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Colonic Metabolism of Polyphenols From Coffee, Green Tea, and Hazelnut Skins

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Abstract: Dietary polyphenolic compounds are poorly absorbed in the small intestine. The absorbed fraction follows the common metabolic pathway of drugs, undergoing phase II enzymatic detoxification with the conjugation of glucuronic acid, sulfate, and methyl groups. However, the unabsorbed fraction can reach the colon, becoming available for the wide array of enzymes produced by the local commensal microbiota. Gut bacteria can hydrolyze glycosides, glucuronides, sulfates, amides, esters, and lactones and are able to break down the polyphenolic skeleton and perform reactions of reduction, decarboxylation, demethylation, and dehydroxylation. These complex modifications generate several low–molecular-weight metabolites that can be efficiently absorbed in situ, subsequently undergoing further phase II metabolism, locally and/or at the liver level, before entering the systemic blood circulation and finally being excreted in urine in substantial quantities that exceed the excretion of phenolic metabolites formed in the upper gastrointestinal tract. This brief work focuses on the phenolic composition and colonic microbial transformation of 2 of the most polyphenol-rich dietary sources, namely, green tea and coffee, and a new interesting and innovative ingredient, hazelnut skin, recently evaluated as one of the richest edible sources of polyphenolic compounds.

Key Words: polyphenols, microbiota, green tea, coffee, hazelnut skin

Dietary phenolic compounds include flavonoids and simple and complex phenolic structures. They are of substantial interest because of their health-related effects and are probably the most studied class of molecules of nutritional interest.[1] Over the last decade, considerable attention had been paid toward understanding the metabolic fate of polyphenols within the human organism. The absorbed phenolic compounds, following the common metabolic pathway of drugs, briefly undergo phase II enzymatic detoxification with the conjugation of glucuronic acid, sulfate, and methyl groups. However, the fraction of phenolic compounds unabsorbed in the upper gastrointestinal tract reaches the colon, thus becoming available for the wide array of enzymes produced by the local microbiota.[2] Gut bacteria can hydrolyze glycosides,

glucuronides, sulfates, amides, esters, and lactones and are able to break down the polyphenolic skeleton and perform reactions of reduction, decarboxylation, demethylation, and dehydroxylation.[3] These complex modifications generate several low–molecular-weight metabolites that can be efficiently absorbed in situ, subsequently undergoing further phase II metabolism, locally and/or at the liver level, before entering the systemic blood circulation and finally being excreted in urine in substantial quantities that highly exceed the excretion of phenolic metabolites formed in the upper gastrointestinal tract.[4]

This brief work will focus on the phenolic composition and colonic microbial transformation of 2 of the most polyphenol-rich dietary sources, namely, green tea and coffee, and a new interesting and innovative ingredient, hazelnut skin, recently evaluated as one of the richest edible sources of polyphenolic compounds.[5]

POLYPHENOLIC COMPOSITION OF GREEN TEA, COFFEE, AND HAZELNUT SKIN

Green Tea

Green tea is one of the major dietary sources of polyphenols, monomeric flavan-3-ols (also known as catechins), being the main subclass present in fresh tea leaves; thus, an infusion of green tea contains up to 200 mg of catechins.[2] Usually, (−)-epigallocatechin-3-*O*-gallate is the most represented compound, occasionally being overtaken by (−)-epicatechin-3-*O*-gallate or (−)-epigallocatechin, together with smaller, but still substantial, amounts of (+)-gallocatechin, (+)-catechin, and (−)-epicatechin. Traces of catechins also exist as gallates, and (−)-epigallocatechin may occur as a digallate, esterified with *p*-coumaric acid or caffeic acid, and with various levels of methylation.[6] Although monomers are by far the most represented form of polyphenols in green tea, oligomeric flavan-3-ols (also called proanthocyanidins or condensed tannins) are also present. In fact, mostly (epi)catechin, (epi)gallocatechin, and their gallate esters can be linked together to form compounds with a high-molecular weight, up to a degree of polymerization (DP) of 7, and also proanthocyanidins isomers containing epiafzelechin-gallate units have also been identified previously in green tea.[7,8] A minor contribution to the phenolic profile of green tea is also made by several flavonol glycosides, comprising monoglycosides, diglycosides, and triglycosides based on kaempferol, quercetin, and myricetin, and various permutations of glucose, galactose, rhamnose, arabinose, and rutinose.[9] With respect to nonflavonoids, green tea also provides several phenolic acids. Among these, gallic acid in free and esterified forms with quinic acid and glucose, in addition to chlorogenic acids, formed by caffeic and *p*-coumaric acids linked to quinic acid at different positions, have been described.[8,9]

Coffee

Coffee is a beverage that is widely consumed across the world. It is one of the richest dietary sources of chlorogenic acids and could be the best dietary sources of these phenolic compounds in regular drinkers. These phenolics are a group of compounds comprising hydroxycinnamates, such as caffeic acid, ferulic acid, and *p*-coumaric acid, esterified to quinic acid to form conjugated structures known as caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), and *p*-coumaroylquinic acids, respectively. 5-*O*-Caffeoylquinic acid is by far the dominant chlorogenic acid, providing up to 350 mg/serving. However, coffee also contains 3-*O*- and 4-*O*-caffeoylquinic acid, the 3 analogous feruloylquinic acids, and 3,4-*O*-, 3,5-*O*-, and 4,5-*O*-dicaffeoylquinic acids,[10] which, if considered together, assume dietary relevance. Furthermore, coffee beans also contain minor chlorogenic acids such as diferuloylquinic, dimethoxycinnamoylquinic, caffeoylferuloylquinic, caffeoyl-dimethoxycinnamoylquinic, and feruloyl-dimethoxycinnamoylquinic acids.[11,12] During the roasting process, the quinic moiety of chlorogenic acids may epimerize and lactonize, forming chlorogenic lactones as caffeoyl quinides.[13]

Hazelnut Skin

The hazelnut (*Corylus avellana* L.), belonging to the family Betulaceae, is one of the most popular tree nuts consumed worldwide, ranking second in tree nut production after almonds. The hazelnut is typically consumed as a whole nut in its raw or roasted form. However, in bakery and confectionery products, the peeled kernels are often used as an ingredient. Thus, the hazelnut skin becomes a byproduct of the hazelnut crop along with the hazelnut hard shell, the hazelnut green leafy cover, and the hazelnut tree leaf.[14] Therefore, it is of great interest to the hazelnut industry to find a suitable way to reuse these waste products, which have been described as good sources of several phytochemicals and potentially useful as new functional food ingredients. Among these hazelnut byproducts, the hazelnut skin could be the best source of polyphenols. In fact, a significant portion of nut antioxidants is located in the pellicle; therefore, the total antioxidant capacity of a hazelnut kernel is significantly reduced by skin removal.[15,16] Furthermore, some works have reported that hazelnut skins contain more phenolic compounds and show a higher antioxidant capacity than all the other hazelnut byproducts.[14,17] In a recent study conducted on peanut, almond, and hazelnut skins, the latter was found to have the highest value of total polyphenols (almost 11 g/100 g).[18] The same study also determined the flavan-3-ol composition, in which the (+)-catechin content was 117 mg/100 g of roasted hazelnut skin, accounting for 90% of the total quantified flavan-3-ols, with the remaining flavan-3-ol fraction constituted by B-type dimers of procyanidin, including procyanidin B3.[18] However, a complete screening of the hazelnut skin phenolics was recently published by our group, reporting a qualitative and quantitative characterization of polyphenolic compounds in 9 samples of hazelnut skin, originating from the major hazelnut-growing areas of the world.[5] The average content of total polyphenols of the 9 samples analyzed by means of HPLC-MS/MS was approximately 675 mg/100 g. The main polyphenolic subclass was mostly monomeric and oligomeric flavan-3-ols, which accounted for >95% of the total polyphenols quantified. Flavonols and dihydrochalcones formed 3.5%, whereas phenolic acids formed <1% of the total identified

phenolics.[5] Although the (+)-catechin was the highest monomer (an average content of 181 mg/100 g), a smaller, but still substantial, amount of (−)-epicatechin was recovered in the skin, whereas the (−)-epicatechin-3-*O*-gallate assumed less dietary relevance because of its low content.[5] The B-type proanthocyanidins formed the main hazelnut skin flavan-3-ols, with procyanidins highly exceeding prodelphinidins. Procyanidin dimers were by far the main oligomeric flavan-3-ol, providing an average of 324 mg/100 g of skin. Among these compounds, several isomers have been identified, including procyanidin B1, B2, and B3. The procyanidin dimers also occurred in galloylated forms, but in a relatively smaller amount.[5] Moreover, hazelnut skin also contained polymeric flavan-3-ols up to DP 9.[18] Our study found a huge variability in the total polyphenol content depending on the growth area, with an average of 9 g/100 g (n = 9),[5] but with the highest sample (13 g/100 g) exceeding >3 times the lowest. On the basis of this observation, hazelnut skin ranks second among the total edible polyphenol sources, overtaking cloves with a polyphenol content of 15 g/100 g.[19] Among the phenolic acids, gallic and protocatechuic acid were the main hydroxybenzoic acids,[5,14] although hexose esters of syringic acid were also detected.[5] Other phenolic acids in the skin were free and esterified ferulic, sinapic, and *p*-coumaric acids.[5,14] Among the flavonols, quercetin was considerably higher than myricetin and kaempferol. Quercetin mainly occurred in glycosylated forms with rhamnose (to form quercitrin), but was also present in unquantifiable amounts as rutinose, together with its methylated counterpart, isorhamnetin.

COLONIC MICROBIOTA: THE KEY ROLE OF GREEN TEA, COFFEE, AND HAZELNUT SKIN POLYPHENOLS IN HUMAN METABOLISM

Green Tea

As mentioned above, the polyphenol-fingerprint of green tea mainly consists (up to 77% of the total phenolics) of monomeric flavan-3-ols.[9] This led us to conduct several studies on human metabolism and the absorption of green tea catechins. The findings in this field show that a relevant fraction of flavan-3-ols reaches the large intestine, thus becoming available to the action of resident microbiota. In a study by Stalmach et al,[20] the ileal fluid of ileostomist volunteers contained 70% of the ingested flavan-3-ols in free and metabolized forms. The microbial metabolism of flavan-3-ols involves ring fission enzymatic reactions leading to the production of valerolactones and phenolic acids.[21,22] These smaller phenolics can be absorbed locally and transported through the portal circulation to the liver, where they are invariably subjected to phase II metabolism before reaching the systemic circulation and then being excreted in urine in substantial quantities that exceed the quantity of flavan-3-ol metabolites absorbed through the small intestine.[22–24]

More than 10 years ago, 2 catechin microbial metabolites were detected in human urine and plasma after the ingestion of green tea: these were (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (named M4) and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (named M6).[25] A third metabolite, (−)-5-(3′,5′-dihydroxyphenyl)-γ-valerolactone (named M6′), was identified 2 years later.[26] The 3 colonic metabolites were mainly excreted conjugated with glucuronic

acid, sulfate, and methyl groups.[21,23] Moreover, several phenolic acids can be formed by colonic microflora action on catechins, and 4-hydroxybenzoic, hippuric, 4-hydroxyphenylacetic, 3-(3-hydroxyphenyl)-3-hydroxypropionic, and 3-methoxy-4-hydroxyphenylacetic acids were all detected in human urine after drinking 300 mL of green tea.[22] The cleavage of the 3-*O*-gallate group possibly resulted in the appearance of pyrogallol and its dehydroxylated counterpart pyrocatechol, as indicated by their detection in human fecal slurries after incubation with (−)-epigallocatechin-3-*O*-gallate. M6, 5-(3,4-dihydroxyphenyl)-γ-valeric and 3-(3-hydroxyphenyl)propionic acid were the main fecal metabolites of epicatechin in vitro, followed by small amounts of 4-hydroxyphenylacetic acid.[22]

A recent study identified the major microbial metabolites of some common polyphenol-rich foods, including green tea.[27] Several fecal metabolites have been linked to their polyphenol precursors. Gallic acid and its decarboxylation product pyrogallol were metabolites formed by bacteria esterase and decarboxylase, which modified catechin-gallates, and galloylquinic acid. Esterase activity explained the presence of free quinic acid, most likely because of the hydrolysis of chlorogenic acids and galloylquinic acid. Dihydrocaffeic acid was linked to dehydrogenase activity toward caffeic acid after its release from caffeoylquinic acids. In terms of phenyl-γ-valerolactones, besides M4 and M6, another ring fission product of green tea flavan-3-ols was 5-(3′-hydroxyphenyl)-γ-valerolactone. This was the sole metabolite present only after 24 hours of fermentation (and not after 5 h, like all the other metabolites), indicating that this specific dehydroxylation could be a late metabolic step of flavan-3-ols degradation.[27] The phloroglucinol detection in this in vitro model could be derived from A-ring fission of quercetin derivatives.[3]

Coffee

Animal models have been used to examine the metabolic fate of 5-caffeoylquinic acid (5-CQA), the major chlorogenic acid in coffee. In rats, the detection of chlorogenic acid in urine was < 1% of the ingested dose, and of the total urinary excretion of caffeic acid released by the hydrolysis of chlorogenic acid and its methylated metabolites (ferulic and isoferulic acids).[28] In contrast, microbial metabolites, such as *m*-coumaric acid and derivatives of phenylpropionic, benzoic, and hippuric acids, were the most represented compounds in both urine and plasma. Hippuric acid largely originated from the transformation of the quinic acid moiety, whereas all other metabolites came from caffeic acid. These colon-derived metabolites accounted for 57% of chlorogenic acid intake, indicating that the bioavailability of chlorogenic acid largely depends on its metabolism by the colon microbiota.[28]

In human subjects drinking instant coffee, the pharmacokinetic data indicate the appearance of free and sulfated dihydrocaffeic and dihydroferulic acids, and feruloylglycine in the blood circulation 5 hours after consumption. This result indicates colonic microflora-mediated conversion of CQAs into caffeic acid and dihydrocaffeic acid, which is further metabolized to dihydro-isoferulic acid. FQAs are metabolized to ferulic and dihydroferulic acid and to feruloylglycine.[29] The conversion of caffeic acid and ferulic acid into their respective dihydro-derivatives could involve either a bacterial reductase or hepatic NADPH-cytochrome P450 phase I metabolism. However, at the time of appearance of feruloylglycine, dihydrocaffeic and dihydroferulic acid in plasma clearly indicates that the colonic microbiota, rather than the liver, was responsible for their formation.[29]

Hazelnut Skin

As mentioned above, the phenolic profile of hazelnut skin was mainly characterized by flavan-3-ols in their monomeric and oligomeric forms. However, the fact that the detailed description of their polyphenolic content is so recent parallels a lack of information on the metabolism of catechins and proanthocyanidins contained in this skin. We can infer that human and colonic metabolism should not be so different from the metabolism described for other sources of the same class of molecules, as the food matrix in which polyphenols are present is known to strongly influence their absorption and their availability to human and microbial enzymes.

We recently used an in vitro fermentation model[27] to evaluate colon-derived metabolites of flavan-3-ols contained in milled hazelnut skin or hazelnut skin extract. It was found that M6 and 3-(3-hydroxyphenyl) propionic acid were the major products 5 hours after incubation (Calani L, Del Rio D, unpublished data). With respect to the specific molecules, many studies have examined the microbiota metabolism of procyanidin dimers, including B1, B2, and B3, which have already been identified in hazelnut skin. In this in vitro experiment, the main metabolites were 2-(3,4-dihydroxyphenyl) acetic acid and valerolactone M6. Other identified metabolites were 3- and 4-hydroxyphenylacetic acid, 3-hydroxyphenylpropionic acid, monohydroxyl- and dihydroxylphenylvaleric acids, monohydroxylated phenyl-γ-valerolactone, and 1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl) propan-2-ol. M6 was formed already after 2 hours of fermentation, unlike phenylvaleric acids and hydroxylphenyl-γ-valerolactone, which were only detected at 6 hours.[30]

Another in vitro study compared the biotransformation of (−)-epicatechin and procyanidin B2 by human fecal microbiota.[31] Ten metabolites were generated from both substrates, including phenylacetic, phenylpropionic, phenylvaleric acids in their monohydroxylated and dihydroxylated forms, and valerolactone M6 and its 3′ monohydroxylated counterpart. In another experiment,[14] C-labeled polymeric proanthocyanidins were incubated with human fecal samples for 48 hours. The fermentation substrate was free from monomers, dimers, and trimers and the average DP of procyanidins was 7.[32] The colonic microbiota broke down the high–molecular-weight procyanidins into low–molecular-weight aromatic acids such as phenylvaleric, phenylpropionic, and phenylacetic acids, monohydroxylated mainly in the meta or the para position. No phenyl-γ-valerolactones and dihydroxylated phenolic acids were detected. On the basis of these results, we can conclude that high DP polymeric flavan-3-ols can also be degraded by colon microflora into lower DP flavan-3-ols, thus forming smaller molecules that may, in turn, be better absorbed. However, the hazelnut skin also contained the monomers (−)-epicatechin and (+)-catechin. Incubation of these molecules with human colonic bacteria led to the generation of valerolactone M6, 5-phenyl-γ-valerolactone, and phenylpropionic acid.[33]

The chemical structure of the main metabolites derived by the interaction of human fecal microbiota with green tea (A and B), coffee (C–E), and hazelnut skin polyphenols is reported in Figure 1.
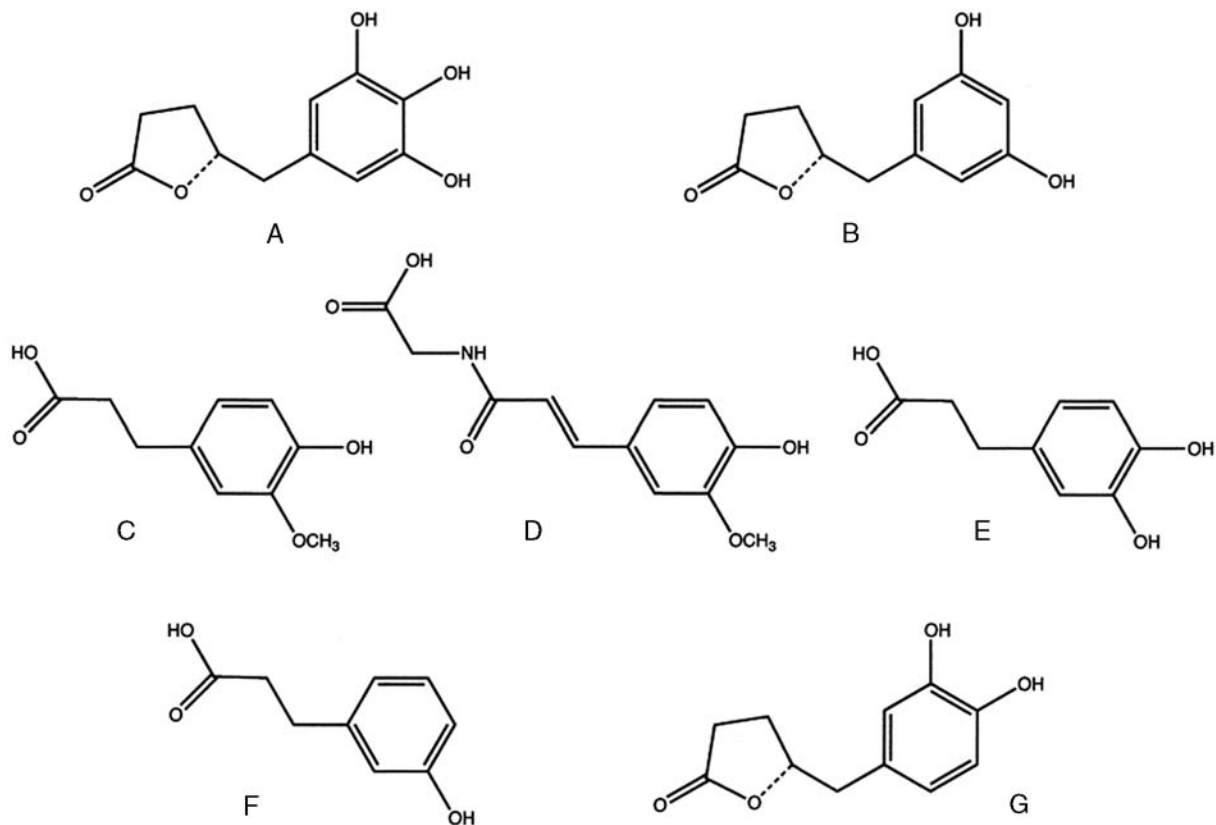
FIGURE 1. Chemical structure of the main metabolites derived by the interaction of human fecal microbiota with green tea (A and B), coffee (C–E), and hazelnut skin polyphenols (F and G). A: (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone or M4; B: (−)-5-(3′,5′-dihydroxyphenyl)-γ-valerolactone or M6′; C: Dihydroferulic acid; D: Feruloylglycine; E: Dihydrocaffeic acid; F: 3-(3-hydroxyphenyl)propionic acid; G: (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone or M6.

CONCLUSIONS

The most known and relevant dietary sources of polyphenols contribute to the presence of several circulating metabolites within the human body, created from interaction with human and/or commensal bacteria enzymes. This plethora of molecules, which interestingly differ from subject to subject in vitro (feces starter donors) and in vivo, may be the best candidates to finally unravel the biochemical and physiological links between the intake of dietary polyphenols and health.

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