Volume 1 | Number 1 | Jan 2013 | Pages 1–100

Food & Function

Linking the chemistry and pyhsics of food with health and nutrition
www.rsc.org/foodfunction

ROYAL SOCIETY OF CHEMISTRY

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Coffee: Biochemistry and Potential Impact on Health

Iziar A. Ludwig[a], Michael N. Clifford[b], Michael E.J. Lean[c], Hiroshi Ashihara[d] and Alan Crozier[a*]

[a]Plant Products and Human Nutrition Group, North Laboratory, Joseph Black Building, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

[b] School of Biosciences and Medicine, University of Surrey, Guildford GU2 5XH, U.K.

[c]University of Glasgow College of Medical, Veterinary and Life Sciences, New Lister Building, Glasgow G31 2ER, U.K.

[d]Department of Biological Sciences, Ochanomizu University, Bunkyo-ku, Tokyo 112-8610, Japan

*Corresponding author: Tel: -44-141-330-4613

E-mail: alan.crozier@glasgow.ac.uk

Abstract

This review provides details on the phytochemicals in green coffee beans and the changes

that occur during roasting. Key compounds in the coffee beverage, produced from the

ground, roasted beans, are volatile constituents responsible for the unique aroma, the

alkaloids caffeine and trigonelline, chlorogenic acids, the diterpenes cafestol and kahweol,

and melanoidins, which are Maillard reaction products. The fate of these compounds in the

body following consumption of coffee is discussed along with evidence of the mechanisms

by which they may impact on health. Finally, epidemiological findings linking coffee

consumption to potential health benefits including prevention of several chronic and

degenerative diseases, such as cancer, cardiovascular disorders, diabetes, and Parkinson's

disease, are evaluated.

1. Introduction

Myth and legend has it that coffee produced from the red berries of a small broad-leaved shrub was discovered by a goat herder around 850 AD in the Kefa region of North Africa, which is now part of Ethiopia.[1] It was spread by the slave trade to the Arabic empires where the drink derived from aqueous infusion of the dry seeds gained in popularity due to the Muslim ban on fermented alcoholic beverages. It was introduced into Europe in the 16th century. The coffee beverage is now an important item in the lives of billions of people and is one of the most traded food products in the world, with the brew being the second most consumed drink after water.[1,2] Coffee is cultivated commercially in plantations in places as widely separated as Hawaii, Jamaica, Ethiopia, Kenya, Brazil and Vietnam. The mature red coffee berries (Fig. 1) are picked and the outer pulp, the pericarp, removed either by soaking in water or, alternatively, the berries are laid out to dry after which the dried outer coating, the parchment, is removed by abrasion. With both methods the removal of the covering reveals the green coffee beans (Fig. 2), the cotyledons of the seed. The beans are roasted to a dark brown/black colour and ground to a powder which is infused with hot water, in an increasing variety of ways, to produce what we know as "a cup of coffee".[1]

The generic name *Coffea* covers approximately seventy species. Small-scale cultivation of *C. liberica*, *C. racemosa* and *C. dewevrei* and other species occurs in some African countries but the resultant beverages are generally of low quality and most of the beans are sold locally rather than exported.[3] Two species are of economic importance, namely *Coffea arabica*, commonly known as Arabica coffee, which accounts for ~60% of world production, with the remaining ~40% coming from *Coffea canephora* var. Robusta,[4] which is used to produce Robusta coffee. Differences between these two species include: ideal growing climate, physical aspects, chemical composition, and characteristics of the beverage. In general, Arabica coffee brew is appreciated for its superior cup quality and

aroma, whereas Robusta brew possesses a more aggressive flavour and contains higher amounts of soluble solids, antioxidants and caffeine.[5] Chemical composition of a coffee brew depends, besides variety, on several other factors, such as climatic and processing conditions, roasting, grinding and barista procedures. The pleasant aroma, taste, and brown colour of brewed coffee are a consequence of the roasting process that leads to profound changes in the chemical composition of coffee.[6]

Although coffee is consumed primarily because of its pleasant flavour and stimulating properties, more recent investigations indicate potential health benefits associated with the beverage, including a reduced incidence of several chronic and degenerative diseases, such as cancer, cardiovascular (CV) disorders, diabetes, and Parkinson's disease.[7–9] There is, therefore, increasing interest in the characterization of specific bioactive compounds in coffee that might serve as physiologically active agents. The bioactive classes of compounds in coffee are considered to be chlorogenic acids (CGAs) (Fig. 3), caffeine, the pentacyclic diterpenes cafestol and kahweol (Fig. 4), trigonelline (Fig 5) and melanoidins.

2. Composition and nutritional characteristics

If coffee beverage in any of its forms is to influence consumer health for better or worse then the effects must be produced by a component(s) in the beverage not otherwise present in the diet or provided by coffee in such quantity that other dietary sources are insignificant by comparison. The composition of the green bean is well documented and has been reviewed on several occasions, [10–13] so only the main points and recent advances are summarised here.

Green coffee is dominated by carbohydrates (~60 % dry weight), including soluble and insoluble polysaccharides (cellulose, arabinogalactan, and galactomannan) slightly higher in Robustas, oligosaccharides (stachyose and raffinose), disaccharides (sucrose) and

monosaccharides (glucose, galactose, arabinose, fructose, mannose, manitol, xylose, and ribose) some of which are associated primarily with contaminating husk and pericarp rather than the bean.[14,15] The lipid content of green coffee accounts for 8-18 % of its dry matter, with Arabicas being significantly higher than Robustas. The coffee lipid fraction consists of 75 % triglyceride, other lipids are sterols (stigmasterol, sitosterol), fatty acids (linoleic, linolenic, oleic, palmitic, stearic, arachidic, lignoceric and behenic), with pentacyclic diterpenes (cafestol, kahweol) and fatty acyl tryptamides in the coffee wax.[16] Proteins, peptides, and free amino acids account for 9-16% of the dry weight of green coffee beans. The main amino acids, both protein-bound and free, are asparagine, glutamic acid, alanine, aspartic acid and lysine.[15] In addition to caffeine and trigonelline, coffee contains a number of other *N*-containing compounds such as theobromine and theophylline (Fig. 5).

Green coffee also contains a variety of (poly)phenols which account for ~6–10% of the dry weight, higher in Robustas than Arabicas. The main components are CGAs among which the caffeoylquinic acids (CQAs), especially 5-CQA, dominate along with lower amounts of feruloylquinic acids and dicaffeoylquinic acids (Fig. 3). They are accompanied by over 50 other structurally related cinnamoylquinic acids and some cinnamoyl-amino acid conjugates and cinnamoyl-glycosides,[17–21] as recently reviewed by Stalmach et al.[13] Some cinnamoylshikimic acids have also been characterised and quantified.[22]

Whereas green coffee has a mild, bean-like aroma, the desirable fragrance associated with coffee beverages develops during roasting. Typical roasting uses air temperatures in the range from 180-250 °C, and roasting time can vary between 25 min at the lower temperatures to 2 min at the higher end of the scale, depending on the technique employed and desired degree of roasting (Parliment, 2000; Belitz et al., 2009).[6,23] The later stages of the roasting process are exothermic and a significant amount of carbon dioxide and other vapours are produced raising the coffee bean internal pressure to an estimated 5 to 7 atmospheres, with an

internal temperature exceeding 180 °C. The coffee bean, thus, functions as a 'pressure vessel' during roasting. As a consequence of these severe reaction conditions, probably the most severe of any major processed dietary component, roasting leads to profound changes in the chemical composition with the transformation of occurring naturally substances in green beans to compounds derived from the Maillard reaction, carbohydrate caramelization, and pyrolysis of organic compounds.[6]

Upon roasting a phlethora of volatile substances are produced, albeit only at trace levels, many being heterocycles rarely found in any quantity elsewhere in the diet. Fourteen of the ~800 volatiles, 2-furfurylthiol, 4-vinylguaiacol, three alkylpyrazines, four furanones and five aliphatic aldehydes, are important determinants of odour.[24] Kahweofuran is a particularly important volatile as it has an aroma characteristic of roasted coffee beans. Structures of key coffee volatiles are presented in Figure 6.

During roasting there is also a decrease in coffee bean carbohydrates, proteins, CGAs, and an almost total loss of free amino acids. Declines also occur in the levels of crude lipids, minerals, and aliphatic acids, although aliphatic acids might actually increase in roasts of intermediate severity [25] Compared to CGAs, caffeine is relatively stable during roasting.

The transformation of the CGAs during roasting and soluble coffee manufacture/brewing is complex. Drastic roasting conditions may produce losses of up to 95% of CGAs with 8–10% being lost for every 1% loss of dry matter.[26,27] The transformations include acyl migration which alters the relative proportions of the isomers, producing 1-acyl and increasing the 3-acyl and 4-acyl at the expense of the 5-acyl isomer.[22] Hydrolysis yields cinnamic acids and quinic acid, and conversion of the released quinic acid to the full range of quinic acid and quinic lactone (quinide) diastereoisomers. The released cinnamic acid can be decarboxylated and converted to methyl-, ethyl- and vinyl-phenols, especially catechols, and 4-vinyl-catechol can 'dimerise' to form hydroxyphenylindans (Fig.

5) (Stadler et al. 1996).[28] Some CGA diastereoisomers, particularly cinnamoyl-*muco*-quinic

acids, chlorogenic lactones and alkyl esters are also produced, as well as conversion of

caffeic acid derivatives to hydroxy-dihydrocaffeic acid and amine derivatives, and

dehydration of cinnamoyl-quinic acids to cinnamoyl-shikimic acids. Formation of novel

CGA acetates and *O*-phenolic quinoyl and shikimoyl esters of CGAs have also been

reported.[29–34]

Although CGAs suffer great losses during roasting, coffee beverage is a variable but

rich, probably the richest, dietary source of CQAs with a single serving of espresso coffee

supplying from 24 to 423 mg (Table 1).[35] Non-espresso brews are also rich sources and

many regular coffee drinkers might easily consume 1 g or even 2 g of CQAs per day, greatly

exceeding intake of CGAs from fruits and vegetables.

Coffee brews contain non-digestible polysaccharides, mainly galactomannans and

type II arabinogalactans that are by definition part of the dietary fibre complex, and these can

be accompanied by sizeable amounts of free galactose and mannose released during brewing

or the manufacture of soluble coffee powder by hydrolysis of polysaccharides. The dietary

fibre content of coffee brews range from 0.14 to 0.65 g/100 mL depending on type of coffee,

the degree of roasting and grinding, and the brewing procedure.[36] As a result of the changes

occurring during the coffee roasting, melanoidins are formed. They account for around 29%

of the dry matter of coffee brew and are generically defined as the heterogeneous, brown-

coloured, nitrogen-containing, high molecular weight end products of the Maillard reaction.[6]

Their exact composition is not known,[37] but interactions of sugar and polysaccharide

degradation products with amino acids, protein and CGAs are indicated, although some

interactions might be non-covalent.[38–42]

The Maillard reaction, thought to be central to melanoidin formation, involves the

condensation of the carbonyl group of the reducing sugars, or other components, with the

NH$_2$ group of amino acids and proteins. Through a complex network of chemical reactions that are modulated by pH, temperature and available moisture, a myriad products are formed, commonly known as Maillard reaction products. The polymeric, brown coloured final products of the Maillard reaction, the melanoidins, result from cyclization, dehydration, retroaldolization, rearrangements, isomerization, and condensation of Maillard reaction products.

Model system studies have led to three compatible proposals for the formation of coffee melanoidins. Tressl et al.[43] suggested that coffee melanoidins are polymers built up of repeating units of furans and/or pyrroles, formed during the advanced stages of the Maillard reaction and linked by poorly defined polycondensation reactions. Hofmann[44] detected low molecular weight coloured substances which were able to link proteins via the ε-amino groups of lysine or arginine to produce high molecular weight coloured melanoidins whereas Caemmerer et al.[45] proposed that the melanoidin skeleton is built up mainly of sugar degradation products, formed in the early stages of a Maillard reaction and/or sugar caramelisation, polymerized through aldol-type condensation reactions, and possibly linked by amino compounds. Despite these proposals, the structural characterization of coffee melanoidins, remains unresolved and a topic of research interest.

It is likely that roasting will also yield products similar to those recently characterised after caramelization of various sugars including glucose, mannose, galactose and arabinose that yielded oligomers with up to six carbohydrate units formed through nonselective glycosidic bond formation, with some oligomers containing hydroxymethyl-furfuraldehyde moieties. Similar studies with cellulose have established the formation of glucose oligomers in which the terminal unit has an unsaturated furan structure.[46,47]

Several micronutrients are present in coffee brew, including magnesium, potassium, and vitamins B3 and E. Depending on the coffee making procedure, filter or espresso, one

cup of coffee provides 7-24 mg of magnesium and 34-116 mg of potassium per cup of coffee.

During roasting trigonelline, which comprises ~1% of the dry weight of the green bean is

degraded substantially being converted to nicotinic acid (aka vitamin B_3 and niacin) and

several pyridine derivatives including 1-methylpyridinium and 1,2-dimethylpyridinium (Fig.

5).[48–50] Coffee has been reported to provide 1-3 mg of nicotinic acid per cup contributing to

6–18% of the recommended dietary allowance of 16 mg/d for adult men.[7]

3. Potential bioactives and possible physiological effects

It is axiomatic that to have a biological effect, whether beneficial or otherwise, the

constituents of coffee beverage and/or their *in vivo* metabolites, must reach an organ or tissue

in a sufficient concentration that is maintained for an adequate period of time. Regrettably,

many claims of benefit are gross over-extrapolations from *in vitro* studies or uncritical

extrapolations from animal studies that take no account of species differences, metabolism or

dose. If carefully designed and critically interpreted, animal and *in vitro* studies can provide

valuable insights that are relevant to human health, but a sense of proportion should be

maintained and common sense prevail.

3.1. Chlorogenic acids

CGAs are frequently referred to as powerful antioxidants, and this might be true *in vitro*.

However, the relative electrode potential at pH 7 of CGAs with a caffeic acid residue is ~540

$\pm$ 20 mV,[51] and significantly higher for those with *p*-coumaroyl or feruloyl residues.

Dimethoxycinnamic acid, although rapidly absorbed from the stomach, is not an

antioxidant.[52–54] After coffee consumption by humans, unmetabolised CQAs achieve only

transient and very low nM peak plasma concentrations (C_{max}) (Table 2),[55] thus, they cannot

realistically compete with the much greater concentrations of the far more powerful ascorbate

(40–70 μM, $E = 282 \pm 50$ mV) and α-tocopherol (5–30 μM, $E = 480 \pm 50$ mV).[56] Moreover, these antioxidant vitamins in concert actually destroy free radicals by the disproportionation of the ascorbate radical anion – phytochemicals such as CGA merely form a comparatively stable phenoxyl radical. That health benefits might arise from this over-simple direct-antioxidant hypothesis have repeatedly been challenged,[56–59] and it has now been discarded. Alternative mechanisms must, therefore, be sought. More recently, (poly)phenols have been found to exert modulatory effects in cells through selective action on multiple cell-signaling pathways involved in pathogenesis of degenerative diseases, indicating that the health effects go beyond simple antioxidant activity.[60] In this regard, it has been suggested that CGAs act as chemopreventive agents by modulating the expression of genes encoding enzymes involved in phase II metabolism which form part of the endogenous antioxidant defences, playing a critical role in conversion of reactive electrophiles and xenobiotics into less toxic products.[61]

An increase in phase II enzyme activity induced by phytochemicals or dietary factors has been linked to cancer chemoprevention.[62] Results obtained by Feng et al.[61] using a mouse epidermal cell model showed that 5-CQA at high μM concentrations induces phase II-enzymes, such as glutathione-*S*-transferase (GST) and NAD(P)H:quinone oxidoreductase. In the same study the anticarcinogenic potential of 5-CQA was demonstrated by a suppression of the proliferation of A549 human cancer cells, but at the non-physiological concentration of 80 μM, more than 1000-fold the plasma C_{max} attained after drinking a cup of coffee.

CGAs might exert anticarcinogenic activity via other mechanisms, such as inhibition of DNA methyltransferase, which catalyzes methylation of DNA at cytosine residues and plays an important role in epigenetic regulation of gene expression, X-chromosome inactivation, genomic imprinting and development, cellular aging and cell differentiation.[63] Errors in DNA methylation contribute to both the initiation and the progression of various cancers.[64] Lee and Zhu[65] analysed the inhibitory effects of 5-CQA and caffeic acid on

mammalian DNA methyltransferase. The results obtained demonstrated that both 5-CQA and its hydrolysis product caffeic acid are strong inhibitors of DNA methylation *in vitro*, with apparent IC_{50} values of 3.0 and 0.75 µM, concentrations greatly in excess of their plasma C_{max}.

Coffee consumption has been linked with reduced risk of CV diseases and the protective effect has been ascribed to the CGAs present in the brew.[66] CGAs may act as antithrombotic agents by inhibiting platelet activity: 5-CQA and caffeic acid show anti-platelet activity with mice platelets *in vitro* at a concentration of 50 nM and, *in vivo* after oral administration to mice at 400 µg/30 g body weight by suppressing the expression of P-selectin, a biomarker for platelet activation.[67] This intake approximates to a human bolus dose of ~1 g. For humans, while this intake is achievable after drinking 2-3 cups of a CQA-rich coffee, it will result in only transient, low nM concentrations of CQAs and caffeic acid derivatives in the bloodstream (Table 2),[55] although higher levels might be anticipated for the few consumers who drink coffee on a regular basis throughout the day.

The fate and mode of action of CQAs in the body following ingestion has turned out to be more complex than originally anticipated mainly because very little of the absorbed molecules retain the structure of the parent CQAs present in the drink.[55] Hence, the biological activities of the ingested CGAs, substantial amounts of which pass from the small to the large intestine,[68] are likely to be confined to the gastrointestinal tract (GIT). Effects elsewhere in the body are probably mediated by hydroxycinnamate metabolites generated in the wall of the small intestine and/or systemically after absorption, as well as colonic catabolites of CQAs absorbed in the distal GIT. The main CGA-related compounds absorbed in the small intestine and appearing transiently in the circulatory systems with C_{max} being achieved ~1 h (T_{max}) after coffee intake, are caffeic acid-3′-O-sulfate and ferulic acid-4′-O-sulfate. More substantial quantities of systemic metabolites originating from the colon, have a delayed T_{max}

of 4.3-5.2 h, and include dihydrocaffeic acid, dihydrocaffeic acid-3′-O-sulfate, dihydroferulic

acid and dihydroferulic acid-4′-O-sulfate (Table 2) (Fig. 4).[55,68]

Urinary excretion of the CGA metabolites suggest that dihydroferulic acid-3′-O-

sulphate and feruloylglycine (Fig. 7) are very good biomarkers of the consumption of

relatively small volumes of coffee, because it is the predominant source of CGAs in the

diet.[35] Trigonelline and 1-methylpyridinium are two further, urinary biomarkers of coffee

intake.[69]

Inhibition *in vitro* of platelet activity has been reported for the CQA colonic catabolites

dihydroferulic acid, dihydrocaffeic acid and 3-(3'-hydroxyphenyl)propionic acid (Fig. 4) at a

concentration of 10 μM.[70] Dihydroferulic acid, in particular, appears in the circulatory system

4.7 h after consumption of a cup of coffee with a C_{max} of 385 nM and an apparent elimination

half-life $(T_{1/2})$[1] of 1.4 h (Table 2).[55] This, coupled with the realistic prospect of repeat

consumption of coffee at, say, 2 to 3 hour intervals, suggests that maximal plasma

concentrations of ~1 μM are feasible, and the involvement of dihydroferulic acid, and other

CQA colonic catabolites (see Table 2) in the potential health benefits of coffee consumption

merits further investigation. In this context Verzelloni et al. [71] measured the protective effect of a

series of colonic catabolites, at physiological high nmol/L to low μmol/L concentrations, against

oxidative stress induced in cultured human neuroblastoma SK-N-MC cells. A mixture of three CQA

catabolites, dihydrocaffeic acid, dihydroferulic acid and feruloylglycine, each at 0.5 μmol/L, induced

significant protection with an increased survival of 16% compared to untreated control cells.

---

[1] A true $T_{1/2}$ value can be determined only by intravenous dosing of a metabolite. Assessments based
on elimination after oral dosing overestimate the $T_{1/2}$ because the metabolite is still entering the
circulatory system when the elimination is being estimated.

Dihydrocaffeic acid shows anti-inflammatory effects in human keratinocyte cell line HaCaT. At 12.5 μM the CQA catabolite was able to down-regulate UV-light-induced production of the pro-inflammatory cytokine interleukin-8.[72] However, it should be noted that *in vivo*, only an extremely small amount of dihydrocaffeic acid is likely to reach skin tissue because of its relatively low concentration in the circulatory system (see Table 2),[55] and arguably, its high rate of metabolism by the gastrocytes/enterocytes, hepatocytes and finally the keratinocytes.

Dihydrocaffeic acid is absorbed by cultured EA.hy926 cells and at a concentration of 12.5 μM it reduces menadione-induced oxidant stress, at 20 μM 2,2′azobis(2-methylpropionamidine)dihydrochloride-induced oxidation of *cis*-parinaric acid is reduced, and at 50 μM endothelial nitric oxide synthase (eNOS) activity is enhanced (Huang et al., 2004).[73] Increased eNOS activity might increase NO bioavailability and so improve endothelial function *in vivo*.

Green coffee extract and 5-CQA administered orally to spontaneously hypertensive rats produced potentially advantageous changes in blood pressure and endothelial function.[74] However, a green coffee extract given to 10 male human volunteers, so as to provide 140 mg of 5-CQA per day for four months, while producing a decrease in the level of plasma total homocysteine did not result in significant differences in either pulse wave velocity or acceleration plethysmograms between the test drink group and the placebo.[75] Circulatory compounds derived from 5-CQA were not monitored in either of these studies.

Some coffee components might interfere with glucose absorption, either directly by blockading the SGLT1 or GLUT2 transporters, or indirectly by impairing release of glucose from disaccharides or polysaccharides, or directly by increasing its rate of removal from plasma. Blockade of the active transporter alone is unlikely to have a major effect because the glucose will then be transported by the facilitative GLUT2. A sweet tasting 1,5-γ-quinide

formed from quinic acid during roasting has been reported in coffee beans at some 0.1–1.7 g/100 g depending on their degree of roast.[29,76] This quinide at 1 μM is a potent inhibitor of SGLT1 *in vitro*,[77] but its stability during coffee brewing and in the GIT, as well as its efficacy *in vivo*, are unknown.

In vitro, 5-CQA at 100 μM is not an inhibitor of the SGLT1 or GLUT2 transporters[78] but at 1 mM has been reported to depolarise SGLT1 and produce an 80% inhibition of glucose transport.[79] Nevertheless, coffee consumption has been shown in some studies to suppress the post-prandial plasma glucose peak,[80–83] but in other studies has resulted in no effect or even a detrimental effect, part of which might be attributable to caffeine.[84,85] Two studies have also looked at effects on incretin hormones, with one showing a significant reduction in the post-prandial concentration of glucose-dependent insulinotropic-polypeptide,[80] and the other proving negative.[86] Further studies are required to clarify the situation. It should be noted that only rarely have investigators attempted to define the composition of the coffee used in these investigations, beyond declaring whether or not it was decaffeinated, and this could be a significant factor in the inconsistent results. It should also be noted that commercial decaffeinated coffee is not absolutely free from caffeine.

In vitro 5-CQA inhibits porcine α-amylase but with an IC_{50} of ~80 μM.[87] Such 5-CQA concentrations might be approached in the duodenum after consumption of a CGA-rich coffee but whether any inhibition would occur in such a matrix is not known. CGA-induced inhibition of sucrase activity appears not to have been investigated. Post-prandial plasma glucose levels can be suppressed by increased clearance and more rapid utilization in muscle. Oral doses of caffeic acid (3 mg/kg b.w.),[88] isoferulic acid (5 mg/kg b.wt.)[89] and 4-hydroxybenzoic acid (5 mg/kg b.wt.)[90] (Fig. 4) have achieved such effects in diabetic rats. These human gut flora CGA catabolites[55,91] have been found at μM concentrations in human fecal water.[92] The form in which 4-hydroxybenzoic acid appears in human plasma is not

known: caffeic acid occurs as a sulphate conjugate (Table 2) and isoferulic acid is excreted

in urine as a 3′-O-sulphate and 3′-O-glucuronide[55] and these phase II catabolites might not

be active even if a sufficient concentration is attained.

Daglia et al.[93] studied the anti-adhesive properties of coffee and its potentially

bioactive components, 5-CQA, caffeine, trigonelline, nicotinic acid, and Maillard reaction

products against *Streptococcus mutans* which is considered to be the major causative agent of

dental caries in humans. Coffee possessed high inhibitory activity against the ability of *S.*

mutans to adsorb onto saliva-coated hydroxyapatite beads, and among the individual coffee

compounds tested 5-CQA, followed by trigonelline, was the most active.

Several phenolic and cinnamic acids which *in vivo* could be derived from CGAs have

been shown *in vitro* to inhibit the opportunistic human pathogen *Listeria monocytogenes*.[94]

Of particular interest in this regard is caffeic acid which has an IC_{50} value of ~40 μM, and

occurs in human fecal water at concentrations in the range 7–126 μM.[92]

It is becoming increasingly evident with *in vitro* and *ex vivo* studies on the protective

effects of coffee, it is crucial to use CGA-derived compounds that appear in the circulatory

system, at real world concentrations, for the assessment of potential health benefits and the

elucidation of the mechanisms involved.

3.2. Caffeine

For many consumers coffee is the principal dietary source of the purine alkaloid, caffeine

(1,3,7-trimethylxanthine)[95] (Fig 1). The caffeine content of green coffee varies between 0.9-

1.3 and 1.5-2.5 % dry matter for Arabica and Robusta coffees, respectively.[5] In contrast to

CGAs, caffeine content of roasted coffee is not markedly reduced, although losses,

presumably due to sublimation, may occur during the heating process, especially if carried

out at higher temperatures.[35] Typical caffeine levels in a cup of coffee vary between 50 and

100 mg.[95] However, more marked variations within different coffee brews have been reported. The caffeine content of 20 specialty coffees purchased at different coffee shops in the United States varied from 58 to 259 mg per cup.[96] Similarly, analysis the caffeine levels in 20 commercial espresso coffees in the UK found that the amounts ranged between 51 and 322 mg per cup (Table 1).[35] These results show that depending on the roasted coffee beans used and barista techniques, the amount of caffeine per serving can vary more than 6-fold, which makes it very difficult to assess caffeine intake on a population basis.

Caffeine is rapidly and almost completely absorbed in the stomach and small intestine and distributed to all tissues, including the brain. C_{max} is reached within 60 min after intake and the apparent $T_{1/2}$ of caffeine is ~5 h.[97] Caffeine metabolism occurs primarily in the liver by the cytochrome P450 isoform CYP1A1. The first metabolic step is the demethylation of caffeine leading to the three dimethylxanthines: paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine). These metabolites are then further demethylated in the liver to the corresponding monomethylxanthines before being oxidised to methyl uric acids and other products[98] as illustrated in Figure 8.

Once absorbed, caffeine exhibits numerous and well-studied physiological effects[95] and exerts most of them through the antagonism of the A_1 and A_2 subtypes of the adenosine receptor. The A_1 receptors are located in all parts of the brain with higher concentrations in the hippocampus, cerebral and cerebellar cortex and certain thalamic nuclei, whereas the A_2 subtypes are located in the dopamine-rich regions of the brain.[99] Adenosine antagonisation elevates the level of dopamine which is responsible for many of the central nervous system stimulant and addictive properties of caffeine. Another mechanism of action of caffeine is the synergistic interaction with adrenalin and noradrenalin, the main neurotransmitters for the sympathetic nervous system.[100] The stimulatory effects of caffeine include enhanced

perception, an increased capacity to remain awake for longer periods, reduced fatigue. More recently, caffeine has shown to posses positive effects on long-term retention by enhancing memory consolidation.[101] It also helps reduce symptoms associated with Parkinson's disease, and has been suggested as a potential contributor to reducing risk factors involved in metabolic syndrome, such as obesity.

Parkinson's disease leads to a progressive loss of dopaminergic neurons of the substantia nigra in the midbrain, which results in the deterioration of gross and small motor skills, and tremors. Caffeine improves the performance of the dopaminergic system by blocking the A_2 adenosine receptors and thereby stimulating the release of dopamine,[102] and via this mechanism, is thought to reduce symptoms associated with Parkinson's disease, such as tremor, and freezing of gait, in animal models and humans.[102,103]

Caffeine consumption increases metabolic rate, energy expenditure, lipid oxidation, and lipolytic and thermogenic activities, and so has potential as an aid in weight loss and reducing the overall risk for developing metabolic syndrome.[95] Consumption of 300 mg caffeine per day has been found to increase energy expenditure by approximately 79 kcal/d.[104] Although this increase is modest, the authors of this study propose that such modifications are sufficient to prevent weight gain and, when consumed regularly as part of a healthy diet and exercise regime, caffeine-containing beverages may provide benefits for weight control.

Caffeine might contribute to the overall antioxidant capacity of coffee. Devasagayam et al.[105] studied caffeine as a protector agent of cell membranes against oxidative damage. The mM concentrations used in this study, however, were more than 40-fold higher than the average blood concentrations observed after consuming coffee in normal dietary amounts.[106] More recently, Lee[107] measured the protective effect of caffeine and its metabolites, at physiological μmolar concentrations, on LDL peroxidation. Neither caffeine nor its

dimethylxanthine metabolites exhibited antioxidant activity while, in contrast, 1-methylxanthine and 1-methyluric acid (Fig. 8), were highly effective antioxidants *in vitro* being able to prevent LDL oxidation.

There are also negative aspects linked to caffeine intake that must be considered. The activation of adenosine receptors by caffeine has negative consequences on CV health, such as increased heart rate and blood pressure.[108] Caffeine in excess, can result in a state of excitement and anxiety including adverse reactions like tachycardia, headache, palpitations, insomnia, restlessness, nervousness, and tremor. Dose-response varies between individuals, and even a single cup may cause sleeplessness with a racing mind for some people, while for others drinking ten times this amount the effects can still be pleasant and not interfere with sleep. The substantial differences in the effects of caffeine between individuals are at least partly the result of genetic variations of susceptibility.[109] In addition, regular coffee drinkers develop a certain tolerance to the adverse impacts of caffeine, especially on adenosine antagonism and sleeplessness. The development of tolerance may have unpleasant consequences. People who drink coffee on a regular basis and decide to interrupt coffee consumption can experience withdrawal effects. The absence of the adenosine antagonist may cause headaches due to intracranial vasodilation[110], and this coupled with reduced catecholamine and serotonin activity may lead to fatigue, mild depression and impaired cognitive function.[111,112]

The typical 2.5-4.5 h apparent $T_{1/2}$ of caffeine in adults can be extended up to 30 h in the body by women taking an oral contraceptive, pregnant women, the developing foetus, young children, and those with liver disease. These groups are, therefore, much more susceptible to the effects of caffeine toxicity. Acute intoxication is characterised by dehydration, tachycardia, extreme anxiety, hallucinations, flushing, and delirium. Further fatal consequences are epileptic seizure, cardiac arrhythmia and ultimately ventricular

fibrillation. The fatal dose for humans depends on individual sensitivity but in rare instances can be as little as 2 g.[100] In general, fatal caffeine overdose involves the ingestion of caffeine-containing medications rather than caffeinated foods or beverages.[7] However, some cases of death after ingestion of high amounts of energy drinks and caffeinated energy mints have been attributed to caffeine.[113-116] Although the amounts present in coffee make it unlikely to be a source for caffeine intoxication, isolated cases of caffeine toxicity have been attributed to excessive coffee intake.[117] Pregnant women have a slower caffeine metabolism with an extended apparent $T_{1/2}$ of up to 10 h. In addition, caffeine freely crosses the placenta and enzymes involved in caffeine metabolism are lacking in both the placenta and fetus.[95] The effect of caffeine consumption on pregnancy complications, such as spontaneous abortion and impaired fetal growth, has been studied extensively but epidemiological data are conflicting. While some studies did not detect a significant association between caffeine intake and the risk of spontaneous abortion[118,119] more recent investigations have found a significant association at intake levels of $\geq$300 mg/d.[120-122] Maternal caffeine intake has also been linked to a higher risk of low birth weight in some[123,124] but not all epidemiological studies.[125,126] Some of the inconsistency in the observations might well arise from using a 'cup of coffee' as a measure of caffeine consumption. Although the threshold at which the risk is significantly higher is not well characterised, results from the CARE study group suggest a reduced fetal growth for women consuming >100 mg/d.[127] Sengpiel et al.[124] reported similar results with a decrease in birth weight of 21-28 g for each daily 100 mg of maternal caffeine intake. Current advice in the UK from the Food Standards Agency is for pregnant women to restrict caffeine to below 200 mg per day.[128]

Summarizing, the actions of caffeine may be seen as a double-edged sword and the balance between beneficial effects and negative impacts on well-being depend largely on the individual susceptibility to caffeine.

3.3. Trigonelline

Trigonelline is present in green coffee at ~1% dry weight, with slightly higher values found in Arabica coffees than in Robustas.[129] During the roasting process trigonelline is partially degraded to nicotinic acid and several pyridine derivatives, as mentioned in Section 2. Depending on coffee variety and degree of roast trigonelline levels in roasted coffee vary between 0.2 and 0.9 mg/g of ground coffee.[130,131] Amounts in coffee brew depends largely on the coffee making procedure and appear to range between 40-110 mg per serving.[5,132,133]

Several studies investigated the bioavailability of trigonelline in humans and animals. Results obtained from a bioavailability study with humans who consumed one cup of coffee, containing 808 μmole of trigonelline, showed that the pyridine alkaloid attained a C_{max} of ~6 μM after ~3 h and had an apparent $T_{1/2}$ of ~5 h.[134] Urinary excretion of trigonelline 0-8 h post-ingestion of coffee was 57% of intake for males and 46% for female volunteers, showing that substantial amounts had passed through the circulation without being subjected to phase II metabolism (Table 3). Trigonelline was detected 15 min after ingestion of coffee, pointing towards absorption occurring initially in the stomach. The coffee also contained 172 μmole of 1-methylpyridinium (Fig. 5) which reached a C_{max} of ~0.8 μM with an earlier T_{max} than that of trigonelline ranging from 1.0-1.7 h. The apparent $T_{1/2}$ of 1-methylpyridinium was also more rapid than that of trigonelline at ~2.2 h while urinary excretion of 1-methylpyridinium was 69% of intake both males for females.[134]

Their levels of urinary excretion (Table 3) indicates that, unlike the CGAs, both trigonelline and 1-methypyridinium pass through the body without undergoing substantial phase II metabolism. In keeping with this possibility, a study on the absorption of trigonelline from the small intestine of germ-free and specific pathogen-free rats demonstrated that the pyridine alkaloid is not degraded by the GIT microflora and that most is absorbed from the small intestine.[135]

Regarding potential biological activities, trigonelline has been shown to possess, hypoglycaemic, neuroprotective, anti-invasive, estrogenic, and antibacterial activities. After a 4 week treatment of diabetic rats with trigonelline (50 mg/day/kg b.w.), blood glucose, total cholesterol, and triglycerides were significantly reduced. Furthermore, there were altered insulin levels, insulin sensitivity index and insulin content in the pancreas, all showing values similar to a non-diabetic control group.[136] Trigonelline regulates key enzymes of glucose and lipid metabolism, such as glucokinase, glucose-6-phosphatase, fatty acid synthase, and carnitine palmitoyl transferase in diabetic rats.[137] In a randomized crossover study with healthy, male humans a 500 mg dose of trigonelline led to significantly lower glucose and insulin levels 15 min after an oral glucose load compared to a placebo.[83]

Trigonelline at 30 and 100 μM prevented, in a dose dependent manner, dendritic and axonal atrophy in rat cortical neurons induced by the neurotoxine amyloyl-ß-peptide [Aß (25-35)]. Moreover, orally administered trigonelline (500 mg/kg b.w.) improved memory in mice treated with Aß (25-35).[138] Recently, Gaur et al.[139] studied the neuroprotective effects of a fenugreek extract, containing 82% trigonelline, in rodent models of Parkinson's disease. There was a significant increase in the number of ipsilateral rotations in 6-hydroxydopamine-induced unilateral lesioned rats that orally ingested the extract (30 mg/kg b.w.) compared to the control group. The extract also induced a significant reversal of motor dysfunction caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced lesions in C57BL/6 mice in pre-treatment (1 h) but not in post-treatment (1 h) experiments.

Cell invasion is one of the key steps in the development of cancer metastasis.[140] The effects of trigonelline on the proliferation and invasion of cancer cells were studied using rat ascites hepatoma cell line AH109A. At the physiological concentrations of 2.5-4.0 μM trigonelline inhibited the invasion of hepatoma cells in a dose responsive manner but did not impact on proliferation. Anti-invasive activity was also determined by incubating AH109A

cells with serum from rats fed 100 μmole trigonelline/100 g b.w. by gavage. Serum obtained

2 h after intake most strongly inhibited invasion of AH109A cells and significant inhibition

was also measured in serum collected 3 h and 6 h after intake. These results suggest that

trigonelline absorbed from the GIT retains its effectiveness in the bloodstream. Both

trigonelline and trigonelline-loaded rat serum suppressed reactive-oxygen-species (ROS)-

potentiated invasive activity of pre-treated cells but did not scavenge intracellular peroxides.

This indicates that a mechanism other than a direct antioxidant effect is involved in

trigonelline-mediated suppressive effects on ROS-potentiated invasion.[141]

Trigonelline stimulates proliferation of MCF-7 breast cancer cells and significantly

enhances cell growth *in vitro* at concentrations as low as 100 pM. This effect was attributed

to the activation of the estrogen receptor which classifies trigonelline as a novel

phytoestrogen. Soy phytoestrogens play a dual role in the development and progression of

breast cancer. Exposure to genistein, prior to tumor formation reduces the risk of developing

breast cancer.[142] In contrast, the isoflavone acts as an estrogen antagonist after tumor

formation and stimulates mammary gland differentiation in rats.[143] It will be interesting to

determine if trigonelline exerts similar effects, not just in rats but also in humans which in

many aspects of their physiology are some what different to rodents.

The transcription factor Nrf2 plays a key role in cancer development and

chemoresistance as its overexpression confers stability to cells and it is thought to be an

adaptive cell reaction to chemical and oxidative insults. This makes Nrf2 activation a

potential target in anticancer therapy[144] as it leads to protection against DNA damage and a

reduction in tumorigenesis[145] but Nrf2 activation is also a means by which cancer cells resist

chemotherapy. Trigonelline is an effective inhibitor of Nrf2 and in the process it has been

reported to increase the sensitivity of chemoresistant pancreatic and colon cell lines to

anticancer drugs.[146]

As noted earlier, trigonelline impacts on the adhesive properties of *Streptococcus mutans*, the major causative agent of dental caries in humans, by reducing the ability of the bacterium to adsorb onto saliva-coated *hydroxyapatite* beads. Trigonelline is also a potential antimicrobial agent against the highly invasive pathogen *Salmonella enterica*.[147]

Summarizing, trigonelline is a pyridine alkaloid, found in substantial amounts in coffee, with therapeutic potential as a hypoglycemic and neuroprotective agent and it also has anticarcinogenic effects. However, at this juncture its use as an anticarcinogenic agent is controversial.

3.4 Kahweol and cafestol

The diterpenes kahweol, cafestol and 16-*O*-methylcafestol (Fig. 2) occur as fatty acyl esters in coffee, contribute to the bitter taste of the beverage where they are found in quantities highly dependent on the brewing technique. They are abundant in boiled and unfiltered coffee but are almost completely removed when the coffee brew is filtered either immediately prior to consumption or during the manufacture of soluble coffee powders. Scandinavian boiled coffee, French press and Turkish coffee contain from 6 to 12 mg per cup, whereas filter coffee, and instant coffee contain less than 0.6 mg per cup. Although espresso coffee has high concentrations of kahweol and cafestol it is considered as an intermediate source of these compounds (~4 mg/cup) due to its small serving sizes.[7] While the presence of these diterpenes has been implicated in the cholesterol-raising effect of coffee,[148] they have also been shown to possess chemopreventive potential.[149] The mode of action includes induction of phase II detoxifying enzymes, and regulation of Nrf2/ARE signalling pathways, thereby enhancing the endogenous defence systems against oxidative damage.[2,150]

3.5. Melanoidins

Coffee brew is considered one of the main sources of melanoidins in the human diet.[151] Although coffee melanoidins, which are products of roasting, do not fall within the strict definition of dietary fibre because they are not true polysaccharides naturally present in the green beans, there is mounting evidence that *in vivo* they actually behave as dietary fibre, being largely indigestible by humans and fermented in the gut. The structural separation between coffee melanoidins and true dietary fiber, i.e. the coffee polysaccharides remaining underivatized after roasting, is very difficult.[152] Comparing melanoidins with the various types of natural polysaccharides, has revealed a structural similarity with cereal arabinoxylans.[153] The overlap between dietary fibre and melanoidins is of interest from a nutritional perspective. According to Fogliano and Morales,[151] the amount of coffee melanoidins reaching the colon ranges from 0.5–2.0 g per day for moderate to heavy coffee drinkers. Considering that the recommended daily intake of soluble dietary fibre is 10 g and that most of people fail to reach this target as estimated from their consumption of the classical sources, melanoidins intake might contribute significantly to the health benefits associated with coffee at the colon level.[154]

Melanoidins have been studied extensively for their possible beneficial effects on health. Different potential biological activities have been ascribed to this heterogenic class of coffee compounds namely, antioxidant and metal chelating activity, antimicrobial activity, anticariogenic activity and the ability to modulate colonic microflora, as well as, antihypertensive, and antiglycative activity. However, there is no evidence to suggest that even the low mass melanoidins are absorbed intact in the circulatory system, and *in vitro* studies where melanoidin fractions have been i) applied to microsomes or cultured liver cells,[42,155,156] ii) tested for their ability to prevent glycation of bovine serum albumin[157] and iii) assayed at concentrations $\geq$ 1 mg/mL for inhibitory activity against angiotensin-I

enzyme,[158] are of little help in evaluating their dietary significance *in vivo*. In contrast, effects exerted in the oro-GIT without the need for absorption might be significant.

Melanoidins could reach high concentrations in the GIT where the data of Tagliazucchi et al.[159] suggest they might exert beneficial effects by preventing the formation of oxidised lipids and decreasing the absorption of these cytotoxic lipid peroxidation products. However, in this study, no effort was made to exclude oxygen from the incubation medium and it is debatable to what extent such oxidation would occur *in vivo*.

High molecular weight melanoidins (HMWMs) isolated from coffee brew has been suggested to be potentially anticariogenic. This property was ascribed to the ability of the HMWMs to inhibit both the adsorption of *Streptococcus mutans* to saliva-coated hydroxyapatite beads and the formation of biofilms on microtiter plates.[160] Studies on melanoidins using model systems have shown that they are able to influence the contractility of gastric smooth muscles with the HMWM fraction from coffee brew being able to evoke contractions of rat circular gastric smooth tissue by activating cholinergic receptors.[161] This finding implies that melanoidins might increase the colon motility, which is linked with reduced colorectal cancer risk

Studies on the effects of coffee melanoidins on different pathogenic microorganisms have shown that they exhibit dose-dependent antimicrobial activity. Moreover, Gram-negative bacteria were more sensitive to the antimicrobial activity of the melanoidins than Gram-positive strains.[162] The antimicrobial activity of coffee melanoidins has been ascribed to their metal chelating properties and Rufian-Henares and de la Cueva[162] proposed three different mechanisms: (i) at low concentrations, melanoidins may exert a bacteriostatic activity mediated by iron chelation from the culture medium; (ii) in bacterial strains that are able to produce siderophores for iron acquisition, melanoidins may chelate the siderophore-Fe^{3+} complex, which could decrease the virulence of such pathogenic bacteria; and (iii)

melanoidins may have bactericide activity at high concentrations by removing Mg^{2+} ions from the outer membrane, promoting the disruption of the cell membrane.

Melanoidins are not digested in the upper GIT and they are mainly recovered in the faeces.[163] Nunes and Coimbra[41] demonstrated that coffee melanoidins have a good prebiotic potential, while Gniechwitz et al.[164] and Reichardt et al.[165] showed that coffee melanoidins behave as a soluble dietary fibre since they are fermented by the microorganisms in the gut and enhance bacterial growth, mainly of the Bacteroides-Prevotella group. Nevertheless, a selective stimulation of bifidobacteria and lactobacilli has so far not been observed with coffee melanoidins.[154]

Taking into account all the properties outlined above, crude melanoidins appear to have potential as health beneficial compounds. Because the various melanoidin fractions used in these studies are crude it is not possible to judge whether the effects are due to novel substances formed during coffee roasting, pre-existing green bean polysaccharides, or non-covalently adsorbed CGAs.

In summary, although *in vitro* and *in vivo* studies using reference compounds have proved that several components occurring in coffee exhibit biological activities, the physiological properties of coffee itself will probably differ and vary because the beverage is a complex and variable mix of hundreds of compounds and its bioactivity may be influenced by possible matrix, synergistic and/or antagonistic effects. As a further complication, with the possible exception of trigonelline and 1-methylpyridine, only a small percentage of the ingested compounds enter the circulatory system and reach tissues, and very little of the absorbed material retains the original structure present in the beverage.

4. Health effects

26

Large literatures exist which mention possible or proposed health implications of coffee

drinking, but there is very little sound evidence for either harm or benefit. Inevitably, cross-

sectional or longitudinal associations with self-reported coffee consumption dominate the

literature, while experimental evidence is more limited. Coffee drinking may simply be a

marker a range of other lifestyle factors. While most epidemiological studies published in the

period 1980 to 2000 reported that coffee consumption may be associated with greater risk of

coronary heart disease and several types of cancer, some more recent investigations have not

confirmed these findings. On balance, the currently available information from

epidemiological research suggest that coffee consumption is associated with a lower risk of

several chronic diseases including type 2 diabetes, CV disease and cancer, as well as

neurodegenerative conditions such as Parkinson disease.[7,166] Whether these associations

relate to biological or social influences of regular coffee consumption is uncertain, although

some experimental evidence would support suggestions of a mechanistic link.

A major difficulty in interpreting epidemiological data is that surveys were not

designed specifically to quantify coffee consumption, so assessing exposure is usually very

crude, and abstainers may have a variety of medical and personal attributes which introduce

confounding factors. The considerable variability in the composition of the coffee beverage

coupled with significant differences in cup size makes it impossible accurately to define the

intake of any of the potentially bioactive constituents unless in prospective studies accurate

analyses are made of what the participating subjects consume. In retrospective studies there

is the further problem of the added imprecision associated with recall. These constraints

make it very difficult to determine the real world biological significance of the statistical

relationships arising from epidemiological studies of coffee consumption. It must also be

recognised that studies focussing on coffee, or indeed any other single commodity or

commodity group, cannot fully take account of possible physiological effects associated with

potential bioactive constituents from other dietary components. A critical assessment of the possible significance of these other dietary components is beyond the scope of the present review.

4.1. Coffee and antioxidant status

Several intervention studies analysed the effect of coffee consumption on the antioxidant activity of plasma. Acute coffee intake resulted in a significant increase in total radical-trapping antioxidant parameter (TRAP) and ferric ion-reducing antioxidant power (FRAP) of plasma when compared to water consumption.[167] These changes in the antioxidant status *in vivo* are unlikely to be a direct consequence of the antioxidant activity of coffee, and are more likely to be the result of coffee compounds being able to augment endogenous antioxidant defences, such as an increase in GST activity and glutathione. After daily consumption of coffee for 2 weeks, significant increases in glutathione concentration (15%) in plasma and in colorectal mucosa (8%) have been observed.[168] Similar findings for plasma glutathione concentration were reported after the daily ingestion of five cups of coffee over a period of 7 days.[169] In a later study consumption of unfiltered coffee showed a weak but significant increase in GST activity.[170] Thus, under some circumstances coffee might contribute to the endogenous systems which prevent oxidative damage to cell components, DNA, proteins, and lipids, which contribute to the pathogenesis of degenerative diseases, such as cancers, cardiovascular and neurodegenerative diseases.

4.2. Coffee and type 2 diabetes

Based on meta-analysis of epidemiological studies there is a relationship between regular coffee intake and a lower risk of type 2 diabetes mellitus (DM).[171,172] A prospective cohort study in the Netherlands including 17,000 men and women reported a lower relative risk of developing type 2 DM of 50% for those who consumed at least seven cups of coffee per day compared to those who drank two cups or less in terms of absolute risk[173] Similar results

were found in a smaller 18 year cohort study of Swedish women with a daily coffee intake of three cups compared with the consumption of two cups or less.[174] The two largest prospective cohort studies to examine the relationship between coffee consumption and type 2 DM were the Health Professionals Follow-up Study (41,934 men) and the Nurses' Health Study (84,276 women) in the USA. Men who drank at least 6 cups of coffee daily had a 54% lower relative risk of developing type 2 DM than men who did not drink coffee, while women who drank ≥6 cups of coffee daily had a 29% relative lower risk than female non-consumers. This inverse relation between coffee consumption and type 2 DM was observed for caffeinated as well as decaffeinated coffee.[7]

A subsequent meta-analysis of 18 prospective studies on coffee intake and type 2 DM found an inverse log-linear relationship between coffee consumption and subsequent risk of diabetes. Every additional cup of coffee consumed per day was associated with a 7% lower risk of diabetes.[175] However, not all prospective cohort studies have observed a significant link between coffee intake and risk of developing type 2 DM. The inverse relation found in epidemiological studies is supported by results obtained in intervention studies that found a positive effect of coffee on diabetic markers such as serum glucose or insulin levels.[80,81] In contrast, other investigators failed to detect an effect of acute coffee intake on these glucose metabolism markers.[176,177]

Although results of the effect of coffee on diabetes are not always clear-cut, and some intervention and epidemiological studies have revealed no effect, most of the more recent studies observed a significant risk reduction of 30-60%, in the same range as observed with pharmacological approaches.[166] Nevertheless, until the relationship between long-term coffee consumption and type 2 DM is better understood and any mechanism involved identified, it is premature to make claims about coffee preventing type 2 DM.

4.3. Coffee and cardiovascular diseases

Several earlier studies have linked coffee consumption with an increased risk in developing

CV disease. However, these findings are controversial and more recent studies indicate that

the risk of CV disease may be related to the ingestion of the diterpenes cafestol and kahweol,

which have been shown to raise serum total and LDL cholesterol reversibly, and can be found

at high amounts in boiled and unfiltered coffee.[148,178,179] The diterpenes are present in much

lower amounts in filter and instant coffee.[7] The overall consumption of boiled coffee has

decreased as of late and a recent meta-analysis including 21 cohort studies report a lower risk

of CV disease in women who were moderate coffee drinkers.[180] Nevertheless, most

prospective cohort studies have not found a significant association between coffee

consumption and CV disease risk[7] while a few have reported negative effects. A prospective

cohort study in Norway analysed the risk of coronary heart disease mortality after 6 years and

12 years of follow up. The authors comment: '*The fairly strong association between coffee*

consumption and mortality from coronary heart disease in Norway has been distinctly

weakened by six more years of follow up. The association was completely absent when the

first six years of observation were excluded and cholesterol concentration was adjusted for'.

They attribute this change in the relationship to an education programme that has led to a

reduced consumption of diterpene-rich boiled coffee.[181]

Results from the Honolulu Heart Study reported a doubling of the risk of thrombotic

stroke in hypertensive men who consumed at least 700 mL of weal American-style coffee per

day.[182] Beside cafestol and kahweol, caffeine might exert negative effect on CV health. Long-

term coffee consumption was not associated with stroke in the Nurses' Health Study but

decaffeinated coffee was associated with lower risk of stroke after adjustment for caffeinated

coffee consumption.[183] One case-control study found a J-shaped relation between coffee

consumption and the risk of developing an acute coronary syndrome (myocardial infarction

or unstable angina). Heavy coffee consumers (>600 mL/day) had a three times higher risk of

developing acute coronary syndrome than those who did not drink coffee, while moderate

coffee consumers (<300 mL/day) had a 30% lower risk than those who did not drink

coffee.[184] Combined positive and negative action of different coffee compounds might

explain the J-shaped correlation found in this study. Results from intervention studies on

coffee and CV risk biomarkers confirm negative effects for caffeinated coffee whereas

beneficial effects are often observed with decaffeinated coffee consumption.[166] In a double-

blind, crossover study with 20 healthy subjects consuming either caffeinated or decaffeinated

espresso coffee at 5 to 7-day intervals resulted in a significant increase in systolic and

diastolic blood pressure but only with caffeinated coffee. Similarly, flow-mediated dilatation

decreased progressively and significantly following ingestion of caffeinated espresso coffee

but not after ingestion of decaffeinated coffee.[185] Natella et al.[186] evaluated the anti-platelet

activity of coffee compared to caffeine in a crossover intervention study on ten healthy

subjects after ingesting either 200 mL of coffee containing 180 mg caffeine or 180 mg

caffeine capsules. Arachidonic acid-induced platelet aggregation was inhibited after coffee

intake but not after caffeine capsules. These results suggest that other compounds, such as

CGAs, might be responsible for the anti-platelet effects observed after coffee intake.

The results of epidemiological and intervention studies on the effects of coffee on CV

risk vary and coffee seems to have a mix of beneficial and harmful effects. Nevertheless,

long-term studies suggest that harmful effects are unlikely for moderate coffee consumption

and it is possible that other compounds in coffee counteract the negative effects of caffeine

and diterpenes on CV health.

4.4. Coffee and cancer

There are numerous epidemiological studies relating coffee consumption to the risk of cancer

and there is currently substantial evidence suggesting that coffee may be associated with

lower risk of some cancers. Strong and consistent protective association has been found

between coffee consumption and reduced risk of endometrial and hepatocellular cancer. In the case of breast and colorectal cancer, a modest or borderline negative association with coffee consumption was reported whereas no association was found for pancreatic, ovarian, prostate or gastric cancer.[8,166,187]

Several case-control studies on coffee and the risk of endometrial cancer conducted in Italy and Japan have produced data indicating 50-60% lower risk for coffee consumption of three or more cups per day and a statistically significant linear association between the amount of coffee consumed and the relative risk.[18-190] Similar results were obtained in a Japanese population-based cohort study of 53,724 women during 15 years of follow-up which also found a 60% lower risk among those consuming at least three cups of coffee per day, compared to those who drank less than two cups.[191]

A significant reduction in risk of hepatocellular cancer (HCC) was observed among habitual coffee consumers in several cohort and case control studies. Two Japanese cohort studies, one including more than 90,000 men and women during 10 years of follow up, and the other including more than 100,000 men and women over 10-12 years, found a significant inverse association between coffee drinking and HCC risk.[192,193] Results from these studies suggest that the consumption of one or more cups of coffee on daily basis reduces the possibility of developing HCC, or death due to HCC, by 50%. Both studies found a clear dose dependent association between the risk of HCC and the amount of coffee consumed. Results of case control studies corroborate these findings.[186] Among these, one hospital-based control study conducted in Italy concluded that compared to non-coffee consumers, the risk of HCC was lower by 20% with an intake of 1-2 cups per day, by 60% for 3-4 cups and by 70% for >5cups.[194] Most of these data were summarised in two meta-analyses that concluded that an increase in coffee consumption of one cup per day was associated with a 22-25% lower risk of liver cancer.[195,196] Bravi et al.[195] noted the concern that subjects with liver

conditions may selectively reduce their coffee consumption. However, the consistency of an inverse relation between coffee drinking and HCC across study design and geographic areas weighs against a major role of bias or confounding factors.

Although a significant amount of potentially anticarcinogenic coffee compounds such as CGAs reaches the colon,[68] controversial results have been reported on coffee consumption and risk of colorectal cancer. A review of 15 case control and three cohort studies concluded that coffee consumption was associated with lower risk for some case-control studies, but that results were inconsistent in the cohort studies.[197] No consistent dose-response was observed among the studies and no relationship emerged for rectal cancer. Subsequently, two meta-analyses, one including 24 case-control studies and the other 25 case-control and 16 cohort studies, revealed a moderate inverse relation between colorectal and colon cancer for case-control studies, but no association was found with rectal cancer.[198,199] Another recent study, on the dose-response effect of coffee consumption and colorectal cancer, confirmed these findings and reported a significant inverse association between colorectal and colon cancer with a daily intake of at least 4 cups of coffee.[200] Although not all studies have produced consistent findings, the accumulated evidence suggests at least a modest inverse association between coffee and the risk of colorectal and colon cancer.

Several studies have analysed the effect of coffee consumption on breast cancer but no association was found for case-control or cohort studies among post-menopausal women,[8,187] but, risk of breast cancer may be lower among premenopausal coffee-drinkers.[201] In addition, a strong negative association was observed between coffee consumption and breast cancer among high-risk women carrying *BRCA* gene mutations. Women with *BRCA* mutations have an estimated risk of developing breast cancer as high as 80% by the age of 70.[202] However, women with *BRCA* mutations who habitually drank six or more cups of coffee per day showed a 70% risk reduction compared to non-coffee drinkers.[203] No

33

association was observed for decaffeinated coffee, suggesting that caffeine might be responsible for the protective effect although an involvement of trigonelline may also be feasible (see Section 3.3).

Overall, mounting evidence supports a protective association between coffee consumption and endometrial and hepatocellular cancer. A modestly favourable effect was observed on the risk of colorectal and colon cancer, whereas protective effects in breast cancer have only been reported among premenopausal women and women carrying *BRCA* mutations.

4.5. Coffee and Parkinson's disease

Results from epidemiological studies reveal a consistent inverse association between coffee consumption and the risk of Parkinson's disease. A meta-analysis of eight case-control and five cohort studies conducted in four countries between 1968 and 2001 concluded that coffee drinkers had a 31% lower risk of Parkinson disease than non-coffee drinkers.[204] Interestingly, the two cohort studies that included only men, the "Honolulu Heart Study" and the "Health Professionals' Follow-up Study", found a strong inverse linear relation between the number of cups of coffee consumed and risk of Parkinson's disease with 49% lower risk per three additional cups per day, whereas the cohort study that included only women (Nurses' Health Study) found no such "three additional cups per day" effect. The sex difference observed could be due to hormonal effects and, in fact, further analysis of the "Nurses' Health Study" revealed that coffee was associated with a lower risk of Parkinson's disease among women who did not use postmenopausal hormones, but a greater risk among hormone users.[204] These findings were corroborated by the results obtained from the "Cancer Prevention Study II" cohort, which showed a significant inverse association between coffee consumption and Parkinson's disease mortality in men. In women, however, this association was dependent on

postmenopausal oestrogen use, with a risk reduction of 53% for women drinking four or

more 600 mL cups of coffee per day compared with non-drinkers and a risk increment of

31% among oestrogen users.[206] Although the cause of this adverse effect of oestrogen is not

yet understood it might be linked with the fact that oestrogen replacement therapy has been

found to inhibit CYP1A-mediated caffeine metabolism.[207] These events might also be related

to the trigonelline content of the ingested coffee (see Section 3.3).

The "protective" effect of association of coffee on Parkinson's disease, if causal,

could be ascribed to its caffeine content. Parkinson's disease is a neuropathological disorder

involving the degeneration of dopaminergic neurons in the substantia nigra, with the

subsequent loss of their terminals in the striatum. Caffeine and other A_2 adenosine receptor

antagonists have been shown to protect against dopaminergic neurotoxicity in animal

models.[208,209] A possible explanation is that caffeine aids improvement of the performance of

dopaminergic system by blocking the A_2 adenosine receptors thereby stimulating the

dopamine release.[102]

Results obtained to-date in epidemiological studies indicate a reasonably consistent

association between coffee consumption and a lower risk of Parkinson's disease. However,

the mechanisms involved are not fully understood and it is premature to recommend

increasing coffee consumption to prevent Parkinson's disease, especially in women taking

postmenopausal hormones. It is possible, however, that this inverse association is spurious, if

people affected by tremor reduce coffee consumption before or after the diagnosis of

Parkinson's disease.

5. Conclusions

Coffee is one of the most popular beverages in the world and, consequently, its impact on human health is of great interest. Viewing the wide range and depth of evidence already collected, it is clear that any impacts of coffee on long-term health, whether protective or detrimental, must be very small indeed, and can only relate to long-term exposure. A large part of the uncertainty, and conflict, in the epidemiological evidence is because research has not specifically focussed on beverages at a design stage so the level of detail collected in surveys is inadequate to quantify and characterise long-term consumptions.

It is possible from mechanistic experiments that some specific constituents of coffee may have negative health impact, while others provide balancing protection. Among the vast array of compounds present in coffee brew, the biologically active classes are usually considered to be CGAs, melanoidins, caffeine, trigonelline and the diterpenes cafestol and kahweol. These compounds have been shown, at least *in vitro*, to possess various properties including antioxidant, chemopreventive, antihypertensive, hypoglycaemic, antiglygative, and anticariogenic activity. However, as they pass along the GIT they are metabolised and, with the possible exception of trigonelline, it is their metabolites, rather than the parent compounds, which predominate in the circulatory system. There may well be other compounds which have a protective role, and their effects need not be very large in order to have public health importance.

Epidemiological studies have associated coffee consumption with beneficial effects including reduced instances of type 2 DM, hepatocellular, endometrial, colorectal and premenopausal breast cancer. Results on the effect on CV disease are conflicting but negative effects of caffeine, kahweol and cafestol seem to be reduced or counteracted by CGAs. A reduced risk of Parkinson's disease was found in men but only in women who never used postmenopausal oestrogen. Before these encouraging epidemiological observations can be confirmed, and used as a sound basis for dietary advice, further research is needed on the

bioavailability and pharmacokinetics of coffee components in order to elucidate the compounds responsible for those effects and mechanisms involved. It is essential also that future prospective epidemiological studies define more accurately the dose of the various potentially bioactive components that are consumed − 'a cup of coffee' is not a well defined measurement. Given the vast consumption of coffee by a huge proportion of the world's population, any small shift towards a more healthful balance could have huge value.

6.   References

1   T. J. Bond in *Teas, Cocoa and Coffee: Plant Secondary Metabolites and Health*, ed. A. Crozier, H. Ashihara and F. Tomás-Barberán, Wiley-Blackwell, Oxford, 2012, pp. 1−24.

2   M. S. Butt and M. T. Sultan, Coffee and its consumption: benefits and risks. *Crit. Rev. Food Sci. Nutr.*, 2011, **51**, 363−373.

3   P. Mazzafera, A. Crozier and A. C. Magalhães, Caffeine metabolism in *Coffea arabica* and other species of coffee. *Phytochemistry* 1991, **30**, 3913−3916.

4   FAS/USDA *Coffee:World Markets and Trade.* Office of Global Analysis, Foreign Agricultural Service/United States Department of Agriculture, 2013.

5   A. Farah, Coffee constituents, in *Coffee: Emerging Health Effects and Disease Prevention*, ed. Y. F. Chu, Blackwell Publishing Ltd., Oxford, 2012, pp. 21−58.

6   H. D. Belitz, M. Grosch, and P. Schieberle, Coffee, tea, cocoa, in *Food Chemistry*, 4th edition, Springer-Verlag, Berlin, 2009, pp. 940−970.

7   J. V. Higdon and B. Frei, Coffee and health: A review of recent human research. *Crit. Rev. Food Sci. Nutr.*, 2006, **46**, 101−123.

8   A. Nkondjock, Coffee consumption and the risk of cancer: An overview. *Cancer Lett.,* 2009, **277**, 121−125.

9   S. K. Bøhn, N. C. Ward, J. M. Hodgson and C. D. Croft, Effects of tea and coffee on cardiovascular disease risk. *Food Funct.*, 2012, **3**, 575−591.

10   R. J. Clark. and R. Macrae, *Coffee 1 Chemistry*. Elsevier Applied Science, London, 1985.

11   M. N. Clifford and K. C. Willson, *Coffee: Botany, Biochemistry And Production Of Beans And Beverage*. Chapman and Hall, London, 1985.

12   R. J. Clarke and O. G. Vitzthum, *Coffee: Recent Developments*. Blackwell Science, Oxford, 2001.

13   A. Stalmach, M. N. Clifford, G. Williamson and A. Crozier, Phytochemicals in coffee and the bioavailability of chlorogenic acids, in *Teas, Cocoa and Coffee: Plant Secondary Metabolites and Health*, ed. A. Crozier, H. Ashihara and F. Tomás-Barberán, Wiley-Blackwell, Oxford, 2012, pp. 143−168.

14   P. Mazzafera, Chemical composition of defective coffee beans. *Food Chem.,* 1999, **64**, 547−554.

15   Q. Hu, T. Zhou, L. Zhang, and Y. Fang, Study of the separation and determination of monosaccharides in soluble coffee by capillary zone electrophoresis with electrochemical detection. *Analyst,* 2001, **126**, 298−301.

16   T. Kurzrock and K. Speer, Diterpenes and diterpene esters in coffee. *Food Rev. Int.*, 2001, **17**, 433−450.

17   R. Jaiswal and N. Kuhnert, Hierarchical scheme for liquid chromatography/multi-stage
        spectrometric identification of 3,4,5-triacyl chlorogenic acids in green robusta coffee
        beans. *Rapid Commun. Mass Spectrom.,* 2010, **24***,* 2283–2294.

18   M. N. Clifford, S. Knight, B. Surucu and N. Kuhnert, Characterization by LC-MS[n] of
        four new classes of chlorogenic acids in green coffee beans:
        dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-
        dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. *J.*
        Agric. Food Chem., 2006*,* **54***,* 1957–1969.

19   M. N. Clifford, S. Marks, S. Knight and N. Kuhnert, Characterization by LC–MS[n] of
        four novel classes of *p*-coumaric acid-containing diacyl chlorogenic acids in green
        coffee beans. *J. Agric. Food Chem.,* 2006, **54***,* 4095–4101.

20   M. N. Clifford and S. Knight, The cinnamoyl–amino acid conjugates of green robusta
        coffee beans. *Food Chem.,* 2004, **87***,* 457–463.

21   R. M. Alonso-Salces, C. Guillou and L. A. Berrueta, Liquid chromatography coupled
        with ultraviolet absorbance detection, electrospray ionization, collision-induced
        dissociation and tandem mass spectrometry on a triple quadrupole for the on-line
        characterization of polyphenols and methylxanthines in green coffee beans. *Rapid*
        Commun. Mass Spectrom., 2009, **23***,* 363–383.

22   R. Jaiswal, M. F. Matei, P. Subedi, and N. Kuhnert, Does roasted coffee contain
        chlorogenic acid lactones or/and cinnamoylshikimate esters? *Food Res. Int.*, 2013, doi:
        10.1016/j.foodres.2013.09.040.

23   T. H. Parliment, An overview of coffee roasting, in *Caffeinated Beverages: Health*
        Benefits, Physiological Effects and Chemistry, ed. T. H. Parliment, C. T. Ho and P.
        Schieberle, Eds., American Chemical Society, Washington, DC, 2000, pp.188–201.

24   W. Grosch in *Coffee: Recent Developments*, ed. R. J. Clarke and O. G. Vitzthum,

Blackwell Publishing Ltd., Oxford, 2001, pp. 68−89.

25   A. Illy and R. Viani, *Espresso Coffee: The Science of Quality.* Elsevier Academic

Press. London, 2005.

26   M. N. Clifford, Chlorogenic acids and other cinnamates-nature, occurrence and dietary

burden. *J. Sci. Food Agric.*, 1999, **79***,* 362−372.

27   M. N. Clifford, Chlorogenic acids and other cinnamates - nature, occurrence, dietary

burden, absorption and metabolism. *J. Sci. Food Agric.*, 2000, **80**, 1033−1043.

28   R. H. Stadler, D.H. Welti, A. A. Stampfli and L. B. Fay, Thermal decomposition of

caffeic acid in model systems: identification of novel tetraoxygenated phenylindan

isomers and their stability in aqueous solution. *J. Agric. Food Chem.,* 1996, **44**,

898−905.

29   B. M. Scholz and H. G. Maier, Isomers of quinic acid and quinide in roasted coffee. *Z.

Lebens.-Unters. Forschung,* 1990, **190***,* 132−134.

30   A. L. Dawidowicz and R. Typek, Thermal stability of 5-*O*-caffeoylquinic acid in

aqueous solutions at different heating conditions. *J. Agric. Food Chem.,* 2010*,* **58***,*

12578−12584.

31   A. L. Dawidowicz and R. Typek, The influence of pH on the thermal stability of 5-*O*-

caffeoylquinic acids in aqueous solutions. *Eur. Food Res. Technol.,* 2011, **23**3,

223−232.

32   A. L. Dawidowicz and R. Typek, Formation of ester and amine derivatives of 5-*O*-

caffeoylquinic acid in the process of its simulated extraction. *J Agric.Food Chem.,*

2012, **60**, 12289−12295.

33   R. Jaiswal, M. F. Matei, A. Golon, M. Witt and N. Kuhnert, Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food Funct.,* 2012, **3***,* 976−984.

34   M. F. Matei, R. Jaiswal and N. Kuhnert, Investigating the chemical changes of chlorogenic acids during coffee brewing - conjugate addition of water to the olefinic moiety of chlorogenic acids and their quinides. *J Agric.Food Chem.*, 2012, **60**, 12105−12115.

35   T. W. M. Crozier, A. Stalmach, M. E. J. Lean and A. Crozier, Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. *Food Funct.,* 2012, **3**, 30−33.

36   D. Gniechwitz, B. Brueckel, N. Reichardt, M. Blaut, H. Steinhart and M. Bunzel, Coffee dietary fiber contents and structural characteristics as influenced by coffee type and technological and brewing procedures. *J. Agric. Food Chem.,* 2007, **56**, 11027−11034.

37   A. S. Moreira, F. M. Nunes, M. R. Domingues, and M. A. Coimbra, Coffee melanoidins: Structure, mechanisms of formation and potential health impacts. *Food Funct.,* **3**, 2012, 903−915.

38   S. Homma, Chemistry II. Non-volatile compounds, Part II, in *Coffee: Recent Developments,* R. J. Clarke and O. G. Vitzthum, Eds., Blackwell Science, Oxford, 2001, pp. 50−67.

39   A. G. W. Bradbury, Chemistry I. Non-volatile compounds, in *Coffee: Recent Developments,* J. Clarke and O. G. Vitzthum, Eds., Blackwell Science, Oxford, 2001, pp. 1−17.

40   D. Perrone, A. Farah and C. M. Donangelo, Influence of coffee roasting on the

incorporation of phenolic compounds into melanoidins and their relationship with

antioxidant activity of the brew. *J. Agric. Food Chem.,* 2012, **60**, 4265−4275.

41   F. M. Nunes and M. A. Coimbra, Role of hydroxycinnamates in coffee melanoidin

formation, *Phytochem. Rev.,* 2010, **9,** 171−185.

42   M. Daglia, A. Papetti, C. Aceti, B. Sordelli, C. Gregotti and G. Gazzani, Isolation of

high molecular weight components and contribution to the protective activity of coffee

against lipid peroxidation in a rat liver microsome system. *J. Agric. Food Chem.,* 2008,

53, 1505−1513.

43   R. Tressl, G. T. Wondrak, L. A. Garbe, R.-P Krüger and D. Rewicki, Pentoses and

hexoses as sources of new melanoidin-like Mallaird polymers. *J. Agric. Food Chem.,*

1998, **46**, 1765−1776.

44   T. Hofmann, Studies on melanoidin-type colorants generated from Maillard reaction of

protein-bound lysine and furan-2-carboxaldehyde – chemical characterisation of a red

colored domain. *Eur. Food Res. Technol*., 1998, **206**, 251−258.

45   B. Caemmerer, W. Jalyschko and L. W. Kroh, Intact carbohydrate structures as part of

the melanoidin skeleton. *J. Agric. Food Chem.*, 2002, **50**, 2083−2087.

46   A. Golon, F. J. Gonzalez, J. Z. Davalos and N. Kuhnert, Investigating the thermal

decomposition of starch and cellulose in model systems and toasted bread using

domino tandem mass spectrometry. *J Agric. Food Chem.,* 2013*,* **61**, 674−684.

47   A. Golon and N. Kuhnert, Characterisation of "caramel-type" thermal decomposition

products of selected monosaccharides including fructose, mannose, galactose, arabinose

and ribose by advanced electrospray ionization mass spectrometry methods. *Food Funct.*, 2013, **4**, 1040−1050.

48   R. H. Stadler, N. Varga, J. Hau, F. A. Vera and D. H. Welti, Alkylpyridiniums. 1. Formation in model systems via thermal degradation of trigonelline. *J. Agric. Food Chem.*, 2002, **50**, 1192−1199.

49   R. H. Stadler, N. Varga, C. Milo, B. Schilter, F. A. Vera and D. H. Welti, Alkylpyridiniums. 2. Isolation and quantification in roasted and ground coffee. *J. Agric. Food Chem.*, 2002, **50**, 1200−1206.

50   R. Viani and I. Horman, Thermal behaviour of trigonelline, *J. Food Sci.,* 1974, **39**, 1216−1217.

51   S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic and M. G. Simic, Flavonoids as antioxidants. *J. Am. Chem. Soc.,* 1994, **116***,* 4846–4851.

52   T. L. Farrell, M. Gomez-Juaristi, L. Poquet, K. Redeuil K. Nagy, M. Renouf and G. Williamson, Absorption of dimethoxycinnamic acid derivatives in vitro and pharmacokinetic profile in human plasma following coffee consumption. *Mol. Nutr. Food Res.,* 2012, **56**, 1413–1423.

53   T. L. Farrell, T. P. Dew, L. Poquet, P. Hansen and G. Williamson, Absorption and metabolism of chlorogenic acids in cultured gastric epithelial monolayers. *Drug Metab Dispos.,* 2011, **39**, 2338−2346.

54   K. Redeuil, C. Smarrito-Menozzi, P. Guy, S. Rezzi, F. Dionisi, G. Williamson, K. Nagy and M. Renouf, Identification of novel circulating coffee metabolites in human plasma by liquid chromatography-mass spectrometry. *J. Chromatogr., A,* 2011, **1218**, 4678−4688.

55   A. Stalmach, W. Mullen, D. Barron, K. Uchida, T. Yokota, C. Cavin, H. Steiling, G. Williamson and A. Crozier, Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after ingestion of coffee by humans: identification of biomarkers of coffee consumption. *Drug Metab. Disp.*, 2009, **37**, 1749−1758.

56   M. N. Clifford, Diet-derived phenols in plasma and tissues and their implications for health. *Planta Medica,* 2004, **12***,* 1103−1114.

57   B. Halliwell, J. Rafter and A. Jenner, Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *Am. J. Clin. Nutr.,* 2005, *81,* 268S−276S.

58   A. Scalbert, I. T. Johnson and M. Saltmarsh, Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr.,* 2005, **81**, 215S−217S.

59   N. Kuhnert, One size does not fit all-bacterial cell death by antibiotics cannot be explained by the action of reactive oxygen species. *Angew. Chem. Int. Ed. Engl.*, 2013, **52**, 10946−10948.

60   D. Del Rio, A. Rodrigues-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges and A. Crozier, Dietary (poly)phenolics in human health and disease: structures, bioavailability, evidence of protective effects and potential mechanisms. *Antioxid. Redox Signal.,* 2013, **18**, 1818−1892.

61   R. Feng, Y. Lu, L. L. Bowman, Y. Qian, V. Castranova and M. Ding, Inhibition of activator Protein-1, NF-κB, and MAPKs and induction of phase 2 detoxifing enzyme activity by chlorogenic acid. *J. Biol. Chem.*, 2005*,* **280**, 27888−27895.

62   S. Ramos, Cancer chemoprevention and chemotherapy: Dietary polyphenols and signalling pathways. *Mol. Nutr. Food Res*., 2008, **52**, 507−526.

63   R. Z. Jurkowska, T. P. Jurkowski and A. Jeltsch, Structure and function of mammalian

DNA methyltransferases. *Chembiochem.*, 2011, **12**, 206−222.

64   A. P. Feinberg and B. Tycko, The history of cancer epigenetics. *Nature Rev. Cancer*,

2004, **4**, 143−153.

65   W. J. Lee and B. T. Zhu, Inhibition of DNA methylation by caffeic acid and

chlorogenic acid, two common catechol-containing coffee polyphenols.

Carcinogenesis, 2006, **27**, 269−277

66   J. S. Bonita, M. Mandarano, D. Shuta and J. Vinson, Coffee and cardiovascular disease:

in vitro, cellular, animal and human studies. *Pharmacol. Res.,* 2007, **55**, 187−198.

67   B. Park, 5-Caffeoylquinic acid and caffeic acid orally administered suppress P-selectin

expression on mouse platelets. *J. Nutr. Biochem.*, 2009, **20**, 800–805.

68   A. Stalmach, H. Steiling, G. Williamson and A. Crozier, Bioavailability of chlorogenic

acids following acute ingestion of coffee by humans with an ileostomy. *Arch. Biochem.

Biophys.,* 2010, **501**, 98–105.

69   R. Lang, A. Wahl, T. and T. Hofmann, Urinary *N*-methylpyridinium and trigonelline as

candidate dietary biomarkers of coffee consumption. *Mol. Nutr. Food Res.*, 2011, **55**,

1613−1623.

70   A. R. Rechner and C. Kroner, Anthocyanins and colonic metabolites of dietary

polyphenols inhibit platelet function. *Thromb. Res.*, 2005, **116**, 327−334.

71   E. Verzelloni, C. Pellacani, D. Tagliazucchi, S. Tagliaferri, L. Calani, L. G. Costa, F.

Brighenti, G. Borges, A. Crozier, A. Conte and D. Del Rio, Antiglycative and

neuroprotective activity of colon-derived polyphenols catabolites. *Mol. Nutr. Food

Res.,* 2011, **55**, S35-S43.

72   L. Poquet, M. N. Clifford and G. Williamson, Effect of dihydrocaffeic acid on UV irradiation of human keratinocyte HaCaT cells. *Arch. Biochem. Biophys.,* 2008, **476**, 196−204.

73   J. Huang, T. de Pauli and J. M. May, Antioxidant effects of dihydrocaffeic acid in human EA.hy926 endothelian cells. *J. Nutr. Biochem*., 2004, **15**, 722−729.

74   A. Suzuki, D. Kagawa, R. Ochiai, I. Tokimitsui and I. Saito, *Hyperten. Res.*, 2002, **25**, 99−107.

75   R. Ochiai, H. Jokura, A. Suzuki, I. Tokimitsu, M. Ohishi, N. Komai, H. Rakugi and T. Ogihara, Green coffee bean extract improves human vasoreactivity. *Hypertens. Res.,* 2004, **27***,* 731**−**737.

76   B. M. Scholz-Böttcher and H. G. Maier, Isomers of quinic acid and quinides in roasted coffee: indicators for the degree of roast? In *Quatorzième Colloque Scientifique International sur le café*, Association Scientifique Internationale du Café, Paris, 1992, pp. 220−229.

77   S. Shamil, G. G. Birch, M. Mathlouthi and M. N. Clifford, Apparent molar volumes and tastes of molecules with more than one sapophore. *Chem. Senses*, 1987, **12***,* 397−409.

78   K. L. Johnston, P. Sharp, M. N. Clifford and M. Morgan, Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. *FEBS Let.,* 2005*,* **579***,* 1653−1657.

79   C. A. Welsch, P. A. Lachance and B. P. Wasserman, Dietary phenolic compounds: Inhibition of Na^+-dependent D-glucose uptake in rat intestinal brush border membrane vesicles. *J. Nutr.,* 1989, **119**, 1698−1704.

80   K. L. Johnston, M. N. Clifford and L. M. Morgan, Coffee acutely modifies

gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects

of chlorogenic acid and caffeine. *Am. J. Clin. Nutr.,* 2003, **78**, 728−733.

81   D. S. Battram, R. Arthur, A. Weekes and T. A. Graham, The glucose intolerance

induced by caffeinated coffee ingestion is less pronounced than that due to alkaloid

caffeine in men. *J. Nutr.,*2006, **136**, 1276−1280.

82   E. Thom, The effect of chlorogenic acid enriched coffee on glucose absorption in

healthy volunteers and its effect on body mass when used long-term in overweight and

obese people. *J. Int. Med. Res,* 2007, **35***,* 900−908.

83   A. E. van Dijk, M. R. Olthof, J. C. Meeuse, E. Seebus, R. J. Heine and R. M. van Dam,

Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid

and trigonelline on glucose tolerance. *Diabetes Care*, 2009, **32**, 1023−1025.

84   L. L. Moisey, S. Kacker and A. C. Bickerton, L. E. Robinson and T. E. Graham,

Caffeinated coffee consumption impairs blood glucose homeostasis in response to high

and low glycemic index meals in healthy men. *Am. J. Clin. Nutr.,* 2008, **87**, 1254−1261.

85   J. A. Greenberg, D. R. Owen, and A. Geliebter, Decaffeinated coffee and glucose

metabolism in young men. *Diabetes Care,* 2010, **33**, 278−280.

86   M. R. Olthof, A. E. van Dijk, C. F. Deacon, R. J. Heine and R. M. van Dam, Acute

effects of decaffeinated coffee and the major coffee components chlorogenic acid and

trigonelline on incretin hormones. *Nutr. Metab.,* 2011, **8***,* 10 (doi: 10.1186/1743-7075-

8-10).

87   Y. Narita and K. Inouye, Kinetic analysis and mechanism on the inhibition of

chlorogenic acid and its components against porcine pancreas α-amylase isozymes I

and II. .*J. Agric. Food Chem.,* 2009, **57***,* 9218–9225.

88   F. L. Hsu, Y. C. Chen and J. T. Cheng, Caffeic acid as active principle from the fruit of

Xanthium strumarium to lower plasma glucose in diabetic rats. *Planta Med.,* 2000, **66**,

228–230.

89   I. M. Liu, F. L. Hsu, C. F. Chen and J. T. Cheng, Antihyperglycemic action of

isoferulic acid in streptozotocin-induced diabetic rats. *Br. J. Pharmacol*., 2000, **129**,

631–636.

90   P. Peungvicha, R. Temsiririrkkul, J. K. Prasain, Y. Tezuka, S. Kadota, S. S.

Thirawarapan and H. Watanabe H., 4-Hydroxybenzoic acid: A hypoglycemic

constituent of aqueous extract of *Pandanus odorus* root. *J. Ethnopharmacol.,* 1998, **62**,

79–84.

91   I. A. Ludwig, M. Paz de Peña, C. Cid, and A. Crozier, Catabolism of coffee

chlorogenic acids by human colonic microbiota. *Biofactors*, 2013, **39**, 623–632.

92   A. M. Jenner, J. Rafter and B. Halliwell, Human fecal water content of phenolics: the

extent of colonic exposure to aromatic compounds. *Free Radic. Biol. Med.,* 2005, **38**,

763–772.

93   M. Daglia, R. Tarsi, A. Papetti, P. Grisoli, C. Dacarro, C. Pruzzo and G. Gazzani, Anti-

adhesive effect of green and roasted coffee on *Streptococcus mutans'* adhesive

properties on saliva-coated hydroxyapatite beads. *J. Agric. Food Chem.,* 2002, **50**,

1225–1229.

94   M. E. Ramos-Niño, C. A. Ramìrez-Rodriguez, M. N. Clifford and A. R. Adam. A

comparison of quantitative structure–activity relationships for the effect of benzoic and

cinnamic acids on *Listeria monocytogenes* using multiple linear regression, artificial

neural networks and fuzzy systems. *J. Appl. Microbiol.,* 1997, **82***,* 168−176.

95   M. A. Heckman, J. Weil and E. Gonzalez de Mejia, Caffeine (1,3,7-trimethylxanthine)

in foods: a comprehensive review on consumption, functionality, safety, and regulatory

matters. *J. Food Sci.,* 2010, **75**, R77−R87.

96   R. R. McCusker, B. A. Goldberg and E. Cone, Caffeine content of specialty coffees. *J.

Anal. Toxicol.,* 2002, **27**, 520−522.

97   R. Lang, N. Dieminger, A. Beusch, Y. M. Lee, A. Dunkel, B. Suess, T. Skurk, A. Wahl,

H. Hauner and T. Hofmann, Bioappearance and pharmacokinetics of bioactives upon

coffee consumption. *Anal. Bioanal. Chem*., 2013, **405**, 8487–8503.

98   M. J. Arnaud, Pharmacokinetics and metabolism of natural methylxanthines in animal

and man. *Handb, Exp. Pharmacol,* 2011, **200**, 33−91.

99   B. B. Fredholm, K. Bäettig, J. Holmén, A. Nehlig and E. E. Zvartau, Actions of

caffeine in the brain with special reference to factors that contribute to its widespread

use. *Pharmacol. Revs.,* 1999, **51**,83−133.

100   M. E. J. Lean, H. Ashihara, M. N. Clifford and A. Crozier, Purine alkaloids: A focus on

caffeine and related compounds in beverages, in *Teas, Cocoa and Coffee: Plant

Secondary Metabolites and Health*, A. Crozier, H. Ashihara, and F. Tomás-Barberán,

Eds., Wiley-Blackwell, Oxford, UK, 2012, pp. 25−44.

101   D. Borota, E. Murray, G. Keceli, A. Chang, J. M. Watabe, M. Ly, J. P. Toscano and M.

A. Yassa, Post-study caffeine administration enhances memory consolidation in

humans. *Nat. Neurosci.*, 2014, doi: 10.1038/nn.3623.

102   J. Trevitt, K. Kawa, A. Jalali and C. Larsen, Differential effects of adenosine

        antagonists in two models of Parkinsonian tremor. *Pharmacol. Biochem. Behav.,* 2009,

        94, 24−29.

103   M. Kitagawa, H. Houzen and K. Tashiro, Effects of caffeine on the freezing of gait in

        Parkinson's disease. *Movement Disorders*, 2007, **22**, 710−712.

104   S. Rudelle, M. G. Ferruzzi, I. Cristiani, J. Moulin, K. Macé, K. J. Acheson and L.

        Tappy, Effect of a thermogenic beverage on 24-hour energy metabolism in humans.

        Obesity, 2007, **15**, 349−355.

105   T. Devasagayam, J. Kamat, H. Mohan and P. C. Kesavan, Caffeine as an antioxidant:

        inhibition of lipid peroxidation induced by reactive oxygen species. *Biochim. Biophys.*

        Acta, 1996, **1282**, 63−70.

106   T. M. MacDonald, K. Sharpe, G. Fowler, D. Lyons, S. Freestone, H. G. Lovell, J.

        Webster and J. C. Pitrie, Caffeine restriction: effect on mild hypertension. *BMJ*, 1991,

        303, 1235−1238.

107   C. Lee, Antioxidant ability of caffeine and its metabolites based on the study of oxygen

        radical absorbing capacity and inhibition of LDL peroxidation. *Clin. Chim. Acta.,* 2000,

        295, 141−154.

108   N. P. Riksen, P. Smits and G. A. Rongen, The cardiovascular effects of

        methylxanthines. *Handbook Expt. Pharmacol*., 2011, **200**, 413−437.

109   P. Rogers, C. Hohoff, S. V. Heatherley, E. L. Mullings, P. J. Maxfield, R. P. Evershed,

        J. Deckert and D. J. Nutt, Association of the anxiogenic and alerting effects of caffeine

        with ADORA2A and ADORA1 polymorphisms and habitual level of caffeine

        consumption. *Neuropsychopharmacology*, 2010, **35**, 1973−1982.

110 R. Guieu, C. Devaux and H. Henry, Adenosine and migraine. *Can. J. Neurol. Sci.,* 1998, **25**, 55-58.

111 L. M. Juliano and R. R. Griffith, A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology,* 2004, **176**, 1−29.

112 L. M. Juliano, S. Ferre and R. R. Griffiths, R.R. The pharmacology of caffeine, in *Principles of Addiction Medicine,* 4th edition, ed. R. K. Ries, D. A. Fiellin, S. C. Miller and R. Saitz, Lippincott, Williams and Wilkins, Philadelphia, 2009, pp. 159−178.

113 Metro, Man killed by 4 cans of Red Bull a day. 2008. http://www.metro.co.uk/news/144543-man-killed-by-4-cans-of-red-bull-a-day.

114 B.B.C. Red Bull in suspected link to deaths. 2001. (http://news.bbc.co.uk/1/hi/1435409.stm).

115 Daily Telegraph. Student died after sharing three cans of Red Bull. 2011. http://www.telegraph.co.uk/health/1374291/Student-died-after-sharing-three-cans-of-Red-Bull.html

116 B.B.C. Man died after eating Hero Instant Energy Mints, says coroner. 2013. http://www.bbc.co.uk/news/uk-england-birmingham-24492833

117 B.B.C. Girl overdoses on espresso coffee. 2007. (http://news.bbc.co.uk/1/hi/england/wear/6944026.stm).

118 J. L. Mills, L. B. Holmes, J. H. Aarons, J. L. Simpson, Z. A. Brown, L. G. Jovanovic-Peterson, M. R. Conley, B. I. Graubard, R. H. Knopp and B. E. Metzger, Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation. *JAMA,* 1993, **569**, 593−597.

119   L. Fenster, A. Hubbard, S. H. Swan, G. C. Windham, K. Waller, R. A. Hiatt, R.A. and

N. Benowitz, Caffeinated beverages, decaffeinated coffee, and spontaneous abortion.

Epidemiology, 1997, **8**, 515–523.

120   M. Giannelli, P. Doyle, E. Roman, M. Pelerin and C. Hermon, The effect of caffeine

consumption and nausea on the risk of miscarriage. *Paediatr. Perinat. Epidemiol.,*

2003, **17**, 316-323.

121   V. Rasch, Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous

abortion. *Acta. Obstet. Gynecol. Scand.,* 2003, **82**, 182–188.

122   W. Wen, X. O. Shu, R. D. Jacobs and J. E. Brown, The association of maternal caffeine

consumption and nausea with spontaneous abortion. *Epidemiology*, 2001, **12**, 38-42.

123   H. D. Vlajinac, R. R. Petrovic, J.M. Marinkovic, S. B. Sipetic and B. J. Adanja, Effect

of caffeine intake during pregnancy on birth weight. *Am. J. Epidemiol.,* 1997, **145**,

335–338.

124   V. Sengpiel, E. Elind, J. Bacelis, S. Nilsson, J. Grove, R. Myhre, M. Haugen, M. H.

Meltzer, J. Alexander, B. Jacobsson and A. L. Brantstaeter, Maternal caffeine intake

during pregnancy is associated with birth weight but not with gestational length: results

from a large prospective observational cohort study. *BMC Med.*, 2013, **11**, 42. (doi:

10.1186/1741-7015-11-42).

125   I. Fortier, S. Marcoux and L. Beaulac-Baillargeon, Relation of caffeine intake during

pregnancy to intrauterine growth retardation and preterm birth. *Am. J. Epidemiol.,*

1993, **137**, 931–940.

126   B. H. Bech, C. Obel, T. B. Henriksen and J. Olsen, Effect of reducing caffeine intake

on birth weight and length of gestation: randomised controlled trial. *BMJ,* 2007, **334**,

409–412.

127   S. Boylan, J. E. Cade, V. A. Dolby, D. C. Greenwood, A. W. Hay et al. (CARE Study
      Group), Maternal caffeine intake during pregnancy and risk of fetal growth restriction:
      a large prospective observational study. *BMJ,* 2008, **337**, a2332.

128   Wadge, A., Food Standard Agency's advice on caffeine. *BMJ,* 2009, **338**, b299.

129   C. L. Ky, J. Louarn, S. Dussert, B. Guyot, S. Hamon, and M. Noirot,
      Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L.
      and *C. canephora* P. Accessions. *Food Chem.*, 2001, **75**, 223−230.

130   S. Casal, B. Oliveira, and A. F. Margarida. HPLC/diode array applied to thermal
      degradation of trigonelline, nicotinic acid and caffeine in coffee. *Food Chem.*, 2000,
      68, 481−485.

131   D. Perrone, A. Farah and C. M. Donangelo, Fast simultaneous analysis of caffeine,
      trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography-mass
      spectrometry. *Food Chem.*, 2008, **110**, 1030−1035.

132   L. Maeztu, S. Andueza, C. Ibañez, M.P. de Peña, J. Bello, and C. Cid, Multivariate
      methods for characterization and classification of espresso coffees from different
      botanical varieties and types of roast by foam, taste, and mouthfeel. *J. Agric. Food
      Chem.* 2001, **49**, 4743−4747.

133   R. Lang, E. F. Yagar, A. Wahl, A. Beusch, A. Dunkel, N. Dieminger, R. Eggers, G.
      Bytof, H. Stiebitz, I. Lantz, and T. Hofmann. Quantitative studies on roast kinetics for
      bioactives in coffee. *J. Agric. Food Chem.* 2013b, doi: 10.1021/jf403846g.

134   R. Lang, A. Wahl, T. Skurk, E. F. Yagar, L. Schmiech, R. Eggers, H. Hauner and T.
      Hofmann, T. Development of a hydrophilic liquid Interaction chromatophiy−high-
      performance liquid chromatography−tandem mass spectrometry based stable isotope

dilution analysis and pharmacokinetic studies on bioactive pyridines in human plasma and urine after coffee consumption. *Anal. Chem.*, 2010, **82**, 1486–1497.

135 S. Yuyama, Absorption of trigonelline from the small intestine of the specific pathogen-free (SPF) and germ-free (GF) rats *in vivo*. *Adv. Exp. Med. Biol.,*1999, **467**, 723–727.

136 J. Zhou, S. Zhou and S. Zeng, Experimental diabetes treated with trigonelline: effect on ß-cell. *Fund. Clin. Pharmacol.*, 2011, **27**, 279–287.

137 O. Yoshinari, H. Sato and K. Igarashi, Anti-diabetic effects of pumpkin and its components, trigonelline and nicotinic acid on Goto-Kakizaki rats. *Biosci. Biotech. Biochem.*, 2009, **73**, 1033–1041.

138 C. Tohoda, T. Kuboyama and K. Komatsu, Search for natural products related to regeneration of neuronal network. *Neurosignals*, 2005, **14**, 34–45.

139 V. Gaur, L. Bodhankar, V. Mohan and P. A. Thakurdesai, Neurobehavioral assessment of hydroalcoholic extract of *Trigonella foenum-graecum* seeds in rodent models of Parkinson's disease. *Pharmaceut. Biol.,* 2013, **51**, 550–557.

140 A. B. Al-Mehdi, K. Tozawa, A. B. Fisher, L. Shientag, A. Lee, and R. J. Muschel, Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. *Nature Medicine*, 2000, **6**, 100–102.

141 N. Hirakawa, R. Okauchi, Y. Miura, and K. Yagasaki,. Anti-invasive activity of niacin and trigonelline against cancer cells. *Biosci. Biotech Biochem.*, 2005, **69**, 653–658.

142 C. A. Lamartiniere, J. B. Moore, M. N. Brown, R. Thompson, M. J. Hardin and S. Barnes, Genistein suppresses mammary cancer in rats. *Carcinogenesis*, 1995, **16**, 2833–2840.

143 W. B. Murril, N. M. Brown, J. X Zhang, P. A. Manzolillo, S. Barnes and C. A.
Lamartiniere, Prepubertal genistein exposure suppresses mammary cancer and
enhances gland differentiation in rats. *Carcinogenesis*, 1996, **17**, 1451−1457.

144 S. Singh, S. Vrishni, B. K. Singh, I. Rahman and P. Kakkar, Nrf2-ARE stress response
mechanism: a control point in oxidative stress-mediated dysfunctions and chronic
inflammatory diseases. *Free Rad. Res.,* 2010, **44**, 1267−1288.

145 W. O. Osbum and T. W. Kensler, Nrf2 signalling: an adaptive response pathway for
protection against environmental toxic insults. *Mutation Res.*, 2008, **659**, 31−39.

146 A. Arlt, S. Sebens, S. Krebs, C. Geismann, M. Grossmann, M. L. Kruse, S. Schreiber
and H. Schaefer, Inhibition of the Nrf2 transcription factor by the alkaloid trigonelline
renders pancreatic cancer cells more susceptible to apoptosis through decreased
proteasomal gene expression and proteasome activity. *Oncogene,* 2013, **32**, 4825–4835.

147 A. A. P. Almeida, A. Farah, D. A. Silva, E. A. Nunan and B. Gloria, Antibacterial
activity of coffee extracts and selected coffee chemical compounds against
enterobacteria. *J. Agric. Food Chem.*, 2006, **54**, 8738−8743.

148 R. Urgert and M. B. Katan, The cholesterol-raising factor from coffee beans. *Annu.
Rev. Nutr.*, 1997, **17**, 305−324.

149 C. Cavin, D. Holzhaeuser, G. Scharf, A. Constable, W. W. Huber and B. Schilter,
Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity.
Food Chem. Toxicol., 2002, **40**, 1155−1163.

150 S. K. Bøhn, R. Blomhoff and I. Paur, Coffee and cancer risk, epidemiological evidence,
and molecular mechanisms. *Mol. Nutr. Food Res.*, 2013, doi: 10.1002/mnfr.201300526.

151 V. Fogliano and F. J. Morales, Estimation of dietary intake of melanoidins from coffee and bread. *Food Funct.,* 2011, **2**, 117−123.

152 J. M. Silvan, F. Morales and F. Saura-Calixto, Conceptual study on Maillardized dietary fiber in coffee. *J. Agric. Food Chem.*, 2010, **58**, 12244−12249.

153 F.J. Morales, V. Somoza and V. Fogliano, Physiological relevance of dietary melanoidins. *Amino Acids*, 2012, **42**, 1097−1109.

154 P. Vitaglione, V. Fogliano, and N. Pellegrini, Coffee, colon function and colorectal cancer. *Food Funct.*, 2012, **3**, 916–922.

155 V. Valls-Bellés, M. C. Torres, P. Muñiz, L. Boix, M. L. González-Sanjose and P. Codoñer-Franch, The protective effects in adriamycin-induced of melanoidins oxidative stress in isolated rat hepatocytes. *J. Sci. Food Agric.*, 2004, **84**, 1701−1707.

156 M. A. Martin, S. Ramos, R. Mateos, J. A. Rufián-Henares, F. J. Morales, L. Bravo and I. Goya, L. Biscuit melanoidins of different molecular masses protect human HepG2 cells against oxidative stress. *J. Agric. Food Chem.,* 2009, **57**, 7250−7258.

157 E. Verzelloni, D. Tagliazucchi, D. Del Rio, L. Calani and A. Conte, Antiglycative and antioxidant properties of coffee fractions. *Food Chem.*, 2011, **124**, 1430−1435.

158 J. A. Rufian-Henares and F. J. Morales Angiotensin-I converting enzyme inhibitory activity of coffee melanoidins. *J. Agric. Food Chem.*, 2007, **55**, 1480−1485.

159 D. Tagliazucchi, E. Verzelloni and A. Conte, Effect of dietary melanoidins on lipid peroxidation during simulated gastric digestion: their possible role in the prevention of oxidative damage. *J. Agric. Food Chem.*, 2010, **58**, 2513–2519.

160 M. Stauder, A. Papetti, D. Mascherpa, A. M. Schito, G. Gazzani, C. Pruzzo and M. Daglai, Antiadhesion and antibiofilm activities of high molecular weight coffee

components against *Streptococcus mutans*. *J. Agric. Food Chem.,* 2010*,* **58**, 11662−11666.

161   M. D. Argirova, I. D. Stevanova, A. D. Krustev and V. I. Turiiski, Testing biological activity of model Maillard reaction products: studies on gastric smooth muscle tissues. *Amino Acids*, 2010, **38**, 797−803.

162   J. A. Rufian-Henares and S. P. de la Cueva, Antimicrobial activity of coffee melanoidins - A study of their metal-chelating properties. *J. Agric. Food Chem.,* 2009, **57**, 432−438.

163   V. Faist and H. F. Erbersdobler, Metabolic transit and *in vivo* effects of melanoidins and precursor compounds deriving from the Maillard reaction. *Ann. Nutr. Metab.,* 2001, **1**, 1−12.

164   D. Gniechwitz, N. Reichardt, E. Meiss, J. Ralph, H. Steinhart, M. Blaut and M. Bunzel, Characterization and fermentability of an ethanol soluble high molecular weight coffee fraction. *J. Agric. Food Chem.,* 2008, **56**, 5960−5969.

165   N. Reichardt, D. Gniechwitz, H. Steinhart,M. Bunzel and M. Blaut, Characterization of high molecular weight coffee fractions and their fermentation by human intestinal microbiota. *Mol. Nutr. Food Res*., 2009, **53**, 287−299.

166   G. Williamson, Coffee and health, in *Teas, Cocoa and Coffee: Plant Secondary Metabolites and Health*, ed. A. Crozier, H. Ashihara and F. Tomás-Barberán, Wiley-Blackwell, Oxford, 2012, pp. 169−181.

167   N. Moura-Nunes, D. Perrone, A. Farah and C. M. Donangelo,The increase in human plasma antioxidant capacity after acute coffee intake is not associated with endogenous non-enzymatic antioxidant components. *Int. J. Food Sci. Nutr*., 2009, **60**, 173−181.

168   M. J. Grubben, C. C. van den Braak, R. Broekhuizen, R. De Jong, L. van Rijt L, E. de Ruijter, W. H. Peters, M. B. Katan and F. M. Nagengast, The effect of unfiltered coffee on potential biomarkers for colonic cancer risk in healthy volunteers: a randomized trial. *Aliment. Pharmacol. Ther.,* 2000, **14**, 1181–1190.

169   F. Esposito, F. Morisco, V. Verde, A. Ritieni, A. Alezio, N. Caporaso and V. Fogliano, Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects. *Aliment. Pharmacol. Ther*., 2003, **17**, 595–601.

170   H. Steinkellner, C. Hoelzl, M. Uhl, C. Cavin, G, Haidinger, A. Gsur, R. Schmid, M. Kundi, J. Bichler and S. Knasmüller, Coffee consumption induces GSTP in plasma and protects lymphocytes against (+/-)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide induced DNA-damage: results of controlled human intervention trials. *Mutat. Res.* 2005, **591**, 264–275.

171   R. M. van Dam and F. B. Hu, Coffee consumption and risk of type 2 diabetes – A systematic review. *JAMA*, 2005, **294**, 97–104.

172   R. M. van Dam, Coffee and type 2 diabetes: From beans to β-cells. *Nutr. Metab. Cardiovasc. Dis.*, 2006, **16**, 69–77.

173   R. M. van Dam and E. J. Feskens, Coffee consumption and risk of type 2 diabetes mellitus. *Lancet,* 2002, **360**, 1477–1478.

174   A. Rosengren, A. Dotevall, L. Wilhelmsen, D. Thelle and S. Johansson, Coffee and incidence of diabetes in Swedish women: a prospective 18-year follow-up study. *J. Int. Med.,* 2004, **255**, 89–95.

175   R. Huxley, C. M. Lee, F. Barzi, L. Timmermeister, S. Czernichow, V. Perkovic, D. E. Grobbee, D. Batty and M. Woodward, Coffee, decaffeinated coffee, and tea

consumption in relation to incident type 2 diabetes mellitus A systematic review with meta-analysis. *Arch. Intern. Med.*, 2009, **169**, 2053−2063.

176   A. Aldughpassi and T. M. S.Wolever, Effect of coffee and tea on the glycaemic index of foods: no effect on mean but reduced variability. *Br. J. Nutr.*, 2009, **101**, 1282−1285.

177   K. Kempf, C. Herder, I. Erlund, H. Kolb S. Martin M. Carstensen M, Koenig W, Sundvall J, Bidel S, Kuha and J. Tuomilehto, Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. *Am. J. Clin. Nutr.,* 2010, **91**, 950−957.

178   D. S. Thelle, S. Heyden and J. G. Fodor, Coffee and cholesterol in epidemiological and experimental studies. *Atherosclerosis*, 1987, **67**, 97−103.

179   S. H. Jee, J. He, L. J. Appel, P. K. Whelton, I. Suh and M. J. Klag, Coffee consumption and serum lipids: A meta-analysis of randomized controlled clinical trials. *Am. J. Epidemiol.,* 2001, **153**, 353−362.

180   J. N. Wu, S. C. Ho, C. Zhou, W. H. Ling, W. Q. Chen, C. L. Wang and Y. M. Chen, Coffee consumption and risk of coronary heart diseases: A meta-analysis of 21 prospective cohort studies. *Int. J. Cardiol.*, 2009, **137**, 216−225.

181   I. Stensvold, A. Tverdal and B. K. Jacobsen, Cohort study of coffee intake and death from coronary heart disease over 12 years. *BMJ,* 1996, **312**, 544−545.

182   A. A. Hakim, G. W. Ross and J. D. Curb, Coffee consumption in hypertensive men in older middle-age and the risk of stroke. The Honolulu Heart Program. *J. Clin. Epidemiol.*, 1998, **51**, 487−494.

183   E. Lopez-Garcia, F. Rodriguez-Artalejo and K. M. Rexrode, Coffee consumption and risk of stroke in women. *Circulation,* 2009, **119**, 1116−1123.

184   D. B. Panagiotakos, C. Pitsavos, C. Chrysohoou, P. Kokkinos, P. Toutouzas and C. Stefanadis, The J-shaped effect of coffee consumption on the risk of developing acute coronary syndromes: the CARDIO2000 case–control study. *J. Nutr.*, 2003, **133**, 3228−3232.

185   S. Buscemi, S. Verga, J. A. Batsis, M.. Donatelli, M. R. Tranchina, S. Belmonte, A. Mattina, A. Re and G. Cerasola, Acute effects of coffee on endothelial function in healthy subjects. *Eur. J. Clin. Nutr*, 2010, **64**, 483−489.

186   F. Natella, M. Nardini, F. Belelli, P. Pignatelli S. Di Santo A. Ghiselli F. Violi and C. Scaccini, Effect of coffee drinking on platelets: inhibition of aggregation and phenols incorporation. *Br. J. Nutr.* 1998, **100**, 1276−1282.

187   L. Arab, Epidemiologic evidence on coffee and cancer. *Nutr. Cancer*, 2010, **62**, 271−283.

188   K. Hirose, Y. Niwa, K. Wakai, K. Matsuo, T. Nakanishi and K. Tajima, Coffee consumption and the risk of endometrial cancer: Evidence from a case-control study of female hormone-related cancers in Japan. *Cancer Sci.,* 2007, **98**, 411−415.

189   T. Koizumi, N. Nakaya, C. Okamura, Y. Sato T. Shimazu, S. Nagase, H. Niikura, S. Kuriyama, T. Tase, K. Ito, Y. Tsubono, K. Okamura, N. Yaegashi and I. Tsuji, Case-control study of coffee consumption and the risk of endometrial endometrioid adenocarcinoma. *Eur. J. Cancer Prev.*, 2008, **17**, 358−363.

190   F. Bravi, L. Scotti, C. Bosetti, A. Zucchetto, R. Talamini, M. Montella, S.Greggi, C. Pelucchi, E. Negri S. Franceschi and C. La Vecchia, Food groups and endometrial cancer risk: a case-control study from Italy. *Am. J. Obst. Gynecol.*, 2009, **200**, 293.e1−7.

191   T. Shimazu, M. Inoue, S. Sasazuki, M. Iwasaki, N. Kurahashi, T. Yamaji and S. Tsugane, Coffee consumption and risk of endometrial cancer: A prospective study in Japan. *Int. J. Cancer*, 2008, **123**, 2406–2410.

192   M. Inoue, I. Yoshimi, T. Sobue and S. Tsugane, Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: A prospective study in Japan. *J. Nat. Cancer Inst.,* 2005, **97**, 293–300.

193   Y. Kurozawa, I. Ogimoto, A.Shibata, T. Nose, T. Yoshimura, H. Suzuki, R. Sakata, Y. Fujita, S. Ichikawa, N. Iwai and A. Tamakoshi, Coffee and risk of death from hepatocellular carcinoma in a large cohort study in Japan. *Br. J. Cancer,* 2005, **93**, 607–610.

194   U. Gelatti, L. Covolo, M. Franceschini, F. Pirali, A. Tagger, M. L. Ribero, P. Trevisi, C. Martelli, G. Nardi and F. Donato, Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: a case-control study. *J. Hepatol.,* 2005, **4**, 528–534.

195   F. Bravi, C. Boscetti, A. Tavani, V. Bagnardi, S. Gallus, E. Negri, S. Franceschi and C. La Vecchia, Coffee drinking and hepatocellular carcinoma risk: A meta-analysis. *Hepatology*, 2007, **46**, 430–435.

196   S. C. Larsson and A. Wolk, Coffee consumption and risk of liver cancer: A meta-analysis. *Gastroenterology,* 2007, **132**, 1740–1745.

197   A. Tavani and C. La Vecchia, Coffee, decaffeinated coffee, tea and cancer of the colon and rectum: a review of epidemiological studies, 1990–2003. *Cancer Causes Control,* 2004, **15**, 743–757.

198   C. Galeone, F. Turati, C. La Vecchia, W. Garavello and A. Tavani, Coffee consumption
and risk of colorectal cancer: a meta-analysis of case–control studies. *Cancer Causes
Cont*rol, 2010, **21**, 1949–1959.

199   G. Li, D. Ma, Y. Zhang and P. Wang, Coffee consumption and risk of colorectal
cancer: a meta-analysis of observational studies. *Public Health Nutr.*, 2013, **16**,
346–357.

200   C. Tian, W. Wang, Z. Hong and X. Zhang, Coffee consumption and risk of colorectal
cancer: a dose-response analysis of observational studies. *Cancer Causes Control*,
2013, **24**, 1265–1268.

201   J. A. Baker, G. P. Beehler, A. C. Sawant, V. Jayaprakash, S. E. McCann and K. B.
Moysich, Consumption of coffee, but not black tea, is associated with decreased risk of
premenopausal breast cancer. *J Nutr*, 2006, **136**, 166–171.

202   D. F. Easton D. Ford and D. T. Bishop, Breast and ovarian cancer incidence in BRCA1-
mutation carriers. Breast Cancer Linkage Consortium. *Am. J. Hum. Genet*., 1995, **56**,
265–271.

203   A. Nkondjock, P. Ghadirian, J. Kotsopoulos, J. Kotsopoulos J. Lubinski, H. Lynch, C.
Kim-Sing, D. Horsman, B. Rosen, C. Isaacs, B. Weber, W. Foulkes, P. Ainsworth, N.
Tung, A. Eisen, E. Friedman, C. Eng, P. Sun and S. A. Narod, Coffee consumption and
breast cancer risk among BRCA1 and BRCA2 mutation carriers. *Int. J. Cancer*, 2006,
118, 103–107, 2006.

204   M. A. Hernán, B. Takkouche, F. Caamaño-Isorna and J. J. Gestal-Otero, A meta-
analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann.
Neurol.*, 2002, **52**, 276–284.

205   A. Ascherio, H. Chen, H., M. A. Schwarzschild, S. M. Zhang, G. A. Colditz and F. E. Speizer, Caffeine, postmenopausal estrogen, and risk of Parkinson's disease. *Neurology*, 2003, **60**, 790–795.

206   A. Ascherio, M. G. Weisskopf, E. J. O'Reilly, M. L. McCullough, E. E. Calle, C. Rodriguez and M. J. Thun, Coffee consumption, gender, and Parkinson's disease mortality in the Cancer Prevention Study II Cohort: the modifying effects of estrogen. *Am. J. Epidemiol.*, 2004, **160**, 977–984.

207   B. G. Pollock, M. Wylie, J. A. Stack, D. A. Sorisio, D. S. Thompson, M. A. Kirshner, M. M. Folan and K. A. Condifer, Inhibition of caffeine metabolism by estrogen replacement therapy in postmenopausal women. *J. Clin. Pharmacol.*, 1999, **39**, 936–940.

208   J. F. Chen, K. Xu, J. P. Petzer, R. Staal, Y. H. Xu, M. Beilstein, P. K. Sonsalla, K. Castagnoli, N. Castagnoli Jr. and M. A. Schwarzschild, Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. *J. Neurosci.*, 2001, **21**, 1–6.

209   K. Ikeda, M. Kurokawa, S. Aoyama and Y. Kuwana, Neuroprotection by adenosine A2A receptor blockade in experimental models of Parkinson's disease. *J. Neurochem.*, 2002, **80**, 262–270.

Figure Legends

Fig. 1.   Coffee fruit at different stages of maturity. Picture supplied by Dr. Tim Bond, AVT Tea Services Ltd., London, UK.

Fig. 2.   Green coffee beans, the cotyledons of the seed after removal of the fleshly pericarp from the mature fruit. Picture supplied by Dr Eduarda Cristovam, Mathew Algie Co. Ltd., Glasgow, UK

Fig. 3.   Structures of the main CGAs in green coffee beans.

Fig. 4.   Structures of the purine alkaloid caffeine and the pentacyclic diterpenes kahweol and cafestol

Fig. 5.   Structures of phenylindans formed during roasting by the degradation of the caffeic acid-containing CGAs; trigonelline, and nicotinic acid and methylated pyridinium derivatives formed from trigonelline during roasting; the mammalian metabolites of caffeine, theobromine, paraxanthine and theophylline

Fig. 6.   Structures of some of the volatile compounds, which have a major impact on the aroma of roasted coffee beans. Kahweofuran is reputed to have a coffee-like small even in isolation.

Fig. 7.   Human metabolites of 5-CQA.

Fig. 8   Pathways for the metabolism of caffeine by humans to di- and monomethylxanthines, the corresponding tri-, di- and monomethyl uric acids and three uracil derivatives formed by opening of the five-membered ring. After Arnaud.[98]

Table 1 Levels of CQA isomers and caffeine in espresso coffees purchased from 20 coffee shops in Glasgow, United Kingdom (after Crozier et al.[35])

Coffee shop	Volume of coffee (mL)	3-CQA (mg)	4-CQA (mg)	5-CQA (mg)	Total CQA per serving (mg)	Caffeine per serving (mg)
Pattiserie Francoise	52	95	112	216	423	322
S'mug	32	62	78	160	300	173
Costa Coffee	25	48	61	118	227	157
Little Italy	23	37	59	121	217	129
Paperino's	50	65	52	99	216	205
Peckhams	70	65	52	99	216	140
Chapter 1	26	45	58	112	215	140
University Cafe	49	40	54	93	187	230
Baguette Express	45	30	40	74	144	140
Kember & Jones	43	37	46	92	141	90
Heart Buchanan	24	22	37	67	126	156
Jellyhill	63	26	29	56	111	151
Coffee @ 491	49	23	31	55	109	98
Beanscene	48	19	25	49	93	77
Tinderbox	25	19	24	46	89	75
Café Cinnamon	59	17	23	41	81	242
Crepe á Croissant	34	17	23	41	81	95
Morton's	35	13	16	27	56	73
Antipasti	36	8	15	21	44	72
Starbucks	27	5	7	12	24	51
Range	**23 – 70**	**5 – 95**	**7 – 112**	**12 – 216**	**24 – 423**	**50 – 322**
Median	**43**	**36**	**37**	**67**	**126**	**140**

Table 2 Pharmacokinetic parameters of CGAs and metabolites circulating in plasma of healthy volunteers, 0-24 h after the ingestion of 412 μmol of CGAs and derivatives contained in a 200 mL serving of instant coffee (after Stalmach et al.[55])[a]

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

[a]Data expressed as mean values ± SE (*n* = 11). C_{max} – peak plasma concentration; T_{max} - time after ingestion to reach C_{max}; Apparent $T_{1/2}$ – apparent elimination half-life after oral dosing

[b] Double C_{max} and T_{max} values are due to the biphasic absorption profile of ferulic acid-4-*O*-sulphate resulting from absorption in both the small and large intestine

Table 3 Plasma pharmacokinetic parameters of trigonelline and 1-methylpyridinium circulating in plasma of male and female volunteers after the ingestion of 350 mL of filter coffee containing 808 µmol (110 mg) of trigonelline and 172 µmol (16 mg) of 1-methylpyridinium contained in a 200 mL serving of instant coffee (after Lang et al.[134]).

Pyrimidine derivatives	C_{max} (µM)		T_{max} (h)		Apparent $T_{1/2}$ (h)		0-8 h urinary excretion (% of intake)	
	Male	Female	Male	Female	Male	Female	Male	Female
Trigonelline	5.5 ± 0.7	6.5 ± 0.1	2.3 ± 1.0	3.2 ± 1.0	4.6 ± 1.6	5.5 ± 2.1	57 ± 4	46 ± 3
1-Methylpyridinium	0.8 ± 0.2	0.8 ± 0.1	1.0 ± 0.4	1.7 ± 0.5	2.3 ± 0.5	2.1 ± 0.3	69 ± 4	62 ± 7

[a]Data expressed as mean values ± SD (males n = 7; females n = 6). C_{max} – peak plasma concentration; T_{max} - time after ingestion to reach C_{max}; Apparent $T_{1/2}$ – apparent elimination half-life after oral dosing
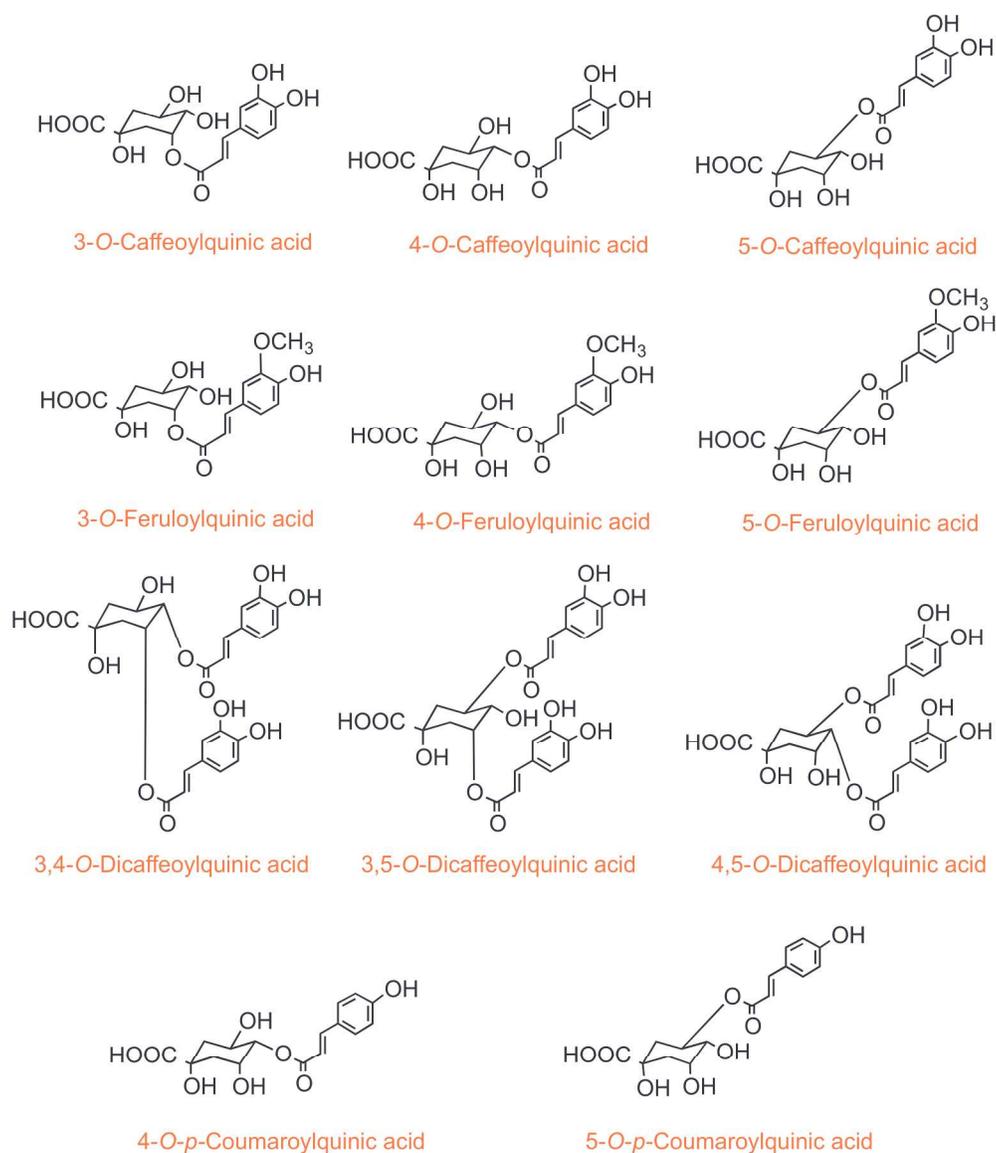
609x431mm (72 x 72 DPI)

Fig. 3.   Structures of the main CGAs in green coffee beans.
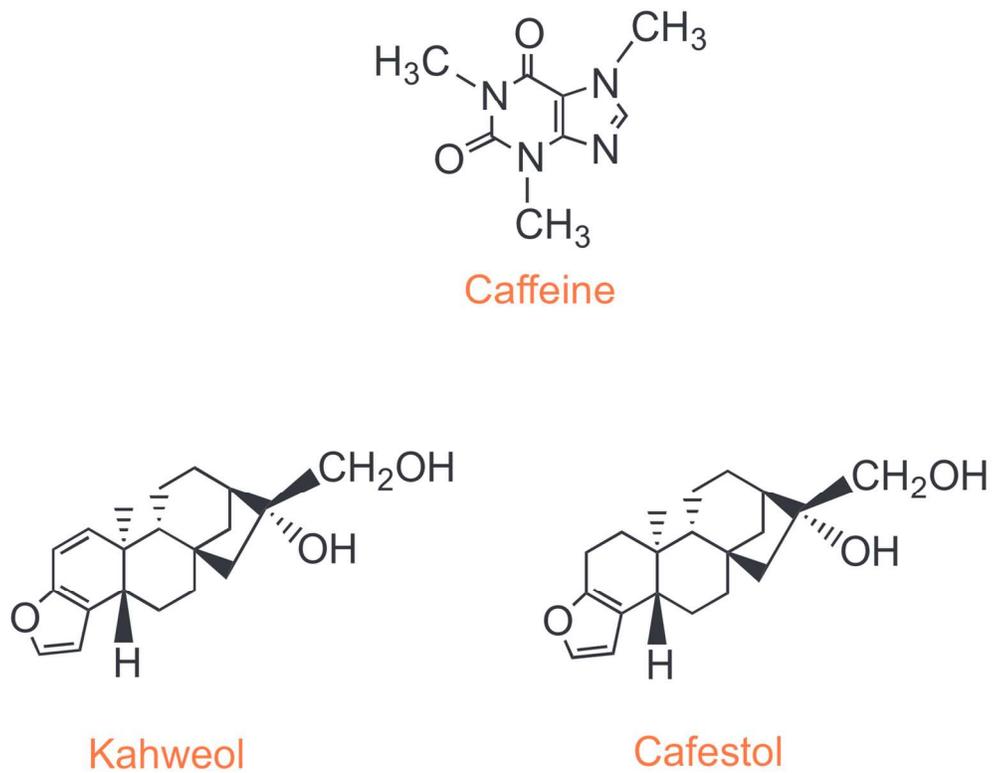158x183mm (600 x 600 DPI)

Fig. 4.   Structures of the purine alkaloid caffeine and the pentacyclic diterpenes kahweol and cafestol
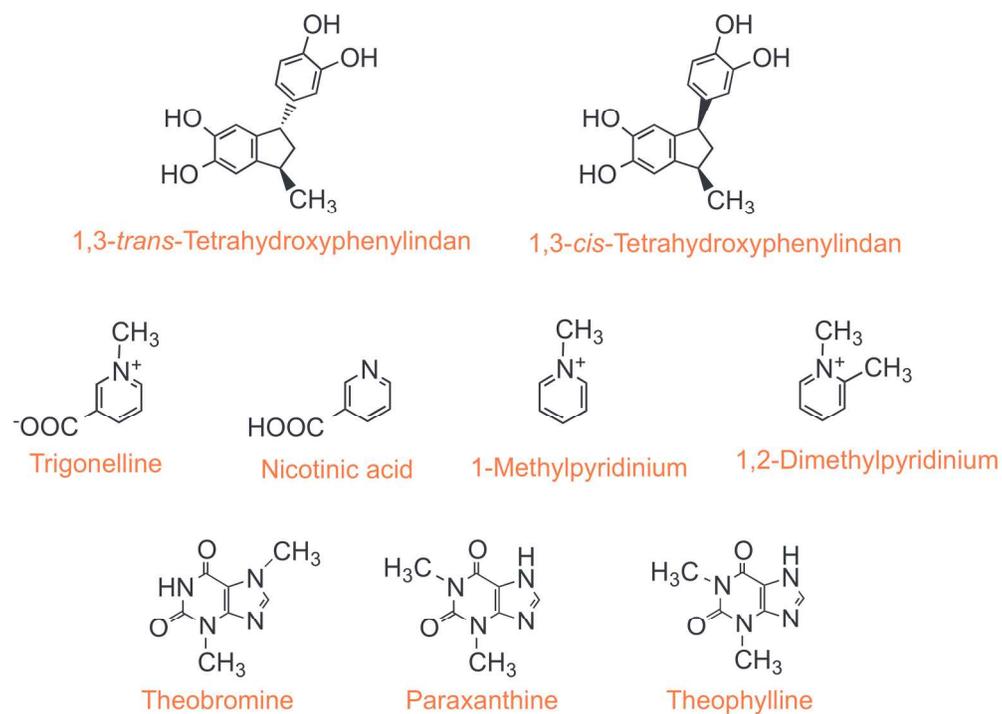67x53mm (600 x 600 DPI)

Fig. 5. Structures of phenylindans formed during roasting by the degradation of the caffeic acid-containing CGAs; trigonelline, and nicotinic acid and methylated pyridinium derivatives formed from trigonelline during roasting; the mammalian metabolites of caffeine, theobromine, paraxanthine and theophylline
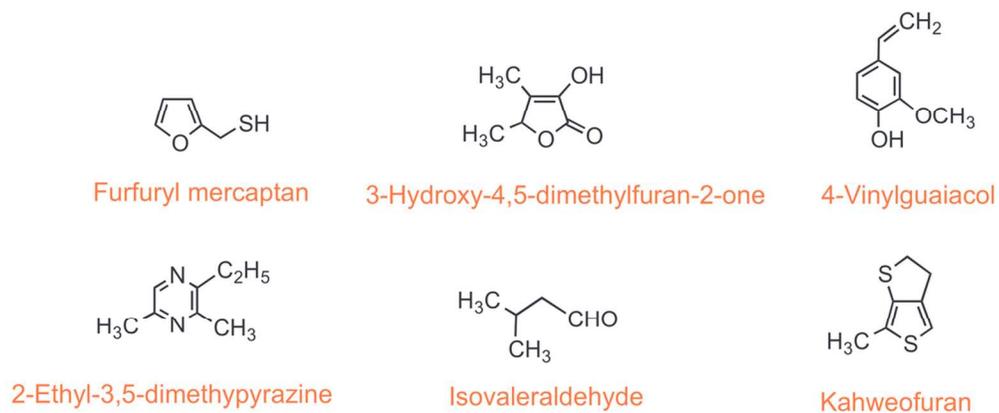96x69mm (600 x 600 DPI)

Fig. 6. Structures of some of the volatile compounds, which have a major impact on the aroma of roasted coffee beans. Kahweofuran is reputed to have a coffee-like small even in isolation.
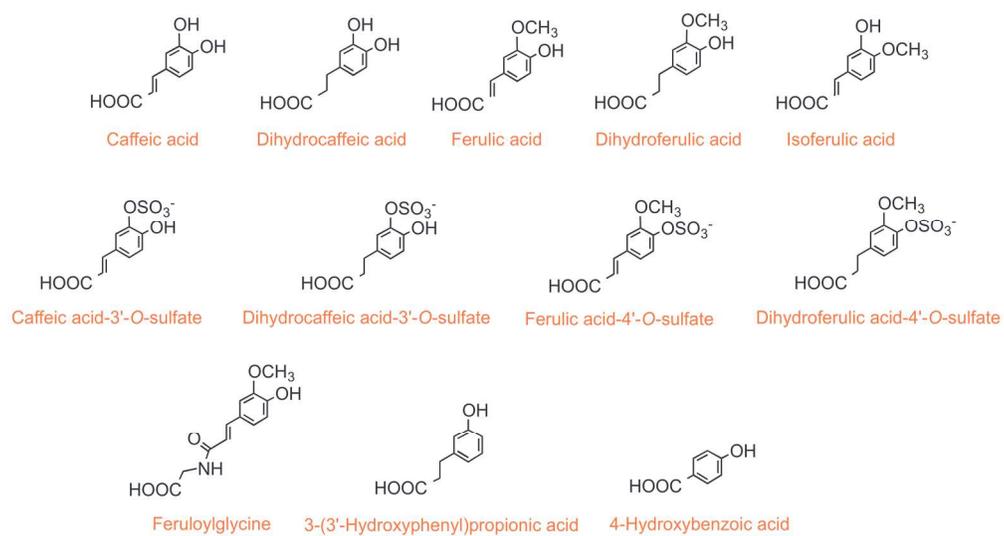51x21mm (600 x 600 DPI)

Fig. 7. Human metabolites of 5-CQA
94x50mm (600 x 600 DPI)

Fig. 8 Pathways for the metabolism of caffeine by humans to di- and monomethylxanthines, the corresponding tri-, di- and monomethyl uric acids and three uracil derivatives formed by opening of the five-membered ring. After Arnaud.98
144x92mm (600 x 600 DPI)

Coffee: Biochemistry and Potential Impact on Health

Iziar A. Ludwig[a], Michael N. Clifford[b], Michael E.J. Lean[c], Hiroshi Ashihara[d] and Alan Crozier[a]

This article reviews the diversity of compounds found in coffee beans, the effect of roasting and the potential impact of coffee beverage on health