Absorption and isomerization of caffeoylquinic acids from different foods using ileostomist volunteers

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Abbreviations

p$K_{a\ (37°C)}$	ionization constant in water
diff(log P^{N-I})	difference between log P^N and log P^I
log P^I	logarithm of the partition coefficient of a given compound in its fully ionized form
log P^N	logarithm of the partition coefficient of a given compound in its neutral form
log $D^{6.8}$	logarithm of the distribution coefficient at pH 6.8
MRP2	multidrug resistance protein 2
CAJ	cloudy apple juice
AS	apple smoothie
CQA	caffeoylquinic acid
diCQA	dicaffeoylquinic acid
QA	quinic acid
CA	caffeic acid
CGA	chlorogenic acids
GIT	gastrointestinal tract

ABSTRACT

Background. Polyphenols are thought to play important roles in human nutrition and health but these health effects are dependent on their bioavailability. This study is one of a series with the aim of determining possible effects of food matrices on caffeoylquinic acid (CQA) bioavailability using ileostomy volunteers.

Methods. After a CQA-free diet, ileostomists consumed coffee (746 *μ*mol total CQA), and CQAs in excreted ileal fluid were subsequently identified and quantified with HPLC–diode array detection and HPLC-ESI-MS/MS. In our previous studies, other food sources such as cloudy apple juice (CAJ) (358 *μ*mol CQA) and apple smoothie (AS) (335 *μ*mol CQA) were investigated with the same model.

Results. Interesterification of CQA from both apple matrices was observed during gastrointestinal passage, whereas CQA consumed in coffee was not influenced by interesterification reactions. In total, 74.3%, 22.4%, and 23.8% of the CQA from CAJ, AS, and coffee, respectively, was absorbed or degraded.

Conclusion. Our results show that variations in food matrices and variations in phenolic composition have a major influence on intestinal bioavailability and interesterification of the investigated subclass of polyphenols, the CQAs.

Keywords

caffeoylquinic acid, apple juice, apple smoothie, coffee, bioavailability, ileostomy

Introduction

Hydroxycinnamic acids, especially CQAs (fig. 1), are a subclass of (poly)phenols. CQAs play an important role in polyphenol intake because of their wide distribution among edible plants [1,2]. The reported health effects of CQAs and of foods with high CQA content are complex; antioxidant activity *in vivo* [3,4] and an increasing plasma-antioxidant capacity after coffee consumption [5] have been described. Several studies have demonstrated the prevention of carcinogenesis by apples (*Malus domestica* Borkh.) and apple constituents *in vitro*, as summarized by Koch *et al.* and Gallus *et al.* [6,7].

Human studies have tried to link the described effects directly to the CQA intake by observing the oral bioavailability, with controversial results, as summarized in [8,9]. In a human study with artichoke leaf extract consumption [10], the renally excreted amount of CQA metabolites was about 4% of the administered CQA dose, independent of amount consumed. Data on coffee CQA bioavailability are difficult to interpret because of the wide spectrum of chlorogenic acids (CGAs) in this beverage. Rechner *et al*. [11] performed a study with a daily ingested CQA intake from six cups of coffee (898.4 ± 5.0 mg CQA in total). They did not find any intact CQAs in the urine and estimated a hypothetical absorption rate of 5.9% for CQAs and derivatives; although recent studies indicate that the absorption rate is higher. For the first time, in 2007 Monteiro *et al*. [12] identified three isomers of CQA, as well as diCQA, in plasma, and identified 5-CQA in urine, after the consumption of coffee containing 1037 ± 35 mg CQA. In another study [13] the administration of green coffee extract in capsules containing 121.0 mg CQA demonstrated a high bioavailability at lower doses. The recoveries of all chlorogenic

acids were calculated as 33 ± 23.1% for plasma and 5.5 ± 10.6% for urine. Stalmach *et al*. [14] were able to confirm a high bioavailability with a similar CQA dose of 94.9 mg from consumption of coffee. However, only traces of 5-CQA were found in plasma and, in total, 29.1 ± 4% of the CQA was recovered renally as metabolites. Administration of different amounts of CGA seems to have minor effects on the percentage absorbed as shown by our recent dose response study [15].

Owing to these controversial results, we conclude that several variables may influence CQA absorption, especially food matrices with a different polyphenol composition. Therefore, we chose three different food sources: in previous studies, CAJ containing 358.2 *μ*mol CQA (126.9 mg) and AS (a beverage containing 60% CAJ and 40% apple puree) containing 335.0 *μ*mol CQA (118.8 mg); and in the study presented here, an instant coffee–beverage containing 746.0 *μ*mol CQA (264.3 mg). For all three studies, we used healthy ileostomy volunteers as in our previously published papers [16-18] and we focused on differences in the ileal excretions of CQA and differences in CQA interesterification depending on the food source.

Materials and Methods

Chemicals

All of the chemicals and solvents used in these experiments were of HPLC or reagent grade. D-(−)-quinic acid and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany) and 3,4,5-trimethoxyphenylacetic acid from Fluka (Steinheim, Germany). Ethanol *p.a*. was from Roth (Karlsruhe, Germany). The HPLC solvent acetonitrile (ACN) and methanol were purchased from J.T. Baker (Deventer, The Netherlands) and water

was purified by bidistillation. Caffeic acid (CA) and 5-caffeoylquinic acid were purchased from Sigma (Steinheim, Germany). 1-Caffeoylquinic acid was synthesized from caffeic acid and D-(−)-quinic acid according to published protocols [19,20]. 3-CQA (99% purity) and 4-CQA (95% purity) were obtained by interesterification of 5-CQA in alkaline medium using the method of Trugo and Macrae [21] and isolated on an analytical HPLC. Characterization was carried out with purified standards from the Department of Food Chemistry, University of Wuerzburg. Chemicals were stored at −80°C prior to use.

Subjects

The criteria for volunteers to participate in the study were to be a non-smoker in good health, with no required medication, and have had a terminal ileostomy. We performed this study with otherwise healthy, female ileostomy volunteers (n = 5) in the Division of Gastroenterology, Department of Medicine II, University of Wuerzburg. Volunteers had an average age of 41 ± 3.6 years, a body mass index of 27.4 ± 2.1 kg m^{-2}, a body-fat content of 33.9 ± 2.6% and a basic metabolism rate of 1404 ± 58 kcal/day. The reason for the colectomy was either Crohn's disease or ulcerative colitis and volunteers had undergone surgery 10.6 ± 6.1 years ago. Anthropometric data of volunteers were measured with a Maltron BF-9 Body Fat Analyser from Juwell medical (Gauting, Germany). The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Wuerzburg (No. 124/04). All participants have signed a written informed consent form before participating at the study.

Study Design

A single dose of decaffeinated instant coffee was administered in proportion to the body weight (BW) of the volunteers (3.46 ± 0.03 mg CQA kg^{-1} BW). Volunteers avoided food containing polyphenols for 48 h before, and during, the study. Instant coffee was dissolved in 650 mL hot water and a volume proportional to body weight was consumed by the ileostomists. Aliquots were stored at −80°C prior to analysis. Coffee was consumed at a temperature of 55 to 60°C within ten minutes. After this, volunteers ate a polyphenol-free breakfast (a white bread bun with artificial honey). Light, polyphenol-free meals were given to the volunteers after five hours (cheese and ham sandwiches). Water was allowed *ad libitum*. Ileostomy effluents were collected 12 hours before, immediately after, and 1, 2, 4, 6, and 8 hours after coffee consumption. Ileostomy bags were weighed, immediately frozen with liquid nitrogen, and stored at −20°C prior to analysis.

Analysis of coffee

The coffee was centrifuged for 5 min at 5000*g* (Centrifuge 5417 R; Eppendorf, Hamburg, Germany) filtered through a membrane filter (0.45 *µm* polyvinylidene fluoride (PVDF); Bio-Rad, Dreieich, Germany) and diluted (20-fold) with distilled water. 3,4,5-trimethoxyphenylacetic acid was added as internal standard (IS) and the samples were quantified in triplicate with HPLC–diode array detection (HPLC-DAD) and identified with HPLC-ESI-MS/MS. CQAs were identified by comparison with retention time, UV spectra, and MS data of references. pH was determined with an HI 2210 pH Meter (Hanna Instruments, Ann Arbor, Michigan, USA).

Ileal fluid extraction

After freeze-drying (Christ Alpha 1–4, Osterode, Germany), the dry weight was determined and samples were carefully homogenized. Aliquots (n = 3) of exactly 20 mg were extracted in an Eppendorf tube with 1 mL extraction solution, H_2O/EtOH/formic acid (70:29.9:0.1, *v/v/v*). Samples were vortexed (MS1 Minishaker, IKA, Staufen, Germany) briefly at 2700 min^{-1} and sonicated (Sonorex Super RK 102 P, Bandelin, Berlin, Germany) for 5 min. Subsequently the samples were centrifuged for 5 min at 4°C and 10000*g* (Centrifuge 5417 R, Eppendorf) so that the supernatant could be poured into a clean Eppendorf tube. The pellet was re-extracted twice more. The supernatants were combined, filtered with PVDF (0.45 µm) and the IS 3,4,5-trimethoxyphenylacetic acid was added before analysis.

Extraction efficiency was checked by spiking a polyphenol-free, redissolved ileal extract with physiological concentrations of 5-CQA, CA, ferulic acid, and D-(−)-quinic acid. No hydrolysis or interesterification was observed during sample preparation. Recovery was between 86% and 90%.

HPLC-DAD analysis

20 µL samples were injected into the HPLC-DAD system (Agilent Technologies 1200 Series). Separation was performed at 30°C using a Synergi Polar-RP 250 × 4.6 mm, 4 µm, 80 Å column (Phenomenex, Aschaffenburg, Germany). Injections were carried out using an autosampler maintained at 4°C. The mobile phase, pumped at a flow rate of 0.5 mL min^{-1}, was a solution of 0.1% formic acid and ACN in water (ACN gradient: 0 min, 5%; 21 min, 17.5%; 27 min, 24%; 30 min, 28%; 60 min, 30%). CQAs and CA were measured at 320 nm and the IS at 270 nm. Data acquisition and evaluation were performed with Agilent Chemstation software. CQA levels were calculated using the

calibration curve for 5-CQA. The limit of detection (LOD) and limit of quantification (LOQ) were defined with signal-to-noise (S/N) ratios of 1:3 and 1:12, respectively. The observed peak-area ratios were plotted versus the concentration ratios. On the basis of the lowest calibration concentrations, absolute LOQs of 0.81 *n*g for 5-CQA and 1.63 *n*g for CA and absolute LODs of 0.20 *n*g for 5-CQA and 0.41 *n*g for CA were calculated.

HPLC-ESI-MS/MS analysis

For HPLC-ESI-MS/MS analysis, a SCIEX API 3200 MS/MS tandem mass spectrometer equipped with an electrospray ionization (ESI) interface (Applied Biosystems, Darmstadt, Germany) coupled to a Jasco HPLC system with two pumps (PU-2080) and a thermostated autosampler (AS-2057) (Jasco, Groß-Umstadt, Germany) was used. Data were acquired and evaluated using Analyst Software 1.4.2 (Applied Biosystems). The chromatographic conditions were the same as those described above. The mass spectrometer was used with ESI operating in full-scan negative-ionization mode (120–900 *m/z*), with a total scan duration of 1.0 s and a dwell time of 2 ms. Tuning of the mass spectrometer was optimized by infusing (syringe pump; Hamilton, Bonaduz, Switzerland) a standard of 5-CQA into the source. ESI settings were as follows: spray capillary voltage, 4.5 kV; curtain gas, nitrogen (450°C at 25 psi); ion source gas 1, 50 psi; ion source gas 2, 40 psi; declustering potential, −25 V; entrance potential, −2.5 V; electron multiplier voltage, 2.2 kV. D-(−)-quinic acid was measured by stable-isotope dilution analysis as described previously [22].

Apple beverages

Data on ileostomy-study performance and quantification of apple CQAs in ileostomy bags, together with methanol and D-(−)-quinic acid quantification of CAJ have been published [18]. As well as data on the study with apple smoothies [17].

Results

CQA content of beverages

The main hydroxycinnamic acid subclass found in the instant-coffee beverage (383 mL consumed) of the study was the caffeoylquinic acids (746 ± 36 μmol) with 1-CQA as a minor compound and 3-CQA, 4-CQA, and 5-CQA as major compounds. Structures are given in fig. 1 and beverage contents in table 1. Free caffeic acid was only detected in low amounts (6.5 ± 0.5 μmol). Other CGAs, such as feruloylquinic acids (109 ± 6 μmol), dicaffeoylquinic acids (67 ± 3 μmol), and caffeoylquinides (125 ± 15 μmol) were also detected in low amounts.

The polyphenol profiles of the apple beverages were similar [17,18]. The CAJ (1000 mL consumed) used in the study had a CQA content of 358 ± 16 μmol and the AS (700 mL consumed) 335 ± 31 μmol (table 1). 5-CQA was the dominant constituent; minor amounts of 4-CQA were detected and 1-CQA and 3-CQA were not detected, but free caffeic acid was present (29.4 ± 9.4 μmol in CAJ, 5.4 ± 1.2 μmol in AS). Coumaroylquinic acids (87.8 ± 1.5 μmol in CAJ, 289 ± 8 μmol in AS) were also present. The apple beverages as expected had lower contents of dihydrochalcones (81.2 ± 3.6 μmol in CAJ, 171 ± 11 μmol in AS) and flavan-3-ols (186 ± 8 μmol in CAJ, 1978 ± 161 μmol in AS) than the coffee sample. The flavonol quercetin and its derivatives were found to be minor constituents of the CAJ, ranging from less than 0.6 to 9.0 (± 0.2) μmol, whereas the content was 72.0 ± 4.9 μmol in the AS sample.

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226 Analysis of CQA in ileal fluid

227 After the consumption of coffee, CAJ, or AS, the ileostomy bags were collected at 0, 1,
228 2, 4, 6, and 8 hours. CQAs were extracted and immediately analyzed at 320 nm with
229 HPLC-DAD. In the ileal fluids excreted following coffee consumption, all four ingested
230 CQAs were detectable (table 1). A comparison of the CQA profile of the administered
231 coffee with that of the excreted fluid is shown in Table 1; no qualitative changes could be
232 observed. Clearly, the upper gastrointestinal tract (GIT) did not affect the coffee CQA
233 composition, so 5-CQA is still the dominating constituent. However, a small increase of
234 free caffeic acid from 6.5 ± 0.5 μmol in the coffee beverage to 13.4 ± 4.3 μmol in the ileal
235 fluid was measured. The recovery rate of the total CQA content excreted into the ileal
236 fluids relative to that ingested was calculated to be 76.2% for coffee.
237 Following CAJ consumption, the CQA composition of the ileal fluids was different to that
238 of the consumed CAJ. Besides 4-CQA and 5-CQA, which were initially present in the
239 CAJ, 1-CQA and 3-CQA could also be detected in the ileal fluids, with 5-CQA and the
240 metabolite 3-CQA dominating (see table 1). The CQA profile of AS was also altered
241 following passage through the GIT, although 5-CQA still dominated. Free caffeic acid
242 was not detected in the ileal fluid following consumption of the apple-based beverages,
243 but a methyl ester was generated during passage through the GIT following CAJ
244 consumption. We recovered 25.7% of the administered CQA in the excreted ileal fluid
245 following CAJ consumption and 77.5% following AS consumption. The time evolution of
246 CQA excretion for the different foods is shown in fig. 2. Within eight hours the CQA
247 content in the ileal fluids decreased to trace amounts. This indicates that the bolus had
248 completely passed the upper GIT of the ileostomy volunteers. The time of maximal

excretion of CQA (T_{max}) was two hours after consumption for CAJ (fig. 2), whereas it was not possible to identify a specific T_{max} for coffee and AS. Following coffee consumption, CQA excretion increased for 1 h, reached a plateau, and then decreased after 4 h. The pattern of CQA excretion following AS consumption was similar. CQA excretion after both coffee and AS ingestion showed high inter-individual differences (fig. 2).

Additionally we determined the content of free D-(−)-quinic acid as it is an indicator of hydrolytic activity on CQAs during GIT passage. We found that 64.5 ± 30.3% of the free D-(−)-quinic acid (QA) in coffee (695 ± 63 μmol) was excreted via ileal fluids. The recovery of QA from AS was determined to be 70.6 ± 47.1%, with the amount ingested being 2680 ± 432 μmol. No free QA was detected in the CAJ, but 67.6 ± 8.1 μmol was excreted.

Discussion

The existing literature on bioavailability of CQA is inconclusive and controversial. The effect of the food matrix and of different polyphenol and CQA compositions has not been considered in most previous studies. Therefore we compared three independent studies of ileostomy subjects who consumed CQA via three different food matrices: CAJ, AS, and coffee. The apple derived beverages had a similar CQA composition, but were different from the coffee CQA composition (table 1). The polyphenol profiles of the beverages were in accordance with the literature [16,14].

The CQA compositions of CAJ and AS changed during GIT passage, with 5-, 4-, 3-, and 1-CQA being found in the excreta whereas only 4- and 5-CQA were initially present in

the beverages. Such interesterification reactions have also been reported in other studies [23,24] and seemed to be caused by the rising pH level, above pH 6, during the passage through the small intestine. The CQA profile of the coffee sample investigated here was closer to the interesterification equilibrium as observed by Trugo and Macrae [21] and thus major differences between the administered CQA profile and the excreted CQA composition were observed neither in our study nor by others [25] (table 1).

Only after CAJ consumption was the compound methyl caffeate observed in the excreted ileal fluids [18], providing evidence of its formation from free caffeic acid and methanol present in CAJ, since methanol can be released from pectin during the processing of apples [26]. The CAJ used in our study contained 734 ± 67 μmol L^{-1} methanol. Despite a methanol concentration of 164 μmol L^{-1} in the AS, no methyl caffeate was observed, suggesting that this amount was not sufficient for the formation of a methyl ester.

The recovery rate of excreted CQA varied considerably across the three food matrices. About 26.1% of the CQA reached the ileal fluids after CAJ consumption whereas 76.2% and 77.5% of the CQA respectively reached the end of the small intestine after consumption of coffee and AS. Recovery of more than 50% of the ingested CQA following coffee consumption has been reported recently by Stalmach *et al.* [25] who found 59 ± 8% excreted into the ileal bags. Furthermore, following administration of pure 5-CQA [27], about two-thirds was recovered in the ileal fluids (summarized in table 2). The question arises why we observed a significantly lower recovery of CQA from CAJ consumption than from coffee or AS consumption. *In vitro* data revealed that the different CQAs showed a similar absorption rate in the upper GIT [28,29]. So the

different CQA composition in the ingested food matrixes might not influence the total CQA recovery.

The total amount of CQA ingested from CAJ was similar to the amount from AS and to the amount reported by Stalmach *et al.* [25] of 278 ± 3 *µ*mol CQA from coffee. Despite comparable CQA doses the recovery rates were different. This means that the main differences in recovery rates cannot be explained by the differences in the amounts ingested (table 3). The higher absorption rate, and thus lower recovery rate in the ileal fluids, of CQA from CAJ could also be due to a longer GIT transit time, but the recorded data provided no evidence for this (fig. 2).

Furthermore, a different chemical or bacterial degradation of CQA in the upper GIT could also lead to different recovery rates in the ileal fluids. During the GIT passage, a moderate hydrolytic activity was observed following CQA consumption via CAJ. This is supported by the fact that free QA (67.6 ± 8.1*µ*mol) was found in the excreted fluid and not in the beverage. In contrast, the QA contents of coffee and AS were only partially recovered in the ileal fluids. On the other hand, free caffeic acid was detectable in all beverages (table 1), but no caffeic acid was found in the ileal fluids after CAJ and AS consumption. Only small amounts of caffeic acid were detected following coffee consumption. Olthof *et al.* [27] showed that a majority of the free caffeic acid is absorbed before reaching the colon.

It is known that esterases which are able to cleave CQA-ester bonds are likely to play a major role only in the colon [30,31], which would rule out overestimation of CQA recovery after coffee consumption resulting from CQA liberation from diCQA during passage through the GIT. A release of CQA from coffee melanoidins in the GIT has not been directly proven so far [32]; further investigations are necessary. We conclude that

parameters such as different degradation rates of CQA in the upper GIT, the food matrix and different polyphenol composition might be responsible for the different CQA recovery rates. Little has been published on absorption mechanisms for CQAs; on the one hand, a passive diffusion is conceivable [33] and, on the other hand, an active transport process has also been discussed [28]. In the latter case, inhibition or amplification of the transport process could be affected by the different polyphenol contents of the matrices. The hydroxycinnamic acids occurring in coffee with structural similarity to CQAs, such as feruloylquinic acid and diCQA, or the high content of free D-(−)-quinic acid, could lead to competitive inhibition and thus reduce absorption of CQA, whereas the polyphenol profile of CAJ is more heterogeneous, with glycosides of dihydrochalcones or quercetin. Quercetin is of importance since its metabolites have been shown to interact with the efflux transporter MRP2 and the aglycone form led to an increased absorption of hesperetin in a Caco-2 cell model [34,35]. Thus, quercetin released by ileal microbiota [36] or by lactase-phlorizin hydrolase [37] could decrease the absorption of other polyphenols. Such interactions with other polyphenols have not been sufficiently investigated, but the influence of quercetin and other polyphenols on *in vivo* bioavailability should have been minor for CAJ, because of its low quercetin content.

One of the major differences between the two apple beverages lies in their production and composition. CAJ is pressed and unfiltered, whereas AS is a beverage containing 60% CAJ and 40% apple puree [18,17]. Where the latter is produced from whole fruits with no pressing. Because of this, AS in general contains a much higher proportion of cell-wall constituents than does CAJ, which seems to reduce the bioavailability considerably. The mechanism of action behind this may be interactions of CQAs with

cell-wall constituents such as lignins. This was clearly the cause of the reduced bioavailability of the hydroxycinnamic acid ferulic acid in rats, with cross-linking of ferulic acid to arabinoxylans and lignins [38].

The physicochemical properties of CQA itself may also play an important role in modulating its bioavailability. If the mechanism of absorption is based on diffusion processes then the lipophilicity is a key parameter [39]. For ionizable compounds such as CQA (pK_a 3.37), lipophilicity is dependent on pH and properties differ between the ionized and neutral forms (*diff*(log P^{N-I}) = 3.53), so the CQAs in the coffee beverage exist in their ionic forms (pH 6.8) and the CQAs in CAJ in their neutral forms (approx. pH 3.2) (table 3). As a consequence, the lipophilicity of the CQAs in the apple beverages was higher (log $D^{3.2}$ = −0.5) than that of the CQAs in the coffee (log $D^{6.8}$ = −3.8). Beverages with higher pH levels, such as pure 5-CQA in hot water [27] and coffee [25], have been shown to have lower bioavailability.

Our results show that the amount of CQA reaching the colon from CAJ consumption is considerably lower than that from coffee or AS consumption. Thus, coffee and AS may be better matrices for delivery of polyphenolic antioxidants to the colon; whereas after CAJ consumption, CQA and it metabolites are more likely to be distributed systemically or degraded during passage of the GIT.

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371 Conflict-of-interest statement

372 The authors declare no commercial or financial conflict of interest.

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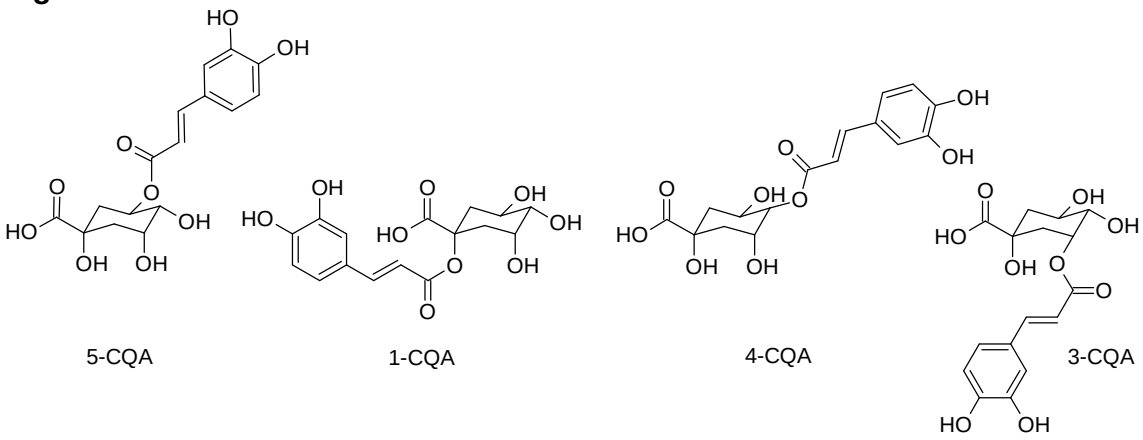
Figure legends:

Figure 1: Structures of caffeoylquinic acids (CQAs), esters of D-(−)-quinic acid and caffeic acid.

Figure 2: Recovery rate of total CQA content of excreted ileal fluid relative to that ingested for 8 h following CAJ, AS, and coffee consumption. Values plotted are mean and standard deviation (*n* = 3).

Figure 1:



499 **Figure 2:**

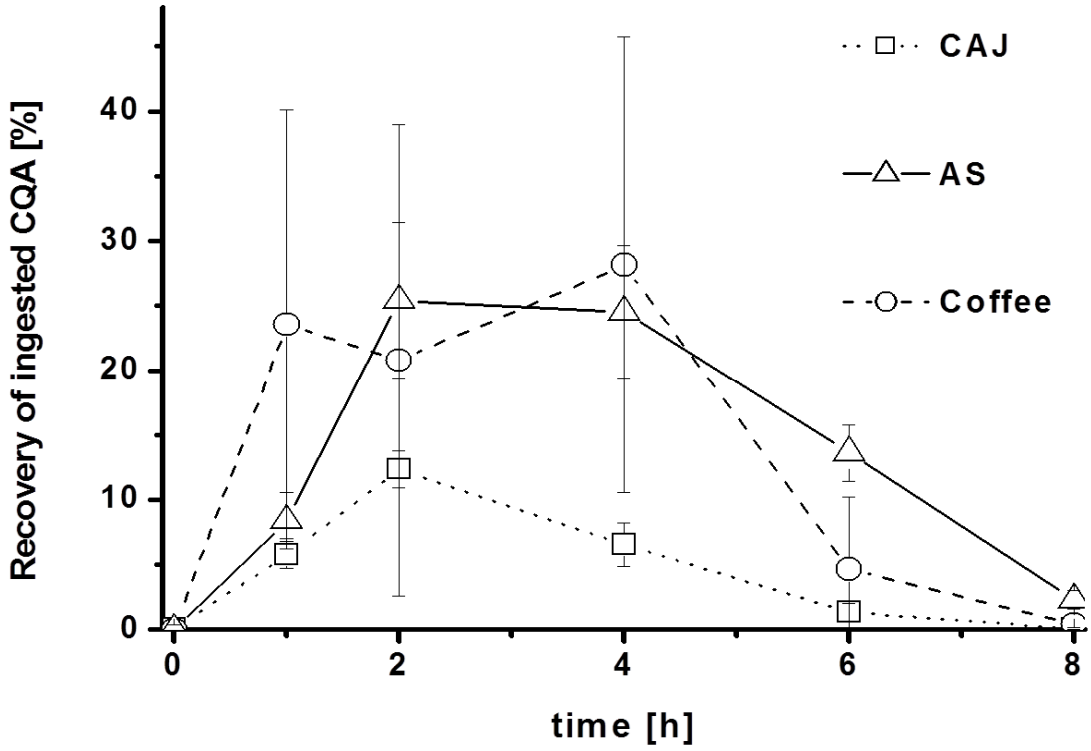


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Table 1: Summary of CQA and D-(−)-quinic acid contents of cloudy apple juice (CAJ), apple smoothie (AS), and coffee. Amounts ingested and detected in ileostomy fluids from consumption of single beverages, reported as mean ± SD in *µ*mol.

	CAJ [17]		AS [16]		Coffee	
	Ingested	Excreted	Ingested	Excreted	Ingested	Excreted
	*µ*mol ± SD[a]	*µ*mol ± SD	*µ*mol ± SD[a]	*µ*mol ± SD	*µ*mol ± SD	*µ*mol ± SD
1-caffeoylquinic acid	ND[b]	16.5 ± 2.4	ND[b]	2.0 ± 1.7	8.0 ± 0.6	5.1 ± 2.1
3-caffeoylquinic acid	ND	33.7 ± 8.2	ND	14.1 ± 13.3	230.9 ± 21.0	178.6 ± 68.9
4-caffeoylquinic acid	39.8 ± 5.6	9.2 ± 3.0	25.7 ± 1.2	27.1 ± 15.5	208.7 ± 17.3	157.1 ± 61.6
5-caffeoylquinic acid	318.4 ± 9.9	32.5 ± 7.7	309.4 ± 30.2	216.5 ± 75.6	298.4 ± 24.1	227.6 ± 88.6
caffeic acid	29.4 ± 9.4	ND	5.4 ± 1.2	ND	6.5 ± 0.5	13.4 ± 4.4
methyl caffeate	ND	11.9 ± 8.5	ND	ND	ND	ND
D-(−)-quinic acid	ND	67.6 ± 8.1	2680 ± 432	1891± 1225	695.3 ± 62.6	439.7 ± 19.9

[a] $n = 3$; [b] ND = not determined

Table 2: Administered amount of caffeoylquinic acids (CQA) in different matrices and corresponding ileal excretion in % observed in the ileostomy model.

Administered amount (*µ*mol)	(mL)	Matrix		Ileal excretion (%)	Reference
2800	200	5-CQA, water	together with light breakfast	67	[27]
358	1000	cloudy apple juice	fasted (light meal after 4h)	26	[18]
335	700	apple smoothie	not specified	78	[17]
278	200	coffee	fasted (light meal after 3h)	59	[25]
746	383	coffee	together with light breakfast	78	current study

Table 3: Properties of the consumed beverages and physicochemical properties of their CQAs calculated with Marvin Sketch 5.3.1 (Chemaxon, Budapest, Hungary).

	Volume (mL)	pH	log $D_{(CQA)}$	$C_{max(CQA)}$ (μmol mL^{-1})
Coffee	382.6 ± 30.2	6.8	−3.8	1.93 ± 0.02
CAJ	1000 ± 0	3.2	−0.5	0.36 ± 0.01
AS	700 ± 0	3.2	−0.5	0.48 ± 0.04