Polyphenols, dietary sources and bioavailability

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Summary. Fruit and beverages such as tea and red wine represent the main sources of polyphenols. Despite their wide distribution, the healthy effects of dietary polyphenols have come to the attention of nutritionists only in the last years. The main factor responsible for the delayed research on polyphenols is the variety and the complexity of their chemical structure. Emerging findings suggest a large number of potential mechanisms of action of polyphenols in preventing disease, which may be independent of their conventional antioxidant activities. To establish evidence for the effects of polyphenol consumption on human health and to better identify which polyphenols provide the greatest effectiveness in disease prevention, it is first of all essential to determine the nature and the distribution of these compounds in our diet, and secondly to better know their bioavailability.

Key words: diet, polyphenols, bioavailability.

Riassunto *(Polifenoli, fonti alimentari e biodisponibilità)*. La frutta e le bevande, come il tè e il vino rosso, costituiscono le principali fonti di polifenoli per l'uomo. A causa della varietà e della complessità della loro struttura chimica, gli effetti benefici dei polifenoli contenuti nella dieta sono stati estesamente studiati solo negli ultimi anni. Recentemente è stato evidenziato che gli effetti protettivi dei polifenoli su importanti patologie umane, come malattie cardiovascolari e cancro, non sono dovuti esclusivamente alle loro proprietà antiossidanti, ma anche alla loro capacità di modulare molteplici attività cellulari. Per chiarire quali siano gli effetti dei polifenoli e per meglio identificare quali tra gli innumerevoli composti polifenolici siano più efficaci nella prevenzione di alcune patologie umane, è necessario sia esaminare la loro natura e distribuzione nella nostra dieta, sia conoscere meglio la loro biodisponibilità.

Parole chiave: polifenoli, dieta, biodisponibilità.

INTRODUCTION

Polyphenols are the most abundant antioxidants in our diet and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages, such as tea, coffee and wine. Despite their wide distribution, the healthy effects of dietary polyphenols have come to the attention of nutritionists only in the last years. The main factor responsible for the delayed research on polyphenols is the variety and the complexity of their chemical structure.

As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress. Experimental studies, in fact, strongly support a role of polyphenols in the prevention of cardiovascular disease, cancer, osteoporosis, diabetes mellitus and neurodegenerative disease [1]. In particular, it has been shown that the consumption of polyphenols limits the development of atheromatous lesions, inhibiting the oxidation of low density lipoprotein [2-5], which is considered a key mechanism in the endothelial lesions occurring in atherosclerosis.

However, emerging findings suggest a variety of potential mechanisms of action of polyphenols in preventing disease, which may be independent of their conventional antioxidant activities. Furthermore, prooxidant effects of polyphenols have been described [6], having opposite effects on basic cell physiological processes: if as antioxidants they improve cell survival, as pro-oxidant they may induce apoptosis and block cell proliferation [7]. On the other hand, accumulating evidence indicates that polyphenols might exert several other specific biological effects such as the inhibition or reduction of different enzymes, among which telomerase [8] cycloxygenase [9, 10], lipoxygenase [11, 12], and the interaction with signal transduction pathways and cell receptors [13-15]. Moreover polyphenols can affect caspase-dependent pathways [16, 17], cell cycle regulation [18] and platelet functions [19]. It is mainly by virtue of these properties that they exert their protective effects and receive more and more attention as potential therapeutic agents against several chronic degenerative diseases [20, 21].

Much of the evidence on the protective effects of polyphenols is derived from experiments performed

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in vitro or in animal models, and moreover by using concentrations much higher than those generally contained in human diet. However the number of human studies investigating the protective effects of polyphenols has rapidly increased over the last decade.

To establish conclusive evidence for the effectiveness of polyphenols in disease prevention and human health improvement, it is essential to determine the nature and distribution of these compounds in our diet and to better identify which of the hundred of existing polyphenols are likely to provide the greatest effects. Furthermore, it is crucial the understanding of the factors involved in polyphenol release from the foods in which they are contained, their extent of absorption and their fate in the organism, in a word their "bioavailability", a term originally used in pharmacology to define the concept of the "rate and extent to which a drug reaches its site of action" [22].

It is worth of note that bioavailability appears to differ greatly among the various polyphenols, and the most abundant ones in our diet are not necessarily those that have the best bioavailability profile. Another difficulty depends on the fact that the active compounds may not be the native polyphenols found in foods, which are generally tested *in vitro* studies, but more likely their, often unknown, metabolites [23, 24].

The knowledge of the bioavailability of the various dietary polyphenols will help us to identify those most likely able to exert protective healthy effects and will allow a more correct evaluation of the real polyphenol intake.

CLASSIFICATION OF POLYPHENOLS AND THEIR DISTRIBUTION IN FOODS

Polyphenols are common constituents of foods of plant origin; they comprise a wide variety of molecules that have a polyphenol structure (*i.e.* several hydroxyl groups on aromatic rings), but also molecules with one phenol ring, such as phenolic acids and phenolic alcohols. Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. The main groups of polyphenols are: flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans *(Figure 1)*.

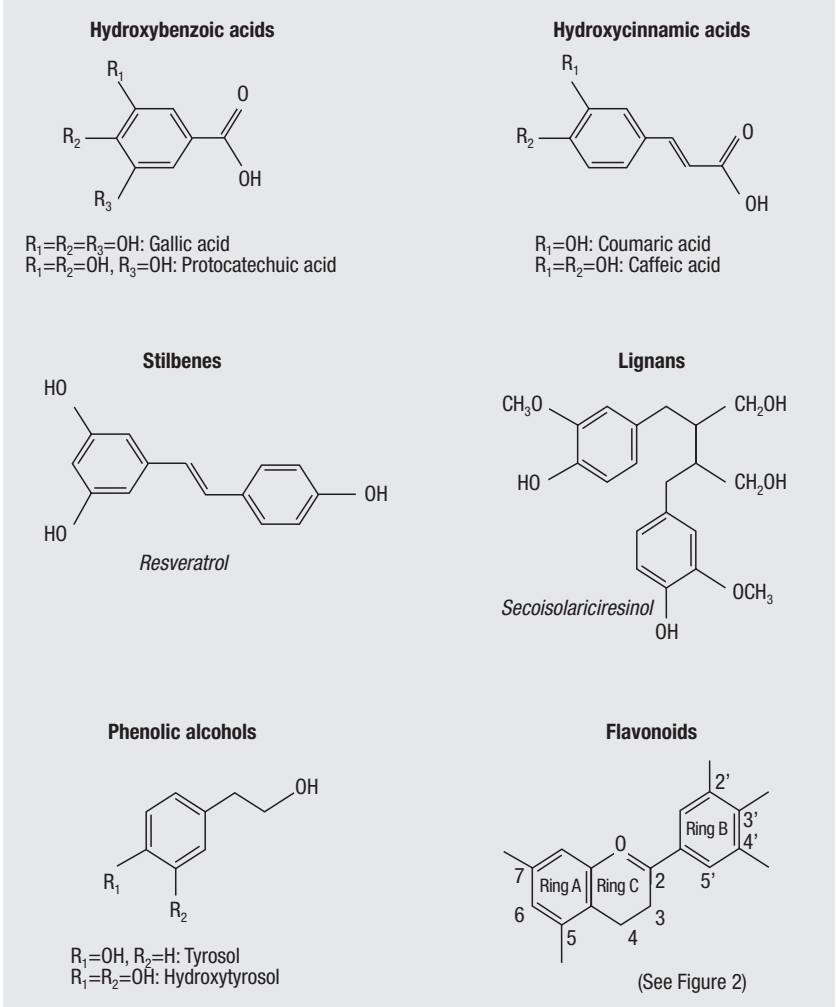

Fig. 1 | *Chemical structures of polyphenols.*

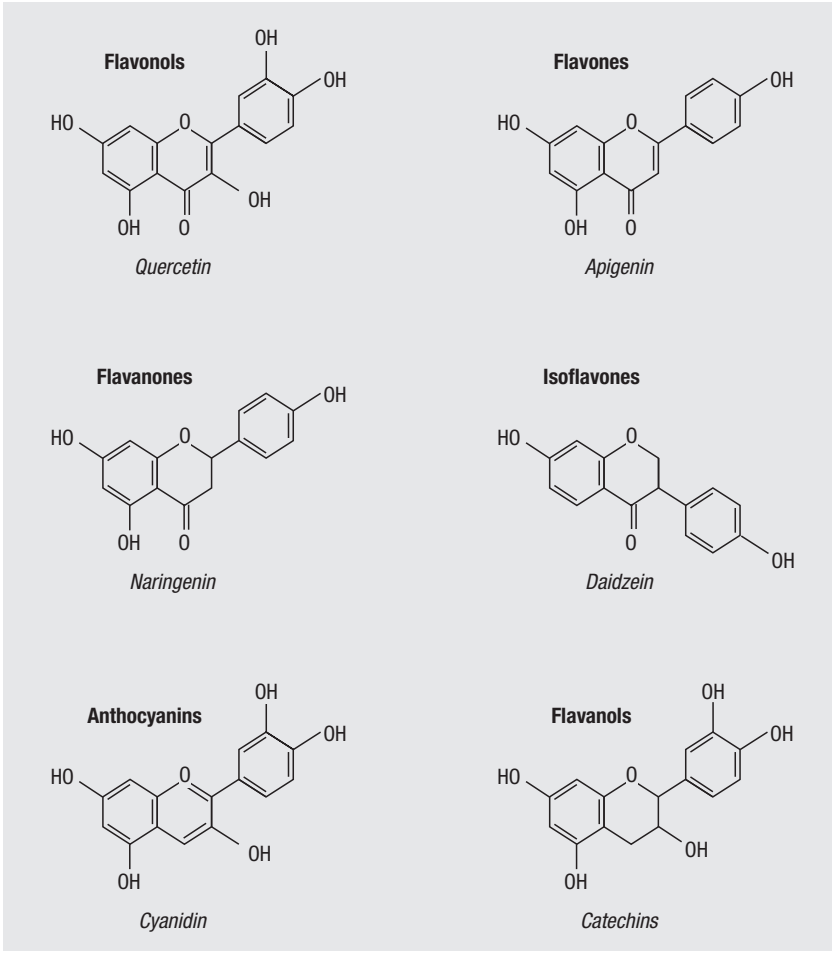

Fig. 2 | *Chemical structures of flavonoids.*

Flavonoids

Flavonoids share a common carbon skeleton of diphenyl propanes, two benzene rings (ring A and B) joined by a linear three-carbon chain. The central three-carbon chain may form a closed pyran ring (ring C) with one of the benzene rings.

Flavonoids are themselves divided into 6 subclasses, depending on the oxidation state of the central pyran ring: flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols (catechins and proanthocyanidins) *(Figure 2)*. More than 4000 flavonoids have been identified in plants, and the list is constantly growing [25]. This is because of the occurrence of numerous substitution patterns in which primary substituents (as hydroxyl group) can themselves be substituted (*i.e.*, additionally glycosylated or acylated), sometimes yielding highly complex structures.

Flavonols have a double bond between C_2 and C_3, with a hydroxyl group in the C_3-position *(Figure 2)*. They represent the most ubiquitous flavonoids in foods, with quercetin as the more representative compound. The main sources of flavonols are onions (up to 1.2 g/kg fresh wt), curly kale, leeks, broccoli, and blueberries *(Table 1)*. Tea and red wine also contain up to 45 mg and 30 mg flavonols/L respectively. It is important to note that flavonols biosynthesis is stimulated by light, so they accumulate in the outer and aerial tissue of fruits. Interestingly, differences in concentration can exist among fruits on the same tree and even between different sides of a single piece of fruit, depending on exposure to sunlight [26].

Flavones have a double bond between C_2 and C_3, and are the less common flavonoids. Parsley and celery represent the only important edible sources of flavones. The skin of fruits contains large quantities of polymethoxylathed flavones: for example in the skin of mandarin their content is up to 6.5 g/L of essential oil of mandarin.

Flavanones are characterized by the presence of a saturated three-carbon chain and an oxygen atom in the C_4. They are generally glycosylated by a disaccharide in C_7. Flavanones are present in high concentrations only in citrus fruit, but they are also found in tomatoes and certain aromatic plants such as mint. The main aglycones are naringenin in grapefruit, hesperetin in oranges, and eriodictyol in lemons. Orange juices contain 470-761 mg/L of hesperidin and 20-86 mg/L of narirutin [27]. The solid parts of citrus fruit, in particular the white spongy portion (albedo) and the membranes separating the

Table 1 | *Bioavailability of polyphenols or polyphenol-containing foods*

Polyphenols	Source	Quantity of polyphenol ingested (mg)	Maximum concentration in plasma (⊠M)	Urinary excretion (% of intake)	Ref.
Anthocyanins					
Cyanidine 3-glucoside	Orange juice (1 L)	71 mg Cy-3-glc	0.002		[43]
Malvidin 3-glucoside	Red wine (500 mL)	68 mg Mal-3-glc	0.001	0.016 6h	[132]
Malvidin 3-glucoside	Red grape juice (500 mL)	117 mg Mal-3-glc	0.003	0.019 6h	[132]
Cyanidine 3-glucoside	Red fruit extract (1.6 g)	2.7 mg Cy-3-glc/kg bw	0.03		[133]
Flavanols					
Epigallocatechin gallate	Green tea infusion (5 g)	105 mg	0.13-0.31		[130]
Catechin	Red wine (120 mL)	34 mg	0.072		[134]
Epicatechin	Chocolate (80 g)	137 mg	0.26		[135]
Catechin	Pure compound	0.36 mg/kg bw	0.14-0.49	1.2-3	[136]
Epigallocatechin gallate	Pure compound	50-1600 mg	0.28-7.4		[137]
Epigallocatechin gallate	Green tea extract	110-328 mg	0.26-0.7		[138]
Catechins	Black tea	140 mg	0.34		[139]
Procyanidin B1	Grapeseed extract	18 mg	0.011		[140]
Flavanones					
Hesperidin	Orange juice	61 mg	0.48	4.1	[141]
Hesperetin	Orange juice	110-220 mg	0.46-1.28	4.1-6.4	[123]
Naringenin	Orange juice	22.6-45 mg	0.06-0.2	7.1-7.8	[123]
Naringenin	Grapefruit juice	199 mg	5.99	30.2	[142]
Naringenin	Pure compound	135 mg	7.4	5.8	[143]
Hesperetin	Pure compound	135 mg	2.7	3.3	[143]
Flavonols					
Quercetin	Apples	107 mg	0.3	3.5	[131]
Quercetin	Onions	100 mg	7.6	6.4	[83]
Quercetin 4'-glucoside	Pure compound	100 mg	7.0	4.5	[83]
Quercetin	Buckwheat tea	200 mg	2.1	1.0	[83]
Quercetin	Pure rutin	200 mg	1.1	0.9	[83]
Isoflavones					
Daidzein	Soy milk	108 mg	0.47	37.3	[144]
Genistein	Soy milk	102 mg	0.41	20.2	[144]
Glycitein	Soy milk	114 mg	0.09		[144]
Daidzein	Pure compound	50 mg	0.76		[121]
Genistein	Pure compound	50 mg	1.26		[121]
Glycitein	Pure compound	25 mg	0.72		[121]
Daidzein	Soy extract	0.28-0.84 mg/kg bw	1.7-9.0	26-42	[145]
Genistein	Soy extract	2-16 mg/kg bw	3.4-25.4	9.5-14	[145]
Daidzein	Soy nuts	6.6-26.4 mg	0.4-1.65	63-44	[146]
Genistein	Soy nuts	9.8-39.2 mg	0.59-2.21	25.2-15.8	[146]
Hydroxybenzoic acids					
Gallic acid	Pure compound	50 mg	1.8GA+2.3 MeGA		[147]
Gallic acid	Assam black tea	50 mg	1.8GA+2.3 MeGA	36.4	[147]
Gallic acid	Red wine (300 mL)	4 mg	1.8GA+2.3 MeGA	39.6	[148]
Hydroxycinnamic acids					
Chlorogenic acid	Coffee (200 mL)	96 mg	0.5 caffeic acid		[149]
Caffeic acid	Red wine (200 mL)	1.8 mg	0.06		[150]
Caffeic acid	Red wine	0.06 mg	0.08		[151]
Hydrocinnamic acids	Apple cider (1.1 L)	15 mg	0.43		[152]

GA: gallic acid; MeGA: methylgallic acid; bw: body weight.

segments, have a very high flavanone content; this is the reason way the whole fruit may contain up to 5 times as much as a glass of orange juice.

Isoflavones have structural similarities to estrogens, *i.e.* hydroxyl groups in the C_7 and C_4, positions, like estradiol molecule. They can bind to estrogen receptors and are classified thus as phytoestrogens. Isoflavones are contained almost exclusively in leguminous plants. Soya and its processed products represent the main source of isoflavones, and contain the three main molecules (genistein, daidzein and glycitein) that occur as aglycones or, more often, as glucose-conjugate forms. Soybeans contain between 140 and 1530 mg isoflavones/kg fresh wt, and soy milk may contain between 12 and 130 mg/L [28, 29].

Isoflavones are sensitive to heat and are often hydrolyzed to glycosides during industrial processing and storage, such as the production of soya milk [29].

Anthocyanins are water-soluble pigments, responsible for most of the red, blue, and purple colours of fruits, vegetables, flowers, and other plant tissues or products [30]. They occur primarily as glycosides of their respective aglycones form, called anthocyanidins, with the sugar moiety mainly attached at the 3-position on the C-ring or at the 5, 7-position on the A-ring. Glycosylation at the 3'-, 4'-, 5'-position on the B-ring, although very rare, has also been observed [31]. The sugar moieties may also be acylated by a range of aromatic or aliphathic acids; the most common acylating agents are cinnamic acids. Anthocyanins are widely distributed in the human diet: they are found in red wine, certain varieties of cereals, and certain vegetables (cabbage, beans, onions, radishes), but they are most abundant especially in fruit. Food contents are generally proportional to colour intensity and reach values up to 2-4 g/kg fresh wt in blackcurrants or blackberries; the contents increase as the fruit ripens. Anthocyanins are found mainly in the skin, except for some red fruits (cherries and strawberries) in which they also occur in the flesh. Wine contains up to 350 mg anthocyanins/L, and these anthocyanins are transformed into various complex structures as the wine ages [32, 33].

Flavanols contain a saturated three-carbon chain with a hydroxyl group in the C_3. They exist in both the monomer and the polymer form (catechins and proanthocyanidins respectively). Unlike other classes of flavonoids, flavanols are not glycosylated in foods. The main representative flavanols in fruit are catechin and epicatechin, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate are found especially in tea [34, 35].

Catechins are found in many fruits such as apricots (250 mg/kg fresh wt) and cherry (250 mg/kg fresh wt). Green tea (up to 800 mg/L), and chocolate (up to 600 mg/L), are by far the richest sources of catechins, which are also present in red wine (up to 300 mg/L).

Proanthocyanidins, also known as condensed tannins, are dimers, oligomers, and polymers of catechins. It is very hard to value the proanthocyanidin content of foods because proanthocyanidins have a wide range of structures and molecular weights: for example in cider apples, the degree of polymerization ranges from 4 to 11 [36]. The only available data concern dimers and trimers, which are as abundant as the catechins themselves [37]. Proanthocyanidins are responsible for the astringent character of fruit (grapes, apples, berries, etc.) and beverages (wine, cider, tea, beer etc) and for the bitterness of chocolate [38]. It is important to note that this astringency changes over the course of maturation and often disappears when the fruit reaches ripeness.

Phenolic acids

These compounds could be divided in two classes: derivatives of benzoic acid and derivatives of cinnamic acid *(Figure 1)*.

The hydroxybenzoic acids, such as gallic acid and protocatechuic acid, are found in very few plants eaten by humans; this is the reason why they are not currently considered to be of great nutritional interest. Their content of edible plants is generally very low, except for certain red fruits, *i.e.* blackberries which contain up to 270 mg/kg fresh wt [39]. Tea is an important source of gallic acid: tea leaves may contain up to 4.5 g/kg fresh wt of gallic acid [40]. Raspberry contain up to 100 mg/kg fresh weight of protocatechuic acid, while in olive oil its concentration is about 0.22 mg/kg [39, 41, 42]. However it should be considered that protocatechuic acid concentration *in vivo* could be higher than the simple quantity ingested, because this compound may represent the major human metabolite of anthocyanins, such as cyanidin-3-glucoside. In fact it has been recently retrieved in human serum and feces after ingestion of cyanidin-rich food [43].

The hydroxycinnamic acids consist chiefly of coumaric, caffeic and ferulic acid, that are rarely found in the free form. The bound forms are glycosylated derivatives or esters of quinic, shikimic or tartaric acid. Caffeic and quinic acid combine to form chlorogenic acid, which is found in many types of fruit and in high concentrations in coffee (a single cup may contain up to 350 mg of chlorogenic acid) [44]. Blueberries contain 2 g hydroxycinnamic acids/kg fresh wt [41].

Caffeic acid is the most abundant phenolic acid, representing between 75% and 100% of the total hydroxycinnamic acids contents in most fruits: kiwis contain up to 1 g caffeic acid/kg fresh wt. Hydroxycynnamic acids are present in all part of fruit, although the highest concentrations are seen in the outer part of ripe fruit. Concentration decrease during the course of ripening, but the total quantity increases as the fruit increases in size.

Ferulic acid is the most abundant phenolic acid found in cereal grains: its content of wheat grain is about 0.8-2 g/kg dry weight, which may represent up to 90% of total polyphenols [45, 46].

Phenolic alcohols

Tyrosol (4-hydroxyphenylethanol) and hydroxytyrosol (3,4-dihydroxyphenylethanol) are the main phenolic alcohols; they are contained mainly in extra virgin olive oil (40.2 and 3.8 mg/kg respectively) [42]. Tyrosol is also present in red and white wines and beer [47], while hydroxytyrosol is also found in red wine and is additionally produced *in vivo* after red wine ingestion [48]. The concentration of total phenols in extra virgin olive oil has a mean value for commercial olive oil of approximately 180 mg/kg [49]. The phenol concentration in olive oil depends on variety, climate, area of growth, latitude, and ripeness of the olive [50]. Despite the wide body of evidence linking the *in vitro* properties of olive oil phenolics with positive health outcomes [51, 52], there are limited data on the absorption and excretion of these compounds [53, 54]. In part, this could be due to the low concentrations of such constituents and, accordingly, the difficulty in detecting the presumptively low concentrations of these compounds in biological systems.

Stilbenes

Low quantities of stilbenes are present in the human diet, and the main representative is resveratrol *(Figure 1)*, that exists in both *cis* and *trans* isomeric forms, mostly in glycosylated forms. It is produced by plants in response to infection by pathogens [55] or to a variety of stress conditions [56]. It has been detected in more than 70 plant species, including grapes, berries and peanuts. The fresh skin of red grapes is particularly rich in resveratrol (50-100 g/kg net weight) [57] which contributes to a relatively high concentration of resveratrol in red wine and grape juice (up to 7 mg aglycones/L and 15 mg glycosides/L in red wine) [58, 59]. Extensive data provide evidence for anticarcinogenic effects of resveratrol [60-62].

Lignans

Lignans are produced by oxidative dimerization of two phenylpropane units *(Figure 1)*; they are mostly present in nature in the free form, while their glycoside derivatives are only a minor form. Linseed represents the main dietary source, containing up to 3.7 g/kg dry wt of secoisolariciresinol [63]. Intestinal microflora metabolizes lignans to enterodiol and enterolactone. The low quantities of lignans normally contained in human diet do not account for the concentrations of the metabolites enterodiol and enterolactone measured in plasma and urine. Thus, there are certainly other lignans of plant origin, precursors of enterodiol and enterolactone, that have not been identified yet [64]. The interest in lignans and their synthetic derivatives is growing because of potential applications in cancer chemotherapy and various other pharmacological effects [65].

POLYPHENOL CONTENT IN HUMAN DIET

As stated above, fruit, tea and red wine constitute the main sources of polyphenols. Some of them are specific to particular foods (flavanones in citrus fruit, isoflavones in soya, phloridzin in apples), whereas others, such as quercetin, are found in all plant products (fruit, vegetables, cereals, leguminous plants, tea, wine, etc.). Generally, foods contain complex mixtures of polyphenols. For instance, apples, that represent a rare example of food for which accurate data on its polyphenol composition are available, contain flavanol monomers or oligomers, chlorogenic acid and small quantities of other hydroxycinnamic acids, several quercetin glycosides, 2 glycosides of phloretin and anthocyanins. The polyphenol profiles of all varieties of apples are practically identical, but concentrations may significantly differ among different varieties (from 0.1 to 10 g total polyphenols/kg fresh wt [36, 66].

On the other hand, for many plant products, the polyphenol composition is much less known. Furthermore numerous factors, such as ripeness at the time of harvest, environmental factors, and storage, may affect the polyphenol content of plants.

Environmental factors, such as climatic (sun exposure, rainfall) or agronomic (different type of culture, fruit yield per tree, etc.) play a key role in determining the polyphenol content. In particular, the exposure to light has a considerable effect on most flavonoids. The degree of ripeness differently affects the concentrations and proportions of the various polyphenols: generally phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase. Storage may also affect the content of polyphenols that are easily oxidized, leading to the formation of more or less polymerized substances, which alter particularly the colour and the organoleptic characteristics of fruits. Cold storage, in contrast, did not affect the content of polyphenols [67, 68].

Polyphenol content of foods is also influenced by the methods of culinary preparation; simple peeling of fruits and vegetables can significantly reduce polyphenol content, because these substances are often present in high concentrations in the outer parts. Cooking, also, have a remarkable effect: for example onions and tomatoes lose about 75% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying. Potatoes contain up to 190 mg chlorogenic acid/kg mainly in the skin [69]; so an extensive loss occurs during cooking, and no remaining phenolic acids were found in French fries [44].

BIOAVAILABILITY OF POLYPHENOLS

Bioavailability can be defined in different ways. The commonly accepted definition of bioavailability is the proportion of the nutrient that is digested, absorbed and metabolised through normal pathways. Consequently, it is not only important to know how much of a nutrient is present in specific food or dietary supplement, but even more important is to know how much of that is bioavailable [70].

The metabolism of several polyphenols is now well understood. Generally, the aglycones can be absorbed

from the small intestine; however most polyphenols are present in food in form of esters, glycosides, or polymers that cannot be absorbed in the native form. Before the absorption, these compounds must be hydrolyzed by intestinal enzymes or by the colonic microflora. During the course of the absorption, polyphenols undergo extensive modification; in fact they are conjugated in the intestinal cells and later in the liver by methylation, sulfation and/or glucuronidation (see below). As a consequence, the forms reaching the blood and tissues are different from those present in food and it is very difficult to identify all the metabolites and to evaluate their biological activity [71-74].

The main goal of bioavailability studies is to determine which are the better absorbed polyphenols, which are the active metabolites and which polyphenols lead to the formation of the active metabolites. The chemical structure of polyphenols, more than the concentration, determines the rate and extent of absorption and the nature of the metabolites circulating in the plasma. The most common polyphenols in our diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues; consequently the biological properties of polyphenols greatly differs from one polyphenol to another.

Evidence, although indirect, of their absorption through the gut barrier is given by the increase in the antioxidant capacity of the plasma after the consumption of polyphenols-rich foods [75, 76]. More direct evidence on the bioavailability of phenolic compounds has been obtained by measuring their concentration in plasma and urine after the ingestion of either pure compounds or foodstuffs with known content of the compounds of interest [2, 53, 77].

In *Table 1* are shown the data from the most recent and relevant studies that investigated the extent of polyphenol absorption in humans, after the ingestion of a single dose of polyphenols provided as pure compound, plant extract or whole food/beverage. These studies show that the quantities of polyphenols found intact in urine vary from one phenolic compound to another. Inter-individual variations have also been observed [78], probably due to the different composition of the colonic microflora which can differently affect their metabolism.

The identification and the quantification of metabolites represent an important field of research; for example specific active metabolites, such as equol, enterolactone and enterodiol, are produced by the colonic microflora. Equol appears to have phytoestrogenic properties even greater than those of the original isoflavone [79]; enterolactone and enterodiol, produced from linseed, have agonistic or antagonistic effects on estrogens [80, 81]. Furthermore, it is to underline that a great inter-individual variability exists in producing these active metabolites, depending on the composition of the intestinal flora [79, 82].

The flavonol quercetin is one of the most extensively studied polyphenols. It serves as a good example because its metabolism in humans is well understood; the flavonol conjugates that have been identified in plasma and urine from persons fed quercetin-containing foods are not those found in food. For example, plasma samples from volunteers receiving quercetin orally (as an onion meal, buckwheat tea, or pure quercetin, quercetin-4'-glucoside, quercetin-3-glucoside, or quercetin-rutinoside supplements) contained conjugated forms of quercetin but not quercetin glucosides, quercetin rutinoside, or quercetin aglycone [71, 83-85].

Intestinal absorption

In foods, all flavonoids except flavanols exist in glycosylated forms. The fate of glycosides in the stomach is not clear yet. Most of the glycosides probably resist acid hydrolysis in the stomach and thus arrive intact in the intestine [86] where only aglycones and few glucosides can be absorbed. Experimental studies carried out in rats [87, 88] showed that the absorption at gastric level is possible for some flavonoids, such as quercetin, but not for their glycosides. Moreover it has been recently shown that, in rats and mice, anthocyanins are absorbed from the stomach [89-91].

However most of the polyphenols are present in food as esters, glycosides or polymers, that cannot be absorbed in the native form. Therefore these substances must be hydrolyzed by intestinal enzymes, such as β-glucosidases and lactase-phlorizin hydrolase, or by the colonic microflora, before they can be absorbed [92, 93].

Glycosylation influences absorption, but generally does not influence the nature of the circulating metabolites. Intact glycosides of quercetin, daidzein and genistein were not recovered in plasma or urine after their ingestion as pure compounds or from complex food [85, 94]. Anthocyanins represent an exception, in fact, intact glycosides are the most representative circulating forms. The explanation for this may lie in the instability of the aglycone forms or in specific mechanisms of absorption or metabolism for anthocyanins [95, 96]. However, recently Felgines *et al.* [97] identified glucuronides and sulfates of anthocyanins in human urine. Glycosylation of resveratrol is known to protect it from oxidative degradation, therefore glycosylated resveratrol is more stable and more soluble and readily absorbed in the human gastrointestinal tract [98].

On the other hand, quercetin glucosylation facilitates its absorption; in fact the efficiency of quercetin glucosides absorption is higher than that of the aglycone itself [99]. It was suggested that glucosides could be transported into enterocytes by the sodium-dependent glucose transporter SGLT1 [100], and then hydrolyzed by a cytosolic β-glucosidase [92]. However the effect of glucosylation on absorption is less clear for isoflavones than for quercetin [24].

Proanthocyanidins differ from most of other plant polyphenols because of their polymeric nature and high molecular weight. This particular feature should

limit their absorption through the gut barrier, and oligomers larger than trimers are unlikely to be absorbed in the small intestine in their native forms [101].

Hydroxycinnamic acids, when ingested in the free form, are rapidly absorbed by the small intestine and are conjugated (in particular glucuronidated) as the flavonoids are [44, 102]. However these compounds are naturally esterified in plant products, and this impairs their absorption because intestinal mucosa, liver and plasma do not possess esterases capable of hydrolyzing chlorogenic acid to release caffeic acid [103, 104], and hydrolysis can be performed only by the colonic microflora [103,105].

The polyphenols that are not absorbed in the small intestine reach the colon, where the microflora hydrolyze glycosides into aglycones and extensively metabolize the aglycones into various aromatic acids [106]. Aglycones are split by the opening of the heterocycle at different points, depending on their chemical structure, and thus produce different acids that are further metabolized to derivatives of benzoic acid.

Intestinal microflora affects the metabolism of isoflavones glucosides, since they are hydrolyzed to aglycones or transformed into active metabolites, such as equol from daidzein [107, 108].

Mechanisms of conjugation and plasma transport

Once absorbed, polyphenols are subjected to the conjugation: this process, that mainly includes *methylation*, *sulfation*, and *glucuronidation*, represents a metabolic detoxication process, common to many xenobiotics, that facilitates their biliary and urinary elimination by increasing their hydrophilicity.

Catechol-*O*-methyl transferase catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to polyphenols such as quercetin, luteolin, caffeic acid, catechins and cyanidin [96]. The methylation generally occurs in the C_3'-position of the polyphenol, but it could occur in the C_4'-position: in fact a notable amount of 4'-methylepigallocatechin was detected in human plasma after tea ingestion [109, 110]. Catechol-*O*-methyl transferase activity is highest in the liver and the kidneys, although it is present in a number of tissues [111, 112].

Sulfotransferases catalyze the transfer of a sulfate moiety from 3'-phosphoadenosine-5'-phosphosulfate to a hydroxyl group on various substrates, among which polyphenols. The sulfation occurs mainly in the liver, but the position of sulfation for polyphenols have not been clearly identified yet [111, 113].

UDP-glucuronosyltransferases are membrane-bound enzymes located in the endoplasmic reticulum in many tissues, which catalyze the transfer of a glucuronic acid from UDP-glucuronic acid to polyphenols as well as to steroids, bile acids and many dietary constituents. Glucuronidation occurs in the intestine and in the liver, and the highest rate of conjugation is observed in the C_3-position [114-116].

The relative importance of these three types of conjugation appears to vary according to the nature of the substrate and the dose ingested. The balance between sulfation and glucuronidation of polyphenols also seems to be affected by species and sex [117].

The conjugation mechanisms are highly efficient, and free aglycones are generally either absent, or present in low concentrations in plasma after consumption of nutritional doses; an exception are green tea catechins, whose aglycones can constitute a significant proportion of the total amount in plasma (up to 77% for epigallocatechin gallate) [118].

It is important to identify the circulating metabolites, including the nature and the positions of the conjugating groups on the polyphenol structure, because the positions can affect the biological properties of the conjugates [119]. However few data on the proportions of the various type of conjugates and the percentages of free forms in plasma are available [74, 109, 120-124].

Polyphenol metabolites circulate in the blood bound to proteins, in particular albumin represents the primary protein responsible for the binding. The affinity of polyphenols for albumin varies according to their chemical structure [125, 126]. The binding to albumin may have consequences for the rate of clearance of metabolites and for their delivery to cells and tissues. It is possible that the cellular uptake of metabolites is proportional to their unbound concentration. Finally, it is still unclear if the polyphenols have to be in the free form to exert their biological activity, or the albumin-bound polyphenols can exert some biological activity, as it has been recently demonstrated for quercetin [127].

Plasma concentrations

The concentrations of polyphenols reached after their consumption vary highly according to the nature of the polyphenol and the food source. In *Table 1* data of the most relevant bioavailability studies of various classes of polyphenols in humans are shown. Moreover the same table indicates the source of polyphenol, the quantity of polyphenol ingested, the maximum concentration in plasma and the urinary excretion when available. The plasma concentrations of intact flavonoids rarely exceed 1μM and the maintenance of a high concentration of the polyphenols in plasma requires repeated ingestion over time [128]; in fact the maximum concentrations are most often reached 1-2 h after ingestion [129, 130], except those polyphenols which require to be degraded prior the absorption [131].

Tissue uptake

Polyphenols are able to penetrate tissues, particularly those in which they are metabolized such as intestine and liver. Determination of the bioavailability of polyphenol metabolites in tissues may be much more important than is the knowledge of their plasma concentrations. However data are still very scarce, not only in humans but even in animals, since few studies reported data on polyphenol concentrations in human tissues. Two studies

measured phytoestrogens and tea polyphenols in human prostate tissue. The first study showed significantly lower prostatic concentrations of genistein in men with benign prostatic hyperplasia than in those with a normal prostate, whereas plasma genistein concentrations were higher in men with benign prostatic hyperplasia [153]. The second one showed that tea polyphenols are bioavailable in human prostate: at the end of the daily consumption of 1.42 L of green tea or black tea for 5 days, in prostate tissue samples epigallocatechin, epicatechin, epigallocatechin gallate, epicatechin gallate reached concentrations ranging from 21 to 107 pmol/g tissue [154].

To determine the systemic bioavailability of curcumin in colorectal tissue, twelve patients with confirmed colorectal cancer received oral curcumin at 0.45, 1.8 or 3.6 g *per die* for 7 days prior to surgery. The concentrations of curcumin in normal and malignant colorectal tissue of patients consuming 3.6 g daily of curcumin were 12.7 ± 5.7 and 7.7 ± 1.8 nmol/g tissue, respectively [155]. Another study showed that equol concentrations in women, who ingested isoflavones, were higher in breast tissue than in serum, whereas genistein and daidzein were more concentrated in serum than in breast tissue [156].

These few studies underline that plasma concentrations of polyphenols are not directly correlated with concentrations in target tissues. Moreover, the distribution between blood and tissues differs between the various polyphenols.

Excretion

Polyphenols and their derivatives are eliminated chiefly in urine and bile. Extensively conjugated metabolites are more likely to be eliminated in the bile, whereas small conjugates, such as monosulfates, are preferentially excreted in urine.

The total amount of metabolites excreted in urine is roughly correlated with maximum plasma concentrations. Urinary excretion percentage is quite high for flavanones from citrus fruit (4-30% of intake) [78, 123, 142, 157], and for isoflavones (16-66% for daidzein and 10-24% for genistein) [73, 158, 159], while for flavonols accounts for 0.3-1.4% of the ingested dose of quercetin and its glycosides [83, 100, 131]. Urinary recovery is 0.5-6% for some tea catechins [160], 2-10% for red wine catechin [161], and up to 30% for cocoa epicatechin [162], while, it ranges from 5.9% to 27% for caffeic and ferulic acids [104, 163].

These percentages may be very low for other polyphenols, such as anthocyanins (0.005-0.1% of intake) [96, 132, 164, 165]. However the low bioavailability of anthocyanins, could be only apparent since they exist in a number of different molecular structures and exists a number of potential metabolites that can be generated [166]. Furthermore, certain metabolites may still be unidentified as a result of analytic difficulties. It has been shown that all the metabolites of the strawberry anthocyanins were very unstable and extensively degraded when urine samples were frozen [97]. A useful approach could be the use of isotopically labelled compounds.

CONCLUSIONS

Fruit and beverages such as tea and red wine represent the main sources of polyphenols, but vegetables, leguminous plants, and cereals are also important sources. The healthy effects of polyphenols depend both on their intake and bioavailability. The concept of bioavailability integrates several variables, such as intestinal absorption, metabolism by the microflora, intestinal and hepatic metabolism, nature of circulating metabolites, binding to albumin, cellular uptake, accumulation in tissues, and biliary and urinary excretion. The main difficulty is to integrate all the informations and relating the variables to health effects at the organ level.

Since the evidence of therapeutic effects of dietary polyphenols continues to accumulate, it is becoming more and more important to understand the nature of absorption and metabolism *in vivo*. Moreover the identification and measurement of the physiologic polyphenol metabolites represent a key prerequisite for the understanding of the role of dietary polyphenols in human health.

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