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Review

The influence of postharvest processing and storage of foodstuffs on the bioavailability of flavonoids and phenolic acids

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Postharvest processing and storage not only influence the content and composition of flavonoids and phenolic acids in foodstuffs, thereby altering the amount of potentially bioavailable bioactive compounds, but can also modify their chemical form. Moreover, due to the intensive metabolism during absorption, the metabolites circulating in blood differ from the parent compounds found in food. Thus, it is difficult to predict potential *in vivo* effects of phenolic compounds merely by their contents in foodstuffs. Their specific bioavailability needs to be determined. This review considers studies regarding the bioavailability of flavonoids and phenolic acids from foodstuffs that meet the following criteria: providing actual concentrations of flavonoids and phenolic acids in blood plasma, body tissues, or urine, comparing differently stored or processed foods (excluding studies that use supplements or pure substances), and considering the high interindividual variability by repeated measurements in the same individuals. Only a few studies meet all of these criteria. In conclusion, processing and storage of food can have either positive or negative effects on the bioavailability of flavonoids and phenolic acids because these treatments may not only change the content, but also the chemical form of these compounds.

Keywords: Bioavailability / Flavonoids / Food processing / Food storage / Phenolic acids

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1 Introduction

Flavonoids and phenolic acids are ingested with the daily diet due to their ubiquitous occurrence in edible plants and foods of plant origin [1] (Fig. 1). The interest in these compounds has been growing continuously due to presumed beneficial effects on several common diseases like cardiovascular diseases, certain cancers, diabetes mellitus, or neurodegenerative diseases [2]. However, an adequate bioavailability of theses substances is a prerequisite for potential *in vivo* effects beyond the gastrointestinal tract. Thus, many studies have investigated the bioavailability of these compounds in humans and in several animal models in the last years. General aspects of the bioavailability of flavonoids and phenolic acids have been reviewed recently [3 – 5].

Postharvest processing and storage play a significant role in determining the ingredients in edible plants and in foods of plant origin. As described in the accompanying review by Amarowicz *et al*. [6], these processes influence the content of flavonoids and phenolic acids in foodstuffs. Consequently, the amount of ingested and potentially bioavailable polyphenols is altered. In addition, food processing might also change the chemical form of the compounds of interest. The latter can have a substantial impact on bioavailability. This was demonstrated in a recent study which com-

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Abbreviations: AUC, area under the curve; **BT,** black tea; **EC,** (−)-epicatechin; **ECG,** (−)-epicatechin-3-gallate; **EGC,** (−)-epigallocatechin; **EGCG,** (−)-epigallocatechin-3-gallate; **GT,** green tea

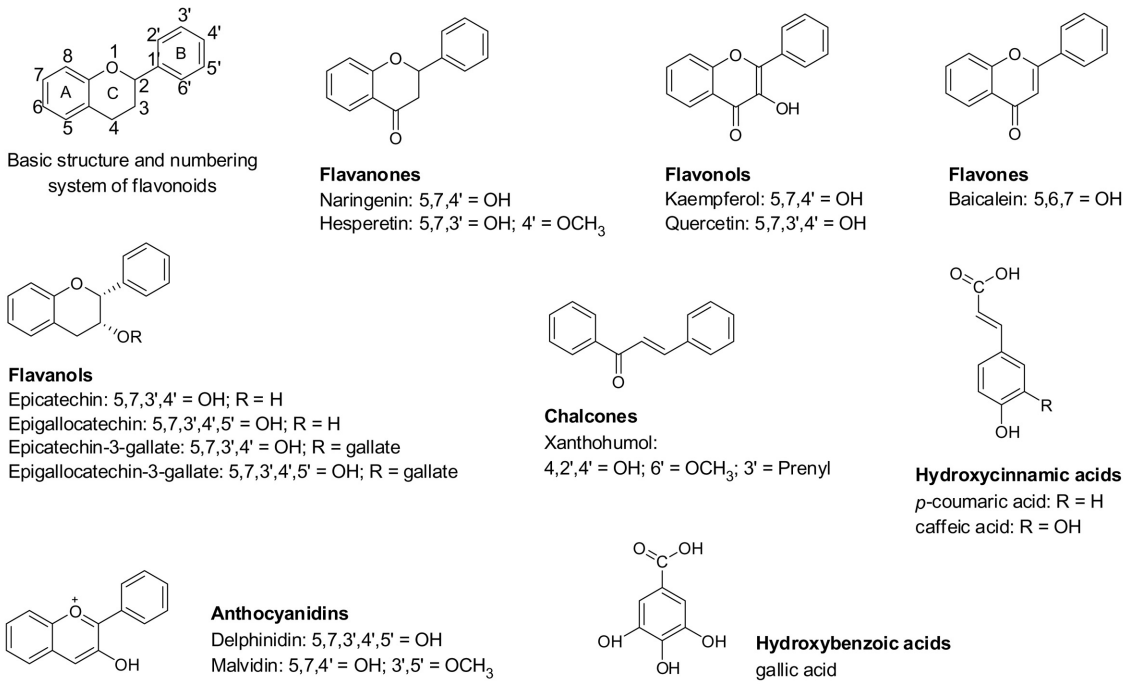

Figure 1. Chemical structures of flavonoids (aglycons) and phenolic acids quoted in the text.

pared the bioavailability of isoflavones from untreated soymilk with that of fermented and enzyme-treated soymilk [7]. In soybeans and nonfermented products like soymilk, the isoflavones daidzein, genistein, and glycitein exist primarily as a complex mixture of glucoside conjugates [8]. In contrast to the sugar-free aglycones, these glucosides cannot be absorbed and, thus, are not directly bioavailable [9]. After ingestion, the glucosides are hydrolyzed by intestinal and bacterial β-glucosidases releasing the aglycones daidzein, genistein, and glycitein. These lipophilic aglycones are next either absorbed or further metabolized by the intestinal microflora in the lower gastrointestinal tract. The bacterial metabolism can either lead to further degradation of the isoflavones or generate even more potent metabolites, for example, in estrogenic activity like equol [10]. On the other hand, fermented soy products contain isoflavones mostly in the form of aglycones. During fermentation, the majority of the isoflavone glucosides are hydrolyzed. These aglycones can usually be absorbed in the upper intestinal tract. A recent study compared the pharmacokinetics of isoflavones from untreated soymilk, enzyme-treated and fermented soymilk in humans [7]. Total isoflavone content hardly differed between the three sources. However, in the latter two, the proportion of isoflavone aglycones was above 90%, whereas in untreated soymilk, it was below 1%. Bioavailability of isoflavones was significantly higher from the enzyme-treated and fermented soymilk. Moreover, absorption from these two products was already maximal within 1 h after ingestion, indicating absorption of the aglycones from the upper part of the digestive tract. In contrast, absorption of isoflavones from untreated soymilk was much slower with a maximum at 6 h after intake. This indicates that the bulk of the isoflavones from untreated soymilk was absorbed from the lower intestinal tract after microbial hydrolysis of the glucosides [7].

Another example of the influence of food processing on the chemical structure of flavonoids is the epimerization of (−)-epicatechin (EC) to (−)-catechin during processing of *Theobroma cacao* beans to cocoa products [11]. In contrast to most foods that contain the native enantiomer (+)-catechin, including *T. cacao* beans, processed cocoa products like chocolate mainly contain the (−)-enantiomer of catechin. Poor intestinal absorption of (−)-catechin in contrast to (+)-catechin explains the rather low catechin bioavailability from chocolate or other cocoa containing products [12]. These examples demonstrate that alterations in the chemical form and structure of phenolic components due to the processing of plant derived foods can have a significant impact on the bioavailability of these compounds.

Moreover, plant material processing for food may change the structure of food matrix. For example, thermal treatment may damage cell walls making compounds more accessible to absorption. It is known that the matrix in which the compounds are ingested is of great importance for the bioavailability of phytochemicals [13, 14]. Previous studies have demonstrated that cooking and processing increased the bioavailability of carotenoids [15, 16]. Thus, it is insufficient to simply measure the contents of flavonoids and phenolic acids in foodstuffs. One must also determine their bioavailability.

However, before we discuss the results of studies in which the bioavailability of polyphenols from stored and processed food were investigated, some preliminary remarks regarding the concept of bioavailability are necessary. Because the term "bioavailability" is not unequivocally defined among human nutritionists and pharmacologists, some misunderstandings may be prevalent regarding the topic of this article.

2 The concept of bioavailability

In human nutrition, the term "bioavailability" generally defines the efficiency with which nutrients are utilized. It comprises the digestion of nutrients as well as their absorption and their metabolic utilization [17]. According to this definition, the postabsorptive utilization of compounds in the diet has to be quantified in order to determine their bioavailability. One way to do this can be to measure specific endpoints of metabolic pathways which the compounds of interest are known to influence.

However, this approach might not be suitable for flavonoids in food. Firstly, the notation of flavonoids and phenolic acids as nutrients is not indisputable. Although they have been designated by Kühnau [18] as "semi-essential food components" and although it is generally presumed that their intake is beneficial for a variety of diseases, final proof of such claims is still missing [19, 20]. Secondly, it is difficult to define *in vivo* effects or biomarkers that are specifically affected by flavonoids or phenolic acids. For instance, the strong antioxidative activity of several flavonoids was believed to be the main principle by which they might counteract several diseases that are associated with oxidative stress. Hence, in several studies the antioxidative capacity in blood plasma was measured after ingestion of polyphenol-rich diets. Postprandial increases in antioxidative capacity were interpreted as *in vivo* effects of the ingested flavonoids. However, this approach was questioned because the observed postprandial increases in plasma antioxidative capacity could hardly be explained by the very minor increases in plasma flavonoid concentration detected [21, 22]. Moreover, a recent study demonstrated that the increase in plasma antioxidative capacity after ingestion of apples rich in flavonoids, exerting a high antioxidative capacity *in vitro*, was evoked by a postprandial rise in plasma urate due to the high fructose content in the apples, but not by the flavonoids themselves [23]. This example shows potential pitfalls that may arise from ascribing *in vivo* effects to the action of flavonoids from complex foods and using such effects as a measure of their bioavailability.

Due to these obstacles, this review will focus on studies in which the bioavailability of flavonoids and phenolic acids was determined as common in pharmacology. The US Food and Drug Administration (FDA) defines bioavailability as "the rate and extent to which the active ingredient or therapeutic ingredient is absorbed from a drug and becomes available at the site of drug action" [24]. Because of the difficulties in measuring drug concentrations at their site of action *in vivo* (which, in the case of the flavonoids, also has to be defined first), bioavailability is usually defined as the rate and extent to which a drug reaches the systemic circulation. According to this definition, the bioavailability of a drug is calculated as the area under the (plasma-concentration) curve (AUC) of the drug that is obtained after its administration. Several pharmacokinetic parameters can be derived from this curve, *e.g.*, among others, peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), or half-life of the drug in blood plasma ($T_{1/2}$) (Fig. 2).

In order to evaluate the proportion of a compound that is absorbed after its ingestion, the compound is administered orally and, after its complete washout, again intravenously (or *vice versa*). The percentage of the AUC value after oral administration *versus* the AUC value after intravenous administration (which, by definition, is 100%) yields the absolute bioavailability of the compound. In order to compare the bioavailability of a compound from a new pharmaceutical formulation with that from a reference formulation, its bioavailability from both formulations (at equal dose) is determined separately. The ratio of the two AUC values describes the bioavailability from the new formulation in proportion to the reference, denoted as relative bioavailability. Accordingly, it is possible to determine the relative bioavailabilities of a compound from two different foods (*e.g.*, processed *vs.* nonprocessed food).

3 Bioavailability of flavonoids and phenolic acids

Several studies have determined the absolute bioavailability of a few flavonoids so far (see Table 1). However, in these studies pure substances or plant extracts were used. Thus, the results cannot be directly extrapolated to complex foodstuffs. Moreover, the data varied significantly, yielding values from a few to over 50%. Some of these differences might be due to the different species and strains used in these experiments. Especially anthocyanins, the only flavonoids which can be absorbed as intact glycosides, seem to have a very limited absolute bioavailability around 1%, which is also reflected in very low C_{max} values after oral intake [3]. The absolute bioavailability for the other investigated flavonoids was, at least in most studies, below 50% (Table 1). This correlates with rather low C_{max} values that are generally in the low micromolar range after intake of foods rich in flavonoids [3]. The situation is similar for phenolic acids [3].

It is important to recognize that in most of these studies conjugated plasma metabolites (*i.e.*, mainly glucuronides and sulfates) were included in the calculation of absolute

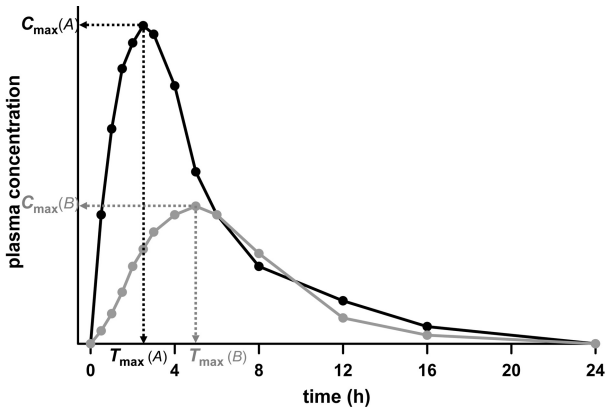

Figure 2. Plasma concentration – time curves of a compound after ingestion from two different dietary sources. The compound was ingested at time 0 in separate experiments from source A (black curve) or source B (gray curve) at equal dose. Dots indicate the plasma concentrations of the compound at the respective time points when blood samples were collected. From these values the plasma concentration *versus* time curves were derived and the following parameters for the respective curves A and B can be determined: C_{max}, time to reach C_{max} (T_{max}) and the AUC. The terminal plasma half-life ($T_{1/2}$) as a parameter for elimination of the compound from the body can be calculated from the terminal exponential part of each curve. In this example, the ratio AUC_B/AUC_A is 0.5. Thus, the relative bioavailability of the compound from source B is only 50% of that from source A.

Table 1. Absolute bioavailability of different flavonoids

Flavonoid[a]	Species	Bioavailability[b] (%)	Total Bioavailability[c] (%)	Ref.
Flavonols				
Quercetin	Rat	n.r.[d]	16 – 27	[66]
	Rat	n.q.[e]	53	[67]
	Rat	5.3	59	[68]
	Pig	0.5	17	[69]
Flavanones				
Naringenin	Rabbit	4	8	[70]
Flavones				
Baicalein	Rat	n.q.	40	[71]
	Rat	n.q.	52	[72]
	Rat	n.q.	36	[73]
Baicalin	Rat	2.2	28	[73]
Anthocyanins				
Delphinidin-3-rutinoside	Rat	0.5	n.r.	[74]
various anthocyanins (from a bilberry extract)	Rat	0.6 – 1.8	n.r.	[75]
Monomeric flavanols				
EGCG	Rat	n.r.	1.6	[76]
	Mouse	12.4	26.5	[77]
ECG	Rat	1 – 3.3	n.r.	[78]

a) Only studies are included in which flavonoids with an intact flavonoid structure in blood plasma were identified and in which absolute bioavailability was determined by comparing oral *versus* intravenous administration.
b) Bioavailability: % of orally administered dose as unchanged parent flavonoid.
c) Total bioavailability: % of orally administered dose including conjugated metabolites.
d) n.r.: not reported.
e) n.q.: not quantifiable (unchanged parent flavonoid near or below LOD).

bioavailability. The molecular structure of the circulating flavonoids in blood is usually different from the ingested chemical forms. In plants and plant products, most flavonoids are linked to sugars except for monomeric flavanols. These glycosides have to be hydrolyzed by intestinal and bacterial β-glycosidases before the released aglycones can be absorbed. Furthermore, flavonoid aglycones as well as phenolic acids are metabolized in the intestinal mucosa and in the liver. *In vivo*, the most relevant metabolic reactions comprise glucuronidation, sulfation, and *O*-methylation [25, 26]. Thus, the forms circulating in blood are mainly glucuronidated, sulfated, and methylated derivatives of the parent compounds. These metabolites may have different biological effects than the original compounds in food [3, 27 – 31]. Exceptions to this general rule are anthocyanins, which can be absorbed as glycosides and appear as such in blood, as well as galloylated monomeric flavanols like epigallocatechin gallate and epicatechin gallate, which, at least to a large extent, may appear unconjugated in the systemic circulation [3, 32, 33].

With regard to the absorption of phenolic acids from the diet it is also important to consider that they, themselves, could be metabolites of larger nonabsorbed or resecreted flavonoids or larger polyphenols that are subject to bacterial degradation in the large intestine [34, 35].

Only a few studies of those which have investigated polyphenol bioavailability have analyzed the exact chemical structure of the circulating metabolites. In the major part of the studies, metabolites were hydrolyzed in order to yield the unconjugated forms prior to analysis by HPLC. Thus, one has to keep in mind that in most of the papers cited below the reported concentrations represent the sum of the conjugated and nonconjugated metabolites (obtained after enzymatic hydrolysis). Taking these preliminary remarks into account, the following criteria were considered for the inclusion of studies in this overview.

Firstly, the focus is on studies in which concentrations of flavonoids and phenolic acids (including all conjugated and methylated metabolites) were actually measured in blood plasma or body tissues after oral intake of foodstuffs. Because differences in urinary excretion of drugs may reflect, at least in some cases, differences in bioavailability [36], studies in which the urinary excretion of flavonoids was measured are also included. However, this assumption

was challenged in one study, which noted that the urinary recovery of the flavanone naringenin after ingestion of orange or grapefruit juice did not match its plasma bioavailability (AUC) [37].

Furthermore, only studies are included in which differently processed or stored foods or beverages were compared; studies in which supplements were used or studies in which pure compounds were added to food are not considered.

Absorption, metabolism, and excretion of xenobiotics are influenced by age, gender, and genetic variability in transporters and metabolic enzymes. Individual dietary and social habits can also influence the metabolism of xenobiotics. For instance, chronic ethanol consumption induces the cytochrome P450 monooxygenase isoform CYP2E1 [38], whereas smoking tobacco induces the isoforms CYP1A1, CYP1A2, and possibly CYP2E1 [39]. Among other cytochrome P450 monooxygenases, these isoforms are able to metabolize certain flavonoids and have also been shown to be induced by flavonoids [31, 40–43]. Because of these manifold factors, resulting in a large interindividual variability regarding the bioavailability of xenobiotics like flavonoids and phenolic acids, preference has been given to studies that compared their bioavailability from different food sources in the same individuals.

3.1 Chalcones

Although chalcones have a central position in the biosynthesis of flavonoids [44] and are common in several foods and beverages [6], there are no data on their bioavailability in humans. The prenylated chalcone xanthohumol is the most abundant chalcone formed in hop cones. During beer brewing, a large portion of xanthohumol is converted to the corresponding isomeric prenylflavanone isoxanthohumol. After administration of xanthohumol to rats by gavage at a very high dose (1 g/kg body weight), conjugated metabolites were detected in plasma [45]. The main metabolite, xanthohumol-4′-*O*-glucuronide, reached its maximal concentration of 3.1 μmol/L 4 h after administration. The C_{max} of unmetabolized xanthohumol was ten-fold lower with the same T_{max} of 4 h [45]. Another rat study detected only conjugates in plasma after oral administration of xanthohumol but failed to detect unmetabolized xanthohumol [46]. However, these studies show that prenylated chalcones are bioavailable, although their bioavailability seems to generally be low.

Gil-Izquierdo *et al*. [47] investigated the potential availability of flavanones in differently processed orange juices by simulating gastric and small intestinal digestion *in vitro*. Besides demonstrating the influence of pasteurization and storage on the content of soluble flavanones, these authors observed that *in vitro* pancreatin ingestion of orange juice in a mild alkaline medium, simulating digestion in the small intestine, transformed 50–60% of the soluble flavanones (mainly hesperidin) to chalcones (mainly hesperidin chalcone). Spontaneous conversion of flavanones to isomeric chalcones in alkaline media is a well-known feature of this class of compounds [48]. No chalcones were detected in the soluble fraction of the orange juice, though. The formed chalcones remained in the cloud fraction of the juice, probably in an insoluble form. Thus, the authors stated that these chalcones were not available for absorption in the small intestine [47]. To our knowledge, studies that investigated the *in vivo* bioavailability of chalcones from differently processed food have not been published so far. Hence, the interpretation of Gil-Izquierdo *et al*. [47] regarding the bioavailability of chalcones from orange juice still needs confirmation by *in vivo* studies.

3.2 Monomeric flavanols (catechins)

A major dietary source of monomeric flavanols (catechins) is green and black tea (BT). Whereas green tea (GT) is manufactured by drying the leaves of *Camellia sinensis*, BT is obtained by an additional fermentation step, which generates theaflavins and thearubigins, oligomeric polyphenolic compounds synthesized from monomeric tea catechins. Thus, the content of the monomeric flavanols is reduced in BT [49]. A recent study compared the bioavailability of the main tea flavanols EC, (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG) (Fig. 1) from GT and BT in a crossover design in human volunteers [50]. In order to standardize the intake of EGCG from both tea preparations, four bags of BT or three bags of GT were added to 426 mL boiling water and steeped for 5 min. The tea was served immediately to the study subjects. Standardizing the EGCG content in the tea preparations (214 mg in GT, 231 mg in BT) affected the content of the other monomeric flavanols. Whereas the ECG content was the same in both infusions, GT contained twice as much EC and 2.5 times the amount of EGC than BT. Blood was collected prior to the intervention and 1, 2, 4, 6, and 8 h after the tea consumption. The flavanol contents in plasma were analyzed by using HPLC with electrochemical detection after treatment of the samples with β-glucuronidase/sulfatase. As expected from its similar content in green and BT, C_{max} and AUC values for ECG were the same after consumption of the two tea varieties. In line with its higher content in GT, the bioavailability of EGC was around 2.5 times higher after ingestion of GT. However, pharmacokinetic parameters of EC did not exactly correspond with its content in the two tea varieties. Although the amount of EC in GT was only twice as much as that in BT, its C_{max} and AUC values were even four times higher after GT consumption. Interestingly, bioavailability of EGCG was significantly higher after intake of BT. T_{max} was similar for all four monomeric flavanols (1.2–1.5 h) and was not different between black and GT [50].

The same group of authors investigated the concentration of tea flavanols in human prostate tissue [51]. Fifteen male

volunteers scheduled for surgical prostatectomy were randomly assigned to the GT group ($n = 8$) or to the BT group ($n = 7$). Prior to the intervention study, a serum sample was collected from each participant. Participants were instructed on tea preparation (one bag brewed with 236 – 284 mL of boiling water for 5 min) and told to consume 284 mL five times spread throughout the day for 5 days. Flavanol concentrations were higher in the GT infusion (concentrations *per* 236 mL in GT or BT, respectively, for EC: 26 or 10 mg; EGC: 91 or 26 mg; ECG: 40 or 31 mg; EGCG: 71 or 58 mg). On day 6, a serum sample was obtained and surgical removal of the prostate took place. No polyphenols were detected by HPLC with electrochemical detection in the serum samples collected at baseline or after tea consumption due to the time elapsed from the last tea administration to the time of blood collection. However, the flavanol concentration in human prostate tissue tended to be higher after consumption of BT, in spite of its lower flavanol content. The tissue concentration of all four flavanols together was 238 pmol/g after BT and 203 pmol/g after GT; the EGCG concentration was 66 ± 10 pmol/g after BT or 40 ± 11 pmol/g after GT [51].

In a previous study on risk factors for ischemic heart disease (IHD) a weak positive correlation between flavonol intake from tea and IHD mortality in Welsh men was found [52]. The authors suggested that the customary addition of milk to the tea might have inhibited flavonol absorption from the drink, thus, canceling out positive health effects. This suggestion was based on a former study in which plasma antioxidative capacity had been measured before and after ingestion of green and BT with or without the addition of milk [53]. In the latter study, it had been observed that the ingestion of 300 mL of either unsweetened BT or GT by human volunteers increased their plasma antioxidant capacity significantly. The addition of 100 mL whole milk to the beverage inhibited the rise in antioxidant capacity after consumption of both GT and BT completely [53].

However, further studies in which plasma concentrations of flavonoids were directly analyzed clearly demonstrated that the addition of milk was not able to alter postabsorptive plasma levels or the bioavailability of either flavonols or monomeric flavanols from BT [54 – 56]. Thus, the addition of milk does not have an effect on the bioavailability of flavanols or flavonols from tea. Instead, increasing the infusion time during tea preparation seems to be an important factor. In BT, total phenol concentration after 3 min infusion time was only 60% of that after 7 min infusion time [56].

Interestingly, in a recent human study, vascular effects of BT *in vivo* were observed which were abolished when the tea was ingested together with 10% skimmed milk [57]. The authors suggested that milk caseins might inhibit the effects of the tea catechins by formation of complexes with the latter. However, due to the experimental evidence that milk does not influence catechin absorption [54 – 56] and due to the fact that the highly digestible casein is enzymatically hydrolyzed before absorption, this is probably not a very likely explanation for the observed effect of milk addition.

3.3 Phenolic acids (hydroxycinnamic acids, hydroxybenzoic acids)

Hydroxybenzoic and hydroxycinnamic acids are abundant in plant foods, the latter mainly occurring as esters and amides and also being bound to cell wall polymers in esterified and etherified forms. One study demonstrated that domestic cooking of cherry tomatoes increased the bioavailability of chlorogenic acid, an ester formed between quinic acid and caffeic acid [13]. Human volunteers ingested a meal consisting of pasta, olive oil, bread, and either 500 g of fresh cherry tomatoes, or 500 g of cherry tomatoes that had been cooked for 15 min at 100°C. Plasma levels for chlorogenic acid were higher after ingestion of the cooked tomatoes, which was significant at 2 and 6 h after intake. Analysis of chlorogenic acid in plasma samples was performed after enzymatic hydrolysis of the circulating conjugates with sulfatase and β-glucuronidase; the presence of chlorogenic acid was confirmed with HPLC-ESI-MS [13].

Serafini *et al*. [58] observed that the plasma concentrations of caffeic and *p*-coumaric acid increased after intake of 250 g of fresh lettuce (*Lactuca sativa*). This increase was significant for at least 3 h after ingestion. In contrast, intake of the same amount of lettuce that had been stored for 3 days under modified-atmosphere packaging conditions failed to alter their baseline plasma levels. Within this storage time, the content of both hydroxycinnamic acids in the lettuce was significantly decreased [58].

3.4 Anthocyanins

A study conducted by Kurilich *et al*. [59] compared the bioavailability of anthocyanins, the glycosides of anthocyanidins, from raw and cooked purple carrots. Anthocyanin bioavailability was not affected by cooking the carrots in a microwave oven (900 W) for 12 min. However, cooking increased the relative urinary recovery (determined by dividing the cumulative 24 h urinary excretion by the ingested anthocyanin dose) of nonacylated anthocyanins but not of the acylated ones. Interestingly, when the amount of ingested cooked carrots was increased from 250 to 500 g, neither C_{max} nor AUC values were affected. Accordingly, the relative urinary recovery of the higher dose was significantly smaller, thus suggesting that anthocyanin absorption capacity was already saturated at the higher dose [59].

Other dietary agents also seem to influence the bioavailability of anthocyanins. Constituents which are produced and/or added during technological processes, in particular

those liable to bind, solubilize or stabilize anthocyanins, might modify the extent of absorption and metabolism. Frank *et al*. [60] compared the bioavailability of a complex mixture of anthocyanins from red wine and red grape juice. Nine volunteers consumed a single dose of either 400 mL of red wine (280 mg total anthocyanins) or 400 mL of red grape juice (284 mg total anthocyanins). Within 7 h, the urinary excretion of total anthocyanins was higher following grape juice ingestion (0.23% of the ingested dose) than following red wine (0.18%). The urinary recovery of five individual anthocyanins, normalized by dividing their values by the administered dose, was also different between the two beverages, although this was not significant for every anthocyanin. The authors speculated that these differences could be partially due to a modification of the *in vivo* metabolism by the ethanol content in the red wine [60].

In an earlier study on human volunteers, Bub *et al*. [61] compared the absorption of malvidin-3-glucoside after ingestion of 500 mL of red grape juice, red wine, or dealcoholized red wine containing 117, 68, and 58 mg of this anthocyanin, respectively. The bioavailability of malvidin-3-glucoside (AUC up to 6 h) was not significantly different after the consumption of either red wine or dealcoholized red wine. After ingestion of the red grape juice, malvidin-3-glucoside bioavailability was about twice as high; however, this was in accordance with its two-fold higher concentration in the juice. The authors concluded that the presence of alcohol did not affect the amount of malvidin-3-glucoside absorbed. Yet, the early absorption of the anthocyanin was probably enhanced by ethanol because the T_{max} value was smaller after red wine (50 min) than after dealcoholized red wine (90 min) or red grape juice (120 min) consumption. The authors suggested that the high glucose content could have been the reason for the significant delay in anthocyanin absorption from the grape juice, due to a possible competition of the glucoside and glucose at the intestinal sodium-dependent glucose transporter 1 [61]. However, the opposite was observed in the study by Frank *et al*. [60], in which the T_{max} value for malvidin-3-glucoside was even shorter after consumption of a sugar containing red grape juice (0.5 h) than after red wine (1.5 h).

In another study, the urinary excretion of anthocyanins was compared when elderberry concentrates were consumed with and without sucrose [62]. Concomitant sucrose consumption lowered urinary recovery of anthocyanins over a period of 6 h and also delayed anthocyanin excretion. In a further study, the addition of a rice cake delayed the absorption of anthocyanins from black currant juice in humans (T_{max} = 90 min with rice cake *vs*. 45 min without) [63]. The authors speculated that the delayed absorption was due to a delay in gastric emptying. However, anthocyanin bioavailability did not seem to be different up to 4 h after ingestion [63].

A similar observation was made by Walton *et al*. [64], who studied the influence of different diets on the absorption of blackcurrant anthocyanins in pigs. The test diets were composed of either a blackcurrant powder with sugar and water, or of a blackcurrant powder with sugar, milk, and wheat-based cereal, or of a blackcurrant powder with sugar, milk, wheat-based cereal, and additional rutin. The total amount of anthocyanins absorbed (AUC up to 8 h) as well as C_{max} values were not different between the three test meals, but T_{max} was delayed from 2 h (test meal without milk and cereal) to 4 h (two test meals with milk, cereal - ± rutin) [64].

3.5 Flavanones

In the previously mentioned study conducted by Bugianesi *et al*. [13], the bioavailability of several compounds after ingestion of fresh and cooked cherry tomatoes was compared (see Section 3.3). Among these compounds, the flavanone naringenin was also investigated, which was analyzed in the plasma samples with HPLC-ED after enzymatic hydrolysis of the circulating conjugates with sulfatase and β-glucuronidase. Plasma concentrations of naringenin tended to be higher up to 6 h after ingestion of the domestically cooked tomatoes in comparison to the fresh tomatoes. The difference was significant at 2 h after intake [13].

3.6 Flavonols

Serafini *et al*. [58] also observed that the plasma concentration of quercetin (plasma samples were enzymatically hydrolyzed prior to flavonol analysis by HPLC-ED) increased after intake of 250 g of fresh lettuce (*L. sativa*). This increase was significant for at least 3 h after ingestion. In contrast, intake of the same amount of lettuce that had been stored for 3 days under modified-atmosphere packaging conditions failed to alter quercetin plasma concentrations. Within this storage time, the quercetin content in the lettuce was decreased by two-third [58].

In one study, the absorption of the flavonols quercetin and kaempferol from BT and GT was compared [55]. Over a 3-day period, human volunteers consumed eight cups of either BT or GT *per* day, providing the same amount of tea solids (4 g) and approximately the same amount of flavonol glycosides. Plasma concentration–time curves for quercetin and kaempferol after BT consumption were similar to those after GT consumption. The authors concluded that the bioavailability of the flavonols was independent of the tea type [55].

4 Concluding remarks

Among the various studies which have investigated the bioavailability of flavonoids and phenolic acids, only few have addressed the influence of postharvest processing and storage on foodstuffs. Thus, it is difficult to make general state-

ments regarding the effects of these processes on bioavailability. Storage conditions that reduce the content of flavonoids and phenolic acids most likely diminish their bioavailability simply by decreasing their amount in the ingested food [58]. Domestic cooking might increase their bioavailability from certain foodstuffs [13]. Moreover, it is well known that not only the amount, but also the sugar moiety to which these polyphenols are linked and which differs between various foods, has an enormous impact on their bioavailability [65]. Thus, any treatment that alters the structure of the glycoside, *i.e.*, that leads to a deglycosylation, very likely, alters their bioavailability. Still, one has to remember that the original plants by themselves show a large variation in terms of their flavonoid content due to their specific variety, region, climate, phytosanitary conditions, *etc.* and this may already explain a large part of the variations observed. In addition, further influences like food matrix, other components in complex meals, or the high interindividual metabolic variability makes it difficult to predict the bioavailability of flavonoids and phenolic acids in each individual case.

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