In vitro bioaccessibility, transepithelial transport and antioxidant activity of *Urtica dioica* L. phenolic compounds in nettle based food products

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Abstract

Nettle (*Urtica dioica* L.) is a well-known plant with a wide historical background uses of stems, roots and leaves. Nettle leaves are excellent source of phenolic compounds, principally 3-Caffeoylquinic Acid (3-CQA), Caffeoylmalic Acid (CMA) and rutin. Bioaccessibility (BAC) of nettle phenolics was evaluated with an *in vitro* dynamic digestion of real food matrices: type of food matrix and chemical characteristic affected kinetics of release and solubilisation, with highest BAC after duodenal digestion. Study of duodenal transepithelial transport highlighted low bioavailability (BAV) of native forms of 3-CQA, CMA and rutin. Simulation of colonic metabolism confirmed that phenolics become fermentable substrate for microflora, indicating the need to expand the study on the impact of phenolic compounds at the level of the large intestine. Photochemiluminescence assay of digestion fluids demonstrated that ingestion of *Urtica* based foods contributes to create an antioxidant environment against superoxide anion radical in the entire gastrointestinal tract (GIT).

Keywords

Phenolic compounds, *Urtica* based food, bioaccessibility, bioavailability, antioxidant activity.

Chemical compounds studied in this article

3-Caffeoylquinic Acid (PubChem CID: 1794427); Caffeoylmalic Acid (PubChem CID: 6124299); rutin (PubChem CID: 5280805)

Abbreviations

Bioaccessibility, BAC; Bioavailability, BAV; 3-Caffeoylquinic Acid, 3-CQA; Caffeoylmalic Acid, CMA; Gastrointestinal tract, GIT.

1. Introduction

Nettle is a green plant, distributed in temperate and tropical regions throughout the world. There are about 100 species of genus *Urtica*, which belong to the family Urticaceae. The most common species are *U. dioica*, dioecious, *U. urens* and *U. pilulifera*, that commonly grow wild in the subtropical areas of Asia, Europa and North America. [1,2]

Nettle leaves are anti-inflammatory, analgesic, antioxidant, antiulcer, diuretic and nutritive[3,4] and has been widely used, for fresh juice or infusion production, in the symptomatic treatment of rheumatoid arthritis, allergic rhinitis and gout.[5] The stinging nettle has been used for many years with food purpose, and in particular for salad, pies, soups and herbal tea preparation.[6,7]

Actually nettle possess a great economic potential due to its potential use in many fields of application, as in food and feed production, and also in medicinal and cosmetic sectors.[8] In the food sector the dried aerial part of leaves can be utilized as food ingredient of the pasta production.

The leaves of the *Urtica* species are an important source of minerals and vitamins and holds also a higher level of proteins[2], chlorophylls and carotenoids as β-carotene and lutein.[9] The phenolic fraction of *U. dioica* leaves is composed principally by the hydroxycinnamic acid derivatives 3-Caffeoylquinic Acid (3-CQA) and Caffeoylmalic Acid (CMA) by the flavonoid rutin,[10] and also by kaempferol, isorhamnetin and quercetin.[11,12] The presence of the CMA is reported in plants such as *Chelidonium majus* L., *Corydalis lutea* L.,[13] lettuce *Lactuca sativa* L.[14] and leaves of *Ficus carica* L.[15] The bioactive compound CMA is considered a distinctive component of *U. dioica* leaves and is absent in leaves of *U. urens*.[16]

The study of bioaccessibility (BAC) and bioavailability (BAV) are important for the evaluation of the potential health benefits of the to a polyphenol rich diet which can provide a significant protection against many degenerative chronically diseases[17]. *In vitro* static and dynamic digestion models are widely used to assess the structural changes, digestibility, and release of food components under simulated gastrointestinal conditions. The dynamic systems are very helpful in food research to

understand the effects of the digestion on food structure, nutrient delivery, nutrient interactions, and compounds bioavailability.[18]

A significant part of the ingested phenolic compounds presents a reduced bioaccessibility in the small intestine but in the large intestine are chemically modified by the colonic microflora getting better the bioactive activity and also the bioavailability.[19]

The aim of this work was to study: a) the bioaccessibility and bioavailability of nettle derived phenolic compounds presents in pasta enriched with dried nettle leaves, nettle tisane and nettle capsules and b) the radical scavenger activity of the simulated gastrointestinal fluid against superoxide anion radical using the Photochem® system.

2. Material and Methods

2.1 Sample and Reagent

Potassium chloride (KCl), potassium thiocyanate (KSCN), sodium dihydrogen phosphate (NaH_2PO_4), sodium sulfate (Na_2SO_4), sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), urea ($CO(NH_2)_2$), alpha-amylase (930 U mg^{-1} A3403), hydrochloric acid (HCl), sodium hydroxide (NaOH), formic acid (HCOOH), pepsin A (674 U mg^{-1} P7000), pancreatin (762 U mg^{-1} P1750), bile salts (B8631), phosphate buffer saline (PBS, pH 7.5) were purchased from Sigma-Aldrich (Madrid, Spain). Acetonitrile, methanol and formic acid were supplied by Fisher Scientific (Madrid, Spain). Deionized water was purchased from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 15 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath.

Standard of rutin hydrate was purchased from Sigma-Aldrich (Milan, Italy) and standard of 3-CQA was purchased from Extrasynthese (Lyon, France).

The reagent grade chemicals and cell culture components used, mainly Dulbecco's Modified Eagle's Medium (DMEM), penicillin, streptomycin, amphotericin B, HEPES, no essential aminoacids

100 (NEAA), Hank's Buffered Salt Solution (HBSS), crude mucin (Type II) were also purchased from
101 Sigma-Aldrich (Madrid, Spain). Fetal calf serum (FCS) was from Cambrex Company (Milan, Italy).
102 Pasta samples were obtained directly from a company located in Ferrara (Italy) and in particular two
103 types of commercial pasta enriched with dried nettle leaves (P1 and P2) and one commercial pasta
104 (PT). The dried leaves used for the tisane production (Ts) and the nettle capsules (Nc) (nutraceutical
105 supplement, Nature's Way®) were provided by an herbal shop (Herbolario Navarro, Valencia, Spain).
106 Forty grams of cooked pasta, 200 mL of tisane and 2 capsules were digested with the *in vitro* dynamic
107 gastrointestinal model. Pasta was cooked as reported in the label, whereas tisane was prepared by an
108 infusion of 5 g of herbs in 500 mL of boiling water during 10 minutes.
109
110 2.2 Extraction of phenolic compounds
111 Total extraction of phenolics from pasta samples and capsules was carried out using a chemical
112 followed by an enzymatic extraction like proposed by Pérez-Jiménez & Saura-Calixto[20] , with some
113 modifications. Briefly, 5 g of cooked pasta or 0,5 g of powder from nettle capsules were placed in a
114 test tube and extracted three times with 15 mL methanol/water (80:20) acidified with 0,1% of formic
115 acid, by using an Ika T18 basic Ultraturrax (Staufen, Germany) for 5 min. After each step, samples
116 were centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 2500 x g for 5 minutes at 4°C and
117 supernatant were recovered and combine. The residual was enzymatically digested as follow: the
118 pellet was suspended in 40 mL of water and after that 0.5 mL of pepsin solution (1g of pepsin
119 dissolved in 25 mL of HCl 0.1 N) and 2 mL of pancreatin and bile salts solution (0.1g of pancreatin
120 – 0.625g of bile salts dissolved in 25mL of $NaHCO_3$ 0.1N) were added. The tube was incubated at
121 37°C during 4h. After that, centrifugation of tubes was followed by the recovery of supernatants.
122 Enzymatic digestion was repeated two times and supernatants combined. The fractions obtained from
123 chemical and enzymatic extractions were filtered through a 0.22 μm filter (Phenomenex, Madrid,
124 Spain) before HPLC analysis. While the Tisane samples were directly filtered and analysed.
125

126 2.3 Bacterial strains and growth conditions

127 Thirteen commercial probiotic strains were used, namely *Lactobacillus animalis* CECT 4060T, *Lb.*
128 *casei* CECT 4180, *Lb. casei rhamnosus* CECT 278T, *Lb. plantarum* CECT 220, *Lb. rhuminis* CECT
129 4061T, *Lb. casei casei* CECT 277, *Bifidobacterium breve* CECT 4839T, *Bif. adolescentes* CECT
130 5781T and *Bif. bifidum* CECT 870T, *Corinebacterium vitaeruminis* CECT 537, *Streptococcus fecalis*
131 CECT 407, *Eubacterium crispatus* CECT 4840 and *Saccharomyces cerevisiae* CECT 1324, were
132 obtained at the Spanish Type Culture Collection (CECT, Valencia, Spain), in sterile 18% glycerol.
133
134 2.4 *In vitro* dynamic digestion model

135 The GI model setup consisted in a reactor, representing the stomach, the small intestine (SI), the
136 ascending colon (AC), the transverse colon (TC) and the descending colon (DC) interconnected by
137 plastic tubing and peristaltic pumps, as previously described by Ferrer, Manyes, Mañes, & Meca [21].
138 The unit was fully computer-controlled (LabVIEW software) for the addition of (a) food to the
139 stomach, (b) buffers to adjust pH of all compartments and (c) pancreatic juice to the SI. The transit
140 time of the flow of intestinal content between reactors was also automatically computer-controlled.
141 Temperature-controlled water flowed between the double glass-jacketed reactors to keep the
142 temperature at 37ºC. The pH was automatically controlled by addition of 0.2 M HCl (AC423795000,
143 Fisher Scientific, Ottawa, ON, Canada) and 0.5 M NaOH (415413, Sigma–Aldrich, Oakville, ON,
144 Canada) to the stomach vessel and SI to keep a pH of 2.0 in the stomach and 6.5 in the SI.
145 Samples prepared as described in section 2.1 were mixed with 60 mL of artificial saliva and placed
146 in a plastic bag containing 1 L of water at 37 °C, homogenized with a Stomacher IUL Instrument
147 (Barcelona, Spain) for 30s and introduced in the fermenter vessel. To simulate gastric digestion 5 g
148 of pepsin (674 U/mg) dissolved in 25 mL of 0.1 N HCl were introduced and the pH was corrected at
149 2. After 2 h of incubation 50 mL of gastric fluid was sampled and centrifuged at 2500 x g for 5 min
150 at 4°C; and the supernatant was recovered filtered with a 0.22 μm filter (Phenomenex, Madrid, Spain)
151 and injected in the LC-DAD.

Small intestine digestion was simulated by increasing the pH to 6.8 with $NaHCO_3$ (0.5 N), Thereafter, 25 mL of pancreatin (8 mg mL^{-1}) and 25 mL of bile salts (50 mg mL^{-1}) dissolved in 200 mL of water, were introduced incubated at 0,11 x g at 37 °C for 2 h. An aliquot of 50 mL of the duodenal fluid was sampled and centrifuged at 2500 x g during 5 min at 4°C, after that the supernatant was recovered and treated for the determination of duodenal phenols content.

To simulate the colonic fermentation, the microbial strains (previously described in section 2.3) were inoculated in the fermenter vessel at 10^{14} CFU/mL and incubated at 37 °C for 48 h. At 2h, 24h and 48h 50mL of the mixture were sampled and centrifuged at 2500 x g for 5 min at 4°C. The supernatant was recovered and filtered for the determination of the phenols colonic content.

2.5 Culture of *Caco-2* cells and duodenal transepithelial transport

Caco-2 cells (HTB-37) were obtained from the American Type Culture Collection (Rockville, MD) at passage 19. Stock cultures were maintained in Dulbecco's modified essential medium (DMEM) supplemented with FBS (16%) and Normocin (InvivoGen, San Diego, USA). The cells were cultured at 37ºC/95% humidified air/5% CO2. The medium was changed every second or third day and the cells were passaged at 80–90% confluence. After passage, the cells were re-seeded at a density of 11,000 cells cm_2. For experiments, cells were seeded at passage 29 in 12-well plates at a density of 200,000 cells per well. All experiments were carried out 14 days post-seeding. The bioaccessibility assays were performed using the method of Meca et al.[22]

2.6 HPLC-UV/DAD and HPLC-ESI-MS analysis

Identification and quantitative analysis of 3-CQA, CMA and rutin were performed with a Merck (Darmstadt, Germany) Hitachi LaChrom Elite HPLC system, consisting of an L-7100 pump, an L-2200 autosampler and an L-7455 diode array detector (DAD) equipped with a Teknokroma Brisa LC C18 column (150 × 4.6 mm, 3μm) . The collection of the data was achieved using the Merck – Hitachi Chromatography Data Station Software D-7000. The mobile phase was composed of (A) 0.1 M

HCOOH in H_2O and (B) ACN. The gradient elution was modified as follows: 0–15 min from 15% to 25% B, 15–25 min from 25% to 50% B. The post-running time was 5 min. The flow rate was 0.6 mL/min. The sample injection volume was 10 μL. The UV/DAD acquisitions were carried out in the range 190–400 nm and chromatograms were integrated at 350 nm. Three injections were performed for each sample.

Stock solutions of rutin and 3-CQA were prepared by dissolving 5 mg of the standard in 5 mL of pure methanol, obtaining a 1000 mg L^{-1} solution. These stock solutions were then diluted with pure methanol, in order to obtain suitable working solutions for the calibration curve. 3-CQA and rutin were determined by using these calibration curves. CMA was quantified with the calibration curve of the reference standard with the same chromophore (3-CQA) with correction by using the molecular weight ratio.

HPLC-ESI-MS and MS^2 analyses were carried out using an Agilent Technologies modular 1200 system (Agilent, Santa Clara, California, USA), equipped with a vacuum degasser, a binary pump, a thermostated autosampler, a thermostated column compartment and a 6310A ion trap mass analyzer with an ESI ion source. The HPLC column and the applied chromatographic conditions were the same as reported for the HPLC-UV/DAD system. The flow rate was split 5:1 before the ESI source. The injection volume in the HPLC system was 5μL. The capillary voltage set to 3500 V, the desolvating temperature was 350 °C. Nitrogen was used as a drying (flow rate 8 L/min) and nebulising gas (pressure 25 psi). The mass spectrometer operated in negative full-scan mode in the scan range 100–800 Da. MS^2 spectra were automatically performed with helium as the collision gas in the m/z range 100–800 with the SmartFrag function.

2.7 Determination of antioxidant capacity by photochemiluminescence (PCL-ACL) method (Photochem®)

The photochemiluminescence assay, based on the methodology of Popov et al.,[23-24] was used to measure the antioxidant activity of digestion fluids against superoxide anion radicals generated from

luminol, a photosensitizer, when exposed to UV light at λ max = 351 nm. The antioxidant activity of extracts was measured using ACL kits (Analytic Jena, Jena, Germany) with a Photochem apparatus (Analytic Jena, Jena, Germany). In the PCL-ACL assay, the photochemical generation of free radicals is combined with the sensitive detection using chemiluminescence. In ACL studies, the kinetic light emission curve was monitored for 3 min and expressed as mM Trolox equivalents. The areas under the curves were calculated using the PCL soft control and analysis software. The presence of Trolox (used as standard for the calibration curve) or any antioxidants from the samples reduce the magnitude of the PCL signal, and hence, the area calculated from the integral. The observed inhibition of the signal was plotted against the concentration of Trolox added to the assay medium. The concentration of the added sample was such that the generated luminescence during the 3-min sampling interval fell within the limits of the standard curve.

For ACL assay, 2.3 mL of reagent 1 (solvent and dilution reagent, methanol), 0.2 mL of reagent 2 (buffer solution), 25 μL of reagent 3 (photosensitizer: luminol 1 mmol/L), and 10 μL of standard or sample solution were mixed and measured. Luminol is used as a photosensitizer and as a detecting substance for free radicals. Trolox were used for the standard calibration curve from 0.25 to 2 nM.

2.8 Statistical analysis

Data sets were statistical analysed by analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) to assess the statistical significance of measured differences and post hoc Tukey's test were used to compare groups. P values < 0.05 were regarded as a significant. All statistical calculations were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1 Bioaccessibility of phenolic compounds after *in vitro* dynamic digestion simulation

Bioaccessibility of a bioactive compound present in a food matrix may be limited by its ability to be released from the matrix and by its interaction with other components presents in the gastrointestinal

tract. The interfering components may arise from the ingested food matrix or may be naturally present within the GIT.[25]

Phenolic compounds presents in pasta samples and in anutraceutical supplement were extracted with a chemical and enzymatic extractions, while Tisane, prepared following label's instruction, was directly analysed as described in section 2.2. Identification of 3-CQA and rutin was carried out by comparison the retention time, the UV spectra and the MS fragmentation with standard solutions using the LC-DAD and the LC-ESI-MS analysis. Identification of CMA was carried out by MS and MS^2 data comparing the mass spectra acquired with other data published in the scientific literature.[12,14,15] The MS spectrum shown in Figure 1 presents a precursor ion at *m/z* 295.0 with MS^2 fragmentation product ions of 178.9 *m/z*, corresponding to the caffeic acid, and another fragment with *m/z* of 133.1, corresponding to the malic acid.

The phenolic compounds presents in the samples studied were digested using a simulated *in vitro* dynamic digestion and analyzed by LC-DAD. As evidenced in the results plotted in Table 1 the maximum of BAC were detected after duodenal and colonic (2h) digestion phases in all the samples analysed after mechanical and enzymatic disruption of food structure. Also in the sample of the tisane, where the phenolic compounds are not linked in a solid structure, the highest level of BAC data were evidenced in the duodenal step, evidencing that the solubility of the bioactive compounds in the digestion fluid affected the BAC of the compounds studied.

The type of matrix presented a great influence on phenols BAC, and in particular for pasta samples after the duodenal phase, the 3-CQA presented the lowest BAC data compared with tisane and the nutraceutical supplement whereas rutin showed a lowest BAC than tisane. Differently, CMA showed comparable levels of BAC in all the matrices. The lowest BAC of 3-CQA and rutin presents in the solids matrices could be due to the complexity of food matrices and also to the gradually release of bound phenols along the digestion progress.[26]

The 3-Caffeoylquinic acid and Caffeoylmalic acid are both esters of caffeic acid, but showed substantial differences on their kinetics of release from food matrix and on their solubilisation and

stability on gastrointestinal fluid. Lee et al.,[27] founded that hydroxycinnamic acids from leaves of *Crepidiastrum denticulatum* were rapidly accessible in the stomach and duodenum with more than 60% of 3-CQA released and the digestive stability were markedly low after ileum phase of simulated digestio. The authors evidenced also that the Caffeoylmalic was less bioaccessible at the end of stomach phase digestion and was less metabolized by colonic microflora if compared with 3-CQA. The simulation of colonic fermentation indicated that polyphenols became fermentable substrates for bacterial microflora, producing a bioconversion of these compounds depending on the microbiota population, chemical structure, and the effect of co-consumed foods.[28] The 3-CQA is more metabolized than CMA, in fact in the samples of pasta after 48h incubation; this compound was not detectable whereas CMA showed a residual BAC percentage of 14.5% and 8.1% respectively In P1 and P2 sample.

These results confirm that the typology of food matrix and also the chemical characteristics of each phenolic compound are important on could influence the BAC of these compounds along the GIT.

3.2 Transepithelial transport study with *Caco-2* cell model

The bioavailability (BAV) of 3-CQA, CMA and rutin was carried out employing an *in vitro* method based on *Caco-2* cell monolayer that simulate the transepithelial transport of small intestine. *Caco-2* cell model is the international validated cell model used to study the passive drug absorption across the intestinal epithelium.[29]

Unidirectional phenolics transport was assessed from the apical to basolateral side of differentiated cell monolayer. Transport efficiencies were expressed as the percentage of phenols concentration initially loaded into the apical side and detected on the basolateral side at incubation of 4h (Table 2). The results showed that the transepithelial transport was generally low for 3-CQA, CMA and rutin in all samples tested. Nc presented the lowest transport and BAV data whereas the rutin was not detectable after 4h of exposure. The BAV data of CMA and rutin presents in P1 and Ts sample do

not present any statistically difference, whereas the 3-CQA presented significant differences between the three types of matrix.

The 3-CQA was also partially metabolized in apical compartment after 4h of exposure to the Caco-2 cells monolayer, in fact in Ts the residual percentage compared with the quantity initially loaded was of 54.32 %. According to previous studies,[27,30] these results confirmed that 3-CQA is poorly absorbed in its native form and can reach the colon where it can be metabolized by colonic microflora. Konishi et. Al[31] demonstrated that 3-CQA is mainly transported via the paracellular pathways and the principal metabolites formed by colonic metabolism can be actively absorbed at the level of large intestine.

CMA showed the same low permeability of 3-CQA, but seemed to be lowly metabolized into the apical compartment where the remaining quantity is higher than 85%.

Yang et. al.[32] demonstrated the low permeability of rutin in Caco-2 cell model and the decrease of transepithelial transport of glycosides and hydrolysate flavonoids. Absorption of rutin was observed in the small intestine and the two absorption metabolites detected in plasma, are quercetin-3-O-glucoronide and isorhamnetin-3-O-glucoronide.[33] Also, in the large intestine the enzymes produced by colonic microflora produced the hydrolysis of rutin with the release of quercetin, 3,4-dihydroxyphenylacetic acid and smaller quantities of 3-hydroxphenylacetic acid. [34]

3.3 Determination of antioxidant capacity by PCL-ACL method

The antioxidant capacity of gastrointestinal simulated fluid was evaluated using the Photochem® device and the ACL kit supplies by Analytic Jena (Jena, Germany). O_2^- is one of the most important free radicals in the body, which has been implicated in the initiation of oxidation reactions associated with ageing, and it plays an important role in the formation of other reactive oxygen species.

In this study, the simulated fluid of each phase of dynamic digestion was sampled for ACL measurements, and results are expressed as μM of Trolox equivalent solution (Fig. 2). Duodenal and colon 2h simulated fluids of each digestion possessed highest antioxidant capacity in according with

307 the highest BAC of major phenolic compounds. However, after 24h and 48h of colonic metabolism,
308 the simulated fluids showed good radical scavenging: that could be due to the residual non-
309 metabolized native phenols and also contribute to radical scavenging from the products of colonic
310 metabolism. These results confirmed that the assumption of foods rich in polyphenols contributes to
311 create an antioxidant environment that can counteracting postprandial oxidative stress in the entire
312 GIT, with potentially health benefits. Polyphenols rich food or beverage can be assumed in their
313 original form, but also can be transformed and alternatively used as functional ingredients of
314 processed foods to provide the health benefits associated with these phytochemicals.[26]
315 The intake of pasta enriched with dried nettle leaves instead of traditional pasta caused a significant
316 increasing of antioxidant capacity of gastrointestinal lumen from stomach to large intestine (Fig 2.A).
317 Furthermore, the assumption of 80g of pasta with nettle studied in this work generated an antioxidant
318 environment in the gastrointestinal lumen comparable to the assumption of 140 mL of nettle tisane
319 and 4 nettle capsules. These results indicated that pasta functionally enriched with dried nettle leaves
320 can substitute the intake of a nutraceutical supplement confirm the promising application of nettle in
321 the food sector.
322 Traditional pasta is a rather poor source of physiologically active compounds; thus, fortification of
323 pasta with phenol-rich ingredients, like nettle leaves, can be used to increase the healthy quality of
324 this product. Studies about fortification of pasta with vegetable leaves were previously performed
325 aimed at expanding the nutritional and nutraceutical quality of this popular dish.[35-36]
326

327 ## 4. Conclusion

328 The addition of dried-powdered nettle leaves to pasta formulations effectively increased significantly
329 the functional properties of this food product, increasing protection against oxidative stress in the
330 gastrointestinal environment. Moreover, further studies are needed to assess the supply of other
331 nutrients, like carotenoids, vitamins and minerals, due to the use of nettle leaves as an ingredient for
332 pasta supplementation.

The influence of the gastrointestinal digestion on the phenolic compounds presents in nettle demonstrated the relation between the chemical structure of these compounds and the BAC.

The study of the duodenal transepithelial transport of the phenolic compounds highlighted the general low bioavailability of 3-CQA, CMA and rutin with the consequence reaching of these compounds in the large intestine. The role of colonic microflora metabolism needs to be fully examined in order to evaluate the impact on phenolic rich diets on diversity and functionality of large intestine microbiota.

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446 **Legend of figures and tables**

447

448 **Fig. 1** Mass spectra (MS) and MS^2 spectra of Caffeoylmalic acid in pasta with nettle (P1) chemical
449 extract.

450

451 **Fig. 2** Antioxidant Capacity (PCL-ACL) of simulated digestion fluids in a) Pasta samples, b) Nettle
452 tisane (Ts) and c) Nettle capsule (Nc). Data are expressed as µM of equivalent Trolox solution for g
453 of sample (Means ± SD, n=6).

454

455 **Table 1**. Bioaccessibility of *Urtica dioica* phenolic compounds in Nettle based foods (P1 and P2=
456 samples of pasta enriched with Nettle, TS=Nettle tisane, NC=Nettle capsules, 1= µg/g of dried pasta
457 P1 and P2, µg/mL of nettle tisane Ts, µg/g of nettle capsules Nc, 2= not detected). Values with no
458 letters in common (in row) are significantly different (p<0,05) (Means ± SD, n=9).

459

460 **Table 2**. Transepithelial transport of *Urtica dioica* phenolic compounds in Nettle based foods after
461 4h of exposure with Caco-2 monolayer model (1= not detected, P1, pasta enriched with Nettle, Ts,
462 Nettle tisane, Nc, Nettle capsules). Values with no small letters in common (in columns, compared
463 for each compound) are significantly different (P<0.05) (Means ± SD, n=9).

464

465 **Table 3**. Bioavailability (Means ± SD, n=9) of *Urtica dioica* phenolic compounds ((1= not detected,
466 P1, pasta enriched with Nettle, Ts, Nettle tisane, Nc, Nettle capsules). Values with no small letters in
467 common (in columns, compared for each compound) are significantly different (P<0.05) (Means ±
468 SD, n=9).

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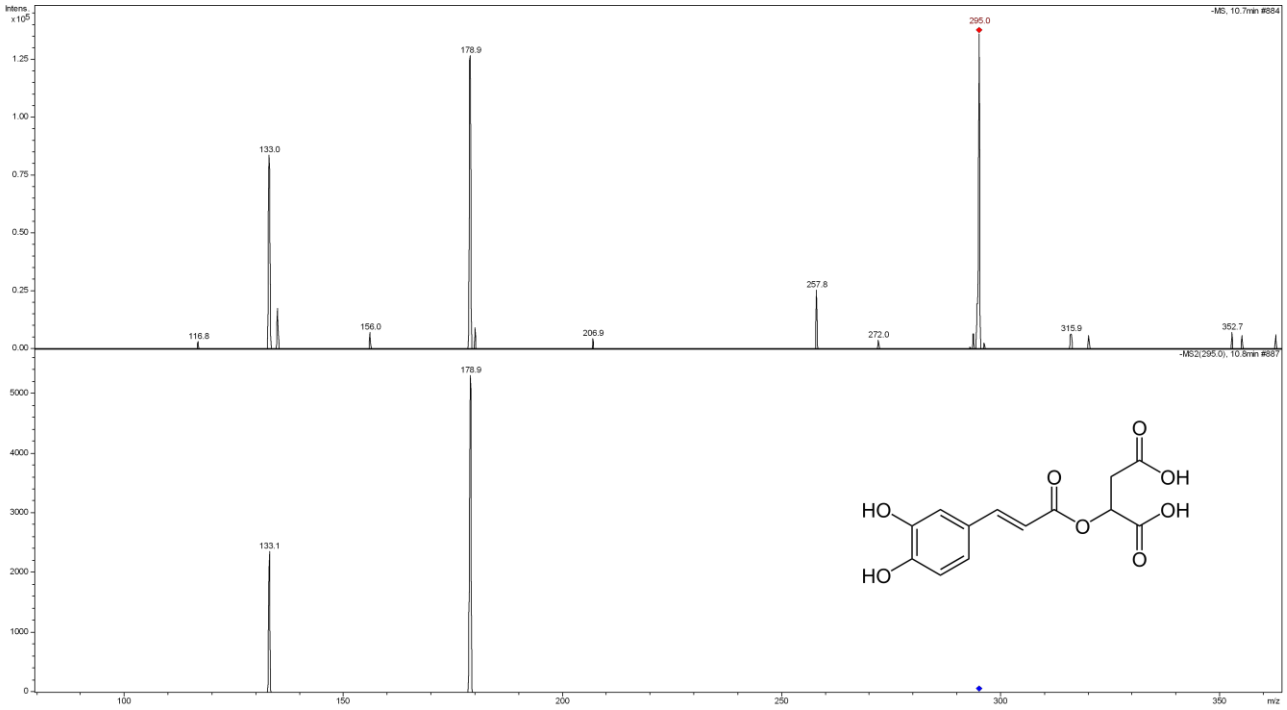
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1.00
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295.0
178.9
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156.0
206.9
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5000
4000
3000
2000
1000
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178.9
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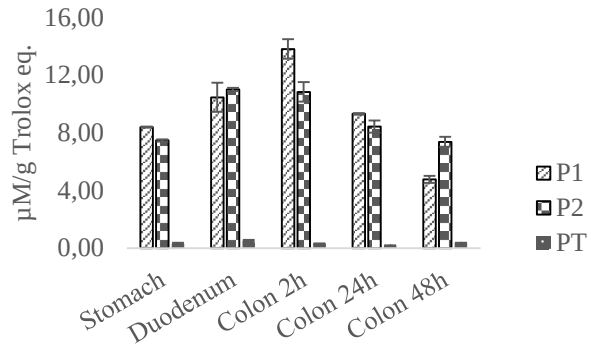
Fig. 1

a)


16,00
12,00
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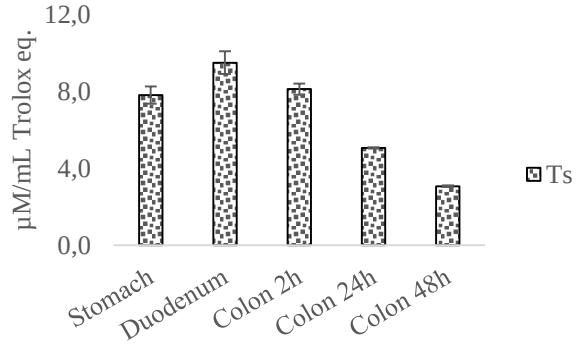
µM/g Trolox eq.
P1
P2
PT
Stomach
Duodenum
Colon 2h
Colon 24h
Colon 48h

b)


12,0
8,0
4,0
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µM/mL Trolox eq.
Ts
Stomach
Duodenum
Colon 2h
Colon 24h
Colon 48h

c)

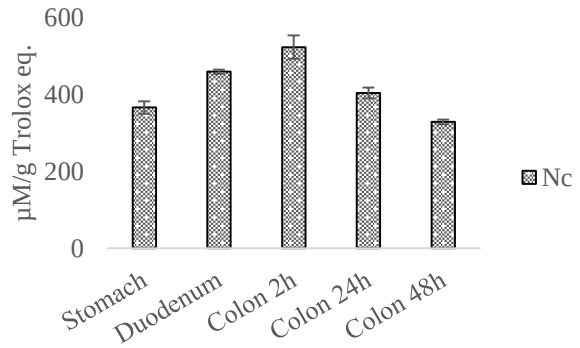
600
400
200
0
µM/g Trolox eq.
Nc
Stomach
Duodenum
Colon 2h
Colon 24h
Colon 48h

Fig. 2

493 **Table 1.**

	Bioaccessibility (%)			
Compound	**P1**	**P2**	**Ts**	**Nc**
3-O-Caffeoylquinic Acid				
Ingested[1]	183.0±5.1	274.5±17.9	28.0±1.2	4813.5±57.3
Stomach	42.1±0.5^{a}	35.0±6.5^{a}	67.9±5.6^{b}	64.6±2.0^{b}
Duodenum	68.5±4.4^{c}	64.6±0.3^{c}	95.9±1.1	85.0±0.8
Colon 2h	58.7±3.9^{d}	59.6±1.2^{d}	77.1±6.0^{c}	77.3±2.4^{c}
Colon 24h	nd[2]	2.9±1.3^{f}	23.2±7.0	6.3±4.8^{f}
Colon 48h	nd[2]	nd[2]	8.4±4.6^{g}	4.2±0.8^{g}
Caffeoylmalic Acid				
Ingested[1]	172.5±6.1	258.7±9.8	57.7±3.2	5563.1±24.6
Stomach	7.4±2.6^{ab}	4.9±3.0^{a}	10.9±2.4^{b}	11.5±2.1^{b}
Duodenum	94.5±3.6^{c}	92.4±0.6^{c}	90.6±3.8^{d}	88.7±2.3^{d}
Colon 2h	84.8±2.2^{e}	87.4±0.4^{e}	77.8±1.3^{d}	79.5±3.8^{f}
Colon 24h	19.4±1.6	30.8±2.7^{g}	13.6±1.4	28.7±0.8^{g}
Colon 48h	14.5±0.6^{h}	8.1±2.2^{i}	7.1±2.1^{i}	13.8±0.7^{h}
Rutin				
Ingested[1]	87.9±2.3	95.2±4.7	6.6±0.4	1415.3±11.3
Stomach	14.0±2.9^{a}	10.4±7.8^{a}	35.4±5.1	11.7±0.3
Duodenum	56.0±3.1	44.4±4.2	69.0±3.8	30.9±0.2
Colon 2h	45.7±0.2	53.6±4.8	70.4±4.6	36.0±0.1
Colon 24h	33.5±0.8	19.5±0.5	48.3±3.4	10.7±1.0
Colon 48h	25.1±0.6	15.0±0.5	36.3±3.9	nd[2]

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505 **Table 2.**

		Transepithelial transport (%)	
Sample	**Compound**	**Apical compartment**	**Basolateral compartment**
P1	3-O-Caffeoylquinic Acid	81.49±1.76[a]	2.48±0.34
	Caffeoylmalic Acid	88.64±1.73[b]	2.17±0.86[ab]
	Rutin	83.55±1.90[c]	2.47±0.65[c]
Ts	3-O-Caffeoylquinic Acid	54.32±0.98	3.08±0.33
	Caffeoylmalic Acid	94.36±2.45[b]	2.81±1.03[b]
	Rutin	94.75±7.37[c]	3.98±1.99[c]
Nc	3-O-Caffeoylquinic Acid	78.13±8.43[a]	0.95±0.16
	Caffeoylmalic Acid	94.36±2.45	1.26±0.31[a]
	Rutin	85.49±2.53[c]	nd[1]

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527 **Table 3.**

Sample	Compound	Bioavailability (%)
P1	3-O-Caffeoylquinic Acid	1.58±0.28
	Caffeoylmalic Acid	2.02±0.45[a]
	Rutin	1.30±0.34[b]
Ts	3-O-Caffeoylquinic Acid	2.89±0.31
	Caffeoylmalic Acid	2.89±1.06[a]
	Rutin	2.91±1.45[b]
Nc	3-O-Caffeoylquinic Acid	0.55±0.14
	Caffeoylmalic Acid	0.92±0.28
	Rutin	nd[1]

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