

Interplay between Anthocyanins and Gut Microbiota

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ABSTRACT: Anthocyanins are naturally occurring compounds abundant in the human diet. Evidence has accumulated regarding the positive association of their intake with healthy biological effects. The microbiota has just been started to be considered as a metabolic organ, hence contributing to the metabolism of phenolic compounds and, consequently, to their bioavailability and the biological effects displayed by them. This review aimed to compile information regarding interaction of anthocyanins with the microbiota, from two perspectives: (i) identification of their colonic metabolites as potential bioactive molecules and (ii) their role as prebiotic agents. These perspectives are key points in anthocyanin metabolomics. Several metabolites have been identified after anthocyanin consumption with potential health benefits, in particular phenolic acids and simple phenols. On the other hand, microbiota modulation is closely related to several physiological impairments, and its modulation has been considered as a possible mechanism by which phenolic compounds may exert their effect.

KEYWORDS: anthocyanins, bioavailability, metabolism, microbiota, phenolic acids

INTRODUCTION

Over the past 10 years, studies have highlighted some key aspects of the mammalian host–gut microbial relationship. The microbiota maintains an important role in human metabolism and is now considered a metabolizing “organ”, with impact on endo- and xenobiotic metabolism beyond its metabolic relevance for vitamin B12 synthesis and carbohydrate breakdown among other important metabolic and immunological functions.

In addition to the obvious role of the intestine in the digestion and absorption of nutrients, the human gastrointestinal (GI) tract contains a huge collection of microorganisms. The microbiota has not been fully described, but it is clear that the human gut is home for an ecosystem of around 10^{13} – 10^{14} bacterial cells. As a whole, the microorganisms that live inside humans are estimated to outnumber human cells by a factor of 10, and the microbiome represents overall >100 times the human genome.¹

The evidence that the gut microbiota composition can be different between healthy and obese or type 2 diabetic patients^{2,3} has led to the study of this factor as a key link between the pathophysiology of metabolic diseases and the gut bacteria composition.

High-fat diet feeding triggers the development of obesity, inflammation, insulin resistance, type 2 diabetes, and atherosclerosis and is also associated with the development of metabolic endotoxemia in human subjects and participates in low-grade inflammation, a mechanism associated with the development of atherogenic markers.^{4,5}

Different polyphenols have been suggested to affect the relative viability of colonic bacterial groups,^{6,7} implying that

dietary modulation with polyphenols may play a role in reshaping the gut microbial community and enhance host microbial interactions to provide beneficial effects such as weight loss.⁸ Thus, polyphenols should be considered to have a prebiotic action.

Anthocyanins, a particular class of flavonoids, naturally occur as glycosides of flavylium (2-phenylbenzopyrylium) salts but differ from them by structural variations in the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, and the nature and number of sugar moieties attached to the phenolic molecule and the position of the attachment, as well as the nature and number of aliphatic or aromatic acids attached to the sugars.⁹ The sugar moieties vary but are usually a mono- or disaccharide unit, frequently glucose, galactose, rhamnose, arabinose, or xylose.¹⁰ Most commonly known anthocyanins are based on six anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (Figure 1), but there are about 700 anthocyanins reported to be isolated from plants.¹¹ The more widespread anthocyanins in fruits are glycosylated in the 3-OH position (3-O-monoglycosides) and, to a lesser extent, in both positions 3-OH and 5-OH (3,5-O-diglycosides).

The majority of dietary anthocyanins are not absorbed at the upper GI level, hence reaching the intestinal microbiota where

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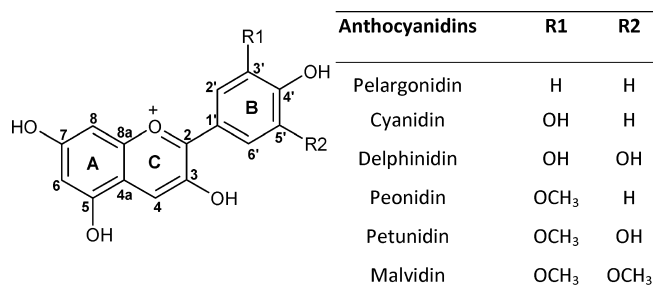


Figure 1. General structure of anthocyanidins (flavylium form).

they are biotransformed into their metabolites, which are absorbed. In the intestinal microbiota, anthocyanins are extensively metabolized, especially by genera and species with enzymes necessary to catalyze these reactions. Dietary polyphenols are substrates for several enzymes located in the small intestine and colon and in the liver (hydrolyzing and conjugating enzymes, phase I and phase II, respectively).^{12–15} Therefore, the colon is being considered as an active site for metabolism rather than a simple excretion route and has been receiving much attention from the scientific community.¹⁶ This conversion is often essential for absorption and modulates the biological activity of these dietary compounds, not only due to the direct bioactivity of the products of microbiota metabolism, which is different from that of the parent compounds, but also because of their prebiotic activity in modulating the microbiota composition.^{17,18}

■ PRODUCTS OF COLONIC METABOLISM OF ANTHOCYANINS

Microbiota catabolism results in the production of new phenolic compounds, which may be absorbed and exhibit different bioactivity from that of the parents, and plays an important role in systemic and local health effects.¹⁹ Information regarding the colonic metabolism of anthocyanins is scarce and diffuse, especially because the metabolism of the microbiota is often studied after an intervention with a whole food or beverage, which contains not only anthocyanins but also other phenolic compounds. In addition, animal and human studies comprise an underlying interindividual variability that can hardly be overcome.

Early *in vitro* studies on the metabolism of anthocyanins by gut microflora have concluded that bacterial metabolism involved the cleavage of glycosidic linkages and breakdown of anthocyanidin heterocycle.²⁰ Protocatechuic acid was indicated as the major metabolite of gut microflora, but the techniques employed did not allow the identification of other metabolites.²⁰ This phenolic acid was also the major human metabolite detected in feces after blood orange juice consumption as a cyanidin-3-glucoside source.²¹ Keppler et al.²² have also confirmed the cleavage of the 3-glycosidic linkage and the instability of the anthocyanidin, which is rapidly converted to phenolic acids. Furthermore, O-demethylation of phenolic acids was also shown.²²

In vitro incubation of phenolic compounds with human gut fecal matter has been a first approach to determine the anthocyanin metabolites formed by microbiota catabolism. Incubation of a red wine extract with a batch of human fecal microbiota resulted in a rapid decline of malvidin-3-glucoside and its acylated forms, but complete degradation of anthocyanins was observed only after 30 h of incubation.²³

The fast catabolism of anthocyanins was attributed to the presence of β -glucosidase activity of the microbiota.²⁰ The acylation forms of anthocyanins also seem to be degraded easily.²³

The breaking of the heterocyclic C-ring of anthocyanins and degradation into phloroglucinol derivatives (from A-ring) and benzoic acids (from B-ring) are usually the result of bacterial metabolism.²² In Sanchez-Patan et al., phloroglucinol was not detected, but instead, the dihydroxylated benzene catechol/pyrocatechol were progressively formed with incubation time. Unlike other studies,^{24,25} in the model used by Sanchez-Patan et al.,²³ only an increase in syringic acid was observed, but no changes in protocatechuic and vanillic acids. However, an increase in benzoic acid was observed, which could be indicative of O-demethylation metabolites being extensively dehydroxylated, giving rise to benzoic acid as the final degradation product.

Two phenolic acids and one aldehyde (3-O-methylgallic acid, syringic acid, and 2,4,6-trihydroxybenzaldehyde) have also been noted as colonic metabolites of anthocyanins from grapes.²⁶ The primary phenolic degradation product detected in the incubation of human gut microflora with malvidin-3-glucoside was syringic acid and transiently gallic acid and pyrogallol.²⁷ Once again, syringic acid demethylation of the B-ring is proposed to account for gallic acid formation.²⁷ When other anthocyanins are incubated, the main metabolites found are gallic, syringic, and *p*-coumaric acids.²⁷

Moreover, the supplementation of human volunteers with raspberry anthocyanins resulted in the increase of catechol, resorcinol, pyrogallol, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), tyrosol, 3-(3'-hydroxyphenyl)propionic acid, and 3-(3',4'-dihydroxyphenyl)propionic acid.²⁵ In this study, an *in vivo* approach was used, and as in other *in vivo* studies, with the advantage of being closer to what happens in regular human consumption of anthocyanins, but it also has the disadvantage of increasing the confounding factors that may affect and influence anthocyanin metabolism by colonic microflora.

An intervention study performed with red wine, which is a very good source of anthocyanins, revealed significant changes in eight metabolites: 3,5-dihydroxybenzoic acid, 3-O-methylgallic acid, *p*-coumaric acid, phenylpropionic acid, protocatechuic acid, vanillic acid, syringic acid, and 4-hydroxy-5-(phenyl)valeric acid.²⁸ It is important to highlight that red wine has several other phenolic compounds, other than anthocyanins, that can give rise to these products, but the O-methyl benzoic acids such as syringic and vanillic acids could arise from methoxylated anthocyanin catabolism^{19,20} and protocatechuic acid from cyanidin. *p*-Coumaric acid may also be a product from *p*-coumaroyl-acylated anthocyanins.²⁹ In addition, alcohol does not seem to affect the performance of the microbial catabolism as there were no changes with red wine and dealcoholized red wine interventions.²⁸

A recent paper described the metabolism of cyanidin-3-glucoside in human microbiota-associated rats and determined protocatechuic acid, 2,4,6-trihydroxybenzaldehyde, and 2,4,6-trihydroxybenzoic acid (gallic acid) as the main colonic metabolites.²⁴ Nevertheless, other metabolites were found but not identified.

In a very recent study that makes use of isotopically labeled cyanidin-3-glucoside (6,8,10,3',5'-¹³C₅-C3G), the human metabolism and pharmacokinetics of this anthocyanin were studied after an ingestion of 500 mg.³⁰ It is very interesting

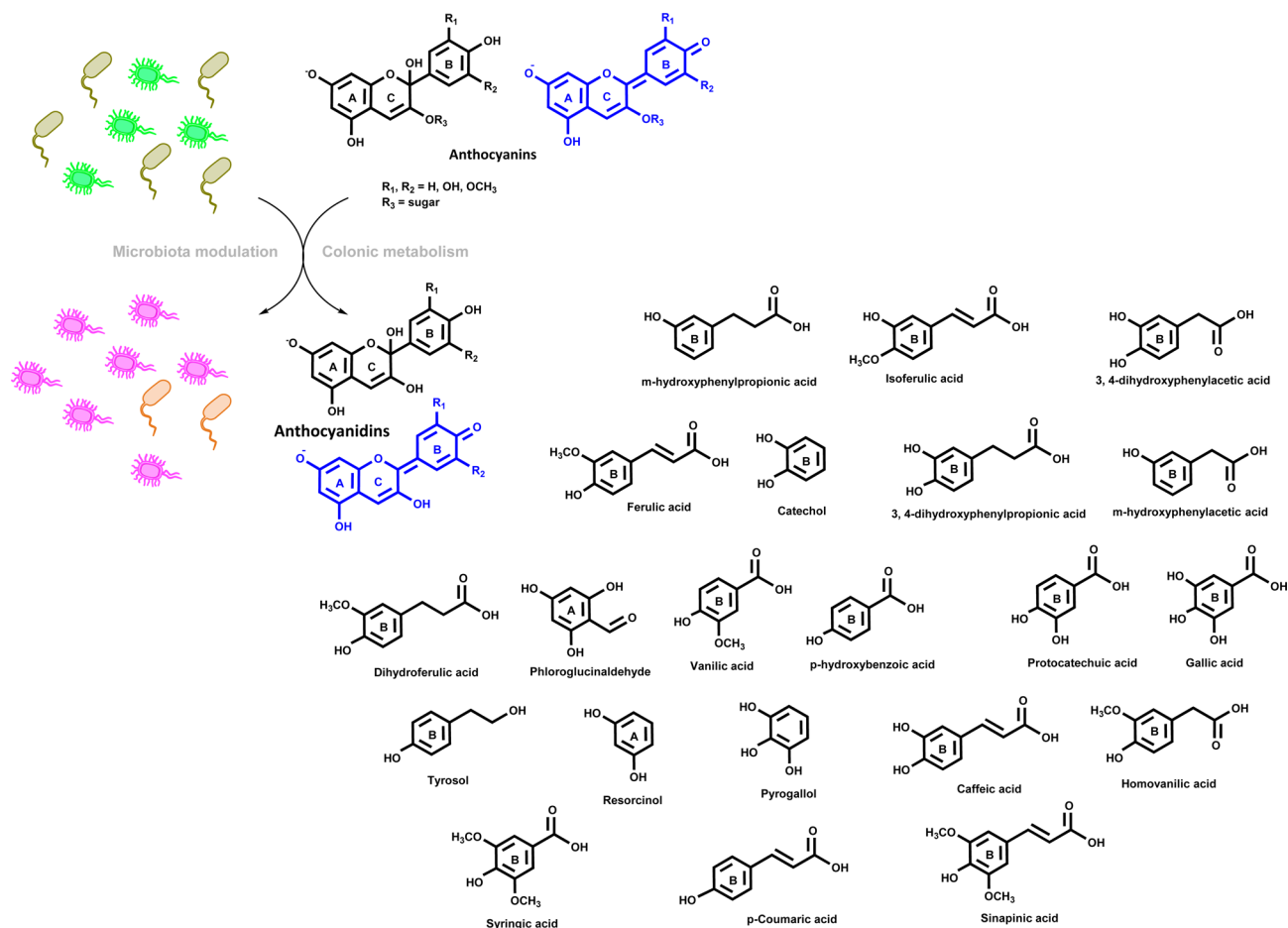


Figure 2. Schematic representation of anthocyanin microbiota modulation and most common detected colonic metabolites. On the one hand, interaction between the microbiota and anthocyanins results in a change of the abundance of certain bacterial groups (prebiotic activity), and, on the other hand, the microbiota is responsible for anthocyanin metabolization giving rise to anthocyanidins and a wide variety of simple phenolics. Anthocyanins are represented, according to the intestine pH range, in their carbinol anionic pseudobase and quinoidal anionic forms.

to observe that the higher ^{13}C -dose recovery was in feces and that the maximal concentration of total metabolites in feces was reached 24 h postconsumption,³⁰ highlighting the importance of colonic metabolization and degradation on the bioavailability and bioactivity of anthocyanins. A thorough analysis of these metabolites showed that B-ring derived ferulic acid was present at the highest concentrations within the feces, followed by the A-ring derived ferulic acid and protocatechuic acid.³¹ Phase II metabolites of protocatechuic acid were also identified in feces by the presence of methyl-3,4-dihydroxybenzoate and methyl vanillate, suggesting that dimethylation is a product of microbial metabolism.³¹ The detection of caffeic acid and its methyl metabolite, ferulic acid, within the feces supports the assumption that phenylpropenoic acids arise from cyanidin-3-glucoside as a result of bacterial cleavage of the C-ring in the colon,³¹ as has already been proposed.^{25,26}

It is worth noting that several factors differ within studies such as the microbiota origin species, the amount of anthocyanins tested in vitro and in vivo, the source of the anthocyanins, or the accuracy and sensitivity of the techniques used to identify the metabolites. Nevertheless, there is an interesting set of studies indicating that anthocyanins are metabolized in the colonic microflora, initially by deglycosylation and in a second phase by degradation into simple phenolic acids. Some of these acids appear to be consistent across studies, such as protocatechuic, vanillic, syringic, gallic, and p-

coumaric, the latter as a product of acylated anthocyanins (Figure 2). These acidic metabolites are probably absorbed through monocarboxylic acids transporters (MCTs) by the epithelial cells.

■ ANTHOCYANIN MODULATION OF MICROBIOTA

No systematic reviews have been undertaken to assess the role of xenobiotic biotransformation in the colon, in particular with regard to anthocyanins. As described earlier, on the one hand, anthocyanins are subjected to metabolism by microbiota, and, on the other hand, they and/or their metabolites may modulate growth of specific bacteria from the microbiota (Figure 2).

There is evidence that the composition of the human intestinal microbiota has an influence on health and the incidence of disease and that gut health is largely determined by the complex interaction between host and gastrointestinal microbiota.³²

Putatively beneficial bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp. are genera which include bacteria that have been observed to contribute to human health at different levels. They have been shown to enhance the gut barrier function, stimulate the host immune system, prevent diarrhea or allergies, participate in the activation of provitamins, and modulate lipid metabolism.^{33,34} However, there are other bacterial species associated with negative implications, such as *Clostridium*

difficile, which has been associated with inflammatory bowel disease.³⁵

In vitro incubation of malvidin-3-glucoside with fecal slurry enhanced the growth of total bacteria including *Bifidobacterium* spp. and *Lactobacillus* spp.²⁷ with no effect observed on *Bacteroides* spp. growth. Interestingly, malvidin-3-glucoside mixed with other anthocyanins yielded a synergistic effect regarding the enhancement of growth of the beneficial bacterial group.²⁷ Gallic acid, one of the microbiota anthocyanin metabolites, was shown to reduce a group of potentially harmful bacteria such as *Clostridium histolyticum*, without negative effect on beneficial bacteria.²⁷ In addition, it significantly reduced *Bacteroides* spp. growth and enhanced total bacterial number and *Atopobium* spp.²⁷ Sanchez-Patan et al. also found decreased growth in the *C. histolyticum* group when incubating a red wine extract with human fecal bacteria, but no other changes were found. A similar result in the *C. histolyticum* group has also been described in human volunteers after red wine consumption.³⁶ It is noteworthy that red wine is a very complex polyphenolic mixture, so it is not possible to attribute these effects directly or solely to anthocyanins.²³

Supplementation of mice with regular apples and genetically engineered apples for high biosynthesis of anthocyanins resulted in changes in the overall population of bacteria and in the number of some individual species.³⁷ Animals fed any of the apple-supplemented diet showed a greater number of bacteria than control mice. Moreover, a significant increase in *Bifidobacterium* spp. was observed.³⁷ Accordingly, a 6 week consumption of a blueberry drink by human volunteers significantly increased *Bifidobacterium* spp.¹⁸

The microbiota was also studied after an intervention in human volunteers with red wine and dealcoholized red wine. The distribution ratio of different genera within Bacteroidetes and Firmicutes phyla was different between baseline and intake periods,³⁶ with increased Firmicutes concentration after red wine consumption. At baseline, *Bacteroides* and Prevotellaceae frequencies were lower than those after the polyphenol intake periods, whereas the *Clostridium* frequency was similar. After the dealcoholized red wine and red wine periods, the *Bacteroides*, *Clostridium*, and Prevotellaceae frequencies were similar when analyzed by denaturing gradient gel electrophoresis (DGGE).³⁶

Major groups of intestinal bacteria possess β -glucosidase activity, including *Bifidobacterium* spp. and *Lactobacillus* spp. They possess the ability to metabolize phenolic compounds during growth, supplying energy to cells and enriching the medium for bacterial growth with the release of glucose. These bacterial groups are associated with beneficial effects in the large intestine including the antimicrobial effect of pathogenic microorganisms by production of short-chain fatty acids, as well as by competition for growth substrate and adhesion sites.³⁸

In vitro, animal and human intervention studies with the purpose of studying anthocyanin effect on gut microbiota are few and, due to the diversity of techniques to study microbiota, the different sources of anthocyanins, and the study designs, it is very difficult to relate them and reach a conclusion. In addition, whether anthocyanins exert a direct or indirect effect (mediated by their microbiota biotransformation products) on bacteria growth remains to be clarified. Nevertheless, there is broad agreement that polyphenols, and in particular anthocyanins, have the ability to modulate colonic bacteria growth.⁷

Several data from experimental models and human subjects support the fact that changing the gut microbiota by means of

prebiotics and/or probiotics may participate in the control of several parameters involved in the development of metabolic diseases associated with obesity.¹ Further research is required to consolidate the prebiotic effects associated with the consumption of anthocyanin and to understand the mechanisms by which the gut microbiota interact with the host to provide new bases for putative dietary intervention.

The bioactivity of anthocyanins is significantly mediated through their several cellular pathways. With regard to all of the described bioactivities it must be assumed that they are absorbed (are bioavailable) and/or that they affect the microbiota, exerting in these ways their biological effects (as microbiota modulators). It is crucial to emphasize the benefits for bioavailability and/or bioactivity from anthocyanin metabolites that are synthesized by the colonic microbiota.

Finally, the present evidence supports the biotechnology applications of anthocyanins in the human diet and the interest of clinical trials to further assess their biokinetics.

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Notes

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REFERENCES

- (1) Cani, P. D.; Delzenne, N. M. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr. Pharm. Des.* **2009**, *15*, 1546–1558.
- (2) Armougom, F.; Henry, M.; Viallettes, B.; Raccach, D.; Raoult, D. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and Methanogens in anorexic patients. *PLoS One* **2009**, *4*, No. e7125.
- (3) Ley, R. E.; Turnbaugh, P. J.; Klein, S.; Gordon, J. I. Microbial ecology: human gut microbes associated with obesity. *Nature* **2006**, *444*, 1022–1023.
- (4) Moreira, A. P.; Teixeira, T. F.; Ferreira, A. B.; Peluzio, M. C.; Alfenas, R. C. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br. J. Nutr.* **2012**, *108*, 801–809.
- (5) Cani, P. D.; Amar, J.; Iglesias, M. A.; Poggi, M.; Knauf, C.; Bastelica, D.; Neyrinck, A. M.; Fava, F.; Tuohy, K. M.; Chabo, C.; Waget, A.; Delmee, E.; Cousin, B.; Sulpice, T.; Chamontin, B.; Ferrieres, J.; Tanti, J. F.; Gibson, G. R.; Casteilla, L.; Delzenne, N. M.; Alessi, M. C.; Burcelin, R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **2007**, *56*, 1761–1772.
- (6) Selma, M. V.; Espin, J. C.; Tomas-Barberan, F. A. Interaction between phenolics and gut microbiota: role in human health. *J. Agric. Food Chem.* **2009**, *57*, 6485–6501.
- (7) Parkar, S. G.; Trower, T. M.; Stevenson, D. E. Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. *Anaerobe* **2013**, *23*, 12–19.
- (8) Rastmanesh, R. High polyphenol, low probiotic diet for weight loss because of intestinal microbiota interaction. *Chem.–Biol. Interact.* **2011**, *189*, 1–8.
- (9) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, FL, USA, 1993; Chapter 1, pp 6–23.

- (10) Francis, F. J. Food colorants: anthocyanins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 273–314.
- (11) Andersen, Ø.; Jordheim, M. Basic anthocyanin chemistry and dietary sources. In *Anthocyanins in Health and Disease*, 1st ed.; Wallace, T., Giusti, M., Eds.; CRC Press: New York, 2014; Vol. 1, pp 13–89.
- (12) Rice-Evans, C. Flavonoid antioxidants. *Curr. Med. Chem.* **2001**, *8*, 797–807.
- (13) Tapiero, H.; Tew, K. D.; Ba, G. N.; Mathe, G. Polyphenols: do they play a role in the prevention of human pathologies? *Biomed. Pharmacother.* **2002**, *56*, 200–207.
- (14) Rechner, A. R.; Kuhnle, G.; Bremner, P.; Hubbard, G. P.; Moore, K. P.; Rice-Evans, C. A. The metabolic fate of dietary polyphenols in humans. *Free Radical Biol. Med.* **2002**, *33*, 220–235.
- (15) Kay, C. D. Aspects of anthocyanin absorption, metabolism and pharmacokinetics in humans. *Nutr. Res. Rev.* **2006**, *19*, 137–146.
- (16) Aura, A.-M. Microbial metabolism of dietary phenolic compounds in the colon. *Phytochem. Rev.* **2008**, *7*, 407–429.
- (17) Landete, J. M. Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 936–948.
- (18) Vendrame, S.; Guglielmetti, S.; Riso, P.; Arioli, S.; Klimis-Zacas, D.; Porrini, M. Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. *J. Agric. Food Chem.* **2011**, *59*, 12815–12820.
- (19) Williamson, G.; Clifford, M. N. Colonic metabolites of berry polyphenols: the missing link to biological activity? *Br. J. Nutr.* **2010**, *104* (Suppl. 3), S48–S66.
- (20) Aura, A. M.; Martin-Lopez, P.; O'Leary, K. A.; Williamson, G.; Oksman-Caldentey, K. M.; Poutanen, K.; Santos-Buelga, C. In vitro metabolism of anthocyanins by human gut microflora. *Eur. J. Nutr.* **2005**, *44*, 133–142.
- (21) Vitaglione, P.; Donnarumma, G.; Napolitano, A.; Galvano, F.; Gallo, A.; Scalfi, L.; Fogliano, V. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. *J. Nutr.* **2007**, *137*, 2043–2048.
- (22) Keppler, K.; Humpf, H. U. Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. *Bioorg. Med. Chem.* **2005**, *13*, 5195–5205.
- (23) Sanchez-Patan, F.; Cueva, C.; Monagas, M.; Walton, G. E.; Gibson, G. R.; Quintanilla-Lopez, J. E.; Lebron-Aguilar, R.; Martin-Alvarez, P. J.; Moreno-Arribas, M. V.; Bartolome, B. In vitro fermentation of a red wine extract by human gut microbiota: changes in microbial groups and formation of phenolic metabolites. *J. Agric. Food Chem.* **2012**, *60*, 2136–2147.
- (24) Hanske, L.; Engst, W.; Loh, G.; Sczesny, S.; Blaut, M.; Braune, A. Contribution of gut bacteria to the metabolism of cyanidin 3-glucoside in human microbiota-associated rats. *Br. J. Nutr.* **2013**, *109*, 1433–1441.
- (25) Gonzalez-Barrio, R.; Edwards, C. A.; Crozier, A. Colonic catabolism of ellagitannins, ellagic acid, and raspberry anthocyanins: in vivo and in vitro studies. *Drug Metab. Dispos.* **2011**, *39*, 1680–1688.
- (26) Forester, S. C.; Waterhouse, A. L. Identification of Cabernet Sauvignon anthocyanin gut microflora metabolites. *J. Agric. Food Chem.* **2008**, *56*, 9299–9304.
- (27) Hidalgo, M.; Oruna-Concha, M. J.; Kolida, S.; Walton, G. E.; Kallithraka, S.; Spencer, J. P.; de Pascual-Teresa, S. Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. *J. Agric. Food Chem.* **2012**, *60*, 3882–3890.
- (28) Jimenez-Giron, A.; Queipo-Ortuno, M. I.; Boto-Ordonez, M.; Munoz-Gonzalez, I.; Sanchez-Patan, F.; Monagas, M.; Martin-Alvarez, P. J.; Murri, M.; Tinahones, F. J.; Andres-Lacueva, C.; Bartolome, B.; Moreno-Arribas, M. V. Comparative study of microbial-derived phenolic metabolites in human feces after intake of gin, red wine, and dealcoholized red wine. *J. Agric. Food Chem.* **2013**, *61*, 3909–3915.
- (29) Monagas, M.; Bartolome, B.; Gomez-Cordoves, C. Updated knowledge about the presence of phenolic compounds in wine. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 85–118.
- (30) Czank, C.; Cassidy, A.; Zhang, Q.; Morrison, D. J.; Preston, T.; Kroon, P. A.; Botting, N. P.; Kay, C. D. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. *Am. J. Clin. Nutr.* **2013**, *97*, 995–1003.
- (31) de Ferrars, R. M.; Czank, C.; Zhang, Q.; Botting, N. P.; Kroon, P. A.; Cassidy, A.; Kay, C. D. The pharmacokinetics of anthocyanins and their metabolites in humans. *Br. J. Pharmacol.* **2014**, DOI: 10.1111/bph.12676.
- (32) Sekirov, I.; Russell, S. L.; Antunes, L. C.; Finlay, B. B. Gut microbiota in health and disease. *Physiol. Rev.* **2010**, *90*, 859–904.
- (33) Gibson, G. R. Prebiotics as gut microflora management tools. *J. Clin. Gastroenterol.* **2008**, *42* (Suppl. 2), S75–S79.
- (34) Burcelin, R.; Garidou, L.; Pomie, C. Immuno-microbiota cross and talk: the new paradigm of metabolic diseases. *Semin. Immunol.* **2012**, *24*, 67–74.
- (35) Rastall, R. A.; Gibson, G. R.; Gill, H. S.; Guarner, F.; Klaenhammer, T. R.; Pot, B.; Reid, G.; Rowland, I. R.; Sanders, M. E. Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. *FEMS Microbiol. Ecol.* **2005**, *52*, 145–152.
- (36) Queipo-Ortuno, M. I.; Boto-Ordonez, M.; Murri, M.; Gomez-Zumaquero, J. M.; Clemente-Postigo, M.; Estruch, R.; Cardona Diaz, F.; Andres-Lacueva, C.; Tinahones, F. J. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am. J. Clin. Nutr.* **2012**, *95*, 1323–1334.
- (37) Espley, R. V.; Butts, C. A.; Laing, W. A.; Martell, S.; Smith, H.; McGhie, T. K.; Zhang, J.; Paturi, G.; Hedderley, D.; Bovy, A.; Schouten, H. J.; Putterill, J.; Allan, A. C.; Hellens, R. P. Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice. *J. Nutr.* **2014**, *144*, 146–154.
- (38) Gibson, G. R.; Wang, X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.* **1994**, *77*, 412–420.