JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Impact of Polyphenols and Polyphenol-Rich Dietary Sources on Gut Microbiota Composition

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ABSTRACT: Gut microbiota plays a key role in host physiology and metabolism. Indeed, the relevance of a well-balanced gut microbiota composition to an individual's health status is essential for the person's well-being. Currently, investigations are focused on analyzing the effects of pre- and probiotics as new therapeutic tools to counteract the disruption of intestinal bacterial balance occurring in several diseases. Polyphenols exert a wide range of beneficial health effects. However, although specific attention has been paid in recent years to the function of this "biological entity" in the metabolism of polyphenols, less is known about the modulatory capacity of these bioactive compounds on gut microbiota composition. This review provides an overview of the latest investigations carried out with pure polyphenols, extracts rich in polyphenols, and polyphenol-rich dietary sources (such as cocoa, tea, wine, soy products, and fruits) and critically discusses the consequences to gut microbiota composition which are produced.

KEYWORDS: *gut microbiota, polyphenols, health, interactions*

INTRODUCTION

Polyphenols are members of a very large family of plant-derived compounds that show an extensive variety of chemical structures. They are classified as flavonoids and nonflavonoids. Among the flavonoids, various groups can be distinguished: flavonols, flavan-3-ols (monomeric and polymeric structures), flavones, isoflavones, flavanones, and anthocyanidins. Among nonflavonoids we find stilbenes, hydrolyzable tannins, and phenolic acids.[1] The polyphenolic profile of vegetables and fruits very much depends on the type of plants, on the conditions under which these plants are grown, on harvest conditions, and how these products are stored.

Polyphenols can have beneficial effects on human health, and thus their study has become an increasingly important area of human nutrition research. A great number of epidemiological studies have shown that the consumption of diets rich in fruits and vegetables is associated with a reduction in the risk of suffering chronic diseases such as cardiovascular diseases, specific cancers, or neurodegenerative diseases. To confirm these observations, numerous intervention trials have been conducted in recent years. The beneficial effects of phenolic compounds on different health issues have been reviewed elsewhere.[2]

Taking into account current scientific evidence about the beneficial effects induced by polyphenol intake, despite the low bioavailability of these molecules, further studies are required to analyze whether polyphenol metabolites contribute to the effects of their parent compounds. However, the investigations reported concerning this topic are scarce. In this context, the role of gut microbiota, which determines to some extent the polyphenol metabolite profile, is an important issue to be addressed.[3,4] The action of gut microbes on polyphenols leading to the production of metabolites with diverse physiological relevance has been also analyzed in the recent years.[5]

This review aims to highlight the impact of phenolic compounds, either as pure compounds or as food constituents, on gut microbiota composition and intends to offer an update of the recent in vitro and in vivo evidence which demonstrates the interaction existing between gut microbes and polyphenols with health impact.

GUT MICROBIOTA AND HEALTH

The intestine is the largest reservoir of human flora, which achieves concentrations of up to 10^{11} or 10^{12} cells/g and consists of a complex microbial community residing in the gut called microbiota. The human body has about 100 trillion microorganisms in the intestine, which is 10 times higher than the total number of human cells in the body. Only a minority of the species that inhabit the human colon has been identified so far, but modern molecular methods, such as broad-range sequencing of 16S rRNA from amplified bacterial nucleic acids extracted from feces or biopsies, are being used nowadays to identify and classify intestinal bacteria.[6]

Bacteria make up most of the flora in the colon, where around 300−500 different species live.[6] The most common bacteria are *Bacteroides*, which constitute around 30% of all

Received: June 7, 2013
Revised: September 12, 2013
Accepted: September 13, 2013
Published: September 13, 2013

dx.doi.org/10.1021/jf402506c | *J. Agric. Food Chem.* 2013, 61, 9517−9533

bacteria in the gut, followed by *Clostridium*, *Prevotella*, *Eubacterium*, *Ruminococcus*, *Fusobacterium*, *Peptococcus*, and *Bifidobacterium*. *Escherichia* and *Lactobacillus* are also present, but to lesser extent.[7] It seems that 99% of the bacteria come from about 30 or 40 species.[7]

It has been reported that diet has a major influence on gut microbiota and is able to modify their impact on health, with either beneficial or deleterious consequences.[8] Thus, levels of *Prevotella* are enriched in children who have had a high-fiber diet,[9] as well as in children and adults whose diet is dominated by plant-derived polysaccharide foods such as maize and cassava. By contrast, the microbiota of people with a long-term diet rich in animal protein and saturated fat has more *Bacteroides*.[10] These changes can be explained by fiber content of the diet. The increase in colonic fermentation results in a decrease in the pH, from 6.5 to 5.5 due to the high concentrations of short-chain fatty acids. This decrease in the pH has a profound selective effect upon the colonic microbial community in fermentor simulations supplied with soluble polysaccharides, with a tendency to suppress *Bacteroides* spp. and to promote butyrate-producing Gram-positive bacteria.[11] However, long-term periods (8−9 weeks) of dietary patterns are needed to induce changes in the microbiota of individuals.[12]

During the metabolism of foods and xenobiotics, the host and its gut microbiota coproduce a large amount of small molecules, many of which play critical roles in shuttling information between host cells and the host's microbial symbionts (cross-talk).

In this context, alterations in the microbiome, dysbiosis, modulate the metabolic phenotype of the host and greatly influence host biochemistry and susceptibility to diseases.[13] Indeed, it has been proposed that gut microbiota is involved in appetite control, energy balance, obesity, diabetes, immune function, allergies, behavioral perturbations, cardiovascular disease, and cancers such as stomach cancer.[8]

POLYPHENOLS AND GUT MICROBIOTA INTERACTIONS

The percentage of polyphenol absorption is very low,[14] and as much as 90% of these compounds persist into the colon. There, they are metabolized via esterase, glucosidase, demethylation, dehydroxylation, and decarboxylation activities of bacteria,[15] resulting in smaller metabolites such as phenolic acids and short-chain fatty acids, some of which can be absorbed across the intestinal mucosa. Interestingly, the microbial bioconversion capacity of each individual influences the final metabolites produced and impacts on their bioavailability. Indeed, because all individuals have their own unique signature of intestinal microbiota, which can make an analogy with a fingerprint, human intestinal microbiota composition can modulate the polyphenol impact on host health.[15,16]

On the other hand, polyphenols and their metabolites can affect the intestinal ecology modulating microbiota.[15] In this sense, several phenolic compounds have been identified as potential antimicrobial agents with bacteriostatic or bactericidal actions. Furthermore, they could also act as inhibitors of infection-causing bacteria within cells of the intestinal and urinary tracts, suggesting that some phenolic compounds have potential to be applied as antimicrobial agents against human infections.[15]

Despite these positive effects, it is important not to forget that excessive amounts of polyphenols may also inhibit the growth of colonic beneficial microbiota, which is responsible for bioconversion of polyphenols and thus indirectly interfere with their own bioavailability. Consequently, dietary supplementation may exert a nondesirable effect on human health instead of supporting it.[16]

INFLUENCE OF PHENOLIC COMPOUNDS IN GUT MICROBIOTA COMPOSITION

The majority of studies encompassing the influence of phenolic compounds on gut microbes have been focused on their antimicrobial activity. However, the concept of polyphenols as potential prebiotic candidates could be considered as a newly emerging concept.

Interestingly, a number of in vitro and in vivo (in animals and humans) studies showing the influence of dietary polyphenols on gut-inhabiting bacteria have been published in recent years.

FLAVONOIDS

Flavanones and Flavonols. The impact of some flavanones (naringenin, naringin, hesperetin, hesperidin) and flavonols (quercetin and rutin), on specific intestinal microbial representatives was screened in vitro by Duda-Chodak.[16] For this purpose, pure cultures of six bacteria species (*Bacteroides galacturonicus*, *Lactobacillus* sp., *Enterococcus caccae*, *Bifidobacterium catenulatum*, *Ruminococcus gauvreauii*, and *Escherichia coli*) were inoculated with pure polyphenols at final concentrations of 4, 20, and 50 μg/mL in the case of quercetin and at 20, 100, and 250 μg/mL for the rest of the compounds. Naringenin and quercetin exerted a complete and dose-dependent inhibitory effect on the growth of all analyzed bacterial species, whereas this effect was weaker for hesperetin. A higher inhibitory effect of the aglycones, compared to that of the glycosides (naringin, rutin and hesperidin), was demonstrated.[16] The fact that flavanone glycosides were unable to exert any antimicrobial activity was explained by the dependency of the potential of these compounds on the sugar presence/absence in the moiety.

In another in vitro study conducted by Kawabata et al.,[17] *Bifidobacterium adolescentis*, one of the probiotic species usually identified in the intestine of both children and adults, was cocultured with different flavonols (galangin, kaempferol, quercetin, myricetin, and fisetin) and the growth rate measured.[17] All the flavonoids studied, except galangin, showed little or no antibacterial effect. In addition, when these conditioned media were exposed to a nitric oxide (NO) production inhibition assay, the coculture of *B. adolescentis* with galangin (54%), quercetin (50%), and fisetin (76%) decreased NO synthesis, suggesting an improvement in flavonol anti-inflammatory capacity by *B. adolescentis*.[17]

Backing up these findings, another study tested pure polyphenols at concentrations ranging from 62.5 to 1000 μg/mL and their influence on the viability of four bacterial strains (*E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Lactobacillus rhamnosus*) was assessed.[18] All polyphenols, except rutin, induced a decrease in bacterial growth, but specifically quercetin (flavonol) and naringenin (flavanone) presented the highest antibacterial activities with the lowest minimum inhibitory concentration (MIC) values. Although Gram-negatives tested had similar sensitiveness to polyphenols, within Gram-positive populations, *S. aureus* was the most sensitive while *Lactobacillus rhamnosus* required a MIC of at least 125 μg/mL.[18]

Flavanols. In a study that investigated the effect of flavanol monomers, namely (−)-epicatechin and (+)-catechin, on the

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

growth of specific bacterial populations, a marked overgrowth of beneficial bacterial groups was noted when microbiota was exposed to 150 or 1000 mg/L of (+)-catechin.[19] Both concentrations promoted the growth of *Eubacterium rectale*−*Clostridium coccoides*, and the lowest concentration was able to induce that of *Lactobacillus* spp. and *Bifidobacterium* spp. Moreover, (+)-catechin inoculation at 150 mg/L induced the growth of *E. coli* and the 1000 mg/L concentration decreased that of *Clostridium histolyticum*. A significant increase in the growth of *Eubacterium rectale*−*Clostridium coccoides* was also reported with the inoculation of (−)-epicatechin at 150 mg/L concentration.[19] The inhibitory potential of (+)- catechin at concentrations ranging 20 to 250 μg/mL on specific intestinal microbial representatives was reported to be powerless as it was only able to slightly slow down the growth of *B. catenulatum* (MIC >250 μg/mL) but promoted the growth of *E. caccae*.[16]

Polyphenon G powder, a purified preparation of tea-derived catechins, was also reported to induce a significant increase in *Lactobacilli* and a marked decrease in *Enterobacteriaceae* in broiler chickens.[20]

The implication of the principal tea phenolic aglycones, epicatechin, catechin, 3-*O*-methylgallic acid, gallic acid, and caffeic acid on pathogenic, commensal, and probiotic intestinal bacteria was investigated in an in vitro study by Lee et al.[21] In agreement with the effects previously mentioned, these compounds suppressed the growth of pathogens like *Clostridium perfrigens*, *Clostridium difficile*, and *Bacteroides* spp., with commensal anaerobes (*Clostridium* spp. and *Bifidobacterium* spp.) and probiotics (such as *Lactobacillus* spp.) being affected to a much lower extent. Caffeic acid was evidenced to be the strongest inhibitor, especially for *E. coli*, *Salmonella*, *Pseudomonas*, *Clostridium*, and *Bacteroides*.[21]

Isoflavones. Gut microbiota composition has been reported to play a key role in the degradation of isoflavones, and studies are being conducted to unveil the bacterial strains responsible. Nonetheless, studies analyzing the effect of isoflavone supplementation on gut microbiota composition are scarce. Clavel et al.[22] found that isoflavone supplementation (100 mg/d) to postmenopausal women for two months produced a bifidogenic effect with increases in *Bifidobacterium* species.[22] In this trial, isoflavones were reported to alter dominant bacterial communities with increases in *Clostridium coccoides*−*Eubacterium rectale* (Erec) cluster, *Faecalibacterium prasnutzii* subgroup, and *Lactobacillus*−*Enterococcus* group. However, unlike *Bifidobacterium* species, the concentrations of the Erec cluster were suggested to be linked to the obtention of equol, an intestinal metabolite from daidzein.

Condensed Tannins (Proanthocyanidins). Condensed tannins, which are also called proanthocyanidins, are present in a broad number of higher plant species. Regarding the effect of these compounds on gut microbiota, the effects of diet supplementation with low-tannin (0.7%) and high-tannin (2.0%) diets were assessed in rats by Smith et al.[23] Shifts in bacterial populations of feces were analyzed by DNA fingerprinting and bacterial cultivation and enumeration. The authors concluded that the most predominant groups in condensed-tannin supplemented animals were those belonging to *Enterobacteriaceae*, *Bacteroides*−*Prevotella*−*Porphyromonas* and the *Bacteroides fragilis* group.[23]

Nonflavonoids. *Stilbenes.* The antimicrobial effects of resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) against several pathogenic agents has been reported in vitro. When this compound was administered to a DSS-induced colitis rat model, a significant increase in *Lactobacilli* and bifidobacteria, as well as decrease of enterobacteria, was observed after 20 days.[24]

Hydrolyzable Tannins (Ellagitannins). Ellagitannins, a type of hydrolyzable tannin, are hydrolyzed in vivo and release ellagic acid, whose metabolism by gut microbiota results in urolithin production.[25] The effect of these tannins on the growth of intestinal bacteria is limited, and generally their antimicrobial potential has been evaluated in vitro. In relation to this, Bialonska et al.[26] analyzed the effect of a commercial extract of pomegranate at 0.01% as well as the effect of its main constituents (0.05%) on the growth of several intestinal bacteria by liquid culturing. A strong inhibition capacity was observed with punicalagins and ellagic acid, especially against *Clostridium* species, while a repression in pathogenic *S. aureus* growth was only obtained with the pomegranate extract and punicalagins. Interestingly, the growth of probiotic *Lactobacilli* and bifidobacteria was less affected.[26] Moreover, the same group aimed to prove whether this trend was maintained using a fermentation batch-culture system inoculated with fecal samples from healthy individuals, which better simulate conditions from colonic region. In this experiment, pomegranate extract was able to produce an increment on total bacterial number, enhancing the growth of *Bifidobacterium* spp., *Lactobacillus* and *Enterococcus* groups, while no effect was observed for the *C. histolyticum* group.[27] In a different study, the growth of *E. coli* (half-maximal inhibitory concentration, IC_{50} = 9.2 μM) and *Pseudomonas aeruginosa* (IC_{50} = 3.2 μM) was suppressed by punicalagins and gallagic acid, while ellagic acid and punicalins did not exhibit any antimicrobial activity. The authors could not correlate these results with the structural differences of the compounds.[28]

Polyphenol-Rich Dietary Sources and Gut Microbiota Composition. *Cocoa.* Cocoa, a product derived from *Theobroma cacao* L. (Sterculiaceae), is rich in flavan-3-ols, in the form of monomeric (−)-epicatechin and (+)-catechin as well as type-B proanthocyanidins. The fact that cocoa polyphenols ingestion could affect diseases such as hypertension, oxidative stress, cancer, atherosclerosis, diabetes, and diverse central nervous system disorders and the fact that these disorders have also been linked to gut microbiota, has opened a research gate to investigating the effect of cocoa or chocolate intake on gut microbiota. In this regard, the fecal microbiota composition was analyzed after a high and continuous (10% w/w) cocoa intake in female Wistar rats.[29] The authors reported significantly lower levels of *Bacteroides*, *Staphylococcus*, and *Clostridium* genera at the end of the intervention. Moreover, reductions in *Clostridium* species were found to correlate with weight loss and body mass index (BMI) *z*-score.[29]

A human intervention study conducted with low-cocoa flavanol (LCF, 29 mg) and high-cocoa flavanol (HCF, 494 mg) drinks over 4 weeks described a significant increase in *Lactobacillus* spp. ($P < 0.001$) and *Bifidobacterium* spp. ($P < 0.001$) when the HCF was compared to the control LCF beverage.[30] This condition was suggested to be partly responsible for the reductions observed in C-reactive protein (mg/mL) (CRP) plasma levels (−30%). On the other hand, a significant decrease in *C. histolyticum* group ($P < 0.001$) was stated, a group that includes *Clostridium perfringens* pathogen, an agent contributing to a wide range of human diseases.[30]

Tea. Flavonoids in tea (from the plant *Camellia sinensis*) occur in large quantities, the major classes being catechins, including epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate. Moreover, flavanols, such as

Table 1. Polyphenolic-Rich Beverages (Cocoa, Tea, Wine) With Potential Effects On Gut Microbiota Composition

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
cocoa	animal study	6 weeks	10% (w/w) (10.62 mg/g polyphenols)	FISH-FCM[a]	*Bacteroides* genus *Clostridium* genus *Staphylococcus* genus		29
cocoa drink	human intervention	4 weeks	HCF[b] (494 mg cocoa flavanols)	FISH	*Clostridium histolyticum* group	*Eubacterium rectale*−*C. coccoides* group *Lactobacillus* spp. *Enterococcus* spp. *Bifidobacterium* spp.	30
			LCF[c] (29 mg cocoa flavanols)			*Eubacterium rectale*−*C. coccoides* group *Clostridium histolyticum* group *Lactobacillus* spp. *Enterococcus* spp.	
	in vitro study	6 h incubation	high-flavanol cocoa powder extract (1 mg/mL; 0.4 mg/mL flavanols)	batch culture fermentation model	*Clostridium histolyticum* group	*Lactobacillus* spp. *Bifidobacterium* spp.	
black tea extract	in vitro study	2 weeks	1000 mg/day	SHIME[d] and qPCR[e]/ pyrosequencing	bifidobacteria *Blautia coccoides Anaeroglobus Victivallis*	*Klebsiella* sp. *Enterococci Akkermansia*	32
tea polyphenols	human intervention	4 weeks	Suphenon (0.4 g/3 times per day)	plate count	*Clostridium* spp. *Clostridium perfringens*	*Bifidobacterium* spp.	35
green tea extract	in vitro study	2 days incubation	10 mg extract/disc	plate count	*Clostridium difficile* ATCC 9689 *Clostridium perfringens* ATCC 13124 *Clostridium perfringens* B-3-7 *Clostridum perfringens* B-3-8 *Clostridium perfringens* B-165-16 *Clostridium perfringens* C-01 *Clostridium paraputrificum* B-3-4 *Clostridium paraputrificum* B-78 *Clostridium paraputrificum* VPI-6372		31
tea polyphenols	animal study	2 weeks	0.2% tea polyphenols	plate count	Bacteroidaceae	*Lactobacilli*	33
green tea leaves powder	animal study	11 and 22 weeks	4% w/w of GT[f] + *Lactobacillus plantarum* DSM 15313	qPCR and T-RFLP[g]		*Lactobacillus*	37
green tea	human intervention	10 days	1000 mL/day	qPCR and T-RFLP		*Bifidobacterium* spp.	36

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

Table 1. continued

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
green tea extracts	in vitro study	2- 3 days incubation	GTE-1[h] (>60% polyphenols)	agar plate dilution method	*Staphylococcus aureus* I-001 (125 μg/mL) *Staphylococcus epidermis* KK108 (125 μg/mL) *Streptococcus* spp. K003 (200 μg/mL) *Corynebacterium suis* 54001 (750 μg/mL) *Escherichia coli* KK88 MN1 (400 μg/mL) *Escherichia coli* KK88 G1253 (400 μg/mL) *Salmonella* spp. O4 type K-011 (200 μg/mL)		34
			GTE-2 (>80% polyphenols)		*Staphylococcus aureus* KK101 (50 μg/mL) *Staphylococcus epidermis* KK108 (50 μg/mL) *Streptococcus uberis* KK204 (200 μg/mL) *Salmonella dublin* L-729 (200 μg/mL) *Salmonella enteriditis* L-58 (200 μg/mL) *Salmonella infantis* L-164 (200 μg/mL) *Salmonella mbandaka* L-743 (200 μg/mL) *Salmonella sofia* L-59 (200 μg/mL) *Salmonella typhimurium* L-413 (200 μg/mL)		
	animal study	4 weeks	GTE-1 (>60% polyphenols)	counting on culture medium	*Clostridium perfringens*	*Bifidobacterium* spp.	
			GTE-2 (>80% polyphenols)			*Lactobacillus* spp.	
wine phenolic extract	in vitro study	48 h incubation	600 mg wine extract	batch culture fermentation model/FISH	*Clostridium histolyticum* group		39
wine polyphenols	human intervention	4 weeks	dealcoholized red wine (272 mL/day)	PCR-DGGE[i]	*Bacteroidetes* *Firmicutes*	*Fusobacteria* *Enterococcus* genus *Blautia coccoides–Eubacterium rectale* *Bifidobacterium* *Eggerthella lenta*	40
			red wine (272 mL/day)			*Proteobacteria*	

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

Table 1. continued

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
						Prevotella	
						Bifidobacterium	
						Eggerthella lenta	
						Fusobacteria	
						Firmicutes	
						Bacteroidetes	
						Enterococcus genus	
						Blautia coccoides– *Eubacterium rectale*	
						Bacteroides genus	
						Bacteroides uniformis sp.	
red wine grape extract	in vitro study	2 weeks	1000 mg polyphenols/day	SHIME and qPCR/pyrosequencing	bifidobacteria	*Klebsiella* sp.	32
					Blautia coccoides	*Alistipes*	
					Anaeroglobus	*Cloacibacillus*	
					Subdoligranulum	*Victivallis*	
					Bacteroides	*Akkermansia*	
wine polyphenols	animal study	15 weeks	50 mg/kg bw[j]	counting on culture medium after fecal inoculation	*Clostridium* spp.	*Bifidobacterium* spp.	38
						Bacteroides	
						Lactobacillus spp.	

[a]FISH-FCM: fluorescence in situ hybridization coupled to flow cytometry. [b]HCF: high-cocoa flavanol. [c]LCF: low-cocoa flavanol. [d]SHIME: simulator of the human intestinal microbiota ecosystem. [e]qPCR: quantitative polymerase chain reaction. [f]GT: green tea powder. [g]T-RFLP: terminal restriction fragment length polymorphism. [h]GTE: green tea extract. [i]DGGE: denaturing gradient gel electrophoresis. [j]bw: body weight.

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

quercetin, kaempferol, myricetin, and their glycosides, are also found. The linkage between tea consumption and gut microbiota as a possible explanation for its contribution to well-being is under investigation. Ahn et al.[31] reported that green tea extracts (GTE) repressed the growth of *Clostridium bifermentans*, *C. difficile*, *Clostridium innocuum*, *Clostridium paraputrificum*, *C. perfringens*, and *Clostridium ramosum* while encouraging the growth of *Bifidobacterium* spp. in vitro.[31] In an in vitro study conducted by Kemperman et al.,[32] the antimicrobial effect of a black tea extract was analyzed by traditional culturing and qPCR, and its impact on microbial community was also assessed by PCR-DGGE and 16S rDNA measurements.[32] Several animal studies conducted in pigs[33] and in calves[34] concluded that tea polyphenols produced an enhancement in animals' intestinal microbiota. Thus, pigs that received 0.2% of tea polyphenols for 2 weeks showed a significant decrease in total bacteria and *Bacteroidaceae* and a tendency to decrease in *C. perfringens*. By contrast, a significant increase in *Lactobacilli* was found.[33] Similarly, two types of GTE were tested in vitro (GTE-1 and GTE-2, polyphenol contents >60% and >80%, respectively) and MICs were determined for diverse pathogenic bacteria. As a result of these in vitro analyses, a stronger inhibitory capacity of GTE-2 was concluded, assuming that the higher amounts of (−)-epigallocatechin gallate, (−)-gallocatechin gallate, and (−)-epicatechin gallate might be responsible for the observed reduction in bacterial growth. Furthermore, within the large variability of MICs detected for the tested species, the total counts of *Bifidobacterium* spp. and *Lactobacillus* spp. were significantly higher in the test group. In the same study, the growth rate reduction of *C. perfringens* was faster in calves supplemented with GTE, supporting the potential of GTE to prevent the growth of pathogenic bacteria and to improve microflora balance.[34]

In humans, a product containing 70% of tea polyphenols (Sunphenon, which included (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-gallocatechin gallate, and (−)-epigallocatechin gallate) was administered (0.4 g/volunteer) three times per day, for 4 weeks, which was equivalent to 10 cups of concentrated green tea. Results indicated that *C. perfringens* and other *Clostridium* spp. were significantly reduced during the tea polyphenol intake periods, whereas percentages of *Bifidobacterium* spp. in total fecal counts markedly increased.[35] More recently, bifidobacteria showed a trend to increase in 10 volunteers who drank green tea for 10 days.[36] In a different study, the effect of 4% green tea powder (GT) supplement with or without the addition of *Lactobacillus plantarun* DSM 15313 (Lp), was evaluated on microbiota of small intestine and cecum of high-fat fed mice. This study showed a synergestic effect with significant increases in *Lactobacillus* group and bacterial diversity, in both small intestine and cecum, after 22 weeks.[37]

Wine. Benefits coming from the moderate consumption of red wine (RW) have been mostly attributed to its phenolic compounds, consisting of a complex mixture of flavonoids, such as flavan-3-ols and anthocyanins, but also of nonflavonoids such as resveratrol, cinnamates, and gallic acid. Similarly to the polyphenols derived from other food products, wine polyphenols have also been stated to display a selective modulation of gut microbiota.[32] Thus, Dolara et al.[38] reported changes in the main bacterial strains of wine polyphenol-treated F344 rats (50 mg/kg for 15 weeks) compared to the control-fed rats. The wine polyphenolic extract contained 4.4% anthocyanins, 0.8% flavonols, 2.0% phenolic acids, 1.4% catechin, 1.0% epicatechin, and 28% proanthocyanidin. *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* spp. were more predominant in feces of polyphenol-treated rats, whereas *Bacteroides*, *Clostridium*, and *Propionibacterium* spp. prevailed in control-fed rats' feces. The authors underlined the potentiality of wine polyphenols to simulate the favorable effects of fibers and prebiotics on the colonic bacterial content.[38] An in vitro batch culture fermentation model carried out with human fecal microbiota aimed to observe the bacteria−polyphenol interactions implicated in the colonic metabolism of RW polyphenols.[39] In this study, the slight inhibition observed in *Clostridium histolyticum* were in concordance with the conclusions drawn with monomeric flavan-3-ols and cocoa flavan-3-ols in previously mentioned batch culture models.[19,30] Nevertheless, the lack of positive effects found on the growth of *Lactobacillus*−*Enterococcus* spp. in this experiment,[39] was ascribed to the lower concentrations of flavan-3-ol compounds provided (20.94 mg/L) compared to the 219 mg/L used by Tzounis et al.[19,30] In an intervention study, Queipo-Ortuño et al.[40] investigated the changes produced in the fecal microbiota of 10 healthy human volunteers after the consecutive intake of RW, dealcoholized red wine (DRW), and gin. After a washout period of two weeks, the small number of participants enrolled in the study was crossed from one treatment to the other, which might help the authors having a greater statistical power. However, the fact that there were three treatments with the absence of washout periods between them was a limitation that required discussion. On the basis of data from the monitorization of urine resveratrol metabolites after each treatment, the authors were able to conclude that there was not any carryover from treatment to treatment, thus this issue was discarded as a confounding factor.[40] Regarding gut microbiota, differences were observed depending on the type of beverage consumed. Briefly, four mayor bacteria phyla (*Proteobacteria*, *Fusobacteria*, *Firmicutes*, and *Bacteroidetes*) were markedly increased after RW intake, and relevant increases in *Bifidobacterium* and *Prevotella* species were detected. In contrast, no changes were found in *Actinobacteria* phyla, and marked decreases were identified in the *Clostridium* genera and *Clostridium histolyticum* group with RW. Nevertheless, these effects were less pronounced or even disappeared with DRW. Interestingly, the induction of gut microbiota remodelling due to the intake of small amounts of ethanol with polyphenols was linked to reductions in blood pressure, triglycerides, total cholesterol, and CRP.[40] Table 1 summarizes the main results from studies analyzing polyphenol-rich beverages and gut microbiota composition.

Soy Products. Soy products, coming from soybeans (members of *Leguminosae*), are rich in phytoestrogens, principally in the form of isoflavones. At this stage, a study that evaluated the impact of diverse soy milk formula consumption on the intestinal ecosystem of human overweight and obese men found a better *Firmicutes* to *Bacteroidetes* ratio in the soy milk-fed groups.[41] Soy milk intake in newborns was found to slightly enlarge the number of *Bifidobacterium* species in the infants.[42] Nevertheless, neither analyzed the isoflavone content of the soy milk or soy formula milk used; therefore, the slight modifications observed could not be attributed to specific components of the milk. The use of the Simulator of the human intestinal microbial ecosystem (SHIME) to prove the influence of a soygerm powder in the fermentative capacity of bacteria from inoculated fecal samples has been focus of interest.[43]

Moreover, in vivo studies with probiotic soy products have been also performed and differences on fecal microbiota have been reported. Bedani et al.[44] analyzed bacterial genus composition in rats feces that were administered the *Enterococcus faecium* CRL 183 probiotic microorganism with a soy product, either as a pure cell suspension or as a fermented product. Data were compared to microbial changes observed in rats treated with the unfermented soy product. In this case, no reductions in pathogenic genus such as, *Clostridium* spp., *Bacteroides* spp., or enterobacteria were concluded for the animals treated with the fermented soy products and only an enhancement in the growth of *Lactobacillus* spp. was obtained for the animals administered the *E. faecium* suspension.[44] Cavallini et al.,[45] in contrast, performed another experiment in which a probiotic soy product was administered to white rabbits and posterior analyses of microbiota were performed in vitro. In this case, the difference in bacterial populations obtained between fermented and unfermented products were more significant regarding *Lactobacillus* spp. and *Bifidobacterium* spp. populations, which might be attributed to the addition of probiotic bacteria (*E. faecium* CRL 183 and *Lactobacillus helveticus* 416). In fact, it could be observed that unfermented soy products are able to reduce *Clostridium* species while fermented soy products, in general, produce an enhancement in the growth of probiotic bacteria.[45]

Fruits. A great deal of evidence suggests that fruits, vegetables, and products coming from them are able to significantly boost the growth of colonic friendly bacteria, as for example *Bifidobacterium* and *Lactobacillus*. Table 2 presents data about the influence of some selected fruits and soy products on gut microbiota composition.

Pomegranate. Scientific research has evidenced the high antioxidant capacity of pomegranate (*Punica granatum* L.) products, which has been attributed to their high content in antioxidants and anti-inflammatory bioactive compounds (ellagitannins and anthocyanins mainly) concentrated in peel, membranes, and piths. In this context, experiments using batch-culture fermentation systems have aimed to examine the potential of pomegranate peel extract (PPE) and punicalagins in the growth of intestinal bacterial strains. In these studies, PPE (0.01%) inhibited the growth of *C. perfringens*, *C. ramosum*, *S. aureus*, and *Clostridium clostridioforme* but significantly enhanced the growth of *Bifidobacterium breve* and *Bifidobacterium infantis* by 275% and 241%, respectively.[26] In a similar study, Bialonska et al.[27] stated a significant increase of total bacteria and beneficial bacteria *Bifidobacterium* spp. and *Lactobacillus*−*Enterococcus* group in media supplemented with PPE, which was accompanied by a marked increase in the production of short chain fatty acids. In contrast, no significant effect was observed in gut bacteria grown in media supplemented with punicalagins (0.2% w/v).[27] Larrosa et al.[46] reported for the first time the prebiotic effect of PPE using a dextran sodium sulfate-induced colitis rat model. The intake of 250 mg PPE/kg/day (equivalent to 2.5 g PPE in a 70 kg person) for 3 weeks increased *Bifidobacterium*, *Lactobacillus*, and *Clostridium* counts, preventing the colonization and invasion of tissues by enterobacteria including *E. coli*. In addition, the same results were obtained when the rats were fed synthetic urolithin A (2.2 mg/kg/day; equivalent to 154 mg in a 70 kg person), which demonstrated that the main gut microbiota-derived metabolite from pomegranate ellagitannins, urolithin A, also showed prebiotic effect.[46] Neyrinck et al.[47] emphasized the enhancement of the cecal pool of bifidobacteria in mice fed a high-fat diet supplemented with the PPE (rich in polyphenols, 30%). In addition, a down-regulation of the expression of inflammatory markers (IL-1β, IL-6, COX-2) in colon and visceral adipose tissue was demonstrated for the latter group. Therefore, a direct relationship between PPE consumption and health improvement through gut microbiota modulation was hypothesized by the authors.[47]

Apples. Apple contains a high amount of pectin, a polysaccharide fiber that has been described to influence intestinal microbiota but is also rich in phenolic compounds with high antioxidant capacity. Licht et al.[48] argued that changes in the microbiota of apple-fed rats should be ascribed to pectin.[48] Rats that received an extraction juice from apples (total polyphenols, 829 mg/L) for 4 weeks, instead of drinking water, showed significantly more *Lactobacillus* and *Bifidobacterium* in fresh feces.[49] The same research group also observed an increase in *Bacteroidaceae* species in Wistar rats offered juice colloids isolated from apple pomace extraction juices, which presented higher contents of dietary fiber and polyphenols.[50] Moreover, as previously reported, in a study where apple pectin (5 g/100 g) and polyphenol-rich apple concentrate (10 g/100 g) was given to rats, even if microbiota analyses were not conducted, a more effective biological effect was demonstrated when both components were administered in combination, implying the important role of the phenolic compounds.[51]

Grapes. Grape fruits are rich in polyphenols, mainly, anthocyanins, flavonols, favan-3-ols, hydroxybenzoates, and phenolic acids. An in vitro study was conducted in order to analyze the potential of two grape seed flavan-3-ol fractions in the growth and metabolism of *Bifidobacterium* spp., *Lactobacillus*−*Enterococcus* spp., *Clostridium histolyticum* group, *Bacteroides* spp., and members of the domain Bacteria.[52] The analyzed grape seed extracts (GSE) differed in their composition of monomers and oligomers. Monomeric-rich fraction of GSE (GSE-M) contained 70% monomers and 28% procyanidins, whereas the oligomeric-rich fraction of GSE (GSE-O) was composed of 21% monomers and 78% procyanidins. In spite of these composition differences, both extracts produced comparable effects on microbiota composition. For instance, a selective inhibitory action on *Clostridium histolyticum* was described for both fractions within 5−10 h of fermentation period, this being statistically significant only for GSE-O at 10 h of fermentation. During these first hours, an increase in *Lactobacillus*−*Enterococcus* group was also found in both cases, although statistical significance was only achieved for the fractions with the highest flavan-3-ol monomers fraction.[52] The chromatographic characterization of grape seed fractions obtained from different extraction methods (Aqualsolv and microwave-assisted extraction) reported that fractions containing mainly monomers, like catechin and epicatechin, presented low antibacterial activity, whereas those fractions containing oligomeric units of catechins and epicatechins were more effective inhibiting the growth of 10 tested pathogens (*S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *E. coli*, *Staphylococcus epidermis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Enterococcus casilliflavus*, and *Pneumococcus*).[53] When comparing the inhibitory capacity of grape seed extracts and grape bagasse extracts in relation to the amounts of total phenolics obtained with two different solvent extraction methods, GSE had the highest total phenolic concentration resulting from the acetone:water:acetic acid extraction.[54] In another study, three commercial GSE, which differed in their flavan-3-ol profiles, were assayed to test their

Table 2. Polyphenol-Rich Fruits and Vegetables (Soy, Pomegranate, Grapes, Berries, Apples) with Effects On Gut Microbiota

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
soygerm powder	in vitro study	14 days	2.5 g/day	SHIME[a] and plate count		*Lactobacillus* spp.	43
aqueous soy extract	animal study	60 days	HUF[b] (2.8 mL/kg bw[c]/day)	plate count after fecal inoculation	enterobacteria	*Enterococcus* spp.	45
					Clostridium spp.		
			HF[d] (2.8 mL/kg bw/day)		enterobacteria	*Bifidobacterium* spp.	
						Lactobacillus spp.	
						Enterococcus spp.	
			HIF[e] (2.8 mL/kg bw−2.1 mg of total isoflavone/kg bw/day)		enterobacteria	*Bifidobacterium* spp.	
					Clostridium spp.	*Lactobacillus* spp.	
						Enterococcus spp.	
fermented soy product	animal study	30 days	fermented soy product (3 mL/kg bw/day)	counting on culture medium after fecal inoculation		*Enterococci*	44
						Lactobacillus spp.	
						Bacteroides spp.	
			unfermented soy product (3 mL/kg bw/day)		*Clostridium* spp.	*Lactobacillus* spp.	
					Bifidobacterium spp.		
soy milk	human intervention	3 months	low glycinin soy milk 500 mL/day	qPCR[f] and FLX pyrosequencing	*Bifidobacterium*	*Bacteroides*− *Prevotella*	41
					Firmicutes	*Bacteroidetes*	
						Proteobacteria	
			conventional soy milk 500 mL/day		*Bifidobacterium*	*Bacteroidetes*	
					Firmicutes	*Proteobacteria*	
soy milk	human intervention	1 month	exclusive feeding	PCR-DGGE[g]		*Bifidobacterium infantis*	42
						Bifidobacterium longum	
						Bifidobacterium breve	
						Bifidobacterium adolescentis	
pomegranate byproduct	in vitro study	0−48 h incubation	1.5 mL of PPE[h]	batch culture fermentation model/FISH[i]		*Lactobacillus*− *Enterococcus* group	27
						Bifidobacterium spp.	
pomegranate extract	animal study	25 days	250 mg/kg	agar plate dilution method	*Escherichia coli*	*Lactobacilli*	46
					enterobacteria	bifidobacteria	
						Clostridium	

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

Table 2. continued

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
pomegranate peel extract	animal study	4 weeks	0.2% (6 mg/day)	qPCR		*Bacteroides*− *Prevotella* spp. *Bifidobacterium* spp.	47
pomegranate byproduct	in vitro study	0−72 h incubation	0.01% (v/v) of PPE	counting on culture medium	*Clostridium perfringens* NRRL B-23584 *Clostridium ramosum* NRRL B-23617 *Staphylococcus aureus* ATCC 29213 *Lactobacillus acidophilus* NRRL B-4495 *Lactobacillus pentosus* NRRL B-227 *Lactobacillus rhamnosus* NRRL B-442 *Bifidobacterium bifidum* NRRL B-41410	*Bifidobacterium infantis* NRRL B-41661 *Bifidobacterium breve* NRRL B-41408	26
grape seed extract	in vitro study	24−48 h incubation	GSE-M[j] (414 mg/g total phenolics) at 0.25−1 mg/mL GSE-O[k] (279 mg/g total phenolics) at 0.25−1 mg/mL	culture medium spectrophotometry (600 nm)	*Streptococcus thermophilus* STY-31 *Lactobacillus fermentum* PNA1 *Lactobacillus fermentum* LC-40 *Lactobacillus acidophilus* LA-5 *Lactobacillus vaginalis* ZL63-22 *Bifidobacterium lactis* BB12	*Bifidobacterium breve* 26M2 *Bifidobacterium bifidum* HDD541 *Lactobacillus plantarum* IFPL93 *Lactobacillus casei* IFPL7190 *Lactobacillus bulgaricus* LBY-27	55
grape seed extract	in vitro study	18−24 h incubation	acetone:water:acetic acid (90:9.5:0.5), 667.87 mg GAE[l]/g at 20% ethyl acetate:methanol:water (60:30:10), 627.98 mg GAE/g at 20%	paper disc diffusion method	*Aeromonas hydrophila* ATCC 7965 *Bacillus brevis* FMC 3 *Bacillus cereus* FMC 19 *Bacillus megaterium* DSM 32 *Bacillus subtilis* IMG 22 *Enterobacter aerogenes* CCM2531 *Enterococcus faecalis* ATCC 15753 *Escherichia coli* DM *Klebsiella pneumoniae* FMC 5 *Listeria monocytogenes* Scott A *Mycobacterium smegmatis* RUT *Proteus vulgaris* FMC 1 *Pseudomonas aeruginosa* ATCC 27853 *Staphylococcus aureus* COWAN 1		54
proanthocyanidin-rich grape seed extract	human intervention	2 weeks	0.5 g/day/subject extract (0.19 g/day/subject as proanthocyanidins)	counting on culture medium after fecal inoculation	*Enterobacteriaceae*	*Bifidobacterium*	58
grape antioxidant dietary fiber	in vitro study	22−26 h incubation	nonextractable polyphenols 2.4 mg/mL	plate count		*Lactobacillus reuteri* ATCC 23272	57

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

Table 2. continued

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
						Lactobacillus acidophilus ATCC 4356	
	animal study	4 weeks	50 g/kg diet (4.23 g/100 g extractable polyphenols; 17.51 g/100 g nonextractable polyphenols)	RT-PCR	*Bifidobacterium* spp.	*Lactobacillus* spp.	
grape pomace cocentrate and grape seed extract	in vitro study	24 h incubation	GSE (7.2 g/kg)	paper disc diffusion method	*Clostridium perfringens* BA05/00439-SB		56
	animal study	21 days	GPC^{m} (60 g/kg)	T-RFLP^{n}	*Pseudomonas/Acinobacter* spp.	*Bacillus/Paenibacillus* spp. *Desulfitobacterium hafniense*	
			GSE (7.2 g/kg)			*Pseudomonas/Acinobacter* spp. *Nocardioides* spp. *Bacillus/Paenibacillus* spp. *Desulfitobacterium hafniense*	
wild blueberry	human intervention	6 weeks	25 g/day	RT-PCR		*Lactobacillus acidophilus* *Bifidobacterium* spp.	63
black currant extract powder	animal study	4 weeks	black currant extract powder 13.4 mg/kg bw/day	FISH	*Bacteroides*	bifidobacteria	62
					Clostridium perfringens/histolyticum sub group	*Lactobacilli*	
			black currant extract powder, lactoferrin, and lutein 30 mg/kg bw/day		*Bacteroides*	bifidobacteria	
					Clostridium perfringens/ histolyticum sub group		
berry extract	in vitro study	12−24 h incubation	1−5 mg/mL cloudberry, raspberry	counting on culture medium	*Staphylococcus aureus* E-70045		60
					Salmonella enterica sv. Typhimurium E-981151		
					Salmonella enterica sv. Infantis E-97738		
berry extracts	in vitro study	12−24 h incubation	0.8−3.5 mg/well blueberry	agar diffusion method	*Escherichia coli* 50		59
					Escherichia coli CM871		
					Lactobacillus rhamnosus E-666		
					Lactobacillus rhamnosus E-800		
					Salmonella enterica SH-5014		
			raspberry		*Lactobacillus rhamnosus* E-666		
					Lactobacillus rhamnosus E-800		
					Lactobacillus reuteri E-849		

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

Table 2. continued

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
					Escherichia coli 50		
					Escherichia coli CM871		
					Salmonella enterica SH-5014		
			cloudberry		*Lactobacillus rhamnosus* E-666		
					Escherichia coli 50		
					Escherichia coli CM871		
					Salmonella enterica SH-5014		
					Enterococcus faecalis E-203		
					Bifidobacterium lactis E-508		
			strawberry		*Escherichia coli* CM871		
					Bifidobacterium lactis E-508		
			lingonberry		*Escherichia coli* 50		
					Escherichia coli CM871		
					Salmonella enterica SH-5014		
			black currant		*Escherichia coli* 50		
					Escherichia coli CM871		
			cranberry		*Escherichia coli* 50		
					Escherichia coli CM871		
					Salmonella enterica SH-5014		
			sea buckthorn berry		*Escherichia coli* CM871		
blueberry extract	in vitro study	1−5 days incubation	Maru cultivar blueberry extract 10−25% (v/v)	plate count		*Bifidobacterium breve* NZRM3932	61
			Centurion cultivar blueberry extract 10−25% (v/v)			*Lactobacillus rhamnosus* NZRM 299	
						Bifidobacterium breve NZRM3932	
		48 h incubation	0.1% (v/v)	fecal batch-culture fermentation/FISH		*Lactobacilli*	
						bifidobacteria	
	animal study	6 days	4 mL/kg bw/day from Maru cultivar	FISH		*Lactobacilli*	
						bifidobacteria	
			4 mL/kg bw/day from Centurion cultivar			*Lactobacilli*	
						bifidobacteria	

dx.doi.org/10.1021/jf402506c | J. Agric. Food Chem. 2013, 61, 9517−9533

Table 2. continued

food or food extracts rich in polyphenols	type of study	duration	dosis	techniques used	antimicrobial activity (AMA)	bacteria growth promoting effect (BGPE)	ref
apples and apple products	animal study	14 weeks	10 g/day raw whole apple	PCR-DGGE and qPCR	*Bacteroides*		48
apple juice	animal study	4 weeks	free access	plate count		*Bifidobacterium* *Lactobacillus*	49
apple juice colloid	animal study	6 weeks	free access	fecal inoculation and plate count		*Bacteroidaceae*	50

[a]SHIME: simulator of the human intestinal microbiota ecosystem. [b]HUF: hypecholesterolemic plus unfermented soy product. [c]bw: body weight. [d]HF: fermented soy product. [e]HIF: hypercholesterolemic plus isoflavone-supplemented fermented soy product. [f]qPCR: polymerase chain reaction. [g]DGGE: denaturing gradient gel electrophoresis. [h]PPE: pomegranate byproduct. [i]FISH: fluorescence in situ hybridization. [j]GSE-M: monomeric-rich fraction grape seed extract. [k]GSE-O: oligomeric-rich fraction grape seed extract. [l]GAE: gallic acid equivalent. [m]GPC: 7grape pomace concentrate. [n]T-RFLP: terminal restriction fragment length polymorphism with specific primer-restriction enzyme system.

effect on the growth of several *Lactobacillus* and bifidobacteria strains.[55] Briefly, a general inhibition of bacterial growth was observed as the most common response. Although dependent on the polyphenol extract composition and the bacterial strain tested, this growth repression increased with the procyanidin content of the extract and was observed not only to be species specific but also strain specific. Interestingly, even if *Lactobacillus* and bifidobacteria were likewise sensitive to the phenolic concentration and to the content of procyanidins, specific strains such as *Lactobacillus casei* and *Lactobacillus plantarum* were able to reach maximal growth with the three extracts.[55] Viveros et al.[56] analyzed and compared cecal and ileal digesta from broiler chicks treated with GSE and grape pomace concentrate (GPC) by plate count and terminal restriction fragment length polymorphism (T-RFLP) method. The impact of both extracts on the bacterial ecosystems of both regions was noticed to differ. Besides, the T-RFLP approach made it possible to confirm that major changes in treated groups were given in uncultured and unidentified species.[56] Moreover, the combination of grape polyphenols with dietary fiber has been demonstrated to improve the desired prebiotic effect. This is the case of grape antioxidant dietary fiber (GADF), a natural product derived from red grapes that was demonstrated to boost *Lactobacilli* growth in rats (50g/kg) but also in vitro, whereby GADF was able to significantly increase the growth of *Lactobacillus acidophilus* and *Lactobacillus reuteri*.[57] Extractable polyphenols (proanthocyanidins) are an important part of GADF, but there is also an important percentage (14.8%) of nonextractable proanthocyanidins (NEPA), which have been demonstrated to be metabolized by intestinal microbiota. The confirmed interaction between grape derived proanthocyanidins and intestinal bacteria has, in turn, given rise to human studies whereby the prebiotic effects of proanthocyanidin-rich extract (0.19 g/day/subject) intake for 2 weeks, significantly increased the number of *Bifidobacterium* in healthy adults and tended to decrease the number of putrefactive bacteria such as *Enterobacteriaceae*.[58]

Berries. Berries contain abundant phenolic compounds, mostly flavonoids (where anthocyanins predominate). Some in vitro studies carried out with diverse berry extracts evaluated their antimicrobial activity on Gram-positive and Gram-negative bacteria. Interestingly, berry extracts inhibited Gram-negative bacteria, such as intestinal pathogen *Salmonella enterica*, but not Gram-positive beneficial probiotic lactic acid bacteria.[59] *Staphylococcus* and *Salmonella* were the most sensitive, and the strongest inhibitory action was observed with cloudberry and raspberry, this outcome being largely attributed to their ellagitannin content (191 and 146 mg/g, respectively). Importantly, the lower pH produced on the media was hypothesized as a possible explanation for the antimicrobial action, as it seems that low pH values promote the growth of probiotic bacteria, while pathogenic bacteria present high variability in their tolerance to acids.[60] Wild blueberry (*Vaccinium angustifolium*) is characterized by its high content in anthocyanidins, whose prebiotic activity has been documented in vitro and in vivo. Thus, soluble extracts of two New Zealand blueberry cultivars promoted the growth of *L. rhamnosus* and *B. breve* in vitro and, more importantly, both extracts were demonstrated to effectively induce the growth of *Lactobacilli* and bifidobacteria in the cecum of rats orally gavaged with these extracts (4 mL/kg/day) for 6 days.[61] Cecal contents of rats treated with supplements, whose major component was the black currant extract powder, were also

estimated using the fluorescent in situ hybridization (FISH) technology. Rats administered the black currant aqueous extract presented a significant increase in bifidobacterial numbers and a significant decrease in bacteroides and clostridial numbers.[62] In an intervention study with 15 healthy male individuals who underwent a dietary intervention where a wild blueberry drink was given before or after a placebo drink, a significant increase in *Bifidobacterium* spp. and *L. acidophilus* group was also detected.[63]

CONCLUSIONS

This review has outlined some of the current work carried out facing the impact of polyphenols or polyphenol-rich dietary sources on gut microbial ecosystem evidencing an interaction between gut microbes and these compounds. When comparing results obtained from in vitro studies with data from in vivo experiments, no direct extrapolations could be made without a special mention to diverse factors acting up on this process.

Type of in Vitro Experiments. In this regard, the in vitro experiments discussed in this work have been performed by different approaches such as in vitro traditional culture techniques or cultivation, in vitro fecal batch culture fermentation systems, and simulator of the human intestinal microbial ecosystem (SHIME).

Bacteria Inoculation with Individual Polyphenols. The simplest experiments where diverse bacteria species were inoculated with pure polyphenols have been useful to confirm that either flavonoids or nonflavonoids could produce an antimicrobial activity against pathogenic bacteria[16] but also a growth promoting effect for some beneficial commensal bacteria.[26] Nevertheless, the biological relevance of these types of outcomes is uncertain, as polyphenol bioavailability is very low and they undergo an extensive modification within the organism.[64] In this sense, special care should be taken as in vitro studies do not consider that some polyphenols are directly absorbed in the small intestine while others reach the colon being degraded by microbiota to produce metabolites which, in some cases, could be more active than the original compounds.[65] There are many review articles that elegantly explain polyphenol biotransformation in the gut, which is not the core of this work.[15,66] Anyway, a basic concept such as the fact that the chemical structure of polyphenols largely influences their absorption and metabolism should be taken into account. In this sense, glycosides that reach the colon will be hydrolyzed into aglycones by microflora and will subsequently suffer other structural modifications such as methylation, sulfation, and glucuronidation processes.[64] This is relevant to understand some data from in vitro experiments, as for instance the work reported by Duda-Chodak et al.,[16] who concluded that aglycones (naringenin, quercetin, and hesperetin) were powerful antibacterial compounds compared to their glycosides (naringin and rutin). Therefore, when trying to draw any conclusive statement about the biological effect of a certain polyphenol, the authors should be aware of the process of compounds' bioconversion in order to avoid the use of erroneous polyphenols in their experiments.[64]

On the other hand, in these studies a limited number of bacterial species were inoculated, ignoring the bacterial complexity found in the intestine. In most cases, a limited number of strains from *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Staphylococcus*, *Salmonella*, *Bacteroides*, and *Escherichia* genus[16−18,26] were incubated with pure polyphenols. Hence, as not all bacterial strains could be evaluated in these assays, those microorganisms that have been proved to be responsible for the hydrolyzation of some of the tested compounds, as for instance *Bacteroides uniformis* and *Bacteroides ovatus*, obligate anaerobes that convert rutin to quercetin,[15] were left without being analyzed. Despite the fact that these types of experiments are considered the initial step for the posterior in vivo experiments, they are essential to gain knowledge for the succeeding human subject's classification on low, medium, and high polyphenol metabolizers. This is the case of daidzein, a soy isoflavone, whose metabolism gives equol (whose biological properties might be beyond its precursor's) when equol-forming bacteria are present in the intestine.[67] Knowledge about the conversion of the mentioned compound has allowed discerning a great variability between individuals regarding their ability to produce its metabolite, attributed to differences in their gut microbiota composition.[68] This sort of information might be useful for personalized nutritional recommendations in the future. Anyway, there are other factors apart from the host's baseline gut microbiota composition such as genetics and the metabolic state of the host that should be considered in order to be able to clarify the exact mechanisms contributing to the health benefits and to distinguish the direct correlation between bacterial strains and the metabolic products resulted from their interaction with polyphenols.

Batch Culture Fermentation System. In this regard, the use of batch culture fermentation systems has been also reported in this work.[27] This approach, characterized to exhibit similar conditions to the human colonic region, aims to assess the effect of polyphenols[27] or polyphenol-rich extracts[27,30,39,61] on the growth of fecal bacteria population using human fecal slurries. Even if these systems are static models that aim to give information about colonic degradation of polyphenols and allow scientists to fix strict conditions regarding oxygen, temperature, and pH of the medium, an important point to bear in mind is that fecal samples used might not entail the same characteristics of daily fecal production. Moreover, one of the main limitations when trying to address the gut microbial polyphenol metabolism in these types of assays is the interindividual variability. Fecal samples obtained from different individuals will exhibit distinct bacterial population for numerous reasons (i.e., diet, exercise, water, absorption/hydratation state, minor infections, stress, and so on), thus they could present a variable metabolic capacity[69] giving rise to different conclusions.

Simulator of the Human Intestinal Microbial Ecosystem (SHIME). Another undeniable factor affecting results is the repercussion of the experiments' duration. In contrast to the aforementioned in vitro static systems, continuous multistaged culture systems that simulate the human microbial community in the large intestine have been developed.[70] Studies carried out in SHIME models[32,43] have allowed scientists to assess in vitro the implication of a two-week continuous treatment period with black tea, wine extract, or a soygerm powder in colonic microbiota composition. As a matter of fact, even if this system is considered to better mimic the real polyphenol−gut microbial interaction happening in the organism, it should be underlined that this approach takes for granted that the extracts reach intact to the colonic region, which is far from the reality.[15] Moreover, owing to the high interrelationship between host factors and the complex intestinal bacteria community, in vitro studies should be supported with further research in vivo and with human intervention trials to be able to elucidate the mechanisms underlying this interaction.

In Vivo Studies and Human Intervention Studies. This review summarizes data from 16 studies conducted in animal models compared to eight human intervention studies. It is widely known that preliminary evidence should be warranted in animal models before human intervention trials. In fact, they contribute to better understanding the mechanisms and biological effects that could be likely to happen in human biology. Furthermore, a good design in human studies is indispensable in order to make as feasible as possible the conclusions drawn. In many cases, it can be difficult to count on volunteers collaboration who meet the criteria and are ready to begin a relatively long-term study (4−10 weeks) so that the number of participants enrolled might be quite poor (8 subjects to 22 subjects). This might lead the researchers to sketch crossover studies[30] in which participants are randomly subjected to a sequence of two or more treatments, and allow removing participants' variation, as well as being more efficient than trials performed in parallel with the same number of subjects. However, their principal disadvantage is that the effects of one treatment might "carryover" and modify the response to the following treatments. In fact, some trials cited in the present work[40] have skipped the washout periods between treatments that might be key in order to favor the continuation of the study by the participants.

Quantification Techniques. The final technique used for the bacteria identification and quantification is of great relevance. It is widely known that 60−80% of bacterial species have been reported to be noncultivable by culture-dependent methods. Thus, these approaches failed to identify a large fraction of gut microbial diversity. Interestingly, at present, the advances gained in molecular techniques have made possible to overcome limitations of culture dependent methods,[71] being able to give a more representative view of all bacterial community in the gut. Nevertheless, these techniques should be complementary, and ideally an interdisciplinary approach comprising several "omics" approaches without the exclusion of culture-dependent techniques should be conducted. An example of this combination could be found in an experiment performed by Kemperman et al.,[32] where data obtained from different microbiological analyses (cultivation, qPCR, PCR-DGGE, and 16S rRNA pyrosequencing) were compared and the previously mentioned limitations of traditional culture techniques became visible. The culture-dependent technique used in this experiment estimated a lower bacterial number and was not able to detect conclusive antimicrobial effect of the extracts tested, whereas the aforementioned techniques were able to distinguish different bacterial clusters depending on the tested compounds.[32] In general, from the articles cited in this review, it could be suggested that culture-dependent techniques were mainly focused on the identification/quantification of six genus (*Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Salmonella*, *Staphylococcus*, and *Bacillus*) belonging to one of three major classes (*Firmicutes*, *Proteobacteria*, *Actinobacteria*),[31,33−35,57,61] while those studies performed with accurate and powerful molecular techniques, such as 16S rRNA pyrosequencing, FISH, FISH-FCM, and PCR-DGGE, enabled the characterization of genus (*Victivallis*, *Akkermansia*) from not so abundant phyla (Chlyamydiae/Verrucomicrobia group) and conduct an in-depth characterization of bacterial populations at species levels. However, briefly, it could be said that even a lot of progress has been made in the last years in the area of gut microbiota, still some challenges regarding parameters such as sampling, the sequencing depth, and setting the limits of the regions to be analyzed remain to be agreed upon so that experiments could be completely reproducible. The advances in sequencing technology will permit detection of all the bacterial species and strains, at a level of detail much more precise than the previously used techniques.

Final Remarks. From a health perspective, the intake of phenolic compounds, either as pure compounds or as part of food constituents, could be an effective approach to modulate gut microbiota, enhancing the growth of specific beneficial bacteria strains while competitively excluding specific pathogenic bacteria. However, further research is needed, especially in humans, to elucidate the specific effects of phenolic compounds on the growth of different gut bacteria, their interactions with other polyphenols and dietary components, the individual genetic, inflammatory, and microbiota background, as well as the involvement of polyphenol−microbiota interactions on the beneficial effects attributed to polyphenols on different chronic diseases.

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Funding

This study was supported by grants from Instituto de Salud Carlos III (PREDIMED, CIBERobn), Government of the Basque Country (IT-512−13), University of the Basque Country (UPV/EHU) (ELDUNANOTEK, UFI11/32), and Línea Especial from University of Navarra (Spain). We wish to thank the Department of Education, Universities and Research of Basque Government for the predoctoral grant given to U.E.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

NO, nitric oxide; MIC, minimum inhibitory concentration; Erec, *Clostridium coccoides*−*Eubacterium rectale*; IC_{50}, half-maximal inhibitory concentration; BMI, body mass index; LCF, low-cocoa flavanol; HCF, high-cocoa flavanol; CRP, c-reactive protein; GTE, green tea extract; GT, green tea powder; Lp, *Lactobacillus plantarum* DSM 15313; RW, red wine; DRW, dealcoholized red wine; SHIME, simulator of the human intestinal microbial ecosystem; PPE, pomegranate peel extract; GSE, grape seed extract; GSE-M, monomeric-rich fraction of grape seed extract; GSE-O, oligomeric-rich fraction of grape seed extract; GPC, grape pomace concentrate; T-RFLP, terminal restriction fragment length polymorphism; GADF, grape antioxidant dietary fiber; NEPA, nonextractable proanthocyanidins; FISH, fluorescent in situ hybridization

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