29
Tannins: Bioavailability and Mechanisms of Action
Fulgencio Saura-Calixto and Jara Pérez-Jiménez

29.1
Introduction

Tannins are a major group of oligomeric and polymeric dietary polyphenols made up of flavanol monomers (proanthocyanidins or condensed tannins) and polyesters of a sugar moiety and gallic acid or ellagic acid (hydrolyzable tannins).

Over the last century, studies on tannins have moved from industrial applications (leather production) and organoleptic properties in foods to the present day research into biological properties associated with disease prevention. Physiological effects of dietary tannins will depend on their release from the food matrix and their absorption and fermentation in the gastrointestinal tract. The action of digestive enzymes may release a fraction of food tannins, which may be partially absorbed through the small intestine mucosa. Unabsorbed tannins and tannins associated with cell walls reach the colon, where they may be fermented by bacterial microflora, releasing some tannins and yielding different metabolites. Tannins absorbed in both the small and the large intestine may exert systemic effects.

Several studies performed through cell culture assays and animal experiments have suggested that tannins may provide some protection against cancer. However, there have not yet been any clinical trials in humans.

Knowledge of the intake of tannins and other polyphenols in common diets may be useful to elucidate their relative contribution to the health effects associated with the intake of antioxidants, especially in relation to gastrointestinal cancer. Note that most data on tannin intake take into account only low molecular weight food tannins extracted with aqueous organic solvents, ignoring the biological activity of a substantial part of nonextractable tannins that are subjected to colonic fermentation.
29.2 Physicochemical Properties

Hydrolyzable tannins are polyesters of a sugar moiety (or other nonaromatic polyhydroxy compounds) and gallic acid (gallotannins) or ellagic acid (ellagitannins) (Scheme 29.1). These compounds undergo hydrolytic cleavage to the respective sugar and acid moiety upon treatment with diluted acids.

Condensed tannins or proanthocyanidins are polyhydroxyflavanol oligomers or polymers. They consist of flavan-3-ol or flavan-3,4-diol monomers that are usually linked by carbon–carbon bonds in the 4 → 6 or the 4 → 8 position (B-type proanthocyanidins). B-type proanthocyanidins can be classified according to the hydroxylation pattern(s) of the chain extender unit(s), and the most commons in plant foods are procyanidins, prodelphinidins, and propelargonidins. The name “proanthocyanidins” is derived from the fact that when treated with acid, they react to form insoluble phlobaphenes and red-colored anthocyanidin solutions (Scheme 29.2).

Tannins – especially proanthocyanidins – have the ability to complex strongly with carbohydrates and proteins. Tannins are sequestered into “pores” in the polysaccharide structure, an association that is influenced by molecular size, conformational mobility and shape, and water solubility. When tannins interact with proteins, the result is usually a precipitation of the complex, but under certain conditions some soluble complexes are formed. Tannin–protein interactions are influenced by characteristics of the protein (size, amino acid composition, pI, etc.), characteristics of the tannin (size, structure), and conditions of the reaction (pH, temperature, solvent, and time) [1].

The major dietary sources of proanthocyanidins are fruits (particularly berries), legumes, nuts and some cereals (sorghum, barley), wine, beer, and other beverages.

In the case of hydrolyzable tannins, ellagitannins have been reported, among others, in almost all varieties of berries, nuts (pecans, walnuts, brazil nuts, peanuts, and cashews), blue plum, pomegranate (fruit and juice), red apples, navel oranges, pink and white grapefruit, tangerine, tangelo, peach, brown and green pear, white and red grapes, oak-aged wines, and kiwi as well as beer.

Scheme 29.1 Structure of an ellagitannin (left) and a gallotannin (right).
29.3 Bioavailability and Metabolism

Very little is known about the metabolic fate and bioavailability of tannins. To exert their biological properties, tannins should be available to some extent in the target tissue. Therefore, the biological properties of tannins may depend on their absorption in the gut and their bioavailability. It must be remembered that molecules from tannins appearing in blood or excreted in urine can be very different from that when ingested.

29.3.1 Proanthocyanidins

It is not yet clearly established whether proanthocyanidins are depolymerized in the stomach or not, and it has been suggested that food bolus may buffer the acid secretion in the stomach, preventing acidic hydrolysis of proanthocyanidins [2, 3].
In small intestine digestion, the main factor that affects the fate of metabolism of proanthocyanidins is their degree of polymerization. It has been observed that the procyanidin dimers B2 and B5 are hydrolyzed to epicatechin in isolated rat small intestine. Also, it has been reported that orally administered procyanidin B2 and B3 dimers were absorbed and excreted by rats and that dimers and trimers from catechin have similar permeability coefficients to that of catechin and close to that of mannitol, a marker of paracellular transport [4].

However, absorption is much lower or entirely absent in the case of higher oligomers, and especially of polymers, which may reach the colon nearly intact [5]. During small intestine digestion, high molecular weight proanthocyanidins may form less digestible complexes with protein, starch, and digestive enzymes.

Another important site where tannins become available in the gastrointestinal tract is the large intestine. Most of the ingested proanthocyanidins reach the colon, where they become fermentable substrate for bacterial microflora along with other nondigestible constituents. The abundant microflora in the colon plays a critical role in the metabolism of tannins. After microbial enzyme metabolism of any tannin that reaches the colon, there are two possible routes available, breakdown of the original tannin structure into absorbable metabolites or a breakdown into nonabsorbable metabolites (probably mid-molecular weight tannins); these remain in the colonic lumen, where they may counteract the effects of dietary pro-oxidants in the colon produced during colonic bacterial metabolism.

Several authors have found that proanthocyanidins are metabolized to a large extent by gut microflora, the main metabolites produced being phenlyacetic, phenylpropionic, and phenylbutyric acids [4, 5]. The higher the degree of polymerization is, the lower the degree of metabolization will be [5]. As to the possibility of depolymerization of proanthocyanidins in the colon to their monomeric units, to our knowledge no intestinal bacteria so far described are capable of performing this transformation.

29.3.2 Hydrolyzable Tannins

Few studies have evaluated the rate of hydrolysis of hydrolyzable tannins into monomers (ellagic acid or gallic acid) during enzymatic digestion in the stomach and small intestine.

The possibility of gastric absorption of ellagic acid has recently been reported in pigs [6], but results of absorption in the small intestine are contradictory [7, 8]. One possible explanation for this discrepancy is the poor water solubility of ellagic acid, which would result in low concentrations of free ellagic acid in plasma, and another is the fact that ellagic acid binds irreversibly to cellular DNA and proteins, which may also account for its limited transcellular absorption and its ability to form poorly soluble complexes with calcium and magnesium ions in the intestine [9]. Following absorption, ellagic acid undergoes conjugation, and conjugated forms with methyl, glucuronyl, and sulfate groups have been found in plasma and excreted in urine in humans.
Two different systems for gastric absorption of gallic acid have been described, a rapid permeation system for intact gallic acid and a slow permeation system for conjugated derivatives [10], while in the small intestine, gallic acid is absorbed, peaking after about 1 h, and is metabolized to other compounds, of which the most abundant in humans is 4-O-methylgallic acid [11].

When hydrolyzable tannins reach the colon, they are subjected to the action of certain lactobacilli with distinct tannase activity, which hydrolyzes hexahydroxydiphenoyl groups in ellagitannins and galloyl groups in gallotannins [12]. A pathway has been proposed for the degradation of ellagitannin by human microbiota via hydrolysis to ellagic acid and its microbial transformation to urolithin A and urolithin B, which are detected in plasma as glucuronides after absorption [13]. A recent study of the complete metabolism of ellagitannins in pig extensively reported conjugated metabolites in bile, which would imply very active enterohepatic circulation of these compounds [6].

29.4 Mechanisms of Protection

Over the last decade, studies on cell cultures and on experimental animals treated with tannins have shown that these compounds may exert several beneficial effects. It is believed that tannins may exert their biological effects in two different ways: (1) as an unabsorbable, complex structure with binding properties that may produce local effects in the gastrointestinal tract (antioxidant, radical scavenging, antimicrobial, antiviral, and antinutrient effects) or (2) as absorbable tannins (probably low molecular weight) and absorbable metabolites from colonic fermentation of tannins that may produce systemic effects in various organs.

Several studies with different cell cultures have reported chemopreventive effects of tannins on breast, oral, prostate, stomach, and skin cancer [14–18]. Most of these studies have tested the molecules of tannins as present in foods, assuming that these polyphenols are absorbed to reach these target tissues to exert the potential anticarcinogenic effect. However, this approach does not take into account the bioavailability of tannins referred above, as a result of which the amount of the tannins as present in the food matrix that would reach the different organs would be negligible and would not even remotely approach the concentration used in the referred studies. It suggests that the original tannins should only be used in cell cultures when studying colorectal cancer, while for other kinds of cancers their corresponding metabolites should be tested.

In the case of colorectal cancer, several studies (with cell cultures and experimental animals) have concluded that tannins – both hydrolyzable tannins and proanthocyanidins – may produce a chemopreventive effect for colorectal cancer through various mechanisms:

1. Induction of apoptosis through, among other processes, activation of initiator caspase-9, and effector caspase-3 [19].
2. Inhibition of the inflammation signaling pathway that increases the risk of colon cancer by inhibiting expression of the enzyme COX-2 (cyclooxygenase-2) [20].

3. Reduction of the levels of MMP-2 and MMP-9 secreted to the extracellular medium [21].

4. Generation of an antioxidant environment in the colon. Since overproduction of free radicals and hence of oxidative stress has been linked to cancer, antioxidants may help to prevent the onset of this disease. Note, in this connection, that tannins are able to inhibit lipid peroxidation and lipoxygenases in vitro, and they are also able to scavenge radicals such as hydroxyl, superoxide, and peroxyl that are known to be important in cellular pro-oxidant states.

5. Inhibition of DNA oxidative damage, another effect associated with the antioxidant properties of tannins [22].

6. Stimulation of the growth of beneficial colonic bacteria, such as Lactobacillus and Bifidobacterium spp., and inhibition of the growth of nondesirable bacteria, such as Clostridium [23].

Interestingly, the observed effects did not take place in normal cells.

As to the products that have been tested, the studies with hydrolyzable tannins focus mainly on punicalagin, present in pomegranate, and ellagic acid, while the studies on proanthocyanidin properties used extracts obtained from grape, wine, or apple. However, these works only consider low molecular weight proanthocyanidins, with a mean degree of polymerization between three and eight units.

This means that the possible chemopreventive effects of high molecular weight proanthocyanidins, which remain in the residues of the common aqueous–organic extractions performed, have not been systematically considered. However, supplementation to rats with a grape product rich in high molecular weight proanthocyanidins (about 20%) revealed a capacity to inhibit proliferation in the colonic epithelium by reducing the total number of crypts per milliliter and producing shorter crypts [24].

In this study, besides high concentrations of high molecular weight proanthocyanidins, the product tested contained numerous low molecular weight proanthocyanidins and other polyphenols. This suggests that mixtures of polyphenols may enhance the health beneficial effects of a single polyphenol or tannin through synergistic action. Similarly, it has been observed that pomegranate juice (rich in polyphenols, including hydrolyzable tannins) induced apoptosis on HT-29 cells, when concentrations of the hydrolyzable tannin punicalagin or total tannins comparable to the concentrations present in pomegranate juice had no effect [19]. Therefore, the possible chemopreventive effect should be evaluated in the context of the overall intake of antioxidants and other chemopreventive compounds in a diet.
29.5 Results of Human Studies

Most intervention studies on the relationship between antioxidants and chronic disease, including gastrointestinal cancer, have tested specific antioxidants (mainly vitamin C, vitamin E, and β-carotene) or specific foods. A more comprehensive view of this field may be gained by examining the antioxidant capacity of whole diets rather than single antioxidants or foods. In this way, epidemiological studies have suggested that dietary antioxidants may play a role in the prevention of a number of diseases [25].

Considering the overall intake in a common diet in the approach to tannins may be useful for epidemiological studies and for the design of intervention studies. A number of authors have estimated the daily intake of tannins based on food composition and consumption survey data. The mean proanthocyanidin intake in the United States, according to the USDA (United States Department of Agriculture) food composition database, is estimated at 53.6 mg/day/person. Recently, Saura-Calixto et al. [26] determined and estimated the intake of highly polymerized proanthocyanidins in the Spanish diet at 450 mg/person/day. The reason why the intake data reported in the United States are low is that USDA composition data do not take into account dietary intake of high molecular weight proanthocyanidins.

There are no data concerning ellagitannins and gallotannins intake in the literature. Saura-Calixto et al. [26] estimated the intake of hydrolyzable polyphenols (polyphenols associated with high molecular weight compounds that includes hydrolyzable tannins and other phenolic acids) in the Spanish population at around 1250 mg/person/day. Nevertheless, these data may, respectively, underestimate and overestimate the hydrolyzable tannin intake.

In the case of the Spanish diet, a total intake of polyphenols (2.8 g/p/day) has been reported, including extractable polyphenols (phenolic acids, flavonoids, and low molecular weight proanthocyanidins), high molecular weight proanthocyanidins, and hydrolyzable phenolics. It is estimated that hydrolyzable phenolics and high molecular weight proanthocyanidins represent about 50% of the total intake of polyphenols.

Clinical trials have failed to show any benefit from antioxidant supplementation in the prevention of gastrointestinal cancer [27]. The reported clinical trials only analyzed isolated antioxidants (e.g., 15–50 mg β-carotene/day, 120–2000 mg vitamin C/day, 30–600 mg vitamin E/day), but the potential effect and the synergistic action of dietary polyphenols that reach the colonic lumen (estimated at 2.5 g/day) [26] are ignored. Note that tannins and other polyphenols, unlike vitamins, remain nearly intact until they reach the colon and are degraded there, releasing absorbable and nonabsorbable metabolites that may play a chemopreventive role. Further studies would be necessary to elucidate such potential action of dietary polyphenols in the colonic lumen.
Impact of Cooking and Processing

There are only a very limited number of references to the influence of storage and processing on stability of tannins. It has been reported that ellagitannins were reduced by up to 40% after 9 months storage at −20 °C [28] and that the amount of ellagic acid in frozen-stored raspberries decreased significantly (14–21%) in the course of 1 year [29].

Proanthocyanidins may be degraded during the drying process or polymerized to more highly polymerized compounds, which can render the extraction and quantification of proanthocyanidins in dried foods incomplete. Similarly, various processing procedures entailing the use of high temperatures have been found to reduce proanthocyanidin content. For example, this has been reported after cooking of faba beans, processing of sorghum bran into cookies and bread, thermal processing, and canning of peaches or heating of grapes [30–33].

References

9 Whitley, A.C., Stoner, G.D., Darby, M.W. and Walle, T. (2003) Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acid extensive binding to protein
and DNA. Biochemical Pharmacology. 66, 907–915.


23 Dolara, P., Luceri, C., De Filippo, C., Femia, A.P., Giovannelli, L., Caderni, G., Cecchini, C., Silvi, S., Orpianesi, C. and


