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REVIEW ARTICLE

The Mechanisms Behind the Biological Activity of Flavonoids

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Abstract: Flavonoids are phenolic compounds widely distributed in the human diet. Their intake has been associated with a decreased risk of different diseases such as cancer, immune dysfunction or coronary heart disease. However, the knowledge about the mechanisms behind their *in vivo* activity is limited and still under discussion. For years, their bioactivity was associated with the direct antioxidant and radical scavenging properties of phenolic compounds, but nowadays this assumption is unlikely to explain their putative health effects, or at least to be the only explanation for them. New hypotheses about possible mechanisms have been postulated, including the influence of the interaction of polyphenols and gut microbiota and also the possibility that flavonoids or their metabolites could modify gene expression or act as potential modulators of intracellular signaling cascades. This paper reviews all these topics, from the classical view as antioxidants in the context of the Oxidative Stress theory to the most recent tendencies related with the modulation of redox signaling pathways, modification of gene expression or interactions with the intestinal microbiota. The use of *C. elegans* as a model organism for the study of the molecular mechanisms involved in biological activity of flavonoids is also discussed.

Keywords: Flavonoids, health implications, bioavailability, metabolites, gut microbiota, antioxidant, gene expression, *C. elegans*.

1. INTRODUCTION

Flavonoids constitute one of the largest groups of plant secondary metabolites. They are phenolic compounds, synthesized through the shikimate/phenylpropanoid and acetate/malonate pathways, which possess a C6-C3-C6 skeleton, where two aromatic rings (A and B in Fig. **1a**) are linked through a three-carbon chain that usually forms a closed pyran ring (ring C), configuring a phenylchromane arrangement. In a few cases, the C3 bridge does not conform a heterocyclic ring but occurs in an open form (chalcone form, Fig. **1b**).

The Flavonoids can be divided into various classes based on the oxidation level of the ring C, being flavones, flavonols, flavan-3-ols, anthocyanins, flavanones, dihydroflavonols and isoflavones the most important ones. The complete structures of more than 8000 naturally-occurring flavonoids have been described [1], although the number of reported compounds is continually increasing. They are widespread in higher plants and widely distributed in the human diet through cereals, pulses, fruits, vegetables and their derived products such as wine, tea or choco-

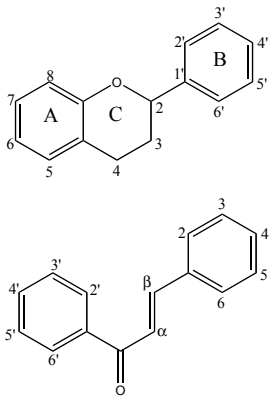

Fig. (1). Basic structure of flavonoids: phenylchromane (a) and chalcone (b) forms.

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late, in which they contribute to sensory, technological and health properties [2]. The intake of these phytochemicals largely varies depending on the country, season and dietary habits, especially related to the consumption of fruits and vegetables. A mean daily flavonoid intake of around one gram was firstly estimated by Kühnau in 1976 [3] although further estimations situate it below this amount, ranging between 140 mg per day in Brazilian population [4] to 350-450 mg/d in the US or different European countries [5-6].

Certain plants containing flavonoids have been used for thousands of years in traditional medicine, so the potential beneficial effects of these phytochemicals were known long before flavonoids were isolated as the effective compounds. In the earlier 1930's Japanese researches [7-8] described the therapeutic action of the flavones, but they received no general recognition until the Hungarian biochemist Albert Szent-Gyorgyi (Nobel Prize in Physiology and Medicine in 1937) and coworkers postulated in 1936 the role of two preparations from lemon peel, "citrin" and "eriodictin" in the treatment of abnormal capillary permeability and fragility [9]. The substances responsible for that activity were identified as flavonoids (flavones or flavonols) and tentatively classed as 'vitamin P' (for permeability). Nevertheless, their essential nature was never demonstrated, so that in 1950 the term vitamin P was finally dropped [10]. The interest for these compounds renewed in the 1990's with the observation of inverse epidemiological associations between the dietary intake of flavonoids and a reduced incidence and mortality from cardiovascular disease (CVD) [11-12]. Currently, numerous epidemiological, clinical and animal studies reveal that flavonoids may exert protective effects not only against cardiovascular disease but also on various disease conditions, including type II diabetes, cancer or neurodegenerative disorders such as Parkinson's [13]. Quite recently, several preclinical trials have pointed to the efficacy of some flavonoids to act as acetylcholinesterase inhibitors, suggesting the potential of these compounds for the symptomatic treatment of Alzheimer's disease [14].

Although the bioactivity of flavonoids has been widely substantiated, the knowledge about the mechanisms behind their *in vivo* activity is limited and still under discussion. The best described property of almost every group of flavonoids is their capacity to act as radical scavenging/antioxidant agents [15], however this activity has been usually established by *in vitro* methods, without considering the low bioavailability and extensive conjugation that occur when the flavonoids are ingested. In fact, the low *in vivo* levels of flavonoids that have been determined for most flavonoids and their metabolites suggest that other mechanisms of action different from a direct antioxidant activity should exist.

This review discusses about the potential working mechanism of flavonoids, from the classical view as antioxidants in the context of the Oxidative Stress theory to the most recent tendencies related with the modulation of redox signaling pathways, modification of gene expression or interactions with the intestinal microbiota.

2. FLAVONOIDS AS ANTIOXIDANTS

For years, most of the beneficial health effects of flavonoids were attributed to their antioxidant and chelating abilities. An antioxidant can be defined as "any substance that delays, prevents or removes oxidative damage to a target molecule" [16]. During normal metabolism, reactive oxygen and nitrogen species (RONS), such as hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), peroxyl ($ROO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$) radicals are constantly formed and believed to contribute to cellular aging. Since Harman proposed in 1950 [17] that organisms age because they accumulate oxidative damage which comes from reactive oxygen species, and Sies formulated in 1985 the "Oxidative Stress theory", according to which the origin of major degenerative diseases could be explained by an imbalance in the dynamic equilibrium between pro-oxidants and antioxidants [18], it was assumed that antioxidants, and among them flavonoids, might help counteract this oxidative damage, thus contributing to the prevention of the age-related diseases.

The antioxidant capacity of flavonoids has been associated to their ability to act as effective scavengers of most types of oxidizing species, such as RONS, through mechanisms that involve the transfer of an H atom (HAT) or of a single electron (SET) to the radical stabilizing it, as well as to their metal chelating activity, as largely shown in *in vitro* studies [19]. Furthermore, *in vivo*, they could also act by indirect mechanisms like the activation of natural antioxidant defenses or the inhibition of oxidases [20].

2.1. Direct scavenging of ROS

Flavonoids are able to scavenge free radicals directly by hydrogen atom donation, a capacity that depends upon the total number of hydroxyl groups, the

substitution pattern and the arrangement of functional groups about the nuclear structure [21]. The presence of a catechol group in the B-ring is the most significant determinant for scavenging of RONS, owing to its ability to donate hydrogen, giving rise to a relatively stable flavonoid-derived radical (aroxyl radical) [22]. Further structural criteria for optimal scavenging activity are the presence of a 2,3-double bond conjugated with a 4-oxo function in the C-ring and a 3- (and 5-) hydroxy group (Fig. **2**), as they provide extensive electron delocalisation over the three-ring system and confer higher stability to the produced radical [15, 23]. According to these criteria, quercetin and myricetin-derived flavonols have been indicated as the most effective radical scavengers in aqueous phase [24]. Some authors, however, have questioned the stability of the formed flavonoid aroxyl radicals, and have described their conversion into more reactive secondary radicals, such as quinones or semiquinones, that may give rise to prooxidant or potentially cytotoxic effects [25]. For instance, it has been shown that quercetin forms an o-semiquinone radical that can be disproportionated to produce o-quinones, as well as to react with O_2 to form superoxide [26].

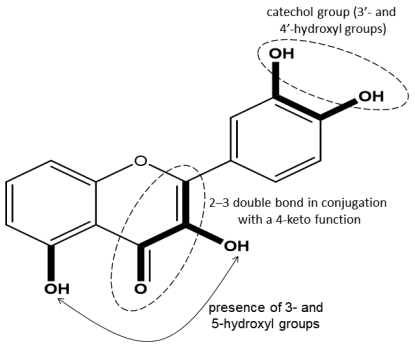

Fig. (2). Structural requirements associated with antioxidant activity of flavonoids as chain breaking antioxidants.

Polymerization of flavonoids, as it occurs in condensed tannins, further increases the antioxidant capacity due to the higher number of hydroxyl groups, whereas substitution of the hydroxyls (e.g. glycosylation or methylation) generally decreases the antioxidant potential compared to the parent molecules [15, 27].

2.2. Metal Chelating Activity

The ability of flavonoids to prevent the toxicity of redox active metal ions, such as iron, cobalt, manganese or copper has been less considered than their scavenging capacity. These cations are believed to catalyze the production of oxidant species leading to oxidation at different cellular levels (lipids, DNA or proteins). Fe(II) catalyzes, in the presence of hydrogen peroxide, the formation of hydroxyl radical $OH^{\bullet}$ by the Fenton reaction, whereas the reaction of Cu(II) with H_2O_2 leads to the formation of both $OH^{\bullet}$ and superoxide ($O_2^{\bullet-}$) radicals [28]. Several flavonoids can form stable metal complexes through their multiple OH groups and the carbonyl moiety, whenever present, removing a causal factor for the development of free radicals [29]. Chelating complexes with divalent cations may be formed between the 3-OH or 5-OH and 4-oxo group, or between the 6- and 7-OH in A ring or 3'- and 4'-OH in B ring (Fig. **3**) [30-31]; this process does not necessarily render the flavonoid inactive, as the complex retains ROS scavenging activity [32]. Both quercetin and rutin (*i.e.,* quercetin 3-*O*-rutinoside) possess iron-chelating and iron-stabilizing properties due to their potential binding sites (α-hydroxy-carbonyl, β-hydroxy-carbonyl or ring-B catechol), suggesting little difference between aglycones and glycosides in the ability to complex metals [33]. The catechol moiety in the B ring has been shown to be the major contributory site for Cu^{2+}-chelate formation [34]. Several examples on the chelating activity of different flavonoids can be found in the papers by Cherrak *et al.* [28] and Leopoldini *et al.* [29].

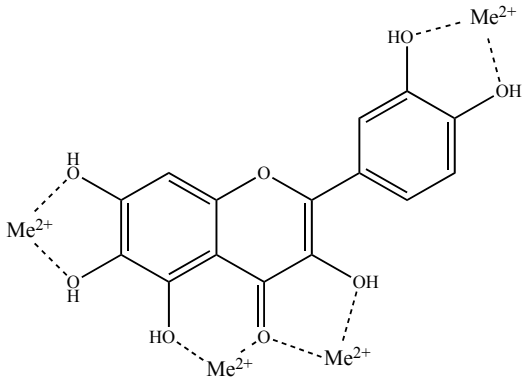

Fig. (3). Structural requirements associated with antioxidant activity of flavonoids as metal chelators.

2.3. Ability to Regulate Enzymes Activities

Other possible mechanisms by which flavonoids can act is through the activation of endogenous defenses or the inhibition of enzymes responsible for superoxide production, such as oxidases.

The potential of different flavonoids to activate endogenous antioxidant enzymes, such as glutathione peroxidase (GPx), catalase (CAT) or superoxide dismutase (SOD) has been described by different authors [35-37]. SOD converts superoxide to H_2O_2, and CAT or peroxidases (*e.g.,* glutathione peroxidase or peroxiredoxins) subsequently convert H_2O_2 to water; peroxi-

dase activity can be further restored through reduction by either thioredoxin (TRX) or glutaredoxin.

Flavonoids can also induce phase II detoxifying enzymes (e.g. NAD(P)H-quinone oxidoreductase, glutathione S-transferase, and UDP-glucuronosyl transferase), which are the major enzymatic line of defense against electrophilic toxicants and oxidative stress [20]. It is known that most phase II genes generally contain cis-acting regulatory elements called the antioxidant response element (ARE) or electrophile-responsive element (EpRE) [38]. The induction of these elements may have origin in the putative toxicity of polyphenols. As commented before, in cellular media, flavonoids and other polyphenols can form potentially toxic quinones that are, themselves, substrates of antioxidant enzymes. That way the flavonoids could be able to activate these enzymes for their own detoxication and, thus, induce a general boosting of body defenses against toxic xenobiotics [39-40]. Flavonoids with a higher intrinsic potential to generate oxidative stress and redox cycling would be the most potent inducers of EpRE-mediated gene expression. Flavonoids containing a free hydroxyl group at position 3 in the ring C, such as quercetin and myricetin, have been indicated to be among the most effective inducers [41]. In the case of tea polyphenols, the presence of a 3-gallate group appears to be an important structural factor for the induction of ARE reporter genes, being epigallocatechin gallate (EGCG) the most active one [38].

Flavonoids are not only able to activate enzymatic defences but they can also regulate the oxidative status of the cell by inhibiting oxidative enzymes responsible for superoxide production, such as xanthine oxidase and protein K [42]. Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal succinoxidase and NADPH oxidase. NADPH oxidase is a membrane-associated system catalyzing the production of $O_2^{\bullet -}$ in activated neutrophils. The inhibition of protein kinase C was suggested to be a mechanism of inhibition of NADPH by flavonoid molecules possessing a planar benzopyrone ring system with free hydroxyl substituents at the 3′, 4′ and 7-positions, such as quercetin, fisetin or luteolin [43].

The interference of flavonoids with nitric oxide-synthase (NOS) activity is another potential mechanism to decrease oxidative damage in the cell. NO, produced by the oxidation of L-arginine catalyzed by NO synthases, interacts with free radicals, especially $O_2^{\bullet -}$, producing peroxynitrites. Although it is not clearly understood yet how flavonoids inhibit induction of NOS and NO production, they would possess ability to directly scavenge molecules of both NO and peroxynitrite once produced. Furthermore, the scavenging ability of flavonoids may hinder the reaction of free radicals with NO, thus resulting in less damage [44]. The most significant determinant for their activity against peroxynitrite is again the presence of a 3',4'-catechol arrangement, followed by an unsubstituted 3-hydroxyl group [45].

2.4. Interaction of Flavonoids with Other Antioxidants

α-Tocopherol (αT) is one of the major lipid-soluble antioxidants in blood, tissues, cell membranes and human low-density lipoprotein (LDL) and it is thought to have an important function in the prevention of degenerative diseases. This compound can scavenge reactive species, originating the corresponding α-T˙ radical. Several studies support the notion of a regeneration of α-T from its α-tocopheroxyl radical at water-lipid interfaces by dietary flavonoids [46]. In particular, the potential of both flavonols and catechins to delay the oxidation of LDL has been pointed out [47].

The recycling of the aroxyl radicals has also been proposed to explain the interactions between flavonoids and other antioxidants, such as ascorbate. Flavonoids that possess a catechol group in the B-ring and a double bond at position 2-3 in the C-ring, such as quercetin, would have a higher redox potential (E_7 = 0,33 V) than ascorbate (E_7 = 0,28 V), thus being capable of oxidizing it to the ascorbyl radical [31], which may be further enzymatically reduced or disproportionate to the non-radical form [48].

Despite the abundant literature about the antioxidant capacity of flavonoids, it is necessary to consider that the compounds of this family are, in general, little bioavailable and largely biotransformed in the organism, so that their levels as such in body fluids, tissues and cells are usually very low and well below those of other physiological antioxidants, like urate, α-tocopherol, ascorbate or glutathione [49].

Interestingly, significant increases in the serum levels of uric acid, the major contributor to plasma total antioxidant capacity, after consumption of flavonoid-rich foods, such as strawberries, spinach or red wine, have been reported [50], although the mechanisms why this effect is produced are not clear. What seems clear is that the notion of these compounds acting as 'systemic' antioxidants and as conventional hydrogen donators is unlikely to be the sole explanation for their putative health effects [1].

Nowadays, other hypotheses, such as the possibility that flavonoids could act as potential modulators of intracellular signaling cascades vital to cellular function independent of their classical antioxidant capacity, are considered more accurate to explain the *in vivo* activity of flavonoids [51].

3. BIOAVAILABILITY OF FLAVONOIDS

Bioavailability can be defined as "the rate and extent to which an active ingredient or active moiety is absorbed from a matrix and becomes available at the site of action". Based on this, the rate of absorption and the availability at the site of action look like the most importance parameters for a flavonoid to be effective within biological systems. The bioavailability of flavonoids is generally low and can vary dramatically among different flavonoid classes, as well as for individual compounds in a particular class [52]. It has been estimated that less than 5-10% of the consumed flavonoids may be absorbed in the small intestine to be further conjugated in the intestinal wall, the liver and peripheral tissues, so that they will be found in plasma in the form of conjugated metabolites (glucuronides, sulfates and methylated derivatives) [53]. Thus, the compounds able to reach the metabolical targets are chemically and, in many instances, functionally distinct from the consumed forms, and such features underlie their bioactivity [54].

In general, the absorption of flavonoids in the digestive tract starts in the ileum (Fig. **4**). Except for flavan-3-ols (*i.e.,* catechins and proanthocyanidins), these compounds mostly exist in plants and foods as glycosides, which cannot be generally absorbed in their native form. Some glycosides, however, can be hydrolyzed by the enzyme lactase phloridzin hydrolase (LPH) in the intestinal epithelium to release the aglycone, which can enter the enterocyte by passive diffusion because of its increased lipophilicity [55]. There is still controversy about the possibility of an active transport of some particular glycosides into epithelial cells by the active sodium-dependent glucose transporter SGLT1 and the subsequent hydrolysis in the cell by a cytosolic β-glucosidase (CBG) [55-56]. It has also been described that hydrolysis of some flavonoid glycosides might already occur in the oral cavity, as both saliva and oral microbiota show ß-glucosidase activity [57]. Compounds with large molecular weight, like polymeric proanthocyanidins, would not be absorbed, while oligomeric proanthocyanidins (dimers, trimers) could be absorbed in the small intestine without previous degradation to lower molecular weight compounds [58]. *In vitro* digestion assays have also suggested that some flavonoids (*e.g.,* procyanidin B1 or anthocyanins) might be also absorbed in a small percentage under the acidic conditions of the stomach [59].

Once absorbed, the flavonoids interact with phase II enzymes, catechol-o-methyltransferase (COMT), UDP-glucuronosyltransferase (UGT) and sulfotransferase, already in the enterocytes and further in the liver and peripheral tissues, to produce conjugated metabolites that will be found circulating in plasma. Except for par-

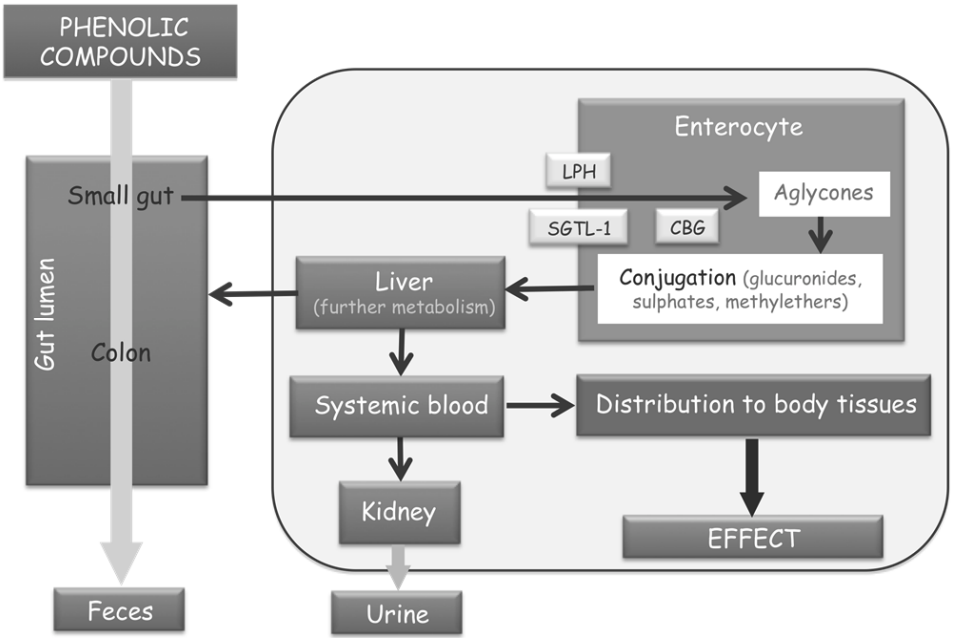

Fig. (4). Scheme of flavonoid metabolism.

ticular flavonoids, like some catechins or certain isoflavones, aglycones are not found as such in the blood [60-61]. It has been suggested that whilst methylation and glucuronidation are produced in both intestinal cells and liver, sulfation would mainly take place in the liver [62]. This metabolic conversion is known to be a major factor affecting flavonoid bioavailability. After conversions in the liver, enterohepatic transport in the bile would also occur and some metabolites are recycled back to the small intestine. Additionally, the conjugated metabolites, especially glucuronide derivatives, can be pumped out into the gut lumen to participate in enteric, and local recycling by efflux transporters such as multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP), decreasing in this way even more the bioavailability of polyphenols and limiting their exposure to target organs [63]. A simplified scheme of human metabolism of flavonoids is presented in Fig. (**4**).

Due to this extensive metabolic process, the final concentration of flavonoid metabolites that can be found in human plasma are in the nanomolar to low micromolar range, and at least ten times lesser amount of aglycones [53]. As above indicated, even at the highest levels reported, the plasmatic concentrations of flavonoids and their conjugated metabolites are far below those of other physiological antioxidants [49], therefore a direct antioxidant effect might be only expected in tissues directly exposed to polyphenols, like the gastrointestinal tract. On the other hand, the process of conjugation involves a decrease in the antioxidant potential of the flavonoids, due to the substitution of the active hydroxyl groups [64].

Some authors have, however, suggested that flavonoids may overcome the challenges posed by their poor bioavailability and decreased activity of their conjugated metabolites, through their accumulation in some body compartments, in view of their capacity to establish H-bonds and hydrophobic interactions with biomembranes [48]. Furthermore, in some tissues [65] or under some physiological situations, such as inflammation [66], deconjugation of the glucuronidated and sulfated forms of flavonoids can occur, so that aglycones and/or their methylated derivatives may be released in some target sites, contributing to the *in vivo* effects of dietary flavonoids [67-69].

Whatever the effects of the compounds absorbed in the small intestine, the large majority of the flavonoids from the oral intake go to the large intestine, where they interact with the gut microflora. Currently these interactions are a major item of interest to explain a relevant part of the potential healthy effects that flavonoids and polyphenols in general may have in the human organism.

4. INTERACTIONS WITH GUT MICROBIOTA

Most of the consumed flavonoids, those that are not absorbed in the small intestine or are recycled after metabolism, are reaching the large intestine where they interact with and may be metabolized by the colonic microflora. Bacterial enzymes may catalyze a wide variety of reactions that occur under anaerobic conditions and are based mainly on processes of reduction and/or hydrolysis, such as dehydroxylation, demethylation, decarboxylation, reduction of double bonds, hydrolysis of glucuronides, sulfates and glycosides, or ring cleavage [20]. In most flavonoid groups, this latter would take place in the heterocyclic C-ring, but cleavage of the aromatic ring A may also occur in flavan-3-ols. The enzymatic cleavage of C-C bonds in flavonoids appears to be characteristic of gut microbiota [70].

It is well stablished that some gut bacteria, especially some *Clostridium* species, can catabolize flavonols. Deglycosylation could take place as a first step of the metabolism, as observed in *in vitro* digestion assays of quercetin glycosides in the presence of rat microflora [71]. After deglycosylation, a breakdown of the C-ring may occur (breaking the bond between the 1- and 2- or 2- and 3-positions) followed by dehydroxylation, leading to the formation of simpler compounds, such as phenolic acids and aldehydes [71]. It has been shown that human intestinal bacteria, such as *Eubacterium ramulus* and *Flavonifractor plautii* (formerly *Clostridium orbiscindens*), are also able to produce the reduction of the double bond in the 2,3- position of the C-ring in quercetin, resulting in the formation of the dihydroflavonol taxifolin, which is further isomerized to the auronol alphitonin by a unique C-ring contraction, and suffer C-ring oxidative cleavage to produce 3,4-dihydroxyphenylacetic acid and phloroglucinol, the latter undergoing further degradation to butyrate and acetate [72]. 3,4-Dihydroxyphenylacetic acid would be degraded into 3,4-dihydroxybenzoic acid (protocatechuic acid, usually the main metabolite in the fermentation media) and subsequently into hydroxybenzoic acid [71]. Other products from the colonic degradation of flavonols that have been described are 3-(3,4-dihydroxyphenyl) propionic acid and 3-(3-hydroxyphenyl) propionic acid resulting from the A ring [73]. The formation of homovanillic acid, found in urine and other body fluids, has been explained by *O*-methylation of 3,4-dihydroxyphenylacetic acid in the

liver after microbial metabolism of quercetin [74]. Indeed, methylation and other conjugation reactions would further take place in the liver after microbial metabolism, as such derivatives have not been observed in the *in vitro* faecal or caecal incubations [75].

Bacterial metabolism of anthocyanins also involves the cleavage of glycosidic linkages and breakdown of the anthocyanidin heterocycle to release B-ring- and A-ring-derived products. Protocatechuic acid has been described as a major metabolite corresponding to the B-ring fragment in the case of cyanidin-derived anthocyanins, while the A-ring generates 2,4,6-trihydroxybenzaldehyde that could further be converted to phloroglucinol [76-77]. A wide range of metabolites was detected in serum and urine by Czank *et al.* (2013) [78] after cyanidin-3-glucoside intake by human volunteers, including phenolic, hippuric, phenylacetic, and phenylpropenoic acids, some of them conjugated with glucuronic acid and sulfate.

Catechins and oligomeric proanthocyanidins undergo the opening of the C-ring followed by different reactions, like lactonization, decarboxylation, dehydroxylation or oxidation. Phenyl-γ-valerolactones and phenylvaleric acids have been described as exclusive microbial metabolites of flavan-3-ols. Phenylvaleric acids would be subsequently biotransformed by successive loss of carbon atoms to give rise to different phenylacetic, phenylpropionic and hydroxybenzoic acids, and in minor extension to hippuric, vanillic and homovanillic acids by further conjugation [79-80].

Other flavonoids families, such as flavanones, are found to be less degraded by the colonic microbiota. Similar to other flavonoids, flavanone glycosides suffer first a deglycosylation to render the aglycone, which through C-ring cleavage gives rise to 3-(3,4-dihydroxyphenyl)propionic acid and sometimes phloroglucinol [81].

Multiple examples of the catabolism of flavonoids and schematic representations of the different processes can be found in the reviews by Del Rio (2013) [13] Stevens (2016) [70] and Selma (2009) [72].

It is important to remark that there is a great interindividual variation both in the amount and the profile of phenolic metabolites produced after flavonoids intake [82]. These differences could be attributed, at least in part, to the existence in the human gut microbiome of the so-called "enterotypes", which are defined as a network of co-abundant microbial populations dominated by the prominent presence of one of these three genera: *Ruminococcus*, *Bacteroides* and *Prevotella* [83], but also due to differences in minority microbial groups related to phenolic metabolism phenotypes ("metabotypes"). The term gut metabotype refers to a metabolic phenotype with specific gut microbiome-derived metabolites that characterize the metabolism of the parent compound. In this regard, a gut metabotype is defined not only by the resulting metabolites but also by the associated gut microbiota both in terms of composition and activity [84].

After the catabolism process, many of the produced catabolites could be efficiently absorbed in the colon, appear in the blood and be transformed by human cell enzymes into phase II conjugates, including methylethers, glucuronides and sulfates, to be ultimately excreted in the urine. Since microbial catabolites and their conjugate derivatives may be present at many sites of the body in higher concentration than the parent compounds, it has been proposed that at least a part of the biological activities ascribed to flavonoids could be due to their colonic catabolites [78, 80].

On the other hand, it is accepted that phenolic compounds and their metabolites may impact on gut microbiota inducing prebiotic-like effects on bacteria [85]. It has been postulated that dietary polyphenols could exert, at less in part, their health effects through changes in the composition of the gut microbiota. Some *in vitro* and *in vivo* studies (humans, pigs, sheep) have suggested that flavonoids or their catabolites might, for example, increase the proportion of bifidobacteria and eubacteria without inhibiting lactic acid bacteria, suppress bacteroides and pathogenic *Clostridium perfringens* and *Clostridium difficile*, or lower the colonic pH value [86-87].

5. *CAENORHABDITIS ELEGANS* AS A MODEL TO STUDY FLAVONOIDS BIOACTIVITY

Nowadays, there is an accepted view that if flavonoids have any preventive or curative activity through their ingestion, it must involve not only their antioxidant potential, but also the modulation of different signaling pathways, such as phosphoinositide 3-kinase, Akt/protein kinase B, mitogen-activated protein kinase, tyrosine kinases, and protein kinase C, that are crucial in the pathogenesis of several diseases [51, 88]. In addition, flavonoids could also modulate various genes expression through activation of diverse transcription factors. Due to their flexibility, model organisms like zebrafish, *Drosophila melanogaster* or the nematode *Caenorhabditis elegans* are increasingly used to perform studies related with genes or cellular pathways.

Caenorhabditis elegans is a well characterized *in vivo* model organism whose genome has been fully sequenced and many of its gene functions characterized by generating mutant strains. It has been widely used because of its easy culture and handling under laboratory conditions, being maintained from 12 to 25 °C on agar with *Escherichia coli* as feed. Besides, approximately 60-80% of *C. elegans* genes have human orthologues, and the presence of tissue and organ systems raises the possibility to consider the metabolism of compounds. Its relatively short lifespan (15-22 days at 25 °C) makes it useful in longevity studies [89-91]. Furthermore, facilities such as information about genes function are available in the wormbase page (http://www.wormbase.org).

This nematode has been used for studying the influence of different flavonoids on the process of ageing (lifespan and oxidative stress).

Some authors have found that catechins are able to extend life duration in wild-type N2 *C. elegans* [92-94], while others did not observe lifespan extension for these flavonoids, although they found it for their methylated metabolites [95]. On the other hand, feeding of the worms with apple polyphenols mainly containing procyanidins, consisting of (epi)catechin units, was reported to increase mean lifespan in wild-type N2 strain and *fem-1* mutants [96]

Significant increases in worm lifespan were also observed for flavonols, like quercetin [97]. In these compounds, the hydroxylation pattern of ring B seems to play a crucial role, the greater the number of hydroxyl groups, the greater the effect: myricetin > quercetin > kaempferol (reviewed by [98]). Lifespan effects are also influenced by aglycone glycosylation and methylation. Greater increase in mean and maximum life duration was produced for isorhamnetin 3-*O*-methylquercetin) than for quercetin in worms fed at the same compound doses (200 μM); however, similar life extension was found for tamarixetin (4-*O*-methylquercetin) and quercetin [97]. The treatment with quercetin-3-*O*-glucoside increased *C. elegans* life at low concentrations (10-25 μM), whereas exposure to greater doses (50-200 μM), at which quercetin showed life extending effects, caused lifespan reduction, suggesting a hormetic response [99]. The type of sugar residue also seems to be important for the biological effects of flavonols; thus, homo-disaccharides (glucose-glucose) were indicated to increase longevity in wild type worms, while hetero-disaccharides (glucose-rhamnose) could no prolong lifespan [100]. This differential behavior could be explained by differences in compound bioavailability, with some glycosides being more efficiently taken up by the nematode than quercetin, to be further deglycosylated, so that higher amounts of the aglycone were accumulated in the worm organism, than following exposure to quercetin itself [99]. These findings suggested that deglycosylation was a key step in the lifespan effects of flavonols and that the aglycone would be the actual responsible for the observed biological activity, as also observed in other *ex vivo* and animal models [101].

Numerous studies have also been conducted in *C. elegans* in order to evaluate the influence of flavonoids on the oxidative stress and the putative mechanisms behind such effects. Flavonoids have the potential to modulate intracellular oxidative stress directly by scavenging free radicals, reducing reactive oxygen species (ROS) basal level and modulating the activity of antioxidant enzymes such as SOD-3, CAT and glutathione-S-transferase (GST) [102-103]. These protective effects were also observed in *C. elegans* submitted to either thermal or oxidative stress and treated with flavonols such as quercetin, myricetin or kaempferol [97, 102], or catechins [95, 104-105], demonstrating the capacity of flavonoids to improve the survival rates of worms under stress conditions.

Studies using different mutant strains have shown that the mechanisms underlying the lifespan and antioxidant effects might differ depending on the flavonoid [106]. Using mutant nematode strains lacking the function of key genes involved in the insulin/IGF-1 signaling pathway, has been a way to study the potential of flavonoids to mediate stress tolerance and extend lifespan [98]. The insulin/insulin like growth factor (IGF-1) signaling pathway is an evolutionary conserved mechanism that influences longevity and metabolism throughout different species. The *daf-2* gene encodes for a receptor tyrosine kinase and is the only member of the insulin receptor family in *C. elegans*. The initiation of the cascade depends on the phosphorylation of DAF-2 (Fig. **5**). The inhibition of the DAF-2 pathway avoids phosphorylation and leads to nuclear localization and activation of the transcription factors (DAF-16/FoxO, HSF-1, SKN-1/Nrf2), which changes the expression of various genes responsible for endogenous stress-response, development, metabolism and longevity [91].

Interestingly, none of the flavonoids or extract tested prolonged further the lifespan in the long-live mutant strain *daf-2* [92, 107]. The results obtained in relation to other genes of the same pathway differ depending on the flavonoid. Quercetin did not prolong

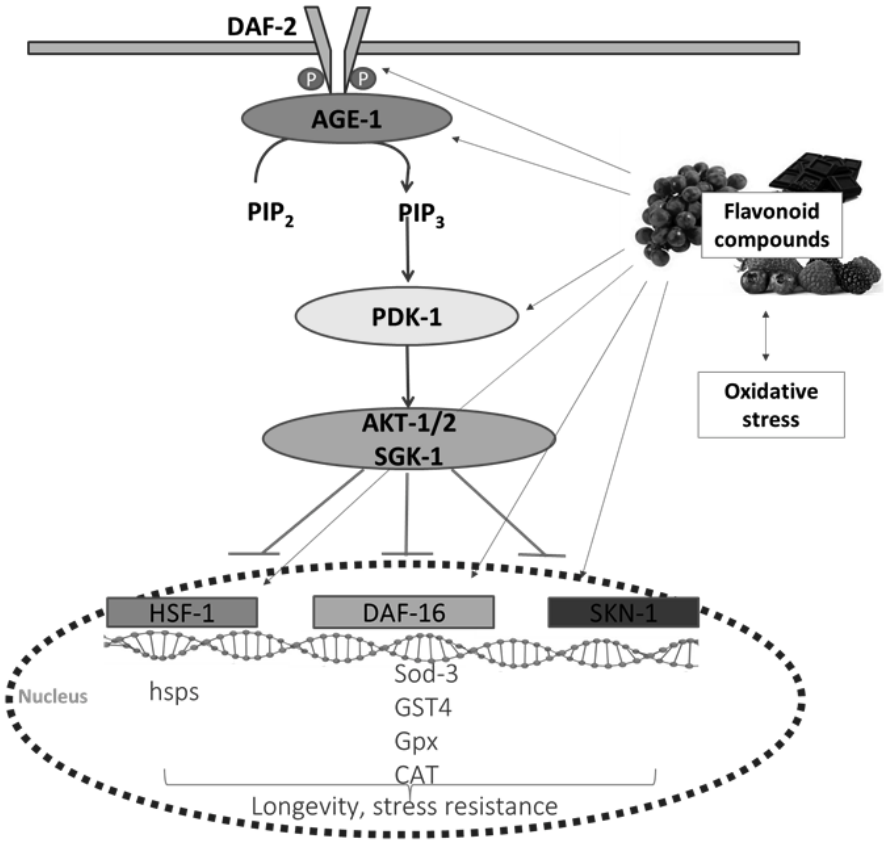

Fig. (5). Abbreviated scheme of the insulin/IGF-1 signaling pathway.

the lifespan of the mutant strain *age-1* (the orthologue of the phosphoinositide 3-kinase (PI3K) in mammals and a central component insulin-like signaling pathway in *C. elegans*) [107], while catechin increased lifespan in the same mutant [92]. By contrast, catechin, but not quercetin, failed to increase lifespan in the *akt-2* mutant [92, 107]; *akt-2* gene encodes for a homologue of the serine/threonine kinase Akt/PKB, AKT-2, and its inactivation causes animals to arrest constitutively at the dauer stage, while having an increased lifespan.

DAF-16 is the *C. elegans* transcription factor homologue to the forkhead box O (FoxO) in the insulin/IGF-1-mediated signaling (IIS) pathway. This factor was found to be essential for lifespan regulation in the action of kaempferol and myricetin, but not for quercetin or catechin [92, 107-108]. DAF-16 has also been related to the increased resistance of *C. elegans* against H_2O_2-induced stress provided by cocoa polyphenols [109], as well as in the decrease in ROS levels in nematodes exposed to myricetin [95]. However, the lowering effect of quercetin and kaempferol on mitochondrial ROS level was observed to be independent of DAF-16 [102].

The *mev-1* gene encodes for the *C. elegans* orthologue of the succinate dehydrogenase cytochrome b560 subunit, and *mev-1* mutations result in abnormal energy metabolism and increased sensitivity to oxidative stress and pathogen infection. It has been shown that epigallocatechin-3,*O*-gallate [94] and myricetin [102] were able to prolong lifespan in *mev-1* mutants, whereas catechin [92], quercetin, kaempferol and naringenin did not [102, 107].

Studies in different mutant strains revealed that *sek-1* and *unc*-43 genes, related to MAP kinase and calmodulin-dependent protein kinase pathways, could be key players in quercetin mediated longevity in *C. elegans*. [107, 110]. SEK-1 is required for the resistance to oxidative stress by assisting the translocation of cytoplasmic DAF-16 into the nucleus.

In *C. elegans,* members of the HSP-16 protein family are known to be involved in thermotolerance and are suggested as predictors for longevity [111]. Different flavonoids have been shown to be able to modulate HSP-16.2 expression induced either thermally or chemically by treatment with the stressor juglone [105, 112-113].

Several authors have also demonstrated that flavonoids possess the ability to increase the expression of *skn-1* and to induce the SKN-1 translocation into the nucleus [92, 113]. This transcription factor is the *C. elegans* homologue of the nuclear factor erythroid-2-related factor 2 (Nrf2) that regulates the gene expression of phase II detoxifying enzymes and antioxidant proteins, such as SOD, GST or NAD(P)H:quinone oxidoreductase (NQO-1). Under physiological conditions in mammals, Nrf2 is bound to Keap1, which retains Nrf2 in the cytoplasm. Oxidative stress induces dissociation of the Nrf2-Keap1 complex and subsequent translocation of Nrf2 into the nucleus, where it binds to antioxidant response elements (ARE) in the promoter region of genes encoding phase II enzymes and antioxidant proteins leading to a changed expression profile [114]. However, this mechanism seems not to be common for all the flavonoids, for instance, the gene expression of *skn-1* appears not to be involved in myricetin, catechin or procyanidins mediated longevity in *C. elegans* [92, 108].

CONCLUDING REMARKS

Notable advances have been made over the last two decades in the knowledge of flavonoids bioavailability and metabolism; however, little is still known about the actual and more active metabolites responsible for their biological effects and the molecular mechanisms finally involved in their activity. It is now accepted that the classical assumption of flavonoids as antioxidants is unlikely to explain their putative health effects, or at least to be the only explanation for them. The interaction with gut microbiota and the possibility that flavonoids or their metabolites could act as potential modulators of intracellular signaling cascades vital to cellular functions are hypotheses that are now gaining place. The studies available in these respects are, however, partial and inconclusive, and point to that not all flavonoids act through the same action mechanisms and on the same molecular targets, but different metabolites and genes may be involved in their biological effects. Further progresses should be expected in the coming years favored by the use of the novel omics approaches (especially transcriptomics and metabolomics) and model organisms like *C. elegans*, and the increased knowledge on human microbiome and its influence on flavonoids/polyphenols metabolism and activity.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Andersen, Ø.M.; Markham, K.R. *Flavonoids: Chemistry, Biochemistry and Applications*; CRC Press: Boca Raton, FL, 2006.

[2] Santos-Buelga, C; González-Paramás, A.M. Flavonoids: Functions, Metabolism and Biotechnology In: *Industrial Biotechnology of Vitamins, Biopigments, and Antioxidants*; Vandamme and Revuelta, Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2016; pp. 469-495.

[3] Kühnau, J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet*., 1976, 24, 117-191

[4] Correa, V.G.; Tureck, C.; Locateli, G.; Peralta, R.M.; Koehnlein, EA.A. Estimate of consumption of phenolic compounds by Brazilian population. *Rev. Nutr*., 2015, *28*, 185–196.

[5] Bai, W.; Wang, C.; Ren, C. Intakes of total and individual flavonoids by US adults. *Int. J. Food Sci. Nutr.*, 2014, *65*, 9–20.

[6] Wang, X.; Ouyang, Y.Y.; Liu, J.; Zhao, G. Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. *Br. J. Nutr.*, 2014, *111*, 1, 1–11.

[7] Akamatsu, K. Uber die Wirkung der Flavonole auf das Froschhers. *Ber. Physiol*, 1931, *62*, 443.

[8] Fukuda, T. Über die pharmakologische wirkung der flavonverbindungen. *Archiv F. Experiment. Pathol. u. Pharmakol*, 1932, *164*, 685-694.

[9] Benthsath, A.; Rusznyak, S.T.; Szent-György, A. Vitamin nature of flavones, *Nature*, 1936, *138*, 798.

[10] Anonymous. Use of the term Vitamin P. *Nature*, 1950, *166*, 543.

[11] Hertog, M.G.; Feskens, E.J.; Hollman, P.C.; Katan, M.B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet*, 1993, *342*, 1007–1011.

[12] Hertog, M.G.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B.S.; Toshima, H.; Feskens, E.J.M.; Hollman, P.C.H.; Katan, M.B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.*, 1995, *155*, 381–386.

[13] Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of

protective effects against chronic diseases. *Antioxid. Redox Signal.*, 2013, *8*, 1818–1892.

[14] Khan, H.; Marya, Amin, S.; Kamal, M.A.; Patel, S. Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. *Biomed. Pharmacother.*, 2018, *101*, 860-870.

[15] Heim, K.E.; Tagliaferro, A.R.; Bobilya, D.J. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*., 2002, *13*, 572–584.

[16] Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. third ed. Oxford University Press: Oxford, 1998.

[17] Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.*, 1956, 11, 298-300.

[18] Sies, H. Oxidative stress: introductory remarks In: *Oxidative Stress*; Sies H. Ed.; Academic Press: London, 1985; pp. 1–8.

[19] Treml, J.; Šmejkal, K. Flavonoids as potent scavengers of Hydroxyl radicals. *Compr. Rev. Food Sci. Food Saf.*, 2016, 720-738.

[20] Procházková, D.; Boušová, I.; Wilhelmová, N. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 2011, *82*, 513–523.

[21] Kumar, S.; Pandey, A.K. Chemistry and biological activities of flavonoids: an overview. *Sci. World J.*, 2013, Article ID 162750.

[22] Bors, W.; Michel, C.; Stettmaier, K. Antioxidant effects of flavonoids. BioFactors, 1997, 6, 399-402.

[23] Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical scavenging efficiencies. *Methods Enzymol.*, 1990, *186*, 343–355.

[24] Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med.*, 1996, *20*, 933–56.

[25] Halliwell, B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies? *Arch. Biochem. Biophys*., 2008, *476*, 107–112.

[26] Metodiewa, D.; Jaiswal, A.K.; Cenas, N.; Dickancaité, E.; Segura-Aguilar, J. Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product. *Free Radic. Biol. Med.*, 1999, *26*, 107–116.

[27] Dueñas, M.; González-Manzano, S.; González-Paramás, A.; Santos-Buelga, C. Antioxidant evaluation of O-methylated metabolites of catechin, epicatechin and quercetin. *J. Pharm. Biomed. Anal*., 2010, *51*, 443–449.

[28] Cherrak, S.A.; Mokhtari-Soulimane, N.; Berroukeche, F.; Bensenane, B.; Cherbonnel, A.; Merzouk, H.; Elhabiri, M. *In vitro* antioxidant versus metal ion chelating properties of flavonoids: A structure-activity investigation. *PLoS ONE*, 2016, 11(10): e0165575.

[29] Leopoldini, M.; Russo, N.; Toscano, M. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem*., 2011, *125*, 288–306.

[30] Cheng, I.F.; Breen, K. On the ability of four flavonoids, baicalein, luteolin, naringenin, and quercetin, to suppress the Fenton reaction of the iron-ATP complex. *Biometals*, 2000, *13*, 77–83.

[31] Pietta, PG. Flavonoids as antioxidants. *J. Nat. Prod*., 2000, *63*, 1035–42.

[32] Arora, A.; Nair, M.G.; Strasburg, G.M. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. *Free Radic. Biol. Med.*, 1998, *24*, 1355–1363.

[33] Soczynska-Kordala, M.; Bakowska, A.; Oszmianski, J.; Gabrielska, J. Metal ion-flavonoid associations in bilayer phospholipid membranes, *Cell Mol. Biol. Lett*., 2001, *6*, 277–281.

[34] Brown, J.E., Khodr, H.; Hider, R.C.; Rice-Evans, C. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochem. J*., 1998, *330*, 1173–1178.

[35] Nagata, H.; Takekoshi, S.; Takagi, T.; Honma, T.; Watanabe, K. Antioxidative action of flavonoids, quercetin and catechin, mediated by the activation of glutathione *peroxidase. Tokai J Exp Clin Med*, 1999, *24*, 1–11.

[36] Masella, R.; Di Benedetto, R.; Varì, R.; Filesi, C.; Giovannini, C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *J. Nutr. Biochem*., 2005, *16*, 577-86.

[37] González-Manzano, S.; González-Paramás, A.M.; Delgado, L.; Patianna, S.; Surco-Laos, F.; Dueñas, M.; Santos-Buelga, C. Oxidative status of stressed *Caenorhabditis elegans* treated with epicatechin. *J. Agric. Food Chem.*, 2012, *60*, 8911−8916.

[38] Chen, Ch.; Yu, R.; Owuor, E.D.; Tony Kong.A.N. Activation of Antioxidant-Response Element (ARE), Mitogen Activated Protein Kinases (MAPKs) and Caspases by Major Green Tea Polyphenol Components during Cell Survival and Death. *Arch. Pharm. Res*., 2000, *23*, 605-612.

[39] Scalbert, A.; Manach, C.; Morand, C.; Remesy, C.; Jimenez, L. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.,* 2005, *45*, 287-306.

[40] Tang, S.Y.; Halliwell, B. Medicinal plants and antioxidants: what do welearn from cell culture and *Caenorhabditis elegans* studies? *Biochem. Biophys. Res. Commun*., 2010, *394*, 1–5.

[41] Lee-Hilz, Y.Y.; Boerboom, A.M.J.F.; Westphal, A.H.; van Berkel, W.J.H.; Aarts, J.M.M.J.G.; Rietjens, I.M.C.M. Prooxidant activity of flavonoids induces EpRE-mediated gene expression. *Chem. Res. Toxicol*., 2006, *19*, 1499–505.

[42] Nijveldt, R.J.; van Nood, E.; van Hoorn, D.E.C.; Boelens, P.G.; van Norren, K.; van Leeuwen, P.A.M. Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr.*, 2001, *74*, 418–25.

[43] Ferriola, P.C.; Cody, V.; Middleton, E. Protein kinase C inhibition by plant flavonoids: kinetic mechanisms and structure–activity relationships. *Biochem. Pharmacol*., 1989, *38*, 1617–24.

[44] Hertzog, D.I.; Tica, O-S. Molecular mechanisms underlying the anti-cancerous action of flavonoids. *Current Health Sci. J.,* 2012, 38, 145-149.

[45] Choi, J.S.; Chung, H.Y.; Kang, S.S.; Jung, M.J.; Kim, J.W.; No, J.K.; Jung, H. A. The structure–activity relationship of flavonoids as scavengers of peroxynitrite. *Phytother. Res*., 2002, *16*, 232–235.

[46] Frank, J.; Budek, A.; Lundh, T.; Parker, R.S.; Swanson, J.E.; Lourenço, C.F.; Gago, B.; Laranjinha, J.; Vessby, B.; Kamal-Eldin A. Dietary flavonoids with a catechol structure increase alpha-tocopherol in rats and protect the vitamin from oxidation *in vitro*. *J. Lipid Res*., 2006, *47*, 2718-2725.

[47] Zhu, Q.Y.; Huang, Y.; Chen, Z.Y. Interaction between flavonoids and α-tocopherol in human low density lipoprotein. *J. Nutr. Biochem*., 2000, *11*, 14–21.

[48] Laranjinha, J. Translation of Chemical Properties of Polyphenols into Biological Activity with Impact on Human Health In: *Recent Advances in Polyphenols Research*, Santos-Buelga, C.; Escribano, M.T.; Lattanzio V. Eds.; Wiley-Blackwell: Chichester, 2010; Vol. 2, pp.269–282.

[49] Hollman, P.C.H. Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism. *Arch. Biochem. Biophys*., 2014, *559*, 100-105.

[50] Cao, G.; Russell, R.M.; Lischner, N.; Prior, R.L. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J. Nutr.*, 1998, *128*, 2383–2390.

[51] Williams, R.J.; Spencer, J.P.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med*., 2004, *36*, 838-49.

[52] Thilakarathna, S.H.; Rupasinghe, H.P.V. Flavonoid bioavailability and attempts for bioavailability enhancement. *Nutrients*, 2013, *5*, 3367-3387.

[53] Clifford, M.N. Diet-derived phenols in plasma and tissues and their implications for health. *Planta Med.*, 2004, *70*, 1103-1114.

[54] Kroon, P.A.; Clifford, M.N.; Crozier, A.; Day, A.J.; Donovan, J.L.; Manach, C.; Williamson, G. How should we assess the effects of exposure to dietary polyphenols *in vitro*? *Am. J. Clin. Nutr.*, 2004, *80*, 15-21.

[55] Day, A. J.; Gee, J.M.; Dupont, M.S.; Johnson, I.T.; Williamson, G. (2003). Absorption of quercetin-3-glucoside and quercetin-4'-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochem. Pharmacol*., 2003, *65*, 1199–1206.

[56] Kottra, G.; Daniel, H. Flavonoid glycosides are not transported by the human Na+/glucose transporter when expressed in *Xenopus laevis* oocytes, but effectively inhibit electrogenic glucose uptake. *J. Pharmacol. Exp. Ther.* 2007, *322*, 829-35.

[57] Requena, T.; Monagas, M.; Pozo-Bayón, M.A.; Martín-Álvarez, P.J.; Bartolomé, B.; del Campo, R.; Ávila, M.; Martínez-Cuesta, M.C.; Peláez, C.; Moreno-Arribas, M.V. Perspectives of the potential implications of wine polyphenols on human oral and gut microbiota. *Trends Food Sci. Technol.,* 2010, *21*, 332-344.

[58] Shoji, T.; Masumoto, S.; Moriichi, N. Apple proanthocyanidin oligomers absorption in rats after oral administration: Analysis of proanthocyanidins in plasma using the porter method and high-performance liquid chromatography/tandem mass spectrometry. *J. Agric. Food Chem*., 2006, *54*, 884–892.

[59] Ariza, M.T.; Reboredo-Rodríguez, P.; Cervantes, L.; Soria, C.; Martínez-Ferri, E.; González-Barreiro, C.; Cancho-Grande, B.; Battino, M.; Simal-Gándara, J. Bioaccessibility and potential bioavailability of phenolic compounds from achenes as a new target for strawberry breeding programs. *Food Chem.*, 2018, *248*, 155–165.

[60] Terao, J. Flavonols: metabolism, bioavailability, and health impacts In: *Plant Phenolics and Human Health: Biochemistry, Nutrition, and Pharmacology*; C. G. Fraga Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA.; 2009, pp.185-196.

[61] Poquet, L.; Clifford, M. N.; Williamson, G. Bioavailability of Flavanols and Phenolic Acids In: *Plant Phenolics and Human Health: Biochemistry, Nutrition, and Pharmacology*; C. G. Fraga Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA.; 2009, pp.51-89.

[62] Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.*, 2000, *130*, 2073S–2085S.

[63] Zhang, X.; Dong, D.; Wang, H.; Ma, Z.; Wang, Y.; Wu, B. Stable knock-down of efflux transporters leads to reduced glucuronidation in UGT1A1-overexpressing HeLa cells: the evidence for glucuronidation-transport interplay. *Mol Pharm*., 2015, *12*, 1268-1278.

[64] Dueñas, M.; Surco-Laos, F.; González-Manzano, S.; González-Paramás; A.M.; Santos-Buelga, C. Antioxidant properties of major metabolites of quercetin. *Eur Food Res Technol*, 2011, *232*, 103–111.

[65] O'Leary, K.A.; Day, A.J.; Needs, P.W.; Sly, W.S.; O'Brien, N.M.; Williamson, G. Flavonoid glucuronides are substrates for human liver β-glucuronidase. *FEBS Letters*, 2001, *503*, 103-106.

[66] Shimoi, K.; Saka, N.; Nozawa, R.; Sato, M.; Amano, I.; Nakayama, T.; Kinae, N. Deglucuronidation of a flavonoid, luteolin monoglucuronide, during inflammation. *Drug Metab. Dispos.*, 2001, *29*, 1521–1524.

[67] Lee-Hilz, Y.Y.; Stolaki, M.; van Berkel, W.J.; Aarts, J.M.; Rietjens, I.M. Activation of EpRE-mediated gene transcription by quercetin glucuronides depends on their deconjugation. *Food Chem. Toxicol*., 2008, *46*, 2128–2134.

[68] Terao, J.; Murota, K.; Kawai, Y. Conjugated quercetin glucuronides as bioactive metabolites and precursors of aglycone *in vivo*. *Food Funct.*, 2011, *2*, 11–17.

[69] Menendez, C.; Duenas, M.; Galindo, P.; Gonzalez-Manzano, S.; Jimenez, R.; Moreno, L.; Zarzuelo, M.J.; Rodríguez-Gómez, I.; Duarte, J.; Santos-Buelga, C.; Perez-Vizcaino, F. Vascular deconjugation of quercetin glucuronide: the flavonoid paradox revealed? *Mol. Nutr. Food Res*., 2011, *55*, 1780–1790.

[70] Stevens, J.F.; Maie, C.S. The chemistry of gut microbial metabolism of polyphenols. *Phytochem. Rev*., 2016, *15*, 425–444.

[71] Serra, A.; Macià, A.; Romero, M.P.; Reguant, J.; Ortega, N.; Motilva, M.J. Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. *Food Chem.*, 2012, *130*, 383–393.

[72] Braune, A.; Engst, W.; Elsinghorst, P.W.; Furtmann, N.; Bajorath, J.; Gütschow, M.; Blaut, M. Chalcone isomerase from *Eubacterium ramulus* catalyzes the ring contraction of flavanonols. *J. Bacteriol.,* 2016, *198*, 2965-2974.

[73] Selma, M.V.; Espín, J.C.; Tomás-Barberán, F.A. Interaction between phenolics and gut microbiota: role in human health. *J. Agric. Food Chem*., 2009, *57*, 6485-501.

[74] Aura, A.M.; O'Leary, K.A.; Williamson, G.; Ojala, M.; Bailey, M.; Puupponen-Pimia, R.; Nuutila, A.M.; Oksman-Caldentey, K.M.; Poutanen, K. Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal microflora *in vitro*. *J. Agric. Food Chem*., 2002, *50*, 1725-1730.

[75] Aura, A.M. Microbial metabolism of dietary phenolic compounds in the colon. *Phytochem. Rev.*, 2008, *7*, 407-429.

[76] Aura, A.M.; Martin-Lopez, P.; O'Leary, K.A.; Williamson, G.; Oksman-Caldentey, K.M.; Poutanen, K.; Santos-Buelga, C. *In vitro* metabolism of anthocyanins by human gut microflora. *Eur. J. Nut.,*. 2005, *44*, 133-142.

[77] Fleschhut, J.; Kratzer, F.; Rechkemmer, G.; Kulling, S.E. Stability and biotransformation of various dietary anthocyanins *in vitro*. *Eur. J. Nutr.*, 2006, *45*, 7–18.

[78] Czank, C., Cassidy, A.; Zhang, Q.; Morrison, D.J.; Preston, T.; Kroon, P.A.; Botting, N.P.; Kay, C.D. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a ^{13}C-tracer study. *Am. J. Clin. Nutr.*, 2013, *97*, 995-1003.

[79] Moco, S.; Martin, F.P.; Rezzi, S. Metabolomics view on gut microbiome modulation by polyphenol-rich foods. *J. Proteome Res*., 2012, *11*, 4781–4790

[80] Williamson, G.; Clifford, M.N. Colonic metabolites of berry polyphenols: the missing link to biological activity? *Br. J. Nutr.*, 2010, *104*, S48–S66.

[81] Rechner, A.R.; Smith, M.A.; Kuhnle, G.; Gibson, G.R.; Debnam, E.S.; Srai, S.K.; Moore, K.P.; Rice-Evans, C.A. Colonic metabolism of dietary polyphenols: Influence of structure on microbial fermentation products. *Free Radical Biol. Med*., 2004, *36*, 212–225.

[82] Feliciano, R.P.; Istas, G.; Heiss, C.; Rodriguez-Mateos, A. Plasma and urinary phenolic profiles after acute and repetitive intake of wild blueberry. *Molecules*, 2016, *21*, 1120.

[83] Arumugam, M.; Raes, J.; Pelletier, E.; Le Paslier, D.; Yamada, T.; Mende, D.R.; Fernandes, G.R.; Tap, J.; Bruls, T.; Batto, J.M.; Bertalan, M.; Borruel, N.; Casellas, F.; Fernandez, L.; Gautier, L.; Hansen, T.; Hattori, M.; Hayashi, T.; Kleerebezem, M.; Kurokawa, K.; Leclerc, M.; Levenez, F.;

Manichanh, C.; Nielsen, H.B.; Nielsen, T.; Pons, N.; Poulain, J.; Qin, J.; Sicheritz-Ponten, T.; Tims, S.; Torrents, D.; Ugarte, E.; Zoetendal, E.G.; Wang, J.; Guarner, F.; Pedersen, O.; de Vos, W.M.; Brunak, S.; Doré, J.; MetaHIT Consortium; Antolín, M.; Artiguenave, F.; Blottiere, H.M.; Almeida, M.; Brechot, C.; Cara, C.; Chervaux, C.; Cultrone, A.; Delorme, C.; Denariaz, G.; Dervyn, R.; Foerstner, K.U.; Friss, C.; van de Guchte, M.; Guedon, E.; Haimet, F.; Huber, W.; van Hylckama-Vlieg, J.; Jamet, A.; Juste, C.; Kaci, G.; Knol, J.; Lakhdari, O.; Layec, S.; Le Roux, K.; Maguin, E.; Mérieux, A.; Melo Minardi, R.; M'rini, C.; Muller, J.; Oozeer, R.; Parkhill, J.; Renault, P.; Rescigno, M.; Sanchez, N.; Sunagawa, S.; Torrejon, A.; Turner, K.; Vandemeulebrouck, G.; Varela, E.; Winogradsky, Y.; Zeller, G.; Weissenbach, J.; Ehrlich, S.D.; Bork, P. Enterotypes of the human gut microbiome. *Nature*, 2011, *473*, 174-180.

[84] Espín, J.C.; González-Sarrías, A.; Tomás-Barberán, F.A. The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochem.. Pharmacol.*, 2017, *139*, 82-93.

[85] Cueva, C.; Gil-Sánchez, I.; Ayuda-Durán, B.; González-Manzano, S.; González-Paramás, A.M.; Santos-Buelga, C.; Bartolomé, B.; Moreno-Arribas, M.V. An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health. *Molecules*, 2017, *22*, 99.

[86] Lee, H.C.; Jenner, A.M.; Low, C.S.; Lee, Y.K. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Res. Microbio.l*, 2006, *157*, 876–884.

[87] Okubo, T.; Ishihara, N.; Oura, A.; Serit, M.; Kim, M.; Yamamoto, T.; Mitsuoka, T. *In vivo* effect of tea polyphenol intake on human intestinal microflora and metabolism. *Biosci. Biotechnol. Biochem.*, 1992, *56*, 588–591.

[88] Mansuri, M.L.; Parihar, P.; Solanki, I.; Parihar, M.S. Flavonoids in modulation of cell survival signalling pathways. *Genes Nutr*., 2014, *9*:400.

[89] The *C. elegans* Sequencing Consortium. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science*, 1998, *282*, 2012–2018.

[90] Tissenbaum, H.A. Genetics, life span, health span, and the aging process in *Caenorhabditis elegans*. *J. Gerontol. A Biol. Sci. Med. Sci*., 2012, *67*, 503–510.

[91] Wormbook: the online review of *C. elegans* biology. http://www.wormbook.org/ (Accessed January 20, 2018).

[92] Saul, N.; Pietsch, K.; Menzel, R.; Stürzenbaum, S. R.; Steinberg, C.E.W. Catechin induced longevity in C. elegans: from key regulator genes to disposable soma. *Mech. Ageing Dev*., 2009, *130*, 477–486.

[93] Saul, N.; Pietsch, K.; Stürzenbaum, S.R.; Menzel, R.; Steinberg, C.E. Diversity of polyphenol action in *Caenorhabditis elegans*: between toxicity and longevity. *J. Nat. Prod*., 2011, *74*, 1713–1720.

[94] Bartholome, A.; Kampkötter, A.; Tanner, S.; Sies, H.; Klotz, L.O. Epigallocatechin gallate-induced modulation of FoxO signaling in mammalian cells and *C. elegans*: FoxO stimulation is masked via PI3K/Akt activation by hydrogen peroxide formed in cell culture. *Arch. Biochem. Biophys*., 2010, *501*, 58-64.

[95] Surco-Laos, F.; Duenas, M.; Gonzalez-Manzano, S.; Cabello, J.; Santos-Buelga, C.; González-Paramás, A.M. Influence of catechins and their methylated metabolites on lifespan and resistance to oxidative and thermal stress of *Caenorhabditis elegans* and epicatechin uptake. *Food Res Int.*, 2012, *46*, 514–521.

[96] Sunagawa, T.; Shimizu, T.; Kanda, T.; Tagashira, M.; Sami, M.; Shirasawa, T. Procyanidins from apples (*Malus pumila* Mill.) extend the lifespan of *Caenorhabditis elegans*. *Planta Med*., 2011, *77*, 122-127.

[97] Surco-Laos, F.; Cabello, J.; Gómez-Orte, E.; González-Manzano, S.; González-Paramás, A.M.; Santos-Buelga, C.; Dueñas, M. Effects of O-methylated metabolites of quercetin on oxidative stress, thermotolerance, lifespan and bioavailability on *Caenorhabditis elegans*. *Food Funct*., 2011, *2*, 445–456.

[98] Koch, K.; Havermann, S.; Büchter, C.; Wätjen, W. *Caenorhabditis elegans* as model system in pharmacology and toxicology: Effects of flavonoids on redox-sensitive signalling pathways and ageing. *Sci. World J.*, 2014, 2014: 920398.

[99] Dueñas, M.; Surco-Laos, F.; González-Manzano, S.; González-Paramás, A.M.; Gómez-Orte, E.; Cabello, J.; Santos-Buelga, C. Deglycosylation is a key step in biotransformation and lifespan effects of quercetin-3-*O*-glucoside in *Caenorhabditis elegans*. *Pharmacol. Res*., 2013, *76*, 41-48.

[100] Ahn, D.; Lee, E.B.; Kim, B.J-; Lee, S.Y.; Lee, T.G.; Ahn, M-S; Lim, H.W.; Cha, D.S.; Jeon, H.; Kim, D.K. Antioxidant and lifespan extending property of quercetin-3-O-dirhamnoside from Curcuma longa L. in *Caenorhabditis elegans*. *J Korean Soc Appl Biol Chem*, 2014, *57*, 709–714.

[101] Perez-Vizcaino, F.; Duarte, J.; Santos-Buelga, C. The flavonoid paradox: conjugation and deconjugation as key steps for the biological activity of flavonoids. *J. Sci. Food Agric.*, 2012, *92*, 1822-1825.

[102] Grünz, G.; Haas, K.; Soukup, S.; Klingenspor, M.; Kulling, S.E.; Daniel, H.; Spanier. B. Structural features and bioavailability of four flavonoids and their implications for lifespan-extending and antioxidant actions in *C. elegans*. *Mech. Ageing Dev*., 2012, *133*, 1-10.

[103] Kampkötter, A.; Timpel, C.; Zurawski, R.F.; Ruhl, S.; Chovolou, Y.; Proksch, P.; Wätjen, W. Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, 2008, *149*, 314-323.

[104] González-Manzano, S.; González-Paramás, A.M.; Delgado, L.; Patianna, S.; Surco-Laos, F.; Dueñas, M.; Santos-Buelga, C. Oxidative status of stressed *Caenorhabditis elegans* treated with epicatechin. *J. Agric. Food Chem.,* 2012, 60, 8911-8916.

[105] Abbas, S.; Wink, M. Epigallocatechin gallate from green tea (*Camellia sinensis*) increases lifespan and stress resistance in *Caenorhabditis elegans*. *Planta Med.*, 2009, *75*, 216–221.

[106] Pallauf, K.; Duckstein, N.; Rimbach, G. A literature review of flavonoids and lifespan in model organisms. *Proc Nutr Soc*., 2017, *76*, 145-162.

[107] Pietsch, K.; Saul, N.; Chakrabarti, S.; Stürzenbaum, S.R.; Menzel, R.; Steinberg, C.E. Hormetins, antioxidants and prooxidants: defining quercetin-, caffeic acid- and rosmarinic acid-mediated life extension in *C. elegans*. Biogerontology, 2011, *12*, 329-47.

[108] Büchter, C.; Ackermann, D.; Havermann, S.; Honnen, S.; Chovolou, Y.; Fritz, G.; Kampkötter, A.; Wätjen, W. Myricetin-mediated lifespan extension in *Caenorhabditis elegans* is modulated by DAF-16. *Int. J. Mol. Sci*., 2013, *14*, 11895–11914.

[109] Martorell, P.; Forment, J. V.; De Llanos, R.; Montón, F.; Llopis, S.; González, N.; Genovés, S.; Cienfuegos, E.; Monzó, H.; Ramón, D. Use of *Saccharomyces cerevisiae* and *Caenorhabditis elegans* as model organisms to study the effect of cocoa polyphenols in the resistance to oxidative stress. *J. Agric. Food Chem*., 2011, *59*, 2077–2085.

[110] Pietsch, K.; Saul, N.; Menzel, R.; Stürzenbaum, S.R.; Steinberg, C.E. Quercetin mediated lifespan extension in *Caenorhabditis elegans* is modulated by age-1, daf-2, sek-1 and unc-43. *Biogerontology*, 2009, *10*, 565-578.

[111] Mendenhall, A.R.; Tedesco, P.M.; Taylor, L.D.; Lowe, A.; Cypser, J. R.; Johnson, T. E. Expression of a single-copy hsp-16.2 reporter predicts life span. *J. Gerontol: Series A*, 2012, *67*, 726–733.

[112] Strayer, A.; Wu, Z.; Christen, Y.; Link, C.D.; Luo, Y. Expression of the small heat-shock protein Hsp16-2 in *Caenorhabditis elegans* is suppressed by Ginkgo biloba extract EGb 761. *FASEB J.*, 2003, 17, 2305–2307.

[113] Zhang, L.; Jie, G.; Zhang, J.; Zhao, B. Significant longevity extending effects of EGCG on *Caenorhabditis elegans* under stress. *Free Radicals Biol. Med.*, 2009, *46*, 414–421.

[114] Surh, Y.J.; Kundu, J. K.; Na, H.K. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med.*, 2008, *74*, 1526–1539.

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