06  | 0.88 ± 0.02 | <LOQ                  | <LOQ          | <LOQ          | 1.22 ± 0.01    | nd             | nd             |

| Apigenin 7-O-Glucoside |             |          |                       |               |               |               |                |                |
| raw          | 0.85 ± 0.04  | 1.20 ± 0.02 | 0.14 ± 0.08           | <LOQ          | nd            | nd            | nd             | nd             |
| sous vide    | 434 ± 1.35   | 4.23 ± 0.38 | 1.55 ± 0.05           | 0.55 ± 0.03   | <LOQ          | nd            | nd             | nd             |

| Apigenin 7-O-Glucononide |             |          |                       |               |               |               |                |                |
| raw          | 2.71 ± 0.24  | 1.48 ± 0.07 | 0.60 ± 0.00           | <LOQ          | <LOQ          | <LOQ          | <LOQ           | <LOQ           |
| sous vide    | 1.16 ± 0.18  | 0.56 ± 0.19 | <LOQ                  | <LOQ          | <LOQ          | <LOQ          | <LOQ           | <LOQ           |

| Apigenin 6,8-Di-C-Glucoside (Vicenin-2) |             |          |                       |               |               |               |                |                |
| raw          | nd          | nd        | nd                    | nd            | nd            | nd            | nd             | nd             |
| sous vide    | 14.14 ± 1.15 | 8.47 ± 0.64 | 4.31 ± 0.01           | 3.66 ± 0.62   | 4.14 ± 0.56   | 2.44 ± 0.12   | nd             | nd             |
| (poly)phenol                              | non-digested | digested | digested and dialyzed | 0 h fermented | 4 h fermented | 8 h fermented | 24 h fermented | 48 h fermented |
|------------------------------------------|--------------|----------|-----------------------|--------------|--------------|--------------|---------------|---------------|
| **Luteolin Derivatives**                 |              |          |                       |              |              |              |               |               |
| Luteolin                                 |              |          |                       |              |              |              |               |               |
| raw                                      | <LOQ         | 1.86 ± 0.05 | 1.41 ± 0.02          | 1.09 ± 0.04  | 2.40 ± 0.07  | 3.88 ± 0.18  | <LOQ          | <LOQ          |
| sous vide                                | 2.60 ± 0.35  | 1.86 ± 0.06 | 1.01 ± 0.12          | 2.62 ± 0.32  | 11.51 ± 0.30 | 21.44 ± 0.22 | 298 ± 0.14    | 1.32 ± 0.09   |
| Luteolin 7-O-Glucoside                   |              |          |                       |              |              |              |               |               |
| raw                                      | 35.62 ± 1.21 | 8.73 ± 0.71 | 3.82 ± 0.30          | 3.93 ± 0.00  | 2.92 ± 0.18  | 0.37 ± 0.03  | 0.22 ± 0.01   | 0.18 ± 0.04   |
| sous vide                                | 105.01 ± 7.30| 98.68 ± 5.13| 48.06 ± 2.81         | 41.72 ± 2.68 | 17.04 ± 0.97 | 4.60 ± 0.04  | 4.43 ± 0.00   | 4.32 ± 0.05   |
| Luteolin Acetylglucoside                 |              |          |                       |              |              |              |               |               |
| raw                                      | 56.44 ± 2.30 | 7.37 ± 0.79 | 2.99 ± 0.18          | 1.40 ± 0.04  | <LOQ         | nd           | nd            | nd            |
| sous vide                                | 41.97 ± 1.09 | 3.51 ± 0.05 | 2.23 ± 0.04          | 0.59 ± 0.14  | nd           | nd           | nd            | nd            |
| **Quercetin Derivatives**                |              |          |                       |              |              |              |               |               |
| Quercetin                                |              |          |                       |              |              |              |               |               |
| raw                                      | nd           | nd        | nd                    | nd           | <LOQ         | nd           | nd            | nd            |
| sous vide                                | nd           | nd        | nd                    | <LOQ         | <LOQ         | 1.65 ± 0.01  | <LOQ          | nd            |
| Quercetin 3-Glucoside (Isoquercitin)     |              |          |                       |              |              |              |               |               |
| raw                                      | 2.72 ± 0.09  | <LOQ      | nd                    | nd           | nd           | nd           | nd            | nd            |
| sous vide                                | 6.63 ± 1.18  | 3.14 ± 0.05| 1.42 ± 0.07          | <LOQ         | <LOQ         | nd           | nd            | nd            |
| **Hesperetin Derivatives**               |              |          |                       |              |              |              |               |               |
| Hesperetin 7-Rutinoside (Hesperidin)     |              |          |                       |              |              |              |               |               |
| raw                                      | <LOQ         | 11.89 ± 0.83| 8.35 ± 0.14          | 7.91 ± 0.17  | <LOQ         | <LOQ         | <LOQ          | nd            |
| **Phenolic Catabolites**                 |              |          |                       |              |              |              |               |               |
| 3-Hydroxyphenylacetic Acid               |              |          |                       |              |              |              |               |               |
| raw                                      | nd           | nd        | nd                    | nd           | 66.61 ± 2.02 | 67.89 ± 1.21 | 83.04 ± 5.14  | 200.29 ± 2.23 |
| sous vide                                | nd           | nd        | nd                    | nd           | 67.93 ± 3.75 | 75.67 ± 2.62 | 254.45 ± 3.25 | 4145.25 ± 51.05|
| 2,5-Dihydroxybenzoic Acid               |              |          |                       |              |              |              |               |               |
| raw                                      | nd           | nd        | nd                    | nd           | 0.45 ± 0.02  | 1.44 ± 0.02  | 2.77 ± 0.18   | 1.64 ± 0.08   |
| sous vide                                | nd           | nd        | nd                    | nd           | 1.53 ± 0.31  | 2.29 ± 0.12  | 6.29 ± 0.08   | 6.98 ± 0.89   |
| 3,4-Dihydroxybenzoic Acid (Protocatechuic Acid) |          |          |                       |              |              |              |               |               |
| raw                                      | nd           | nd        | nd                    | nd           | 1.85 ± 0.41  | 3.89 ± 0.16  | 2.49 ± 0.06   | 3.61 ± 0.93   |
| **3-(3-Hydroxyphenyl)propionic Acid**    |              |          |                       |              |              |              |               |               |
| raw                                      | nd           | nd        | nd                    | nd           | 1.79 ± 0.08  | 5.04 ± 0.18  | 2.56 ± 0.04   | 1.37 ± 0.03   |
| sous vide                                | nd           | nd        | nd                    | nd           | 37.31 ± 9.14 | 227.39 ± 4.59| 275 ± 0.01    | 4.89 ± 0.45   |

*Journal of Agricultural and Food Chemistry* pubs.acs.org/JAFC

https://doi.org/10.1021/acs.jafc.1c03014

*J. Agric. Food Chem.* 2021, 69, 9270 − 9286
Table 1. continued

| Phenolic compound         | Raw     | Digested | 0 h fermented | 4 h fermented | 8 h fermented | 24 h fermented | 48 h fermented |
|---------------------------|---------|----------|---------------|---------------|---------------|----------------|---------------|
| **4-Hydroxybenzoic Acid** |         |          |               |               |               |                |               |
| Raw                       | nd      | nd       | nd            | nd            | nd            | 3.86 ± 0.43    | 5.70 ± 0.43   |
| Sous-vide                 | nd      | nd       | nd            | nd            | nd            | 3.83 ± 0.00    | 1.71 ± 0.06   |
| **Phenylacetic Acid**     |         |          |               |               |               |                |               |
| Raw                       | nd      | nd       | nd            | nd            | nd            | 77.14 ± 3.74   | 596.09 ± 14.66|
| Sous-vide                 | nd      | nd       | nd            | nd            | nd            | Nd             | 71.30 ± 24.27 |

Results are expressed as mean μg (poly)phenolic compound per g red cardoon sample dry matter ± SD. nd, not detected; <LOQ, below the limit of quantification.

upon further fermentation. Both phenolic acids and flavonoids were mainly catabolized during the first 8 h of colonic fermentation, remaining bioaccessible for 0.1–2.2% of phenolic acids and 4.1–16.9% of flavonoids (Table 2). During the first 4 h of colonic fermentation, five catabolites were detected [i.e., 3-hydroxyphenylactic, 2,5-dihydroxybenzoic, protocatechuic, 3-(3-hydroxyphenyl)propionic and dihydrocaffeic acids], and their contents gradually increased throughout the 48 h of colonic fermentations, except for dihydrocaffeic acid whose content diminished after 8 h (Table 1). The catabolite 4-hydroxybenzoic acid was produced after 8 h of fermentation of both samples; and phenylactic acid and 1,2-dihydroxybenzene were produced after 24 h. Small amounts of 3-hydroxybenzoic acid were quantified at 48 h of fermentation of both raw and sous-vide-cooked cardoon. The highest total content of catabolites occurred after 48 h of fermentation of both raw and sous-vide-cooked cardoon (863.13 and 5899 μg/g dm, respectively) (Table 2). Finally, the total content of catabolites was higher in sous vide than in raw cardoon at different time points (1.7-fold at 4 h, 5.8-fold at 8 h, 8-fold at 24 h, and 6.8-fold at 48 h) (Table 2), mainly due to the increase of the most abundant catabolites [3-hydroxyphenylactic and 3-(3-hydroxyphenyl)propionic acids] (Table 1).

In Vitro Anti-inflammatory Activity. The anti-inflammatory activity in the small intestine was evaluated by measuring the impact of GI-digested raw and sous-vide cardoon on basal and LPS-stimulated cytokine secretion in differentiated Caco-2 cells. GI-digested raw cardoon neither significantly modified the basal secretion of the tested cytokines (IL-8, IL-6, IL-1β, TNF-α, and IL-10) in cells at any of the concentrations (i.e., 2.42 and 9.68 mg dm/mL), as compared to the control (untreated cells) (Figure 1a–e), nor did the digested sous-vide-cooked cardoon induced significant differences in the basal secretion of the tested cytokines at 2.42 mg dm/mL but caused a significant increase of IL-8 (p < 0.001), IL-1β (p = 0.001), TNF-α (p = 0.002), and IL-10 (p < 0.001) at 9.68 mg dm/mL, as compared to control. In addition, the increased secretion of IL-1β and TNF-α induced by digested sous-vide-cooked cardoon at 9.68 mg dm/mL was not significantly different to that caused by LPS.

Differentiated Caco-2 cells stimulated with 5 μg/mL LPS for 48 h (called “control + LPS”) showed a significant rise in IL-8, IL-6, TNF-α, IL-10 (p < 0.001), and IL-1β (p = 0.001) secretions (Figure 1a–e). Control + LPS was used as the control to assess the impact of GI-digested cardoon on the LPS-induced secretion of cytokines. At 2.42 mg dm/mL, digested raw cardoon significantly decreased the LPS-induced secretion of IL-8 (p = 0.020), whereas at 9.68 mg dm/mL it induced a significant reduction in the LPS-induced secretion of IL-8 (p < 0.001), IL-6 (p = 0.026), TNF-α (p = 0.014), and IL-10 (p = 0.004) to levels that were not significantly different from the control without LPS stimulation. On the contrary, digested sous-vide-cooked red cardoon did not significantly change the LPS-induced secretion of IL-8, IL-6, IL-1β, TNF-α, or IL-10 at the tested concentrations.

The anti-inflammatory activity in the colon was evaluated by measuring the impact of GI-digested and colonic-fermented raw and sous-vide cardoon on basal and LPS-stimulated cytokine secretion in HT-29 cells. Untreated HT-29 cells were used as the blank control (called “control”), and cells incubated with supernatants from 8 or 24 h NFC were used as a carrier control (called “NFC”) for the assessment of the
Table 2. Total (Poly)phenols and Phenolic Catabolites from Raw and Sous-Vide-Cooked Red Cardoon before and after Simulated Oral GI Digestion In Vitro, after Subsequent Dialysis and during a Simulated Colonic Fermentation In Vitro (0, 8, 24, and 48 h), as Analyzed by HPLC—MS/MS

| (poly)phenol | red cardoon | nondon digested | digested | digested and dialyzed | 0 h fermented | 4 h fermented | 8 h fermented | 24 h fermented | 48 h fermented |
|--------------|-------------|----------------|----------|----------------------|--------------|--------------|--------------|--------------|--------------|
| Phenolic Acids | Monocaffeoylquinic Acids (CQAs) and Derivatives | | | | | | | | |
| raw | 5707.33 | 55.27 (1%) | 41.04 (0.7%) | 29.10 (0.5%) | 15.60 (0.3%) | 16.73 (0.3%) | 0.78 (<0.1%) | 0.19 (<0.1%) |
| Sous vide | 6402.44 | 4393.86 (68.6%) | 3318.66 (51.8%) | 2033.07 (31.8%) | 1560.62 (24.4%) | 37.56 (0.6%) | 6.82 (0.1%) | 0.82 (<0.1%) |
| Fusicaffeoylquinic acids (dCQAs) and Derivatives | | | | | | | | | |
| raw | 7308.38 | 13.09 (0.2%) | 8.00 (0.1%) | 3.51 (<0.1%) | 1.11 (<0.1%) | 1.01 (<0.1%) | <LOQ | <LOQ | <LOQ |
| Sous vide | 6464.06 | 3295.71 (51%) | 1602.48 (24.8%) | 794.63 (12.3%) | 760.73 (11.8%) | 251.38 (3.9%) | 6.43 (0.1%) | 2.33 (<0.1%) | |
| Other Hydroxycinnamic Acids | | | | | | | | | |
| raw | 7.40 | 1.01 (13.6%) | 1.16 (15.7%) | 0.49 (6.7%) | 2.92 (39.4%) | <LOQ | <LOQ | <LOQ | <LOQ |
| Sous vide | 7.09 | 8.20 (11.4%) | 1.60 (2.2%) | 7.39 (10.3%) | 49.27 (68.4%) | 1.30 (1.8%) | 2.76 | 2.02 | |
| Total phenolic Acids | | | | | | | | | |
| raw | 13,023.11 | 69.37 (0.5%) | 50.21 (0.4%) | 33.10 (0.3%) | 19.63 (0.2%) | 17.74 (0.1%) | 0.78 (<0.1%) | 0.19 (<0.1%) | |
| Sous vide | 12,938.58 | 7697.77 (59.5%) | 4922.74 (38%) | 2837.09 (21.9%) | 2370.62 (18.3%) | 290.24 (2.2%) | 16.01 (0.1%) | 5.17 (<0.1%) | |
| Flavonoids | Apigenin Derivatives | | | | | | | | |
| raw | 3.56 | 2.68 (75.3%) | 0.74 (20.8%) | <LOQ | <LOQ | 0.98 (25.7%) | <LOQ | <LOQ | <LOQ |
| Sous vide | 2.09 | 14.13 (68.6%) | 5.98 (29%) | 4.20 (20.4%) | 4.14 (20.1%) | 3.65 (17.8%) | <LOQ | <LOQ | <LOQ |
| Luteolin Derivatives | | | | | | | | | |
| raw | 119.81 | 20.77 (17.3%) | 9.77 (8.2%) | 7.23 (6%) | 5.32 (4.4%) | 4.24 (3.5%) | 0.22 (0.2%) | 0.18 (0.2%) | |
| Sous vide | 158.58 | 135.60 (85.5%) | 69.08 (43.6%) | 50.08 (31.6%) | 28.55 (18%) | 26.04 (16.4%) | 7.41 (4.7%) | 5.64 (3.6%) | |
| Quercetin Derivatives | | | | | | | | | |
| raw | 2.72 | <LOQ | 0.00 | 0.00 | 0.00 | <LOQ | <LOQ | 1.65 (24.9%) | <LOQ | 0.00 |
| Sous vide | 6.63 | 3.14 (47.3%) | 1.42 (21.4%) | <LOQ | <LOQ | 1.65 (24.9%) | <LOQ | <LOQ | 0.00 |
| Total Flavonoids | | | | | | | | | |
| raw | 126.09 | 23.45 (18.6%) | 10.51 (8.3%) | 7.23 (5.7%) | 5.32 (4.2%) | 5.22 (4.1%) | 0.22 (0.2%) | 0.18 (0.1%) | |
| Sous vide | 197.70 | 161.21 (82.3%) | 84.39 (41.2%) | 54.28 (29.2%) | 32.68 (17.6%) | 31.34 (16.9%) | 7.41 (4%) | 5.64 (3%) | |
| Total (Poly)phenols | | | | | | | | | |
| raw | 13,146.20 | 92.82 (0.7%) | 60.72 (0.5%) | 40.33 (0.3%) | 24.95 (0.2%) | 22.96 (0.2%) | 1.00 (<0.1%) | 0.37 (<0.1%) | |
| Sous vide | 13,136.28 | 7858.98 (58.9%) | 5007.13 (38.1%) | 2891.37 (22%) | 2403.30 (18.3%) | 321.58 (2.5%) | 23.42 (0.2%) | 10.81 (0.1%) | |
| Phenolic Catabolites | | | | | | | | | |
| raw | 0.00 | 0.00 | 0.00 | 67.05 | 73.95 | 100.84 | 238.22 | 863.13 | |
| Sous vide | 0.00 | 0.00 | 0.00 | 72.08 | 123.73 | 584.65 | 1918.16 | 5899.00 | |

*Results are expressed as total μg (poly)phenolic compound per g red cardoon sample dry matter. Total (poly)phenol bioaccessibility (%) is included in brackets. <LOQ below the limit of quantification.
and at 8 and 24 h of sous-vide-cooked cardoon (p = 0.018 and 0.023) and FOS (p = 0.009 and 0.019) fermentations compared with NFC (Figure 4d). Bacteroides–Prevotella spp. showed the highest growth during fermentation when compared with the other tested populations of gut microbiota. There was no significant change in the total bacterial numbers with sous-vide-cooked cardoon and FOS, while a significant
increase was observed at 8 h of raw cardoon fermentation ($p = 0.046$) (Figure 4e).

**Impact on Lactic Acid and SCFA Production by Gut Microbiota.** Significant increases of acetate were detected during the fermentation of raw and sous-vide-cooked cardoon compared to the baseline (0 h) and NFC ($p < 0.05$) (Table 3). The highest concentration of acetate was detected at 48 h of fermentation with raw cardoon and FOS and at 24 and 48 h of fermentation with sous-vide cardoon. Propionate was the second more abundant SCFA produced during the fermentation of FOS and cardoon samples (Table 3). Similar to acetate, propionate production showed an increasing trend during fermentation but the highest increase occurred from 8 h to 24 h of fermentation of cardoon samples. The propionate
concentration from raw and sous-vide-cooked cardoon fermentations was not significantly higher than that in NFC at any time point, and it reached a statistical significance after 48 h of FOS fermentation ($p = 0.003$). Although the production of butyrate was lower than acetate and propionate, a slight but significant increase in the butyrate concentration was observed at 24 and 48 h of fermentation with raw and sous-vide-cooked cardoon compared to the baseline (0 h) ($p < 0.05$). However, this increase did not reach a statistical significance compared to NFC. The highest amount of butyrate was detected with FOS after 48 h of fermentation and this increase was significant compared to NFC ($p = 0.014$). The lactate concentration was very low in all the samples and did not reach a statistical significance compared to NFC.

Figure 3. Impact of 24 h fermented raw and sous-vide red cardoon on the basal and LPS-induced secretion of cytokines (a) IL-8, (b) IL-6, (c) IL-1β, (d) TNF-α, and (e) IL-10 in HT-29 cells (Luminex multiplex assay). Cells were pretreated with supernatants from 24 h of fermentation of cardoon samples or NFC at 0.25 or 1% v/v for 1 h, and treatment was continued for further 48 h in the absence or presence of 0.1 μg/mL LPS. Results are expressed as the mean of picograms of cytokine secretion per milliliter (pg/mL) ± SD ($n = 3$ experiments). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ indicate significantly different from control (untreated cells). Lack of asterisk indicates nonsignificant differences ($p \geq 0.05$) (both in comparison with control and NFC values, either with or without LPS stimulation, and between raw and sous-vide cardoon).
Concentrations of isobutyric and isovaleric acids were either below the detection limit or, in many cases, were detected only in a single donor (data not shown). The concentration of total SCFAs increased significantly (p < 0.05) at 8, 24, and 48 h of FOS fermentation, and in the case of raw and sous-vide-cooked cardoon fermentation at 48 h compared to NFC (p < 0.05). The highest concentrations of total SCFAs were found at 48 h of fermentation of FOS and cardoon samples, mainly due to acetate production but also propionate production. No significant differences were found between raw and sous-vide-cooked cardoon regarding their impact on lactic acid and SCFA production.

**DISCUSSION**

The application of culinary heat treatments to (poly)phenolic-rich plant foods, such as cultivated cardoon stalks, induces wall and cell ruptures and consequently the release of those (poly)phenols bound to the food matrix. However, some (poly)phenols remained linked to other macromolecules, such as dietary fiber during boiling, or are included into melanoidin structures when formed at high temperatures. Moreover, the inactivation of polyphenol oxidases by heat inhibits (poly)phenol degradation, and the use of little water and vacuum bags in the sous vide cooking avoids the (poly)phenols leaching into the water. All these reasons may explain the similar total content of (poly)phenols in raw and sous-vide-cooked red cardoon. After GI digestion, (poly)phenols were almost totally degraded by the digestive enzymes.
Table 3. Impact of Raw and Sous-Vide-Cooked Red Cardoon on the Production of Major SCFAs (Acetic, Propionic, and Butyric Acids) and Lactic Acid at Different Time Points (0, 8, 24, and 48 h) of In Vitro Colonic Fermentation, as Analyzed by GC-FID\textsuperscript{a}

| SCFA      | NFC      | FOS      | raw cardoon | sous vide-cooked cardoon |
|-----------|----------|----------|-------------|--------------------------|
| Acetate   |          |          |             |                          |
| 0 h       | 0.29 ± 0.29 | 0.26 ± 0.26 | 1.24 ± 0.07* | 1.59 ± 0.05**            |
| 8 h       | 4.88 ± 1.48 | 17.68 ± 1.51## ** | 16.65 ± 1.38## ** | 19.91 ± 0.64## ***       |
| 24 h      | 6.96 ± 4.49 | 32.28 ± 3.63## ** | 25.27 ± 3.89## * | 33.41 ± 2.84## **       |
| 48 h      | 8.67 ± 4.68 | 38.77 ± 2.69## ** | 34.22 ± 2.92## ** | 33.76 ± 4.69## **       |
| Propionate|          |          |             |                          |
| 0 h       | nd       | nd       | nd          | Nd                       |
| 8 h       | 1.02 ± 0.52 | 5.40 ± 4.06 | 2.75 ± 0.93 | 2.64 ± 0.80             |
| 24 h      | 1.61 ± 0.92 | 14.48 ± 7.13 | 9.80 ± 3.25 | 12.92 ± 4.72           |
| 48 h      | 2.14 ± 1.00 | 26.80 ± 2.39## ** | 11.65 ± 2.89 | 12.81 ± 5.28         |
| Butyrate  |          |          |             |                          |
| 0 h       | nd       | nd       | nd          | Nd                       |
| 8 h       | 0.36 ± 0.35 | 0.87 ± 0.44 | 0.10 ± 0.10 | 0.10 ± 0.10             |
| 24 h      | 0.52 ± 0.52 | 3.94 ± 1.76 | 1.74 ± 0.46# | 1.35 ± 0.15#           |
| 48 h      | 1.05 ± 0.61 | 6.83 ± 1.81* | 2.06 ± 0.36# | 1.70 ± 0.36#         |
| Lactate   |          |          |             |                          |
| 0 h       | nd       | nd       | nd          | Nd                       |
| 8 h       | 2.17 ± 2.16 | 3.01 ± 0.52# | 3.42 ± 1.05 | 4.71 ± 1.92             |
| 24 h      | 0.01 ± 0.00 | 6.76 ± 5.18 | 2.10 ± 2.10 | 2.30 ± 2.30           |
| 48 h      | nd       | nd       | 1.60 ± 1.60 | 2.07 ± 2.07            |
| Total     |          |          |             |                          |
| 0 h       | 0.29 ± 0.29 | 0.26 ± 0.26 | 1.24 ± 0.07* | 1.59 ± 0.05**            |
| 8 h       | 8.43 ± 1.87# | 26.96 ± 5.53* | 22.92 ± 2.98# | 27.36 ± 5.28## *        |
| 24 h      | 9.09 ± 5.88 | 57.46 ± 14.98# * | 38.92 ± 6.82# | 49.98 ± 7.61##         |
| 48 h      | 11.86 ± 6.29 | 72.40 ± 4.30## ** | 49.53 ± 5.19# | 50.33 ± 9.33## *        |

\textsuperscript{a}The effect of NFC and prebiotic FOS is also included. Results are expressed as mean ± SEM of lactic acid or SCFA of three independent fermentations with fecal samples from three different donors, \( p < 0.05 \) and \( ** p < 0.01 \), significantly different from the 0 h value within the same treatment. \( * p < 0.05 \), \( ** p < 0.01 \), and \( *** p < 0.001 \), significantly different from NFC at the same time point. nd, not detected.

and conditions in raw red cardoon, whereas they remained substantially bioaccessible in sous-vide-cooked red cardoon. A similar behavior in (poly)phenol bioaccessibility has been observed in cooked white cardoon,\textsuperscript{3} blanched\textsuperscript{2,26} and sous-vide-cooked globe artichoke,\textsuperscript{4} which are rich in phenolic acids, mainly CQAs. However, less degradation of (poly)phenols, particularly phenolic acids, was shown in raw \textit{Opuntia} cactus cladodes, which is rich in pectins, mucilages, and other dietary fibers.\textsuperscript{27} Then, the hydration of the dietary fiber during boiling may favor the retention of phenolic acids into macromolecules, inducing less degradation by digestive enzymes and conditions. This may also explain the positive effect of sous vide heat treatment on the bioaccessibility of red cardoon (poly)phenols and particularly CQAs.

The \textit{in vitro} anti-inflammatory activity of red cardoon stalks, in two culinary ways of consumption (raw and sous-vide-cooked), was assessed at both the small intestine (differentiated Caco-2 cells) and colon (HT-29 cells) level. LPS was used to induce a proinflammatory phenotype in both cell lines and thereby to evaluate the ability of red cardoon to protect against LPS-induced secretion of IL-8, IL-6, IL-1β, TNF-α, and IL-10. No previous studies that evaluate the \textit{in vitro} immunomodulatory activity of cardoon stalks, whether undigested, GI-digested, or colonic-fermented have been found.

The bioaccessible fraction of GI-digested raw red cardoon, but surprisingly not the sous-vide-cooked one, exerted \textit{in vitro} anti-inflammatory capacity in the human enterocyte-like cell line Caco-2 (Figure 1). This inflammatory protection was stronger at the highest concentration tested (9.68 mg dm/mL), which significantly \(( p < 0.05)\) counteracted the LPS-induced secretion of IL-8, IL-6, TNF-α, and IL-10. Contrarily, the bioaccessible fraction of digested sous-vide-cooked cardoon showed strong proinflammatory effects at the highest tested concentration (9.68 mg dm/mL) in Caco-2 cells in the absence of LPS stimulation (Figure 1). The most abundant (poly)phenol of digested red cardoon samples (5-CQA) was able to inhibit TNF-α- and H2O2-induced IL-8 secretions in a dose-dependent manner (0.5–2 mM) in differentiated Caco-2 cells.\textsuperscript{28} However, both anti- and proinflammatory effects have been reported in (poly)phenols, and their biological effects depend on the state of the target cells (e.g., resting vs activated), cell ontogeny and pathological conditions (e.g., normal vs cancer cells or macrophages), and the (poly)phenol concentration, exposure times, and pharmacokinetics.\textsuperscript{28} The higher bioaccessibility of (poly)phenols (and potentially other phytochemicals not identified in the current study) in digested sous-vide-cooked red cardoon might have led to an increased absorption of these compounds in the enterocyte-like cells,\textsuperscript{29} causing a proinflammatory response at high concentrations. This study corroborates that the consumption of higher amounts of (poly)phenols in a diet does not necessarily increase inflammatory protection.\textsuperscript{28} Considering a consumption of 150 g of cardoon, the tested concentrations (i.e., 2.42 and 9.68 mg cardoon dm/mL) would correspond to around 4.7 and 1.2 L of fluid in the small intestine, respectively. The
small intestine receives about 9.3 L of fluid each day and absorbs around 8.3 L during the day, so the tested concentrations were considered physiologically achievable. It would also be interesting to assess whether, in a context of a meal in which other non-phenolic-rich foods are included, lower concentrations of (poly)phenols from digested sous-vide-cooked cardoon could exert anti-inflammatory activity at the small intestine level. Also, further studies on the intestine inflammatory protection of other potential bioactive compounds different than (poly)phenols present in raw red cardoon would be of interest.

In a colon cell model (HT-29), after colonic fermentation, the high proinflammatory activity of NFC at the baseline (p < 0.05) may be related to the presence of some harmful compounds or metabolites derived from the gut microbiota activity of volunteers, which is in agreement with the highly reported adverse effects of fecal water on intestinal cells (cytotoxicity, mutagenicity, and genotoxicity). Gut microbiota action induced the formation of phenolic catabolites, nine of which were identified and quantified in the bioaccessible fractions of colonic fermented raw and sous-vide-cooked red cardoon. In the study reported by Júaniz et al., 3-(3-hydroxyphenyl)propionic acid was the most abundant catabolite produced during white cardoon stalks in vitro fermentation, whereas in the current study that catabolite was the second most abundant one after 3-hydroxyphenylacetic acid. Júaniz et al. did not identify 3-hydroxyphenylacetic acid, which might come from the α-oxidation of 3-(3′-hydroxyphenyl)propionic acid by gut microbiota. According to the proposed catabolic pathway for CQA degradation by Ludwig et al., red cardoon (poly)phenols, followed a major pathway that involved the formation of caffeic acid, followed by dihydrocaffeic acid, 3-(3′-hydroxyphenyl)propionic acid and 3-hydroxyphenylacetic acid as the final compounds identified. Despite the fact that sous-vide-cooked cardoon had a higher total content of (poly)phenols and catabolites (7–8-fold) than the raw one (Table 2), no response was observed in the secretion of cytokines induced by the NFC or LPS (Figures 2 and 3). The tested concentrations of colonic fermented cardoon samples (23.8 and 95.3 µg dm cardoon/mL) were around 100-fold lower than those of the digested vegetable due to cytotoxicity issues and are equivalent to an intake of 312 and 1250 mg of cardoon, respectively, for a fluid volume of 1 L in the colon. These data raise the question of whether higher concentrations of colonic fermented red cardoon (raw and sous-vide-cooked) may have the ability to modulate cytokine secretion in HT-29 cells, as digested cardoon did in differentiated Caco-2 cells.

To the best of the authors’ knowledge, this study also reports for the first time the impact of red cardoon (raw and sous-vide-cooked) on gut microbiota composition and SCFA production. Red cardoon showed a potential prebiotic effect on the human microbiota comparable to the well-established prebiotic FOS used as a reference control. Raw and sous-vide-cooked cardoon led to a beneficial shift of the microbiota composition by increasing health-promoting bacteria such as Bifidobacterium spp. and Lactobacillus/Enterococcus (Figure 4) as well as stimulating the production of SCFAs (Table 3). Moreover, increases in other bacterial populations such as Bacteroides—Prevotella spp. and E. rectale—C. cocoides were detected (Figure 4). Bacteroides—Prevotella spp. (8.5%) and E. rectale—C. cocoides (28%) are two of the most predominant groups in the human fecal microbiota. Some species of these groups are related with some pathological conditions (Bacteroides are increased in IBD and some species of Clostridium are pathogenic), but others may be considered potentially beneficial due to their saccharolytic metabolism that results in the production of SCFAs, which are known to have beneficial effects on the host health. Hence, the E. rectale—C. cocoides group includes many acetate- and/or lactate-converting butyrate producers, and an increase in the production of butyric acid was observed with raw cardoon, sous-vide-cooked cardoon, and FOS fermentations at 24 and 48 h, although it did not reach statistical significance due to great variability in the butyrate production among volunteers (Table 3). In addition, Bacteroides/Prevotella mainly produce acetic, succinic, and propionic acids, and a significantly high production of propionic acid with the raw and sous-vide-cooked red cardoon fermentations was observed at 24 and 48 h (p < 0.05), as compared to the baseline. Acetic acid was the main SCFA produced by both raw and sous-vide-cooked cardoon fermentations. This correlates with the fact that acetic acid is the most abundant SCFA produced, representing around 60–75% of the total SCFA in feces, as acetate synthesis is a widespread ability in the human gut microbiota.

Overall, slight differences were observed between the raw and the sous-vide-cooked vegetable. For instance, sous-vide-cooked cardoon as the substrate had a higher bifidogenic effect and longer effect in the stimulation of Bacteroides—Prevotella than raw cardoon. The application of culinary treatments has been shown to impact not only bioactive compounds such as (poly)phenols but also dietary fibers. Culinary treatments on vegetables cause the breakdown of macrostructures and mesostructures and increase the solubility of dietary fibers, making them more digestible. Thus, the differences between raw and sous-vide-cooked red cardoon on the stimulation of different bacterial populations may be related to their different contents of soluble fibers and (poly)phenols (and potentially other bioactive compounds). There is evidence about the ability of both fibers and (poly)phenols to positively modulate the composition and function of gut microbiota. Specifically, dietary fiber (mainly inulin-type fructans) obtained from artichoke, a vegetable that belongs to the same genus as cardoon, showed a prebiotic effect by stimulating the growth of different strains of Lactobacillus and Bifidobacterium spp. In addition, the coffee sample with high levels of chlorogenic acids [the main type of (poly)phenols in coffee that occurs in cardoon] induced a significant increase of Bifidobacterium spp. growth, and an equivalent quantity of chlorogenic acids alone induced the same effects along with an increase of the C. cocoides—E. rectale group. According to this, the stimulation of Bifidobacterium spp. by red cardoon may be due to the action of both fibers and (poly)phenols.

In summary, the application of a culinary treatment, and the GI digestion and gut microbiota action, following a realistic intake in vitro approach affects the (poly)phenol bioaccessibility and bioactivity of red cardoon stalks in the GI tract. Digested raw red cardoon showed anti-inflammatory activity against LPS-induced secretion of IL-8, IL-1β, TNF-α, and IL-10, while digested sous vide red cardoon with a higher content of (poly)phenols showed proinflammatory effects in enterocytes-like cells. However, colonic fermented raw and sous-vide-cooked cardoon did not show anti-inflammatory activity in HT-29 cells but both induced a beneficial effect on gut microbiota by stimulating Bifidobacterium spp. (especially sous vide cardoon) and Lactobacillus/Enterococcus spp. growth and...
by increasing the production of acetic acid mainly. Therefore, this study provides insights into mechanisms through which red cardoon stalk consumption might influence digestive health and provides evidence for further investigation in health-induced benefits of Cynara vegetables.

**ASSOCIATED CONTENT**

*Supporting Information*

The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acs.jafc.1c03014](https://pubs.acs.org/doi/10.1021/acs.jafc.1c03014).

MS characteristics of (poly)phenolic compounds identified in this study and cytotoxicity (MTT assay) of raw and sous-vide-cooked red cardoon after GI digestion in differentiated Caco-2 cells and after 8 and 24 h of colonic fermentation in HT-29 cells, with and without LPS stimulation (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

Maria-Paz de Peña – Departamento de Ciencias de la Alimentación y Fisiología, Facultad de Farmacia y Nutrición, Universidad de Navarra, 31008 Pamplona, Spain; IdiSNNA, Navarra Institute for Health Research, 31008 Pamplona, Spain; orcid.org/0000-0002-9994-4268; Phone: +34 948 425600 (806580); Email: mpdepena@unav.es; Fax: +34 948 425740

**Gessica Serra** – Department of Food and Nutritional Sciences, University of Reading, RG6 6AP Reading, U.K.

**Andrea Monteagudo-Mera** – Department of Food and Nutritional Sciences, University of Reading, RG6 6AP Reading, U.K.

**Jeremy Spencer** – Department of Food and Nutritional Sciences, University of Reading, RG6 6AP Reading, U.K.

**Concepción Cid** – Departamento de Ciencias de la Alimentación y Fisiología, Facultad de Farmacia y Nutrición, Universidad de Navarra, 31008 Pamplona, Spain; IdiSNNA, Navarra Institute for Health Research, 31008 Pamplona, Spain; orcid.org/0000-0001-6464-5412

Complete contact information is available at: [https://pubs.acs.org/doi/10.1021/acs.jafc.1c03014](https://pubs.acs.org/doi/10.1021/acs.jafc.1c03014)

**Funding**

This research was funded by the Spanish Ministry of Science and Innovation (AGL2014-52636-P) and Plan de Investigación de la Universidad de Navarra (PIUNA 2018-09). E. Huarte thanks Asociación de Amigos de la Universidad de Navarra and Banco Santander and the Government of Navarra for the PhD and mobility grants received, respectively.

**Notes**

The authors decline no competing financial interest.

**ABBREVIATIONS**

CQAs, caffeoylquinic acids; diCQAs, dicaffeoylquinic acids; FISH, fluorescence in situ hybridization; FOS, fructooligosaccharide; GC-FID, gas chromatography with flame ionization detection; GI, gastrointestinal; HPLC–MS/MS, high-performance liquid chromatography with tandem mass spectrometry; IL, interleukin; SCFAs, short-chain fatty acids; SCI, systemic chronic inflammation; LOQ, limit of quantification; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NFC, negative fermentation control; TNF, tumor necrosis factor

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