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Chlorogenic acids and the acyl-quinic acids: discovery, biosynthesis, bioavailability and bioactivity†

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Covering: 2000 up to late 2017

This review is focussed upon the acyl-quinic acids, the most studied group within the *ca.* 400 chlorogenic acids so far reported. The acyl-quinic acids, the first of which was characterised in 1846, are a diverse group of plant-derived compounds produced principally through esterification of an hydroxycinnamic acid and 1L-(−)-quinic acid. Topics addressed in this review include the confusing nomenclature, quantification and characterisation by NMR and MS, biosynthesis and role *in planta*, and the occurrence of acyl-quinic acids in coffee, their transformation during roasting and delivery to the beverage. Coffee is the major human dietary source world-wide of acyl-quinic acids and consideration is given to their absorption and metabolism in the upper gastrointestinal tract, and the colon where the microbiota play a key role in the formation of catabolites. Evidence on the potential of the *in vivo* metabolites and catabolites of acyl-quinic acids to promote the consumer's health is evaluated.

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1   Discovery of chlorogenic acids

The term 'chlorogenic acids' (CGAs) encompasses a large group of naturally-occurring compounds of which the majority are synthesised *in planta* by esterification of a C_6–C_3 *trans*-hydroxycinnamic acid with 1L-(−)-quinic acid (**1**). The main hydroxycinnamates are *p*-coumaric (**2**), caffeic (**3**), ferulic (**4**) and sinapic acids (**5**).

1L-(−)-Quinic acid (**1**)   *p*-Coumaric acid (**2**)   Caffeic acid (**3**)

Ferulic acid (**4**)   Sinapic acid (**5**)
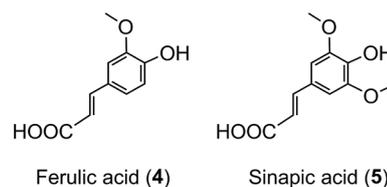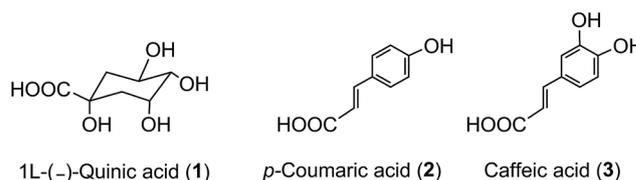
A wider definition of CGAs includes compounds formed with various quinic acid epimers, quinic acid methyl ethers, alkyl

quinates, deoxyquinic acid, 2-hydroxyquinic acid and shikimic acid and its epimers, plus the analogous compounds that are esterified with a hydroxybenzoic acid (**6**), hydroxyphenylacetic acid (**7**) or 3-(4′-hydroxyphenyl)propionic acid (**8**). Aliphatic acid substituents may also be present, occasionally in the absence of an aromatic residue. A fuller account of these less common CGAs is available.[1]

The origin of 'chlorogenic acid' is probably related to the use of the term 'chlorogen acid' by Payen[2,3] in 1846 (1846a, 1846b) who reported the isolation of a crystalline potassium caffeine chlorogenate that formed up to 5% of green coffee (*Coffea arabica*) beans. He proposed an empirical formula of $C_{14}H_8O_7$ which is now known to be $C_{16}H_{18}O_9$, and described its conversion to a green pigment on alkaline oxidation. In retrospect, one can deduce that there were a few earlier studies with the same or a very similar fraction prepared from green coffee beans. The earliest of these appears to be a paper by Robiquet and Boutron in 1837,[4] in which they reported the isolation of an acidic substance that turned green when treated with ferric chloride. Rochleder[5] isolated an acidic substance from green coffee beans in 1844 that associated with caffeine and which precipitated as a lead salt. In 1846 Rochleder further reported that this fraction

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Iziar Ludwig obtained a bachelors degree in Food Science and Biotechnology at the University of Natural Resources and Life Sciences (BOKU, Vienna) in 2009 and her PhD in Physiology, Nutrition and Health at the University of Navarra (Pamplona) in 2013. She then work in Alan Crozier's group at the University of Glasgow and is currently a Research Associate at the Antioxidants Group, Department of Food Technology, at the University of Lleida. Her research is focussed on (poly)phenolic compounds in fruits and beverages, their metabolism, bioavailability and potential health effects.

Indu Jaganath is a Principal Research Officer at the Malaysian Agricultural Research and Development Institute. She obtained her Master of Science from the University of California, Riverside and her PhD at University of Glasgow. Her research is focused on the discovery of chemically and biologically active natural 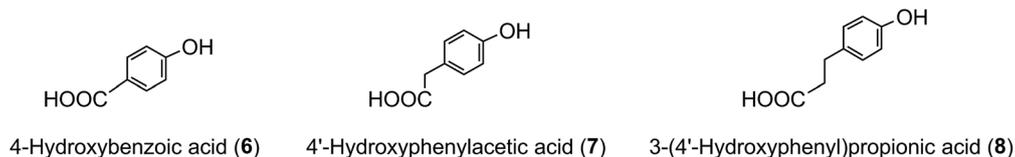compounds in tropical plants. Her main expertise lies in the area of metabolomics and nutrigenomics where she incorporates in multidisciplinary approach utilizing transcriptomics, metabolomics, pharmacokinetics and in vivo animal models to gain a holistic approach on how dietary compounds alters metabolism and affect various cellular pathways through gene regulation.

Alan Crozier is Senior Researcher in the Department of Nutrition at the University of California, Davis and a consultant for Mars Inc. He is also an Honorary Senior Research Fellow in the School of Medicine, Dentistry and Nursing at the University of Glasgow where he was previously Professor of Plant Biochemistry and Human Nutrition. He has published over 300 papers and edited nine books. While retaining an interest in purine alkaloids, his research has centred on dietary flavonoids and related phenolic compounds in fruits, vegetables and beverages and their bioavailability following ingestion. He was elected an Eminent Scientist of RIKEN, the Institute of Physical and Chemical Research in Japan, in 1999 for his achievements in the field of plant hormones and secondary metabolites. In 2013 he was awarded the Mars Prize for his research on "Flavonoid Metabolism/Chemistry" at the Sixth International Conference on Polyphenols and Health, in Buenos Aires, Argentina. He was a Thomson Reuters Highly Cited Researcher in 2014, 2015, 2016 and 2017.

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4-Hydroxybenzoic acid (**6**) 4'-Hydroxyphenylacetic acid (**7**) 3-(4'-Hydroxyphenyl)propionic acid (**8**)

was yellow in ammoniacal solution and became green on exposure to oxygen, and suggested $C_{16}H_9O_8$ for the free acid.[6] It was not until 1907 that pure white crystals were obtained which had a melting point of 206–207 °C, and from which quinic acid (**1**) and caffeic acid (**3**) were released by alkaline hydrolysis. An empirical formula of $C_{32}H_{38}O_{19}$ was proposed in 1908 and to reconcile this with the evidence from hydrolysis Gorter[7] proposed that quinic acid combined with caffeic acid to form hemichlorogenic acid, two molecules of which condensed losing one molecule of water to produce chlorogenic acid. This would

development of higher resolution NMR led to a steady increase in the number of CGAs and in the number of species in which they were found.[21–29] With the arrival of the ion trap mass spectrometer coupled to HPLC in the first decade of the 21st century the total number of identified naturally occurring CGAs has increased markedly.[30–33] Taking the wider definition of CGAs, and including non-food sources, over 400 CGAs have been reported in over 400 genera embracing some 44 botanical orders.[34] This review will focus on the acyl-quinic acids which are the most studied of the extended CGA family.

5-*O*-Caffeoylquinic acid (**9**) (chlorogenic acid) 4-*O*-Caffeoylquinic acid (**10**) (cryptochlorogenic acid) 3-*O*-Caffeoylquinic acid (**11**) (neochlorogenic acid)

1-*O*-Caffeoylquinic acid (**12**) 1,3-*O*-Dicaffeoylquinic acid (**13**) (cynarin) 5-*O*-Caffeoylshikimic acid (**14**)
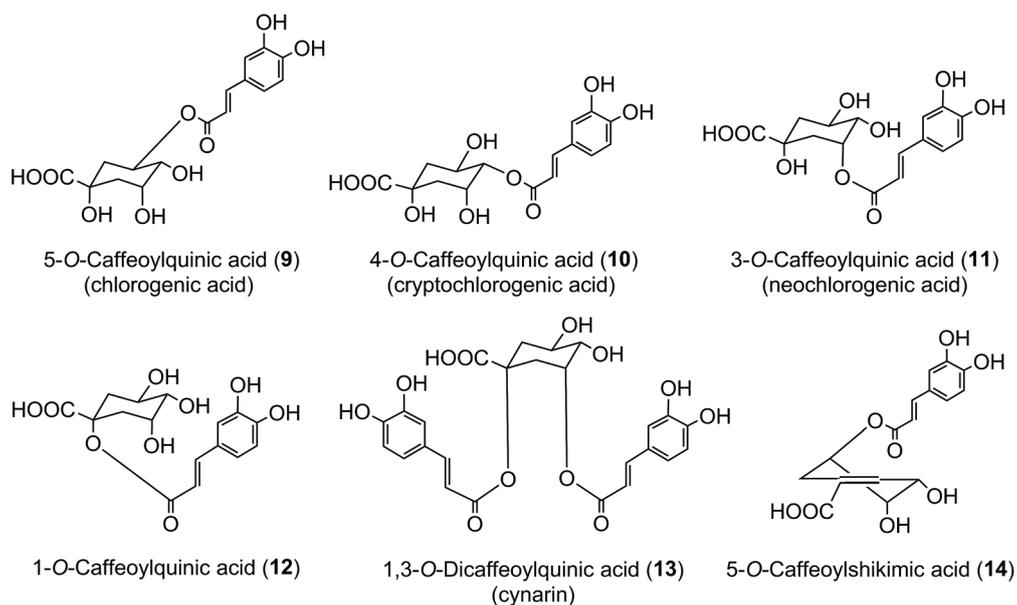
correspond to an empirical formula of $C_{16}H_{20}O_{10}$. Freudenberg[8] reported in 1920 that chlorogenic acid was a substrate for the enzyme tannase and that hydrolysis released equimolar amounts of quinic acid and caffeic acid. In 1932 Fischer and Dangschat[9] proposed that this substance was 3-*O*-caffeoylquinic acid (3-CQA).

In 1950 Barnes *et al.*[10] reported the presence in coffee of 5-*O*-caffeoylquinic acid (**9**) (5-CQA), and over the next 15 years other isomers (**10**, **11**, **12**) and several related dicaffeoylquinic acids (diCQAs) were reported from various sources. Cynarin(e), now known to be (1,3-*O*-dicaffeoylquinic acid (**13**, 1,3-di-CQA)) from artichoke, characterised in 1954, was the first 1-acyl quinic acid to be reported.[11] The same period saw the first reports of galloyl,[12,13] *p*-coumaroyl,[14,15] feruloyl,[16] caffeoyl-feruloyl,[17] caffeoyl-succinoyl[18] and sinapoyl[19] esters of quinic acid. The first papers referring to caffeoylshikimic acids (**14**) were published by Maier *et al.* in 1964.[20] During the last quarter of the 20th century, improvements in chromatographic procedures and the

Acyl-quinic acids are widespread dietary components being found, for instance, in coffee, cherries (*Prunus avium*), blueberries (*Vaccinium* spp.), aubergine (*Solanum melongena*), apples (*Malus pumila*) oregano (*Origanum vulgare*), spearmint (*Mentha spicata*), chicory (*Cichorium intybus*) and sunflower (*Helianthus annus*) seeds[28,35–39] with high levels in globe artichoke.[40,41] The herbal tea maté, made from infusion of dry leaves of *Ilex paraguariensis*, contains substantial amounts of CQAs and diCQAs (Table 1).[42] Coffee beverage, rather than fruits and vegetables is probably the main dietary source of CQAs for many people with intakes in excess of 1 g per day being readily attained.[43]

The hydroxycinnamic acid moiety of acyl-quinic acids is predominantly in the *trans* form. Some *cis* isomers are known, with *cis*-5-*O*-*p*-coumarylquinic acid (**15**) being detected in flower buds of herbal aster (*Aster ageratoides* Turcz).[47] and a wide range of *cis*-isomers have now been detected in other species.[34] It has been suggested that the *cis* derivatives originate from plant

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Table 1 Acyl-quinic acid content of coffees, apples juices, globe artichoke and maté[a]

Product	Serving	Dose		Reference
		μmol	mg	
Espresso coffee	27 mL	68	24	Crozier et al.[43]
	52 mL	1195	422	Crozier et al.[43]
Instant coffee	200 mL	412	146	Stalmach et al.[44]
Cloudy apple juice	200 mL	72	25	Kahle et al.[45]
Apple smoothie	200 mL	96	34	Hagl et al.[46]
Globe artichoke	100 g	762	268	Pandino et al.[41]
Maté	200 mL	270–320	94–111	Clifford and Ramìrez-Martìnez[42]

[a] Data expressed as mean values ($n = 3$).

tissues where the *trans* isomer has been exposed to relatively strong UV-irradiation which induces geometric isomerisation.[48] There is good experimental evidence to support this view.[49] However, direct synthesis cannot be ruled out, and there is some evidence from research with cell culture to suggest that UV-irradiation is not essential.[50]

cis-5-*O*-*p*-Coumaroylquinic acid (**15**)
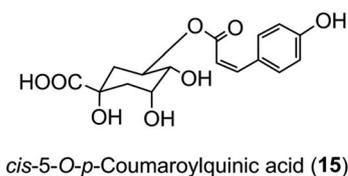
2 Nomenclature

Acyl-quinic acids exhibit configurational isomerism, conformational isomerism and regio-isomerism, plus geometric isomerism for those containing a cinnamic acid residue. Consequently, it is not easy to describe unambiguously the structures of acyl-quinic acids that may appear almost identical when drawn in 2D or projected in 3D. Various systems for cyclitols were evaluated by IUPAC who recommended that the most common natural form of quinic acid be described as 1L-1(OH),3,4/5-tetrahydroxycyclohexanecarboxylic acid, with the trivial names (−)-quinic acid or L-quinic acid.[51] In the IUPAC system, Fischer and Dangschatt's 3-CQA[9] became 5-CQA (**9**).

Unfortunately, papers are still published in which non-IUPAC numbering is used, the numbering system used is not stated, or worse, the numbering is IUPAC but the structure shown is not, or *vice versa*. Kremr et al.[52] commented that some published structures make no attempt to depict the spatial arrangement of the substituents, and Clifford recorded that some authors discuss previously published data unaware that different numbering systems have been used, for example 3-CQA (non-IUPAC) and 3-CQA (IUPAC) are treated as the same compound.[34,53] Note that Wikipedia and many other online sources, plus many catalogues listing acyl-quinic acid preparations, use non-IUPAC nomenclature. Many examples of misleading or incorrect descriptions have been compiled.[1,34,53] For the avoidance of doubt, the IUPAC and non-IUPAC structures are presented in Fig. 1. From this point onwards the
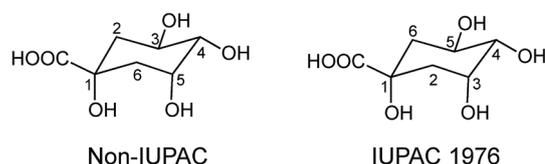

Fig. 1 Non-IUPAC and IUPAC numbering of 1L-(−)-quinic acid.

IUPAC numbering system will be used in this review. Trivial names further complicate the literature but a glossary is available,[1,54] and this is presented as Table S1 in the ESI.† To this must be added chrysanthemorimic acids, a series of recently discovered diCQAs, in which one caffeic acid residue has undergone a [5 + 2] cycloaddition of D-glucose.[55]

The IUPAC system, however, has limitations when applied to acyl-quinic acids and Abrankó and Clifford[54] have proposed a combination of IUPAC cyclitol numbering[51] the Cahn–Ingold–Prelog (CIP) sequence rules[56] plus the use of α to define a hydroxyl *trans* to the quinic acid carboxyl (and β to define a hydroxyl *cis* to the quinic acid carboxyl) to describe the orientation of a substituent on a carbon atom which is not a centre of chirality, as favoured by Eliel and Ramirez.[57] This approach can accommodate all eight quinic acid stereo-isomers, with, for example, IUPAC (−)-quinic acid described as 1L-(−)-quinic acid 3*R*,5*R*-(1α,3α,4α,5β).[54] ESI Table S2† provides a comprehensive set of structures for the various quinic acids, which in addition to using different styles of presentation (Fischer–Tollens, 2D, alternative chair conformations), also considers the perspective from which a structure is viewed.

3 Characterisation and quantification of acyl-quinic acids

This section of the review draws on three open-access documents which contain extensive tabulations and discussion of the topics presented here.[1,53,56]

3.1 Extraction

Extraction of acyl-quinic acids from plant material generally employs aqueous alcohol, usually 70% MeOH. Artefacts may

arise from acyl migration, alkylation, hydrolysis, water addition across the cinnamic acid double bond and/or *trans–cis* iso-merisation.[59] Acyl migration is favoured by higher water content in the solvent and sample, and when extracting undried plant material 100% MeOH is advisable for the first stage, especially if 1,5-diCQA might be present, as it rapidly converts to 1,3-diCQA. In contrast, controlled acyl migration and partial hydrolysis can usefully generate regio-isomers that are not otherwise conveniently available (*e.g.* 1,4-diCQA, 1-CQA),[26,60,61] as can UV-irradiation for preparing *cis*-isomers.[48] Any novel acyl-quinic acid should be examined by controlled acyl migration in order to access associated regio-isomers.

3.2   ^{1}H-NMR

Acyl-quinic acids can be characterised by ^{1}H-NMR, but this has limitations.[53,63–65] The definitive studies of Pauli et al.,[63,64] and the data tabulated by Clifford,[53] show that if best practice is followed, mono- and di-acyl-quinic acids can be successfully identified – d_4-MeOH is the preferred solvent, to which a small amount of D_2O or d_6-DMSO (not exceeding 10%) can be added, followed by analysis at not less than 500 MHz. Identification of the acyl residue(s) is straightforward, assignment of the quinic acid configuration and the position(s) of acylation less so, because signals for H2 and H6 methylenes and H3 and H5 methines often overlap, especially in tri- or tetra-acyl-quinic acids, and/or when an aliphatic substituent is present.

Temperature, analyte concentration, and especially solvent influence the proton chemical shifts, the order in which the shifts occur, and the conformation of the quinic acid moiety, and hence the coupling constants for the relevant protons.[63,64,66] Incomplete signal resolution, producing inaccurate coupling constants, has generated unconvincing structural assignments, notably reports of acyl-quinic acids containing a stereo-isomer of 1L-(−)-quinic acid.[67–73]

These novel compound(s) are rarely observed in company with a full set of the commoner 1L-(−)-quinic acid derivatives. This does not *per se* refute the claim, but might suggest that one of the commoner acyl-quinic acids has been wrongly identified. Reports by Wang *et al.*[74,75] of an acyl-*iso*-quinic acid (= 4,5-diC-*epi*-QA IUPAC) are convincing because the quinic acid moiety released by saponification did not co-chromatograph with 1L-(−)-quinic acid, and subsequent 500 or 600 MHz NMR in d_4-MeOH is distinctive.[75,76] The biosynthesis of 1L-(−)-*epi*-quinic acid requires only that D-threose-4-phosphate replace D-erythrose-4-phosphate in the pathway to 1L-(−)-quinic acid.[58] There are LC-MS data for four incompletely characterised CQA,[32,77] which plausibly are derivatives of a quinic acid isomer. NMR data are available for 3-C-*muco*-QA and 3-F-*muco*-QA,[78] plus (±)-*epi*-quinic acid and *scyllo*-quinic acid.[79–81]

3.3   Chromatography

A reverse-phase LC column packing and shallow linear solvent gradient providing structure-diagnostic relative capacity factors or relative retention times (RRTs) is the best strategy for characterising acyl-quinic acids. Once characterised, a revised

gradient may speed up the separation for routine use. The behaviour of acyl-quinic acids is determined by (i) orientation of the free quinic acid hydroxyls, and (ii) number and hydrophobicity of the acyl moieties. For a given acyl moiety, 1-acyl and 3-acyl regio-isomers (two free equatorial hydroxyls) are well-resolved from the 4-acyl and 5-acyl regio-isomers (two free axial hydroxyls). 1-Acyl regio-isomers almost always elute first, but 4-acyl and 5-acyl regio-isomers vary with column packing. Similarly 1,3-diacyl regio-isomers (two free equatorial hydroxyls) elute first and 4,5-diacyl regio-isomers elute last (two free axial hydroxyls). The other four diacyl regio-isomers elute close together just before the 4,5-diacyl isomer, but their sequence varies with column packing.[31,82] For diacyl-quinic acids with different substituents, the isomer with the more hydrophilic substituent more equatorially positioned, elutes first.[58]

Relative to free quinic acid, acylation delays elution. Cinnamic and benzoic acids with more ring hydroxyls elute faster, and for a given number of ring substituents, methylation of the hydroxyl slows elution, *i.e.* for a given regio-isomer the sequence is CQA, *p*-coumaroylquinic acid (*p*CoQA), feruloylquinic acid (FQA), and (3′,4′-dimethoxycinnamoyl)quinic acid (DQA), *etc.* *cis*-3-Cinnamoyl and *cis*-4-cinnamoyl elute before their *trans*-counterparts but *cis*-5-cinnamoyl elute later. The methyl esters of the mono-acyl-quinic acid regio-isomers, but not the diacyl-quinic acid regio-isomers, elute in the reverse order compared with the free acids.[83] Glycosidation of the acyl moiety markedly speeds the elution. Although the presence of an aliphatic dicarboxylic acid, *e.g.* succinic acid, may speed elution the behaviour is less predictable because of internal hydrogen bonding in some regio-isomers.[84]

With that exception, these RRTs are sufficiently consistent to be used as a structure-diagnostic tool, easily locating *cis*-5-CQA which is often reported as *trans*-1-CQA,[85–89] and early-eluting CQA-glycosides which are often reported as diCQA.[90,91] RRT values should always be considered when assigning the structure of a novel acyl-quinic acid.

3.4   LC-MS

Mass spectroscopy was long considered blind to isomers but the development of LC-ion trap-MS changed perceptions,[92] especially with regard to acyl-quinic acids,[58] for which negative ion MS is preferable. The methods and hierarchical keys developed by Clifford and Kuhnert using mild fragmentation conditions produce distinctive patterns at MS[2], MS[3] and MS[4], allowing regio-isomers to be assigned confidently. Critically, these methods have been applied successfully to previously unknown acyl-quinic acids. The geometric isomers of cinnamoyl-quinic acids fragment identically,[48] but the *cis*-isomers can be distinguished by UV-irradiation,[62] sodium-adduct MS,[93] and ion mobility-MS.[94] Using the hierarchical keys it is possible to identify at regio-isomer level in excess of 20 acyl-quinic acids in a single run subject to adequate analyte concentration.[31,77,78,83,95–115] It is false economy to characterise CGAs and flavonoids in a single run, because flavonoids require harsher MS conditions which mask the structure-diagnostic fragmentations of the acyl-quinic acids, and invalidate the hierarchical keys.

The hydrogen-bonding networks responsible for these distinctive fragmentations have been deduced.[58] For some regio-isomers the charge may be on a phenolic hydroxyl rather than the quinic acid carboxyl.[116] Accurate mass may be helpful, but is not essential to distinguish diCQA from CQA-glycosides because the glycosides yield characteristic MS[2] ions (m/z 341 and 323)[84] the latter as the base peak indicating a 3′-glycoside.[103,107] TriCQA, diCQA-glycosides and CQA-biosides, as well as acyl-isocitric acids and acyl-quinic acids, can be distinguished similarly, but only fragmentation can distinguish between isobaric methyl-CQ, FQA and isoFQA, and acyl-quinides and acyl-shikimic acids.[58] Methyl-CQ and methyl-diCQ fragment very differently from CQA and diCQA,[83] which coupled with the 'reversed' order of elution for the methyl CQ, necessitates care in regio-isomer assignment. Reports of FQA and caffeoyl-feruloylquinic acids (CFQA) producing fragments at m/z 179 or m/z 161, but not m/z 193 or m/z 367, might be methyl-CQ and methyl-diCQ.[117–120]

Full assignment of tri-acyl- and tetra-acyl-quinic acids requires MS[4] and perhaps MS[5] spectra, particularly where there are two or more different acyl moieties (*e.g.* dicaffeoyl-feruloylquinic acids or caffeoyl-sinapoyl-feruloylquinic acids). MS[8] is required to characterise depsidic GQA bearing up to eight galloyl residues.[98] Targeted rather than automated fragmentation may be essential with more complex structures such as 4-methoxyoxalyl-1,5-dicaffeoylquinic acid and 4-methoxyoxalyl-3,5-dicaffeoylquinic acid,[112] or when a 'dehydrated' base peak (*e.g.* m/z 349 rather than m/z 367) occurs, more frequent with increasing methylation of the acyl moieties.[58,102]

These structure-diagnostic protocols were developed using a ThermoFinnigan LCQ deca+ or a Bruker Daltronics HCT Ultra ion trap mass spectrometer, but should be easily transferred to similar instruments provided that the ionisation potential and fragmentation energy are appropriate. Even on the instruments originally used changes to the instrument parameters, such as increasing the ion spray voltage from 3.5 kV to 4.5 kV, generates many additional fragments and invalidates the hierarchical keys as published.[121,122]

Some investigators have reported that with QTOF-MS all CQA regio-isomers fragment identically.[123,124] However, Madala and co-workers have demonstrated that with careful control of the collision energy it is possible to obtain the same MS[2] and MS[3] fragmentation data as an ion trap.[125,126] When adapting the ion trap-MS hierarchical key methods to a non-ion trap-MS, a surrogate standard, such as a green coffee extract,[127] should be analysed and operating parameters adjusted until identical fragmentations are achieved.[58] Whatever equipment is used there is scan-to-scan variation in fragment intensity, being most prominent for weak signals, *i.e.* low analyte concentrations, and higher order spectra, and for a reliable assignment at least 20 scans should be taken. If necessary use a more concentrated extract or larger injection.

Only limited success has been achieved with triple quadrupole instruments because of the much increased fragmentation energy employed. Matsui *et al.* successfully fingerprinted several CQA, FQA and diCQA regio-isomers using two different collision energies in positive ion mode (15 and 30 eV) and three different collision energies in negative ion mode (20, 40 and 60 eV),[128] but this is too cumbersome for routine use. Similar limitations are apparent in the methods reported by Lin and Harnly,[129,130] and Willems *et al.*[131]

Ion-trap-MS has its limitations, most apparent where there is an aliphatic dicarboxylic acid substituent (*e.g.* succinic acid) because the fragmentations can be driven also by the distal carboxyl of that substituent in addition to the quinic acid carboxyl.[84,95,111,112] Reproducible fingerprints are obtained, but even with targeted fragmentations, full regio-isomeric assignment is not always possible.[58] Scopoletin (**16**) and the CQAs are indistinguishable by accurate mass, and there are currently insufficient fragmentation data for scopoletin to judge whether it could easily be distinguished.[58]
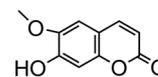
Scopoletin (**16**)

3.5 Calibrants

Quantification requires one or more pure calibrants. The molar absorbance of any set of acyl-quinic acid regio-isomers differs comparatively little, *e.g.* CQA ± 4% of mean (18 500), pCoQA ± 2.5% of mean (20 400), FQA ± 2.5% of mean (19 000) and diCQA ± 6% of mean (33 300). A good quality 5-CQA (for which the molar absorbance should be quoted) can be used for all of the foregoing with arithmetic corrections for the subgroups if required, although this is only significant for the diCQA and other acyl-quinic acids with two or more aromatic substituents.

In contrast to molar absorbance values obtained after meticulous purification with corrections for ash content, water content, *etc.*, published calibration curve response factors for acyl-quinic acids can vary by some 300%, indicating that these commercial preparations are far from pure, containing non-UV-absorbing salts, solvents, *etc.*[58] Such preparations are unsuitable for quantitative analysis. Some commercial standards are incorrectly described at regio-isomer level,[1] and use of surrogate standards such as a green coffee extract, an alcoholic cider, or artichoke extract, characterised by LC-MS fragmentation and relative retention times should be considered.[130]

4 Biosynthesis of acyl-quinic acids

The initial steps in the biosynthesis of CQAs are *via* the phenylpropanoid pathway and the enzymes catalysing the conversions that produce 5-CQA (**9**) are well established but there is less clarity about the later stages of the pathway leading from 5-CQA to other acyl-quinic acids.

The conversion of phenylalanine to *p*-coumaroyl-CoA, with cinnamic acid and *p*-coumaric acid acting as intermediates, is catalysed sequentially by phenylalanine ammonia lyase (PAL), cinnamate 4′-hydroxylase (C4H) and 4-cinnamoyl-CoA ligase (4CL) (Fig. 2). PAL is encoded by a multi-gene family and typically exists as multiple isoforms in plants, ranging from a few members, such as three in Arabica coffee (*Coffea canephora*),[132] four in *Arabidopsis thaliana*, and nine in rice (*Oryza sativa*)[133] to

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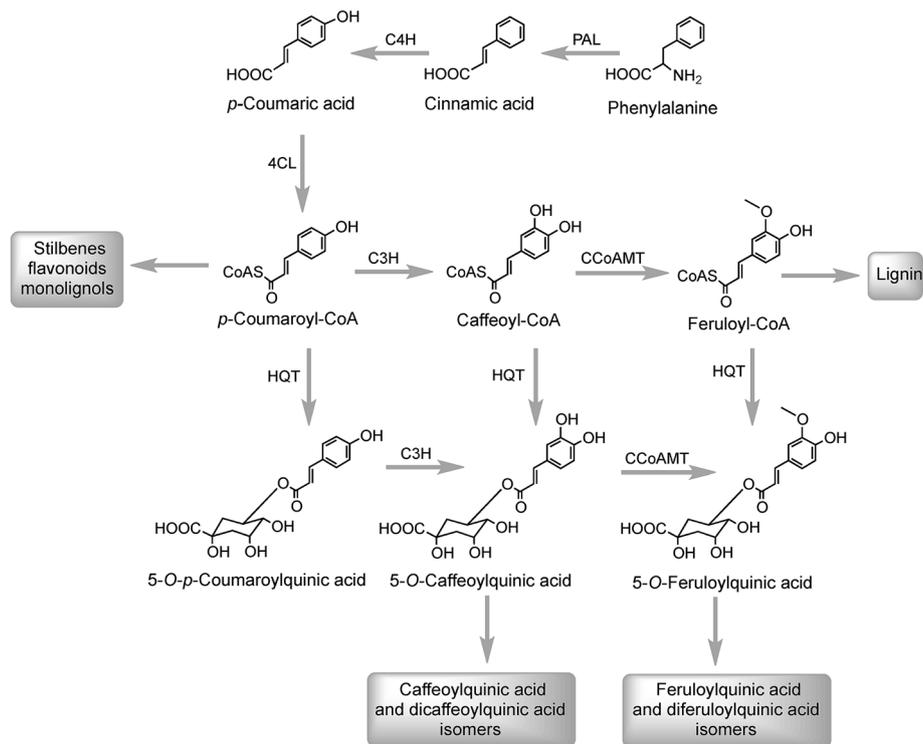This journal is © The Royal Society of Chemistry 2017

Fig. 2 5-O-Caffeoylquinic acid biosynthesis pathways. Enzymes: phenylalanine ammonia lyase (PAL), cinnamate 4′-hydroxylase (C4H), 4-cinnamoyl CoA ligase (4CL), hydroxycinnamoyl CoA:quinate hydroxycinnamoyl transferase (HQT), p-coumaroyl-3′-hydroxylase (C3H), and caffeoyl-CoA-3-O-methyltransferase (CCoAMT).

more than a dozen copies in tomato (*Lycoperscion esculentum*).[134] Individual PAL isoforms might be associated with specific metabolic activity during plant growth and development, and in plant–environment interactions.[135] In western balsam poplar (*Populus trichocarpa*), there are five genes (PtrPAL1–5) that are differentially expressed. PtrPAL2, 4 and 5 are mainly expressed in xylem and root tips, predominantly responsible for the production of lignin while PtrPAL1 and 3 are responsible principally for the production of condensed tannins.[136] However, in plants such as tomato, most of the isoforms of PAL present are functionally redundant and underutilized. There are 20 PAL-encoding genes in tomato but only one is expressed strongly throughout the plant with others being silenced.[134] Since PAL is the entry point enzyme, its control and regulation is crucial to mediate carbon flux from primary metabolism into biosynthesis of acyl-quinic acids. The regulation of PAL have been shown to occur at multiple levels, and has been reviewed in detail by Zhang and Liu.[135]

In *Arabidopsis thalania* the C4H catalysing the 4′-hydroxylation of cinnamic acid to p-coumaric acid (Fig. 2) is encoded by a single gene, mutation of which causes a dwarf phenotype, male sterility and swellings at branch junctions, and also results in the accumulation of cinnamoylmalate (17), which is not found in wild-type plants.[137] However, when the gene encoding C4H in sweet sagewort (*Artemisia annua*) was silenced through RNAi technology, it caused accumulation of cinnamic acid accompanied with significant reductions in p-coumaric acid (1), total phenolics and anthocyanins, highlighting its role as

a major flux controlling enzyme in the phenylpropanoid pathway (Kumar *et al.* 2016).[138] The next step in the pathway is the conversion of p-coumaric acid to p-coumaroyl-CoA catalysed by a small gene family of 4CL ligases (Fig. 2). p-Coumaroyl-CoA is a key branch point in phenylpropanoid biosynthesis acting as the immediate precursor of flavonoids and stilbenes, as well as being channeled into the production of methoxy guaiacyl- and syringyl-monolignols.[139] Four isoforms of 4CL have been identified in *Morus notabilis* (mulberry)[140] and *Arabidopsis*.[141] The subcellular localization of specific isoforms of 4CL plays a central role in redirecting the biosynthetic pathway either towards the p-coumaroyl-CoA or towards other branch points in the phenylpropanoid pathway.[141]
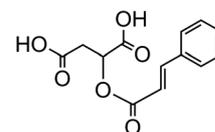
Cinnamoylmalate (17)

The conversion of p-coumaroyl-CoA to 5-CQA involves the enzymes hydroxycinnamoyl CoA:quinate hydroxycinnamoyl transferase (HQT) and a cytochrome P450 oxidase p-coumaroyl-3′-hydroxylase (C3H) (Fig. 2). The HQT-catalysed metabolism of p-coumaroyl-CoA produces 5-O-p-coumaroylquinic acid (5-pCoQA) which then undergoes C3H-mediated 3′-hydroxylation to yield 5-CQA. Alternatively, C3H-mediated hydroxylation of p-coumaroyl-CoA produces caffeoyl-CoA which is then converted

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to 5-CQA *via* the action of HQT (Fig. 2). Two HQT-encoding genes have been isolated from globe artichoke and when expressed in *Escherichia coli* each produced a recombinant protein, HQT1 and HQT2, with acyltransferase activity. Kinetic data and *in silico* homology modeling and docking analyses suggested that the two enzymes may be involved in different steps in the 5-CQA biosynthesis pathway with HQT1 catalysing the esterification of caffeoyl-CoA with quinic acid to produce 5-CQA, and HQT2 being involved in the conversion of *p*-coumaroyl-CoA to 5-*p*CoQA (Fig. 2).[142] This esterification is reversible. However, as a result of the genome-wide identification of BAHD acyltransferases in globe artichoke, a third isoform of HQT enzyme was detected.[143] When *HQT1* was subjected to virus-induced gene silencing, a marked reduction of both CQAs and diCQAs occurred. In contrast, transient overexpression of all three isoforms in leaves of *Nicotiana benthamiana* had the opposite effect,[63] highlighting the role of all three isoforms in the production of acyl-quinic acids.

5-CQA (**9**) is the dominant regio-isomer in most plants. However, most species also contain 4-CQA (**10**) and 3-CQA (**11**), while 1-CQA (**12**) is present in a limited number of plants, and 3-CQA is the dominant isomer in some species, most notably in plums (*Prunus* sp.) belonging to the Rosaceae family,[27] and some Brassicaceae.[144] From an enzymic perspective information is available only on the biosynthesis of 5-CQA (**9**) and the perceived wisdom is that other CQAs are derived from 5-CQA although there are no data on the seemingly specific isomerases involved in such conversions. Little is also known about the biosynthesis of di- and triCQAs or acyl-quinic acids containing substituents other than caffeic acid (**3**), although the *in vitro* synthesis of diCQAs from 5-CQA and CoA, mediated by a recombinant HCT enzyme cloned from coffee, has been reported.[145] In tomato, the enzyme HQT converted 5-CQA to diCQAs *in vitro*. It was proposed that the HQT enzyme has a dual role *in vivo* catalysing different reactions in two subcellular compartments: in the cytoplasm acting as a quinate transferase while in the vacuole favouring chlorogenate:chlorogenate transferase (acyl-quinate:acyl-quinate transferase) activity producing diCQA.[146]

Research with globe artichoke, switchgrass (*Panicum virgatum*) and chicory has established a further route to acyl-quinic acids that contains a shikimic acid shunt[142,147] It involves an hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT1; EC 2.3.1.133), or possibly two such enzymes in switchgrass[148] that catalyse the conversion of *p*-coumaroyl-CoA to 5-*O*-*p*-coumaroylshikimic acid, which is further converted to 5-*O*-caffeoylshikimic acid (dactylifric acid) by *p*-coumaroyl-shikimate-3′-hydroxylase (C3H) (Fig. 3). The caffeoylshikimate so generated could be converted to caffeoyl-CoA by an HCT acting in the reverse direction. *In vivo* functional analysis of the gene encoding HCT from chicory when transiently overexpressed in tobacco confirmed the involvement and the role of HCT in this step.[147] Alternatively a caffeoylshikimate esterase could release caffeic acid which is converted to caffeoyl-CoA by a ligase. Conversion of caffeoyl-CoA to 5-CQA in globe artichoke and other dicots probably involves an HQT.[143,147] The gene encoding this enzyme is not present in switchgrass and phylogenetic analysis suggests that in monocots the conversion may be catalysed by enzymes more closely related to HCT than HQT (Fig. 3).[148]

As far as the production of FQAs, such as 5-FQA is concerned, cDNA encoding *S*-adenosyl-L-methionine:caffeoyl-CoA-3-*O*-methyltransferase (CCoAOMT) an enzyme which catalyses the conversion of caffeoyl-CoA to feruloyl-CoA (Fig. 2) has been
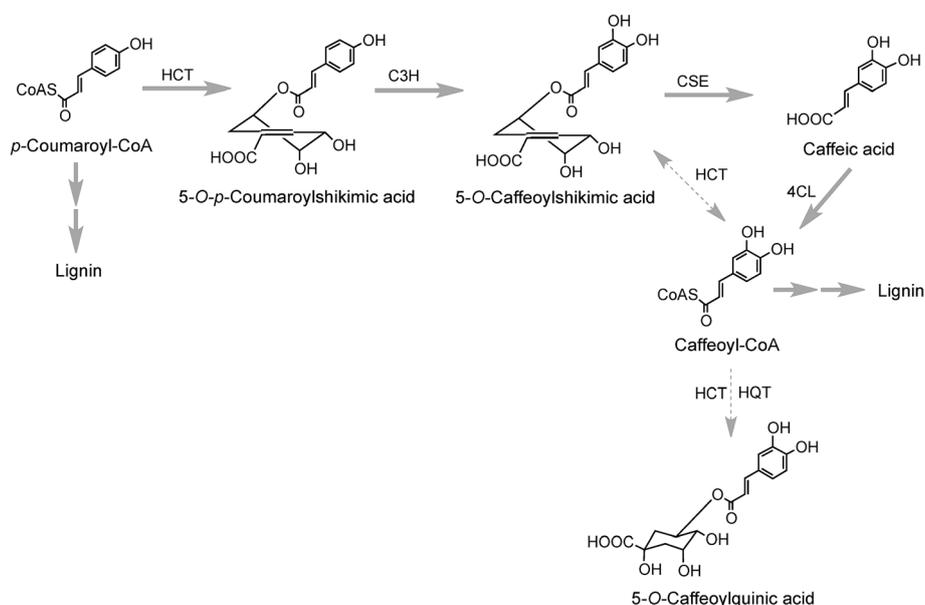

Fig. 3 5-*O*-Caffeoylquinic acid biosynthesis by a shikimate shunt. The likely main routes are indicated with bold arrows and minor routes with dotted arrows. Enzymes: hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT), *p*-coumaroyl-3′-hydroxylase (C3H), caffeoylshikimate esterase (CSE), 4-cinnamoyl CoA ligase (4CL), hydroxycinnamoyl CoA:quinate hydroxycinnamoyl transferase (HQT).

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cloned from oats (*Avena sativa*)[149] and other plants, including *Coffea* species.[150] Although the main role of the CCoAOMT family was initially ascribed to lignin biosynthesis,[151] other studies have indicated its multifunctional role in catalysing steps in other biosynthetic pathways including those producing the coumarin, scopoletin (**16**),[152] anthocyanins[153] and other flavonoids.[154] In the scopoletin biosynthetic pathway, a recombinant CCoAOMT1 protein possessed methylating activity *in vitro* targeting caffeoyl-CoA and converting it to feruloyl-CoA.[152]

A third pathway to 5-CQA from cinnamic acid has been proposed on the basis of data obtained with tubers of sweet potato (*Ipomoea batatas* Lam.) which converted [2-^{14}C]cinnamic acid to a radiolabeled intermediate that was metabolized to 5-CQA and further converted to 3,5-diCQA[155] both of which are major native acyl-quinic acids in sweet potato.[156] The radio-labeled intermediate was identified as 1-*O*-cinnamoyl-glucose[157] and it was proposed that it was converted to 5-CQA *via* 1-*O*-*p*-coumaroyl-glucose and 1-*O*-caffeoyl-glucose as illustrated in Fig. 4.[157,158] Enzymes which catalyse the first three steps in this postulated pathway, a UDP-glucose:cinnamate glucosyltransferase,[159] a *p*-coumaroyl-glucose hydroxylase[160] and a hydroxycinnamoyl glucose:quinate hydroxycinnamoyltransferase have been detected in sweet potato roots.[158,161] In addition, a partially purified enzyme has been isolated from sweet potato which catalyses the single step conversion of 5-CQA to 3,5-diCQA.[162]

The production of cinnamoyl-glucose has also been reported in strawberry (*Fragaria × ananassa*) where a cDNA encoding a UDP-glucose:cinnamate preferentially catalysed cinnamic acid *in vitro*. The cDNA transcript was found to accumulate during strawberry fruit ripening and this positively correlated with the *in planta* concentration of cinnamoyl, *p*-coumaroyl-, and caffeoyl-glucose.[163] Similar results were obtained when the gene encoding this enzyme was overexpressed in transgenic *Populus* where it led to accumulation of hydroxycinnamate glucose esters which further increase under *N*-limiting conditions.[164]

It has long been known that exposure of *trans*-cinnamoyl-quinic acids to UV light readily produces the associated *cis* isomers, and such isomers have been found in tissues, such as the leaves of tobacco and coffee, that are exposed to comparatively strong UV-irradiation, suggesting that such exposure might explain their presence.[48] 3,5-DiCQA has been shown to undergo isomerisation upon UV exposure where the naturally occurring 3-*trans*, 5-*trans*-diCQA isomer (**18**) gives rise to the 3-*cis*, 5-*trans*-diCQA (**19**), 3-*trans*, 5-*cis*-diCQA (**20**), and 3-*cis*, 5-*cis*-diCQA (**21**) isomers, allowing them to have a wider synergistic biological activity when present together.[165] Recent studies of 120 samples of *Stevia rebaudiana* leaves have established a strong positive correlation between sunshine hours prior to harvesting and the content of *cis* isomers in the harvested leaves,[166] effectively confirming this hypothesis. It has also been shown that treatment of cultured tobacco cells with several inducers of plant stress leads to an approximate doubling of *cis* 5-CQA (**22**) in the absence of UV irradiation.[50]

There are many other details yet to be elucidated and several intriguing questions remain to be answered regarding the factors that control the biosynthesis of acyl-quinic acids and the significance of the observed variations.

5   Role of acyl-quinic acids *in planta*

With one or two notable exceptions, the *in planta* role(s) of acyl-quinic acids are not known with any certainty. It seems almost certain that their function in tissues containing *ca.* 100 g kg^{-1} is different to their function in tissues containing only mg kg^{-1} concentrations. Radiotracer studies have shown that 5-CQA (**9**) is incorporated into lignin in *Xanthium*[167] which suggests that 5-CQA may accumulate as a store of cinnamic acid for lignin biosynthesis. It has also been proposed that FQAs are important indirect plant defence agents acting as lignin precursors, the lignin *per se* making tissues more resistant to attack or invasion.[168]
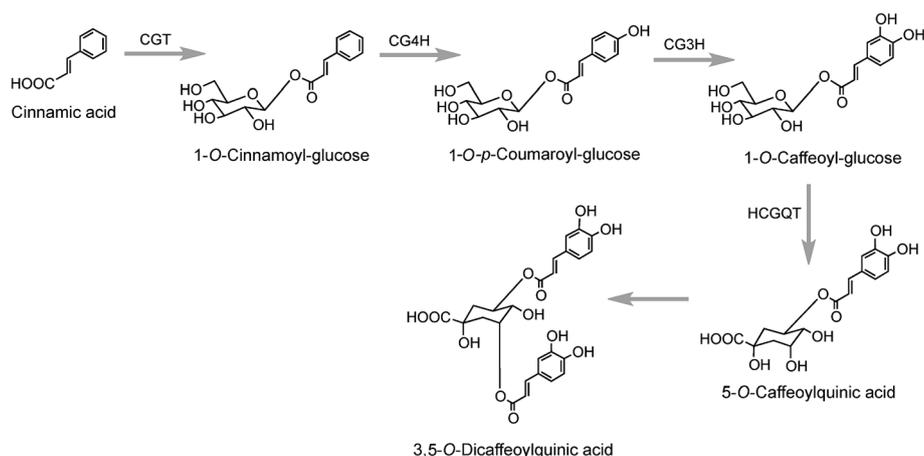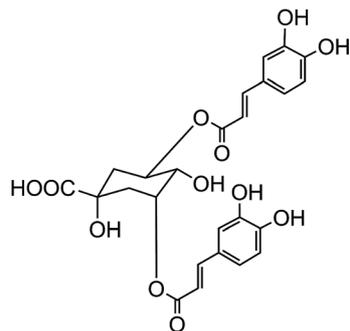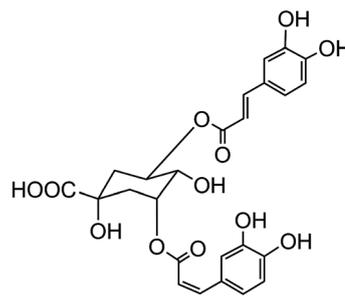

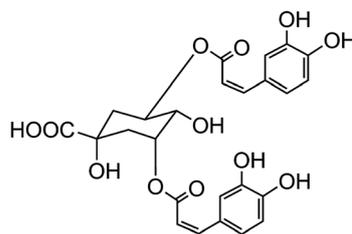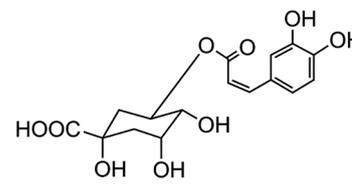Fig. 4   Proposed pathway for the biosynthesis of 5-*O*-caffeoylquinic acid in sweet potato. Enzymes: UDP-glucose:cinnamate glucosyltransferase (CGT), cinnamate–glucose 4′-hydroxylase (CG4H), *p*-coumaroyl-glucose 3′-hydroxylase (CG3H) and hydroxycinnamoyl glucose:-quinate hydroxycinnamoyltransferase (HCGQT).

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3-*trans*, 5-*trans*-O-Dicaffeoylquinic acid (**18**)   3-*cis*, 5-*trans*-O-Dicaffeoylquinic acid (**19**)
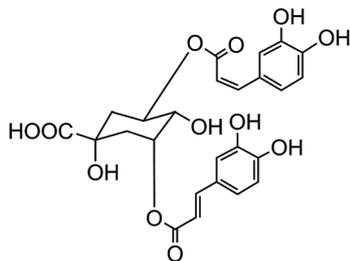
3-*trans*, 5-*cis*-O-Dicaffeoylquinic acid (**20**)   3-*cis*, 5-*cis*-O-Dicaffeoylquinic acid (**21**)   5-*cis*-O-Caffeoylquinic acid (**22**)

A high acyl-quinic acid content has been linked to the allelopathic potential and resistance to root disease of some sweet potato clones,[169] *via* an inhibition of fungal toxin production.[170] An elevated 5-CQA content of transgenic tomato plants has been associated with enhanced tolerance to paraquat-induced oxidative stress and resistance to infection by the pathogen *Pseudomonas syringae*.[171] Acyl-quinic acids acting as defence metabolites may have possible links to phytohormone-activated defense signaling networks as implied in *Nicotiana tabacum* cells treated with salicylic acid (**23**) and methyl-jasmonate (**24**).[172] The defense signaling networks produce a range of acyl-quinic acids to combat pathogens and some are capable of inhibiting cell wall degrading enzymes, such as polygalacturonase and cutinase, produced by the pathogens.[173]
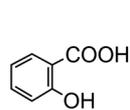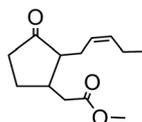
Salicylic acid (**23**)   Methyl jasmonate (**24**)

In tomato plants CQA has been associated with a protective ability against oxidative damage when exposed to salinity or a combination of salinity and heat.[174] Studies with transgenic tobacco plants have demonstrated that inhibition of acyl-quinic acid biosynthesis makes the plants more susceptible to oxidative damage and accelerated cell death,[175] while in tomato, globe artichoke and chamomile (*Matricaria chamomilla*), acyl-quinic acids appear to protect against UV damage.[176–178] There is evidence that these compounds also protect against predators because those which are substrates for polyphenol oxidase and/or peroxidase, are readily converted to quinones which react readily with nucleophiles and link covalently to protein.[179–182] This interaction is thought to have anti-feedant activity, for example, limiting the growth of herbivorous insect pests by impairing digestibility of the hosts' proteins.[183–185]

6 Acyl-quinic acids in coffee

Much of the literature on acyl-quinic acids focuses on coffee and green beans which contain substantial amounts. In addition to the major CQAs, FQAs, diCQAs and *p*-CoQAs (Fig. 5) many quantitatively minor acyl-quinic acids have also been identified.[33,102,114,186,187] Currently, green coffee, with 72 acyl-quinic acids, is second only to *Lonicera japonica* (Japanese honeysuckle) which contains a record 111 in the form of 35 *trans*-cinnamoyl-quinic acids, 17 *cis* isomers and 49 acyl-quinic acid glucosides.[188]

The principal component in green coffee beans is always 5-CQA (**9**) and is accompanied by numerous diacyl-quinic acids and some triacyl-quinic acids. All the cinnamic acid derivatives so far characterised in green coffee have the cinnamate moiety in the *trans* configuration. To date, no 1-acyl-quinic acids have been found in the green coffee bean. The triacyl-quinic acids have so far been found only in Robusta coffee beans.[33] Robustas usually have a greater content of any isomer but there are exceptions, the best known being a tendency for Arabicas to contain more 5-*p*-CoQA (**25**) than Robustas. From a chemotaxonomic standpoint it is interesting to note that acyl-quinic acids containing 3′,4′-dimethoxycinnamic acid (**26**), 3′,4′,5′-trimethoxycinnamic acid (**27**), 3′-methoxy-4′,5′-dihydroxycinnamic acid (5-hydroxyferulic
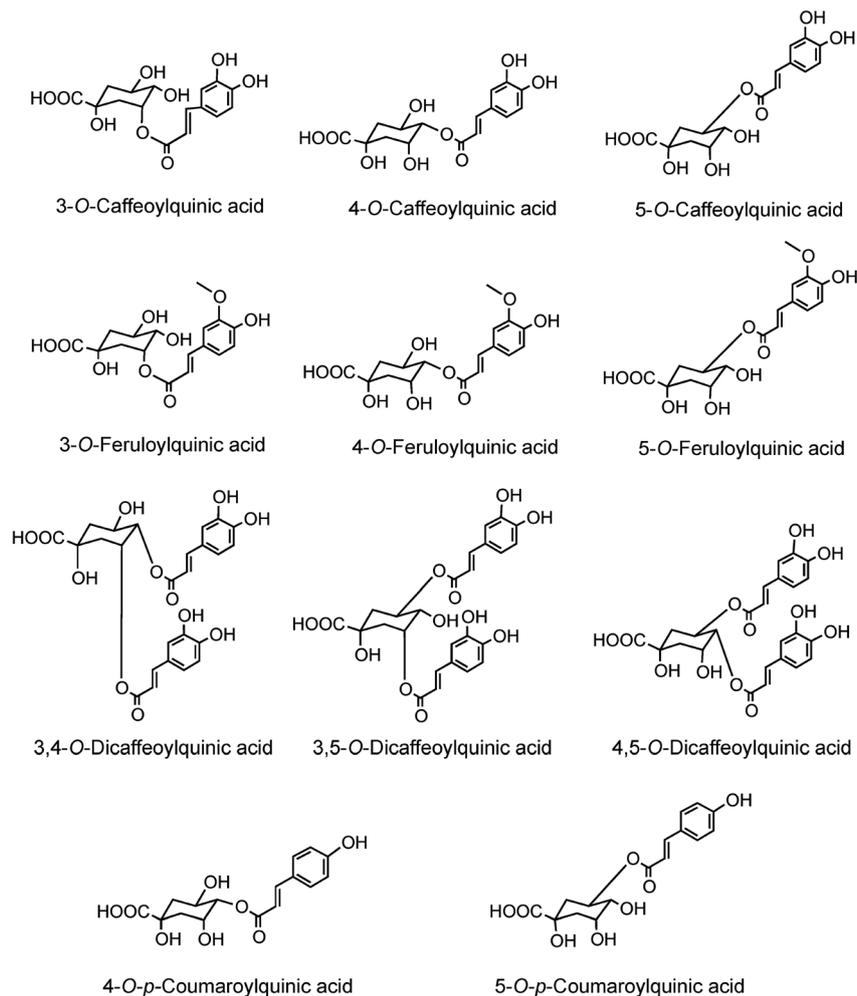
3-O-Caffeoylquinic acid   4-O-Caffeoylquinic acid   5-O-Caffeoylquinic acid

3-O-Feruloylquinic acid   4-O-Feruloylquinic acid   5-O-Feruloylquinic acid

3,4-O-Dicaffeoylquinic acid   3,5-O-Dicaffeoylquinic acid   4,5-O-Dicaffeoylquinic acid

4-O-p-Coumaroylquinic acid   5-O-p-Coumaroylquinic acid

Fig. 5   The main acyl-quinic acids in coffee beans.

acid) (**28**), and 3′,5′-dihydroxy-4′-methoxycinnamic acid (**29**) are scarce in nature and may be unique to coffee. Cinnamoyl-amino acid conjugates also have been characterised,[26,187,189,190] and together with the acyl-quinic acids have been used as criteria in chemo-taxonomic studies[191,192] for distinguishing Robustas from Arabicas,[193] and to some extent the profile can be used to define the geographic origin of green coffee beans, especially Robustas from Angola,[25,197] Ethiopia,[194] Uganda, Vietnam, Cameroun and Indonesia, and for distinguishing South American from African Arabicas.[193]

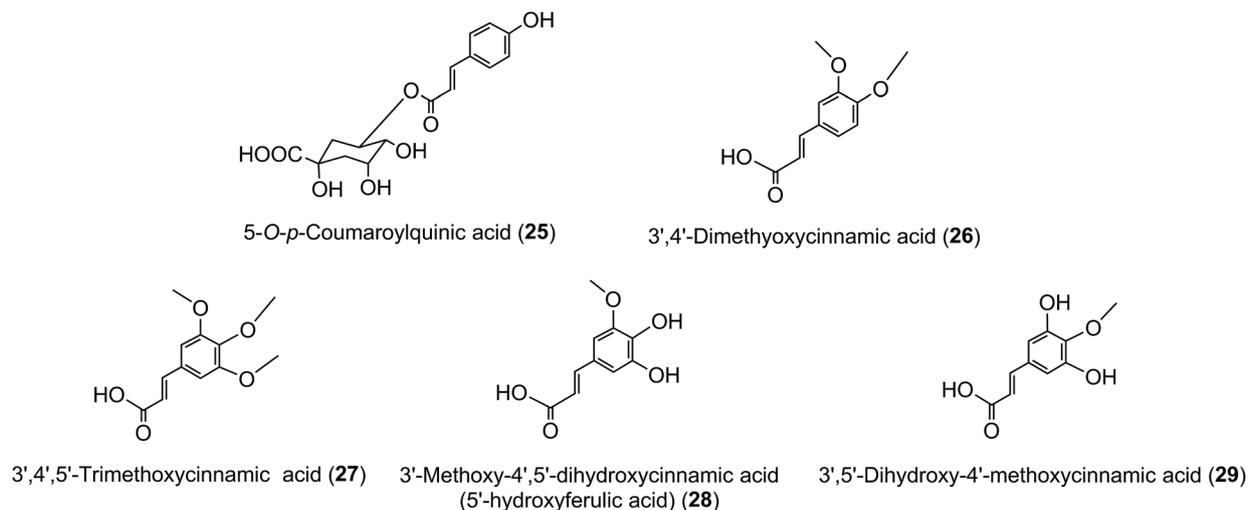5-O-p-Coumaroylquinic acid (**25**)   3′,4′-Dimethyoxycinnamic acid (**26**)

3′,4′,5′-Trimethoxycinnamic acid (**27**)   3′-Methoxy-4′,5′-dihydroxycinnamic acid (5′-hydroxyferulic acid) (**28**)   3′,5′-Dihydroxy-4′-methoxycinnamic acid (**29**)

Table 2   Cinnamoyl–quinic acids and other cinnamic acid derivatives occurring in green coffee beans

Caffeic acid[114]	Caffeoyl-hexose conjugate[a][114]
Ferulic acid[114]	Dicaffeoyl-hexose conjugate[a][114]
3,4-Dimethoxcinnamic acid[114]	Dimethoxycinnamoyl-hexose conjugate[a][114]
3-O-p-Coumaroylquinic acid[30]	3-O-Caffeoylquinic acid[30]
4-O-p-Coumaroylquinic acid[30]	4-O-Caffeoylquinic acid[30]
5-O-p-Coumaroylquinic acid[30]	5-O-Caffeoylquinic acid[30]
3-O-Feruloylquinic acid[29]	3-O-Dimethoxycinnamoylquinic acid[102]
4-O-Feruloylquinic acid[29]	4-O-Dimethoxycinnamoylquinic acid[102]
5-O-Feruloylquinic acid[29]	5-O-Dimethoxycinnamoylquinic acid[102]
3-O-Sinapoylquinic acid[101]	3,4-O-Di-p-coumaroylquinic acid[111]
4-O-Sinapoylquinic acid[101]	3,5-O-Di-p-coumaroylquinic acid[111]
5-O-Sinapoylquinic acid[101]	4,5-O-Di-p-coumaroylquinic acid[111]
3,4-O-Dicaffeoylquinic acid[30]	3,4-O-Diferuloylquinic acid[110]
3,5-O-Dicaffeoylquinic acid[30]	3,5-O-Diferuloylquinic acid[110]
4,5-O-Dicaffeoylquinic acid[30]	4,5-O-Diferuloylquinic acid[110]
3-O-Caffeoyl-4-O-feruloylquinic acid[30]	3-O-Caffeoyl-4-O-p-coumaroylquinic acid[101]
3-O-Feruloyl-4-O-caffeoylquinic acid[30]	3-O-p-Coumaroyl-4-O-caffeoylquinic acid[101]
3-O-Caffeoyl-5-O-feruloylquinic acid[30]	3-O-Caffeoyl-5-O-p-coumaroylquinic acid[101]
3-O-Feruloyl-5-O-caffeoylquinic acid[30]	3-O-p-Coumaroyl-5-O-caffeoylquinic acid[101]
4-O-Caffeoyl-5-O-feruloylquinic acid[30]	4-O-Caffeoyl-5-O-p-coumaroylquinic acid[101]
4-O-Feruloyl-5-O-caffeoylquinic acid[30]	4-O-p-Coumaroyl-5-O-caffeoylquinic acid[101]
3-O-Dimethoxycinnamoyl-4-O-caffeoylquinic acid[102]	3-O-Dimethoxycinnamoyl-4-O-feruloylquinic acid[102]
3-O-Caffeoyl-4-O-dimethoxycinnamoylquinic acid[190]	3-O-Dimethoxycinnamoyl-5-O-feruloylquinic acid[102]
3-O-Dimethoxycinnamoyl-5-O-caffeoylquinic acid[102]	4-O-Dimethoxycinnamoyl-5-O-feruloylquinic acid[102]
3-O-Caffeoyl-5-O-dimethoxycinnamoylquinic acid[190]	
4-O-Dimethoxycinnamoyl-5-O-caffeoylquinic acid[102]	
4-O-Caffeoyl-5-O-dimethoxycinnamoylquinic acid[190]	
3-O-p-Coumaroyl-4-O-feruloylquinic acid[101]	3-O-Caffeoyl-4-O-sinapoylquinic acid[33]
3-O-p-Coumaroyl-5-O-feruloylquinic acid[101]	3-O-Sinapoyl-5-O-caffeoylquinic acid[33]
4-O-p-Coumaroyl-5-O-feruloylquinic acid[101]	3-O-Sinapoyl-4-O-caffeoylquinic acid[33]
3-O-p-Coumaroyl-4-O-dimethoxycinnamoylquinic acid[33]	3-O-Sinapoyl-5-O-feruloylquinic acid[33]
3-O-p-Coumaroyl-5-O-dimethoxycinnamoylquinic acid[33]	3-O-Feruloyl-4-O-sinapoylquinic acid[b][33]
4-O-Dimethoxycinnamoyl-5-O-p-coumaroylquinic acid[101]	4-O-Sinapoyl-5-O-feruloylquinic acid[33]
3-O-Trimethoxycinnamoyl-5-O-caffeoylquinic acid[33]	3-O-Trimethoxycinnamoyl-4-O-feruloylquinic acid[33]
4-O-Trimethoxycinnamoyl-5-O-caffeoylquinic acid[33]	3-O-Trimethoxycinnamoyl-5-O-feruloyl-quinic acid[33]
	4-O-Trimethoxycinnamoyl-5-O-feruloylquinic acid[33]
3-O-(3′,5′-dihydroxy-4′-methoxy)cinnamoyl-4-O-feruloylquinic acid[33]	3,4,5-O-Tricaffeoylquinic acid[33]
3,4-O-Dicaffeoyl-5-O-feruloylquinic acid[33]	3,4-O-Dicaffeoyl-5-O-sinapoylquinic acid[33]
3,5-O-Dicaffeoyl-4-O-feruloylquinic acid[33]	3-O-Sinapoyl-4,5-O-dicaffeoylquinic acid[33]
3-O-Feruloyl-4,5-O-dicaffeoylquinic acid[33]	
3-O-Dimethoxycinnamoyl-4-O-feruloyl-5-O-caffeoylquinic acid[b][33]	3,4-O-Diferuloyl-5-O-caffeoylquinic acid[33]
	3-O-Caffeoyl-4,5-O-diferuloylquinic acid[33]

[a] Tentative identification. [b] Identification correct but structure as illustrated in original paper is incorrect.

Tables 2 and 3 provide a comprehensive list of the acyl-quinic acids and cinnamoyl-amino acid conjugates occurring in green coffee beans. Although Robusta coffees contain all six CFQA and all six p-coumaroyl-caffeoylquinic acids, intriguingly, they only contain three caffeoyl-(3′,4′-dimethoxycinnamoyl)-quinic acids, feruloyl-(3′,4′-dimethoxycinnamoyl)quinic acids, p-coumaroyl-feruloylquinic acids and p-coumaroyl-(3′,4′-dimethoxycinnamoyl)quinic acids highlighting some subtle control of biosynthesis.

Roasting of green coffee beans can result in acyl-quinic acid losses of ~90%.[43] Substantial transformations also occur and the number of acyl-quinic acids detected in the roasted bean increases to over 200 as judged by the number of characteristic fragments observed in tandem mass spectrometry-based

Table 3 Cinnamoyl-amino acid conjugates in green and roasted coffee beans

Cinnamoyl-amino acid conjugates
p-Coumaroyl-N-tyrosine[186]
Caffeoyl-N-tyrosine[186]
Feruloyl-N-tyrosine[186]
p-Coumaroyl-N-tryptophan[186]
Caffeoyl-N-tryptophan[186]
Feruloyl-N-tryptophan[186]
Caffeoyl-N-phenylalanine[186]
p-Coumaroyl-N-aspartic acid[187]
Caffeoyl-N-aspartic acid[187]

analyses, in comparison with the 72 derivatives detected in the green coffee bean.[30,31,33,102,114,115,186,187,190] Acyl migration and hydrolysis during coffee roasting changes the relative proportions of each subgroup, being most obvious within the CQAs, FQAs and diCQAs, with 3-CQA being very susceptible to hydrolysis. For example, it has been reported that after 5 min of roasting the content of 5-CQA (**9**) decreased substantially while the levels of 4-CQA (**10**) and 3-CQA (**11**) increased to twice their original values. The same behavior was observed for FQAs. It is also noted that partial hydrolysis of diCQAs to CQAs occurs in addition to isomerization.[22,195] The triacyl-quinic acid derivatives of the green coffee bean have not been found in the roasted bean, and presumably are lost *via* partial hydrolysis. The clearest evidence for acyl migration during coffee roasting is the appearance of 1-acyl derivatives, absent from the green beans,[196] either as the free acids or as the lactones 1-O-caffeoylquinic-1,5-lactone (1-CQL) (**30**) and 1-O-feruloylquinic-1,5-lactone (1-FQL) (**31**)[197] with similar transformations also occurring during brewing and instant coffee manufacture.[198,199]
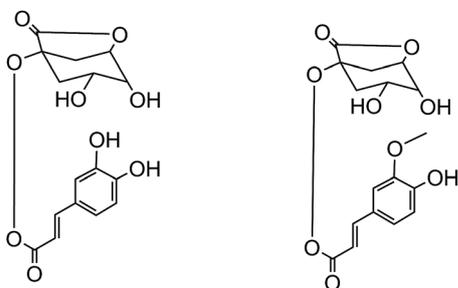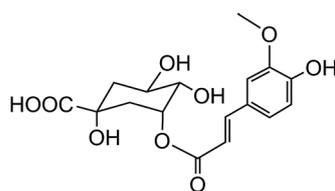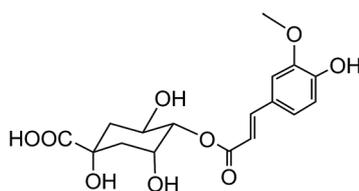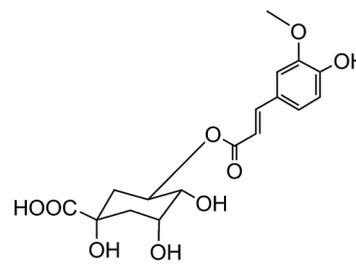
Quantitatively, the main acyl-quinic acids in the roast bean and, instant coffee powder and beverage are CQAs, FQAs and diCQAs (Fig. 5).[23] Despite the losses of acyl-quinic acids that occur during roasting, coffee beverage is still a rich, probably the richest, dietary source.[200] However, because of the use of a diversity of roasting methods and infusion/manufacturing protocols, a 'cup of coffee' is an extremely variable commodity which is best illustrated by modern data for the acyl-quinic acids contents. Espresso coffees bought at 20 commercial outlets near the University of Glasgow in the UK delivered between 24 mg and 423 mg per serving (Table 4).[43] A similar but more extensive study of coffees from 104 commercial outlets in Scotland, Italy and Spain observed that one cup delivered between 6 mg and 188 mg of acyl-quinic acids.[201] Standardised brewing of eight commercial roast and ground coffees (20 g/900 mL) delivered between 27 and 95 mg of acyl-quinic acids per cup, whereas standardised preparation of ten commercial instant coffees available in the UK (1.8 g/200 mL) delivered between 37 and 121 mg.[202] An individual preferring weak instant coffee would likely consume rather less than these figures suggest. It is well known that coffee brews consumed on the American west coast are appreciably weaker than on the American east coast. This variation is such that, conceivably, a person consuming 10 cups per day might actually receive less acyl-quinic acids than another consuming only one cup per day. While this is undoubtedly an extreme and rare event, a four to five-fold variation can surely be expected. Under such circumstances data from epidemiological studies that link 'cups consumed' with an effect on health, whether advantageous or otherwise, can only be treated as a hypothesis until further investigations are performed under conditions that are as controlled as possible.

7 Bioavailability and metabolism of acyl-quinic acids in humans

Because of the dominance of coffee as the source of dietary acyl-quinic acids it has been used in most human studies on their absorption and metabolism, supported by *in vitro* investigations using cultured human cells. A study using cultured gastric epithelial cells established that CQAs, FQAs and CQLs cross the epithelium. The 3-acyl and 5-acyl regio-isomers probably cross by passive diffusion *via* the paracellular route, whereas facilitated transport might operate for 4-CQA and 4-FQA.[203] DiCQAs crossed the membrane even more rapidly, a behaviour that could be attributable to their greater hydrophobicity, although in the case

1-O-Caffeoyl-1,5,-lactone (**30**)   1-O-Feruloyl-1,5,-lactone (**31**)

3-O-Feruloylquinic acid (**32**)   4-O-Feruloylquinic acid (**33**)   5-O-Feruloylquinic acid (**34**)

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Table 4 Levels of caffeoylquinic acids (6, 14, 15) in espresso coffees purchased from 20 coffee shops in Glasgow, United Kingdom (after Crozier et al.[43])[a]

Coffee shop	Volume of coffee (mL)	3-CQA (mg)	4-CQA (mg)	5-CQA (mg)	Total CQA per serving (mg)
Pattiserie Francoise	52	95	112	216	423
S'mug	32	62	78	160	300
Costa coffee	25	48	61	118	227
Little Italy	23	37	59	121	217
Paperino's	50	65	52	99	216
Peckhams	70	65	52	99	216
Chapter 1	26	45	58	112	215
University Cafe	49	40	54	93	187
Baguette express	45	30	40	74	144
Kember & Jones	43	37	46	92	141
Heart Buchanan	24	22	37	67	126
Jellyhill	63	26	29	56	111
Coffee @ 491	49	23	31	55	109
Beanscene	48	19	25	49	93
Tinderbox	25	19	24	46	89
Café Cinnamon	59	17	23	41	81
Crepe á croissant	34	17	23	41	81
Morton's	35	13	16	27	56
Antipasti	36	8	15	21	44
Starbucks	27	5	7	12	24
Range	23–70	5–95	7–112	12–216	24–423
Median	43	36	37	67	126

[a] Data expressed as mean values (n = 3). SE = <10% of mean value.

of 3,5-diCQA (18) there was evidence for a carrier-mediated efflux.[203] These observations may help to explain the results of feeding studies, discussed below, where minor acyl-quinic acids in the coffee beverage had a higher peak plasma concentration (C_{max}) than the dominant regio-isomers. For example, Stalmach et al.[44] observed that 3-FQA (32) and 4-FQA (33) had C_{max} values of 16 ± 2 nmol L^{-1} and 14 ± 2 nmol L^{-1}, respectively, while the beverage dominant 5-FQA (34) peaked at 6 ± 2 nmol L^{-1}.

In vitro studies have also shown that acyl-quinic acids differ in their susceptibility to intestinal chlorogenate esterase (acyl-quinate esterase), with 5-CQA (9) being hydrolysed more readily than 3-CQA (11), and 4-CQA (10) being particularly resistant to hydrolysis.[204] Consistent with this enzymic hydrolysis occurring in the stomach or upper gastrointestinal (GI) tract, significant amounts of 1L-(−)-quinic acid, in excess of the amount consumed in the free form, were found in the ileostomy effluent of volunteers who consumed cloudy apple juice, apple smoothie, or coffee.[205] Such in vivo resistance to enzymic and chemical hydrolysis post-absorption would also predispose to greater plasma concentrations of 4-acyl-quinic acids. It follows that commodities with acyl-quinic acid profiles substantially different from that of coffee beverage are likely to produce plasma profiles of acyl-quinic acid metabolites that differ quite markedly from those produced when coffee is consumed, as recently reported for maté which contains proportionately more 3-CQA (11) and diCQAs.[206]

The presence of low nmol L^{-1} concentrations of CQAs in plasma and their low level excretion in urine after oral intake of coffee,[44,207–209] artichoke,[210] and 5-CQA (9)[211] suggest that the bioavailability of acyl-quinic acids per se is limited. In contrast, Monteiro et al.[212] reported the presence of unmetabolised CQAs in the circulatory system with a C_{max} of 7.7 µmol L^{-1}, 2.3 h (T_{max}) after acute ingestion of a coffee containing 3395 µmol of acyl-quinic acids. Despite the high C_{max} of the CQAs, CQAs were not detected in urine collected 0–24 h after coffee intake. In a subsequent study by the same group, in which volunteers consumed a green coffee extract containing a much lower 451 µmol of acyl-quinic acids, 5-CQA (9) and 4-CQA (10) were detected in urine from some, but not all, subjects.[213] The CQAs were also detected in plasma although there were unusually marked variations in the plasma pharmacokinetic profiles of the individual subjects with, in two instances, an exceedingly high C_{max} of >20 µmol L^{-1} being recorded. The coffee used in this study contained 43.2 µmol of diCQAs which after consumption were reported to have attained a C_{max} of 6.6 µmol L^{-1}. Assuming an average plasma volume of 3 L per person, this corresponds to 45.8% of intake. Despite there being some evidence from in vitro studies that diCQAs are more rapidly absorbed than monoacyl-quinic acids,[203] it is difficult to reconcile these studies with the results obtained by other investigators. After volunteers consumed maté delivering 210 µmol of diCQAs no diacyl-quinic acids were detect in plasma.[206]

Stalmach et al.[44,214] carried out coffee feeding studies with healthy humans and ileostomists who have had their colon removed surgically for medical reasons, typically Crohn's disease or ulcerated colitis. In these investigations, samples were analysed using HPLC-MS[2] without recourse to the use of

the more typical glucuronidase/sulfatase treatments prior to analysis. It was observed that during passage through the body extensive metabolism of acyl-quinic acids occurs, with some compounds being absorbed in the stomach and/or small intestine and others in the colon. Studies with ileostomists have made a major contribution to our understanding of colonic (poly)phenol metabolism in humans with an intact colon. Viewed simply, a transformation associated with the colonic gut microflora will not be seen in ileostomists. In reality, it is important to recognise some colonisation of the upper GIT can occur in ileostomists and, as a consequence, some limited microbial transformations may persist. An ileostomy involves major surgery and is likely to have some patho-physiological

effects on other organs and tissues, and although the consequences have barely been studied, it would not be surprising if there were some alteration to (poly)phenol metabolism in, for example, the upper GIT, liver and kidney of ileostomists.

7.1 Studies involving volunteers with and without a functioning colon

Stalmach et al.[44] carried out a study in which healthy humans with an intact colon consumed a 200 mL serving of instant coffee, containing 412 μmol (146 mg) of acyl-quinic acids, with CQAs comprising 65% of the total (Table 5), after which plasma and urine samples were collected over a 24 h period. A total of 12 hydroxycinnamate derivatives, of which four were unchanged acyl-quinic acids and eight were metabolites, two of which were unconjugated, were identified and quantified in plasma. Their pharmacokinetic parameters are summarized in Table 6 and pharmacokinetic profiles are illustrated in Fig. 6.

A similar array of metabolites has been detected in plasma after the consumption of coffee by Scherbl et al.[209] Post-ingestion C_{max} values in the Stalmach study ranged from 2.2 nM for 5-CQA (9) to 385 nM for dihydroferulic acid (35)[44] with the duration for T_{max} extending from 0.6 h (ferulic acid-4′-sulfate [36] and a 3-CQL-sulfate) to 5.2 h (dihydroferulic acid [35]). The compounds detected in highest concentrations in plasma were free and sulfated conjugates of dihydroferulic acid (35) and dihydrocaffeic acid (37) with C_{max} values ranging from 41 to 385 nmol L^{-1}. The T_{max} for these compounds was in a narrow range from 4.7 to 5.2 h, implying absorption in the large intestine. Much shorter T_{max} values of 0.6 to 1.0 h, indicative of stomach and/or small intestine absorption, were obtained with 5-CQA (9) and three FQAs (32–34) which had not been subject to phase II metabolism, plus ferulic acid-4′-sulfate (36), caffeic acid-3′-sulfate (38), and two CQL-sulfates, and all of which had relatively low C_{max} values (Fig. 6 and Table 6).

As noted by Stalmach et al.[44] most of the acyl-quinic acid-derived compounds were rapidly removed from the circulatory

Table 5 Quantities of acyl-quinic acids in a 200 mL serving of instant coffee fed to volunteers (after Stalmach et al.[44])[a]

Chlorogenic acids	μmol
3-O-Caffeoylquinic acid	72 ± 1.3
4-O-Caffeoylquinic acid	78 ± 1.5
5-O-Caffeoylquinic acid	119 ± 2.1
Total caffeoylquinic acids	*269 ± 4.9*
3-O-Feruloylquinic acid	20 ± 1.6
4-O-Feruloylquinic acid	22 ± 1.8
5-O-Feruloylquinic acid	25 ± 1.9
Total feruloylquinic acids	*67 ± 5.3*
3-O-Caffeoylquinic acid lactone	34 ± 2.9
4-O-Caffeoylquinic acid lactone	23 ± 2.0
Total caffeoylquinic acid lactones	*57 ± 4.9*
3,4-O-Dicaffeoylquinic acid	5.8 ± 0.6
3,5-O-Dicaffeoylquinic acid	2.8 ± 0.3
4,5-O-Dicaffeoylquinic acid	4.0 ± 0.4
Total dicaffeoylquinic acids	*12.6 ± 1.2*
4-O-p-Coumaroylquinic acid	3.8 ± 0.1
5-O-p-Coumaroylquinic acid	3.1 ± 0.1
Total p-coumaroylquinic acids	6.8 ± 0.1
Total acyl-quinic acids	**412 ± 9.3**

[a] Data expressed as mean values ± SEM ($n = 3$).

Table 6 Pharmacokinetic parameters of acyl-quinic acid derivatives and metabolites circulating in plasma of healthy volunteers, 0–24 h following the ingestion of 412 μmol of acyl-quinic acids and derivatives contained in a 200 mL serving of instant coffee (after Stalmach et al.[44])[a]

Chlorogenic acids and metabolites	C_{max} (nmol L^{-1})	T_{max} (h)	$T_{1/2}$[c] (h)
5-O-Caffeoylquinic acid	2.2 ± 1.0	1.0 ± 0.2	0.3 ± 0.3
3-O-Caffeoylquinic lactone-sulfate	27 ± 3	0.6 ± 0.1	0.5 ± 0.1
4-O-Caffeoylquinic lactone-sulfate	21 ± 4	0.7 ± 0.1	0.4 ± 0.1
3-O-Feruloylquinic acid	16 ± 2	0.7 ± 0.1	0.9 ± 0.1
4-O-Feruloylquinic acid	14 ± 2	0.8 ± 0.1	0.9 ± 0.1
5-O-Feruloylquinic acid	6.0 ± 1.5	0.9 ± 0.1	0.8 ± 0.1
Caffeic acid-3′-sulfate	92 ± 11	1.0 ± 0.2	1.9 ± 0.4
Ferulic acid-4′-sulfate[b]	76 ± 9	0.6 ± 0.1	4.9 ± 1.0
	46 ± 13	4.3 ± 0.3	
Dihydroferulic acid	385 ± 86	4.7 ± 0.3	1.4 ± 0.4
Dihydroferulic acid-4′-sulfate	145 ± 53	4.8 ± 0.5	4.7 ± 0.8
Dihydrocaffeic acid	41 ± 10	5.2 ± 0.5	1.0 ± 0.4
Dihydrocaffeic acid-3′-sulfate	325 ± 99	4.8 ± 0.6	3.1 ± 0.3

[a] Data expressed as mean values ± SEM ($n = 11$). [b] Double C_{max} and T_{max} values are due to the biphasic absorption profile of ferulic acid-4′-sulfate (see Fig. 4). [c] Apparent $T_{1/2}$ estimated after oral intake rather than intravenous dosing.

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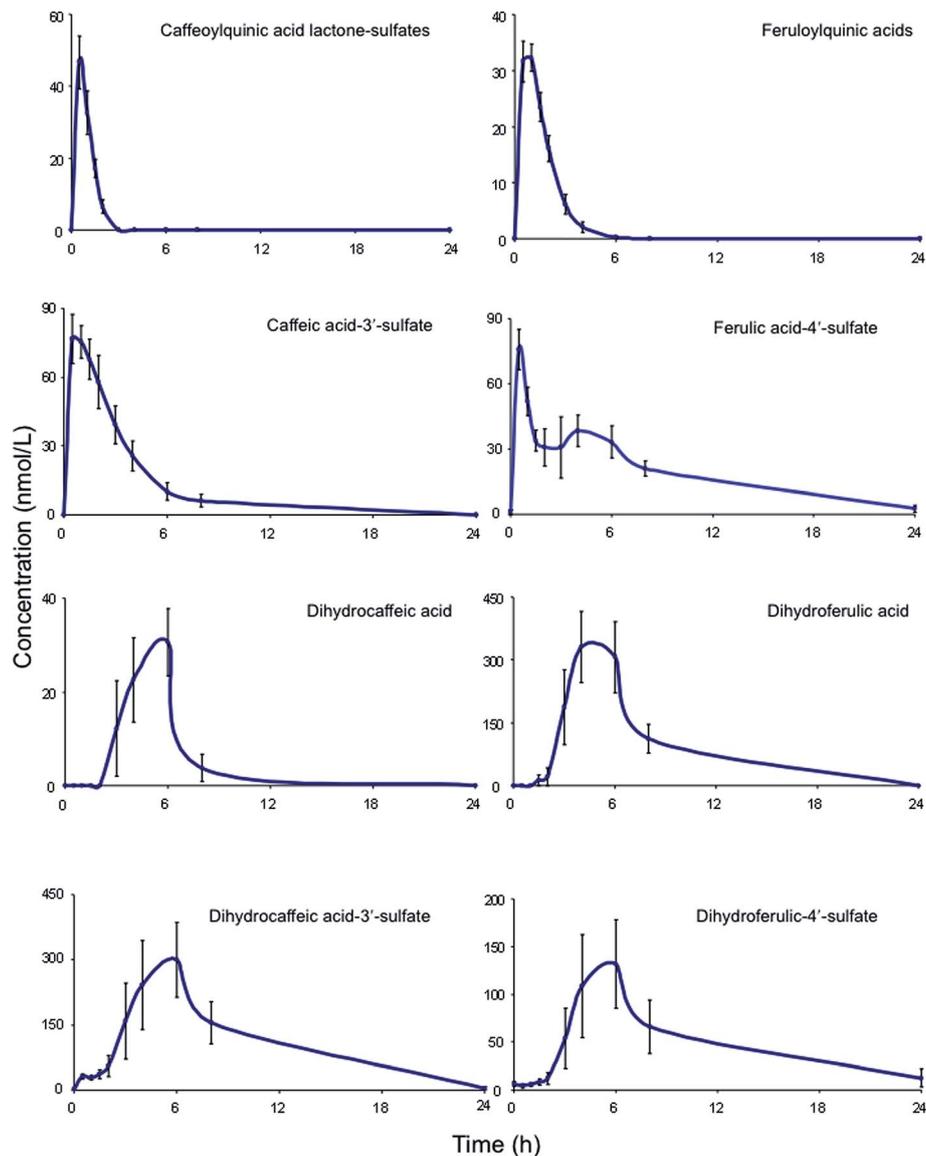
Fig. 6 Plasma pharmacokinetic profiles of circulating acyl-quinic acids and metabolites, following the ingestion of 200 mL of coffee by healthy human subjects (based on Stalmach *et al.*[44]).
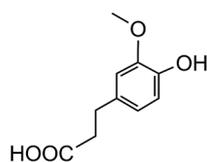
Dihydroferulic acid (**35**)
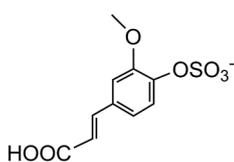
Ferulic acid-4'-sulfate (**36**)
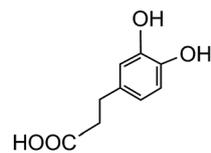
Dihydrocaffeic acid (**37**)
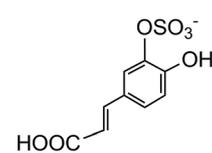
Caffeic acid-3'-sulfate (**38**)
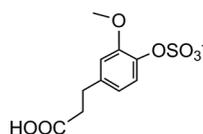
Dihydroferulic acid-4'-sulfate (**39**)
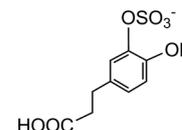
Dihydrocaffeic acid-3'-sulfate (**40**)

system with apparent elimination half-life ($T_{1/2}$) values of 0.3 to 1.9 h (Table 6). The only compounds with an extended $T_{1/2}$ were dihydroferulic acid-4'-sulfate (**39**) (4.7 h), dihydrocaffeic acid-3'-sulfate (**40**) (3.1 h) and ferulic acid-4'-sulfate (**36**) which had an unusual biphasic plasma profile with dual T_{max} values at 0.6 h and 4.3 h. It is of note that the free acid, dihydroferulic acid (**35**), as opposed to the more typical glucuronide and sulfate metabolites, was the principal component to accumulate in plasma which also contained dihydrocaffeic acid (**38**) in a lower concentration.

The true $T_{1/2}$ values can be determined only by intravenous dosing of the metabolite. Estimates based on elimination after oral intake overestimate the true T_{max} because the metabolite is still entering the plasma when the elimination is being estimated. A further complication arising with gut flora metabolites for which absorption, and hence elimination, cannot commence until several hours after substrate ingestion, is the lack of sufficient data points in the declining period between 8 and 24 hours. The lack of a reliable value for the true T_{max} effectively precludes modelling of multiple doses at say three hour intervals, *i.e.* the manner in which many people drink coffee. Nevertheless, with such a pattern of repeat consumption

there is clearly a potential for a sizable accumulation of gut flora metabolites in the plasma because a subsequent dose of substrate will have entered the colon before the previous intake has been eliminated.

In a further study, ileostomists drank an instant coffee with a very similar 385 μmol (136 mg) acyl-quinic acid content and profile to that ingested by the healthy subjects.[214] Plasma metabolites were not investigated, but data on the amounts of acyl-quinic acids and their metabolites in ileal fluid collected over a 0–24 h period after the ingestion of coffee are presented in Table 7. The highest recovery of unmetabolised acyl-quinic acids compared to their intake was FQAs (77%), followed by CQAs (59%) and *p*-CoQAs and diCQAs (46%). The recoveries of acyl-quinic acid metabolites were much lower, ranging from 3.6 to 8.8%, except for CQL metabolites which corresponds to 56% of CQL intake.

Of the 385 μmol of acyl-quinic acids ingested by the ileostomists, 274 μmol (71%) was recovered in the 0–24 h ileal fluid as the parent compounds and metabolites (Table 7). This indicates that ~30% of intake is absorbed in the stomach and/or small intestine, and that in subjects with a functioning colon ~70% of the ingested acyl-quinic acids pass from the small to the large intestine where they will be subjected to the action of the colonic microflora. These observations are in line with the findings of Olthof *et al.*[211] who fed 2.8 mmol of 5-CQA (9) to humans with an ileostomy and recovered ~70% of the CQA intake in ileal fluid. The data from both studies, albeit at substantially different doses, imply that around one third of ingested 5-CQA is absorbed and enters the bloodstream from the small intestine. *In vitro* studies support this conclusion as 5-CQA is not extensively degraded when incubated with gastric juice, duodenal fluid and ileostomy effluent[211,215] although as noted below interesterification can occur.[205] In keeping with these findings, when 3,5-, 3,4-, and 4,5-diCQAs (18, 41, 42) from *Ilex kudingcha* were incubated with artificial saliva, gastric and pancreatic fluids, they were not degraded. When incubated with a human fecal slurry under anaerobic conditions, the diCQAs were hydrolysed to CQAs and caffeic acid, which was then further catabolised to dihydrocaffeic acid.[216]

Table 7 Quantities of acyl-quinic acids and metabolites recovered in ileal fluid collected 0–24 h after the consumption of 200 mL of instant coffee containing 385 μmol of acyl-quinic acids by humans with an ileostomy (after Stalmach *et al.*[214])[a]
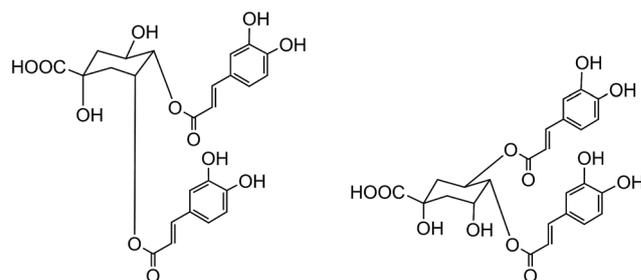
Acyl-quinic acids and metabolites	Ileal fluid (μmol)	Recovery of amount ingested (%)
3-*O*-Caffeoylquinic acid	55 ± 6	
4-*O*-Caffeoylquinic acid	45 ± 6	
5-*O*-Caffeoylquinic acid	64 ± 10	
Total caffeoylquinic acids	*164 ± 22*	*59 ± 8*
3-*O*-Caffeoylquinic acid-sulfate	3.5 ± 0.5	
4-*O*-Caffeoylquinic acid-sulfate	5.1 ± 0.6	
5-*O*-Caffeoylquinic acid-sulfate	0.7 ± 0.2	
3-*O*-Caffeoylquinic acid-*O*-glucuronide	0.7 ± 0.2	
4-*O*-Caffeoylquinic acid-*O*-glucuronide	0.4 ± 0.2	
Total caffeoylquinic acid metabolites	*10 ± 1*	*3.6 ± 0.4*
3-*O*-Feruloylquinic acid	12 ± 1	
4-*O*-Feruloylquinic acid	12 ± 1	
5-*O*-Feruloylquinic acid	13 ± 1	
Total feruloylquinic acids	*37 ± 4*	*77 ± 8*
3-*O*-Feruloylquinic acid-sulfate	0.7 ± 0.2	
4-*O*-Feruloylquinic acid-sulfate	0.8 ± 0.2	
5-*O*-Feruloylquinic acid-sulfate	0.3 ± 0.1	
3-*O*-Feruloylquinic acid-*O*-glucuronide	0.4 ± 0.1	
4-*O*-Feruloylquinic acid-*O*-glucuronide	2.0 ± 0.3	
Total feruloylquinic acid metabolites	*4.2 ± 0.6*	*8.8 ± 1.3*
3-*O*-Caffeoylquinic lactone	1.0 ± 0.3	
4-*O*-Caffeoylquinic lactone	1.5 ± 0.1	
Total caffeoylquinic lactones	*2.5 ± 0.3*	*6.4 ± 0.8*
3-*O*-Caffeoylquinic lactone-sulfate	13 ± 2.4	
4-*O*-Caffeoylquinic lactone-sulfate	8.2 ± 1.7	
3-*O*-Caffeoylquinic lactone-*O*-glucuronide	0.6 ± 0.2	
4-*O*-Caffeoylquinic lactone-*O*-glucuronide	0.4 ± 0.1	
Total caffeoylquinic lactone metabolites	*22 ± 4.4*	*56 ± 11*
4-*O*-*p*-Coumaroylquinic acid	1.1 ± 0.3	
5-*O*-*p*-Coumaroylquinic acid	1.9 ± 0.2	
Total p-coumaroylquinic acids	*3.0 ± 0.2*	*46 ± 3*
3,4-*O*-Dicaffeoylquinic acid	3.0 ± 0.3	
3,5-*O*-Dicaffeoylquinic acid	1.3 ± 0.2	
4,5-*O*-Dicaffeoylquinic acid	2.2 ± 0.2	
Total dicaffeoylquinic acids	*6.5 ± 0.7*	*46 ± 5*
Caffeic acid	9.0 ± 3.1	
Caffeic acid-3′- and 4′-sulfates	11 ± 2	
Ferulic acid	0.8 ± 0.3	
Ferulic acid-4′-sulfate	4.2 ± 1.0	
Total caffeic and ferulic acids	*25 ± 5*	*-*
Total acyl-quinic acids and metabolites	**274 ± 28**	**71 ± 7**

[a] Data presented as mean values ± standard error ($n = 5$). n.d – not detected.

3,4-*O*-Dicaffeoylquinic acid (41)   4,5-*O*-Dicaffeoylquinic acid (42)

Animal and *in vitro* cell culture studies indicate that post-absorption acyl-quinic acids are subjected to the action of epithelial esterases in the stomach and small intestine.[204,217,218] In incubations with cultured gastric epithelial cells, there is some release of caffeic acid (3), ferulic acid (4) and 3′,4′-dimethoxycinnamic acid (26) from CQAs, FQAs and dimethoxycinnamoylquinic acids (DQAs). Caffeic acid is metabolised primarily to isoferulic acid (43) and a lesser amount of ferulic acid but is not transported

unchanged to the basal side. In contrast, ferulic, isoferulic and dimethoxycinnamic acids are transported, and this must be the primary source of these substrates in ileostomists.[219,220]
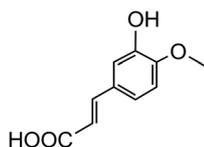
Isoferulic acid (**43**)

The absorption of ferulic acid and subsequent conjugation in the liver[221] is consistent with the early plasma T_{max} (0.6 h) for ferulic acid-4′-sulfate (**36**) observed in feeding studies[44] while the later secondary T_{max} (4.3 h) (Fig. 6 and Table 6) plausibly reflects the absorption and sulfation of ferulic acid released by

(Table 7). The necessary hydrolysis of FQAs and hydrogenation of ferulic acid to dihydroferulic acid could involve either microbial and/or human enzymes post absorption.[223]

3′,4′-Dimethoxycinnamic acid (**26**) and 3′,4′-dimethoxy-cinnamoylquinic acid (**44**) are minor components in coffee. However, when a coffee containing ∼400 nmol of these compounds was ingested by volunteers free 3′,4′-dimethoxy-cinnamic acid was detected in plasma with a 500 nmol L^{-1} C_{max} and a T_{max} of ∼0.5 h.[219] The C_{max} is higher than that of the metabolites derived from CQAs that occur in coffee in much higher quantities (see Fig. 6). 3-(3′,4′-Dimethoxyphenyl)propionic acid (**45**) appeared later, predominantly 8–12 h after coffee intake with a C_{max} of 97 nmol L^{-1}. The high C_{max} relative to dose probably reflects the comparatively high hydrophobicity of dimethoxy-cinnamic acid derivatives facilitating passive absorption.[225]

5-O-3′,4′-Dimethoxycinnamoylquinic acid (**44**)

3-(3′,4′-Dimethoxyphenyl)propionic acid (**45**)

gut microflora-mediated hydrolysis of unabsorbed FQA. *Escherichia coli*, *Bifidobacterium lactis* and *Lactobacillus gasseri* have the requisite cinnamoyl esterase activity.[222] The plasma profile was not recorded for the ileostomists and, thus, it is not known whether that too was biphasic, but because ileostomists could not be absorbing ferulic acid released in the colon there would have to be another compensatory source to maintain ferulic acid-4′-sulfate excretion. The plasma profile (Fig. 6) and the excretion of substantial amounts of dihydroferulic acid (**35**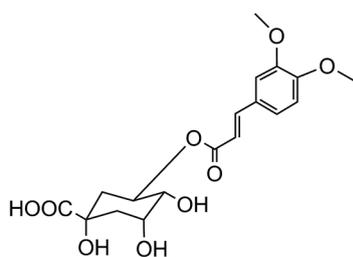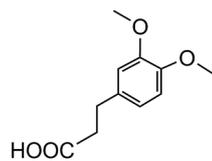) by volunteers with an intact colon continues until at least 8 h after coffee consumption,[44] indicating that a significant part of this is derived from 77% of FQA intake that reaches the colon

In contrast to the behaviour of ferulic acid and dimethoxy-cinnamic acid, the failure of cultured gastric cells to transport caffeic acid (**3**)[219] seemingly conflicts with the ∼1 h plasma T_{max} for caffeic acid-3′-sulfate (**39**) (Table 6).[44] The most plausible explanation is that absorbed CQAs may be hydrolysed and the caffeic acid conjugated in hepatocytes.

The quantities of acyl-quinic acids and their metabolites excreted in urine by healthy subjects and ileostomists over a 24 h period after ingestion of coffee are summarised in Table 8. It is apparent that absence of a colon had minimal impact on urinary excretion of CQL-sulfates and FQAs, as well as caffeic, ferulic and isoferulic acid sulfates. The ileostomists
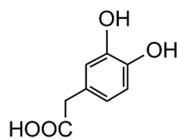
3′,4′-Dihydroxyphenylacetic acid (**46**)
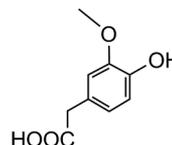
3′-Methoxy-4′-hydroxyphenylacetic acid (**47**)
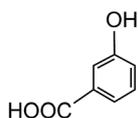
3-Hydroxybenzoic acid (**48**)
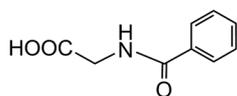
Hippuric acid (**49**)
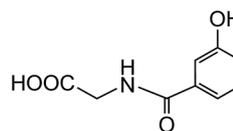
3′-Hydroxy-hippuric acid (**50**)

Table 8 Urinary excretion of acyl-quinic acids and their conjugated metabolites in 0–24 h urine of healthy subjects ($n = 11$) and ileostomists ($n = 5$) following the ingestion of 200 mL of coffee (after Stalmach et al.[44,214])[a]

Chlorogenic acid and metabolites	Subjects without a colon (385 μmol ingested)	Subjects with a colon (412 μmol ingested)
3-O-Caffeoylquinic lactone-sulfate	0.6 ± 0.1	1.1 ± 0.1
4-O-Caffeoylquinic lactone-sulfate	0.4 ± 0.1	1.0 ± 0.1
3-O-Feruloylquinic acid	0.9 ± 0.2	1.2 ± 0.1
4-O-Feruloylquinic acid	0.9 ± 0.2	1.1 ± 0.1
5-O-Feruloylquinic acid	1.1 ± 0.2	1.0 ± 0.2
Ferulic acid-4′-O-sulfate	9.9 ± 1.9	11.1 ± 1.6
Feruloylglycine	2.1 ± 0.3[a]	20.7 ± 3.9[b]
Dihydroferulic acid	n.d.[a]	9.7 ± 2.0[b]
Dihydroferulic acid-4′-sulfate	0.8 ± 0.2[a]	12.4 ± 3.4[b]
Dihydroferulic acid-4′-O-glucuronide	n.d.[a]	8.4 ± 1.9[b]
Isoferulic acid-3′-sulfate	0.2 ± 0.0	0.4 ± 0.1
Isoferulic acid-3′-O-glucuronide	3.9 ± 0.8	4.8 ± 0.5
Dihydroisoferulic acid-3′-O-glucuronide	n.d.[a]	2.5 ± 0.4[b]
Caffeic acid-3′-sulfate	6.2 ± 1.2	6.4 ± 0.8
Caffeic acid-4′-sulfate	0.6 ± 0.1	0.6 ± 0.1
Dihydrocaffeic acid-3′-sulfate	3.2 ± 0.9[a]	37.1 ± 8.2[b]
Dihydrocaffeic acid-3′-O-glucuronide	n.d[a]	0.7 ± 0.2[b]
Total	**30.8 ± 4.3 (8.0%)**[a]	**120.2 ± 17.0 (29.2%)**[b]

[a] Data represent mean values in μmol ± standard error. n.d. not detected. Different superscripts within rows indicate a statistical difference between the two sets of volunteers (two-sample t-test, P-value < 0.05). [b] Figures in bold italicised parentheses indicate excretion as a percentage of acyl-quinic acids intake.

excreted in urine 30.8 μmol of acyl-quinic acid metabolites equivalent to 8.0% of the amount ingested. In contrast, the volunteers with an intact colon excreted a total of 120.2 μmol which corresponds to 29.2% of intake. This is almost certainly an under estimate of acyl-quinic acid bioavailability because in this study phenolic catabolites, such as C_6–C_2 hydroxy- and methoxy-phenylacetic acids (**46**, **47**), C_6–C_1 benzoic acids (**48**) and hippuric acids (**49**, **50**) were not quantified. As well as these phenolics being catabolites of caffeic acid,[215,224–226] there is growing evidence that they are also a feature of the catabolism of many flavonoids including flavonols,[224,227,228] anthocyanins,[229–231] flavanones[232–234] and flavan-3-ols.[224,235,236]

The data in Table 7 show that after coffee consumption a total of 46.2 μmol of ferulic acid-based compounds (ferulic acid [**3**], FQAs [**32–34**], ferulic acid-4′-sulfate [**36**], FQA-O-glucuronides FQA-sulfates) were present in the 0–24 h ileal fluid. In healthy subjects these compounds would pass to the large intestine and the quantity of ferulic acid metabolites excreted in the urine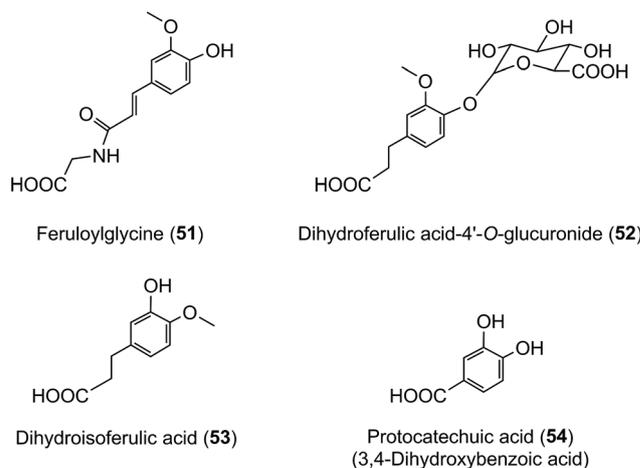 of volunteers with a functioning colon (feruloylglycine [**51**], and dihydroferulic acid [**35**] and its 4′-sulfate [**39**] and 4′-O-glucuronide [**52**]) totalled 51.2 μmol (Table 8). This is not greatly in excess of the 46.2 μmol of ferulic acid-based compounds entering the large intestine (Table 8). In contrast to in vitro cell culture studies,[203] arguably, this suggests that in vivo the ferulic acid metabolites may be derived principally from the ingested FQAs rather than via 3′-methylation of caffeic acid derivatives, formed from CQAs, diCQAs and CQLs. However, the presence of isoferulic acid [**43**] and dihydroisoferulic acid [**53**] metabolites in urine signifies that caffeic acid undergoes 4′-methylation. Excretion of these metabolites by subjects with and without a colon (Table 9) points to 4′-methylation of caffeic acid [**3**] producing isoferulic acid [**43**] occurring

in the upper GI tract. The human gastric epithelium is capable of such a methylation,[203] while formation of dihydroisoferulic acid (**54**) takes place in the distal GI tract.

Feruloylglycine (**51**)

Dihydroferulic acid-4'-O-glucuronide (**52**)

Dihydroisoferulic acid (**53**)

Protocatechuic acid (**54**) (3,4-Dihydroxybenzoic acid)

On the basis of coffee feeding studies with healthy volunteers and ileostomists, Stalmach et al.[44,214] proposed a series of metabolic pathways. These have been extended to incorporate data obtained when coffee was incubated with fecal slurries and the breakdown of acyl-quinic acids monitored.[226] The proposed pathways are illustrated in Fig. 7 and 8. In the preparation of these pathways the following points were taken into consideration. Some, but not complete hydrolysis of CQAs and CQLs can occur in the small intestine, catalysed by mammalian esterases

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Table 9 Impact of acyl-quinic acid dose on ileal and urinary excretion of acyl-quinic acids and conjugated metabolites as a percentage of intake, and on metabolite sulfate : glucuronide (S : GlcUA) ratio, after ingestion of coffees and a fruit drink containing apple juice by ileostomists[a] (after Stalmach et al.[214] and Erk et al.[205])

Acyl-quinic acids intake	Ileal excretion			Urinary excretion		
	% of intake	% conjugated metabolites	S : GlcUA ratio	% of intake	% conjugated metabolites	S : GlcUA ratio
385 μmol	71 ± 7	22.3	15.5	8.0 ± 1.1	90	5.6
1053 μmol	77 ± 2	8.9	13.4	14.6 ± 3.0	70	1.3
2219 μmol	72 ± 2	7.7	10.4	12.1 ± 3.0	69	1.1
4525 μmol	69 ± 4	6.7	8.2	8.0 ± 2.2	67	0.7

[a] Data presented as mean values $\pm$ standard error.

releasing caffeic acid.[237] Further, hydrolysis in the colon is probably due to the action of bacterial esterases. Small amounts of CQAs and more substantial amounts of FQAs and CQLs are absorbed in the small intestine with the CQLs appearing in the circulatory system as sulfates (Fig. 6). The released caffeic acid is subjected to sulfation forming caffeic acid-4′-sulfate and smaller quantities of caffeic acid-3′-sulfate. Caffeic acid is also methylated producing isoferulic acid, but also possibly ferulic acid, both of which in turn form glucuronide and sulfate derivatives.

FQAs are absorbed in the small intestine and are also hydrolysed to some degree with ferulic acid-4′-sulfate [**36**] appearing in the bloodstream with an initial C_{max} of 0.6 ± 0.1 h.

Ferulic acid (**4**) undergoes glycination forming feruloylglycine (**51**) and although this involves mammalian enzymes the conversion is reduced by 90% in ileostomists indicating that it is primarily colonic in origin. Methyl, glucuronyl, sulfate and glycine conjugation steps involve mammalian enzymes. Dehydroxylation and demethoxylation are almost certainly mediated by the gut microflora while demethylation and hydrogenation steps can be mediated by both microbial and mammalian enzymes. For convenience, the pathways in Fig. 7 and 8 show C_6–C_3 catabolites being converted by two α-oxidations to C_6–C_1 compounds by microflora and/or mammalian enzymes. However, it is possible the C_6–C_3 catabolites progress directly to
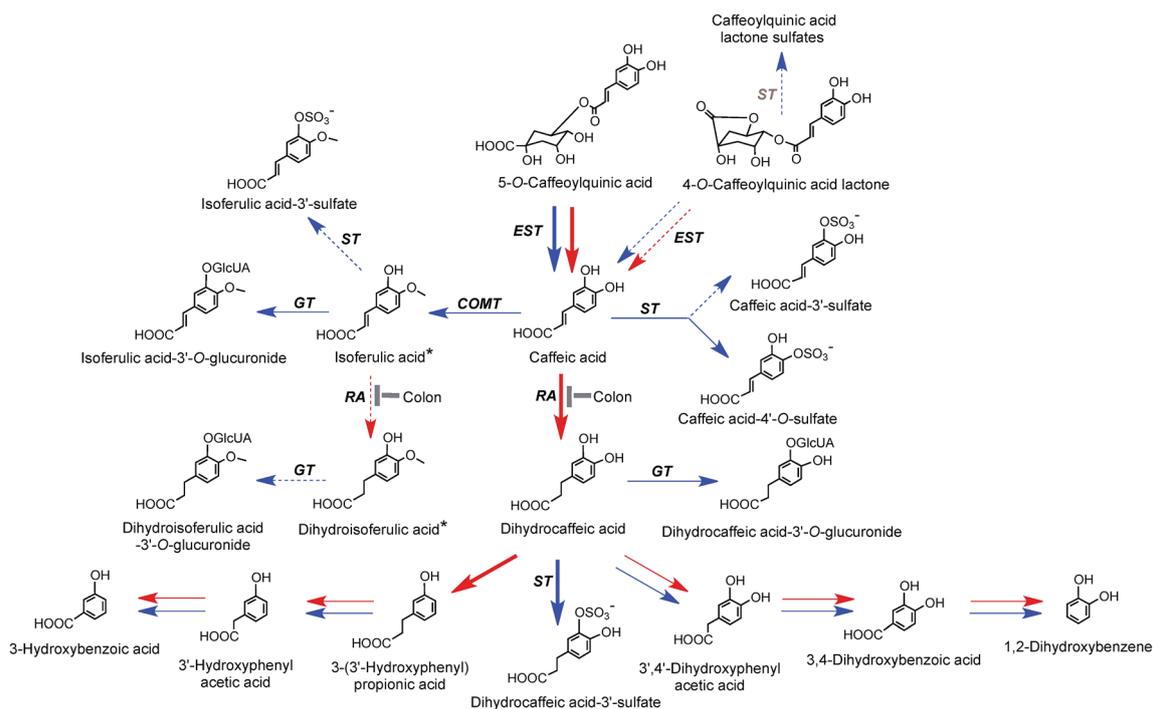
Fig. 7 Proposed metabolism of caffeoylquinic acids and caffeoylquinic lactones following the ingestion of coffee by volunteers. 5-CQA is the illustrated structure but the respective 3′- and 4′-isomers and diCQAs would be metabolized in a similar manner. Likewise 4-CQAL is the illustrated lactone but 3-CQAL would be metabolised in a similar manner. COMT, catechol-O-methyltransferase; ET, esterase; RA, reductase; GT, UDP-glucuronyltransferase; ST, sulfuryltransferase; Co-A, co-enzyme A. Arrows: bold – major routes, dotted – minor pathways; red – microbial enzymes, blue – mammalian enzymes. Steps blocked in subjects with an ileostomy and hence occurring principally, but not necessarily exclusively, in the colon are indicated. * Intermediates that did not accumulate in detectable amounts (based on data of Stalmach et al.[44,139] and Ludwig et al.[226]).
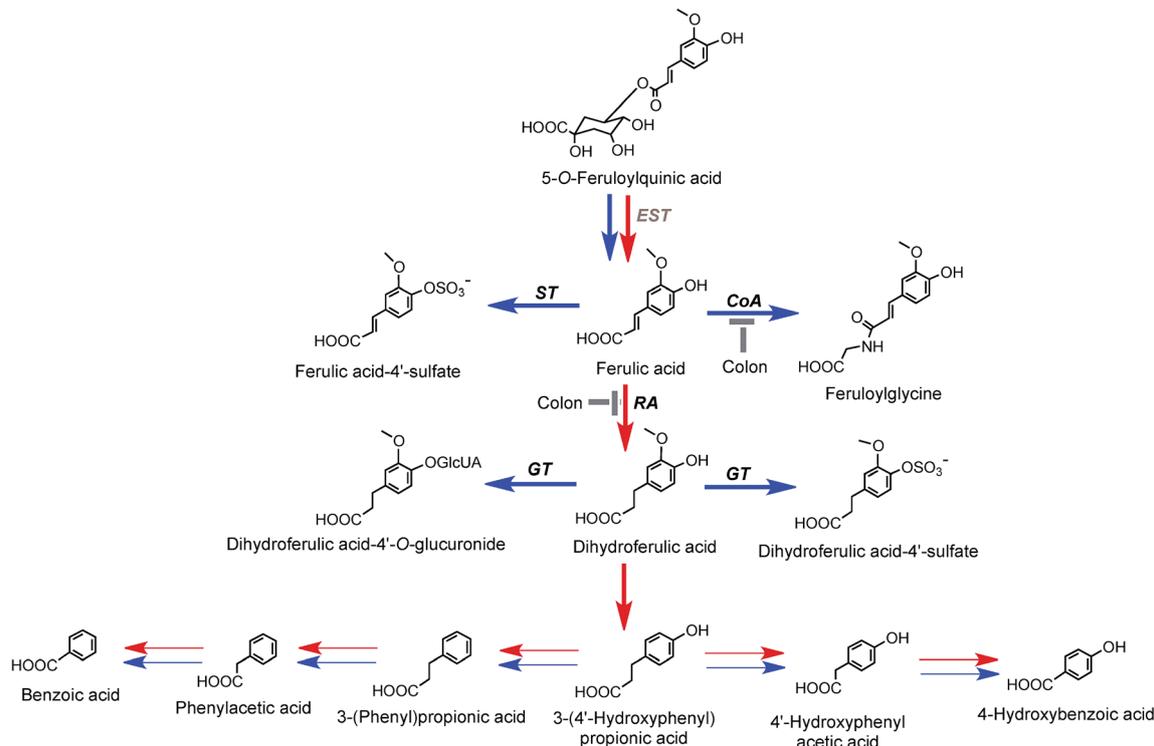
Fig. 8 Proposed metabolism of feruloylquinic acids following the ingestion of coffee by volunteers. 5-FQA is the illustrated structure but the respective 3′- and 4′-isomers would be metabolized in a similar manner. EST, esterase; RA, reductase; GT, UDP-glucuronyltransferase; ST, sulfuryltransferase; Co-A, co-enzyme A. Arrows: bold – major routes; red – microbial enzymes, blue – mammalian enzymes. Steps blocked in subjects with an ileostomy and hence occurring principally, but not exclusively, in the colon are indicated (based on data of Stalmach et al.[44,139] and Ludwig et al.[226]).
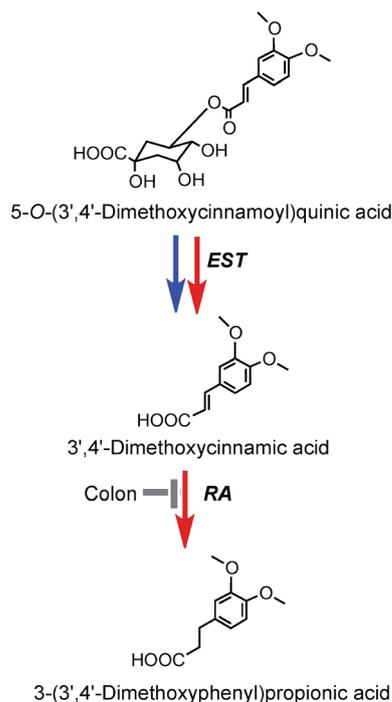
Fig. 9 Potential metabolism of 5-O-(3′,4′-dimethoxycinnamoyl) quinic acid following the ingestion of coffee by volunteers. The 5-O-ester is the illustrated structure but the respective 3-O- and 4-O-isomers would be metabolized in a similar manner. EST, esterase; RA, reductase. Blue arrows mammalian enzymes, red arrow – microbial enzymes (based on data of Farrell et al.[144]).

C_6–C_1 structures via a mammalian catalysed β-oxidation and that C_6–C_2 catabolites arise independently. In reality, further complexity is introduced as there are multiple points at which catabolites might be absorbed. For example, a percentage of some C_6–C_3 catabolites could be absorbed and undergo β-oxidation and/or mammalian phase II conjugation while the balance is subjected to microbial hydrogenation and/or α-oxidation prior to absorption and mammalian conjugation. Also for some catabolites mammalian conjugation either does not occur or is incomplete.

As noted above, after feeding coffee containing trace levels of 3′,4′-dimethoxycinnamic acid (26) and dimethoxycinnamoylquinic acids to volunteers with a functioning colon, 3′,4′-dimethoxycinnamic acid appears rapidly in plasma and this is followed by a delayed appearance of a smaller amounts of 3-(3′,4′-dimethoxyphenyl)propionic acid (45).[219] These observations are in keeping with esterases in the small intestine hydrolysing the dimethoxycinnamoylquinic acids releasing free dimethoxycinnamic acid which is readily absorbed in the upper GI tract, while the unabsorbed methoxy acid reaching the colon it is converted to 3-(3′,4′-dimethoxyphenyl)propionic acid. A potential pathway involved in such conversions is illustrated in Fig. 9. Currently there is no evidence for further phase II metabolism of these products.

Sulfates are the main acyl-quinic acid metabolites and glucuronides are relatively minor components (Fig. 6 and Table

9). Transformations that occur in the colon facilitate the absorption, as microbial catabolites, of 70% or more of the acyl-quinic acids consumed, clearly highlighting the importance of the colon in their bioavailability. It is also apparent that ileostomists are less able to utilise acyl-quinic acids and other dietary phenolics and are, thus, less able to access any benefits that might otherwise accrue.

Further clarification of the proposed pathways in Fig. 7–9 will require feeding studies, not with coffee, but with substrates such as 5-CQA (9) and 5-FQA (34) in which the hydroxy-cinnamate moiety is labelled with ^{13}C. This approach has been used successfully with $[^{13}C_5]$cyanidin-3-O-glucoside to obtain detailed profiles of the metabolism and catabolism of the anthocyanin in the small and large intestine and in the process provided novel details of involvement of the microbiota in the lower bowel.[229,230] Human feeding studies are the gold standard and while animal studies can be helpful, because of species differences in gut microbiota and endogenous metabolism, they can produce data that are difficult to apply to human studies as has recently been shown with the radiolabelled flavan-3-ol $[2\text{-}^{14}C](-)$-epicatchin.[236] Likewise, data obtained *in vitro* with isolated cells and tissue extracts should be treated with caution as substrates can come into contact with enzymes and conditions to which they would not be exposed *in vivo* when cellular compartmentation is maintained.

7.2 Impact of dose on acyl-quinic acid bioavailability

The impact of dose on acyl-quinic acid absorption in the small intestine has been assessed in a study by Erk *et al.* on coffee consumption by ileostomists where acyl-quinic acid-derived compounds were analysed in plasma, urine and ileal fluid after the ingestion of coffees containing 1053, 2219 and 4525 μmole of CQAs, diCQAs and FQAs.[238] The profile of acyl-quinic acids and their metabolites identified in ileal fluid and urine by HPLC-MS was very similar to that detected by Stalmach *et al.*[214] after the ingestion of a coffee containing 385 μmol of acyl-quinic acids. Ileal excretion reported by Erk *et al.*[238] was ∼70% of intake irrespective of dose (Table 9), which was also the level reported by Stalmach *et al.*[214] In keeping with these observations the ∼3 : 1 ratio of urinary excretion of metabolites absorbed in the distal and proximal GI tract was not influenced to any extent by dose following coffee acyl-quinic acid intakes of 412–795 μmol (Table 10).[239] Dose did, however, have a noticeable impact on the amount of conjugated metabolites appearing in ileal fluid where they were equivalent to 22.3% of the acyl-quinic acids at the lowest dose and 6.7–8.9% at the three higher intakes.[238] Sulfated metabolites were

Table 10 Urinary excretion of acyl-quinic acid metabolites absorbed in the upper and lower gastrointestinal tract by volunteers with a functioning colon after the consumption of 200 mL of coffee beverage containing 412, 635 and 795 μmol of acyl-quinic acids. (after Stalmach *et al.*[239])[a]

	Acyl-quinic acids intake		
	412 μmol	635 μmol	795 μmol
Upper gastrointestinal tract	29 ± 2 (27%)	37 ± 4 (24%)	37 ± 4 (21%)
Lower gastrointestinal tract	80 ± 14 (73%)	119 ± 21 (76%)	137 ± 23 (79%)

[a] Data expressed as mean values in μmol ± SE ($n = 11$). Italicised figures in parentheses represent the percentage absorption taking place in the upper and lower gastrointestinal tract.

Table 11 Impact of acyl-quinic acid dose on plasma C_{max} of acyl-quinic acids and metabolites absorbed in the small intestine after ingestion of coffees by healthy volunteers with a colon (lowest dose) (Stalmach *et al.*[44]) and ileostomists (three higher doses) (after Erk *et al.*[238])[a]

	Acyl-quinic acid intake			
Acyl-quinic acids and metabolites	385 μmol	1053 μmol	2219 μmol	4525 μmol
3-O-Caffeoylquinic acid	n.d.	14 ± 3	33 ± 7	43 ± 5
4-O-Caffeoylquinic acid	n.d.	20 ± 4	57 ± 12	73 ± 4
5-O-Caffeoylquinic acid	2.2 ± 1.0	14 ± 3	30 ± 6	44 ± 4
3-O-Feruloylquinic acid	16 ± 2	23 ± 5	56 ± 13	96 ± 20
4-O-Feruloylquinic acid	14 ± 2	32 ± 5	62 ± 12	117 ± 16
5-O-Feruloylquinic acid	6.0 ± 1.5	16 ± 3	41 ± 12	45 ± 4
Caffeic acid	92 ± 11	77 ± 12	162 ± 26	214 ± 11
Ferulic acid	76 ± 9	147 ± 14	214 ± 20	518 ± 38
Dihydrocaffeic acid	n.d.	53 ± 7	35 ± 6	63 ± 9
Dihydroferulic acid	n.d.	22 ± 8	34 ± 6	85 ± 6
Dimethoxycinnamic acid	n.a.	58 ± 11	129 ± 23	305 ± 32

[a] Data in nM ± standard error. Analysis of acyl-quinic acids at the three higher doses was after enzyme hydrolysis[162] and without enzyme hydrolysis for the lowest dose.[139] n.d., not detected; n.a., not analysed.

15.5 times more prevalent than glucuronides at the lowest dose but as intake increased this changed and with the ingestion of 4525 µmol of acyl-quinic acids the ratio of sulfated metabolites to glucuronides in ileal fluid fell to 8.2 (Table 10). In keeping with these observations, in a further study in which ileostomists consumed a (poly)phenol-rich drink containing 46 µmol of 5-CQA derived from apples, only sulfate metabolites of caffeic acid, dihydrocaffeic acid and ferulic acid were detected in urine 0–24 h after intake.[240]

These dose-related changes presumably reflect enzyme saturation, limited transport capacities into and out of the enterocyte in the small intestine and/or differences in GI tract transit times. The increasing proportion of glucuronidation with increasing acyl-quinic acid dose was even more marked in urine (Table 9), arguably indicative of phase II UDP-glucuronyltransferase activity in the small intestine epithelium and/or in the liver and kidneys.

Data on the effect of dose on plasma C_{max} of acyl-quinic acids and metabolites absorbed in the small intestine after ingestion of coffee by healthy subjects with a colon (lowest dose) and ileostomists are presented in Table 11. This reveals a clear trend towards higher C_{max} values with increasing acyl-quinic acids intakes.

7.3 Matrix effects and acyl-quinic acid bioavailability

There is one report on the consumption by humans of black coffee and black coffee made with milk rather than water in which urinary excretion of acyl-quinic acids and metabolites was 68% of intake with the black coffee and 40% after ingestion of coffee with milk. The two figures were not statistically different but it was hypothesized that milk may have impaired the absorption of coffee acyl-quinic acids.[241] Although an *in vitro* investigation suggests that the addition of milk fat to coffee may increase acyl-quinic acids bioavailability,[242] a feeding study by Renouf *et al.*[243] revealed no difference in the pharmacokinetic profiles of plasma acyl-quinic acid metabolites after drinking black coffee with or without 10% whole milk. Thus, although 5-CQA has been reported to bind to certain proteins *in vitro*, such as albumin and casein,[244,245] milk would appear not to have a significant impact on the overall absorption of coffee acyl-quinic acids. However, adding a mixture of sugar and non-dairy creamer to the black coffee resulted in lower C_{max} values

for caffeic acid (**3**) and isoferulic acid (**43**) accompanied by longer T_{max} times for ferulic acid (**4**) and isoferulic acid.[243] Sugar[246] and lipids[247] are known to delay gastric emptying and this may have delayed absorption of the coffee acyl-quinic acids resulting in an extended T_{max} for two of the three metabolites.

Changes in the CQA profile in the upper GI tract have been monitored by feeding ileostomists coffee, cloudy apple juice and an apple smoothie and analysing the CQAs excreted in ileal fluid over an 8 h period.[205] The data obtained are summarised in Table 12. With coffee, in keeping with other investigations, there was an overall recovery of 76% of intake and the ratios of the individual CQA isomers were not affected by inter-esterification reactions during passage through the proximal GI tract.[214,238] There was a similar 77% recovery of apple smoothie CQAs but whereas the drink contained only 5-CQA (**9**) and 4-CQA (**10**) while the ileal fluid also contained 3-CQA (**11**) and 1-CQA (**12**). The same occurred following consumption of cloudy apple juice but in this instance the overall recovery of CQAs was only 26%.

The matrix in which the CQAs were ingested, therefore, appears to have had an impact on their fate as they pass through the proximal GI tract with interesterification of CQAs occurring with the apple products but not coffee. Such reactions have been reported previously[248,249] and seemingly are caused by the pH increasing to above pH 6 during passage through the small intestine. Erk *et al.*[238] suggest that the CQA profile of the coffee was closer to the interesterification equilibrium reported by Trugo and Macrae[250] and so did not alter substantially during gastric transport (Table 12). The susceptibility of individual acyl-quinic acids to inter-esterification and hydrolysis was discussed in Section 3.1.

There was also a ~3-fold lower recovery of CQAs in ileal fluid after cloudy apple juice consumption compared with the apple smoothie (Table 12).[205] The CQA content of the two beverages was similar and the drinks differed only in that cloudy apple juice was pressed and unfiltered whereas the smoothie comprised 60% cloudy juice and 40% apple purée which contains a much higher proportion of cell wall constituents. How this resulted in the enhanced ileal recovery of CQAs from the apple smoothie is unclear. Although neither plasma nor urine were analysed in this study to confirm the point, it appears unlikely that it is a consequence of enhanced absorption in the small intestine of the cloudy apple juice CQAs. It is also unclear as to what constituents

Table 12 Summary of caffeoylquinic acid intake by ileostomists and 0–8 h CQA ileal excretion after the ingestion of coffee, apple smoothie and cloudy apple juice. (after Erk *et al.*[205])[a]

Caffeoylquinic acids	Coffee		Apple smoothie		Cloudy apple juice	
	Intake	Ileal excretion	Intake	Ileal excretion	Intake	Ileal excretion
1-*O*-Caffeoylquinic acid	8.0	5.1	n.d.	2.0	n.d.	16
3-*O*-Caffeoylquinic acid	231	179	n.d.	14	n.d.	34
4-*O*-Caffeoylquinic acid	209	157	26	27	40	9.2
5-*O*-Caffeoylquinic acid	298	227 (**76%**)	309	216 (**77%**)	318	32 (**26%**)

[a] Data presented in µmoles as mean values. Bold figures in parentheses represent total caffeoylquinic acid recovery in ileal fluid as a percentage of intake. n.d., not detected.

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Table 13 Summary of the quantities of total acyl-quinic acids and their metabolites, excreted in urine 0–24 h after the consumption of 200 mL of coffee beverage containing 412, 635 and 795 µmol of acyl-quinic acids by 11 volunteers with an intact functioning colon (based on unpublished data of Stalmach et al.[239])[a]

Acyl-quinic acid intake	Volunteers											Mean ± SE	Range
	1	2	3	4	5	6	7	8	9	10	11		
412 µmol	12	17	16	58	29	24	22	30	28	28	23	26 ± 4	12–58
635 µmol	10	12	11	35	25	17	52	29	36	19	31	25 ± 4	10–52
795 µmol	18	14	15	47	19	16	48	20	28	25	50	27 ± 4	14–48

[a] Data expressed as a percentage of intake.

in the smoothie result in the CQAs being less prone to degradation and/or irreversible binding in the apple matrix.

Scherbl et al. reported on a study in which volunteers consumed (i) black coffee, (ii) a carbohydrate-rich black coffee and two bread rolls and honey (∼626 kcal) and (iii) a fat-rich black coffee and one bread roll and peanut butter (∼626 kcal).[209] Plasma and urine collected 0–24 h post-ingestion were analysed and a detailed spectrum of acyl-quinic acid metabolites was obtained. The fat-and carbohydrate-rich supplements resulted in a statistically significant, but relatively small, reduction in the levels of acyl-quinic acid metabolites. This contrast with more marked effects obtained in an anthocyanin study in which a strawberry drink was ingested either 2 h before or 2 h after a breakfast meal which comprised a croissant with butter and apple jelly, cereal, whole milk and sausages (838 kcal).[251] Co-ingestion of the drink with the meal resulted in the C_{max} of the main anthocyanin, a pelargonidin-O-glucuronide, falling significantly to 12.8 ± 2.1 nmol L^{-1} compared to 38.0 ± 6.6 and 35.5 ± 2.1 nmol L^{-1} when the drink was ingested, respectively, before and after the meal. Co-ingestion with the meal also extended the T_{max} from ∼1.8 h to 2.9 h.

7.4 Inter-individual variations in acyl-quinic acids excretion after coffee intake

Data obtained on urinary excretion of acyl-quinic acids and their metabolites following coffee consumption showed substantial inter-individual variation. With a 412 µmole intake by 11 volunteers the mean excretion was equivalent to $26 \pm 4\%$ of intake while individual values ranged from 12 to 58% urinary recoveries. Similar figures were obtained with intakes of 635 and 793 µmole (Table 13). It is noteworthy that while there was some variation, high excretors such as volunteer 4, maintained the condition at all three doses as did low excretors such as volunteers 1–3. Much of this person-to-person variation probably reflects variation in the composition and biochemical competence of the gut microbiota.[239] The inter-individual variation in the bioavailability of dietary (poly)phenols in general, is an area of increasing interest because the knowledge of the variation in the capacity to absorb and metabolize these compounds is thought to be a key factor in obtaining a better knowledge of the beneficial effects of plant bioactive compounds against diseases,

and particularly, an understanding of their role in healthy ageing and cardiometabolic risk reduction.[252]

8 Biomarkers of acyl-quinic acids intake

The data presented in Table 8, and that of Gomez-Juaristi et al.[206] on urinary excretion of hydroxycinnamate metabolites after the respective ingestion of coffee and maté by healthy volunteers clearly show that dihydrocaffeic acid-3′-sulfate (**40**) and feruloylglycine (**51**) would serve as very sensitive biomarkers for the consumption of relatively small amounts of acyl-quinic acids. A more detailed fingerprint for coffee could be obtained by the additional analysis of 3′,4′-dimethoxycinnamic acid (**26**), dihydroferulic acid (**35**), dihydroferulic acid-4′-sulfate (**39**), ferulic acid-4′-sulfate (**36**) and dihydroferulic acid-4′-O-glucuronide (**52**). However, whether coffee and/or maté consumption can be accurately assessed in population studies from measurements of urinary biomarkers is doubtful. These colonic catabolites are not unique to acyl-quinic acids as some can be derived from other (poly)phenols including orange juice flavanones and berry anthocyanins. Intake of CQAs from coffee or maté is, however, likely to yield much higher levels of the catabolites than consumption from alternative sources. Of more importance, is the 4–5-fold inter-individual variation in the quantity of acyl-quinic acids metabolites excreted by volunteers who consume the same coffee (Table 13) which is further compounded by the large variations in the acyl-quinic acids content of seemingly similar coffees consumed by the general public, as noted in Section 6.[43,201,202]

9 Bioactivity

Potential health benefits have been associated with coffee consumption, including a reduced incidence of several chronic and degenerative diseases, such as cancer, cardiovascular disorders, diabetes, and Parkinson's disease and the protective effects have been ascribed, at least in part, to the acyl-quinic acids present in the brew.[253–255] However, relatively little information exists about their mechanisms of action. For many years, mechanistic studies linked the protective effects of dietary (poly)phenols to their antioxidant activity. Acyl-quinic acids, particularly the dominating CQAs, are frequently referred to as powerful antioxidants, and this might be true in

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vitro. After coffee consumption by humans, unmetabolised CQAs achieve transient and very low nmol L^{-1} peak plasma concentrations (Table 6), thus, they cannot realistically compete with the much more substantial concentrations of other dietary antioxidants, such as the far more powerful ascorbate and α-tocopherol.[256] As discussed in Section 7.1, after ingestion acyl-quinic acids are extensively metabolised and the concept that health benefits might arise from direct-antioxidant activity has been challenged repeatedly,[256–258] and it has now been discarded and alternative mechanisms are being investigated.

(Poly)phenols have been found to exert modulatory effects in cells through selective action on multiple cell-signalling pathways involved in the pathogenesis of degenerative diseases, indicating that the health effects go beyond simple antioxidant activity.[259,260] However, most *in vitro* studies investigating the biological activity of acyl-quinic acids have used the un-metabolised parent compounds present in foods and beverages and, in many cases, at supra-physiological concentrations that are never attained in the circulatory system, and organs or tissues, except perhaps in the GI tract. This section will, therefore, cover studies using catabolites of acyl-quinic acids and their phase II metabolites that have been tested at physiologically relevant sub- and low μmol L^{-1} concentrations. As noted in Section 8, the relevant catabolites are not unique to acyl-quinic acids so studies that originally focussed on other dietary polyphenols are included.

Several such studies have investigated beneficial effects, on markers of cardiovascular diseases and the possible molecular mechanisms involved., Van Rymenant *et al.*[261] compared the effects of ferulic acid (4) and ferulic acid-4′-sulfate (36) on vasorelaxation *ex vivo* using mice saphenous artery, femoral artery and aorta. While ferulic acid was inactive, its ferulic acid-4′-sulfate, caused a concentration-dependent relaxation in all three tissues that were significant at sub-μmol L^{-1} concentrations. Further analyses demonstrated that soluble guanylate cyclase (sGC) and voltage-gated K$^+$-channels, are involved in the ferulic acid-4′-sulfate-dependent vasorelaxation. Whether these effects also occur with human test systems remains to be determined. It is of interest, however, that following human consumption of coffee containing 412 μmol of acyl-quinic acids, ferulic acid-4′-sulfate, but not ferulic acid, appears in the circulatory system with a dual C_{max} of 76 nmol L^{-1} after 0.6 h and 46 nmol L^{-1} after 4.3 h (Fig. 6 and Table 6).[44]

Amin *et al.*[262] studied the anti-inflammatory effects of ferulic acid, and protocatechuic acid (3,4-dihydroxybenzoic acid) (54) and its glucuronide and sulfate conjugates in human umbilical vein endothelial cells (HUVECs) stimulated with either oxidized LDL or a cluster of differentiation 40 ligand. Protocatechuic acid and its phase II metabolites were effective in modulating the production of key inflammatory mediators IL-6 and vascular cell adhesion molecule-1 (VCAM-1) at dietary relevant concentrations as low as 100 nmol L^{-1}, with maximum reduction observed for the sulfate conjugates. In keeping with the data of Van Rymenant *et al.*[261] these results indicate that phase II conjugation may unexpectedly increase bioactivity. However, in a similar study by Warner *et al.*[263] that analysed the effects of phenolic catabolites on secretion of VCAM-1 in HUVEC-

stimulated with tumor necrosis factor alpha (TNF-α), a statistically significant reduction of 17.2% was observed with 1 μmol L^{-1} protocatechuic acid, but not its phase II metabolites. Protocatechuic acid at 0.5 μmol L^{-1} has also been shown to significantly reduce adhesion of monocytes to HUVECs by 28.7%. Protocatechuic acid-3-sulfate (55) and protocatechuic acid-4-sulfate (56) also significantly reduced VCAM-1 secretion but at the much higher concentrations of 10 and 100 μmol L^{-1}, respectively.[264] In the same study, 1 μmol L^{-1} ferulic acid (4) significantly reduced monocyte adhesion by 21.3%. Adhesion of monocytes to endothelial cells and their subsequent *trans*-endothelial migration into the vascular wall initiate the atheroma formation which leads to cardiovascular diseases.
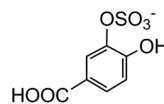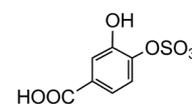
Protocatechuic acid-3-sulfate (55)   Protocatechuic acid-4-sulfate (56)

A study using lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells, demonstrated that dihydrocaffeic acid (37) and 3′,4′-dihydroxyphenylacetic acid (48) at 1 μmol L^{-1} significantly reduced secretion of the main pro-inflammatory cytokines TNF-α, IL-1β and IL-6 by 84.9–97.9% compared to control cells.[265] In a study in which 300 g of raspberries were consumed by volunteers, after 6 h, 3′4′-dihydroxyphenylacetic acid (46) attained a plasma C_{max} of 180 nmol L^{-1}, while after consuming coffee, dihydrocaffeic acid (37) reached a C_{max} of 41 nmol L^{-1} (Table 6).

Baeza *et al.*[266] studied the inhibitory effects of caffeic acid (3), ferulic acid (4), dihydrocaffeic acid (37), and dihydroferulic acid (35) on platelet activation in human blood samples by measuring ADP-induced P-selectin expression. Excessive platelet activation has been associated with development of chronic inflammation and has, therefore, been proposed as a risk factor for cardiovascular diseases.[267] A significant decrease in P-selectin expression was observed with dihydrocaffeic acid at 1 μmol L^{-1} while ferulic acid and dihydroferulic acid were without effect at concentrations up to 10 and 20 μmol L^{-1}, respectively.

González-Sarrías *et al.*[268] evaluated the potential neuroprotective effects of 19 (poly)phenol-derived metabolites, including dihydrocaffeic acid (37) and 3′,4′-dihydroxyphenylacetic acid (46). At 1 μmol L^{-1} no protective effects were observed but at 5 μmol L^{-1} both CQA catabolites significantly attenuated the H$_2$O$_2$-induced cytotoxicity in SH-SYS neuroblastoma cells. The strongest neuroprotective effects occurred at 10 μmol L^{-1} and at this concentration they were also able to significantly reduce ROS levels in the SH-SY5Y cells and decrease oxidative stress-induced apoptosis by preventing caspase 3-activation *via* mitochondrial apoptotic pathway.

In a similar study Verzelloni *et al.*[269] investigated the ability of an array of (poly)phenol catabolites generated *in vivo* from different food sources, including coffee, to counteract neurotoxicity linked to oxidative stress in human SK-N-MC neuroblastoma cells. The compounds were tested at concentrations ranging from 0.1 to 20 μmol L^{-1}. Dihydrocaffeic acid (37)

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exhibited significant protective effects at 0.5 μmol L^{-1}, while at the highest tested concentration dihydroferulic acid (**35**) and feruloylglycine (**51**) also protected neurons against 2,3-dimethoxy-1,4-naphthoquinone-induced toxicity, with increased survival after oxidative stress ranging from 6.5 to 17.0%. The possible synergistic effects were investigated by evaluating the neuroprotective effects of different combinations of catabolites. When assayed in this manner at concentrations of 0.5 μmol L^{-1}, the three acyl-quinic acids catabolites induced a statistically significant 16% increase in cell viability.

It is unfortunate that there are still comparatively few studies of relevant *in vitro* metabolites and catabolites at concentrations close to those known to occur in humans consuming real-world diets. Nevertheless, the results summarised here suggest that at least some gut flora catabolites and their phase II metabolites may indeed have anti-inflammatory and neuroprotective effects at such concentrations. Although the consumption of acyl-quinic acids is not an absolute pre-requisite for the production of these catabolites and metabolites, the fact that acyl-quinic acids-rich beverages such as coffee and maté are typically consumed repeatedly at short intervals throughout the day, day after day, makes the acyl-quinic acids particularly important. This frequent and long term consumption results in several bolus doses being available simultaneously to the gut microbiota and greatly increasing the period of time during which catabolite absorption can occur. Accordingly there is a potential for a somewhat higher plasma C_{max} than would be achieved by a single bolus dose, but more importantly, a significant concentration is achieved for several hours rather than a few minutes.[270] For these reasons plasma AUC values and 24 hour urinary excretion values are better indicators of physiological relevance than C_{max} values *per se*. Such data for free-living healthy and clinically compromised volunteers, in addition to volunteers given defined supplements, would be a valuable adjunct to epidemiological and *in vitro* studies.

10   Conflicts of interest

There are no conflicts to declare.

11   Acknowledgements

12   References

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