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REVIEW

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Dietary phenolics: chemistry, bioavailability and effects on health

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In this issue...

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Dietary phenolics: chemistry, bioavailability and effects on health

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There is much epidemiological evidence that diets rich in fruit and vegetables can reduce the incidence of non-communicable diseases such as cardiovascular diseases, diabetes, cancer and stroke. These protective effects are attributed, in part, to phenolic secondary metabolites. This review summarizes the chemistry, biosynthesis and occurrence of the compounds involved, namely the C_6–C_3–C_6 flavonoids – anthocyanins, dihydrochalcones, flavan-3-ols, flavanones, flavones, flavonols and isoflavones. It also includes tannins, phenolic acids, hydroxycinnamates and stilbenes and the transformation of plant phenols associated with food processing (for example, production of black tea, roasted coffee and matured wines), these latter often being the major dietary sources. Events that occur following ingestion are discussed, in particular, the deglycosylation, glucuronidation, sulfation and methylation steps that occur at various points during passage through the wall of the small intestine into the circulatory system and subsequent transport to the liver in the portal vein. We also summarise the fate of compounds that are not absorbed in the small intestine, but which pass into the large intestine where they are degraded by the colonic microflora to phenolic acids, which can be absorbed into the circulatory system and subjected to phase II metabolism prior to excretion. Initially, the protective effect of dietary phenolics was thought to be due to their antioxidant properties which resulted in a lowering of the levels of free radicals within the body. However, there is now emerging evidence that the metabolites of dietary phenolics, which appear in the circulatory system in nmol/L to low μmol/L concentrations, exert modulatory effects in cells through selective actions on different components of the intracellular signalling cascades vital for cellular functions such as growth, proliferation and apoptosis. In addition, the intracellular concentrations required to affect cell signalling pathways are considerably lower than those required to impact on antioxidant capacity. The mechanisms underlying these processes are discussed.

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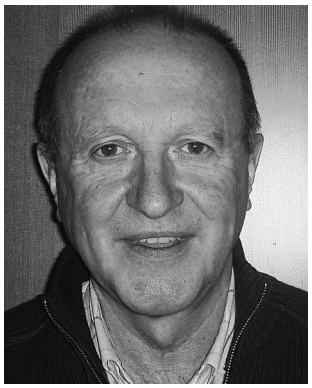
1 Introduction

Current dietary advice is that for optimum health people should consume on a daily basis five portions of fruit and vegetables each comprising at least 80 grams.[1] The epidemiological evidence for the benefit of consuming a diet that is high in fruit and vegetables is quite compelling. The evidence for specific vegetables, and indeed specific phytochemicals, is less convincing and the best simple advice that can be given is to recommend as much variety as possible. Phytochemicals are plant secondary metabolites, *i.e.* substances that *in planta* have little or no role in photosynthesis, respiration or growth and development, but which may accumulate in surprisingly high concentrations.[2]

Unlike the traditional vitamins, phytochemicals as dietary components are not essential for short-term well-being, and whereas the body has specific mechanisms for the accumulation and retention of vitamins, in contrast, phytochemicals are treated as non-nutrient xenobiotics and metabolised so as to eliminate them efficiently. The flavonoids and allied phenolic and polyphenolic compounds, including tannins and derived polyphenols, form one major group of phytochemicals. We here review data relating to those dietary commodities that make a particular contribution to the intake of phenols and polyphenols, either because the commodity is unusually rich, or consumed in large quantities, or is otherwise of note. However, it cannot be overstressed that published analytical data may not be truly representative of the individual component in a particular diet.

The lack of comprehensive and reliable data for the phytochemical content of raw foods severely limits the insights that can be obtained from epidemiological studies. This is compounded by a lack of information relating to changes in content and character, *i.e.* the production of derived polyphenols caused by food processing, and the physiological consequences of the gut microbial and mammalian metabolism of both native and derived phytochemicals once consumed.



Alan Crozier

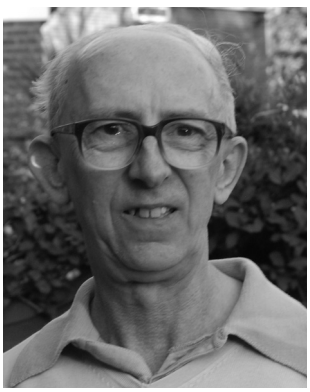
Alan Crozier is a Botany graduate of the University of Durham. He obtained a PhD at Bedford College, University of London, carried out post-doctoral research at the University of Calgary and lectured at the University of Canterbury in New Zealand before joining the University of Glasgow where he is currently Professor of Plant Biochemistry and Human Nutrition. He has published extensively on plant hormones and purine alkaloids in coffee and teas. The activities of his research group are currently focussed on dietary flavonoids and phenolic compounds in fruits, vegetables and beverages, including teas and fruit juices, and their fate within the body following ingestion in relation to their potentially beneficial effects on health.

Indu Jaganath

Indu Jaganath is a senior research officer at The Biotechnology Research Centre at the Malaysian Agricultural Research and Development Institute. She obtained a Master of Science from the University of California, Riverside, and a PhD at the University of Glasgow. Her earlier research areas include pollination biology and ethnobotany. Currently her research is focused on the discovery of chemically and biologically active natural products in tropical plants. Her research group uses metabolomics and genomics approaches to understand and delineate the metabolic pathways involved in the production of these biologically active compounds.

2 Classification of phenolic compounds

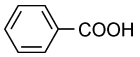
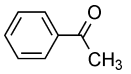
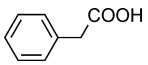
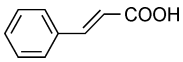
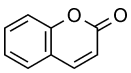
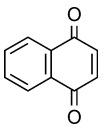
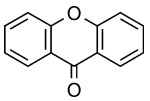
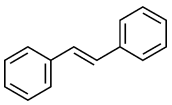
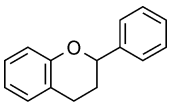
Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. In excess of 8000 phenolic structures have been reported and they are widely dispersed throughout the plant kingdom[3] – many occur in food. Phenolics range from simple, low molecular weight, single-aromatic-ring compounds to the large and complex tannins and derived polyphenols. They can be classified by the number and arrangement of their carbon atoms (Table 1) and are commonly found conjugated to sugars and organic acids. Phenolics occurring naturally in healthy plant tissue can be classified into two groups, the flavonoids and the non-flavonoids: traditionally processed foods and beverages, such as black tea, matured red wine, coffee and cocoa, may contain phenolic transformation



Mike Clifford

Mike Clifford is a Food Science graduate of the University of Reading. He obtained a PhD at the University of Strathclyde. He taught at Grimsby College of Technology for eight years and joined the University of Surrey in 1979, where he founded and led the Food Safety Research Group. He is now Emeritus Professor of Food Safety. He has published extensively on the analysis and characterisation of phenols, especially those in coffee (chlorogenic acids) and black tea (thearubigins), and their metabolism by the gut microflora and their effects on the consumer.

Table 1 Basic structural skeletons of phenolic and polyphenolic compounds.

Skeleton	Classification	Basic structure
C_6–C_1	Phenolic acids	
C_6–C_2	Acetophenones	
C_6–C_2	phenylacetic acid	
C_6–C_3	Hydroxycinnamic acids	
C_6–C_3	Coumarins	
C_6–C_4	Naphthoquinones	
C_6–C_1–C_6	Xanthones	
C_6–C_2–C_6	Stilbenes	
C_6–C_3–C_6	Flavonoids	

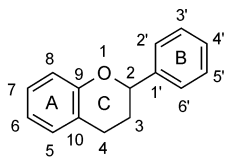
products that are best described as 'derived polyphenols'. Tannins are the active ingredients of traditional plant extracts used to convert hides to leather and occur widely in foods and beverages but at concentrations too low to tan hides.

2.1 Flavonoids

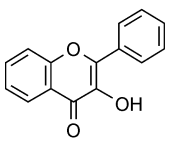
Flavonoids are polyphenolic compounds comprising 15 carbons, with two aromatic rings connected by a three carbon bridge, hence C_6–C_3–C_6 (**1**). They are the most numerous of the phenolics and are found throughout the plant kingdom.[4] They are present particularly in the epidermis of leaves and the skin of fruits.

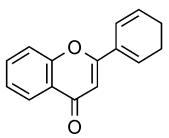
The main sub-classes of dietary flavonoids are flavonols (**2**), flavones (**3**), flavan-3-ols (**4**), anthocyanidins (**5**), flavanones (**6**) and isoflavones (**7**), while those that are comparatively minor components of the diet are dihydroflavonols (**8**), flavan-3,4-diols (**9**), coumarins (**10**), chalcones (**11**), dihydrochalcones (**12**) and aurones (**13**). The basic flavonoid skeleton can have numerous substituents. Hydroxyl groups are usually present at the 4′-, 5- and 7-positions. Sugars are very common, with the majority of flavonoids existing naturally as glycosides. Whereas both sugars and hydroxyl groups increase the water solubility of flavonoids, other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic.

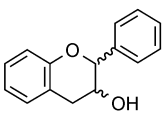
Flavonols (**2**) are the most widespread of the flavonoids, being dispersed throughout the plant kingdom with the exception of algae. They are also not found in fungi. The distribution and structural variations of flavonols are extensive and have been well documented.[5] The main dietary flavonols, kaempferol (**14**), quercetin (**15**), isorhamnetin (**16**) and myricetin (**17**), are most commonly found as *O*-glycosides. Conjugation occurs most

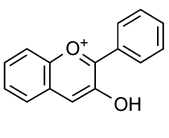


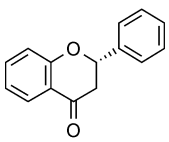
Flavonoid skeleton (**1**)

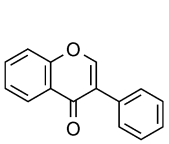

Flavonol (**2**)

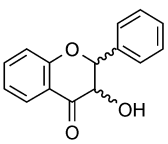

Flavone (**3**)

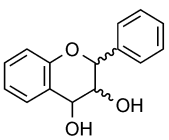

Flavan-3-ol (**4**)

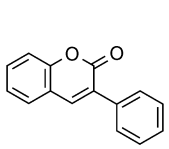

Anthocyanidin (**5**)

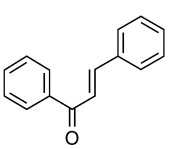

Flavanone (**6**)

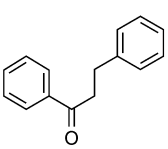

Isoflavone (**7**)

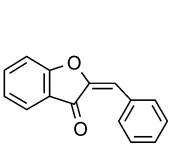

Dihydroflavonol (**8**)

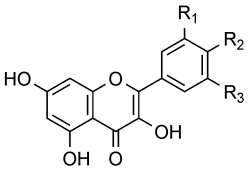

Flavan-3,4-diol (**9**)


Coumarin (**10**)

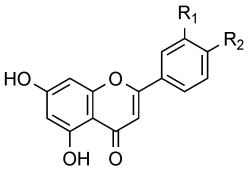

Chalcone (**11**)


Dihydrochalcone (**12**)

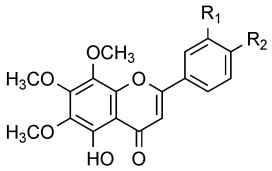

Aurone (**13**)



Kaempferol (**14**) R_1 = H, R_2 = OH, R_3 = H
Quercetin (**15**) R_1 = OH, R_2 = OH, R_3 = H
Isorhamnetin (**16**) R_1 = OCH_3, R_2 = OH, R_3 = H
Myricetin (**17**) R_1 = OH, R_2 = OH, R_3 = OH



Apigenin (**18**) R_1 = H, R_2 = OH
Luteolin (**19**) R_1 = OH, R_2 = OH



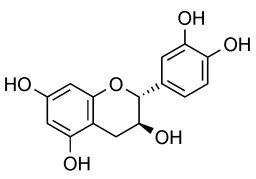
Tangeretin (**20**) R_1 = H, R_2 = OCH_3
Nobiletin (**21**) R_1 = OCH_3, R_2 = OCH_3

frequently at the 3-position of the C-ring but substitutions can also occur at the 5-, 7-, 4′-, 3′- and 5′-carbons. Although the number of aglycones is limited there are numerous flavonol conjugates, with more than 200 different sugar conjugates of kaempferol alone. The levels of flavonols found in commonly consumed fruits, vegetables and beverages are well documented.[6] However, sizable differences are found in the amounts present in seemingly similar produce, possibly due to seasonal changes and varietal differences;[7] effects of processing will also have an impact.

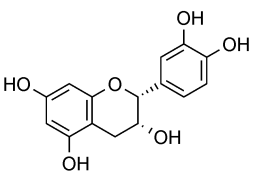
Flavones (**3**), such as apigenin (**18**) and luteolin (**19**), lack oxygenation at C3 but otherwise may have a wide range of substitutions including hydroxylation, methylation, *O*- and *C*-alkylation and glycosylation. Most flavones occur as 7-*O*-glycosides. Flavones are not distributed widely, with significant occurrences being reported in only celery, parsley and some herbs. Polymethoxylated flavones, such as tangeretin (**20**) and nobiletin (**21**), have been found in citrus species.

Flavan-3-ols (**4**) are non-planar by virtue of their saturated C3 element and are the most structurally complex subclass of flavonoids, ranging from the simple monomers (+)-catechin (**22**) and its isomer (−)-epicatechin (**23**), which can be hydroxylated to form gallocatechins (**24**, **25**) and also undergo esterification with gallic acid (**26**, **27**), through to complex structures including the oligomeric and polymeric proanthocyanidins, which are also known as condensed tannins. The two chiral centres at C2 and C3 of the flavan-3-ols produce four isomers for each level of B-ring hydroxylation, two of which, (+)-catechin and (−)-epicatechin, are widespread in nature whereas (−)-catechin (**28**) and (+)-epicatechin (**29**) are comparatively rare.[8] The oligomeric and polymeric proanthocyanidins have an additional chiral centre at C4 of each additional flavan-3-ol unit. Pairs of enantiomers are not resolved on the commonly used reverse-phase HPLC columns, and so are easily overlooked. Although difficult to visualise, these differences in chirality have a significant effect on the 3-D structure of the molecules, as illustrated in Fig. 1 for the (epi)gallocatechin-3-*O*-gallates. Although this has little, if any, effect on their redox properties or ability to scavenge small unhindered radicals,[9] it can be expected to have a more pronounced effect on their binding properties and hence any phenomenon to which the 'lock and key' concept is fundamental, *e.g.* enzyme–substrate, enzyme–inhibitor or receptor–ligand interactions. Humans fed (−)-epicatechin (**23**) excrete some (+)-epicatechin (**29**), indicating ring opening and racemisation, possibly in the gastrointestinal tract.[10] Transformation can also occur during food processing.[11]

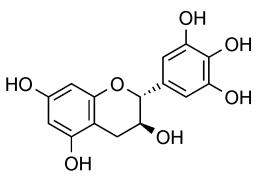
Type B proanthocyanidins are formed from (+)-catechin (**22**) and (−)-epicatechin (**23**) with oxidative coupling occurring between the C4 of the heterocycle and the C6 (**30**) or C8 positions (**31**) of the adjacent unit to create oligomers or polymers. Type A proanthocyanidins have an additional ether bond between C2 and C7 (**32**). Proanthocyanidins can occur as polymers of up to 50 units. Proanthocyanidins that consist exclusively of (epi)catechin units are called procyanidins, and are the most abundant type of proanthocyanidins in plants. The less common proanthocyanidins containing (−)-epiafzelechin (**33**) and (+)-afzelechin (**34**) or (epi)gallocatechin (**24**, **25**) sub-units are called propelargonidins and prodelphinidins, respectively. Many condensed tannins



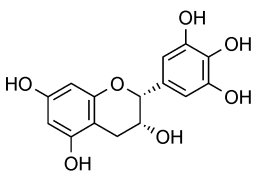
(+)-Catechin (**22**)



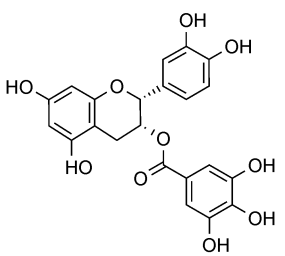
(–)-Epicatechin (**23**)



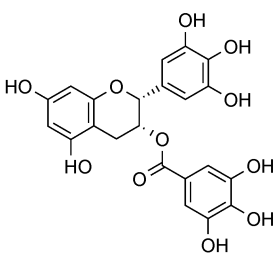
(+)-Gallocatechin (**24**)



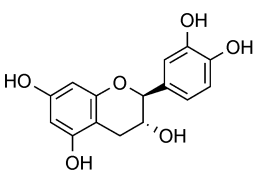
(–)-Epigallocatechin (**25**)



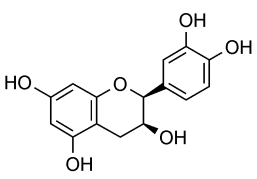
(–)-Epicatechin-3-*O*-gallate (**26**)



(–)-Epigallocatechin-3-*O*-gallate (**27**)



(–)-Catechin (**28**)



(+)-Epicatechin (**29**)

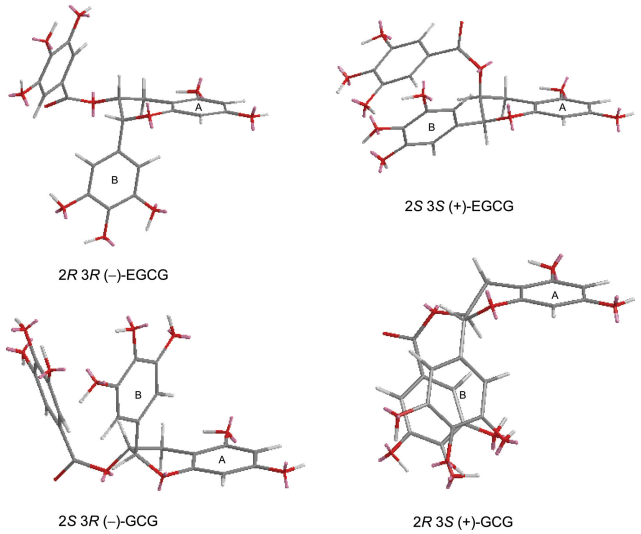
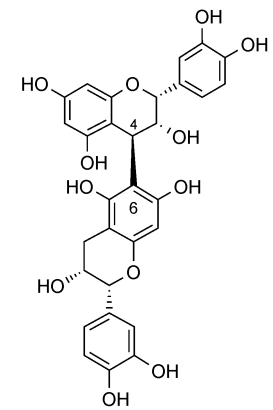


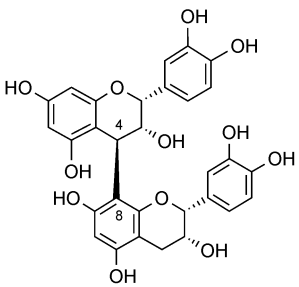
Fig. 1 Computer-generated stereochemical projections for flavan-3-ol diastereoisomers. epigallocatechin-3-*O*-gallate (EGCG) and gallocatechin-3-*O*-gallate (GCG). Three-dimensional structures computed by Mr J. Warren Dryman, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.

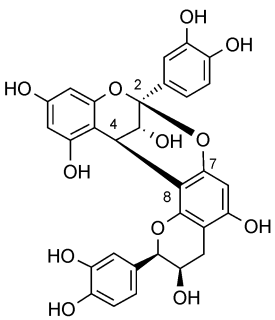
contain more than one monomer. Flavan-3-ol monomers are extensively transformed during the traditional processing of wines, cocoa and black tea, in the latter case yielding theaflavins, theacitrins and thearubigins (see Section 3.1.1)

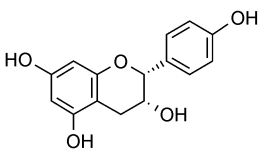
Anthocyanidins (**5**) are widely dispersed throughout the plant kingdom, being particularly evident in fruit and flower tissue where they are responsible for red, blue and purple colours. They are also found in leaves, stems, seeds and root tissue. The most common anthocyanidins are pelargonidin (**35**), cyanidin (**36**), delphinidin (**37**), peonidin (**38**), petunidin (**39**) and malvidin (**40**). In plant tissues these compounds are invariably found as sugar conjugates that are known as anthocyanins, which may also be conjugated to hydroxycinnamates and organic acids such as acetic acid (**41**–**44**). Although glycosylation can take place on carbons 3, 5, 7, 3′ and 5′, it occurs most often on C3. In certain products, such as matured red wines and ports, chemical and enzymic transformations occur, and an increasing number of 'anthocyanin-derived polyphenols' are being found (see Section 3.1.4).

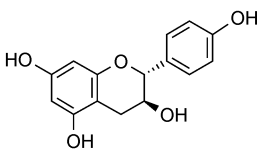
The flavanones (**6**) are non-planar and have a chiral center at C2. In the majority of naturally occurring flavanones, ring C is attached to the B-ring at C2 in the α-configuration. Flavanones are present in especially high concentrations in citrus fruits. The most common flavanone glycoside is hesperetin-7-*O*-rutinoside

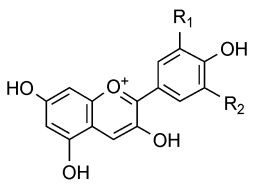

Proanthocyanidin B_5 dimer (**30**)

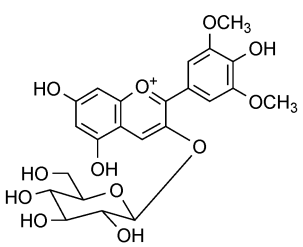

Proanthocyanidin B_2 dimer (**31**)

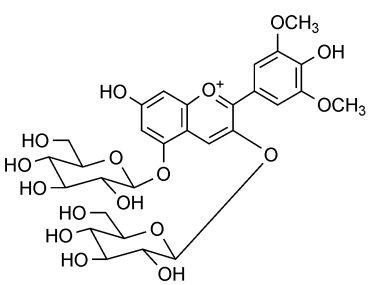

Proanthocyanidin A_2 dimer (**32**)

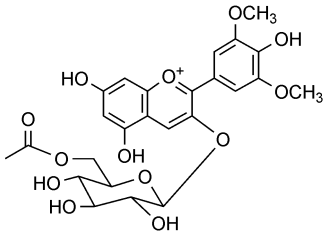

(–)-Epiafzelechin (**33**)


(+)-Afzelechin (**34**)

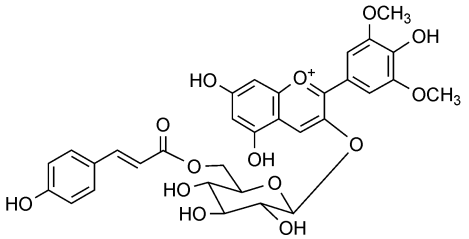

Pelargonidin (**35**) $R_1 = H, R_2 = H$
Cyanidin (**36**) $R_1 = OH, R_2 = H$
Delphinidin (**37**) $R_1 = OH, R_2 = OH$
Peonidin (**38**) $R_1 = OCH_3, R_2 = H$
Petunidin (**39**) $R_1 = OCH_3, R_2 = OH$
Malvidin (**40**) $R_1 = OCH_3, R_2 = OCH_3$


Malvidin-3-*O*-glucoside (**41**)

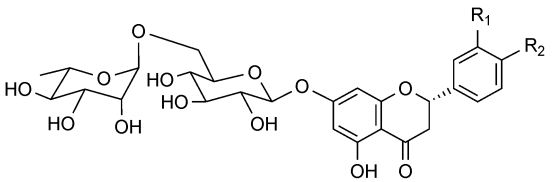

Malvidin-3,5-di-*O*-glucoside (**42**)



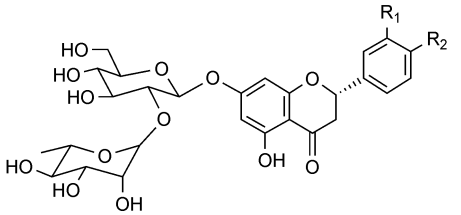
Malvidin-3-*O*-(6''-*O*-acetyl)glucoside (**43**)



Malvidin-3-*O*-(6''-*O*-*p*-coumaroyl)glucoside (**44**)



Hesperetin-7-*O*-rutinoside (**45**) R_1 = OH, R_2 = OCH_3
Naringenin-7-O-rutinoside (**46**) R_1 = H, R_2 = OH



Hesperetin-7-*O*-neohesperidoside (**47**) R_1 = OH, R_2 = OCH_3
Naringenin-7-O-neohesperidoside (**48**) R_1 = H, R_2 = OH

(hesperidin) (**45**), which along with narigenin-7-*O*-rutinoside (narirutin) (**46**) is found in citrus peel. Flavanone rutinosides are tasteless. In contrast, flavanone neohesperidoside conjugates such as hesperetin-7-*O*-neohesperidoside (neohesperidin) (**47**) from bitter orange (*Citrus aurantium*) and naringenin-7-*O*-neohesperidoside (naringin) (**48**) from grapefruit peel (*Citrus paradisi*) are intensely bitter.

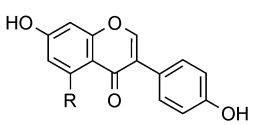
Isoflavones (**7**) have the B-ring attached at C3 rather than C2. They are found almost exclusively in leguminous plants, with the highest concentrations occurring in soy bean (*Glycine max*).[12] The isoflavones, daidzein (**49**) and genistein (**50**), and the coumestan, coumestrol (**51**) from lucerne and clovers (*Trifolium* spp.), have sufficient oestrogenic activity to seriously affect the reproduction of grazing animals such as cows and sheep, and are termed phyto-oestrogens. These isoflavonoids appear to mimic the steroidal hormone oestradiol (**52**) which blocks ovulation. The consumption of legume fodder by animals must, therefore, be restricted, or low-isoflavonoid producing varieties selected. This is clearly an area where it would be beneficial to produce genetically modified isoflavonoid-deficient legumes.

Dietary consumption of genistein and daidzein from soy products is thought to reduce the incidence of prostate and breast cancers in humans. However, the mechanisms involved are different. Growth of prostate cancer cells is induced by and dependent upon the androgen testosterone (**53**), the production of which is suppressed by oestradiol. When natural oestradiol is insufficient, the isoflavones can lower androgen levels and, as a consequence, inhibit tumour growth. Breast cancers are dependent upon a supply of oestrogens for growth, especially during the early stages. Isoflavones compete with natural oestrogens, restricting their availability and thereby suppressing the growth of the cancerous cells. There was concern that neonates and infants could be adversely affected by excessive intakes of isoflavones in soy-protein-based human milk-replacers, and the levels have been voluntarily reduced by industry as a precaution.[13]

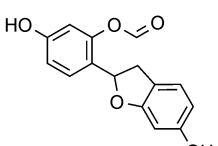
2.2 Non-flavonoids

The main non-flavonoids of dietary significance are the C_6–C_1 phenolic acids, most notably gallic acid (**54**), which is the biosynthetic precursor of hydrolysable tannins, the C_6–C_3 hydroxycinammates and their conjugated derivatives, and the polyphenolic C_6–C_2–C_6 stilbenes (Table 1).

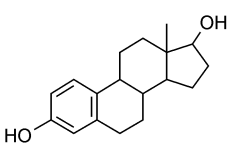
Gallic acid is the commonest phenolic acid, and occurs widely as complex sugar esters in gallotannins such as 2-*O*-digalloyl-tetra-*O*-galloyl-glucose (**55**) but these are found only to a limited extent in dietary components. Non-sugar galloyl esters in grapes, wine, mangoes, green tea and black tea are the major source of gallic acid in the human diet. The related ellagic acid (**56**) and ellagitannins, such as sanguiin H-10 (**57**), which is found in raspberries (*Rubus idaeus*) and strawberries (*Fragaria* × *ananassa*), are also present in a number of fruits including pomegranate (*Punica granatum*), blackberries (*Rubus* spp.), persimmon (*Diospyros kaki*) as well as walnuts (*Juglans regia*), hazelnuts (*Corylus avellana*) and oak-aged wines.



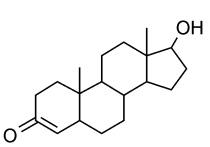
Daidzein (**49**) R = H
Genistein (**50**) R = OH



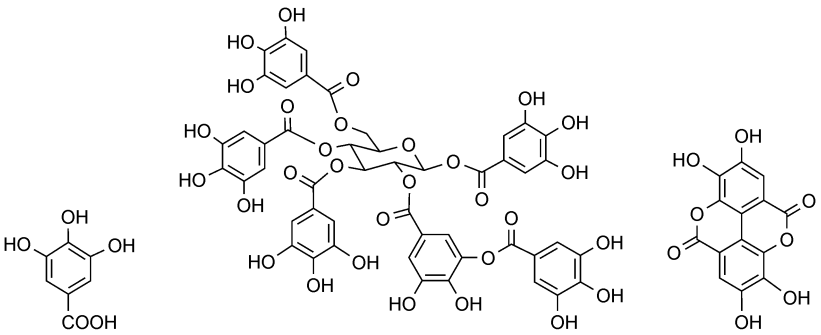
Coumestrol (**51**)



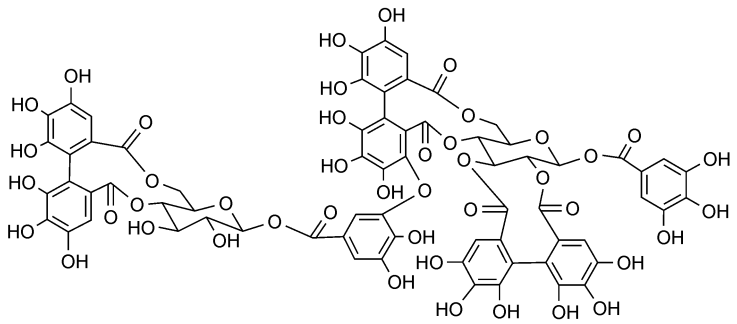
Oestradiol (**52**)



Testosterone (**53**)



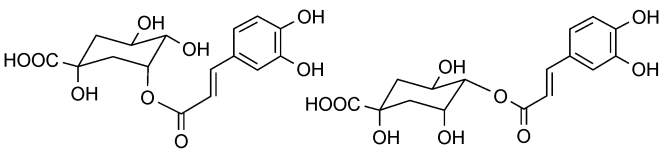
Gallic acid (**54**) 2-*O*-Digalloyl-tetra-*O*-galloyl-glucose (**55**) Ellagic acid (**56**)



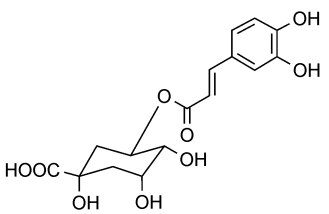
Sanguiin H-10 (**57**)

The most common hydroxycinnamates are *p*-coumaric acid (**58**), caffeic acid (**59**), ferulic acid (**60**) and sinapic acid (**61**), with caffeic acid dominating. These occur as conjugates, for example with tartaric acid or quinic acid, collectively referred to as chlorogenic acids. Chlorogenic acids, principally 3-*O*-, 4-*O*- and 5-*O*-caffeoylquinic acids (**62–64**), form *ca.* 10% of green robusta coffee beans (processed seeds of *Coffea canephora*). Regular consumers of coffee may have a daily intake in excess of 1 g, and these for many people will be the major dietary phenols.

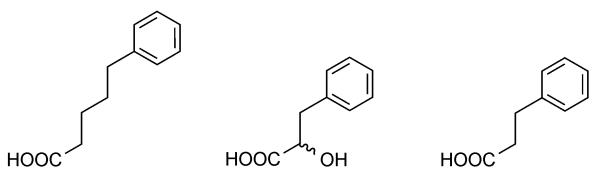
Derivatives of phenylvaleric acid (**65**), phenyl-lactic acid (**66**), phenylpropionic acid (**67**), phenylmandelic acid (**68**) and phenylhydracrylic acid (**69**) rarely occur preformed in food but are colonic microflora metabolites of many dietary phenols and polyphenols that are readily absorbed, and may in part be responsible for some biological effects associated with diets rich in polyphenols (see Section 7.0).



3-*O*-Caffeoylquinic acid (**62**) 4-*O*-Caffeoylquinic acid (**63**)

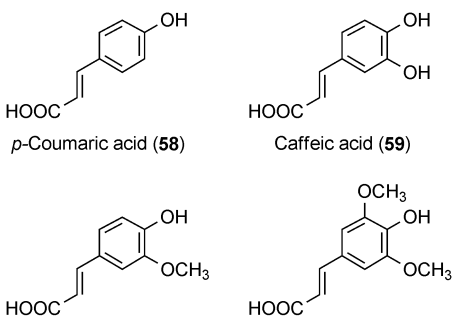


5-*O*-Caffeoylquinic acid (**64**)



Phenylvaleric acid (**65**) Phenyl-lactic acid (**66**) Phenylpropionic acid (**67**)

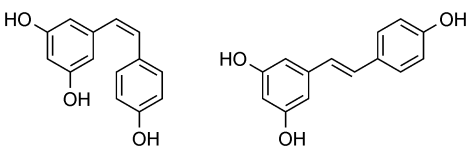
Phenylmandelic acid (**68**) Phenylhydracrylic acid (**69**)



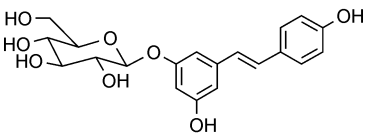
p-Coumaric acid (**58**) Caffeic acid (**59**) Ferulic acid (**60**) Sinapic acid (**61**)

Stilbenes have a C_6–C_2–C_6 structure (Table 1) and are phytoallexins produced by plants in response to disease, injury and stress.[14] The main dietary source of stilbenes is resveratrol (3,5,4′-trihdroxystilbene) from red wine and peanuts (*Arachis*

hypogaea)[15] with lesser amounts found in berries, red cabbage (*Brassica oleracea*), spinach and certain herbs. Resveratrol occurs as *cis* and *trans* isomers (**70**, **71**), and *trans*-resveratrol and *trans*-resveratrol-3-*O*-glucoside (*trans*-piceid) (**72**) have been detected in pistachio nuts (*Pistacia vera*).[16] The woody root of the noxious weed *Polygonum cuspidatum* (Japanese knotweed or Mexican bamboo) has been shown to contain very high levels of *trans*-resveratrol and its glucoside, with concentrations of up to 377 mg per 100 g dry weight.[17] As well as resveratrol, red wines can also contain *trans*-piceatannol (3,3′,4,5′-tetrahydroxystilbene) (**73**) and *trans*-astringin, its 3-*O*-glucoside (**74**).[18] *trans*-Resveratrol is transformed by *Botrytis cinerea*, a fungal grapevine pathogen, to pallidol (**75**) and resveratrol *trans*-dehydrodimer (**76**), and both these compounds have been detected in grape cell cultures along with the 11-*O*- and 11′-*O*-glucosides of resveratrol *trans*-dehydrodimer (**77**, **78**).[19] Viniferins are another family of oxidised resveratrol dimers, and *trans*-δ-viniferin (**79**) and smaller amounts of its isomer *trans*-ε-viniferin (**80**) have been detected in *Vitis vinifera* leaves infected with *Plasmopara viticola* (downy mildew).[20]

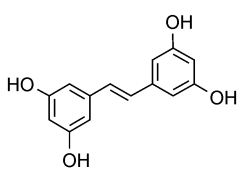


cis-Resveratrol (**70**) *trans*-Resveratrol (**71**)

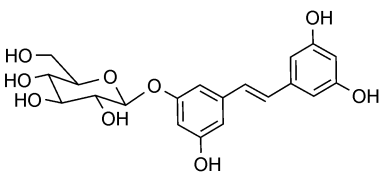


trans-Resveratrol-3-*O*-glucoside (**72**)

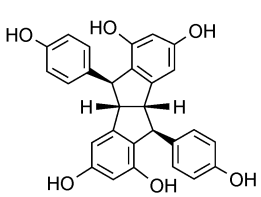
trans-Resveratrol (**71**) has gained significant worldwide attention because of its ability to inhibit or retard a wide variety of animal diseases[21] that include cardiovascular disease[22] and cancer.[23] It has also been reported to increase stress resistance and enhance longevity.[24] The protective effects of red wine consumption are regularly attributed to resveratrol.[25] However, this is highly unlikely as the levels of resveratrol in red wines are low, and for humans to ingest the quantity of resveratrol that affords protective effects in animals they would have to drink in excess of 100 L of red wine per day.[26]



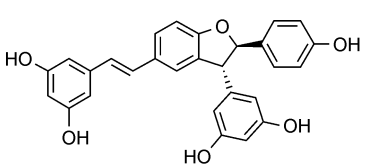
trans-Piceatannol (**73**)



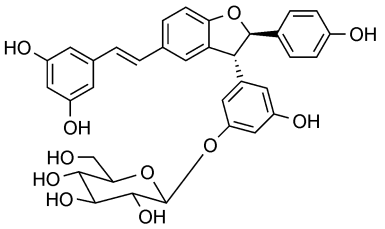
trans-Piceatannol-3-*O*-glucoside (**74**)



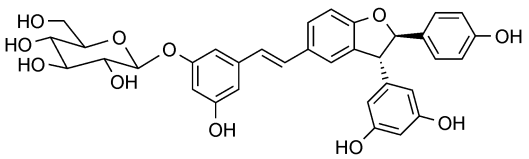
Pallidol (**75**)



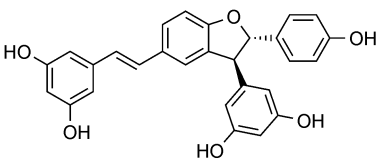
Resveratrol *trans*-dehydrodimer (**76**)



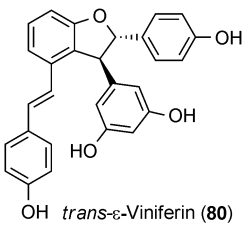
Resveratrol *trans*-dehydrodimer-11-*O*-glucoside (**77**)



Resveratrol *trans*-dehydrodimer-11′-*O*-glucoside (**78**)



trans-δ-Viniferin (**79**)



trans-ε-Viniferin (**80**)

3 Significant dietary sources of phenolics and polyphenolics

It is not possible to rank commodities in terms of their production of phenols and polyphenols per annum. However, on a global scale, the most important commodities are those that are rich in polyphenols and widely consumed in large quantities such as green tea, black tea, red wine, coffee and cocoa/chocolate. Generally, fruits, and especially vegetables, are a poor second because of their much lower contents. No staples are rich in these phytochemicals, but along with herbs and spices, nuts, algae and olive oil, they are potentially significant for supplying certain phenols and polyphenols of restricted botanical occurrence.[2] Because of constraints on space, our emphasis will be on those commodities making the greatest quantitative contribution, but this is not to suggest that the minor dietary components are unimportant.

3.1 Beverages

3.1.1 Tea. Tea prepared from the leaves of *Camellia* spp. is one of the most widely consumed beverages in the world. Approximately 3.2 million metric tons of dried leaf are produced annually, of which 20% is green tea and 2% is oolong, the remainder being black tea. In all cases the raw material is young leaves, the tea flush, which are preferred as they have a higher flavan-3-ol content and elevated levels of active enzymes. The highest quality teas utilise 'two leaves and a bud', with progressively lower quality taking four or even five leaves.[27] Although

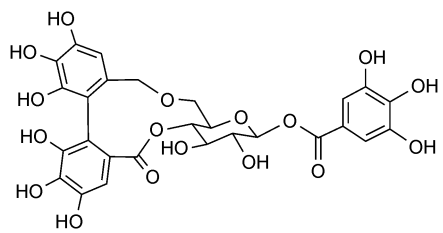
produced from similar plant material, these teas differ markedly in the nature of phenols and polyphenols that they contribute to the diet because of differences in their manufacture.

There are basically two types of green tea.[28] The Japanese type utilises a shade-grown hybrid leaf with comparatively low flavan-3-ol levels and high amino acid content, including theanine. After harvesting the leaf is steamed rapidly to inhibit polyphenol oxidase and other enzymes. Chinese green tea traditionally uses selected forms of *Camellia sinensis* var. *sinensis* and dry heat (firing) rather than steaming, giving a less efficient inhibition of the polyphenol oxidase activity and allowing some transformation of the flavan-3-ols.

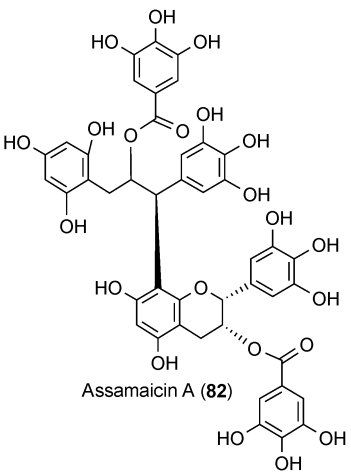
In the production of black tea there are two major processes – the 'orthodox' and the 'cut–tear–curl' processes.[29,30] In both the objective is to achieve efficient disruption of cellular compartmentation bringing phenolic compounds into contact with polyphenol oxidases and activating many other enzymes. A detailed account of the processes is beyond the scope of this article (see ref. 30) but oxidation for 60–120 min at about 40 °C before drying is representative.

When harvested, the fresh tea leaf is unusually rich in polyphenols (*ca.* 30% dry weight) and this changes with processing even during the manufacture of commercial green tea, and progressively through semi-fermented teas to black teas and those with a microbial processing stage. Flavan-3-ols are the dominant polyphenols of fresh leaf. Usually (−)-epigallocatechin-3-*O*-gallate (**27**) dominates, occasionally taking second place to (−)-epicatechin-3-*O*-gallate (**26**), together with smaller but still substantial amounts of (+)-catechin (**22**), (−)-epicatechin (**23**), (+)-gallocatechin (**24**), (−)-epigallocatechin (**25**) and (−)-epiafzelchin (**33**). The minor flavan-3-ols also occur as gallates, and (−)-epigallocatechin (**25**) may occur as a digallate, esterified with *p*-coumaric acid or caffeic acid, and with various levels of methylation.[31] There are at least 15 flavonol glycosides, comprising mono-, di- and tri-glycosides based upon kaempferol (**14**), quercetin (**15**) and myricetin (**17**), and various permutations of glucose, galactose, rhamnose, arabinose and rutinose.[32–34] Three *C*-glycosides of apigenin (**18**),[35] several caffeoyl- and *p*-coumaroylquinic acids (chlorogenic acids) and galloylquinic acids and at least 27 proanthocyanidins, including some with (−)-epiafzelchin units, also occur.[36] In addition, some forms have a significant content of hydrolysable tannins, such as strictinin (**81**),[37] perhaps indicating an affinity with *C. japonica*, *C. sasanqua* and *C. oleifera*,[38] whereas others contain chalcan–flavan dimers known as assamaicins (**82**).[39]

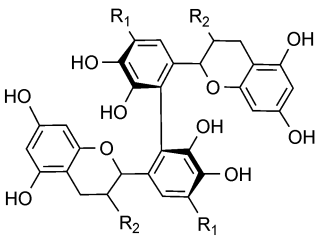
In green teas, especially those of Japanese production, most of these various polyphenols survive and can be found in the marketed product. In Chinese green teas and the semi-fermented teas such as oolong, some transformations occur, for example leading to the production of theasinensins (**83**) (flavan-3-ol dimers linked 2→2′), oolong homo-bis-flavans linked either 8→8′ (**84**) or 8→6′, oolongtheanin (**85**) and 8*C*-ascorbyl-epigallocatechin-3-*O*-



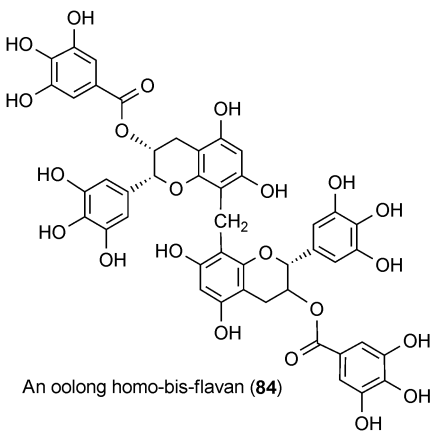
Strictinin (**81**)



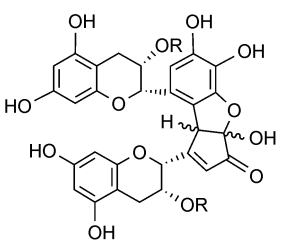
Assamaicin A (**82**)



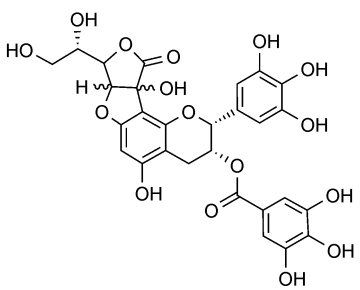
Theasinensins (**83**)
(R_1 = H or OH,
R_2 = H or gallate)



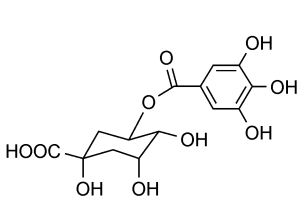
An oolong homo-bis-flavan (**84**)



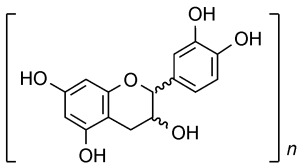
Oolongtheanin (**85**)
(R = H or gallate)



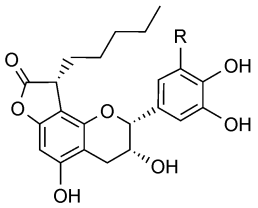
8*C*-Ascorbyl-epicatechin-3-*O*-gallate (**86**)



5-*O*-Galloylquinic acid (**87**)



Thearubigins (**88**)



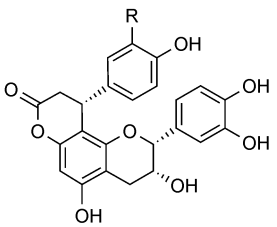
Puerin A (**89**) R = H
Puerin B (**90**) R = OH

gallate (**86**).[40] In black tea production the transformations are much more extensive, with some 90% destruction of the flavan-3-ols in orthodox processing and even greater transformation in cut–tear–curl processing. Some losses of 5-*O*-galloylquinic acid (theogallin) (**87**), quercetin glycosides and especially myricetin glycosides have been noted, and recent studies on thearubigins (**88**) suggest that theasinensins (**83**) and possibly proanthocyanidins may also be transformed. Pu'er tea is produced by a microbial fermentation of black tea. Some novel compounds have been isolated and it is suggested that they form during the fermentation.[41] These include two new 8-*C*-substituted flavan-3-ols, puerins A and B (**89**, **90**), two known cinchonain-type phenols, epicatechin-[7,8-*bc*]-4-(4-hydroxyphenyl)-dihydro-2(3*H*)-pyranone (**91**) and cinchonain Ib (**92**), and 2,2′,6,6′-tetrahydroxydiphenyl (**93**). However, various cinchonains have previously been reported in unfermented plant material.[42]

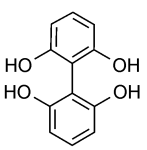
It is generally considered that polyphenol oxidase, which has at least three isoforms, is the key enzyme in the fermentation processes that produce black teas, but there is also evidence for an important contribution from peroxidases, with the essential hydrogen peroxide being generated by polyphenol oxidase.[43] The primary substrates for polyphenol oxidase are the flavan-3-ols which are converted to quinones. These quinones react further, and may be reduced back to phenols by oxidising other phenols, such as gallic acid (**54**), flavonol glycosides and theaflavins (**94**), that are not direct substrates for polyphenol oxidase.[44]

Many of the transformation products are still uncharacterised. The best known are the various theaflavins and theaflavin gallates (**94**), characterised by their bicyclic undecane benztropolone nucleus, reddish colour and solubility in ethyl acetate. These form through the Michael addition of a B-ring trihydroxy (epi)gallocatechin quinone to a B-ring dihydroxy (epi)catechin quinone prior to carbonyl addition across the ring and subsequent decarboxylation.[45] However, it is now accepted that the theasinensins (**83**) form more rapidly and may actually be theaflavin (**94**) precursors.[46,47] Theaflavonins (**95**) and theogallinin (**96**) (2→2′-linked theasinensin analogues formed from (−)-epigallocatechin (**23**)/(−)-epigallocatechin-3-*O*-gallate (**27**) and isomyricetin-3-glucoside (**97**) or 5-*O*-galloylquinic acid (**87**), respectively) have also been found in black tea.[46]

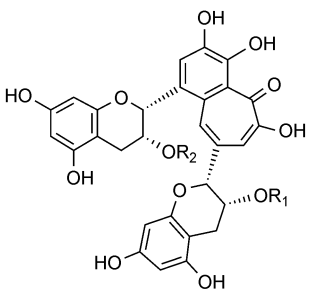
Coupled oxidation of free gallic acid or ester gallate produces quinones that can replace (epi)gallocatechin quinone leading to



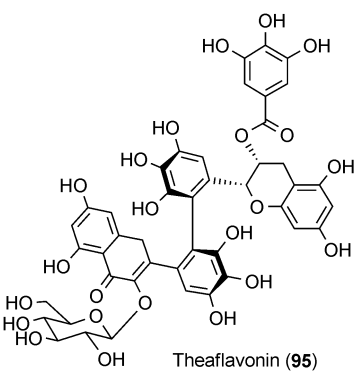
Epicatechin-[7,8-*bc*]-4-(4-hydroxyphenyl)-dihydro-2(3*H*)-pyranone (**91**) R = H
Cinchonain Ib (**92**) R = OH



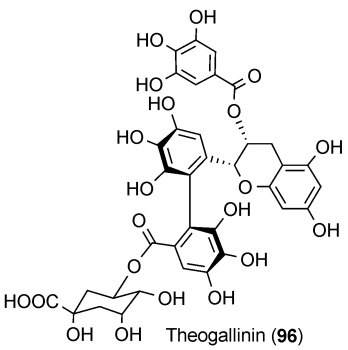
2,2′,6,6′-Tetrahydroxydiphenyl (**93**)



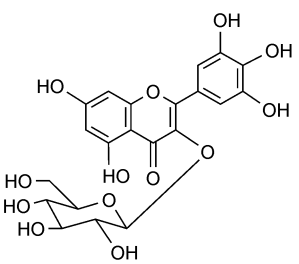
Theaflavins (**94**)
Theaflavin R_1 and R_2 = H
Theaflavin-3-gallate R_1 = H, R_2 = gallate
Theaflavin-3′-gallate R_1 = gallate, R_2 = H
Theaflavin-3,3′-digallate R_1 and R_2 = gallate



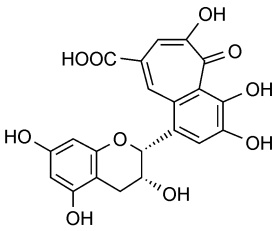
Theaflavonin (**95**)



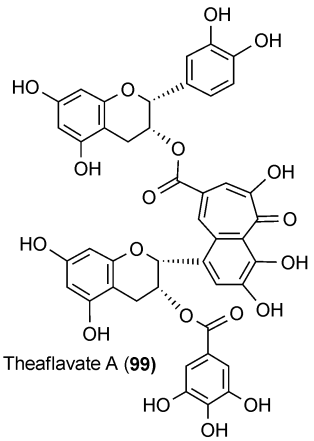
Theogallinin (**96**)



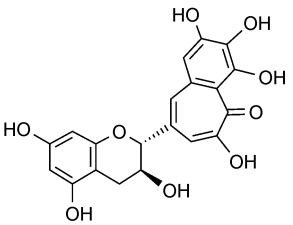
Isomyricetin-3-*O*-glucoside (**97**)



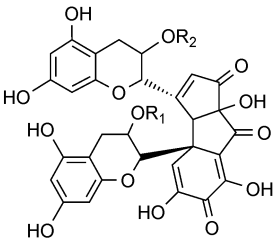
Epitheaflavic acid (**98**)



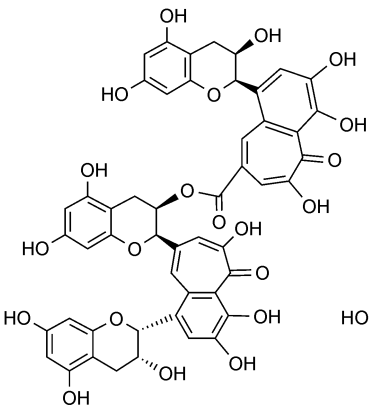
Theaflavate A (**99**)



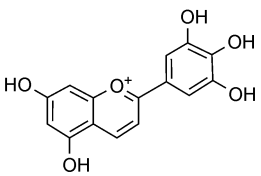
Theaflagallin (**100**)



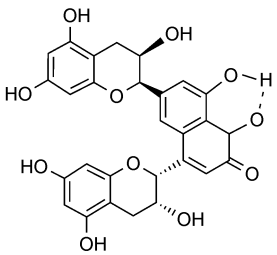
Theacitrins (**101**)
(Theacitrin R_1 and R_2 = H)
(Theacitrin-3-gallate R_1 = H, R_2 = gallate)
(Theacitrin-3'-gallate R_1 = gallate, R_2 = H)
Theacitrin-3,3'-digallate R_1 and R_2 = gallate)



Theadibenztropolone A (**102**)



Tricetanidin (**103**)

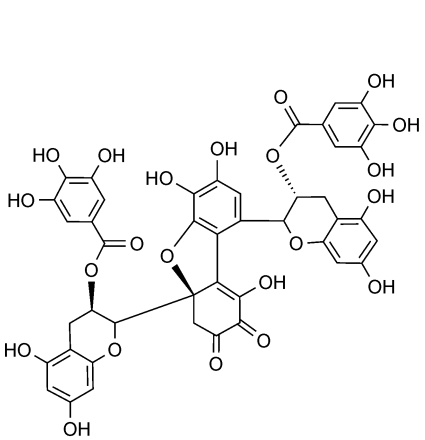


Theanaphthoquinone (**104**)

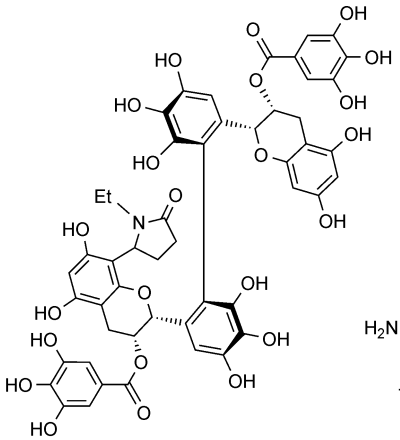
(epi)theaflavic acids (**98**) and various theaflavates (**99**).[48] Interaction between two quinones derived from trihydroxy precursors can produce benztropolone-containing theaflagallins (**100**)[49] or yellowish theacitrins (**101**) that have a tricyclic dodecane nucleus.[50] Mono- or di-gallated analogues are similarly formed from the appropriate gallated precursors, and in the case of theaflavins coupled oxidation of benztropolone gallates can lead to theadibenztropolones (**102**) (and higher homologues at least in model systems). Oxidative degallation of (−)-epigallocatechin-3-*O*-gallate (**27**) produces a pinkish-red desoxyanthocyanidin, tricetanidin (**103**).[51]

The brownish water-soluble thearubigins (**88**) are the major phenolic fraction of black tea, and these have been only partially characterised. Masses certainly extend to *ca.* 2000 daltons. Early reports that these were polymeric proanthocyanidins[52] probably arose through detection of proanthocyanidins that had passed through from the fresh leaf unchanged. The few structures that have been identified include dibenztropolones (**102**) where the 'chain extension' has involved coupled oxidation of ester gallate,[53] theanaphthoquinones (**104**) formed when a bicyclo-undecane benztropolone nucleus collapses back to a bicyclo-decane nucleus[54] and dehydrotheasinensins (**105**).[55] Production of higher-mass thearubigins could involve coupled oxidation of gallate esters yielding tribenztropolones, *etc.*, coupled oxidation of large mass precursors such as proanthocyanidin gallates or theasinensin gallates (**83**) rather than flavan-3-ol gallates,[56] or interaction of quinones with peptides and proteins. Though long anticipated, 8′-ethylpyrrolidinonyl-theasinensin A (**106**), the first such product containing an *N*-ethyl-2-pyrrolidinone moiety, was only isolated from black tea in 2005.[57] It is probably formed from a theasinensin (**83**) and the quinone-driven Strecker aldehyde produced by decarboxylation of theanine (**107**). Much remains to be done in this area, and it is interesting to note that for consumers of black tea, consumption of these uncharacterised derived polyphenols at *ca.* 100 mg per cup greatly exceeds their consumption of chemically-defined polyphenols such as flavonoids.[58]

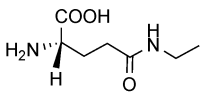
Green and semi-fermented teas retain substantial amounts of the flavan-3-ols, but they decline progressively with increased fermentation and are lowest in cut–tear–curl black teas. Beverages from green, semi-fermented and black teas also have significant contents of flavonol glycosides and smaller amounts of chlorogenic acids, flavone-*C*-glycosides (including luteolin-8-*C*-glucoside (**108**)) and 5-*O*-galloylquinic acid (**87**), which are less affected by processing but may vary more markedly with the origin of the fresh leaf.[32,34,59] The black tea beverage uniquely contains theaflavins (**94**) and to a greater extent the high molecular weight thearubigins (**88**), which are responsible for the astringent taste of black tea and the characteristic red-brown colour. Thearubigins are difficult to analyse, since they either do not elute from or are not resolved on reverse-phase HPLC columns. Indirect estimates indicate that they comprise around 80% of the phenolic components in black tea infusions.[60] Details of how some of the phenolic



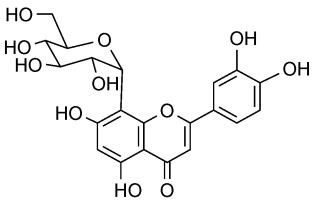
Dehydrotheasinensin AQ (**105**)



8′-Ethylpyrrolidinonyl-theasinensin A (**106**)



Theanine (**107**)



Luteolin-8-*C*-glucoside (**108**)

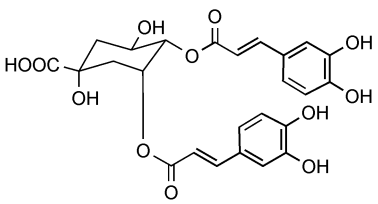
compounds in green tea are modified by fermentation to produce black tea are presented in Table 2.[61] Further changes may occur during the domestic brewing process and production of instant tea beverages. The flavan-3-ols may epimerise, producing for example (−)-catechin (**28**) and (+)-epicatechin (**29**).[62]

3.1.2 Coffee. In economic terms, coffee is the most valuable agricultural product exported by third-world and developing countries, amounting to *ca.* six million metric tonnes per annum.[27] The green coffee bean is the processed, generally non-viable, seed of the coffee cherry. Commercial production exploits

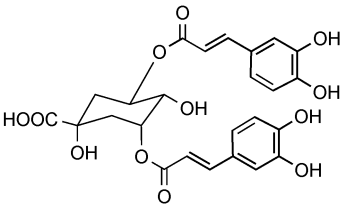
Table 2 Concentration of the major phenolics in infusions of green and black tea manufactured from the same batch of *Camellia sinensis* leaves.[61,a]

Compound	Green tea	Black tea	Black tea content as a percentage of green tea content
Gallic acid (**54**)	6.0 ± 0.1	125 ± 7.5	2083
5-*O*-Galloylquinic acid (**87**)	122 ± 1.4	148 ± 0.8	121
Total gallic acid derivatives	**128**	**273**	**213**
(+)-Gallocatechin B (**24**)	383 ± 3.1	n.d.	0
(−)-Epigallocatechin (**25**)	1565 ± 18	33 ± 0.8	2.1
(+)-Catechin (**22**)	270 ± 9.5	12 ± 0.1	4.4
(−)-Epicatechin (**23**)	738 ± 17	11 ± 0.2	1.5
(−)-Epigallocatechin-3-*O*-gallate (**27**)	1255 ± 63	19 ± 0.0	1.5
(−)-Epicatechin-3-*O*-gallate (**26**)	361 ± 12	26 ± 0.1	7.2
Total flavan-3-ols	**4572**	**101**	**2.2**
3-*O*-Caffeoylquinic acid (**62**)	60 ± 0.2	10 ± 0.2	17
5-*O*-Caffeoylquinic acid (**64**)	231 ± 1.0	62 ± 0.2	27
4-*O*-*p*-Coumaroylquinic acid (**149**)	160 ± 3.4	143 ± 0.2	89
Total hydroxycinammate quinic esters	**451**	**215**	**48**
Quercetin-*O*-rhamnosylgalactoside	15 ± 0.6	12 ± 0.2	80
Quercetin-3-*O*-rutinoside (**153**)	131 ± 1.9	98 ± 1.4	75
Quercetin-3-*O*-galactoside (**150**)	119 ± 0.9	75 ± 1.1	63
Quercetin-*O*-rhamnose-hexose-rhamnose	30 ± 0.4	25 ± 0.1	83
Quercetin-3-*O*-glucoside (**117**)	185 ± 1.6	119 ± 0.1	64
Kaempferol-rhamnose-hexose-rhamnose	32 ± 0.2	30 ± 0.3	94
Kaempferol-galactoside	42 ± 0.6	29 ± 0.1	69
Kaempferol-rutinoside	69 ± 1.4	60 ± 0.4	87
Kaempferol-*O*-glucoside	102 ± 0.4	69 ± 0.9	68
Kaempferol-arabinoside	4.4 ± 0.3	n.d.	0
Unknown quercetin conjugate	4 ± 0.1	4.3 ± 0.5	108
Unknown quercetin conjugate	33 ± 0.1	24 ± 0.9	73
Unknown kaempferol conjugate	9.5 ± 0.2	n.d.	0
Unknown kaempferol conjugate	1.9 ± 0.0	1.4 ± 0.0	74
Total flavonols	**778**	**570**	**73**
Theaflavin (**94**)	n.d.	64 ± 0.2	∞
Theaflavin-3-gallate (**94**)	n.d.	63 ± 0.6	∞
Theaflavin-3′-gallate (**94**)	n.d.	35 ± 0.8	∞
Theaflavin-3,3′-digallate (**94**)	n.d.	62 ± 0.1	∞
Total theaflavins	**n.d.**	**224**	∞

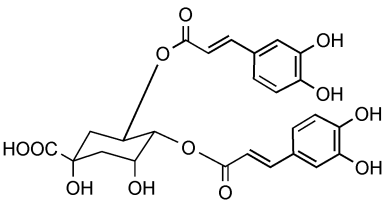
[a] Data expressed as mg/L ± standard error ($n = 3$). n.d. – not detected. Green and black teas prepared by infusing 3 g of leaves with 300 mL of boiling water for 3 min.



3,4-*O*-Dicaffeoylquinic acid (**109**)



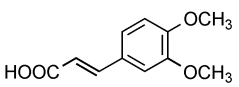
3,5-*O*-Dicaffeoylquinic acid (**110**)



4,5-*O*-Dicaffeoylquinic acid (**111**)

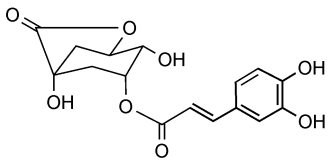
the seeds of *Coffea arabica* (so-called arabica coffees) accounting for *ca.* 70% of the world market and *C. canephora* (so-called robusta coffees) accounting for *ca.* 30%. Although the method of processing the coffee cherry and the extracted beans has subtle effects on the sensory properties of the beverage obtained, the effects on the delivery of polyphenols are comparatively slight,[27,63] and will not be described here.

Green coffee beans are one of the richest dietary sources of chlorogenic acids comprising 6–10% on a dry-weight basis. 5-*O*-Caffeoylquinic acid (**62**) is by far the dominant chlorogenic acid, accounting for some 50% of the total. This is accompanied by significant amounts of 3-*O*- and 4-*O*-caffeoylquinic acid (**63**, **64**), the three analogous feruloylquinic acids and 3,4-*O*-, 3,5-*O*- and 4,5-*O*-dicaffeoylquinic acids (**109–111**).[64] Recently, many minor mono-acyl and diacyl chlorogenic acids involving also *p*-coumaric acid (**58**) and 3,4-dimethoxycinnamic acid (**112**) have been characterised in green coffee beans[65] along with a series of amino acid conjugates.[66] Robustas, with the possible exception of those from Angola, have a significantly greater content of chlorogenic acids than arabicas.[67]

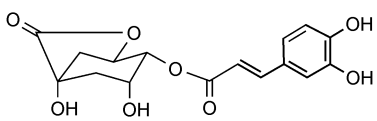


3,4-Dimethoxycinnamic acid (**112**)

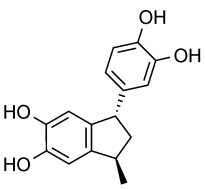
The commercial beans are roasted at air temperatures as high as 230 °C for a few minutes, or at 180 °C for up to *ca.* 20 min. During roasting there is a progressive destruction and transformation of chlorogenic acids with some 8–10% being lost for every 1% loss of dry matter, but substantial amounts survive to be extracted into domestic brews and commercial soluble coffee powders. For many consumers coffee beverage must be the major dietary source of chlorogenic acids.[63] Regular coffee drinkers will almost certainly have a greater intake of chlorogenic acids than flavonoids.[58] While a portion of the green bean chlorogenic acids is completely destroyed, some is transformed during roasting. Early in roasting when there is still adequate water content, isomerisation (acyl migration) occurs accompanied by some hydrolysis, releasing the cinnamic acids and quinic acid. Later in roasting the free quinic acid epimerises and lactonises, and several chlorogenic lactones including 3-*O*- and 4-*O*-caffeoyl-1,5-quinide (**113**, **114**)† also form.[68] The cinnamic acids may be decarboxylated and transformed to a number of simple phenols and a range of phenylindans, probably *via* decarboxylation and cyclisation of the vinylcatechol intermediate.[69] Two of these rather unstable compounds, 1,3-*trans*- and 1,3-*cis*-tetrahydroxyphenylindan (**115**, **116**), have been found in roasted and instant coffee at 10–15 mg/kg.



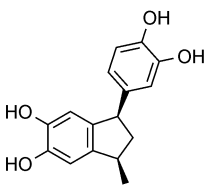
3-*O*-Caffeoyl-1,5-quinide (**113**)



4-*O*-Caffeoyl-1,5-quinide (**114**)



1,3-*trans*-Tetrahydroxyphenylindan (**115**)



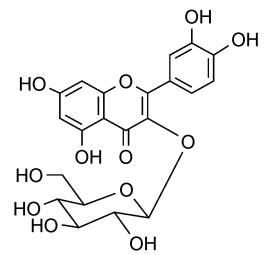
1,3-*cis*-Tetrahydroxyphenylindan (**116**)

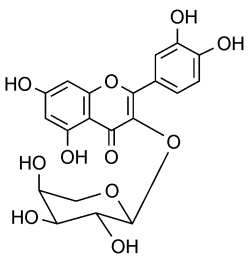
3.1.3 Cocoa. Cocoa (*Theobroma cacao*) is a tree which originated in the tropical regions of South America. There are two forms sufficiently distinct as to be considered subspecies. Criollo developed north of the Panama isthmus and Forastero in the Amazon basin, the latter accounting for 90% of world production. Extraction of the seeds, fermentation and conversion to chocolate and cocoa are described elsewhere.[27,70] These processes will cause some transformation of the native polyphenols, but although there is a role for polyphenol oxidase and a roasting at *ca.* 150 °C, these transformations are even less well characterised than those of tea or coffee processing. Cocoa and chocolate as consumed have been characterised only with regard to the surviving untransformed flavan-3-ols and proanthocyanidins.

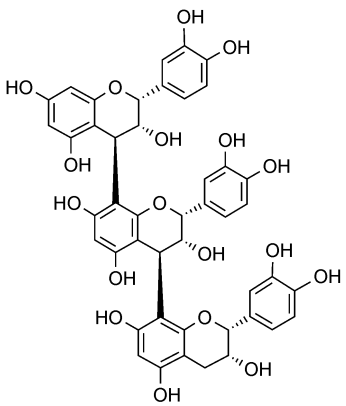
The major polyphenols in fresh beans are (+)-catechin (**22**), (−)-epicatechin (**23**) and oligomeric procyanidins ranging from dimers to decamers. Trace quantities of quercetin-3-*O*-glucoside (**117**) and quercetin-3-*O*-arabinoside (**118**) also occur.[71] Individual procyanidins that have been identified include the B5 and B2 dimers (**30**, **31**) and the trimer C1 (**119**).[72] *N*-Caffeoyl-3-*O*-hydroxytyrosine (clovamide) (**120**) and *N*-*p*-coumaroyl-tyrosine

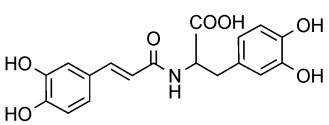
† To avoid confusion, non-IUPAC numbering is used for the quinide moiety in **113** and **114**.

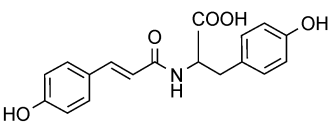
(deoxyclovamide) (**121**) are also present.[73] These compounds along with the proanthocyanidins contribute to the astringent taste of unfermented cocoa beans and roasted cocoa nibs, but not to the same degree as other amides, in particular cinnamoyl-L-aspartic acid (**122**) and caffeoyl-L-glutamic acid (**123**).[74] During fermentation and processing, the conversion of many of the phenolic components to insoluble brown polymeric compounds takes place and the level of soluble polyphenols can fall by *ca.* 90%. As a consequence, there are large variations in the flavan-3-ol monomer and procyanidin content of commercial cocoas, and many brands of milk chocolate are largely depleted of flavan-3-ols.[75] As a further complication it has recently been reported that chocolate has a significant content of (−)-catechin (**28**), which is absorbed less readily than its (+)-isomer (**22**).[76]

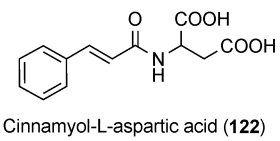

Quercetin-3-*O*-glucoside (**117**)

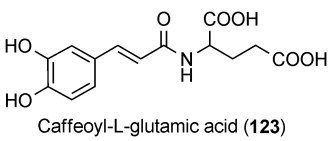

Quercetin-3-*O*-arabinoside (**118**)


Proanthocyanidin C_1 trimer (**119**)


N-Caffeoyl-3-*O*-hydroxytyrosine (**120**)


N-*p*-Coumaroyl-tyrosine (**121**)


Cinnamoyl-L-aspartic acid (**122**)


Caffeoyl-L-glutamic acid (**123**)

3.1.4 Wines. Wine is basically the fermented juice of *Vitis vinifera* grapes with a minimum alcohol level of 8.5% by volume. The wild grapevine originated in the Far East (Mesopotamia) and Egypt, and evidence for wine production dates from Neolithic times. Today, wines are produced from numerous varieties of grapes, including Cabernet Sauvignon, Merlot, Pinot Noir, Syrah, Cinsault, Rondinella, Sangiovese, Nebiolo, Grenache, Tempranillo, Tannat and Carignan. The main commercial producers are located in France, Italy, Australia, New Zealand, Spain, Chile, Argentina, California and South Africa, as well as Bulgaria, Romania, southern Brazil, and (more recently) China and India.

A wide variety of processes are used in the making of red wine. Typically, however, black grapes are pressed and the juice ('must'), together with the crushed grapes, undergo alcoholic fermentation for 5–10 days at *ca.* 25–28 °C. The solids are removed and the young wine subjected to a secondary or malo-lactic fermentation during which malic acid is converted to lactic acid and carbon dioxide. This softens the acidity of the wine and adds to its complexity and stability. The red wine is then matured in stainless steel vats or (in the case of higher quality vintages) in oak barrels for varying periods, before being filtered and bottled.

White wines are produced from both black and, more traditionally, white varieties of grapes. The berries are crushed gently rather than pressed to prevent breaking of stems and seeds. Solid material is removed and the clarified juice fermented typically between 16 °C and 20 °C for 5 days. The resultant must then undergoes malo-lactic fermentation, before maturation, filtration and bottling.

Wines are produced from an assortment of grape cultivars grown under climatic conditions that can vary substantially not only in different geographical regions but also locally on a year-to-year basis. To complicate matters further, grapes at different stages of maturity are used, and vinification and ageing procedures are far from uniform. It is hardly surprising, therefore, that wines are extremely heterogeneous in terms of their colour, flavour, appearance, taste and chemical composition.[72,77] In general, however, red wines, and to a much lesser extent white wines, are an extremely rich source of a variety of phenolic and polyphenolic compounds.

In the making of red wine, with prolonged extraction, the fermented must can contain up to 40–60% of the phenolics originally present in the grapes. Subtle changes in these grape-derived phenolic components occur during the ageing of the wines, especially when carried out in oak barrels or, as in recent years, during exposure to chips of oak wood. Consequently, there is a wide range in the level of phenolics between different red wines, the concentration of flavonols, for instance, varying by more than 10-fold and the overall level of phenolics by almost 5-fold (Table 3).[78] Information on variations in the levels of a number of phenolic compounds in comprehensive range of French red wines have been published.[79,80]

The phenolics in red wines are the hydroxycinnamate–tartaric acid conjugates, coutaric acid (**124**), caftaric acid (**125**) and fertaric acid (**126**), malvidin-3-*O*-glucoside (**41**) and other anthocyanins with lower levels of gallic acid (**54**), stilbenes and flavonols. From the data presented in Table 3 it is evident that the levels of the flavan-3-ol monomers (+)-catechin (**22**) and (−)-epicatechin (**23**) are not high and that there is a large discrepancy between the levels of phenolics measured by HPLC and the total phenolics determined by the Folin-Ciocalteau assay. Among the 'missing ingredients' that were not measured

Table 3 Range of concentrations of phenolic compounds in 15 red wines of different geographical origin.[77,a]

Phenolic	Range (mg/L)
Total flavonols	5–55
trans-Resveratrol (**71**) and *trans*-resveratrol-3-*O*-glucoside (**72**)	1–18
Gallic acid (**54**)	8–71
Total hydroxycinnmates	66–124
(+)-Catechin (**22**) and (−)-epicatechin (**23**)	8–60
Free and polymeric anthocyanins	41–150
Total phenols	824–4059

[a] Total phenols measured by colorimetric Folin-Ciocalteau assay; other estimates based on HPLC analyses that did not detect proanthocyanidins.

Table 4 Average concentration of selected phenolic compounds in 34 red, 11 dry white and 7 sweet white French wines.[79,a]

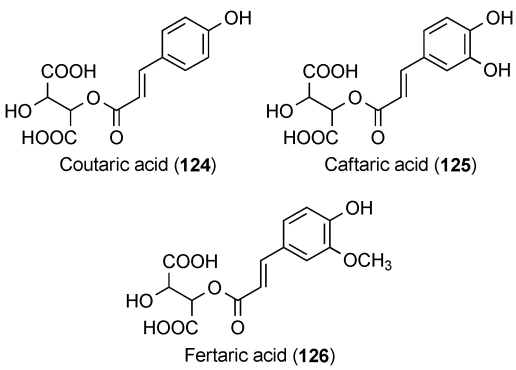
	Red wine	Dry white wine	Sweet white wine
Flavan-3-ols			
(+)-Catechin (**22**)	41 ± 6	15 ± 8	4.2 ± 0.7
(−)-Epicatechin (**23**)	29 ± 3	12 ± 9	1.4 ± 0.3
Procyanidin B_1 (**127**)	15 ± 2	5.1 ± 2.3	3.4 ± 0.4
Procyanidin B_2 (**31**)	27 ± 5	8.9 ± 4.9	3.0 ± 0.5
Procyanidin B_3 (**128**)	59 ± 7	13 ± 5	10 ± 2
Procyanidin B_4 (**30**)	5.2 ± 1.0	4.0 ± 2.5	2.0 ± 1.1
Total flavan-3-ols	177 ± 22	59 ± 31	24 ± 1
Gallic acid (**54**)	30 ± 2	4.0 ± 2.1	5.8 ± 1.1
Caffeic acid (**59**)	11 ± 1	3.4 ± 0.5	1.6 ± 0.3
Caftaric acid (**125**)	51 ± 4	33 ± 6	14 ± 3
Anthocyanins	22 ± 19	n.d.	n.d.

[a] Data expressed as in mg/L as mean values ± standard error. n.d. – not detected.

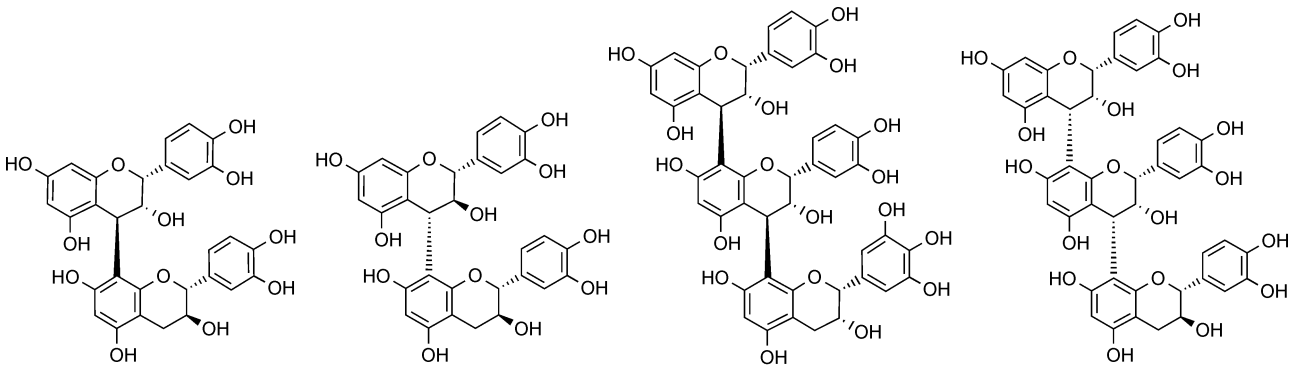
by HPLC are proanthocyanidin B_{1-4} dimers (**127**, **31**, **128**, **30**), the C_1 and C_2 trimers (**129**, **130**)[81] and oligomeric and polymeric forms with respective mean degrees of polymerisation of 4.8 and 22.1.[82] The equivalent mean degrees of polymerisation of proanthocyanidins in grapes were 9.8 and 31.5, indicating that substantial changes in flavan-3-ol composition occur during fermentation and aging of the wines. Among the processes involved is the formation of compounds corresponding to malvidin-3-*O*-glucoside (**41**) linked through a vinyl bond to either (+)-catechin, (−)-epicatechin or the procyanidin dimer B_3 (**131–133**).[83] Similar blue-coloured compounds with the flavan-3-ols linked to malvidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside (**44**) have also been detected in red wines (**134–136**).[84] The production of pyruvate and acetaldehyde by yeast during fermentation of Tempranillo grapes has been associated with the formation of malvidin-3-*O*-glucoside-pyruvic acid (vitisin A) (**137**) and malvidin-3-*O*-glucoside-4-vinyl (vitisin B) (**138**).[85]

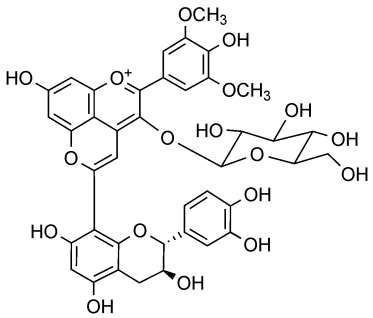
The production of white wine results in either low levels or an absence of skin- and seed-derived phenolics, so the overall level of phenolics can be much lower than that found in many red wines.[86] This observation is reflected in a more detailed comparison of the constituents of French red wines, dry white wines and sweet white wines summarised in Table 4.[80]

3.1.5 Beer. Beer is an alcoholic beverage made from malted grains, usually barley (*Hordeum vulgare*) or wheat (*Triticum vulgare*), hops (*Humulus lupulus*), yeast (*Saccharomyces* spp.) and water. It contains a range of phenolic and polyphenolic compounds, derived partly from the barley (70%) and partly from the hops (30%). Flavan-3-ols are found in both hops and malt. These include monomers such as (+)-catechin (**22**) and (−)-epicatechin (**23**), and the dimers procyanidin B_3 (**128**) and prodelphinidin B_3 (**139**). Trimers also occur, although a more recent study, albeit with one unnamed American beer, reported an absence of high molecular weight polymeric proanthocyanidins and an average degree of polymerisation of only 2.1.[87] The malt contributes most of the simple phenolics such as 3,4-dihydroxybenzoic acid (protocatechuic acid) (**140**), caffeic acid (**59**) and ferulic acid (**60**), with small amounts of these compounds also being found in hops.



Coutaric acid (**124**) Caftaric acid (**125**) Fertaric acid (**126**)


Procyanidin B_1 dimer (**127**) Procyanidin B_3 dimer (**128**) Procyanidin C_1 trimer (**129**) Procyanidin C_2 trimer (**130**)



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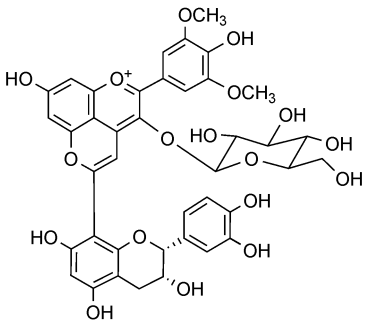

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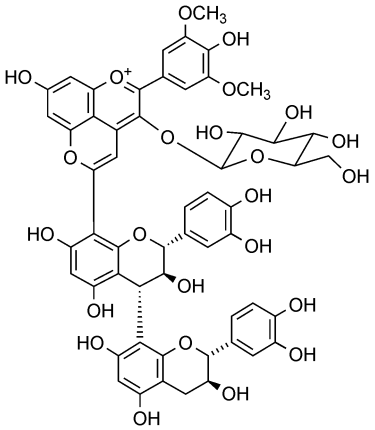

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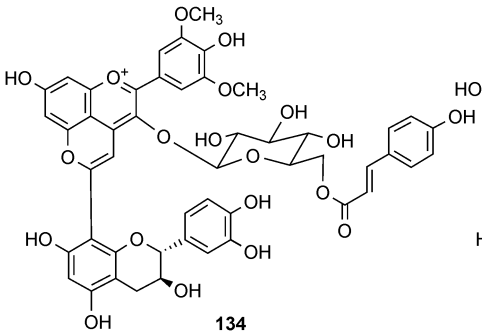

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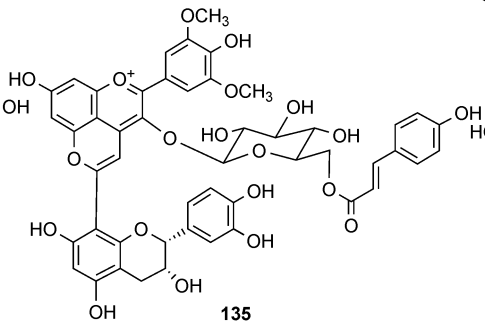

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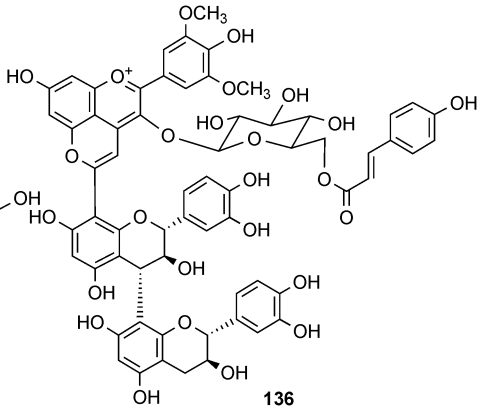

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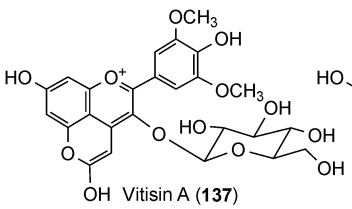
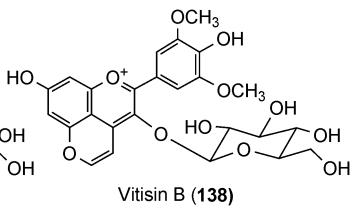
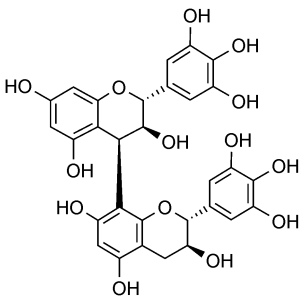


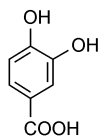
Vitisin A (**137**)



Vitisin B (**138**)

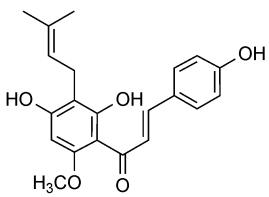


Prodelphindin B_3 dimer (**139**)

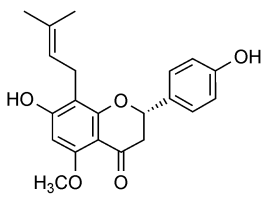


3,4-Dihydroxybenzoic acid (**140**)

Hops contain quercetin conjugates and the prenylflavonoid xanthohumol (**141**), which during the brewing process undergoes substantial conversion to the flavanone isoxanthohumol (**142**), which predominates in most beers. Other prenylflavonoids found in beers include desmethylxanthohumol (**143**), 6- and 8-prenylnaringenin (**144**, **145**) and 6-geranylnaringenin (**146**).[88] The quantity of phenolics in beer has not been widely studied, but in general low and sub-mg quantities per litre are present. De Pascual-Teresa *et al.*[89] determined the flavan-3-ol content of a red wine and a beer and found 17.8 and 7.3 mg/L, respectively. Considering serving size, the potential flavan-3-ol intake from these two sources is similar.

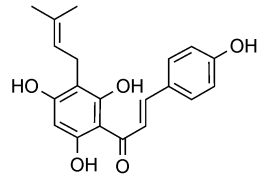


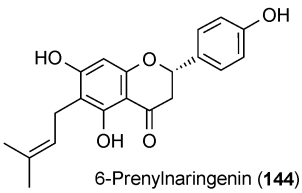
Xanthohumol (**141**)

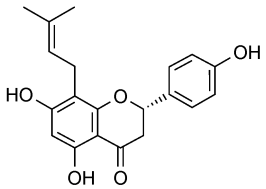


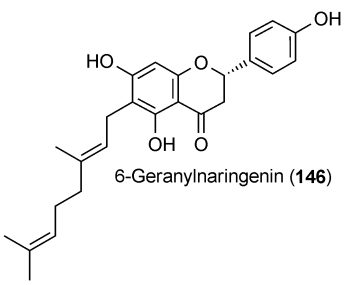
Isoxanthohumol (**142**)

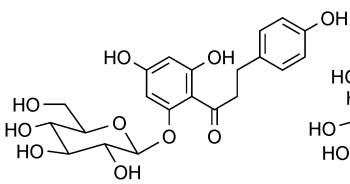
3.1.6 Cider. Cider is an alcoholic beverage produced by yeast fermentation of apples (*Malus domestica*), typically specific cider varieties rather than dessert apples, the varieties often but not always blended. A survey of English ciders found that the overall level of phenolics ranged from 44 to 1559 mg/L. The principal components were 5-*O*-caffeoylquinic acid (**62**), and procyanidins. Also present were (+)-catechin (**22**), (−)-epicatechin (**23**), the dihydrochalcones phloretin-2′-*O*-glucoside (phloridzin) (**147**) and phloretin-2′-*O*-(2″-*O*-xylosyl)-glucoside (**148**), hydroxycinnamates and trace amounts of quercetin glycosides.[90,91]

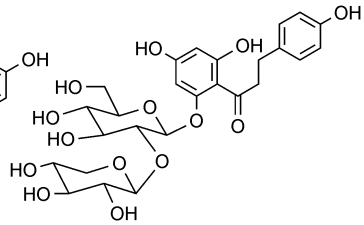

Desmethylxanthohumol (**143**)


6-Prenylnaringenin (**144**)


8-Prenylnaringenin (**145**)


6-Geranylnaringenin (**146**)


Phloretin-2'-*O*-glucoside (**147**)

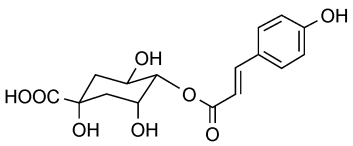

Phloretin-2'-*O*-(2''-*O*-xylosyl)glucoside (**148**)

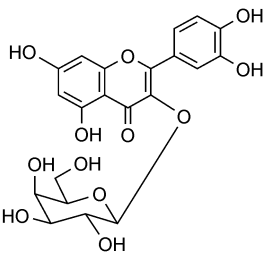
Chlorogenic acids and flavan-3-ols are the two classes that are important in the cider industry due to their physiochemical properties. 5-*O*-Caffeoylquinic acid (**64**) is a key substrate for endogenous polyphenol oxidases, with further reactions from the products formed giving cider its yellow-brown colouring.[92] Phenolics of apples are implicated in cider quality, being involved in astringent and bitter tastes. The degree of polymerisation of procyanidins is directly involved in the balance of bitterness and astringency. Bitterness is due to oligomeric procyanidins with a degree of polymerisation of 2–5, whereas procyanidins with a degree of polymerisation of 6–10 are more involved in astringency.[93]

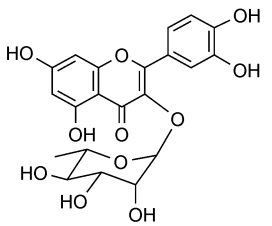
The method of production has been shown to affect the phenolic content, with modern techniques of pneumatically pressed cider fermented in stainless steel vats decreasing levels more slowly than the more traditional methods of pressing and fermenting in wooden barrels.[94] Oxidation which occurs during the juice extraction has also been linked with a reduction in the level of polymeric procyanidins.[95] Fining to clarify the cider has been shown to decrease the procyanidin content.[90]

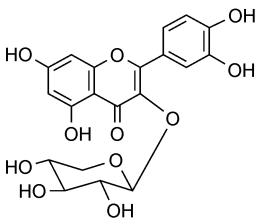
3.2 Fruits

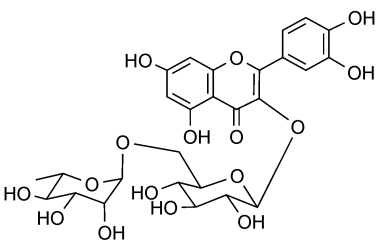
Apples (*Malus domestica*) and pears (*Pyrus communis*) are among the main sources of proanthocyanidins in the diet.[96] Apples and apple products are extensively consumed. They are a good source of flavonoids and phenolic compounds, containing 2310–4880 mg/kg.[97] The principal ingredients include 5-*O*-caffeoylquinic acid (**64**) which occurs together with small quantities of phloretin-2′-*O*-glucoside (**146**), phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside (**148**) and 4-*O*-*p*-coumaroylquinic acid (**149**). Apples are an important source of flavonols, containing quercetin-3-*O*-glucoside (**117**), quercetin-3-*O*-galactoside (**150**), quercetin-3-*O*-rhamnoside (**151**), quercetin-3-*O*-xyloside (**152**), quercetin-3-*O*-rutinoside (**153**), quercetin-3-*O*-arabinopyroside (**154**) and quercetin-3-*O*-arabinofuranoside (**155**). They also contain flavan-3-ols, including (−)-epicatechin (**23**) and its procyanidin dimers (B_1 (**127**) and B_2 (**31**)) and oligomers, these latter especially in cider apples.[98] The procyanidins have been shown to have an average degree of polymerisation of between 3.1 and 8.5.[99] An anthocyanin, cyanidin-3-*O*-galactoside (**156**), is found in the skin of red apple varieties.

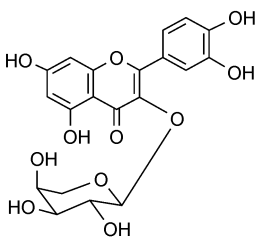

4-*O*-*p*-Coumaroylquinic acid (**149**)

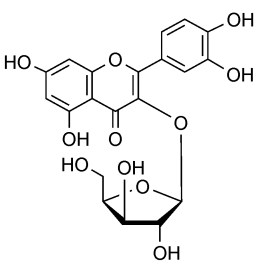

Quercetin-3-*O*-galactoside (**150**)

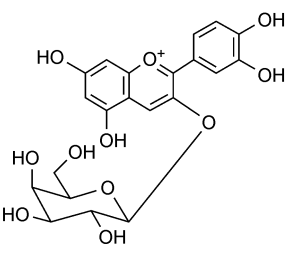

Quercetin-3-*O*-rhamnoside (**151**)


Quercetin-3-*O*-xyloside (**152**)


Quercetin-3-*O*-rutinoside (**153**)


Quercetin-3-*O*-arabinoside (**154**)

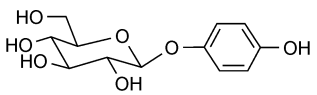

Quercetin-3-*O*-arabinofuranoside (**155**)


Cyanidin-3-*O*-galactoside (**156**)

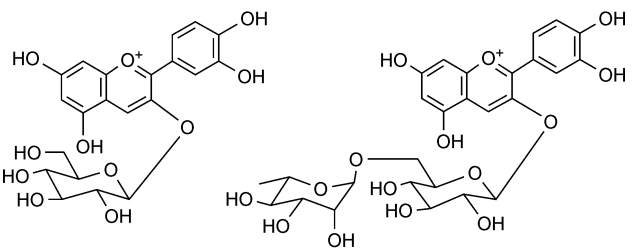
The total phenolic content of some cultivars of pears is between 1235 and 2500 mg/kg in the peel and 28–81 mg/kg in the flesh.[100] The phenolic composition of pears is very similar to that of apples, containing 5-*O*-caffeoylquinic acid (**64**), 4-*O*-*p*-

coumaroylquinic acid (**149**), procyanidins and quercetin glycosides. The main difference in the phenolic content of apples and pears is the presence of 1-hydroxyphenyl-4-*O*-glucoside (arbutin) (**157**) in pears and the dihydrochalcones in apples.[101] The average degree of polymerisation of procyanidins in some varieties of pears has been shown to be as high as 44.[102]

There is increasing usage of nectarines (*Prunus persica* var. *nectarina*) a smooth-skinned variety of peach. Peaches and nectarines contain cyanidin-3-*O*-glucoside (**158**), cyanidin-3-*O*-rutinoside (**159**), quercetin-3-*O*-glucoside (**117**) and quercetin-3-*O*-rutinoside (**153**), and other stone fruits (cherries, plums, prunes) are not greatly different. Stone fruits are characterised by a greater content of 3-*O*-caffeoylquinic acid (**62**) than 5-*O*-caffeoylquinic acid (**64**).[103] Canning and storage of nectarines causes a reduction in the contents of (+)-catechin (**22**), (−)-epicatechin (**23**) and proanthocyanidins including procyanidin B_1 (**127**).[104]



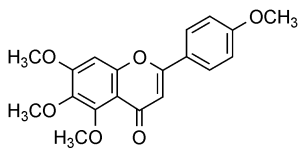
1-Hydroxyphenyl-4-*O*-glucoside (**157**)



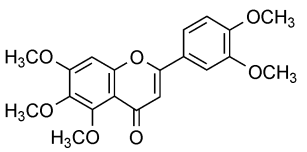
Cyanidin-3-*O*-glucoside (**158**) Cyanidin-3-*O*-rutinoside (**159**)

Citrus fruits are significant sources of flavonoids, principally flavanones, which are present in both the juice and the tissues that are ingested when fruit segments are consumed. These tissues are a particularly rich source but are only consumed as an accidental adjunct to the consumption of the pulp. It is difficult to estimate dietary intake in such cases because it is so heavily dependent on the amount of tissue surrounding the segments after peeling, and on the method of analysis.[105] Citrus peel, and to a lesser extent the segments, also contain the conjugated flavanone naringenin-7-*O*-rutinoside (**46**) as well as hesperetin-7-*O*-rutinoside (**45**), which is included in dietary supplements and is reputed to prevent capillary bleeding. Naringenin-7-*O*-neohesperidoside (**48**) from grapefruit peel and hesperetin-7-*O*-neohesperidoside (**47**) from bitter orange are intensely bitter flavanone glycosides. Orange juice contains polymethoxylated flavones such as tangeretin (**20**), nobiletin (**21**), scutellarein (**160**) and sinensetin (**161**), which are found exclusively in citrus species. The relative levels of these compounds can be used to detect the illegal adulteration of orange juice with juice of tangelo fruit (*Citrus reticulata*).

The red colour of ripe mango (*Mangifera indica*) peel is due to cyanidin-3-*O*-galactoside (**156**). The peels also contains several

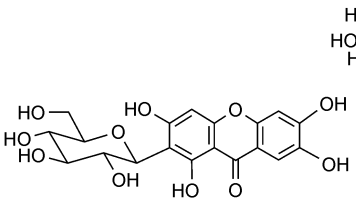


Scutellarein (**160**)

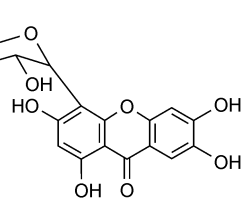


Sinensetin (**161**)

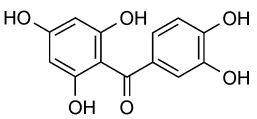
quercetin and kaempferol glycosides, the principal flavonols being quercetin-3-*O*-glucoside (**117**) and quercetin-3-*O*-galactoside (**150**), a xanthone *C*-glucoside, mangiferin (**162**), and smaller amounts of its isomer isomangiferin (**163**), an array of gallotannins, and *C*-glucosides and galloyl derivatives of the benzophenones maclurin (**164**) and iriflopheone (**165**).[106] Mango latex also contains the contact allergen 5-(12-heptadecenyl)resorcinol (**166**),[107] which may contaminate the peel but not normally the fruit itself. Mango extracts are used widely in traditional medicines for treating a number of conditions including diarrhoea, diabetes and skin infections,[108] and mangiferin is reported to inhibit bowel carcinogenesis in rats.[109]



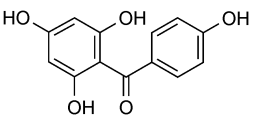
Mangiferin (**162**)



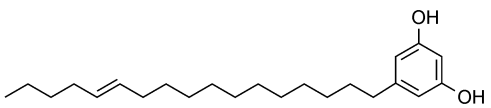
Isomangiferin (**163**)



Maclurin (**164**)

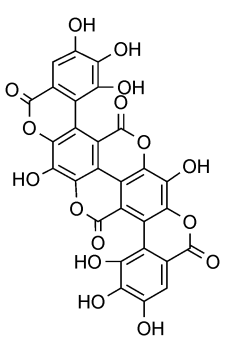


Iriflopheone (**165**)

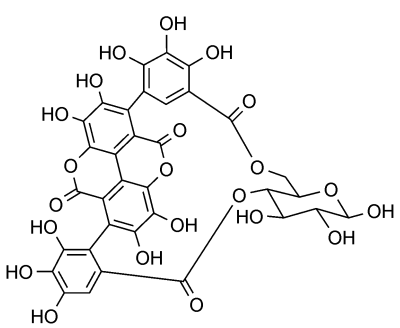


5-(12-Heptadecenyl)resorcinol (**166**)

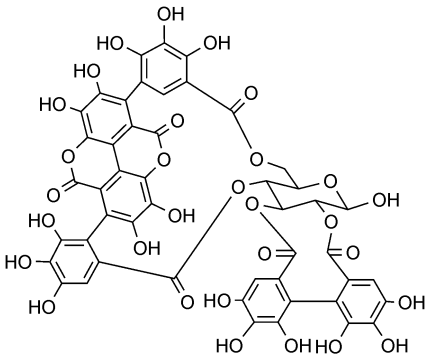
Commercial pomegranate juice is being consumed in increasing quantities. Some, but not all, of the products have a high antioxidant content attributable to gallagic acid (**167**), an analogue of ellagic acid (**56**) containing four gallic acid (**54**) residues, and punicalin (**168**), the principal monomeric ellagitannin in which gallagic acid is bound to glucose. Punicalagin (**169**) is a further ellagitannin in which ellagic acid, as well as gallagic acid, is linked to the glucose moiety. Pomegranates contain 3-*O*-glucosides and 3,5-*O*-diglucosides of cyanidin (**36**) and delphinidin (**37**) and several ellagic acid derivatives.[110] There is evidence that consumption of pomegranate juice can have a favourable impact on cardiovascular disease[111] and have protective effects against colon and prostate cancer.[112]



Gallagic acid (**167**)



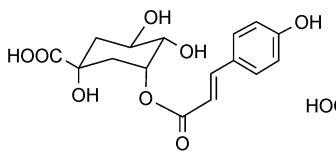
Punicalin (**168**)



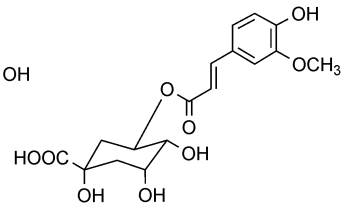
Punicalagin (**169**)

3.3 Vegetables

Carrots contain a range of chlorogenic acids including 3-*O*- and 5-*O*-caffeoylquinic acids (**62**, **64**), 3-*O*-*p*-coumaroylquinic acid (**170**), 5-*O*-feruloylquinic acid (**171**) and 3,5-*O*-dicaffeoylquinic acid (**110**). The chlorogenic acids are found in orange, purple, yellow and white carrots, the level of 5-*O*-caffeoylquinic acid in purple carrots, at 540 mg/kg, being almost 10-fold higher than the amounts present in the other varieties.[113] Chlorogenic acids are considered a carrot root fly attractant, and varieties resistant to this pest have been bred to have low caffeoylquinic acid contents.[114]



3-*O*-*p*-Coumaroylquinic acid (**170**)

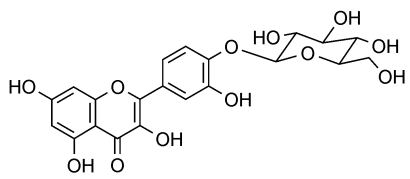


5-*O*-Feruloylquinic acid (**171**)

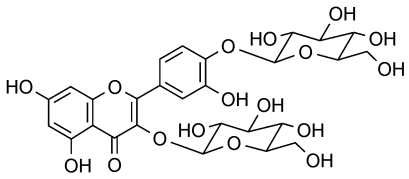
Onions provide a range of flavonols that are of comparatively restricted occurrence, principally quercetin-4′-*O*-glucoside (**172**) and quercetin-3,4′-*O*-diglucoside (**173**) with smaller amounts of isorhamnetin-4′-*O*-glucoside (**174**) and other quercetin conjugates.[115] Yellow onions form one of the main sources of flavonols in the Northern European diet, the edible flesh containing between 280 and 490 mg/kg.[7] Even higher concentrations are found in the dry outer scales.[116] By contrast, leeks have been found to contain only 10–60 mg/kg of kaempferol and no quercetin. White onions are all but devoid of flavonols. Red onions like their yellow counterparts are rich in flavonols and also contain up to 250 mg/kg of anthocyanins,[117] among the major components being cyanidin-3-*O*-(6″-malonyl)glucoside (**175**) and cyanidin-3-*O*-(6″-malonyl)laminaribioside (**176**).[118] Flavonols are of chemotaxonomic interest, and many commodities, *e.g.* broccoli[119] and spinach,[120] contain distinctive forms, but in most cases intake is low. Flavonols are generally concentrated in the leaf or peel, and cherry tomatoes, because of their high skin:volume ratio, are an especially rich source of quercetin-3-*O*-rutinoside (**153**).[121] The extent to which this is transferred to processed products is largely unknown.

Although widely consumed in fresh and processed forms, there is relatively little information on the phytochemicals present in most legumes of dietary significance. The notable exception is soybean (*Glycine max*) which contains the isoflavones diadzein-7-*O*-(6″-*O*-malonyl)glucoside (**177**) and genistein-7-*O*-(6″-*O*-malonyl)glucoside (**178**), and their aglycones (**49**, **50**).[122] The levels of isoflavones in soybeans have been reported to range from 560–3810 mg/kg, which is two orders of magnitude higher than the amounts detected in other legumes. Fermented soya products can be comparatively rich in the aglycones as hydrolysis of the glycosides can occur.[123] Products whose manufacture involves heating at 100 °C, such as soy milk and tofu, contain reduced quantities of isoflavones, the principal components being daidzein and genistein glucosides, which form as a result of degradation of the malonyl- and acetylglucosides.[124]

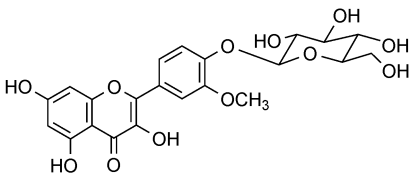
As far as other legumes are concerned, peanuts (*Arachis hypogaea*) contain 5,7-dimethoxyisoflavone (**179**), broad beans (*Vicia faba*) are a relatively rich source of flavan-3-ols (containing more than 150 mg/kg[89]), while French beans (*Phaseolus vulgaris*) can contain substantial quantities of quercetin-3-*O*-glucuronide (**180**).[125] Pinto beans and red kidney beans contain in excess of 5 g/kg of proanthocyanidins, principally as prodelphinidins and propelargonidins, most with a degree of polymerisation >4.[126]



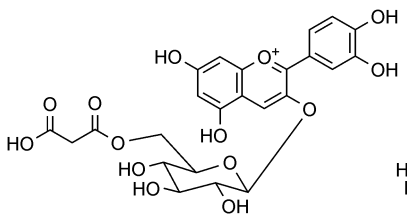
Quercetin-4'-*O*-glucoside (**172**)



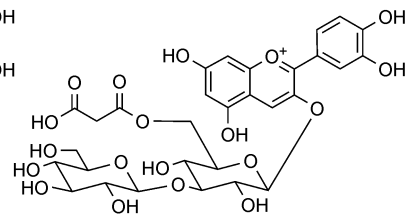
Quercetin-3,4'-*O*-diglucoside (**173**)



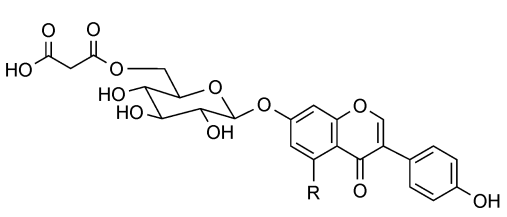
Isorhamnetin-4'-*O*-glucoside (**174**)



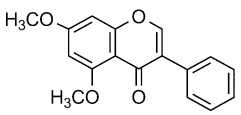
Cyanidin-3-*O*-(6''-malonyl)glucoside (**175**)



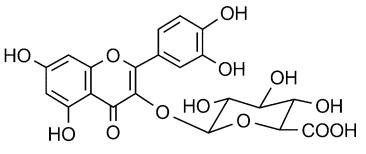
Cyanidin-3-*O*-(6''-malonyl)laminaribioside (**176**)



Daidzein-7-*O*-(6''-*O*-malonyl)glucoside (**177**) R = H
Genistein-7-*O*-(6''-*O*-malonyl)glucoside (**178**) R = OH



5,7-Dimethoxyisoflavone (**179**)



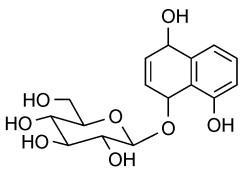
Quercetin-3-*O*-glucuronide (**180**)

3.4 Miscellaneous minor commodities

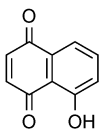
Minor commodities are those consumed in small quantities, perhaps less than a gram per day on average, for example nuts, algae, herbs and spices. Compositional data are scarce and have been reviewed in detail elsewhere.[2,127] Attention is drawn here to their provision of unusual phenols and polyphenols.

Nuts encompass a botanically diverse collection of fruits that contain an edible and usually rather hard and oily kernel within a hard or brittle outer shell. They are consumed raw or roasted as snack foods or decorative/comparatively minor ingredients in baked goods and confectionary. Proanthocyanidins are frequently present at quite high concentrations, for example hazelnuts (*Corylus avellana*) and pecans (*Carya illinoensis*) are particularly rich with *ca.* 5 g/kg, whereas almonds (*Prunus dulcis*) and pistachios (*Pistachio vera*) contain 1.8 to 2.4 mg/kg, walnuts *ca.* 0.67 g/kg, roasted peanuts *ca.* 0.16 g/kg and cashews (*Anacardium occidentale*) only *ca.* 0.09 g/kg. Fruits of black walnut (*Juglans nigra*) and buttermilk walnut (*J. cinerea*) contain 1,5-dihydroxynaphthalene-4-*O*-glucoside (**181**), from which 5-hydroxy-1,4-naphthoquinone (juglone) (**182**) is produced during ripening by hydrolysis and oxidation. This quinone is responsible for the yellow-brown staining and irritation of the hands that can occur after handling these nuts.[127]

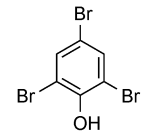
Marine algae are utilised to a limited extent for food and as a source of polysaccharides used as food additives, but are increasingly being investigated for their novel, potentially bioactive components. Red algae which are consumed as laver bread and 'nori' synthesise a substantial range of halogenated phenols. 2,4,6-Tribromophenol (**183**) predominates and the total bromophenols content ranges from 8 to 180 μg/kg. Herbs and spices are botanically heterogeneous, phytochemically complex, and extremely variable in composition geographically. Frequently, herbs and spices contain phytochemicals not found in other foodstuffs and may sometimes resemble herbal medicines. Rosmarinic acids (**184**, **185**), cinnamaldehyde (**186**), 2-hydroxycinnamaldehyde (**187**), curcuminoids (cinnamoyl-methanes or diaryl-heptenoids) (**188–190**), piperidine (**191**), capsaicin (**192**) and gingerols (**193**) are variously found.[2]



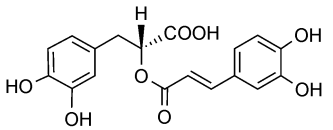
1,5-dihydroxynaphthalene-4-*O*-glucoside (**181**)



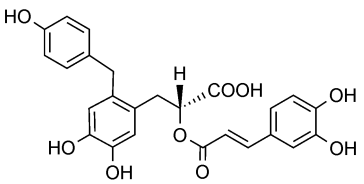
5-hydroxy-1,4-naphthoquinone (**182**)



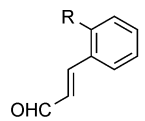
2,4,6-Tribromophenol (**183**)



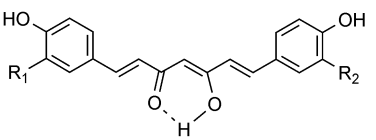
Rosmarinic acid (**184**)



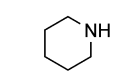
2'-(4-Hydroxybenzyl)-rosamarinic acid (**185**)



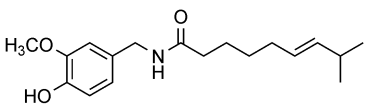
Cinnamaldehyde (**186**) R = H
2-Hydroxycinnamaldehyde (**187**) R = OH)



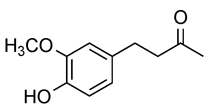
Curcumin (**188**) R_1, R_2 = OCH_3
Demethoxycurcumin (**189**) R_1 = H, R_2 = OCH_3
Bisdemethoxycurcumin (**190**) R_1, R_2 = H



Piperidine (**191**)



Capsaicin (**192**)



Zingerone (**193**)

4 Biosynthesis of phenolics and polyphenolics

The biosynthesis of flavonoids, stilbenes, hydroxycinnamates and phenolic acids involves a complex network of routes based principally on the shikimate (C_6–C_1 compounds), phenylpropanoid (C_6–C_3 compounds) and flavonoid pathways. The non-flavonoids are formed *via* the shikimic and phenylpropanoid pathways (Fig. 2). The C_6–C_3–C_6 flavonoid structure is formed from the interaction of two separate biosynthesis pathways. The bridge and the aromatic B-ring constitute a phenylpropanoid unit synthesized from *p*-coumaroyl-CoA. The six carbons of ring A originate from the condensation of three acetate units *via* the malonic acid pathway (Fig. 3). Much of the recent information on these pathways, the enzymes involved, and the encoding genes has come from molecular biology-based studies with *Arabidopsis thaliana* which are discussed in detail elsewhere.[128] This knowledge has opened up the possibility of using genetic engineering to produce fruits and vegetables containing elevated levels of key

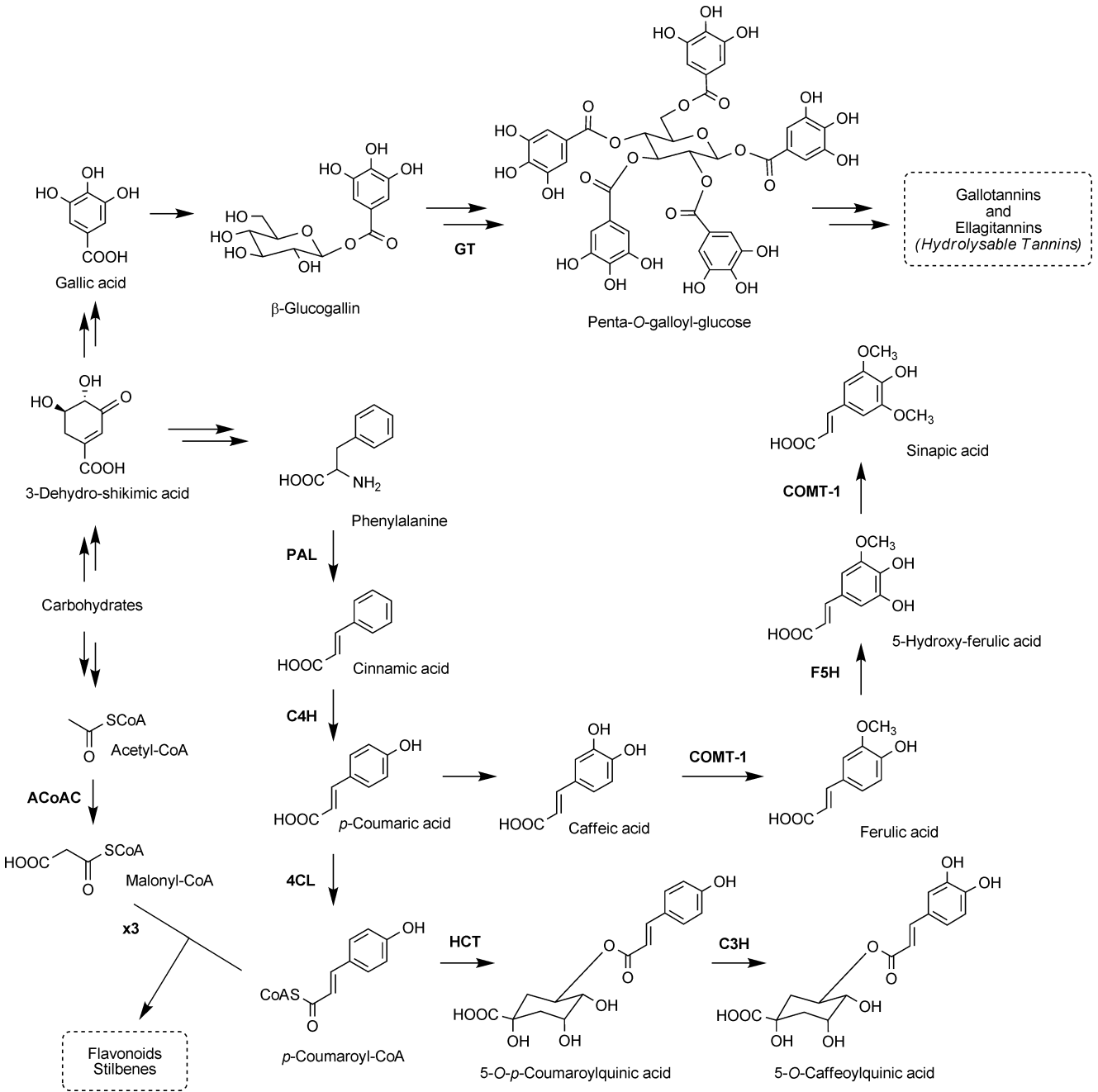


Fig. 2 Schematic diagram of the main pathways and key enzymes involved in the biosynthesis of hydrolysable tannins, hydroxycinnamates and 5-*O*-caffeoylquinic acid (**64**). Enzyme abbreviations: PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; COMT-1, caffeic/5-hydroxyferulic acid *O*-methyltransferase; 4CL, *p*-coumarate:CoA ligase; F5H, ferulate 5-hydroxylase; GT, galloyltransferase; ACoAC, acetylCoA carboxylase.

phenolic and polyphenolic compounds that may enhance protection of human health.

5 Potential for genetically-modified produce

Recognising that genetic control plays a most crucial role in the production of functional metabolites has made plants producing beneficial phytochemicals an attractive target for manipulation and augmentation using biotechnological breeding strategies. The goal is to increase the production of specific bioactive ingredients in the normal producing plant species or to transfer a pathway, or part of a pathway, to other plant species.[129] Both structural genes and transcription factors have been manipulated for this purpose. Transcriptional factors play an important role in regulating secondary metabolism, and since they are able to control multiple steps within a pathway, they are potentially more powerful than structural genes (which control only a single step) when attempting to manipulate metabolic pathways in plants.[130]

Conveniently from a nutritional perspective, the flavonoid biosynthesis pathway is the best-studied route at the genetic level, and to date most of the structural and several regulatory genes

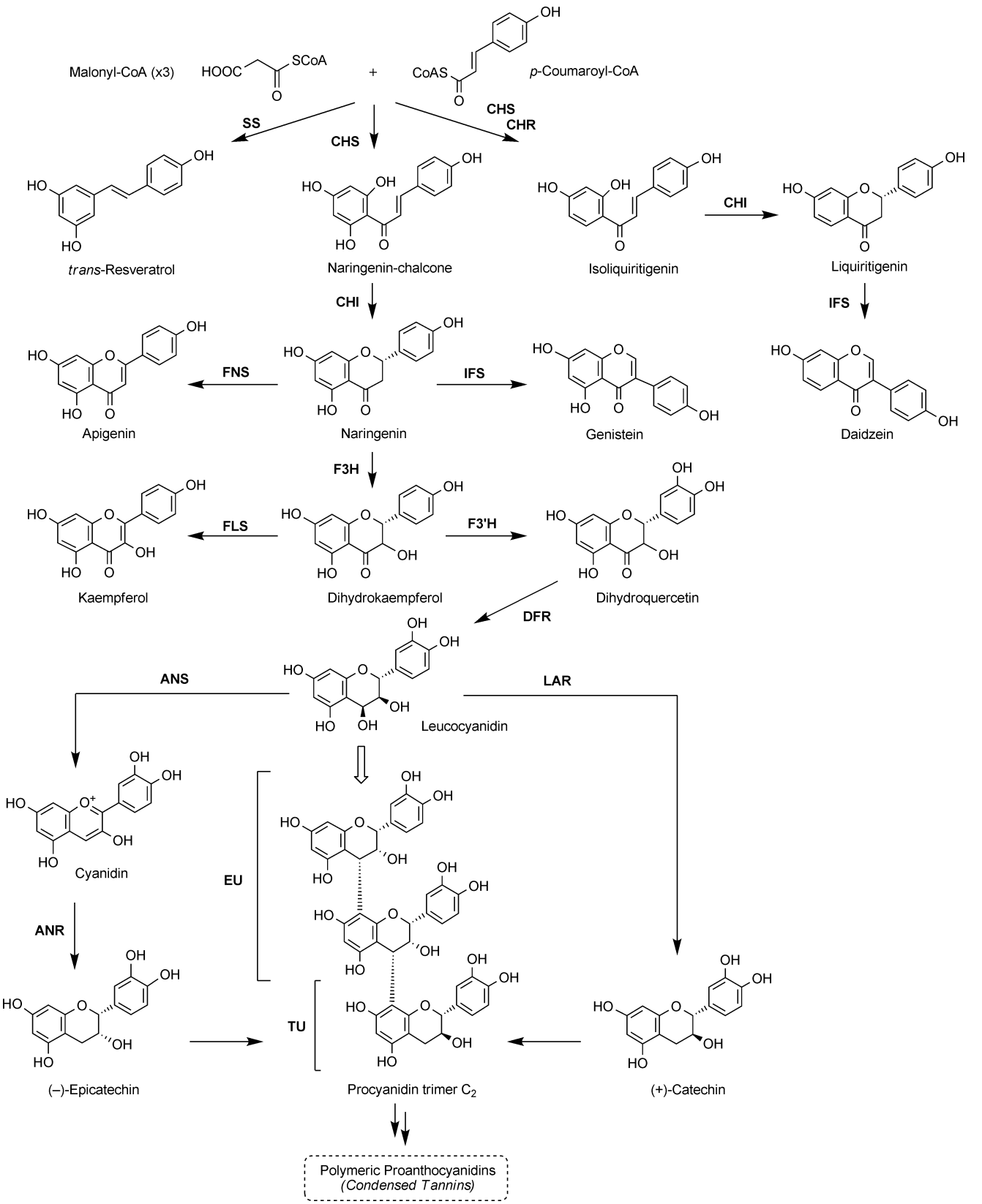
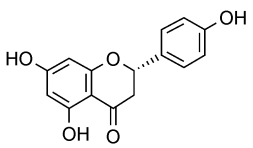
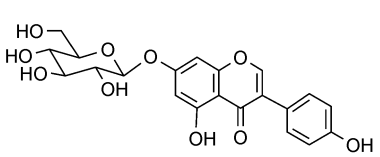


Fig. 3 Schematic diagram of the stilbene and flavonoid biosynthetic pathways. Enzyme abbreviations: SS, stilbene synthase; CHS, chalcone synthase; CHR, chalcone reductase; CHI, chalcone isomerase; IFS, isoflavone synthase; FNS, flavone synthase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; F3′H, flavonoid 3′-hydroxylase; DFR, dihydroflavonol 4-reductase; LAR, leucoanthocyanidin 4-reductase; ANS, anthocyanidin synthase; ANR, anthocyanidin reductase; EU, extension units; TU, terminal unit.

have been cloned (see Fig. 3). The use of structural genes in metabolic engineering was the means by which Jung *et al.*[131] introduced the isoflavone synthase gene into the non-legume *Arabidopsis* in order to convert naringenin (**194**) to the isoflavone genistein (**50**). The same gene was also introduced into tomato plants under the control of the cauliflower mosaic virus *35S* promoter and this resulted in the accumulation of up to 90 nmol/g (fresh weight) of genistein-7-*O*-glucoside (**195**) in leaves of the transgenic plants.[132] In another study, chalcone isomerase, the key enzyme to enhance flavonol production, was over-expressed in tomato, resulting in a 78-fold increase in the flavonol levels in the skin of the fruit.[133] In contrast, over-expression of regulatory genes, such as the *LC* and *C1*, to control multiple pathways, also resulted in an increase in flavonols in the flesh of tomato fruit. Total flavonol content of ripe transgenic tomatoes over-expressing *LC* and *C1* was *ca.* 20-fold higher than that of the controls where flavonol production occurred only in the skin.[134] In a further study, *AtMYB12*, a flavonol-specific transcriptional factor in *Arabidopsis thaliana*, was over-expressed in a tissue-specific manner in tomato. This activated both caffeoylquinic acid biosynthetic production and the flavonol biosynthetic pathway, and as a result a transgenic tomato fruit with extremely high levels of multiple, polyphenolic compounds was produced.[135]



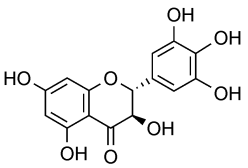
Naringenin (**194**)



Genistein-7-*O*-glucoside (**195**)

Butelli *et al.*[136] attempted to increase the anthocyanin content of tomatoes by over-expressing two transcription factors from snapdragon. The fruit of the plants accumulated substantial concentrations of anthocyanins at levels significantly higher than those found in blackberries and blueberries. Subsequently, cancer-susceptible Trp53(−/−) mice were fed with a diet supplemented with the high-anthocyanin tomatoes, and results revealed that these mice exhibited significant extension of the average life span from 142 to 182 days.

Because of the complexity of the pathways involved and variation between plant species, metabolic engineering to enhance levels of desired dietary phytochemicals remains a challenging task. In practice, the final result is dependent upon a number of factors, including the approach used, the encoded function of the introduced gene and the type of promoter used as well as the regulation of the endogenous pathway,[130,137] and the anticipated results may not always be achieved. In several cases, over-expression has resulted in the accumulation of unexpected products, demonstrating the complexity of the metabolic pathways and our lack of knowledge of these networks and their regulation. This was the case in a study by Bovy *et al.*[138] in which the *C1* and *R* transcriptional factors were introduced into tomato. This led to the induction of several flavonoid genes, but was not sufficient to induce flavonoid-3′,5′-hydroxylase activity, and thereby bring about anthocyanin production by the fruit. Sometimes, the host plant or tissue may be 'incapable' of producing certain compounds due to the substrate specificity of endogenous enzymes. This was reported for the tomato dihydroflavonol-4-reductase that was restricted in its substrate specificity to dihydromyricetin (**196**), and thus only gave rise to the production of delphinidin (**37**)-type anthocyanins.[134] The picture that evolves from the studies on biosynthetic pathways and metabolic engineering is that once the plant cell factory has been assembled, based on the genetic information, the important determinants controlling the fluxes through the pathways are the post-translational regulation of enzyme activity and enzyme and metabolite compartmentation and transport.[129]



Dihydromyricetin (**196**)

In addition to the complexities mentioned above, another huge hurdle to clear is moving from research to practical application. Commercialization of the transgenic products assumes that at some point their consumption would become acceptable to the general public in the UK and Europe, which is certainly not the case at the moment. It is also critical to ascertain the impact and potential market for a given target trait and to ensure that the expression system in the desired organelle, organ, or tissue at each developmental stage will achieve a high accumulation level of the desired compound or groups of compounds. In most cases, a relatively constant and predictable product accumulation level must be established to be sure that the introduced trait does not adversely affect the host plant under agronomic conditions.[139] Another important criterion, and one that would be much more difficult to achieve, is to ascertain whether the increased levels of desired phytochemicals remain palatable and are sufficient to achieve discernable health benefits.

6 Bioavailability of phenolics and polyphenolics

Following the ingestion of dietary flavonoids which, with the notable exception of flavan-3-ols, exist *in planta* predominantly as glycoside conjugates, absorption of some but not all components into the circulatory system occurs in the small intestine.[140] Typically, this is associated with hydrolysis, releasing the aglycone, as a result of the action of lactase phloridizin hydrolase (LPH) in the brush-border of the small intestine epithelial cells. LPH exhibits broad substrate specificity for flavonoid-*O*-β-D-glucosides, and the released aglycone may then enter the epithelial cells by passive diffusion as a result of its increased lipophilicity and its proximity to the cellular membrane.[141] An alternative site of hydrolysis is a cytosolic β-glucosidase (CBG) within the epithelial cells. In order for CBG-mediated hydrolysis to occur the polar glucosides must be transported into the epithelial cells, possibly with the involvement of the active sodium-dependent glucose transporter SGLT1.[142] Thus, it has been accepted that there are two possible routes by which the glucoside conjugates are hydrolysed and the resultant aglycones appear in the epithelial cells, namely 'LPH/diffusion' and 'transport/CBG'. However, a recent investigation, in which SGLT1 was expressed in *Xenopus laevis* oocytes, indicated that

SLGT1 does not transport flavonoids and that glycosylated flavonoids, and some aglycones, have the capability to inhibit the glucose transporter.[143]

Prior to passage into the blood stream the aglycones undergo metabolism, forming sulfate, glucuronide and/or methylated metabolites through the respective action of sulfotransferases (SULT), uridine-5′-diphosphate glucuronosyltransferases (UGTs) and catechol-*O*-methyltransferases (COMT). There is also efflux of at least some of the metabolites back into the lumen of the small intestine and this is thought to involve members of the adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters including multidrug resistance protein (MRP) and P-glycoprotein (P-gp). Once in the bloodstream, metabolites can be subjected to phase II metabolism with further conversions occurring in the liver, where enterohepatic transport in the bile may result in some recycling back to the small intestine.[140]

Flavonoids and their metabolites not absorbed in the small intestine can be absorbed in the large intestine but will be subjected to the action of the colonic microflora, which will cleave conjugating moieties and subject the resultant aglycones to ring fission, leading to the production of phenolic acids and hydroxycinnamates. These can be absorbed and ultimately excreted in urine in substantial quantities that, in most instances, are well in excess of the flavonoid metabolites that entered the circulatory system *via* the small intestine.

A detailed review on the bioavailability of polyphenols in humans was published in 2005 by Manach and colleagues.[144] Much of the research covered involved feeding volunteers a single supplement and monitoring the levels of flavonoids in plasma and urine over a 24 h period. As flavonoid metabolites were rarely available, analysis almost invariably involved treatment of samples with mollusc glucuronidase/sulfatase preparations and subsequent quantification of the released aglycones by HPLC using either absorbance, fluorescence or electrochemical detection. While at the time such studies provided valuable insights, it is important to appreciate their potential shortcomings. Namely, information yielded on the metabolites produced is very indirect, and quantitative estimates, although precise, are not necessarily accurate as there are few data on the efficiency with which the enzymes hydrolyse individual metabolites and release the aglycone.[145] Indeed, the only study on the subject to date reports that the use of enzyme hydrolysis results in an underestimation of isoflavone metabolites.[146] Our review will focus on post-2005 bioavailability studies in which HPLC with mass spectrometric (MS) detection, in the single ion or selective reaction monitoring mode, has been used to identify or characterise polyphenol metabolites in plasma and urine.

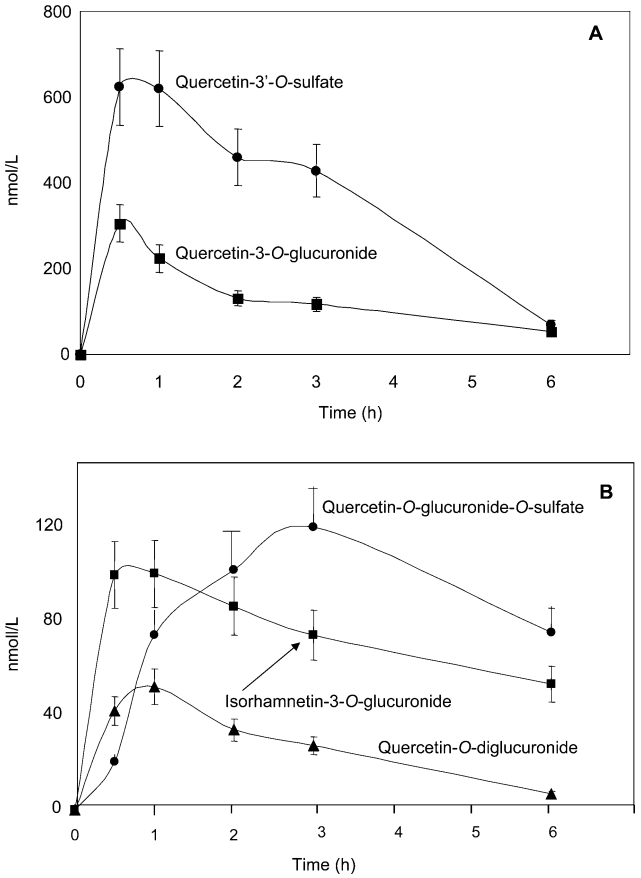


Fig. 4 Concentration of (**A**) quercetin-3′-*O*-sulfate (**197**) and quercetin-3-*O*-glucuronide (**180**), (**B**) a quercetin-*O*-glucuronide-*O*-sulfate, isorhamnetin-3-*O*-glucuronide (**198**) and a quercetin-*O*-diglucuronide in plasma from six healthy human volunteers collected 0–6 h after the ingestion of onions containing 275 μmol of flavonol glucosides. Data expressed as mean values in nmol/L ± standard error ($n = 6$). Note that no quercetin metabolites were present in detectable amounts in plasma collected 24 h after supplementation.

6.1 Flavonols

6.1.1 Onion quercetin-*O*-glucosides. As noted in Section 3.3, onions are a rich source of quercetin-4′-*O*-glucoside (**172**) and quercetin-3,4′-*O*-diglucoside (**173**), and Mullen *et al.*[147] have reported on an acute human feeding study with 270 g of lightly fried onions containing a total of 275 μmol of flavonol glucosides with the main constituents being 143 μmol of the 4′-*O*-glucoside and 107 μmol of the 3,4′-*O*-diglucoside. The volunteers were on a low flavonoid diet for two days prior to ingestion of the meal, after which they continued on a low flavonoid diet for a further 24 h during which time plasma and urine samples were collected for analysis by HPLC-MS^2. Five principal quercetin metabolites, quercetin-3′-*O*-sulfate (**197**), quercetin-3-*O*-glucuronide (**180**), a quercetin-*O*-glucuronide-*O*-sulfate, isorhamnetin-3-*O*-glucuronide (**198**) and a quercetin-*O*-diglucuronide,‡ were detected in plasma; their quantitative 0–6 h profiles are illustrated in Fig. 4. No quercetin metabolites were detected in plasma collected either prior to (0 h) or 24 h after supplementation. A pharmacokinetic analysis of the five plasma metabolites is summarised in Table 5 with information on maximum post-ingestion plasma

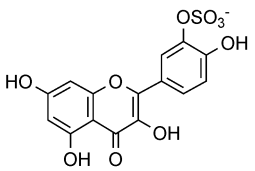
‡ The availability of reference compounds enables specific metabolites to be identified by HPLC-MS^2 and MS^3. In the absence of standards it is not possible to distinguish between isomers and to ascertain the position of conjugating groups on the flavonoid skeleton. Thus, the quercetin-*O*-diglucuronide and the quercetin-*O*-glucuronide-*O*-sulfate, along with other metabolites detected in the onion study, could only be partially identified on the basis of their MS fragmentation pattern. Nonetheless, the use of MS in this way represents a powerful HPLC detection system, as with low ng quantities of sample it provides structural information on analytes of interest that is not obtained with absorbance, fluorescence or electrochemical detectors.

Table 5 Pharmacokinetic analysis of quercetin metabolites in the plasma of healthy human volunteers after the consumption of 270 g of fried onions containing 275 μmol of flavonol glucosides.[147,a]

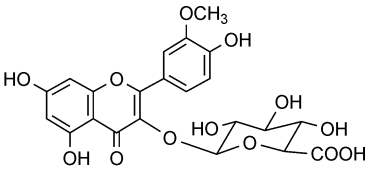
Metabolites	C_{max} (nmol/L)	T_{max} (h)	$T_{1/2}$ (h)
Quercetin-3′-*O*-sulfate (**197**)	665 ± 82	0.75 ± 0.12	1.71
Quercetin-3-*O*-glucuronide (**180**)	351 ± 27	0.60 ± 0.10	2.33
Isorhamnetin-3-*O*-glucuronide (**198**)	112 ± 18	0.60 ± 0.10	5.34
Quercetin-*O*-diglucuronide	62 ± 12	0.80 ± 0.12	1.76
Quercetin-*O*-glucuronide-*O*-sulfate	123 ± 26	2.5 ± 0.22	4.54

[a] Data presented as mean values ± standard error ($n = 6$).

concentration (C_{max}), the time to reach C_{max} (T_{max}) and the elimination half-life ($T_{1/2}$).



Quercetin-3′-*O*-sulfate (**197**)



Isorhamnetin-3-*O*-glucuronide (**198**)

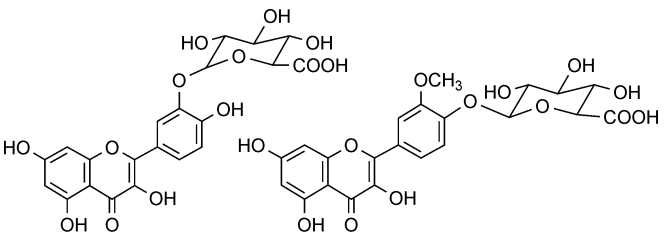
The two major metabolites, quercetin-3′-*O*-sulfate (**197**) (C_{max} 665 nmol/L) and quercetin-3-*O*-glucuronide (**180**) (C_{max} 351 nmol/L) appeared in plasma within 30 min of the ingestion of onions, both had T_{max} values of under 1 h and $T_{1/2}$ values of 1.71 and 2.33 h respectively (Fig. 4, Table 5).[147] The quercetin-*O*-diglucuronide had a lower C_{max} and similar T_{max} and $T_{1/2}$ values. The pharmacokinetic profiles of isorhamnetin-3-*O*-glucuronide (**198**) and the quercetin-*O*-glucuronide-*O*-sulfate were somewhat different in that both had a much longer $T_{1/2}$ and the glucuronide sulfate also had a much delayed T_{max} (Table 5). However, the total contribution of these two compounds to the overall absorption profile was minimal, having no effect on the T_{max} and only extending the $T_{1/2}$ to 2.61 h. This $T_{1/2}$ is much shorter than values obtained in earlier flavonol absorption studies,[148] which is almost certainly a consequence of the enhanced accuracy of analytical data available with the advent of HPLC-MS^2.

Table 6 Quercetin metabolites detected in plasma and urine after the consumption of 270 g of fried onions containing 275 μmol of flavonol glucosides by six healthy human volunteers.[147,a]

Metabolites	Plasma C_{max} (nmol/L)	0–24 h Urinary excretion (nmol)
Quercetin-3-*O*-glucuronide (**180**)	351 ± 27	912 ± 149
Quercetin-3′-*O*-sulfate (**197**)	665 ± 82	Trace
Isorhamnetin-3-*O*-glucuronide (**198**)	112 ± 18	1789 ± 27
Quercetin-O-glucuronide-*O*-sulfate	123 ± 26	1229 ± 190
Quercetin-*O*-diglucuronide	51 ± 13	2223 ± 417
Quercetin-3′-*O*-glucuronide (**199**)	Trace	1845 ± 193
Isorhamnetin-4′-*O*-glucuronide (**200**)	Trace	700 ± 11
Quercetin-*O*-glucuronide-*O*-sulfate	n.d.	1384 ± 163
Quercetin-*O*-glucoside-*O*-sulfates	n.d.	1214 ± 156
Quercetin-*O*-glucoside-*O*-glucuronide	n.d.	163 ± 23
Methylquercetin-*O*-diglucuronides	n.d.	1429 ± 156

[a] Data presented as mean values ± standard error ($n = 6$). n.d. – not detected. Trace – compound detected but not in sufficient amounts for routine quantification.

In keeping with the rapid T_{max} and short plasma $T_{1/2}$ values, most urinary excretion of quercetin metabolites occurred within the first 8 h after ingestion of the onions and over the 0–24 h collection period a total of 12.9 μmol of metabolites were excreted, which corresponds to 4.7% of intake.[147] The urinary metabolite profile was very different from that of the plasma as shown in Table 6. The main plasma metabolite, quercetin-3′-*O*-sulfate (**197**), was excreted in only trace quantities while isorhanmetin-3-*O*-glucuronide (**198**) and the quercetin-*O*-diglucuronide that were minor components in plasma were major urinary metabolites. Several other metabolites, including quercetin-3′-*O*-glucuronide (**199**) and isorhamnetin-4′-*O*-glucuronide (**200**), which were present in only trace quantities or absent from plasma, were excreted in urine in substantial amounts (Table 6).



Quercetin-3′-*O*-glucuronide (**199**) Isorhamnetin-4′-*O*-glucuronide (**200**)

The data obtained in this investigation[147] indicate absorption in the proximal part of the small intestine. However, it provides no information on the mechanisms involved or the efficiency with which quercetin-4′-*O*-glucoside and quercetin-3,4′-*O*-diglucoside are hydrolysed and the resultant aglycone transported into the enterocyte. On the basis of the plasma metabolite profile, it is evident that following the release of the aglycone, quercetin is subjected to sulfation, glucuronidation and methylation. Arguably, the short T_{max} times of quercetin-3′-*O*-sulfate (**197**), quercetin-3-*O*-glucuronide (**180**) and the quercetin-*O*-diglucuronide may be indicative of sulfation and glucuronidation taking place in the entercyte prior to passage of the metabolites into the circulatory system. The longer plasma $T_{1/2}$ value of isorhamnetin-3-*O*-glucuronide (**198**) could reflect post-absorption 3′-*O*-methylation of quercetin-3-*O*-glucuronide (**180**) in the liver. Likewise, the delayed $T_{1/2}$ of the quercetin-*O*-glucuronide-*O*-sulfate could be a consequence of post-absorption sulfation of quercetin-3-*O*-glucuronide (**180**) and/or glucuronidation of quercetin-3′-*O*-sulfate (**197**). The marked differences in the plasma and urinary metabolite profiles are suggestive of extensive phase II metabolism and rapid turnover and removal from the circulatory system *via* the kidneys, but where in the body these conversions occur remains to be determined.[147]

6.1.2 Tomato juice quercetin-3-*O*-rutinoside. In a human study parallel to that carried out with quercetin glucosides in onions, the bioavailability of quercetin-3-*O*-rutinoside (**153**) was investigated by feeding 250 mL of tomato juice containing 176 μmol of the quercetin rhamnose-glucose disaccharide.[149] In this instance only two metabolites were detected in plasma, quercetin-3-*O*-glucuronide (**180**) and isorhamnetin-3-*O*-glucuronide (**198**) (Fig. 5). They were present in *ca.* 25-fold lower quantities

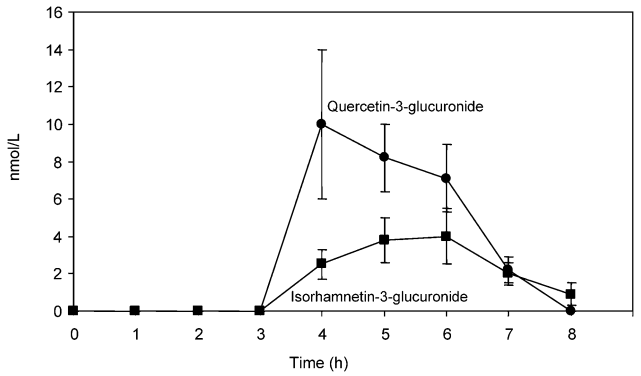


Fig. 5 Concentration of quercetin-3-*O*-glucuronide (**180**) and isorhamnetin-3-*O*-glucuronide (**198**) in the plasma of six healthy human subjects 0–8 h after the consumption of tomato juice containing 176 μmol of quercetin-3-*O*-rutinoside (**153**). Data expressed as mean values in nmol/L ± standard error ($n = 6$). Note that neither metabolite was present in detectable amounts in plasma collected 24 h after supplementation.

Table 7 Pharmacokinetic analysis of quercetin metabolites in the plasma of six healthy human volunteers after the consumption of 250 mL of tomato juice containing 176 μmol of quercetin-3-*O*-rutinoside (**153**).[148,a]

Metabolites	C_{max} (nmol/L)	T_{max} (h)	$T_{1/2}$ (h)
Quercetin-3-*O*-glucuronide (**180**)	12 ± 2	4.7 ± 0.3	1.67
Isorhamnetin-3-*O*-glucuronide (**198**)	4.3 ± 1.5	5.4 ± 0.2	1.66

[a] Data presented as mean values ± standard error ($n = 6$).

than in the onion study, with respective C_{max} values of 12 and 4.3 nmol/L. The T_{max} times extended to *ca.* 5 h (Table 7), indicating absorption in the large rather than the small intestine. A total of nine methylated and glucuronidated quercetin metabolites were detected in urine but some volunteers excreted only 3–4 metabolites. The overall level of metabolite excretion ranged from 0.02 to 2.8% of quercetin-3-*O*-rutinoside intake, which is probably a reflection of variations in the colonic microflora of the individual volunteers. Confirmation of large intestine absorption was obtained in a separate feeding study using subjects with an ileostomy *i.e.* who have had their colon removed surgically. Unlike the healthy subjects with a functioning colon, neither plasma nor urinary metabolites were detected, and ileal fluid collected after tomato juice consumption contained 86% of the ingested quercetin-3-*O*-rutinoside (**153**). The urine collected from the ileostomy volunteers, as well as not containing quercetin metabolites, also lacked the phenolic acid catabolites 3,4-dihydroxyphenylacetic acid (**201**), 3-hydroxyphenylacetic acid (**202**), and 3-methoxy-4-hydroxyphenylacetic acid (**203**). These catabolites were present in the urine of the healthy volunteers with a functioning colon in quantities corresponding to 22% of quercetin-3-*O*-rutinoside (**152**) intake, having probably originated from colonic bacteria-mediated deglycosylation of the rutinoside, and ring fission of the released aglycone followed by the conversions illustrated in Fig. 6.[149]

The data indicate that the rhamnose-glucose disaccharide sugar moiety of quercetin-3-*O*-rutinoside (**153**) is not cleaved by the action of either LPH or CBG during passage through the small intestine and that, as a consequence, following the release of quercetin through the action of colonic bacteria, low-level production and absorption of methylated and glucuronidated quercetin metabolites takes place in the large intestine. However, most of the quercetin is degraded by the colonic microflora, releasing substantial quantities of 3,4-dihydroxyphenylacetic acid (**201**) and smaller quantities of 3-hydroxphenylacetic acid (**202**). These are absorbed into the portal vein, and (probably during passage through the liver) 3,4-dihydroxyphenylacetic acid (**203**) undergoes methylation to yield 3-methoxy-4-hydroxyphenylacetic acid, with all three catabolites being excreted in urine.[149]

It is of interest to note that after feeding tomato juice containing quercetin-3-*O*-rutinoside (**152**) where transformations are restricted to the large intestine, no quercetin sulfates were detected either in plasma or urine. In marked contrast, after feeding onions containing quercetin glucosides that are transformed in the small intestine, quercetin-3′-*O*-sulfate (**197**) was the major plasma metabolite and other sulfated metabolites accumulated in urine, as described in Section 6.1.1. This indicates that

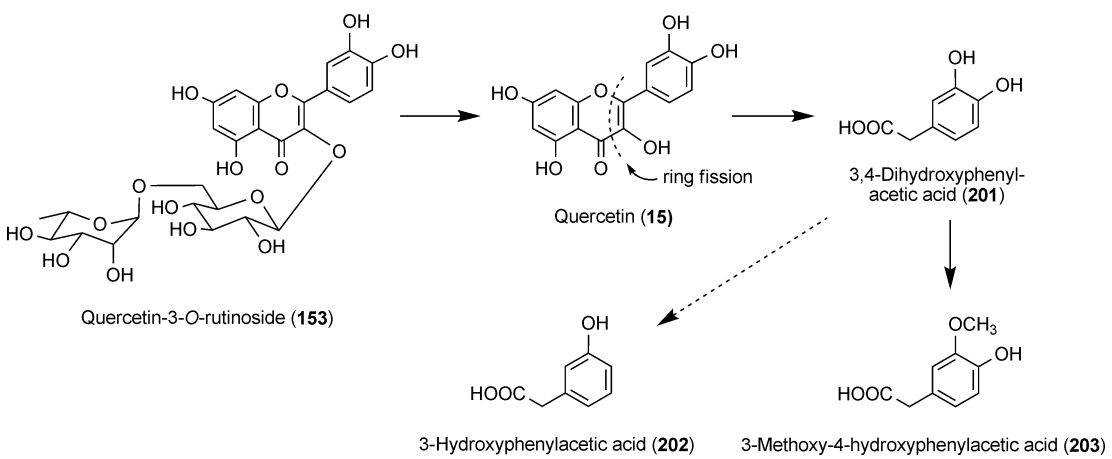


Fig. 6 Proposed pathway for colon bacteria-mediated catabolism of quercetin-3-*O*-rutinoside (**153**) in the large intestine resulting in the production of 3,4-dihydroxyphenylacetic acid (**201**) and smaller quantities of 3-hydroxyphenylacetic acid (**202**), with the subsequent hepatic conversion of 3,4-dihydroxyphenylacetic acid to 3-methoxy-4-hydroxyphenylacetic acid (**203**) prior to urinary excretion. The dotted arrow indicates a minor pathway.

sulfation of quercetin occurs exclusively in the wall of the small intestine and that data obtained in *ex vivo* studies in which quercetin-3-*O*-glucuronide (**180**) was converted to quercetin-3′-*O*-sulfate (**197**) by liver cell-free preparations[150] may not accurately reflect *in vivo* sulfation. It also suggests that formation of mixed conjugates such as quercetin-*O*-glucuronide-*O*-sulfate might occur following glucuronide excretion in bile and reabsorption in the large intestine, and this would be consistent with T_{max} values of <1 h for quercetin-3-*O*-glucuronide compared with *ca.* 3 h for the mixed conjugate.[147]

6.2 Orange juice flavanones

Several early studies, where analyses involve the use of enzyme hydrolysis, have shown that the orange juice flavanone rutinosides are absorbed in the large intestine.[151] In a more recent study, metabolites were analysed by HPLC-MS^2 after volunteers consumed 250 mL of orange juice containing 168 μmol of hesperetin-7-*O*-rutinoside (**45**) and 12 μmol of naringenin-7-*O*-rutinoside (**46**).[145] The hesperetin-7-*O*-rutinoside dose was therefore very similar to that of quercetin-3-*O*-rutinoside (**152**) in the study outlined in Section 6.1.2. Plasma contained hesperetin-7-*O*-glucuronide (**204**) and a second unassigned hesperetin-*O*-glucuronide, and the combined C_{max} for both metabolites was 922 nmol/L at a T_{max} of 4.4 h (Fig. 7). The two hesperetin metabolites were also excreted in urine along with a third hesperetin-*O*-glucuronide, two hesperetin-*O*-glucuronide-*O*-sulfates and a hesperetin-*O*-diglucuronide. These marked differences in the plasma and urinary hesperetin metabolite profiles demonstrate that substantial post-absorption phase II metabolism is occurring. The quantities of these metabolites excreted 0–24 h after ingestion corresponded to 6.5% of hesperetin-7-*O*-rutinoside intake. Although no naringenin metabolites were detected in plasma, urine contained naringenin-7-*O*-glucuronide (**205**), narigenin-4′-*O*-glucuronide (**206**) and a naringenin-*O*-diglucuronide

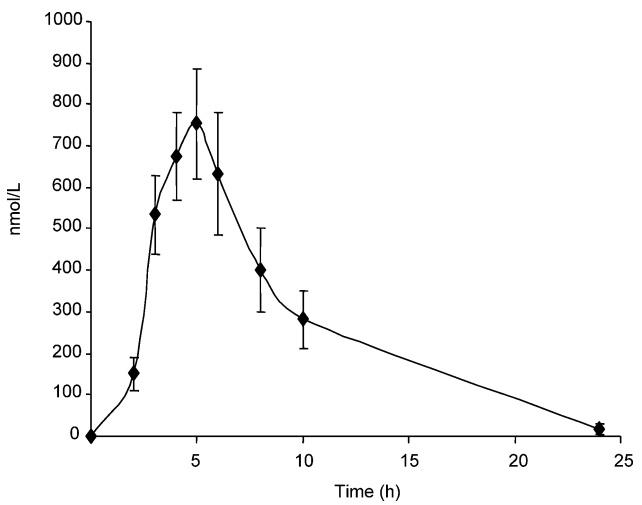


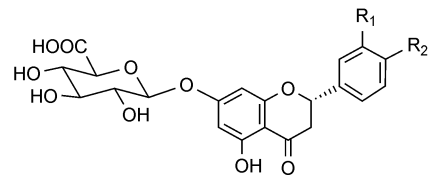
Fig. 7 Combined concentration of hesperetin-7-*O*-glucuronide (**204**) and an unassigned hesperetin-*O*-glucuronide in the plasma of eight healthy human subjects 0–24 h after ingesting 250 mL of orange juice containing 168 μmol of hesperitin-7-*O*-rutinoside (**45**) and 12 μmol of naringenin-7-*O*-rutinoside (**46**). Data expressed as mean values in nmol/L ± standard error. Note that no metabolites were present in detectable amounts in plasma collected 24 h after supplementation.

Table 8 Quantities of hesperetin and naringenin metabolites excreted in the urine of eight healthy human volunteers 0–24 h after the consumption of 250 mL of orange juice containing 168 μmol of hesperetin-7-*O*-rutinoside and 12 μmol of naringenin-7-*O*-rutinoside.[145,a]

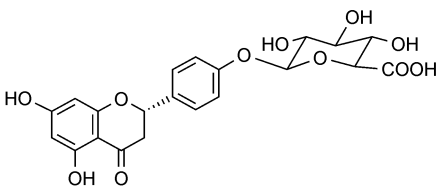
Metabolites	Quantity (nmol)
Hesperetin-7-*O*-glucuronide (**204**)	1373 ± 471
Hesperetin-*O*-glucuronide	3662 ± 1483
Hesperetin-*O*-glucuronide	2319 ± 420
Hesperetin-*O*-diglucuronide	767 ± 361
Hesperetin-*O*-glucuronide-*O*-sulfates	2841 ± 699
Total hesperetin metabolites	**10962** (6.5%)
Naringenin-7-*O*-glucuronide (**205**)	1001 ± 344
Naringenin-4′-*O*-glucuronide (**206**)	976 ± 389
Naringenin-*O*-diglucuronide	98 ± 46
Total naringenin metabolites	**2075** (17.3%)

[a] Data expressed as mean values ± standard error ($n = 8$). Figures in parentheses indicate excretion of hesperetin and naringenin metabolites expressed as a percentage of intake.

in amounts equivalent to 17.1% of the ingested naringenin-7-*O*-rutinoside (Table 8).[145] The differing levels of excretion of hesperetin and naringenin metabolites relative to the amounts ingested is a trend that has been observed in some but not all flavanone feeding studies.[144] While it could be a dose effect reflecting the higher intake of the hesperetin conjugate, it is more likely to be due to naringenin-7-*O*-rutinoside (**46**) being more bioavailable than hesperetin-7-*O*-rutinoside (**45**), indicating that the 3′ and 4′ substituents have an impact on absorption.



Hesperetin-7-*O*-glucuronide (**204**) R_1 = OH, R_2 = OCH_3
Naringenin-7-*O*-glucuronide (**205**) R_1 = H, R_2 = OH



Naringenin-4′-*O*-glucuronide (**206**)

Although both are absorbed in the large intestine, the 922 nmol/L C_{max} of the hesperetin-*O*-glucuronides is more than 50-fold higher than that of the quercetin-3-*O*-rutinoside metabolites (Table 7). This, coupled with the higher level of excretion of the orange juice metabolites, indicates that hesperetin-7-*O*-rutinoside is absorbed from the large intestine much more effectively than quercetin-3-*O*-rutinoside. This may be a consequence of the hesperetin-7-*O*-rutinoside being converted to glucuronides in the large intestine more efficiently than quercetin-3-*O*-rutinoside, perhaps because it is less prone to degradation by colonic bacteria. Among the flavanone metabolites excreted in quantity is a hesperetin-*O*-glucuronide-*O*-sulfate (Table 8).[145] This contrasts with the absence of sulfated naringenin metabolites and with metabolites derived from large intestine absorption of

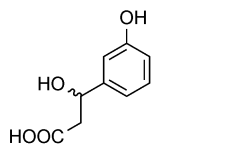
Table 9 Quantities of key phenolic acids excreted in human urine 0–24 h after drinking 250 mL of water or 250 mL of orange juice, containing 168 μmol hesperetin-7-*O*-rutinoside and 12 μmol naringenin 7-*O*-rutinoside.[152,a]

	0–2 h	2–5 h	5–10 h	10–24 h	Total (0–24 h)
Water	1.8 ± 0.4	1.1 ± 0.2	1.2 ± 0.2	2.7 ± 0.5	6.7 ± 1.8
Orange juice	0.5 ± 0.0	1.7 ± 0.2	26 ± 2	34 ± 12	62 ± 18

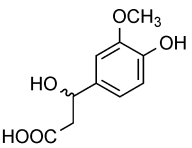
[a] Data were expressed in μmol as mean values ± standard error ($n = 5$). Quantifications based on the combined levels of 3-hydroxyphenylacetic acid (**202**), 3-hydroxyphenylhydracrylic acid (**207**), 3-methoxy-4-hydroxyphenylhydracrylic acid (**208**) dihydroferulic acid (**209**) and 3-hydroxyhippuric acid (**210**) presented in Table 2. Within each of the last three columns, the values for water and orange juice are significantly different at $p < 0.05$.

quercetin-3-*O*-rutinoside in the tomato juice feed (Section 6.1.2).[149] Thus, there appear to be clear differences in the substrate specificity of flavonoid SULTs in the large intestine and/or the liver.

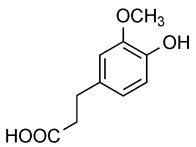
Analysis of phenolic acids excreted in urine after the ingestion of orange juice indicates that the hesperetin, released through colonic bacteria-mediated deglycosylation, as well as being glucuronidated, undergoes ring fission and is catabolised, producing 3-hydroxyphenylacetic acid (**202**), 3-hydroxyphenylhydracrylic acid (**207**), 3-methoxy-4-hydroxyphenylhydracrylic acid (**208**), dihydroferulic acid (**209**) and 3-hydroxyhippuric acid (**210**). The chirality of the hydracrylic acids was not determined. The 62 μmol of these phenolic acids excreted 0–24 h after orange juice ingestion corresponds to 37% of hesperetin-7-*O*-rutinoside intake (Table 9). The main compounds excreted, 3-hydroxyphenylhydracrylic acid (**207**) and 3-methoxy-4-hydroxyphenylhydracrylic acid (**208**), are potential biomarkers of orange juice consumption.[152]



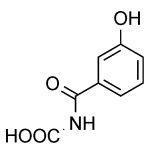
3-Hydroxyphenylacrylic acid (**207**)



3-Methoxy-4-hydroxyphenyl-hydracrylic acid (**208**)



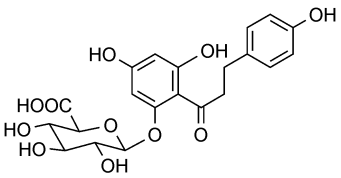
Dihydroferulic acid (**209**)



3-Hydroxyhippuric acid (**210**)

6.3 Dihydrochalcones

Dihydrochalcones (**12**) are a minor group of flavonoids with a ring-opened structure that occur almost exclusively in apples and apple products, including cider, as phloretin derivatives. In a human feeding study, 500 mL of cider containing 5% alcohol was consumed, 0–24 h plasma and urine collected and HPLC-MS^2 used to analyse dihydrochalcone metabolites.[153] The drink had a total dihydrochalcone content of 46 μmol, comprised mainly of 14 μmol of phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside (**148**) and 31 μmol of phloretin-2′-*O*-glucoside (**147**). The sole quantifiable metabolite in plasma was phloretin-2′-*O*-glucuronide (**147**), which reached a C_{max} of 73 nmol/L 0.6 h after ingestion and had a $T_{1/2}$ of 0.7 h, indicative of absorption in the proximal part of the small intestine and rapid elimination from the circulatory system. The 0–24 h urine contained a total of 2.3 μmol of phloretin metabolites, equivalent to 5.0% of intake, which consisted mainly of phloretin-2′-*O*-glucuronide (**211**) (1.9 μmol) and trace quantities of two additional phloretin-*O*-glucuronides and a phloretin-*O*-glucuronide-*O*-sulfate. There was, therefore, relatively little evidence of phase II metabolism.



Phloretin-2′-*O*-glucuronide (**211**)

In keeping with absorption in the small intestine, similar plasma and urine data were obtained when the cider was consumed by subjects with an ileostomy. Of the two major phloretin-*O*-glycosides in cider, only phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside (**148**) was recovered in ileal fluid in quantities corresponding to 22% of intake. The absence of phloretin-2′-*O*-glucoside (**147**) in ileal fluid suggests it is more readily absorbed than phloretin-2′-*O*-(2″-*O*-xylosyl)glucoside. Phloretin-2′-*O*-glucuronide (**211**), two other phloretin-*O*-glucuronides, one phloretin-*O*-glucuronide-*O*-sulfate, two phloretin-*O*-sulfates and the aglycone were also detected in the ileal fluid. This implies that the wall of the small intestine contains β-glycosidase, SULT and UGT activities and that, as well as being absorbed, sizable amounts of the phloretin metabolites that are formed efflux back into the lumen of the gastrointestinal tract. The overall recovery of the dihydrochalcones and their metabolites in the ileal fluid was equivalent to 38.6% of intake.[153]

6.4 Flavan-3-ols

6.4.1 Green tea and flavan-3-ol monomers. Green tea, as outlined in Section 3.1.1, is an extremely rich source of flavan-3-ol monomers. In a recent study 500 mL of a bottled green tea was given as an acute supplement to ten volunteers after which plasma and urine were collected over a 24 h period. The tea contained a total of 648 μmol of flavan-3-ols, principally in the form of 257 μmol of (−)-epigallocatechin (**25**), 230 μmol of (−)-epigallocatechin-3-*O*-gallate (**27**), 58 μmol of (−)-epicatechin (**23**), 49 μmol of (−)-epicatechin-3-*O*-gallate (**26**) and 36 μmol of (+)-gallocatechin (**24**).[154]

Two of the native green tea flavan-3-ols, (−)-epicatechin-3-*O*-gallate (**26**) and (−)-epigallocatechin-3-*O*-gallate (**27**), were identified by HPLC-MS^3 in plasma along with glucuronide, methyl-glucuronide and methyl-sulfate metabolites of (epi)-gallocatechin and glucuronide, sulfate and methyl-sulfate metabolites of (epi)catechin§. The pharmacokinetic profiles of

§ Note that without reference compounds and chiral chromatography,[76] reverse-phase HPLC (even with MS^3 detection) is unable to distinguish between the four possible enantiomers of any (epi)catechin or any (epi)gallocatechin derivative.

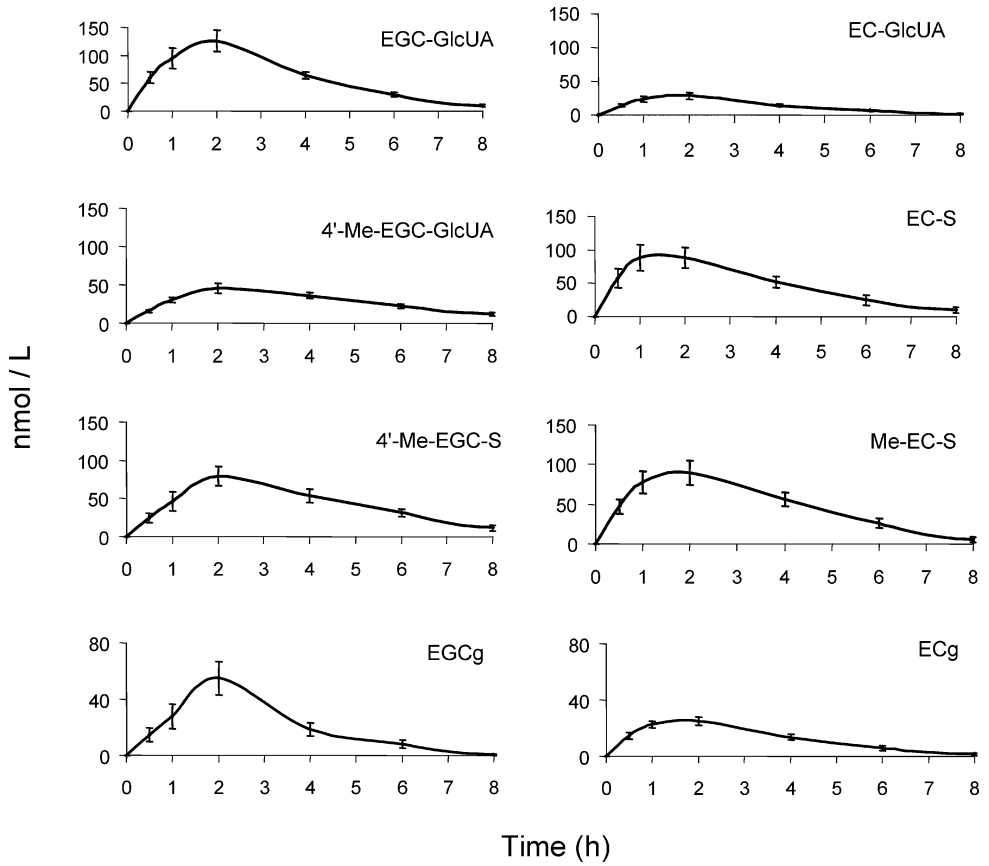


Fig. 8 Concentrations of (epi)gallocatechin-*O*-glucuronide (EGC-GlcUA), 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide (4′-Me-EGC-GlcUA), 4′-*O*-methyl-(epi)gallocatechin-*O*-sulfates (4′-Me-EGC-S), (epi)catechin-3′-*O*-glucuronide (EC-GlcUA), (epi)catechin-*O*-sulfates (EC-S), 3′- and 4′-*O*-methyl-(epi)catechin-*O*-sulfates (Me-EC-S), (−)-epigallocatechin-3-*O*-gallate (**27**) (EGCg) and (−)-epicatechin-3-*O*-gallate (**26**) (ECg) in the plasma of healthy human subjects 0–8 h after the ingestion of 500 mL of green tea containing 648 μmol of flavan-3-ol monomers. Data expressed as mean values in nmol/L ± standard error ($n = 10$) Note that no flavan-3-ols or their metabolites were detected in plasma collected 24 h after ingestion of the green tea.

the eight groups of flavan-3-ols and their metabolites are illustrated in Fig. 8. The C_{max} values ranged from 25 to 126 nM and T_{max} values from 1.6 to 2.3 h (Table 10). These T_{max} values and the pharmacokinetic profiles are indicative of absorption in the small intestine. The appearance of unmetabolised flavonoids in plasma is unusual. The passage of (−)-epicatechin-3-*O*-gallate (**26**) and (−)-epigallocatechin-3-*O*-gallate (**27**) and through the wall of the small intestine into the circulatory system without

Table 10 Pharmacokinetic analysis of flavan-3-ols and their metabolites detected in plasma of ten healthy human volunteers following the ingestion of 500 mL of green tea containing 648 μmol of flavan-3-ol monomers.[154,a]

Flavan-3-ols	C_{max} (nmol/L)	T_{max} (h)	$T_{1/2}$ (h)
(Epi)gallocatechin-*O*-glucuronide	126 ± 19	2.2 ± 0.2	1.6
4′-*O*-Methyl-(epi)gallocatechin-*O*-glucuronide	46 ± 6.3	2.3 ± 0.3	3.1
(Epi)catechin-*O*-glucuronide	29 ± 4.7	1.7 ± 0.2	1.6
(Epi)catechin-*O*-sulfates	89 ± 15	1.6 ± 0.2	1.9
4′-*O*-Methyl-(epi)gallocatechin-*O*-sulfates	79 ± 12	2.2 ± 0.2	2.2
O-Methyl-(epi)catechin-*O*-sulfates	90 ± 15	1.7 ± 0.2	1.5
(−)-Epigallocatechin-3-O-gallate (**27**)	55 ± 12	1.9 ± 0.1	1.0
(−)-Epicatechin-3-*O*-gallate (**26**)	25 ± 3.0	1.6 ± 0.2	1.5

[a] Data expressed as mean values ± standard error ($n = 10$).

Table 11 Quantification of the major groups of flavan-3-ol metabolites excreted in urine 0–24 h after the ingestion of 500 mL of green tea containing 648 μmol of flavan-3-ol monomers by ten healthy human volunteers.[154,a]

Flavan-3-ol metabolites	Quantity (μmol)
(Epi)gallocatechin-*O*-glucuronide	6.5 ± 1.2
4′-*O*-Methyl-(epi)gallocatechin-*O*-glucuronide	4.4 ± 1.5
(Epi)gallocatechin-*O*-sulfates	2.6 ± 0.3
4′-*O*-Methyl-(epi)gallocatechin-*O*-sulfates	19.8 ± 3.0
Total (epi)gallocatechin metabolites	33.3 (11.4%)
(Epi)catechin-*O*-glucuronide	1.5 ± 0.3
(Epi)catechin-*O*-sulfates	6.7 ± 0.7
O-Methyl-(epi)catechin-*O*-sulfates	10.9 ± 1.2
Total (epi)catechin metabolites	19.1 (28.5%)
Total flavan-3-ol metabolites	**52.4** (8.1%)

[a] Data expressed as mean values ± standard error ($n = 10$). Figures in parentheses indicate amount excreted as a percentage of intake.

metabolic modification could be a consequence of the presence of the 3-*O*-galloyl moiety, as gallic acid *per se* is readily absorbed with a reported urinary excretion of 37% of intake.[155]

Urine collected 0–24 h after green tea ingestion contained an array of flavan-3-ol metabolites similar to that detected in plasma except for the presence of minor amounts of three additional (epi)gallocatechin-*O*-sulfates and the absence of (−)-epicatechin-3-*O*-gallate (**26**) and (−)-epigallocatechin-3-*O*-gallate (**27**) (Table 11). This indicates that the flavan-3-ols do not undergo extensive phase II metabolism, in contrast to quercetin and flavanone metabolites (see Sections 6.1.1, 6.1.2 and 6.2). In total, 52.4 μmol of metabolites were excreted, which is equivalent to 8.1% of the ingested green tea flavan-3-ols. When the urinary (epi)gallocatechin and (epi)catechin metabolites are considered separately, a somewhat different picture emerges. The 33.3 μmol excretion of (epi)gallocatechin metabolites is 11.4% of the ingested (−)-epigallocatechin and (+)-gallocatechin while the 19.1 ± 2.2 μmol recovery of (epi)-catechin represents 28.5% of intake (Table 11). These figures are in keeping with high recoveries obtained in earlier studies with green tea and cocoa products,[144,156] confirming that (−)-epicatechin (**23**) and (+)-catechin (**22**) in particular are highly bioavailable, being absorbed and excreted to a much greater extent that other flavonoids, with the possible exception of isoflavones.[144,157]

The absence of detectable amounts of (−)-epigallocatechin-3-*O*-gallate (**27**) in urine, despite its presence in plasma (an event observed by several investigators[158]), is difficult to explain. It is possible that the kidneys are unable to remove (−)-epigallocatechin-3-*O*-gallate from the bloodstream, but if this is the case there must be other mechanisms that result in its rapid decline after reaching C_{max}. Studies with rats have led to speculation that (−)-epigallocatechin-3-*O*-gallate may be removed from the bloodstream in the liver and returned to the small intestine in the bile.[159] To what extent enterohepatic recirculation of (−)-epigallocatechin-3-*O*-gallate (**27**), and also (−)-epicatechin-3-*O*-gallate (**26**), occurs in humans remains to be established.

Summing the C_{max} values for the individual plasma flavan-3-ols and metabolites in Table 11 results in an overall maximum plasma concentration of 538 nmol/L being attained after the ingestion of green tea.[154] This is lower than the 1313 nmol/L C_{max} of quercetin metabolites obtained following the ingestion of onions containing 250 μmol of quercetin-4′-*O*-glucoside (**172**) and quercetin-3,4′-*O*-diglucoside (**173**) (Table 5),[147] and is also less than the 922 nM C_{max} of the hesperetin-*O*-glucuronides that appear in plasma after the ingestion of orange juice containing 168 μmol of hesperetin-7-*O*-rutinoside (**45**).[145] Despite the relatively low concentration of the green tea flavan-3-ol metabolites in plasma (Table 10, Fig. 8), the data on urinary excretion (Table 11) demonstrate that they are absorbed in substantial quantities, especially (−)-epicatechin (**23**). Their failure to accumulate in comparable concentrations in plasma suggests that they are in a state of flux, are more rapidly turned over in the circulatory system, and, rather than accumulating, are excreted *via* the kidneys. In the circumstances, urinary excretion provides a more realistic assessment of absorption, but as this does not include the possibility of metabolites being sequestered in body tissues, this too is theoretically an underestimate of absorption, but to what degree remains to be determined. However, the fact that tissue sequestration has yet to be convincingly demonstrated suggests that it can only be at low levels, if at all.

A further point of note is that the plasma T_{max} times of the (epi)catechin metabolites following absorption in the small intestine are all in excess of 1.6 h (Table 10) while with one exception the T_{max} of the flavonol metabolites absorbed in the small intestine and derived from onion quercetin glucosides was 0.6–0.8 h (Table 5). This is unexpected, as in contrast to the onion flavonols, the green tea flavan-3-ols were already in solution and did not have to be solubilised post-ingestion. Furthermore, they were aglycones not conjugates, and therefore did not have to be hydrolysed prior to absorption and metabolism. The delayed T_{max} of the green tea flavonols is unlikely to be due to a slower rate of absorption, as their excretion is well in excess of the quantity of flavonol metabolites that appear in urine. Although further investigation is required, this does raise the possibility that (i) the flavan-3-ols may be absorbed in the distal part of the small intestine, and flavonols in the proximal part, or (ii) some component(s) in the green tea may be slowing transport through the gastrointestinal tract, something which could be checked by drinking the tea with a small amount of lactulose and measuring breath hydrogen to ascertain the time taken for the head of the drink to reach the colon.[145]

The comparatively high lipophilicity of quercetin aglycone is another factor that might, in part, explain its more rapid attainment of T_{max} compared with green tea flavan-3-ols absorbed at the same site. Experimentally determined octanol–water partition coefficients ($\log P$) or the analogous theoretical quantum-mechanical calculated values are widely considered to indicate the relative lipophilicity of a xenobiotic and/or its metabolites. The more lipophilic a compound, the greater its affinity for the lipid-rich cell membrane, and its potential to diffuse passively through such a membrane and to bind to proteins such as serum albumin. In contrast, the transfer of a compound of low lipophilicity is likely to require interaction with a transporter. In some instances there is a good statistical correlation between a set of $\log P$ values and the magnitude of a biological effect where cellular uptake is a prerequisite. For example, Sergediene *et al.* observed that cellular cytotoxicity increased as a function of lipophilicity, although it must be noted the test compound concentrations used were much higher than would be encountered in the alimentary canal,[160] and $\log P$ increases with concentration when π–π stacking occurs. Similarly, Crespy *et al.*, using much lower concentrations, reported that the net transfer of the tested flavonoids across the intestinal brush border of rats ranged from 70–80% for flavonols to 38% for flavan-3-ols, and appeared to be linked to the lipophilicity of the compound defined by $\log P$.[161]

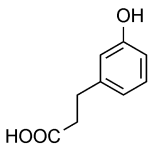
However, there are exceptions and contradictions in the published data, in part because some experimental procedures for determining $\log P$ are now known to be inadequate.[162] The literature does not provide an appropriate set of experimental octanol–water $\log P$ values obtained under identical conditions and encompassing the flavan-3-ols and flavonols required. Calculated values suggest that quercetin ($\log P = 2.075$) is much more lipophilic than (epi)catechins (0.491) and (epi)-gallocatechins (−0.096) but identical to (epi)gallocatechin gallates (2.082).[163] However, this ranking by calculated $\log P$ does not match the ranking by T_{max} for substances absorbed

proximally, *i.e.* quercetin from quercetin glucosides is <1 h and green tea flavan-3-ols, including gallates, is *ca.* 1.5–2.5 h, (see Tables 5 and 11). This discrepancy indicates that these simple models fail to take account of all factors that operate *in vivo*, for example metabolism and efflux by active transporters, *etc.* To overcome these limitations some investigators have determined an 'apparent partition coefficient' (P_{app}) by measuring transfer across Caco-2 cell membranes and reported a correlation between P_{app} and log *P* for flavones, isoflavones and dihydroflavones, but not for flavonols.[164] Thus, while the use of an isolated organ or cultured cells takes account of some factors that the simple octanol–water partition cannot, even these models cannot predict perfectly the pharmacokinetics that would occur in a whole organism. The marked divergence between log *P* and pharmacokinetic behaviour observed with green tea flavan-3-ols and onion flavonols reinforces our contention that proper understanding of the significance of dietary polyphenols will not be possible without recourse to volunteer studies.

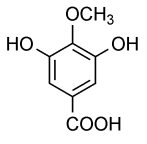
The necessity of volunteer studies is also indicated by claims in the literature that green tea flavan-3-ols are poorly bioavailable because of instability under digestive conditions, with >80% losses being observed with *in vitro* digestion models simulating gastric and small intestine conditions.[165] It is clear that the data obtained in these investigations do not accurately reflect the *in vivo* fate of flavan-3-ols following ingestion, as they are at variance with the high urinary excretion observed in green tea feeding studies[144,154] and the substantial recovery of flavan-3-ols in ileal fluid after the consumption of Polyphenon E, a green tea extract.[156]

6.4.2 Procyanidins. Procyanidins are major components in the human diet because of their widespread occurrence in fruits, berries nuts, beans, cocoa-based products, wine and beer.[166] *In vivo*, their consumption has been implicated in improved antioxidant status[167] and decreased DNA damage in humans,[168] and reduced development of aortic atherosclerosis[169] and delayed tumour production[170] in animal test systems. Because of these and other biological effects of procyanidins,[171] principally derived from the ingestion of grape seed extracts or consumption of cocoa-derived food stuffs, information on the bioavailability of procyanidins and the compounds responsible for these effects *in vivo* is of importance.

There are numerous feeding studies with animals and humans indicating that polymeric procyanidins are not absorbed.[172] Most pass unaltered to the large intestine where they are catabolised by the colonic microflora yielding a diversity of phenolic acids[144,173] including 3-(3-hydroxyphenyl)propionic acid (**212**) and 4-*O*-methylgallic acid (**213**)[174] which are absorbed in to the circulatory system and excreted in urine. There is one report, based on data obtained in an *in vitro* model of gastrointestinal conditions, that procyanidins degrade yielding more readily absorbable flavan-3-ol monomers.[175] Subsequent studies have not supported this conclusion.[176] There are two reports of minor quantities of procyanidin dimers B_1 (**127**) and B_2 (**31**) being detected in human plasma after the respective consumption of a grape seed extract[177] and a flavan-3-ol-rich cocoa.[178] In the latter study, the levels of the B_2 dimer (**31**) in plasma were *ca.* 100-fold lower than those of flavan-3-ols monomers.



3-(3-Hydroxyphenyl)propionic acid (**212**)



4-*O*-Methylgallic acid (**213**)

The biological effects of procyanidins are generally attributed to their more readily absorbed colonic breakdown products, the phenolic acids, although there is a lack of detailed study in this area. There is, however, a dissenting view as trace levels of procyanidins, in contrast to (−)-catechin (**22**) and (+)-epicatechin (**23**), inhibit platelet aggregation *in vitro* and suppress the synthesis of the vasoconstriction peptide endothelin-1 by cultured endothelial cells.[179] Supporting this view is a study in which individual procyandins were fed to rats after which dimers through to pentamers were detected in plasma which was extracted with 8 mol/L urea, rather than the more traditional methanol/acetonitrile, which it was proposed prevented the irreversible binding of procyanidins to plasma proteins.[180] The procyanidins were, however, administered by gavage at an extremely high dose, 1 g/kg body weight, and it remains to be determined if procyanidins can be similarly detected in urea-extracted plasma after the ingestion of more nutritionally relevant quantities.

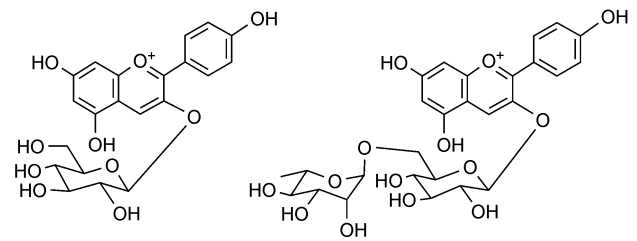
6.5 Anthocyanins

Anthocyanins, for people who eat berries and drink red wine on a routine basis, are major dietary components. Although there are exceptions, unlike other flavonoids that are absorbed and excreted, most anthocyanins do not appear to undergo extensive metabolism of the parent glycosides to glucurono, sulfo or methyl derivatives.[181,182] In feeding studies with animals and humans, typically *ca.* 0.1% of the quantities ingested, and sometimes much less, has been detected in urine. The available data imply that the determinants of absorption and excretion are influenced not only by the nature of the sugar moiety but also by the structure of the anthocyanidin aglycone.[181,183]

The complex array of information on anthocyanin bioavailability obtained with human and animal test systems has been reviewed by Prior and Wu.[184] One of the reasons for the complicated picture that has emerged is that many feeds have involved berry or fruit supplements containing several structurally diverse anthocyanins. For instance, black raspberries contain five cyanidin-3-*O*-sugar conjugates ranging from mono to trisaccharides while blueberries contain a total of 12 anthocyanins, principally 3-*O*-glucosides, galactosides and arabinosides of cyanidin (**36**), delphinidin (**37**), petunidin (**39**) and malvidin (**40**).[181,185] This makes the complex anthocyanin content of plasma and urine exceedingly difficult, if not impossible, to assess in terms of absorption, excretion and potential phase I and phase II metabolism, especially when 3′-*O*-methylation can convert cyanidin to peonidin, and delphinidin to petunidin, and 5′-*O*-methylation converts petunidin to malvidin. Much simpler anthocyanin profiles are found in strawberries and blackberries, both of which contain one predominant anthocyanin, pelargonidin-3-*O*-glucoside (**214**) in the former and cyanidin-3-*O*-glucoside (**158**) in the latter.[186] As a consequence, data on

anthocyanin bioavailability after ingestion of these berries by humans are potentially more straightforward to interpret.

In a recent human study, 200 g of strawberries containing 222 μmol of pelargonidin-3-*O*-glucoside (**214**) and trace quantities of pelargonidin-3-*O*-rutinoside (**215**) (13 μmol) and cyanidin-3-*O*-glucoside (**158**) (6 μmol) were consumed by six subjects, after which plasma and urine were collected over a 24 h period.[187] The plasma contained a pelargonidin-*O*-glucuronide in substantial quantities along with non-quantifiable amounts of three other pelargonidin-*O*-glucuronides and pelargonidin-3-*O*-glucoside, the latter perhaps derived from removal of the 6″-rhamnose moiety from pelargonidin-3-*O*-rutinoside (**215**). The main pelargonidin-*O*-glucuronide had a C_{max} of 274 ± 24 nmol/L, a T_{max} of 1.1 ± 0.4 h, in keeping with small intestine absorption, and $T_{1/2}$ of 2.1 ± 0.7 h. All the plasma anthocyanins also appeared in urine along with small quantities of pelargonidin aglycone and a pelargonidin-*O*-sulfate. The pelargonidin-*O*-glucuronide that was the main metabolite in plasma was by far the predominant component in urine, accounting over 0–24 h for 1498 nmol of a total of 1672 nmol of anthocyanins excreted. This corresponds to 0.75% of pelargonidin-3-*O*-glucoside intake.



Pelargonidin-3-*O*-glucoside (**214**) Pelargonidin-3-*O*-rutinoside (**215**)

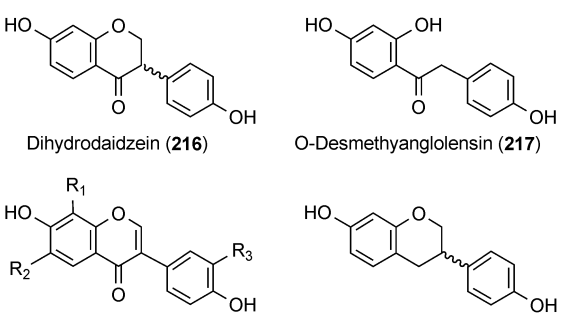
There is, therefore, no evidence of substantive post-absorption metabolism prior to excretion.

In an earlier feeding study with strawberries, Felgines and co-workers[188] reported a urinary excretion equivalent to 1.8% of the 179 μmol of ingested pelargonidin-3-*O*-glucoside (**214**) and this is also similar to values obtained in a 15–60 μmol dose study with strawberries.[189] These urinary recoveries are high for anthocyanins, and suggest that pelargonidin-3-*O*-glucoside (**214**) is absorbed more readily that other anthocyanins. In a separate human feeding study with 200 g of blackberries containing 960 μmol of cyanidin-3-*O*-glucoside (**158**), 12 anthocyanins were excreted including unmetabolised cyanidin-3-*O*-glucoside, a cyanidin-*O*-glucuronide and a peonidin-*O*-glucuronide in quantities equivalent to 0.16% of intake.[190] This suggests that pelargonidin-3-*O*-glucoside (**214**), while it is metabolised to fewer products, may be absorbed more readily than cyanidin-3-*O*-glucoside (**158**). However, the high cyanidin-3-*O*-glucoside content of the blackberry supplement may have had an impact on absorption and/or excretion. In the circumstances, it would be of interest to carry out a feeding study and to determine not only the urine but also the plasma anthocyanin profile after ingestion of blackberries and strawberries containing similar quantities of anthocyanins

A point of note is that anthocyanins are readily distinguished from other flavonoids as they undergo re-arrangements in response to pH. The red flavylium cation predominates at pH 1–3 but as the pH increases to 4 and above the colourless carbinol pseudobase is the major component along with smaller amounts of the colourless chalcone pseudobase and the blue quinoidal base.[191] Anthocyanins are traditionally extracted and analysed in acidic medium as the red flavylium cation is the most stable form. However, it is not known what forms predominate *in vivo*. The limited available experimental evidence indicates that in the acidic conditions that prevail in the stomach anthocyanins are in the red flavylium form but once they enter more basic conditions in the small intestine the carbinol pseudobase is likely to predominate. It could be that the colourless carbinol pseudobase is the main form in the small intestine where it undergoes limited absorption, possibly being metabolised to conjugates that are overlooked because they cannot be converted to red flavylium forms prior to the eventual analysis. It is also possible that significant amounts of the carbinol pseudobase might pass into the large intestine where degradation, to as-yet undetermined products, occurs due to the action of colonic bacteria. More subtle scenarios may exist, and detailed information is unlikely to be forthcoming until ring-labelled ^{14}C-anthocyanins become available.

6.6 Isoflavones

Isoflavones, though not a major component of the European diet, are one of the better absorbed dietary flavonoids with urinary excretion of metabolites typically being 20–50% of intake.[140,144] A study by Rüfer *et al.*[192] in which seven male volunteers were given either pure daidzein (**49**) or pure daidzein-7-*O*-glucoside (**195**), both at a dose of 3.9 μmol/kg body weight, has demonstrated that the plasma C_{max}, at *ca.* 8–9 h, was three to six times longer after consumption of the glucoside, which is dominant in soya compared with the aglycone, the main component in fermented soya products. The metabolites, quantified after deconjugation, included dihydrodaidzein (**216**), *O*-desmethylangolensin (**217**), 6-hydroxydaidzein (**218**), 8-hydroxydaidzein (**219**) and 3′-hydroxydaidzein (**220**). One of the seven volunteers also produced equol (**221**).[192] The bioavailability reported in this study contrasts markedly with the results obtained when tablets containing a crude preparation of soya saponins and either daidzein (**49**) and genistein (**50**) aglycone or their mixed glycosides was given to eight volunteers weighing 51–87 kg at doses of 0.11 and 1.7 mmol.[193] At the higher dose, the isoflavone aglycone mixture produced plasma C_{max} concentrations up to five times higher than the preparation containing the diadzein and genistein glycosides. The T_{max} in this study was *ca.* 4 h,[193] which is much earlier than the 8–9 h reported by Rüfer



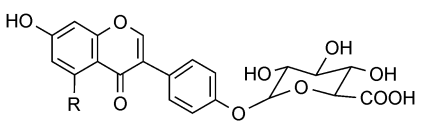
Dihydrodaidzein (**216**) O-Desmethyanglolensin (**217**)

6-Hydroxydaidzein (**218**) R_1 = H, R_2 = OH, R_3 = H
8-Hydroxydaidzein (**219**) R_1 = OH, R_2 = H, R_3 = H
3'-Hydroxydaidzein (**220**) R_1 = H, R_2 = H, R_3 = OH

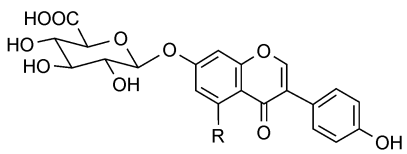
Equol (**221**)

et al.[192] These differences are not easily explained but a possible role for the saponins is suggested.

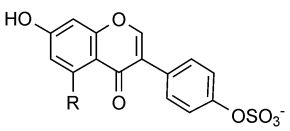
A study in which two volunteers consumed 50 g of kinako (baked soya bean powder) containing 66 μmol of daidzein (**49**), 106 μmol of genistein (**50**), 120 μmol of diadzein-7-glucoside (**222**) and 205 μmol of genistein-7-*O*-glucoside (**195**) suspended in 300 ml of cow's milk, used HPLC-MS to establish the presence of daidzein (**49**), genistein (**50**), daidzein-4′-*O*-glucuronide (**222**), genistein-4′-*O*-glucuronide (**223**), daidzein-7-*O*-glucuronide (**224**), genistein-7-*O*-glucuronide (**225**), daidzein-4′-*O*-sulfate (**226**), genistein-4′-*O*-sulfate (**227**), daidzein-7-*O*-sulfate (**228**) and genistein-7-*O*-sulfate (**229**) in plasma in the 1–7 h period post-consumption.[194] Traces of the glucosides of genistein and daidzein were also detected in plasma 1 h post-consumption. The short duration of the study prevented determination of T_{max}, C_{max} and $T_{1/2}$. The aglycone concentration never exceeded *ca.* 200 nmol/L with genistein (**50**) exceeding daidzein (**49**) for one volunteer but the reverse for the other. Within the period studied the isoflavone metabolites never exceeded *ca.* 3 μmol/L in total, and no single metabolite exceeded 0.8 μmol/L. Conjugation for both isoflavones occurred preferentially at the C7 position, but the ratio of glucuronides to sulfates varied with time.[194]



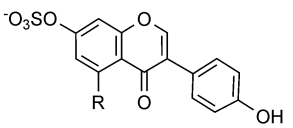
Daidzein-4′-*O*-glucuronide (**222**) R = H
Genistein-4′-O-glucuronide (**223**) R = OH



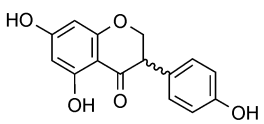
Daidzein-7-*O*-glucuronide (**224**) R = H
Genistein-7-*O*-glucuronide (**225**) R = OH



Daidzein-4′-*O*-sulphate (**226**) R = H
Genistein-4′-*O*-sulphate (**227**) R = OH



Daidzein-7-*O*-sulphate (**228**) R = H
Genistein-7-*O*-sulphate (**229**) R = OH



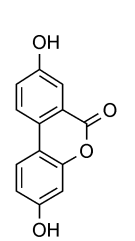
Dihydrogenistein (**230**)

Although, as mentioned above, traces of the glucosides have been detected in plasma, most absorption occurs after deconjugation. The first phase of absorption, up to 1 hour, is impaired in lactose malabsorbers, suggesting a role for LPH, but overall this is compensated by microbial hydrolysis, and total absorption was not significantly affected by lactose malabsorption.[195] The ability of ileostomists to absorb isoflavone glycosides, not significantly different from healthy volunteers with an intact colon, confirms that absorption occurs in the upper gastrointestinal tract. However, urinary excretion of the microbial metabolites equol (**221**), dihydrodaidzein (**216**) and dihydrogenistein (**230**) by ileostomists was lower than that of healthy subjects, and the ileostomy group contained fewer equol-producers. Equol was characterised as the *S*-enantiomer.[196]

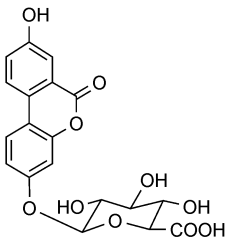
6.7 Ellagitannins

Studies into the bioavailability of ellagitannins following ingestion by humans have been carried out mainly with pomegranate, which contains punicalin (**168**) and punicalagins (**169**),[197–199] but the fate of ellagitannins in strawberries, raspberries, walnuts and oak-aged wines has also been investigated.[200]

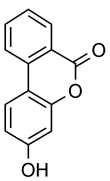
After drinking pomegranate juice containing 318 mg of punicalagins (**169**), ellagic acid (**56**) was detected in plasma with C_{max} of 60 nmol/L at a T_{max} of 0.98 h, suggesting acid hydrolysis of at least some of the ellagitannins releasing free ellagic acid which is absorbed directly from the stomach or the proximal small intestine.[198] Also detected in the plasma of some but not all volunteers, mainly 6 h after supplementation, were 3,8-dihydroxy-6H-dibenzo[*b*,*d*]pyran-6-one (urolithin A) (**231**), urolithin A-3-*O*-glucuronide (**232**), 3-hydroxy-6*H*-dibenzo[*b*,*d*]pyran-6-one (urolithin B) (**233**) and a methylated urolithin B. Urinary metabolites which began to appear after 12 h included urolithin A-3-*O*-glucuronide (**232**), urolithin B-3-*O*-glucuronide (**234**) and 2-(1-*O*-glucuronyl)-3,8-di-*O*-methylellagic acid (3,8-*O*-dimethylellagic acid -2-*O*-glucuronide) (**235**), and excretion continued for



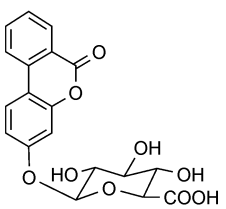
Urolithin A (**231**)



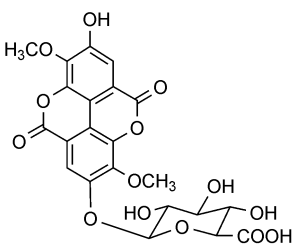
Urolithin A-3-*O*-glucuronide (**232**)



Urolithin B (**233**)



Urolithin B-3-*O*-glucuronide (**234**)



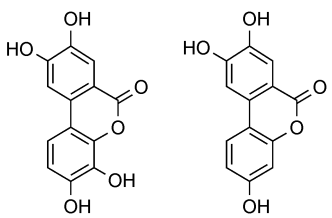
3,8-*O*-Dimethylellagic acid-2-*O*-glucuronide (**235**)

up to a further 36 h. None of these compounds were quantified and there was much subject-to-subject variation in the spectrum of metabolites produced. This implies that when the ellagitannins

and/or ellagic acid reach the distal part of the small intestine and the colon they are metabolised by the gut microflora, producing urolithins A and B which are then absorbed along with ellagic acid (**56**) and subjected to the action of Phase II UGTs and/or methyltransferases before being excreted in urine.[198]

In a separate study in which volunteers ingested one litre of pomegranate juice containing 4.37 g of punicalagins on a daily basis for five days, circulating urolithin levels reached a concentration of 18.6 μmol/L.[197] Feeding human subjects a single dose of strawberries, raspberries, walnuts and oak-aged red wine, all of which contain ellagitannins, resulted in excretion of urolithin A-3-*O*-glucuronide (**232**) in quantities equivalent to 2.8% (strawberries), 3.4% (raspberries), 6.5% (oak-aged red wine) and 16.6% (walnuts) of intake.[200]

The most detailed study on ellagitannins to date has been carried out with Iberian pigs which in their natural habit feed on oak acorns, which are a further source of ellagitannins.[201] The pigs were given an average of 4.04 kg of acorns on a daily basis for 117 days after which tissues and body fluids were processed and analysed by HPLC-MS^3. A total of 31 ellagitannin-derived metabolites were detected, including 25 urolithin and six ellagic acid derivatives. A summary of the complex picture that emerges is that in the jejunum the acorn ellagitannins release ellagic acid (**156**), which the intestinal microflora metabolise, sequentially producing 3,8,9,10-tetrahydroxy-6*H*-dibenzo[*b*,*d*]pyran-6-one (urolithin D) (**236**), 3,8,9-trihydroxy-6*H*-dibenzo[*b*,*d*]pyran-6-one (urolithin C) (**237**), urolithin A (**231**) and urolithin B (**233**). These urolithins are absorbed preferentially as their lipophilicity increases with plasma containing mainly urolithin A-3-*O*-glucuronide (**232**) and urolithin B-3-*O*-glucuronide (**234**) with traces of a urolithin C-*O*-glucuronide and 3,8-*O*-dimethylellagic acid -2-*O*-glucuronide (**235**). The urolithin A and C glucuronides were the major components in urine. Among the 26 conjugated metabolites detected in bile were glucuronides and methyl glucuronides of ellagic acid and substantial quantities of urolithin A, C and D derivatives. This indicates extensive hepatic metabolism and active enterohepatic circulation, and also explains the persistence of urinary urolithin metabolites observed in the human studies. No ellagitannins or their metabolites were detected in body tissues outside the gastrointestinal tract, which is interesting as the meat and fat of Iberian pigs fed on acorns is resistant to rancidity.[201] Perhaps this may be attributable to other potential ellagitannin colonic breakdown products such as phenolic acids, which have yet to be investigated.



Urolithin D (**236**) Urolithin C (**237**)

7 Paradigm shift on the possible mode of action of phenolics

Flavonoids and phenolic compounds in foods have attracted great interest since the 1990s owing to growing evidence of their beneficial effects on human health. The interest was stimulated mainly by epidemiological studies indicating an inverse association between intake of foods rich in these compounds and the incidence of non-communicable diseases such as cardiovascular diseases, diabetes mellitus, and cancer.[202] For example, studies carried out by various investigators suggested a protective effect of flavonol (**2**) and flavone (**3**) intake on (i) the risk of fatal or non-fatal coronary artery diseases,[203] (ii) the risk of lung cancer,[204,205] (iii) the incidence of asthma[204] and (iv) the impairment of pulmonary functions.[206]

Since the phenolics in fruits and vegetables, cocoa, chocolate, red wine, green tea and other dietary sources exhibit potent free radical-scavenging properties *in vitro*, their main role *in vivo* was thought to be as antioxidants involved in protection against lipid peroxidation. However, in the last decade, the fate and mode of action of these compounds has turned out to be more complex than originally expected.[144,207,208] Generalisations are invidious, and there are always exceptions. Nevertheless, one can say that only a small percentage of the phenols and polyphenols consumed ever reaches the tissues, and very little of this absorbed material retains the structure found in the plant.

The percentage of intake that is absorbed varies with structure (for example being significantly modulated by which sugars are attached to a flavonoid aglycone), and the food matrix. Any single phenol or polyphenol generates several metabolites, perhaps as many as 20 in the case of quercetin glycosides although two or three usually dominate.[147] The exact yields and proportions of metabolites from any substrate will vary not only with the individual's genetic profile but also with the composition and competence of that individual's intestinal microflora. Any biological effects produced by these metabolites will be a function of the concentration achieved at the relevant site and the susceptibility of the organelle (receptor, enzyme, transporter, *etc.*) that again might vary with the individual's genetic profile. It is not possible to quantify the magnitude of the variation produced by these factors, but it would not be unreasonable to assume an order of magnitude overall.

As discussed in Section 6, most dietary polyphenolics are modified during absorption from the small intestine, with the formation of glucuronide, methyl and sulfate metabolites, while in the large intestine breakdown to phenolic acid and non-phenolic catabolites occurs. Consequently, the compounds that reach cells and tissues are chemically, biologically and (in many instances) functionally distinct from the dietary form, and such features underlay their bioactivity. This, in addition to the fact that very low levels of dietary flavonoids and related compounds are actually absorbed and appear in the bloodstream (generally the maximum is <10 μmol/L in total, and such maxima are transient), implies that their mechanism of action goes beyond the modulation of oxidative stress.[209] The capacity of flavonoids and their metabolites to bind to proteins is another factor that must be considered when determining the overall bioactivity. Manach *et al.*[210] have reported the existence of intermolecular bonds between serum albumin and quercetin metabolites, which supports its slow elimination from the body. Similarly, (−)-epigallocatechin-3-*O*-gallate (**27**) possesses a high affinity for blood proteins[211] which, potentially, could extend its half-life in the circulatory system. However, volunteer studies indicate that in normal dietary circumstances the half-life is shorter than the

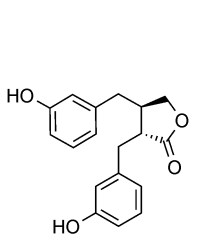
interval between repeat consumption, indicating that there is little opportunity for metabolite concentrations to increase during the day, and any slight daytime accumulation is likely to be eliminated overnight. Many of the commodities so far examined in volunteer studies might only be consumed once a day, but the significance of this rapid elimination is well illustrated by the data in for green tea (Fig. 8 and Table 10) that would often be consumed several times a day.

There is now emerging evidence that some phytochemicals, at concentrations that might be achieved under normal dietary circumstances, can exert modulatory effects in cells through selective actions on multiple intracellular signalling cascades, which are vital for cellular functions such as growth, proliferation and death (apoptosis).[207] For example, *trans*-resveratrol (**71**) has been shown to target many components of intracellular signaling pathways, including pro-inflammatory mediators, regulators of cell survival and apoptosis, and tumor angiogenic and metastatic switches, by modulating a distinct set of upstream kinases, transcription factors and their regulators.[212] The identification of these molecular targets is an important first step that must be attained before the molecular mode(s) of action can be elucidated and understood, and this understanding is essential in order to formulate dietary strategies that might manage or even prevent certain non-communicable diseases. Emphasis on the mode of action in subsequent sections will cover investigations that either deal with clinical and preclinical studies or *in vitro* investigations that use either phenolic compounds and/or their main *in vivo* metabolites at concentrations that, at least, approach what might be achieved through diet.

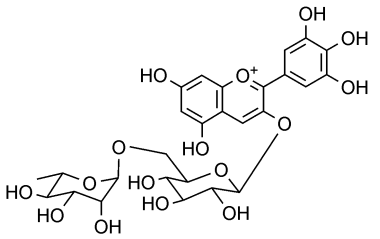
7.1 Significance of phenolic metabolites for human health

There are a large number of reports on *in vitro* studies into the effect of individual phenolics or plant extracts on various aspects of human health. However, many of these investigations lack any physiological significance because of the high doses used, typically of parent compounds, such as quercetin, rather than their conjugated mammalian metabolites or microbial degradation products (see Section 6). In order for any metabolite of any dietary phenolic to exert *in vivo* a biologically significant effect, then it must be sufficiently potent to exert that effect at 50% of its transient plasma C_{max}. So far as we are aware, such potency has yet to be demonstrated, and as a consequence only a handful of *in vitro* studies have used concentrations sufficiently low to have any relevance to potential bioactivities *in vivo*.

Enterolactone (**238**), a phytoestrogenic lignan metabolite formed by the action of colonic microflora, has been ascribed various health-benefiting properties. A high concentration of plasma enterolactone is associated with a lower risk of acute coronary events,[213] breast cancer,[214,215] colorectal adenomas[216] and prostate cancer.[217] More recently, enterolactone has been shown to have direct inhibitory effects associated with cancer cell growth and is able to modulate the cell-signaling pathway.[218] Enterolactone (**238**) competes with E2 for the type II estrogen receptor, induces sex-hormone-binding globulin[219] and influences steroid-metabolising enzymes and synthesis, thus potentially reducing proliferation of hormone-dependant cancer.[215]



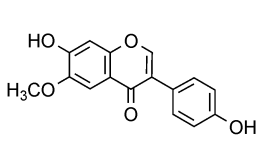
Enterolactone (**238**)



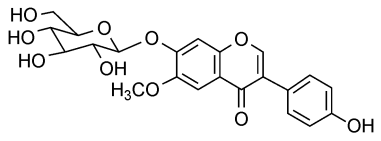
Delphinidin-3-*O*-rutinoside (**239**)

Platelet activation and subsequent aggregation play a major role in the pathogenesis of myocardial infarction and ischaemic heart disease. Hence, promoting an optimal platelet function *via* the reduction of platelet hyper-reactivity using dietary solutions is considered a feasible approach for the maintenance of cardiovascular health. An investigation on this topic has assayed the anti-platelet activity of physiologically relevant concentrations of the anthocyanins delphinidin-3-*O*-rutinoside (**239**), cyanidin-3-*O*-glucoside (**158**), cyanidin-3-*O*-rutinoside (**159**), and malvidin-3-*O*-glucoside (**41**), and their putative colonic metabolites, dihydroferulic acid (**209**), 3-(3-hydroxyphenyl)propionic acid (**212**), 3-hydroxyphenylacetic acid (**202**) and 3-methoxy-4-hydroxyphenylacetic acid (**203**), both separately and in combination. Anti-thrombotic properties were exhibited by 10 μmol/L dihydroferulic acid, and 3-(3-hydroxyphenyl)propionic acid, 1 μmol/L delphinidin-3-rutinoside and a mixture of all the test compounds.[220]

The isoflavones daidzein (**49**) and genistein (**50**) are known to have estrogenic properties because of their structural similarity to estradiol (**52**), and both compounds elicit or selectively modulate estrogenic responses by binding estrogen receptors ERα and ERβ, with greater affinity for ERα.[221] However, equol (**221**), a gut bacterial metabolite of daidzein (**49**), exhibits greater binding affinity to ERα and posses higher antioxidant capacity than its parent molecule.[222] Furthermore, equol at μmol/L concentrations is more active than soy isoflavones in competing for binding to thromboxane A2 receptor in human platelets, thus eliciting its anti-platelet activity. The order of the relative affinity in competing for binding was equol (**221**) > genistein (**50**) > daidzein (**49**) > glycitein (**240**) > genistein-7-*O*-glucoside (**195**) > daidzein-7-*O*-glucoside (**222**) > glycitein-7-*O*-glucoside (**241**).[223] However, only 20–30% of the human population are equol producers.[224] Thus, the use of dietary sources of isoflavones as a treatment for those at risk of thrombus formation and atherosclerosis will require evaluation of the patient's ability to convert daidzein (**49**) to equol (**221**).[223]



Glycitein (**240**)

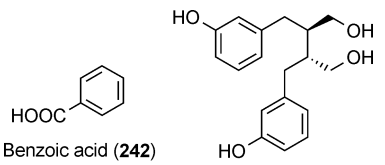


Glycitein-7-*O*-glucoside (**241**)

7.2 Tissue or organ targets of phenolics

To understand the mechanism of action of dietary phenolics and their derivatives, it is necessary to identify their target sites. However, data on tissue distribution are very scarce even in experimental animals. Microautoradiography of mice tissues

after administration of either radiolabeled (−)-epigallocatechin-3-*O*-gallate (**27**) or resveratrol (**71**) indicated that radioactivity is unequally incorporated into the cells of organs.[225] Phenolics have also been detected in the brain,[226] heart, kidney, spleen, pancreas and reproductive organs of mice and rats.[227] After acute ingestion of a nutritionally appropriate dose of the flavonol [2-^{14}C]quercetin-4′-glucoside (**172**) by rats, a number of glucuronide and methylated quercetin metabolites formed in the small intestine and subsequently small amounts, *ca.* 4% of intake, were excreted in urine. Once the flavonols reached the caecum and colon they were rapidly degraded to phenolic acids, principally 3-hydroxyphenylacetic acid (**202**) and benzoic acid (**242**), most of which (over a 72 h period) were rapidly excreted in urine without any noticeable build-up in the circulatory system. In this instance there is, therefore, no evidence of significant quantities of quercetin-derived compounds binding to albumin and elimination from the body being slowed, as proposed by Manach and colleagues.[210] There was also no marked accumulation of radioactivity in any of the body tissues, including the brain.[228]



Benzoic acid (**242**) Enterodiol (**243**)

Following the ingestion of isoflavones by humans, the presence of daidzein (**49**) and genistein (**50**) and their metabolites equol (**221**), enterolactone (**238**) and enterodiol (**243**) have been detected in prostate tissue.[229] In another study, after ingesting *ca.* 300 μmol of daidzein (**49**), concentrations up to 6 μmol/L of equol (**221**) accumulated in the breast tissue.[230] When 20 men scheduled for surgery consumed 1.42 L of green tea and black tea on a daily basis for 5 days before radical prostatectomy, tea flavan-3-ols, specifically (−)-epigallocatechin (**25**) and (−)-epigallocatechin-3-*O*-gallate (**27**), were found to accumulate in prostate samples.[231]

Recently, immunochemistry has been used successfully to demonstrate the target sites of a flavan-3-ol in humans. A monoclonal antibody specific for (−)-epicatechin-3-*O*-gallate (**27**) was developed, and this antibody detected immunoreactive materials in human atherosclerotic lesions, specifically localized in the macrophage-derived foam cells, but not in the normal aorta.[232] This is especially interesting as it is known that unmetabolised (−)-epicatechin-3-*O*-gallate (**27**) appears in the human circulatory system after the ingestion of green tea,[154] but why the concentration is higher in damaged tissue than in healthy tissue is not known. Similar methodology was also used to reveal that quercetin-3-*O*-glucuronide (**180**), a known plasma flavonol metabolite,[147] accumulates in macrophage-derived foam cells in human atherosclerotic lesions where it is converted to the aglycone quercetin (**15**) and is associated with a subsequent reduction in lesion size.[233] This suggests a role for human β-glucuronidase which is released at sites of inflammation, but the exact specificity of this enzyme towards the flavonoids and non-flavonoid conjugates is not known, and certain glucuronide conjugates may have greater potential than others. In this regard it is interesting to note that quercetin-3-*O*-glucuronide (**180**), one of the major human metabolites of some quercetin glycosides, inhibits angiogenesis *in vitro*, whereas quercetin-3′-*O*-sulfate (**197**), another major metabolite, promotes angiogenesis.[234] These results may provide insights into a mechanism for the anti-atherosclerotic actions of flavan-3-ols and flavonols.

7.3 Potential mode of action of phenolic compounds and their metabolites

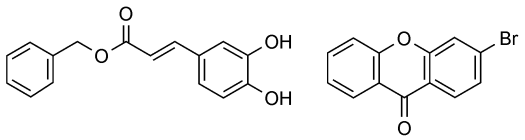
Many phenolic compounds are potent effectors of biologic processes and have the capacity to influence disease risk *via* several complementary and overlapping mechanisms. In this section, current knowledge on mechanisms by which dietary phenolic compounds play a role in preventing degenerative pathologies will be summarized. In particular, the complex interactions between these dietary molecules and their molecular targets including the cell signaling pathways and response will be discussed.

7.3.1 NF-κB signaling pathway. NF-κB (nuclear factor kappa B) is a redox-sensitive transcription factor that regulates numerous physiological functions and is involved in the pathogenesis of various diseases. NF-κB regulates the expression of cytokines, inducible nitric oxide synthase (iNOS), cyclo-oxgenase 2 (COX-2), growth factors and inhibitors of apoptosis. Pathological dysregulation of NF-κB is associated with inflammatory disease such as asthma,[235] Crohn's disease and ulcerative colitis,[236] and is also involved in the pathophysiology of autoimmune disorders, such as rheumatoid arthritis, as well as neurodegenerative diseases and cancer. Potent inhibitors of NF-κB include curcumin (**188**),[237] resveratrol (**71**),[238] ellagic acid (**56**), and (−)-epigallocatechin-3-*O*-gallate (**27**).[239] Curcumin at doses of 10 to 30 μmol/L inhibits NF-κB activation of human prostate cancer cells.[240]

7.3.2 Activator protein-1 (AP-1). AP-1 is another class of redox-sensitive transcriptional factor with important roles in normal development and the response to stress. AP-1 activation is linked to growth regulation, cell transformation, inflammation, and innate immune response. AP-1 has been implicated in regulation of genes involved in apoptosis and proliferation, and may promote cell proliferation by activating the cyclin *D1* gene, and repressing tumor-suppressor genes, such as *p53*, *p21cip1/waf1* and *p16*. Several phenolic compounds, such as green tea flavan-3-ols, quercetin (**15**), *trans*-resveratrol (**71**) and curcumin (**188**), have been shown to suppress the AP-1 activation process.[241]

7.3.3 Phase II enzyme activation and Nrf2. Anthocyanins were shown to induce phase II antioxidant and detoxifying enzymes in cultured cells.[242] Treatment of rat liver clone 9 cells and non-cancerous breast cells with anthocyanins, albeit at high concentrations (20–50 μM), enhanced antioxidant capacity through the activation of both NADPH:quinone reductase and three glutathione-related enzymes, glutathione reductase, glutathione peroxidase, and glutathione *S*-transferase.[243] Other phenolic compounds have also been implicated in the induction of phase II enzymes and they can be considered as potential candidates for preventing tumour development.[244] 5-*O*-

Caffeoylquinic acid (**64**) increases the activity of the phase II detoxifying enzymes GST and NADPH quinone oxidoreductase in mouse epidermal cells.[245] Similar results were also obtained with drinks rich in phenolic compounds such as tea and maté.[246] The mechanism by which phenolic compounds exhibited these effects is through activation of the antioxidant response element upstream of genes that code for these enzymes. Recently, nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) has been shown to be a critical transcription factor that binds to the antioxidant response element in the promoter region of a number of genes encoding for antioxidant enzymes in several types of cells and tissues.[247] Phenolic compounds such as gallic acid (**54**), *p*-coumaric acid (**58**) and ferulic acid (**60**), albeit at a high dosage of 100 mg/kg body weight, significantly increase levels of Nrf2 and thus up-regulate the gene expression of cardiac antioxidant enzymes in the heart of male Sprague-Dawley rats.[248] Similarly, curcumin (**188**), *trans*-resveratrol (**71**), and the synthetic analogues caffeic acid phenethyl ester (**244**) and 4′-bromoflavone (**245**), exhibit chemopreventive properties through stimulating Nrf2.[249]



Caffeic acid phenethyl ester (**244**) 4'-Bromoflavone (**245**)

7.3.4 Mitogen-activated protein kinase (MAPK) signaling pathway. MAPKs are a family of highly conserved groups of signalling proteins; they are divided into three main groups, (i) the extracellular signal-regulated protein kinase (Erk), (ii) c-Jun *N*-terminal kinase/stress-activated protein kinases (JNK) and (iii) $p38^{MAPK}$. Typically, JNK and $p38^{MAPK}$ cascades are activated by environmental stresses and pro-inflammatory cytokines such as tumour necrosis factor (TNF), interleukin-1 (IL-1), IL-2 and IL-17, and they are largely associated with the promotion of inflammation, pain and programmed cell death.[250] MAPKs have also been implicated in the regulation of Phase II enzyme gene expression and induction of apoptosis.[251] Green tea flavan-3-ols, specifically 25 μmol/L (−)-epigallocatechin-3-*O*-gallate (**27**), which appears in the bloodstream (albeit at *ca.* 100 nM concentrations[154]) suppress tumorigenesis through induction of the antioxidant-response element and MAPK (ERK, JNK and p38) in several chemical-induced animal carcinogenesis models in a dose- and time-dependent manner.[251]

(+)-Catechin (**22**) and quercetin (**15**) exhibit cardiovascular protection through suppressing PAI-1 expression in human coronary artery endothelial cells *in vitro* through activating the ERK, JNK and p38 signalling pathways.[252] In a more recent study, quercetin (**15**) at μM concentrations exerted anti-adipogenesis activity in a dose-dependent manner by inducing apoptosis of mature adipocytes through the modulation of the ERK and JNK pathways, which play pivotal roles during apoptosis.[253] 5-*O*-Caffeoylquinic acid (**64**) exhibits chemopreventive effects on A549 human cancer cells *in vitro* in a dose-dependent manner at μmol/L concentrations, mainly through its up-regulation of the MAPK signaling pathway. In addition, it also suppressed the ROS-mediated NF-κB and AP-1 signalling pathways, thus providing protection against environmental carcinogen-induced carcinogenesis.[245]

8 Concluding comments

The current interest in dietary polyphenols has been driven primarily by epidemiological studies that suggest diets rich in these phytochemicals are beneficial to human health. Epidemiology is a valuable tool that has featured significantly in studies of health, nutrition and toxicology, ranging historically from essentially *ad hoc* but painstaking observations by individuals¶ to the large and systematic studies used today. The objective is to establish a statistically valid association between the health of a population and one (or more) factors impacting upon it. Establishing associations is easier where the effect is acute and the outcome dramatic and unequivocal. It is much more difficult where phenomena are determined by multiple independent factors and an observable effect takes years to become apparent, as is the situation with the less healthy diet. Moreover, the demonstration of a strong statistical relationship does not establish cause-and-effect. It may suggest causality but in fact it merely defines a problem requiring further study. To demonstrate causality it is essential to define a logical chain of events based on sound biochemistry, chemistry and physiology, and ideally to support this by controlled and randomised intervention studies. Intervention studies may be impractical, and in many cases the additional support inevitably comes from animal and *in vitro* studies.

Statistical relationships can be misleading. For example, a relationship was established between the traditional consumption of maté and the incidence of oesophageal cancer. On the basis of an imperfect chemical analysis this effect was attributed initially to tannins in maté, leading to speculation about the risks of drinking black tea.[255] Subsequently it was established that maté does not contain tannins,[256] and in due course the primary driving force was defined as chronic damage to the oesophageal tissues caused by scalding hot water and subsequent exposure to a carcinogen during the error-prone stage of tissue repair.[257]

Consistent outcomes from independent epidemiological studies greatly strengthen the likelihood of a robust and potentially causal relationship, for example, the statistical link between increased coffee consumption and reduced likelihood of developing Type II diabetes.[258] The explanation could be the ability of coffee constituents, chlorogenic acids and their transformation products (see Section 3.1.2) to blunt the post-prandial surge in plasma glucose with associated effects on the incretin hormones.[259] In contrast, inconsistent outcomes, as in the case of the Zutphen study investigating flavonol intake and coronary heart disease mortality[260] and the Caerphilly study investigating flavonol and flavone intake and the incidence of ischaemic heart disease,[261] suggest the existence of confounding factors or at least one outcome having occurred purely by chance.

¶ It is often said that John Snow was the first epidemiologist. In London, in 1854, he prepared a map recording all cases of cholera and the location of wells supplying water. The cases were clustered around a well in Broad Street, and it is said that the outbreak of cholera was ended by removing the handle of the pump, rendering it inoperable. The infectious agent, *Vibrio cholerae*, was not unequivocally identified for a further 50 years.[254]

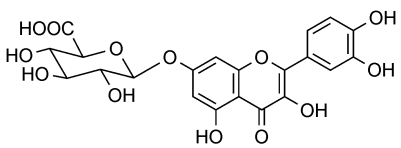
Furthermore, although the Zutphen study has been a very important catalyst for research into the protective effects of dietary phenolics, the statistics suggested that diets richer in tea, apples and onions might protect against cardiovascular disease. The statistical data *per se* did not focus on the flavones and flavonols – that was an inference drawn by the investigators – and other components in these commodities (see Sections 3.1.1, 3.2 and 3.3) might have a role. Subsequent events suggest that the inference has some merit, but cause and effect have still not been proven. This statement is not meant as a criticism only of the seminal Zutphen study, or of the scientists who made the investigation, but rather of the over-simple extrapolations to which the general public are exposed.

Epidemiological studies fall in to several categories.[262] Prospective studies are the more powerful and follow one or more groups (cohorts) of individuals, who have not yet had the outcome event in question, and who are monitored for the number of such events that occur over time. In a cohort study, two (or more) defined groups, such as exposed and non-exposed, are followed and a comparison is made of the disease rates emerging during the study period. In some prospective studies diets are sampled and stored frozen for future chemical analysis. Alternatively, the intake of phytochemicals might be assessed from tabulated composition data for particular commodities recorded in diet-diaries. For this approach to be satisfactory much more detailed compositional databases are required. The current lack of data for the composition of commodities after cooking or processing is a major deficiency that must be addressed before reliable statistical data can be obtained.[263] Even when adequate compositional data are available for the commodity as consumed (*i.e.* after cooking or processing) this cannot provide quantitative data for gut microflora metabolites that might in some cases be the active principle. This weakness has not yet been addressed in any epidemiological study so far as we are aware.

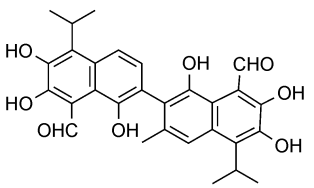
In contrast, a retrospective study involves questioning, or otherwise investigating, cases where individuals who had a particular outcome, and analysing the collected data after the outcomes have occurred. One such design is the case-control study where the retrospective comparison involves the history of persons with disease (cases) with those of persons without the disease (controls). With retrospective studies there can be significant problems of recall especially if it is necessary to define the diet consumed, levels of exercise, *etc.* for the preceding 20 years. It is even more difficult if the subject has died, although in this case there is usually unequivocal information on the cause of death (as compared with information derived from controversial biomarkers). Whatever the study type, the inferences obtained apply to populations and not necessarily to particular individuals.

Epidemiological studies are expensive, and if prospective may take many years. Intervention studies are even more expensive, costing millions of pounds. Intervention studies seem ideal, but have particular limitations. It is vital that the study sample is representative of the target population. It is essential that clinical data are sound, and the value of data can be severely compromised by injudicious choice of the biomarkers of effect that will be monitored. Biomarkers that are difficult to reproduce across study centres, or are of questionable predictive value for ultimate disease outcome, can ruin a study. An intervention may have to be maintained for a very long time, and may so curtail a participant's freedom of action, as to be unacceptable. It is vital to have sufficiently large study groups to provide statistical power. This is especially important in a stratified study (having subgroups such as age, gender, *etc.*). Excessive drop-out arising from mounting inconvenience can erode the statistical power and ruin a study.

Animal studies may be unpopular with society but overcome some of the difficulties associated with human intervention trials. For example, animals may be used in a three-generation study, allowing investigation of reproductive effects and effects on offspring, something requiring over 60 years in humans and obviously totally impractical for many reasons. However, animal studies have their peculiarities and limitations. The animals in a study tend to be genetically similar. The human population is anything but. On the one hand, the genetic standardisation may make it easier to achieve statistical significance for a relatively weak effect; on the other it may completely miss something of great human importance simply because the selected strain of animal is insufficiently sensitive to whatever challenge or treatment is being investigated. Differences between the human and animal genomes may also lead to potential problems of extrapolation. For example, rodents methylate dietary phenols far more extensively than humans. The three major human metabolites of quercetin glycosides are quercetin-3-*O*-glucuronide (**180**), quercetin-3′-*O*-sulfate (**197**) and isorhamnetin-3-*O*-glucuronide (**198**), but quercetin-7-*O*-glucuronide (**246**) is not detected.[147] This is, however, a major rat metabolite.[264] Ruminants are not susceptible to the toxicity of gossypol (**247**), a polyphenol peculiar to cotton, but monogastric species are. Many species, including rats, can synthesise ascorbate, but humans cannot.



Quercetin-7-*O*-glucuronide (**246**)



Gossypol (**247**)

In vitro studies utilising tissue slices or cultured cells can provide extremely valuable biochemical information. However, great care is required when interpreting and extrapolating the data obtained. Again, the species of origin must be considered – obviously slices of animal liver are more readily available than slices of human liver, but biochemical differences must not be overlooked. Many cells that are amenable to culturing are derived from human tumours, which may differ from healthy tissue in important ways; *e.g.* individual clones of Caco-2 differ dramatically in their production of particular enzymes and transporters. It is clearly unwise to draw hasty conclusions from a study where a tissue such as liver was exposed directly to tea brew. If the tissue had been the buccal epithelium, gastric epithelium, or even intestinal Caco-2 cells, the experimental conditions might have better reflected real life. When the test

substance is more appropriate to the tissue, valuable data can be generated and the extrapolations that follow are much more likely to be sound – for example, the studies in which HepG2 cells were exposed to quercetin glucuronides establishing that these could be absorbed, deglucuronidated, methylated and converted to sulfates[150] (although as noted in Section 6.1.2 the conversion of glucuronide to sulfate seems not to occur in humans *in vivo*).

The dose used should also reflect real life. In a paper reporting that urokinase could be inhibited *in vitro* by epigallocatechin-3-*O*-gallate (**27**) (IC_{50} *ca.* 4 μmol/L), it was suggested that this might explain why drinking green tea prevents cancer.[265] This unwise comment was severely criticised by others who pointed out that plasma levels of epigallocatechin-3-*O*-gallate even following heavy green tea consumption were lower by a factor of 10 000.[266]

Elevated doses can be used to 'force' an outcome but such results must be extrapolated with great care. Elevated doses can sensibly be used when comparing potency of a range of related substances and numerical data are required for the potency of the less powerful compounds, and when determining a dose–response relationship. It is well known from toxicological studies that while a linear dose–response relationship may occur over a certain range of concentrations, the effect may have a threshold, and extrapolation to very low concentrations may over-estimate the toxic effect. This could also be true for beneficial effects, and downwards extrapolation from a 'forced' effect at high concentration *in vitro* must be interpreted with caution.

With regard to dietary polyphenols and human health, much yet remains to be investigated. We are convinced that further human studies will be essential to unravelling the complexities. As yet, claims for benefit outstrip understanding, and some claims have clearly been based on methodology that has severe limitations. Nevertheless, we remain optimistic that there is a real possibility that some dietary polyphenols will be shown to contribute positively to health and well-being, but at the present state of knowledge feel that the only sound advice that one can give is to have as much variety as possible, so as to maximise the range of phytochemicals that are consumed.

9 References

1 C. L. Williams, *Br. Med. J.*, 1995, **310**, 1453–1455.
2 *Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet*, ed. A. Crozier, M. N. Clifford and H. Ashihara, Blackwell Publishing, Oxford, 2006.
3 D. Strack, in *Plant Biochemistry*, ed. P. M. Dey and J. B. Harborne, Academic Press, London, 1997, pp. 387–416.
4 *The Flavonoids: Advances in Research Since 1986*, ed. J. B. Harborne, Chapman-Hall, London, 1993; *Flavonoids. Chemistry, Biochemistry and Applications*, ed. Ø. Andersen and K. R. Markham, CRC Press, Boca Raton, FL, 2006.
5 K. M. Valant-Vetschera and E. Wollenweber, in *Flavonoids. Chemistry, Biochemistry and Applications*. ed. Ø. Andersen and K. R. Markham, CRC Press, Boca Raton, FL, 2006, pp. 617–748; C. A. Williams, in *Flavonoids. Chemistry, Biochemistry and Applications*. ed. Ø. Andersen and K. R. Markham, CRC Press, Boca Raton, FL, 2006, pp. 749–856.
6 M. G. L. Hertog, P. C. H. Hollman and M. B. Katan, *J. Agric. Food Chem.*, 1992, **40**, 2379–2383; M. G. L. Hertog, P. C. H. Hollman and B. van de Putte, *J. Agric. Food Chem.*, 1993, **41**, 1242–1246.
7 A. Crozier, M. E. J. Lean, M. S. McDonald and C. Black, *J. Agric. Food Chem.*, 1997, **45**, 590–595.
8 M. N. Clifford, in *Interactions of Food Components*, ed. G. G. Birch and M. G. Lindley, Elsevier Applied Science, London, 1986, pp. 143–164.
9 T. Unno, A. Sugimoto and T. Kakuda, *J. Sci. Food Agric.*, 2000, **80**, 601–606.
10 B. Yang, K. Arai and F. Kusu, *Anal. Biochem.*, 2000, **283**, 77–82.
11 R. Seto, H. Nakamura, F. Nanjo and Y. Hara, *Biosci. Biotech. Biochem.*, 1997, **61**, 1434–1439.
12 http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html.
13 L. I. Mennen, R. Walker, C. Bennetau-Pelissero and A. Scalbert, *Am. J. Clin. Nutr.*, 2005, **81**, 326S–329S.
14 P. Langcake and R. J. Pryce, *Phytochemistry*, 1977, **16**, 1193–1196.
15 J. Burns, T. Yokota, H. Ashihara, M. E. J. Lean and A. Crozier, *J. Agric. Food Chem.*, 2002, **50**, 3337–3340.
16 F. Grippi, L. Crosta, G. Aiello, M. Tolomeo, F. Oliveri, N. Gebbia and A. Curione, *Food Chem.*, 2008, **107**, 483–488.
17 B. C. Vastano, Y. Chen, N. Zhu, C. T. Ho, Z. Zhou and R. T. Rosen, *J. Agric. Food Chem.*, 2000, **48**, 253–256.
18 X. Vitrac, A. Bornet, R. Vanderlinde, J. M. Valls, T. Richard, J.-C. Delaunay, J.-M. Mérillon and P.-L. Teissédre, *J. Agric. Food Chem.*, 2005, **53**, 5664–5669.
19 P. Waffo-Téguo, D. Lee, M. Cuendet, J. Mérillon, J. M. Pezzuto and A. D. Kinghorn, *J. Nat. Prod.*, 2001, **64**, 136–138.
20 R. Pezet, C. Perret, J. B. Jean-Denis, R. Tabacchi, K. Gindro and O. Viret, *J. Agric. Food Chem.*, 2003, **51**, 5488–5492.
21 J. A. Baur and D. A. Sinclair, *Nat. Rev. Drug Discov.*, 2006, **5**, 493–506.
22 S. Bradamante, L. Barenghi and A. Villa, *Cardiovasc. Drug Rev.*, 2004, **22**, 169–188.
23 M. Jang, L. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon and J. M. Pezzuto, *Science*, 1997, **275**, 218–220.
24 J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Kalra, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R. G. Spencer, E. G. Lakatta, D. L. Couteur, R. J. Shaw, P. Navas, P. Puigserver, D. K. Ingram, R. de Cabo and D. A. Sinclair, *Nature*, 2006, **444**, 337–342; D. Valenzano, E. Terzibasi, T. Genade, A. Cattaneo, L. Domenici and A. Celerino, *Curr. Biol.*, 2006, **16**, 296–300.
25 M. Kaeberlein and P. S. Rabinovitch, *Nature*, 2006, **444**, 280–281.
26 R. Corder, A. Crozier and P. A. Kroon, *Nature*, 2003, **426**, 119.
27 K. S. Willson, *Coffee, Cocoa and Tea*, CABI Publishing; Wallingford, UK, 1999.
28 T. Takeo, in *Tea: Cultivation to Consumption*, ed. K. C. Willson and M. N. Clifford, Chapman and Hall, London, 1992, pp. 413–457.
29 M. G. Hampton, in *Tea: Cultivation to Consumption*, ed. K. C. Willson and M. N. Clifford, Chapman and Hall, London, 1992, pp. 459–512.
30 *Tea: Cultivation to Consumption*, ed. K. C. Willson and M. N. Clifford, Chapman and Hall, London, 1992.
31 F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1992, **40**, 1383–1389.
32 U. H. Engelhardt, A. Finger, B. Herzig and S. Kuhr, *Deutsche Lebensmittel Rundschau*, 1992, **88**, 69–73.
33 A. Finger, U. H. Engelhardt and V. Wray, *Phytochemistry*, 1991, **30**, 2057–2060; C. Lakenbrink, T. M. L. Lam, U. H. Engelhardt and V. Wray, *Nat. Prod. Lett.*, 2000, **14**, 233–238.
34 K. R. Price, M. J. C. Rhodes and K. A. Barnes, *J. Agric. Food Chem.*, 1998, **46**, 2517–2522.
35 Y. Sakamoto, *Agric. Biol. Chem.*, 1967, **31**, 1029–1034; Y. Sakamoto, *Agric. Biol. Chem.*, 1969, **33**, 959–961; Y. Sakamoto, *Agric. Biol. Chem.*, 1970, **34**, 919–925.
36 G.-I. Nonaka, O. Kawahara and I. Nishioka, *Chem. Pharm. Bull.*, 1983, **31**, 3906–3914; G.-I. Nonaka, R. Sakai and I. Nishioka, *Phytochemistry*, 1984, **23**, 1753–1755; C. Lakenbrink, U. H. Engelhardt and V. Wray, *J. Agric. Food Chem.*, 1999, **47**, 4621–4624.
37 G.-I. Nonaka, R. Sakai and I. Nishioka, *Phytochemistry*, 1984, **23**, 1753.
38 T. Hatano, S. Shida, L. Han and T. Okuda, *Chem. Phar. Bull.*, 1991, **39**, 876–880; L. Han, T. Hatano, T. Yoshida and T. Okuda, *Chem. Pharm. Bull.*, 1994, **42**, 1399–1409; T. Yoshida, T. Nakazawa, T. Hatano, R. C. Yang, L. L. Yang, K. Y. Yen and T. Okuda, *Phytochemistry*, 1994, **37**, 241–244.

39 F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1989, **37**, 77–85.
40 F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1988, **36**, 1676–1684; F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1989, **37**, 3255–3263; F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1989, **37**, 77–85.
41 Z. H. Zhou, Y. J. Zhang, M. Xu and C. R. Yang, *J. Agric. Food Chem.*, 2005, **53**, 8614–8617.
42 G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1982, **30**, 4268–4276; G.-I. Nonaka, O. Kawahara and I. Nishioka, *Chem. Pharm. Bull.*, 1982, **30**, 4277–4282; H.-F. Chen, T. Tanaka, G.-I. Nonaka, T. Fujioka and K. Mihashi, *Phytochemistry*, 1993, **33**, 183–187.
43 R. Subramanian, P. Venkatesh, S. Ganguli and V. P. Sinkar, *J. Agric. Food Chem.*, 1999, **47**, 2571–2578.
44 S. C. Opie, M. N. Clifford and A. Robertson, *J. Sci. Food Agric.*, 1993, **63**, 435–438; S. C. Opie, M. N. Clifford and A. Robertson, *J. Sci. Food Agric.*, 1995, **67**, 501–505.
45 C. W. Goodsall, A. D. Parry and D. S. Safford, in *Polyphenols 96*, ed. J. Vercauteren, C. Chèze, M. C. Dumon and D. S. Safford, *XVIIIe Journeés Internationales Groupe Polyphénols*, Vol. 2, INRA Editions, Paris, 1996, pp. 287–288.
46 F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1992, **40**, 1383–1389.
47 T. Tanaka, C. Mine, S. Watarumi, T. Fujioka, K. Mihashi, Y. J. Zhang and J. Kouno, *J. Nat. Prod.*, 2002, **65**, 1582–1587.
48 X. C. Wan, H. E. Nursten, Y. Cai, A. L. Davis, J. P. G. Wilkins and A. P. Davies, *J. Sci. Food Agric.*, 1997, **74**, 401–408.
49 F. Hashimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1986, **34**, 61–65.
50 A. L. Davis, J. R. Lewis, Y. Cai, C. Powell, A. P. Davies, J. P. G. Wilkins, P. Pudney and M. N. Clifford, *Phytochemistry*, 1997, **46**, 1397–1402.
51 P. Coggon, G. A. Moss, H. N. Graham and G. W. Sanderson, *J. Agric. Food Chem.*, 1973, **21**, 727–733.
52 A. G. Brown, W. B. Eyton, A. Holmes and W. D. Ollis, *Nature*, 1969, **221**, 742–744.
53 S. Sang, W. Tian, S. Meng, R. E. Stark, R. T. Rosen, C. S. Yang and C.-T. Ho, *Tetrahedron. Lett.*, 2002, **43**, 7129–7133; S. Sang, S. Tian, R. E. Stark, C. S. Yang and C.-T. Ho, *Bioorg.Med. Chem.*, 2004, **12**, 3009–3017.
54 T. Tanaka, Y. Betsumiya, C. Mine and J. Kouno, *Chem. Commun.*, 2000, 1365–1366; T. Tanaka, K. Inoue, Y. Betsumiya, C. Mine and J. Kouno, *J. Agric. Food Chem.*, 2001, **49**, 5785–5789.
55 T. Tanaka, Y. Matsuo and I. Kouno, *J. Agric. Food Chem.*, 2005, **53**, 7571–7578.
56 M. C. Menet, S. Sang, C. S. Yang, C.-T. Ho and R. T. Rosen, *J. Agric. Food Chem.*, 2004, **52**, 2455–2461.
57 T. Tanaka, S. Watarumi, M. Fujieda and I. Kouno, *Food Chem.*, 2005, **93**, 81–87.
58 S. L. Gosnay, J. A. Bishop, S. A. New, J. Catterick and M. N. Clifford, *Proc. Nutr. Soc.*, 2002, **61**, 125A; E. Woods, M. N. Clifford, M. Gibbs, S. Hampton, J. Arendt and L. Morgan, *Proc. Nutr. Soc.*, 2003, **62**, 60A.
59 W. Shao, C. Powell and M. N. Clifford, *J. Sci. Food Agric.*, 1995, **69**, 535–540; J.-K. Lin, C.-L. Lin, Y.-C. Liang, S.-Y. Lin-Shiao and I.-M. Juan, *J. Agric. Food Chem.*, 1998, **46**, 3635–3642; C. Lakenbrink, S. Lapczynski, B. Maiwald and U. H. Engelhardt, *J. Agric. Food Chem.*, 2000, **48**, 2848–2852; A. Luximon-Ramma, T. Bahorun, A. Crozier, V. Zbarsky, K. P. Datla, D. T. Dexter and O. I. Aruma, *Food Res. Int.*, 2005, **38**, 357–367.
60 A. J. Stewart, W. Mullen and A. Crozier, *Mol. Nutr. Food Res.*, 2005, **49**, 52.
61 D. Del Rio, A. J. Stewart, W. Mullen, J. Burns, M. E. J. Lean, F. Brighenti and A. Crozier, *J. Agric. Food Chem.*, 2004, **52**, 2807–2815.
62 H. Wang and K. Helliwell, *Food Chem.*, 2000, **70**, 337–341; R. Ito, A. Yamamoto, S. Kodama, K. Kato, Y. Yoshimura, A. Matsunaga and H. Nakazawa, *Food Chem.*, 2003, **83**, 563–568.
63 *Coffee: Botany, Biochemistry and Production of Beans and Beverage*, ed. M. N. Clifford and K. S. Willson, Croom Helm, London, 1985.
64 M. N. Clifford, *J. Sci. Food Agric.*, 1999, **79**, 362–372.
65 M. N. Clifford, S. Knight, B. Surucu and N. Kuhnert, *J. Agric. Food Chem.*, 2006, **54**, 1957–1969.
66 M. N. Clifford and S. Knight, *Food Chem.*, 2004, **87**, 457–463.
67 M. N. Clifford and T. Jarvis, *Food Chem.*, 1988, **9**, 291–298.
68 B. M. Scholz and H. G. Maier, *Z. Lebens.-Unters. Forschung*, 1990, **190**, 132–134; C. Bennat, U. H. Engelhardt, A. Kiehne and F. M. Wirries, *Z. Lebens.-Unters. Forschung*, 1994, **199**, 17–21; K. Schrader, A. Kiehne, U. H. Engelhardt and H. G. Maier, *J. Sci. Food Agric.*, 1996, **71**, 392–398; A. Farah, T. de Paulis and L. C. Trugo, *J. Agric. Food Chem.*, 2005, **53**, 1505–1513.
69 R. H. Stadler, D. H. Welti, A. A. Stämpfli and L. B. Fay, *J. Agric. Food Chem.*, 1996, **44**, 898–905.
70 G. G. Duthie and A. Crozier, in *Plants: Diet and Health*, ed. G. Goldberg, British Nutrition Foundation, Blackwell Publishing, Oxford, 2003, pp. 147–182.
71 J. F. Hammerstone, S. A. Lazarus, A. E. Mitchell, R. Rucker and H. H. Schmitz, *J. Agric. Food Chem.*, 1999, **47**, 490–496.
72 E. Haslam, *Practical Polyphenols – From Structure to Molecular Recognition and Physiological Action*, Cambridge University Press, Cambridge, 1998.
73 C. Sanbongi, N. Osakabe, M. Natsume, T. Takizawa, S. Gomi and T. Osawa, *J. Agric. Food Chem.*, 1998, **46**, 454–457.
74 T. Stark and T. Hofmann, *J. Agric. Food Chem.*, 2005, **53**, 5419–5428.
75 L. Gu, S. E. House, X. Wu, B. Ou and R. L. Prior, *J. Agric. Food Chem.*, 2006, **54**, 4057–4061; K. B. Miller, W. J. Hurst, M. J. Payne, D. A. Stuart, J. Apgar, D. S. Sweigart and B. Ou, *J. Agric. Food Chem.*, 2008, **56**, 8527–8533; R. Corder, *The Red Wine Diet*, Sphere, London, 2007; K. A. Cooper, E. Campos-Gimenez, A. D. Jimenez, K. Nagy, J. L. Donovan and G. Williamson, *J. Agric. Food Chem.*, 2007, **55**, 2841–2847.
76 J. L. Donovan, V. Crespy, M. Oliveira, K. A. Cooper, B. B. Gibson and G. Williamson, *Free Rad. Res.*, 2006, **40**, 1029–1034.
77 V. L. Singleton, in *Proceedings of the University of California, Davis, Grape and Wine Centennial Symposium*, ed. A. D. Webb, University of California Press, Davis, 1982, pp. 215–227.
78 J. Burns, P. T. Gardner, D. B. McPhail, J. O'Neil, S. Crawford, I. Morecroft, C. Lister, D. Matthews, M. R. MacLean, M. E. J. Lean, G. G. Duthie and A. Crozier, *J. Agric. Food Chem.*, 2000, **48**, 220–230.
79 S. Carando, P.-L. Teissedre, L. Pascual-Martinez and J.-C. Cabanis, *J. Agric. Food Chem.*, 1999, **47**, 4161–4166.
80 N. Landrault, N. Poucheret, P. Ravel, G. Gasc, G. Cros and P. L. Teissedre, *J. Agric. Food Chem.*, 2001, **49**, 3341–3348.
81 J. M. Ricardo da Silva, J.-P. Rosec, M. Bourzeix and N. Hereda, *J. Sci. Food Agric.*, 1990, **53**, 85–92.
82 B. Sun, C. Leandro, J. M. Ricardo da Silva and I. Spranger, *J. Agric. Food Chem.*, 1998, **46**, 1390–1396.
83 N. Mateus, A. M. S. Silva, C. Santos-Buelga, J. C. Rivas-Gonzalo and V. de Freitas, *J. Agric. Food Chem.*, 2002, **50**, 2110–2116.
84 N. Mateus, E. Carvalho, A. R. F. Carvalho, A. Melo, A. M. Gonzalez-Paramas, C. Santos-Buelga, A. M. Silva and V. de Freitas, *J. Agric. Food Chem.*, 2003, **51**, 277–282.
85 A. Morata, M. C. Gómes-Cordovés, B. Colomo and J. A. Suarez, *J. Agric. Food Chem.*, 2003, **51**, 7402–7409.
86 A. Waterhouse and P.-L. Teissedre, in *Wine: Nutritional and Therapeutic Benefits*, ed. T. R. Watkins, ACS Symposium Series 661, American Chemical Society, Washington, DC, 1997, pp. 12–23.
87 L. Gu, M. A. Kelm, J. F. Hammerstone, G. Beecher, J. Holden, D. Haytowitz and R. L. Prior, *J. Agric. Food Chem.*, 2003, **51**, 7513–7521.
88 J. F. Steven, A. W. Taylor and M. L Deinzer, *J. Chromatogr. A*, 1999, **832**, 97–107.
89 S. de Pascual-Teresa, C. Santos-Buelga and J. C. Rivas-Gonzalo, *J. Agric. Food Chem.*, 2000, **48**, 5331–5337.
90 A. G. H. Lea, in *Bitterness in Foods and Beverages*, ed. R. L. Rouseff, Elsevier Science, Amsterdam, 1990, pp. 123–144.
91 S. C. Marks, W. Mullen and A. Crozier, *J. Agric. Food Chem.*, 2007, **55**, 8723.
92 S. Guyot, N. Marnet, D. Laraba, P. Sanoner and J.-F. Drilleau, *J. Agric. Food Chem.*, 1998, **46**, 1698–1705.
93 A. G. H. Lea and G. M. Arnold, *J. Sci. Food Agric.*, 1978, **29**, 478–483.
94 G. del Campo, J. I. Santos, I. Berregi, S. Velasco, I. Ibarburu, M. T. Duenas and A. Irastorza, *J. Inst. Brewing*, 2003, **109**, 342–348.
95 A. G. H. Lea, *Ann. Nutr. l'Aliment.*, 1978, **32**, 1051–1061.
96 C. Santos-Buelga and A. Scalbert, *J. Sci. Food Agric.*, 2000, **80**, 1094–1117.
97 A. Podsedek, J. Wilskajeszka and B. Anders, *Polyphenol Commun.*, 1998, 319–320.

98 M. N. Clifford, K. L. Johnston, S. Knight and N. Kuhnert, *J. Agric. Food Chem.*, 2003, **51**, 2900–2911; K. Kahle, M. Kraus and E. Richling, *Mol. Nutr. Food Res.*, 2005, **49**, 797–806; S. C. Marks, W. Mullen and A. Crozier, *J. Sci. Food Agric.*, 2007, **87**, 719–728.
99 P. Sanoner, S. Guyot and J. F. Drilleau, *Polyphenol Commun.*, 1998, 401–403.
100 A. C. Galvis-Sánchez, A. Gil-Izquierdo and M. I. Gil, *J. Sci. Food Agric*, 2003, **83**, 995–1003.
101 G. A. Spanos and R. E. Wrolstad, *J. Agric. Food Chem.*, 1992, **40**, 1478–1487.
102 D. Ferreira, S. Guyot, N. Marnet, J. Delgadillo, C. M. Renard and M. A. Coimbra, *J. Agric. Food Chem.*, 2002, **50**, 4537–4544.
103 M. N. Clifford, in *Methods in Polyphenol Analysis*, ed. G. Williamson and C. Santos-Buelga, Royal Society of Chemistry, London, 2003, pp. 314–337.
104 Y.-J. Hong, D. M. Barrett and A. E. Mitchell, *J. Agric Food Chem.*, 2004, **52**, 2366–2371.
105 F. A. Tomás-Barberán and M. N. Clifford, *J. Sci. Food Agric.*, 2000, **80**, 1073.
106 N. Berardini, R. Carle and A. Schieber, *Rapid Comm. Mass Spec.*, 2004, **18**, 2208–2216.
107 M. Cojocaru, S. Droby, A. Glotter, A. Goldman, H. E. Gottlieb, B. Jacoby and D. Prusky, *Phytochemistry*, 1986, **25**, 1093–1095.
108 A. J. Núñez-Sellés, H. T. Vélez Castro, J. Agüero-Agüero, J. Gonzalez-Gonzalez, F. Naddeo, F. de Simone and L. Rastrelli, *J. Agric. Food Chem.*, 2002, **50**, 762–766.
109 N. Yoshimi, K. Matsunaga, M. Katayama, Y. Yamada, T. Kuno, Z. Qiao, A. Hara, J. Yamahara and H. Mori, *Cancer Lett.*, 2001, **163**, 163–170.
110 M. I. Gil, F. A. Tomás-Barberán, B. Hess-Pierce, D. M. Holcroft and A. A. Kader, *J. Agric. Food Chem.*, 2000, **48**, 4581–4589.
111 M. Aviram, M. Rosenblat, D. Gaitini, S. Nitecki, A. Hoffman, L. Dornfeld, N. Volkova, D. Presser, J. Attias, H. Liker and T. Hayek, *Clin. Nutr.*, 2004, **23**, 423–433; M. D. Sumner, M. Elliott-Eller, G. Weidner, J. J. Daubenmeir, M. H. Chew, R. Marlin, C. J. Raisin and D. Ornish, *Am. J. Cardiol.*, 2005, **96**, 810–814.
112 A. J. Pantuck, J. T. Leppert, N. Zomorodian, J. Hong, J. R. Barnard, N. Seeram, H. Lier, H. Wang, R. Elashoff, D. Heber, M. Aviram, L. Ignarro and A. Belldegrun, *Clin. Cancer Res.*, 2006, **12**, 4018–4026; M. Larrosa, F. A. Tomás-Barbéran and J. C. Espin, *J. Nutr. Biochem.*, 2006, **17**, 611–625.
113 C. Alasalvar, J. M. Grigorr and D. Zhang, *J. Agric. Food Chem.*, 2001, **49**, 1410–1416.
114 R. A. Cole, *Ann. Appl. Biol.*, 1985, **106**, 211–217.
115 W. Mullen, A. Boitier, A. J. Stewart and A. Crozier, *J. Chromatogr. A.*, 2004, **1058**, 163–168.
116 Y.-H. Chu, C.-L. Chang and H.-F. Hsu, *J. Sci. Food Agric.*, 2000, **80**, 561–566.
117 M. N. Clifford, *J. Sci. Food Agric.*, 2000, **80**, 1063–1072.
118 H. Donner, L. Gao and G. Mazza, *Food Res. Int.*, 1997, **30**, 637–643.
119 G. W. Plumb, K. R. Price, M. J. C. Rhodes and G. Williamson, *Free Rad. Res.*, 1997, **27**, 429–435; F. Vallejo and F. A. Tomás-Barberán, *J. Chromatogr. A*, 2004, **1054**, 181–193.
120 U. Kidmose, P. Knuthsen, M. Edelenbos, U. Justesen and E. Hegelund, *J. Sci. Food Agric.*, 2001, **81**, 918–923; E. Zane and S. H. Wender, *J. Org. Chem.*, 1961, **26**, 4718–4719.
121 A. J. Stewart, S. Bozonnet, W. Mullen, G. I. Jenkins, M. E. J. Lean and A. Crozier, *J. Agric. Food Chem.*, 2000, **48**, 2663–2669.
122 S. Barnes, M. Kirk and L. Coward, *J. Agric. Food Chem.*, 1994, **42**, 2466–2474.
123 L. Coward, N. C. Barnes, K. D. R. Setchell and S. Barnes, *J. Agric. Food Chem.*, 1993, **41**, 1961–1967.
124 J. Liggins, L. J. C. Bluck, S. Runswick, C. Atkinson, W. A. Coward and S. A. Bingham, *Brit. J. Nutr.*, 2000, **84**, 717–725; R. J. Fletcher, *Brit. J. Nutr.*, 2003, **89**, S30–S43.
125 J. Hempel and H. Böhm, *J. Agric. Food Chem.*, 1996, **44**, 2114–2116.
126 L. Gu, M. A. Kelm, J. F. Hammerstone, G. Beecher, J. Holden, D. Haytowitz, S. Gebhardt and R. L. Prior, *J. Nutr.*, 2004, **134**, 613–617.
127 M. N. Clifford, *J. Sci. Food Agric.*, 2000, **80**, 1126–1137.
128 N. Shimada, T. Aoki, S. Sato, Y. Nakamura, S. Tabata and S.-I. Ayabe, *Plant Physiol.*, 2003, **131**, 941–951; G. J. Tanner, K. T. Franki, S. Abrahams, J. M. Watson, P. J. Larkin and A. R. Ashton, *J. Biol. Chem.*, 2003, **278**, 31647–31656; J. Boatright, F. Negre, X. Chen, C. M. Kish, B. Wood, G. Peel, I. Oriova, D. Gang, D. Rhodes and N. Dudareva, *Plant Physiol.*, 2004, **135**, 1993–2011; L. Hoffman, S. Besseau, P. Geoffroy, C. Ritzenthaler, D. Meyer, C. Lapierre, B. Pollet and M. Legrand, *Plant Cell*, 2004, **16**, 1446–1465; R. A. Dixon, D. Y. Xie and S. B. Sharma, *New Phytol.*, 2005, **165**, 9–28; R. Niemetz and G. G. Gross, *Phytochemistry*, 2005, **66**, 2001–2011; D. Y. Xie and R. A. Dixon, *Phytochemistry*, 2005, **66**, 2127–2144; A. Crozier, I. B. Jaganath and M. N. Clifford, in *Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet*, ed. A. Crozier, M. N. Clifford and H. Ashihara, Blackwell Publishing, Oxford, 2006, pp. 1–24; J.-L. Ferrer, M. B. Austin, C. Stewart and J. P. Noel, *Plant Physiol. Biochem.*, 2008, **46**, 356–370.
129 R. Verpoorte and J. Memelink, *Curr. Opin. Biotechnol.*, 2002, **13**, 181–187.
130 P. Broun, *Curr. Opin. Plant Biol.*, 2004, **7**, 202–209.
131 W. Jung, O. Yu, S. M. Lau, D. P. O'Keefe, J. Odell, G. Fader and B. McGonigle, *Nat. Biotechnol.*, 2000, **18**, 208–212.
132 C.-H. Shih, Y. Chen, M. Wang, I. K. Chu and C. Lo, *J. Agric. Food Chem.*, 2008, **56**, 5655–5661.
133 S. R. Muir, G. J. Collins, S. Robinson, S. Hughes, A. Bovy, R. de Vos, A. J. van Tunen and M. E. Verhoeyen, *Nat. Biotechnol.*, 2001, **19**, 470–474.
134 A. G. Bovy, R. de Vos, M. Kemper, E. Schijlen, M. Almenar Pertejo, S. R. Muir, J. Collins, S. Robinson, M. E. Verhoeyen, S. G. Hughes and A. J. van Tunen, *Plant Cell*, 2002, **14**, 2509–2526.
135 J. Luo, E. Butelli, L. Hill, A. Parr, R. Niggeweg, P. Bailey, B. Weisshaar and C. Martin, *Plant J.*, 2008, **56**, 316–326.
136 E. Butelli, L. Titta, M. Giorgio, H. P. Mock, A. Matros, S. Peterek, E. G. Schijlen, R. D. Hall, A. G. Bovy, J. Luo and C. Martin, *Nat. Biotechnol.*, 2008, **26**, 1301–1308.
137 P. A. Lessard, H. Kulaveerasingam, G. M. York, A. Strong and A. J. Sinskey, *Metab. Engin.*, 2001, **4**, 67–79.
138 A. G. Bovy, J. Luo and C. Martin, *Nat. Biotechnol.*, 2008, **26**, 1301–1308.
139 K. Yonekura-Sakakibara and K. Saito, *Biotechnol Lett.*, 2006, **28**, 1983–1991.
140 J. L. Donovan, C. Manach, R. M. Faulks and P. Kroon in *Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet*, ed. A. Crozier, M. N. Clifford and H. Ashihara, Blackwell Publishing, Oxford, 2006, pp. 303–351.
141 A. J. Day, F. J. Canada, J. C. Diaz, P. A. Kroon, R. McLauchlan, C. B. Faulds, G. W. Plumb, M. R. Morgan and G. Williamson, *FEBS Lett.*, 2000, **468**, 166–170.
142 J. M. Gee, S. M. DuPont, A. J. Day, G. W. Plumb, G. Williamson and I. T. Johnson, *J. Nutr.*, 2000, **130**, 2765–2771.
143 G. Kottra and H. Daniel, *J. Pharmacol. Exp. Ther.*, 2007, **322**, 829–835.
144 C. Manach, G. Williamson, C. Morand, A. Scalbert and C. Rémésy, *Am. J. Clin. Nutr.*, 2005, **81**, 230S–242S.
145 W. Mullen, M.-A. Archeveque, C. A. Edwards, H. Matsumoto and A. Crozier, *J. Agric. Food Chem.*, 2008, **56**, 11157–11164.
146 L. Gu, M. Laly, H. C. Chang, R. L. Prior, N. Fang, M. J. J. Ronis and T. M. Badger, *J. Agric. Food Chem.*, 2005, **53**, 6858–6863.
147 W. Mullen, C. A. Edwards and A. Crozier, *Br. J. Nutr.*, 2006, **96**, 107–116.
148 P. C. H. Hollman, M. S. van der Gaag, M. J. B Mengelers, J. M. P. van Trjip, J. H. M. de Vries and M. B. Katan, *Free Rad. Biol. Med.*, 1996, **21**, 703–707; P. C. H. Hollman, J. M. P. van Trjip, M. N. C. P. Buysman, M. S. van der Gaag, M. J. B Mengelers, J. H. M. de Vries and M. B. Katan, *FEBS Lett.*, 1997, **48**, 152–156; E. U. Graefe, J. Wittig, S. Mueller, A.-K. Riethling, B. Ueheke, B. Drewelow, H. Pforte, G. Jacobasche, H. Derendorf and M. Veit, *J. Clin. Pharmacol.*, 2001, **41**, 492–499.
149 I. B. Jaganath, W. Mullen, C. A. Edwards and A. Crozier, *Free Rad. Res.*, 2006, **40**, 1035–1046.
150 K. A. O'Leary, A. J. Day, P. W. Needs, F. A. Mellon, N. M. O'Brien and G. Williamson, *Biochem. Pharmacol.*, 2003, **65**, 479–491.
151 B. Ameer, R. A. Weintraub, J. V. Johnson, R. A. Yost and R. L. Rouseff, *Clin. Pharm. Ther.*, 1996, **60**, 34–40; I. Erlund, E. Meririnne, G. Alfthan and A. Aro, *J. Nutr.*, 2001, **131**, 235–241; C. Manach, C. Morand, A. Gil-Izquierdo, C. Bouteloup-Demange and C. Rémésy, *Eur. J. Clin. Nutr.*, 2003, **57**, 235–242.
152 S. Roowi, W. Mullen, C. A. Edwards and A. Crozier, *Mol. Nutr. Food Res.*, 2009, **53**, in press.

153 S. C. Marks, W. Mullen, G. Borges and A. Crozier, *J. Agric. Food Chem.*, 2009, **57**, 2009–2015.
154 A. Stalmach, S. Troufflard, M. Serafini and A. Crozier, *Mol. Nutr. Food Res.*, 2009, **53**, in press.
155 S. Shahrzad and I. Bitsch, *J. Chromatogr. B*, 1998, **705**, 87–95; S. Shahrzad, K. Aoyagi, A. Winter, Koyama and I. Bitsch, *J. Nutr.*, 2001, **131**, 1207–1210.
156 C. Auger, W. Mullen, Y. Hara and A. Crozier, *J. Nutr.*, 2008, **138**, 1535S–1542S.
157 J. L. Donovan, V. Crespy, M. Oliveira, K. A. Cooper, B. B. Gibson and G. Williamson, *Free Rad. Res.*, 2006, **40**, 1029–1034.
158 S. M. Henning, Y. Niu, Y. Liu, N. H. Lee, Y. Hara, G. D. Thames, R. R. Minutti, C. L. Carpenter, H. Wang and D. Heber, *J. Nutr. Biochem.*, 2005, **16**, 610–616; H. H. Chow, Y. Cai, D. S. Alberts, I. Hakim, R. Dorr, F. Shai, J. A. Crowell, C. S. Yang and Y. Hara, *Cancer Epidemiol. Biomarkers Prev.*, 2001, **10**, 53–58; T. Unno, K. Kondo, H. Itakura and T. Takeo, *Biosci. Biotechnol. Biochem.*, 1996, **60**, 2066–2068.
159 T. Kida, Y. Suzuki, N. Matsumoto, F. Nanjo and Y. Hara, *J. Agric. Food Chem.*, 2000, **48**, 4151–4155; T. Kohri, F. Nanjo, M. Suziki, R. Seto, N. Matsumoto, M. Yamakawa, H. Hojo, Y. Hara, D. Desai, S. Amin, C. C. Conaway and F.-L. Chung, *J. Agric. Food Chem.*, 2001, **49**, 1042–1048.
160 E. Sergediene, K. Jonsson, H. Szymusiak, B. Tyrakowska, J. M. Rietjens and N. Cenas, *FEBS Lett.*, 1999, **462**, 392–396.
161 V. Crespy, C. Morand, C. Besson, N. Cotelle, H. Vezin, C. Demigne and C. Remesy, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2003, **284**, G980–G988.
162 J. A. Rothwell, A. J. Day and M. R. Morgan, *J. Agric. Food Chem.*, 2005, **53**, 4355–4360.
163 http://www.emolecules.com/cgi-bin/search.
164 X. J. Tian, X. W. Yang, X. Yang and K. Wang, *Int. J. Pharm.*, 2009, **367**, 58–64.
165 Q. Y. Zhu, A. Q. Zhang, D. Tsang, Y. Huang and Z. Y. Chen, *J. Agric. Food Chem.*, 1997, **45**, 4624–4628; K. Yoshino, M. Suzuki, K. Sasaki, T. Miyase and M. Sano, *J. Nutr. Biochem.*, 1999, **10**, 223–229; R. Record and J. M. Lane, *Food Chem.*, 2001, **73**, 481–486; R. J. Green, A. S. Murphy, B. Schulz, B. A. Watkins and M. G. Ferruzzi, *Mol. Nutr. Food Res.*, 2007, **51**, 1152–1162.
166 L. Gu, M. A. Kelm, J. F. Hammerstone, G. Beecher, J. Holden, D. Hayytowitz, S. Gebhardt and R. L. Prior, *J. Nutr.*, 2004, **134**, 613–617.
167 L. Packer, G. Rimbach and F. Virgili, *Free Rad. Biol. Med.*, 1999, **27**, 704–724.
168 P. Simonetti, S. Ciappellano, C. Gardana, L. Bramati and P. Pietta, *J. Agric. Food Chem.*, 2002, **50**, 6217–6221.
169 J. Yamakoshi, S. Kataoka, T. Koga and T. Ariga, *Atherosclerosis*, 1999, **142**, 139–149.
170 J. A. Bomser, K. W. Sinletary, M. A. Wallig and M. A. Smith, *Cancer Lett.*, 1999, **135**, 151–157.
171 G. Williamson and C. Manach, *Am. J. Clin. Nutr.*, 2005, **81**, 243S–255S.
172 J. C. Espín, M. T. García-Conesa and F. A. Tomás-Barberán, *Phytochemistry*, 2007, **68**, 2986–3008.
173 S. Déprez, C. Brezillon, S. Rabot, C. Phiippe, I. Mila, C. Lapierre and A. Scalbert, *J. Nutr.*, 2000, **130**, 2733–2738; M.-P. Gonthier, J. L. Donovan, O. Texier, T. Felgines, C. Rémésy and A. Scalbert, *Free Rad. Biol. Med.*, 2003, **35**, 837–844; M. M. Appeldoorn, V. P. Vincken, A. M. Aura, P. C. Hollman and H. Gruppen, *J. Agric. Food Chem.*, 2009, **57**, 1084–1092.
174 N. C. Ward, K. D. Croft, I. B. Pudley and J. M. Hodgson, *J. Agric. Food Chem.*, 2004, **52**, 5545–5549.
175 J. P. E. Spencer, F. Chaudry, A. S. Pannala, S. K. Srai, E. Debnam and C. Rice-Evans, *Biochem. Biophys. Res. Commun.*, 2000, **272**, 236–241.
176 J. L. Donovan, C. Manach, L. Rios, C. Morand, A. Scalbert and C. Rémésy, *Br. J. Nutr.*, 2002, **87**, 299–306; L. Y. Rios, R. N. Bennett, S. A. Lazarus, C. Rémésy, A. Scalbert and G. Williamson, *Am. J. Clin. Nutr.*, 2002, **76**, 1106–1110; C. Tsang, C. Auger, A. Bornet, J.-M. Rouanet, A. Crozier and P.-L. Teissedre, *Br. J. Nutr.*, 2005, **94**, 170–181.
177 A. Sano, J. Yamakoshi, S. Tokutake, K. Tobe, Y. Kubota and M. Kikuchi, *Biosci. Biotechnol. Biochem.*, 2003, **77**, 1149–1143.
178 R. R. Holt, S. A. Lazarus, M. C. Sullards, Q. Y. Zhu, D. D. Schramm, J. F. Hammerstone, C. G. Fraga, H. H. Schmitz and C. L. Keen, *Am. J. Clin. Nutr.*, 2002, **76**, 798–804.
179 R. Corder, *Heart*, 2008, **94**, 821–823.
180 T. Shoji, S. Masumoto, N. Moriichi, H. Akiyama, T. Kanad, Y. Ohtake and Y. Goda, *J. Agric. Food Chem.*, 2006, **54**, 884–892.
181 T. K. McGhie, G. D Ainge, L. E. Barnett, J. M. Cooney and J. D. Jensen, *J. Agric. Food Chem.*, 2003, **51**, 4539–4548.
182 T. Miyazawa, K. Nakagawa, M. Kudo, K. Muraishi and K. Someya, *J. Agric. Food Chem.*, 1999, **47**, 1083–1091; P. Milbury, G. Cao, R. L. Prior and J. Blumberg, *Mech. Ageing Develop.*, 2002, **123**, 997–1006; J. M. Cooney, J. D. Jensen and T. K. McGhie, *J. Sci. Food Agric.*, 2004, **84**, 237–245; T. Ichiyanagi, Y. Shida, M. M. Rahman, Y. Hatano and T. Konishi, *J. Agric. Food Chem.*, 2005, **53**, 7312–7319.
183 X. Wu, H. E. Pittman, S. McKay and R. L. Prior, *J. Nutr.*, 2005, **135**, 2417–2424.
184 R. L. Prior and X. Wu, *Free Rad. Res.*, 2006, **40**, 1014–1028.
185 M. J. Cho, L. R. Howard, R. L. Prior and J. R. Clark, *J. Sci. Food Agric.*, 2004, **84**, 1771–1782.
186 X. Wu, G. Beecher, J. M. Holden, D. B. Haytowitz, S. E. Gebhardt and R. L. Prior, *J. Agric. Food Chem.*, 2006, **54**, 4069–4075.
187 W. Mullen, C. A. Edwards, M. Serafini and A. Crozier, *J. Agric. Food Chem.*, 2008, **56**, 713–719.
188 C. Felgines, S. Talavéra, M.-P. Gonthier, O. Texier, A. Scalbert, J.-L. Lamaison and C. Rémésy, *J. Nutr.*, 2003, **133**, 1269–1301.
189 C. Carkeet, B. A. Clevidence and J. A. Novotny, *J. Nutr.*, 2008, **138**, 897–902.
190 C. Felgines, S. Talavéra, O. Texier, A. Gil-Izquierdo, J.-L. Lamaison and C. Rémésy, *J. Agric. Food Chem.*, 2005, **53**, 7721–7727.
191 M. N. Clifford, *J. Sci. Food Agric.*, 2000, **80**, 1063–1072.
192 C. E. Rüfer, A. Bub, J. Möseneder, P. Winterhalter, M. Stürtz and S. E. Kulling, *Am. J. Clin. Nutr.*, 2008, **87**, 1314–1323.
193 T. Izumi, M. K. Piskula, S. Osawa, A. Obata, K. Tobe, M. Saito, S. Kataoka, Y. Kubota and M. Kikuchi, *J. Nutr.*, 2000, **130**, 1695–1699.
194 K. Hosoda, T. Furuta, A. Yokokawa, K. Ogura, A. Hiratsuka and K. Ishii, *Drug Metab. Dispos.*, 2008, **36**, 1485A–1495A.
195 A. Tamura, T. Shiomi, S. Hachiya, N. Shigematsu and H. Hara, *Clin. Nutr.*, 2008, **27**, 248–253.
196 K. R. Walsh, S. J. Haak, T. Bohn, Q. Tian, S. J. Schwartz and M. L. Failla, *Am. J. Clin. Nutr.*, 2007, **85**, 1050–1056; X. L. Wang, H. G. Hur, J. H. Lee, K. T. Kim and S. I. Kim, *Appl. Environ. Microbiol.*, 2005, **71**, 214–219.
197 B. Cerdá, J. C. Espín, S. Parra, P. Martínez and F. A. Tomás-Barberán, *Eur. J. Nutr.*, 2004, **43**, 205–220.
198 N. P. Seeram, S. M. Henning, Y. Zhang, M. Suchard, Z. Li and D. Heber, *J. Nutr.*, 2006, **136**, 2481–2485.
199 S. U. Mertens-Talcott, P. Jilma-Stohlawetz, J. Rios, L. Hingorani and H. Derendorf, *J. Agric. Food Chem.*, 2006, **54**, 8956–8961.
200 B. Cerdá, F. A. Tomás-Barberán and J. C. Espín, *J. Agric. Food Chem.*, 2005, **53**, 227–235.
201 J. C. Espin, R. González-Barrio, B. Cerdá, C. López-Bote, A. I. Rey and F. A. Tomás-Barberán, *J. Agric. Food Chem.*, 2007, **55**, 10476–10485.
202 M. G. Hertog, D. Kromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli, A. Jansen, A. Menotti and S. Nedeljkovic, *Arch. Intern. Med.*, 1995, **155**, 381–386.
203 M. G. Hertog, E. J. Feskens and D. Kromhout, *Lancet*, 1997, **349**, 699; I. C. Arts and P. C. Hollman, *Am. J. Clin. Nutr.*, 2005, **81**, 317S–325S.
204 T. Hirvonen, J. Virtamo, P. Korhonen, D. Albanes and P. Pietinen, *Cancer Causes Control*, 2001, **12**, 789–796.
205 P. Knekt, J. Kumpulainen, R. Jarvinen, H. Rissanen, M. Heliovaara, A. Reunanen, T. Hakulinen and A. Aromaa, *Am. J. Clin. Nutr.*, 2002, **76**, 560–568.
206 C. Tabak, I. C. W. Arts, H. A. Smit, D. Heederik and D. Kromhout, *Am. J. Respir. Crit. Care Med.*, 1999, **164**, 61–64.
207 R. J. Williams, J. P. E. Spencer and C. Rice-Evans, *Free Rad. Biol. Med.*, 2004, **6**, 838–849.
208 B. Halliwell, J. Rafter and A. Jenner, *Am. J. Clin. Nutr.*, 2005, **81**, 268S–276S; T. Schewe, Y. Steffen and H. Sies, *Arch. Biochem. Biophys.*, 2008, **476**, 102–106.
209 M. N. Clifford, *Planta Med.*, 2004, **12**, 1103–1114; A. Johnson, I. T. Johnson and M. Saltmarsh, *Am. J. Clin. Nutr.*, 2005, **81**, 215S–217S.
210 C. Manach, A. Scalbert, C. Morand, C. Remesy and L. Jimenez, *Am. J. Clin. Nutr.*, 2004, **79**, 727–747.

211 M. Sazuka, T. Itoi, Y. Suzuki, S. Odani, T. Koide and M. Isemura, *Biosci. Biotechnol. Biochem.*, 1996, **60**, 1317–1319.
212 J. K. Kundu and Y.-K. Surh, *Cancer Lett.*, 2008, **269**, 243–261.
213 M. Vanharanta, S. Voutilainen, T. A. Lakka, M. van der Lee, H. Adlercreutz and J. T. Salonen, *Lancet*, 1999, **354**, 2112–2115.
214 H. Adlercreutz, *Lancet Oncol.*, 2002, **3**, 364–373; A. L. Webb and M. L. McCullough, *Nutr. Cancer*, 2005, **51**, 117–131.
215 K. Hultén, A. Winkvist, P. Lenner, R. Johansson, H. Adlercreutz and G. Hallmans, *Eur. J. Nutr.*, 2002, **41**, 168–176.
216 A. Kuijsten, I. C. W Arts, P. C. H. Hollman, P. van't Veer and E. Kampman, *Cancer Epidemiol. Biomarkers Prev.*, 2006, **15**, 1132–1136.
217 P. Stattin, H. Adlercreutz, L. Tenkanen, E. Jellum, S. Lumme, G. Hallmans, S. Harvei, L. Teppo, K. Stumpf, T. Luostarinen, M. Lehtinen, J. Dillner and M. Hakama, *Int. J. Cancer.*, 2002, **99**, 124–129.
218 A. M. Kivela, E. Kansanen, H. K. Jyrkkanen, T. Nurmi, S. Yla-Herttuala and A. L. Levonen, *J. Nutr.*, 2008, **138**, 1263–1268; L. H. Chen, J. Fang, H. Li, W. Demark-Wahnefried and X. Lin, *Mol. Cancer Ther.*, 2007, **6**, 2581–2590.
219 L. Q. Wang, *J. Chromatogr. B*, 2002, **777**, 289–309.
220 A. R. Rechner and C. Kroner, *Thromb. Res.*, 2005, **116**, 327–334.
221 K. D. R. Setchell, C. Clerici, E. D. Lephart, S. J. Cole, C. Heenan, D. Castellani, B. E. Wolfe, L. Nechemias-Zimmer, N. M. Brown, T. D. Lund, R. J. Handa and J. E. Heubi, *Am. J. Clin. Nutr.*, 2005, **81**, 1072–1077.
222 R. S. Muthyala, Y. H. Ju, S. Sheng, L. D. Williams, D. R. Doerge, B. S. Katzenellembogen, W. G. Helferich and K. A. Katzenellenbogenet, *Bioorg. Med. Chem.*, 2004, **12**, 1559–67.
223 Y. Muñoz, A. Garrido and L. Valladares, *Thrombosis Res.*, 2008, **123**, 740–744.
224 K. D. Setchell, M. S. Faughnan, T. Avades, L. Zimmer-Nechemias, N. M. Brown, B. E. Wolfe, W. T. Brashear, E. Desai, M. R. Oldfield, N. E. Botting and A. Cassidy, *Am. J. Clin. Nutr.*, 2003, **77**, 411–419.
225 M. Suganuma, S. Okabe, M. Oniyama, Y. Tada, H. Ito and H. Fujiki, *Carcinogenesis*, 1998, **19**, 1771–1776; X. Vitrac, A. Desmouliere, B. Brouillaud, S. Krisa, G. Deffieux, N. Barthe, J. Rosenbaum and J.-M. Merillon, *Life Sci.*, 2003, **72**, 2219–2233.
226 K. P. Datla, M. Christidou, W. W. Widmer, H. K. Rooprai and D. T. Dexter, *Neuroreport*, 2001, **12**, 3871–3875; M. M. Abd El Mohsen, G. Kuhnle, A. R. Rechner, H. Schroeter, S. Rose, P. Jenner and C. A. Rice-Evans, *Free Radic. Biol. Med.*, 2002, **33**, 1693–1702.
227 N. G. Coldham and M. J. Sauer, *Toxicol. Appl. Pharmacol.*, 2000, **164**, 206–215; S. Kim, M. J. Lee, J. Hong, C. Li, T. J. Smith, G. Y. Yang, D. N. Seril and C. S. Yang, *Nutr. Cancer*, 2000, **37**, 41–48.
228 W. Mullen, J.-M. Rouanet, C. Auger, P.-L. Teissedre, S. T. Caldwell, R. C. Hartley, M. E. J. Lean, C. A. Edwards and A. Crozier, *J. Agric. Food Chem.*, 2008, **56**, 12127–12137.
229 S. J. Hong, S. I. Kim, S. M. Kwon, J. R. Lee and B. C. Chung, *Yonsei Med. J.*, 2002, **43**, 236–241.
230 J. Maubach, M. E. Bracke, A. Heyerick, H. T. Depypere, R. F. Serreyn, M. M. Mareel and D. de Keukeleire, *J. Chromatogr. B*, 2003, **784**, 137–144.
231 S. M. Henning, W. Aronson, Y. Niu, F. Conde, N. H. Lee, N. P. Seeram, L. Ru-Po, J. Lu, D. M. Harris, A. Moro, J. Hong, L. Pak-Shan, R. J. Barnard, H. G. Ziaee, G. Csathy, V. L. W. Go, H. Wang and D. Heber, *J. Nutr.*, 2006, **136**, 1839–1843.
232 Y. Kawai, H. Tanaka, K. Murota, M. Naito and J. Terao, *Biochem. Biophys. Res. Commun.*, 2008, **374**, 527–532.
233 Y. Kawai, T. Nishikawa, Y. Shiba, S. Saito, K. Murota, N. Shibata, M. Kobayashi, M. Kanayama, K. Uchida and J. Terao, *J. Biol. Chem.*, 2008, **283**, 9424–9434.
234 S. Donnini, F. Finetti, L. Lusini, L. Morbidelli, V. Cheynier, D. Barron, G. Williamson, J. Waltenberger and M. Ziche, *Br. J. Nutr.*, 2006, **95**, 1016–1023.
235 Y. Yamamoto and R. B. Gaynor, *J. Clin. Invest.*, 2001, **107**, 135–142; J. W. Christman, R. T. Sadikot and T. S. Blackwell, *Chest*, 2000, **117**, 1482–1487.
236 M. F. Neurath, I. Fuss, G. Schurmann, S. Pettersson, K. Arnold, H. Muller-Lobeck, W. Strober, C. Herfarth and K. H. Buschenfelllde, *Ann. N.Y. Acad. Sci.*, 1998, **859**, 149–159; S. Schreiber, S. Nikolaus and J. Hampe, *Gut*, 1998, **42**, 477–484.
237 S. Singh and B. B. Aggarwal, *J. Biol. Chem.*, 1995, **270**, 4995–5000.
238 S. K. Manna, A. Mukhopadhyay and B. B. Aggarwal, *J. Immunol.*, 2000, **64**, 6509–6519.
239 F. Yang, H. S. Oz, S. Barve, W. J. de Villiers, C. J. McClain and G. W. Varilek, *Mol. Pharmacol.*, 2001, **60**, 528–533.
240 S. Krishnan, S. K. Sandur, S. Shentu and B. B Aggarwal, *Int. J. Rad. Oncol. Biol. Phys.*, 2006, **66**, S547.
241 B. B. Aggarwal and S. Shishodia, *Biochem. Pharm.*, 2006, **71**, 1397–1421.
242 P. H. Shih, C. T. Yeh and G. C. Yen, *Food Chem. Toxicol.*, 2005, **55**, 1557–1566.
243 P. H. Shih, C. T. Yeh and G. C. Yen, *J. Agric. Food Chem.*, 2007, **55**, 9427–9435; K. W. Singletary, K. J. Jung and M. Giusti, *J. Med. Food.*, 2007, **10**, 244–251.
244 S. Ramos, *Mol. Nutr. Food Res.*, 2008, **52**, 507–526.
245 R. Feng, Y. Lu, L. Bowman, Y. Qian, V. Castranova and M. Ding, *Biol. Chem.*, 2005, **280**, 27888–27895.
246 S. Chandra and E. Gonzalez de Mejia, *J. Agric. Food Chem.*, 2004, **52**, 3583–3589.
247 A. Kobayashi, T. Ohta and M. Yamamoto, *Methods Enzymol.*, 2004, **378**, 273–86.
248 C.-T. Yeh, L.-C. Ching and G. C. Yen, *J. Nutr. Biochem.*, 2009, **20**, 163–171.
249 J. S. Lee and Y. J. Surh, *Cancer Lett.*, 2005, **224**, 171–184.
250 L. Chang and M. Karin, *Nature*, 2001, **410**, 37–40; Z. Chen, T. B. Gibson, F. Robinson, L. Silvestro, G. Pearson, B. Xu, A. Wright, C. Vanderbilt and M. H. Cobb, *Chem. Rev.*, 2001, **101**, 2449–2476; G. M. Thomas and R. L. Huganir, *Nat. Rev. Neurosci.*, 2004, **5**, 173–183.
251 C. Chen, R. Yu, E. D. Owuor and A. N. Kong, *Arch. Pharm. Res.*, 2000, **23**, 605–612.
252 C. Pasten, N. C. Olave, L. Zhou, E. M. Tabengwa, P. E. Wolkowicz and H. E. Grenett, *Thrombosis Res.*, 2007, **121**, 59–65.
253 J. Ahn, H. Lee, S. Kim, J. Park and T. Ha, *Biochem, Biophys. Res. Comm.*, 2008, **373**, 545–549.
254 Anonymous, *Epidemiology*, 2003.
255 G. J. Kapadia, G. S. Rao and J. F. Morton, *Carcinogens and Mutagens in the Environment*, 1983, **3**, 3–12; J. F. Morton, in *Chemistry and Significance of Condensed Tannins*, ed. R. W. Hemingway and J. J. Karchesy, Plenum Press, New York, 1989, pp. 403–415; J. F. Morton, *Basic Life Sci.*, 1992, **59**, 739–765.
256 M. N. Clifford and J. R. Ramìrez-Martìnez, *Food Chem.*, 1990, **35**, 13–21.
257 *Coffee, Tea, Maté, Methylxanthines and Glyoxal*, IARC Monographs: The Evaluation of Carcinogenic Risks to Humans, Vol 51, IARC, Lyons, France, 1991.
258 R. M. van Dam and E. J. Feskens, *Lancet*, 2002, **360**, 1477–1478; A. Saremi, M. Tulloch-Reid and W. C. Knowler, *Diabetes Care*, 2003, **26**, 2211–2212; E. E. Agardh, S. Carlsson, A. Ahlbom, S. Efendic, V. Grill, N. Hammar, A. Hilding and C. G. Ostenson, *J. Intern. Med.*, 2004, **255**, 645–652; A. Rosengren, A. Dotevall, L. Wilhelmsen, D. Thelle and S. Johansson, *J. Intern. Med.*, 2004, **255**, 89–95; E. Salazar-Martinez, W. C. Willett, A. Ascherio, J. E. Manson, M. F. Leitzmann, M. J. Stampfer and F. B. Hu, *Ann. Internal Med.*, 2004, **140**, 1–8; J. Tuomilehto, G. Hu, S. Bidel, J. Lindstrom and P. Jousilahti, *J.A.M.A.*, 2004, **291**, 1213–1219.
259 K. L. Johnston, M. N. Clifford and L. M. Morgan, *Am. J. Clin. Nutr.*, 2003, **78**, 728–733; M. F. McCarty, *Med. Hypotheses*, 2005, **64**, 848–853; T. Ranheim and B. Halvorsen, *Mol. Nutr. Food Res.*, 2005, **49**, 274–284.
260 M. G. L. Hertog, E. J. M. Feskens, P. C. H. Hollman, M. B. Katan and D. Kromhout, *Lancet*, 1993, **342**, 1007–1011.
261 M. G. L. Hertog, P. M. Sweetman, A. M. Fehily, P. C. Elwood and D. Kromhout, *Am. J. Clin. Nutr.*, 1997, **65**, 1489–1494.
262 Anonymous, *Clinical Epidemiology Glossary*, 2003; http://www.med.ualberta.ca/ebm/define.htm.
263 M. N. Clifford and J. E. Brown, in *Flavonoids: Chemistry, Biochemistry and Applications*, ed. Ø. Andersen and K. R. Markham, CRC Press, Boca Raton, FL, 2005, pp. 320–370.
264 A. J. Day, F. Mellon, D. Barron, G. Sarrazin, M. R. A. Morgan and G. Williamson, *Free Rad. Res.*, 2001, **35**, 941–952.
265 J. Jankun, S. H. Selman, R. Swiercz and E. Skrzypczak-Jankun, *Nature*, 1997, **387**, 561–561.
266 C. S. Yang, *Nature*, 1997, **389**, 134–135.