



## Review

# Why is it important to understand the nature and chemistry of tannins to exploit their potential as nutraceuticals?



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## ABSTRACT

Tannins comprise a large group of polyphenols that can differ widely in chemical composition and molecular weight. The use of tannins dates back to antiquity, but it is only in recent years that their potential use as nutraceuticals associated with the human diet is beginning to be exploited. Although the biological effects of these phytocomplexes have been studied for many years, there are still several open questions regarding their chemistry and biotransformation. The vastness of the molecules that make up the class of tannins has made their characterisation, as well as their nomenclature and classification, a daunting task.

This review has been written with the aim of bringing order to the chemistry of tannins by including aspects that are sometimes still overlooked or should be updated with new research in order to understand the potential of these phytocomplexes as active ingredients or technological components for nutraceutical products. Future trends in tannin research should address many questions that are still open, such as determining the exact biosynthetic pathways of all classes of tannins, the actual biological effects determined by the interaction of tannins with other molecules, their metabolism, and the best extraction methods, but with a view to market requirements.

## 1. Introduction

It is not certain what the origin of the term tannins is. Its original sense was “to convert skins to leather” and it is normally attributed to the French word ‘tanin’ (1802). However, it could have more ancient origins, going back to the Celtic source indicating an oak tree. From that, the term was acquired by the Medieval Latin tannare “tan, dye a tawny colour” (c. 900), from tannum “crushed oak bark,” used in tanning leather (Harper, 2020). Whatever the etymology of the term, the meaning of the word tannins has always been associated with the use of these substances since ancient times.

Tannins can be found in nature in numerous different families of the higher plants all over the world, such as in chestnut, quebracho and oak wood, sumac or plant galls. Depending on their origin, they can rise to a molar mass of up to 20,000 D, thus their chemistry could differ widely.

These molecules are concentrated in almost every part of the plant, from the roots to fruit. It is accepted that the biological role of tannins in the plant is associated to growth regulation, as well as the preservation against external perturbations, such as infection, insects, or animal herbivory. Tannins can exhibit many different appearances, as light yellow or white amorphous powders or as shiny, nearly colourless, loose masses, with a characteristic smell and astringent taste (Molino et al., 2019).

It is considered that the oldest application of these vegetal compounds was the stabilization of animal skin protein against putrefaction, thanks to their property of precipitating proteins. Thanks to the expansion of their use, tannins have also started to be experimented with and applied in different fields, from medicinal application to food industry. In particular Asian (Chinese and Japanese) natural medicine used actives from tannin-containing plant extracts to treat different

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disorders (i.e. diarrhoea and inflammation) and pathologies (Baba et al., 2021; Li et al., 2021; Ren et al., 2021).

In time the nomenclature of tannins underwent multiple interpretations, misunderstandings, and changes, due to the progress in the field. After the oversimplified description of Seguin (1796), in the 20th century Bate-Smith and Swain and then Griffith tried to give a definition, including also some of the tannin properties. However, each of these presented some restrictions, leaving out tannin groups with high (above 3000 D) or low (below 1000 D) molar mass, respectively (Das et al., 2020).

To formulate a definition that would take into account all the tannins, their structural characteristics and their properties is *a priori* not an easy mission. Finally, in 2001, Khanbabaee & van Ree formulated an updated definition based on the molecular structures of the currently known tannins, and their origin and role in plant life (Khanbabaee & van Ree, 2001):

*“Tannins are polyphenolic secondary metabolites of higher plants, and are either galloyl esters and their derivatives, in which galloyl moieties or their derivatives are attached to a variety of polyol-, catechin- and triterpenoid cores (gallotannins, ellagitannins and complex tannins), or they are oligomeric and polymeric proanthocyanidins that can possess different interflavanyl coupling and substitution patterns (condensed tannins).”*

According to our research, there is no more up-to-date official definition in the scientific literature. Despite this, the scientific community has shown great interest in this category of polyphenols by carrying out research to understand how to universally and adequately describe and group such a wide class of compounds. During the ninth edition of the tannin conference held in Madison (USA) in 2018, several exponents expressed the need to arrive at a consensus, which as yet has not been defined.

In 2011, Salminen's research group has proposed to adopt a broader approach to describing tannins by relying on chemical ecology. They claim that tannins should be defined in light of the plant-herbivore interactions, including determination of the oxidative capacity, the protein precipitation capacity and the tannin moieties responsible for these two types of activities. According to the authors, molecules cannot be studied only from a chemical point of view, because their functionality is strongly influenced by different conditions (such as concentration) and interactions with the environment (such as animal species that may ingest them, pH in which they can be implemented, etc.) (Salminen & Karonen, 2011).

Last, but not least, it is of utmost importance to understand the chemistry of tannins to exploit their potential as nutraceuticals. For example, companies like Silvateam or Tanin d.d. Sevnica produce different tannin extracts that are widely used for wine, beer and spirits production; depending on tannins chemistry, they enhance wine flavor and stability during vinification (Sevnica, 2023a; Silvateam, 2023a), improve beer physical and organoleptic stability due to their antioxidant capacity and interaction with proteins (Silvateam, 2023b) and add special oak flavor and oldness (Sevnica, 2023b). By the way, tannins are also used in animal nutrition to improve milk and meat production, as well as gut function and overall animal health in cows, cattle, calves, goats, sheep and pigs (Sevnica, 2023c, Silvateam, 2023c). Even tannins can be used for food safety and preservation due to their antimicrobial and antioxidant capacity (Sevnica, 2023d). In fact, part of these uses are under development to produce nutraceuticals by improving human well-being though and increase on antioxidant capacity and short chain fatty acids production, microbiota balancing and intestinal permeability improvement (Silvateam, 2023d). It should be emphasized that the identification of the structure-activity relationship of tannins will contribute to a more fruitful and widespread use of them as nutraceuticals, as this would allow to choose the most effective compounds.

## 2. Classification

Due to the challenge of giving a comprehensive definition of tannins

based on their biochemistry, it is not so obvious to reach a universal consensus on classification. Indeed, based on the definition given in the previous paragraph, tannins can vary greatly in their affinity for proteins, and some tannin compounds may exhibit very low activities. On the other hand, some types of non-tannin polyphenols, in appropriate concentrations, are able to partially bind and precipitate proteins (Salminen & Karonen, 2011).

Given this, an unambiguous definition and classification of tannins could be that based on their chemical properties or structural characteristics. As regard the chemical properties, Serrano and co-workers (2009) proposed to classify them into extractable and non-extractable tannins (Serrano et al., 2009). The former group includes tannins that can be readily extracted with aqueous-organic solvents, so they would include relatively low-molecular-weight tannins, which could be analysed by spectrophotometric and chromatographic techniques. Non-extractable tannins would cover all the high-molecular-weight tannins or tannins complexed with proteins or cell-wall polysaccharides, which are not easily extractable with the above-mentioned extraction method. These insoluble compounds would therefore need acid and basic hydrolysis, which allow quantification of monomers.

Another basic classification of tannins was proposed by Okuda & Ito (2011), who reported the grouping of tannins and other polyphenols related to tannins as Type A polyphenols, which include those with constant chemical structure, and Type B, characterized by a variable composition (Okuda & Ito, 2011).

However, the most common way to classify tannins is the analytical subdivision based on their structural characteristics. A simplistic grouping adopted by several authors divides tannins into two large categories: hydrolysable tannins and condensed tannins. Treatment of many tannins with hot water or tannases enables hydrolysis into monomeric products, and leads to classifying them as 'hydrolysable tannins', which comprise two further sub-groups: the gallotannins and the ellagitannins. The remaining non-hydrolysable oligomeric and polymeric proanthocyanidins have been classified as 'condensed tannins'. Khanbabaee & van Ree (2001) proposed a new sorting including also partially hydrolysable tannins, so far called 'unclassified tannins', which contain characteristic structural elements of both ellagitannins and condensed tannins (Khanbabaee & van Ree, 2001). Therefore, the authors divided tannins into four major groups: gallotannins, ellagitannins, complex tannins, and condensed tannins. A major shortcoming of this nomenclature is the fact that many authors have focused on considering only the tannins produced by terrestrial plants. However, in 2009, Serrano et al. proposed to add also the category of phlorotannins, which are mainly produced by marine organisms, such as brown algae (Serrano et al., 2009) (Fig. 1).

### 2.1. Gallotannins

Gallotannins are scarcely found in nature and are the most basic hydrolysable tannins, consisting of gallic acid derivatives which contain six or more galloyl groups (Fig. 2). Moreover, they are further characterized by the presence of one or more digalloyl groups (Gross, 1999). Even though there is a wide variety of polyol residues, most of the gallotannins isolated from plants contain a polyol residue derived from a saccharide, in particular d-glucose. The hydroxyl functions (-OH) of the polyol residues may be entirely or partially replaced by galloyl units. In the latter, the -OH groups can be substituted or not by other residues (Kiss & Piwowarski, 2016).

Even though the described compounds are key intermediates in the biosynthesis of nearly all hydrolysable plant polyphenols, it is quite infrequent to find gallotannins in nature compared to ellagitannins. However, gallotannins, especially hexa to decagalloyl glucoses, are exploited extensively at industrial level as commercially available tannic acid, and principally used for protein precipitation (Salminen & Karonen, 2011).

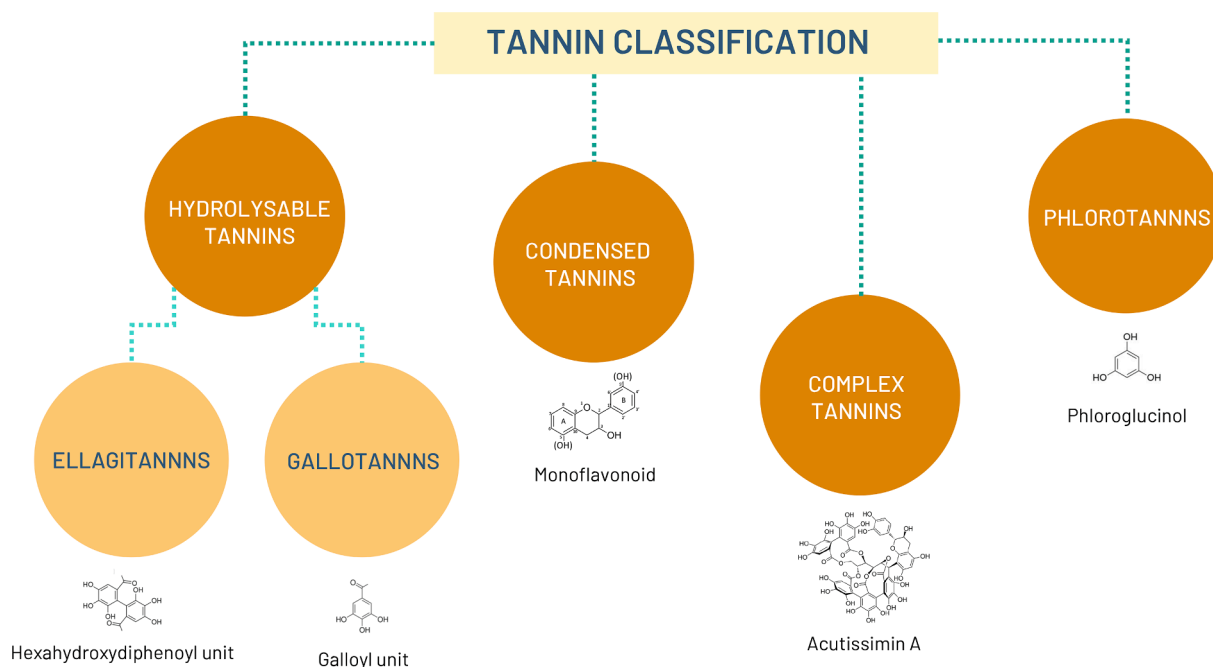


Fig. 1. Tannin classification.

## 2.2. Ellagitannins

Ellagitannins, widespread in many plant families, are the largest group of known tannins including more than 1000 natural compounds. Ellagitannins are constituted from gallotannins by at least two galloyl units which are C–C linked to each other, leading to the axially chiral monomer hexahydroxydiphenyl (HHDP), and they do not contain glycosidically linked catechin units (Fig. 2). The chirality is given by the large substituents located in the ortho positions of the biaryl axis and the restricted rotation around this axis, resulting in isomerism. In many cases this chirality is triggered by the esterification in the ortho positions of the two carboxyl groups with a polyol (commonly D-glucopyranose) (Khanbabaee & van Ree, 2001; Kiss & Piwowarski, 2016).

The multiple possibilities in which HHDP units can be linked to sugar moieties and the potential to produce oligomeric derivatives determine the presence of a very large variability in the structures of different ellagitannins. For this reason, some authors have proposed to further divide ellagitannins into different subgroups, given the different chemical structures: HHDP esters, dehydro-HHDP esters and their modifications, nonahydroxy-triphenyl esters, flavanoellagitannins, and oligomers with both varying degree of oligomerization and types of bonds between the monomers (Salminen & Karonen, 2011).

A common feature frequently used to describe all of these compounds is the release of ellagic acid as a result of hydrolysis of the HHDP group and the consequent lactonization of the parent acid. However, it should be specified here that some of the chemical structures of ellagitannins exhibit a modification of HHDP groups, due to oxidation processes in the biosynthetic pathway. In this case, the above molecules are unable to produce ellagic acid when hydrolysed and would be recognised as ellagitannins only by their biosynthetic origin.

## 2.3. Condensed tannins

Condensed tannins are the most common group of naturally occurring tannins and represent almost 90% of the worldwide production. These tannins are generally found in nature complexed with proteins, depending on their chemical structure and consequently on their affinity. To be called condensed tannins, these compounds must be formed by the repetition of 3 to 8 units (or building blocks) and the precursors must

be a flavan-3-ol (catechin) or a flavan-3,4-diol (leucoanthocyanidin) (Fig. 2). Each flavonoid is composed of two phenolic rings (A and B) having different reactivities and two possible configurations comprising or not a hydroxyl group in positions 5 and 5'. The different configurations set up four different possibilities of basic building blocks to form condensed tannins (Sallam et al., 2021).

Monomeric catechins and leucoanthocyanidins do not possess tanning properties, but they have then the interesting capacity of being converted into oligomers and polymers that do have tanning capacities, by the action of acids or enzymes. The type of connection between the various flavanoid units depends on the nature of the rings. All oligomeric and polymeric proanthocyanidins are formed by the linkage of the C-4 of one monomer with the C-8 or C-6 of the next building block. In particular, C4–C6 bonds are typically found in proflisetidins and pro-robinetidins, while C4–C8 are found in procyanidins and prodelfphinidins (Meikleham et al., 1994).

Monomers can polymerise from a degree of two up to over fifty. In particular, catechin oligomers and polymers with a degree of polymerization from two to ten are also called flavolans. Beside the size, also the coupling pattern of the catechin units in condensed tannins can vary considerably (Arbenz & Averous, 2015; Sienawska & Baj, 2017).

## 2.4. Complex tannins

Complex tannins contain both condensed and hydrolysable tannins. In their structure a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit (Fig. 2). The ones most commonly found are flavano-ellagitannins, procyanidin-ellagitannin, and flavano-ellagitannins. An example from this substance class is acutissimin A, containing a flavogallonyl unit (nonahydroxytriphenyl unit) bound glucosidically to C-1, and linked via three further hydrolysable ester bridges to the d-glucose derived polyol (Khanbabaee & van Ree, 2001).

## 2.5. Phlorotannins

Phlorotannins are recognised as the simplest tannin group from a structural point of view. These molecules mainly produced by brown seaweeds, such as *Ecklonia stolonifera*, *Ecklonia cava*, and *Ecklonia bicyclis*, or macroalgae (Phaeophyceae) occur as hydrolytic compounds in

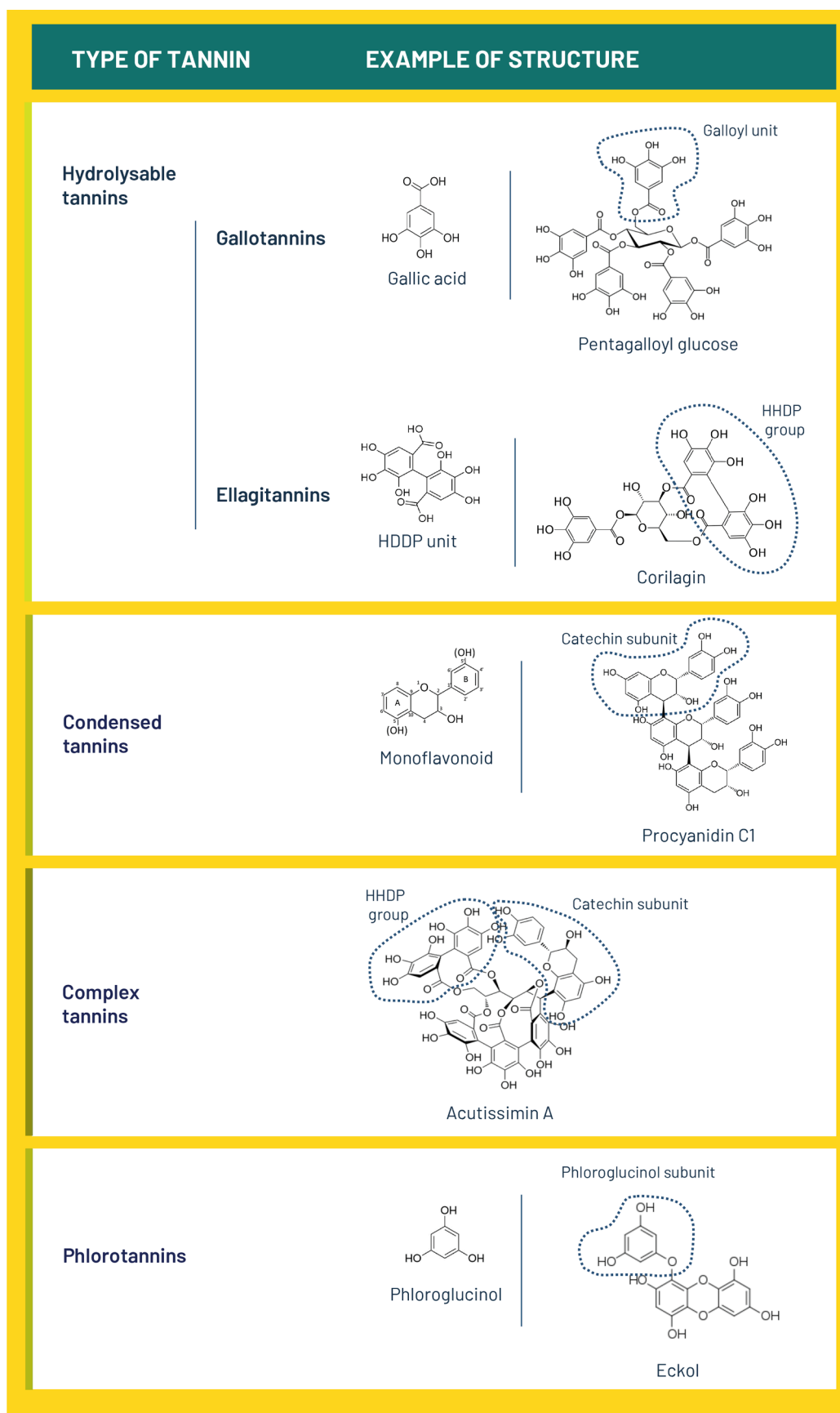


Fig. 2. Structures of tannins.

nature with a relatively low molecular weight ranging from 126 Da–650 kDa. Chemically, phlorotannins exhibit phloroglucinol (1,3,5-benzenetriol) monomer units, which constitute polymers mainly by combining through C–C and/or C–O–C bonds. There could be further structural variations including additional OH-groups in the molecules or additional bonds between the monomers (Meng et al., 2021; Salminen & Karonen, 2011).

Thus, based on the number and type of bonds (aryl-aryl or diaryl-ether) between monomers, these tannins could be divided into six subgroups: phloretols, fucols, fuhalols, fucophloretols, isofuhalols, and eckols (Kumar et al., 2022). This specific chemical organization distinctly distinguishes phlorotannins not only from other type of tannins but also from other phenolic compounds.

### 3. Tannins in nature

Tannins are highly hydroxylated secondary compounds, synthesized through plant secondary metabolism. As mentioned earlier, tannins produced by terrestrial plants can be accumulated in any part of the plant, and especially in the growing zones of trees. At cellular level, tannins can be found in the sap vacuole of some parenchymatic cells (i.e. pith and cortical parenchyma) or in more specialized cells such as idioblasts. For example, once the condensed tannins are synthesised, they are stored in a chlorophyll organelle, which is called the tannosome. This ensures that these compounds do not interact with the plant's proteins or its metabolism. Their metabolic action only begins after the cell dies and then disintegrates (Brillouet et al., 2013). Tannins can also be found as smaller molecules, in plant organs with intense physiological activity (Montes-Ávila et al., 2017).

In general, plants which have a strong capacity to synthesise proanthocyanidins do not metabolise substantial quantities of hydrolysable tannins, and vice-versa. As regards complex tannins, they are widely present as monomers in Fagaceae, Combretaceae and Myrtaceae, as well as in some species of Melastomataceae and Theaceae (Haslam, 2007).

The analogous marine tannins are mainly found within the physodes in brown algae. Physodes are cell vacuoles that constitute the main cytoplasmic components in adult cells lining the mucosal channels of the cortex. When phlorotannins are released by physodes, they can establish bonds with the structural polysaccharide alginic acid, and thus contribute to the structuring of the brown algae cell wall (Imbs & Zvyagintseva, 2018).

The content of phlorotannins may vary in different parts of the alga, but in the Fucaceae the vegetative parts are particularly rich compared to the reproductive ones (Catarino et al., 2017).

The function of tannins has been related to plant-environmental interactions. Being secondary metabolites, tannins have been commonly identified as protection agents against oxidative stress and external insults, such as infections, solar radiation and dryness. Moreover, they are able to prevent plant-herbivore interactions thanks to their acid taste and the property of precipitating proteins. Some particular pathological conditions increase tannin production by the plant. A clarifying example are plant galls produced as a result of insect attack. Similarly, it has been reported that the production of phlorotannin is increased by certain environmental factors such as salinity and incidence of solar radiation.

The tannin content may vary depending on the plant species, the specific part of the plant and external factors (i.e. harvest time, time of the year, intensity of illumination). It has been estimated that the level of terrestrial plant tannins normally found in most plant tissues is in the range 2–5% of the fresh weight (Furlan et al., 2011). However, the content could increase up to 25% of dry weight (Cuong et al., 2020). The same ratio was reported also for the content of phlorotannins in dry seaweed, with maximum values in brown algae from temperate and northern latitudes, in summer (Imbs & Zvyagintseva, 2018; Maheswari & Babu, 2022).

Given the great antioxidant potential of hydrolysable tannins, and

their almost ubiquitous presence in plants, it has been proposed to use them as chemotaxonomic markers, especially for Angiosperm orders and families. In fact, Okuda et al. (2000) re-designed the Cronquist system of plant classification, organizing the families and genera based on the progressive oxidative structural transformation of hydrolysable tannins (Fig. 3).

Gallotannins are the starting point of the biogenetic sequence that goes through 4 steps: (I) galloyl group in gallotannin → (II) HHDP group in ellagitannin → (III) dehydrohexahydroxydiphenoyl (DHHDP) group in dehydroellagitannin → (IV) transformed DHHDP groups in transformed dehydroellagitannin.

From this classification it could be deduced that tannins are present in all plants, except for the subclass of the Asteriidae, comprising mainly herbaceous plants. This has been then confirmed by the new proposal of angiosperm systematics, the Angiosperm Phylogeny Group, based on DNA sequencing techniques (Chase et al., 2016).

### 4. Biosynthesis of tannins

The biosynthetic pathways of all tannins rely on products that come from the Calvin cycle, a fundamental process that occurs in chloroplasts and allows carbon dioxide to be fixed into glyceraldehyde-3-phosphate, and then further modified and accumulated as carbohydrates (eg, sucrose and starch). Calvin cycle products can enter either the glycolytic or the oxidative pentose phosphate pathways, which return precursor molecules for tannin synthesis via the acetate/malonate and shikimate pathways. Hydrolysable tannins rely exclusively on the shikimate pathway, phlorotannins only on the acetate/malonate pathway, while for the biosynthesis of condensed tannins both pathways are essential (Fig. 4).

The shikimate pathway is found mostly in the plant world but also in fungi and bacteria, giving as final products phenylalanine and other building blocks that are essential for the synthesis of metabolites, such as the precursors of terrestrial plant tannins (i.e. p-coumaric acid and gallic acid). This route is located in plastids and starts with phosphoenolpyruvate (a metabolite from the glycolytic pathway) and erythrose-4-phosphate (resulting from the pentose-phosphate pathway) (Fig. 4). Given the importance of this route, numerous regulatory pathways have developed at different levels, from transcriptional to enzymatic.

The precursor of flavonoids is obtained after the action of three different enzymes: PAL (phenylalanine ammonia lyase), C4H (cinnamic acid 4-hydroxylase), and C4L (4-coumaric acid: CoA ligase), which convert phenylalanine to p-coumaryl-CoA. In plants, PAL has several isogenes, which are activated in distinct ways to produce metabolites of different type, such as tannins or lignin (Vogt, 2010).

#### 4.1. Biosynthesis of hydrolysable tannins

The esterification of gallic acid with glucose starts the synthesis of hydrolysable tannins and the first intermediate is  $\beta$ -glucogallin (1-O-galloyl- $\beta$ -D-glucose) (Fig. 5). This reaction has been demonstrated with different techniques of molecular biology and by *in vitro* assays, using *Escherichia coli* (Mittasch et al., 2014). Subsequently,  $\beta$ -glucogallin, without any additional cofactor, acts as donor and acceptor of galloyl groups to produce further substituted glucose. The substitution of glucose hydroxyls by galloyl groups follows the sequence:  $\beta$ -glucogallin → 1,6-digalloylglucose → 1,2,6-trigalloylglucose → 1,2,3,6-tetragalloylglucose → 1,2,3,4,6-pentagalloylglucose (PGG). PGG is the basic compound needed to synthesise both gallotannins and ellagitannins (Montes-Ávila et al., 2017).

Biosynthesis of gallotannins derives from the galloylation of PGG, and forms structures characterised by the presence of moieties of two galloyl groups joined by an ester bond.  $\beta$ -glucogallin is fundamental in the control of the galloyltransferases, responsible of the catalysis of the depsidic bond (Mora et al., 2022).

Conversely, the generation of ellagitannins depends on intra- and

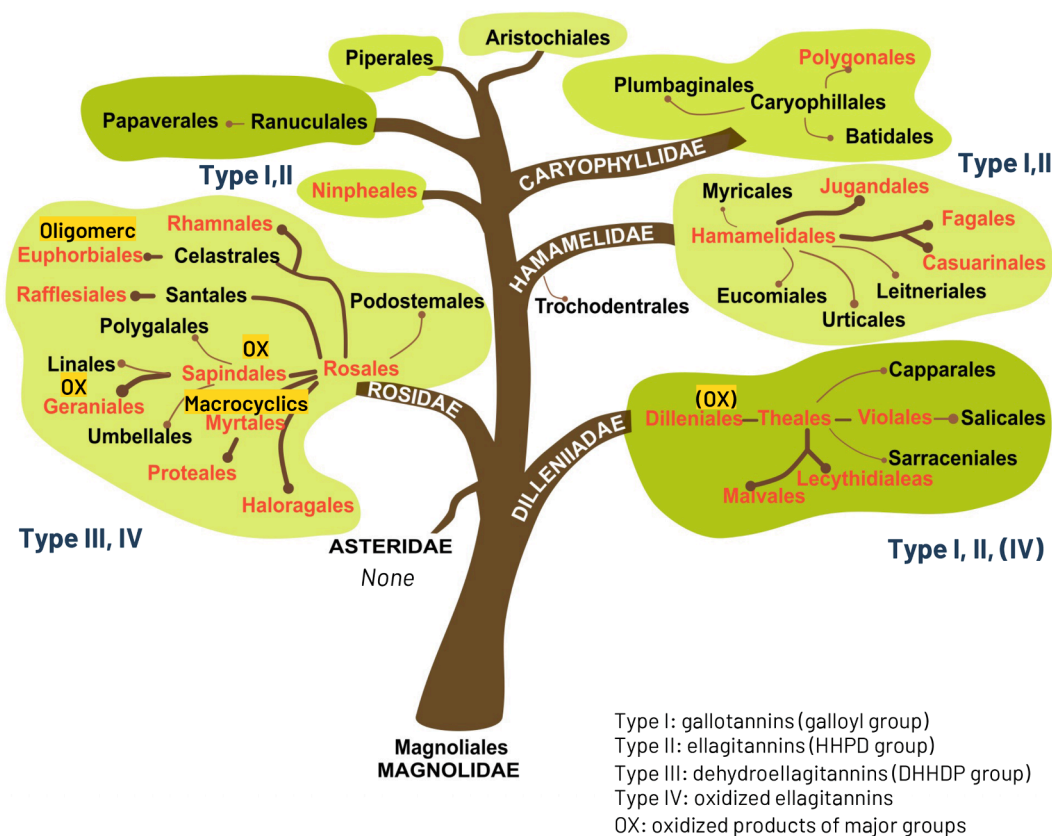


Fig. 3. Polyphenolics evolution - Ellagitannins; Cronquist's evolutionary representation applied to this schema.

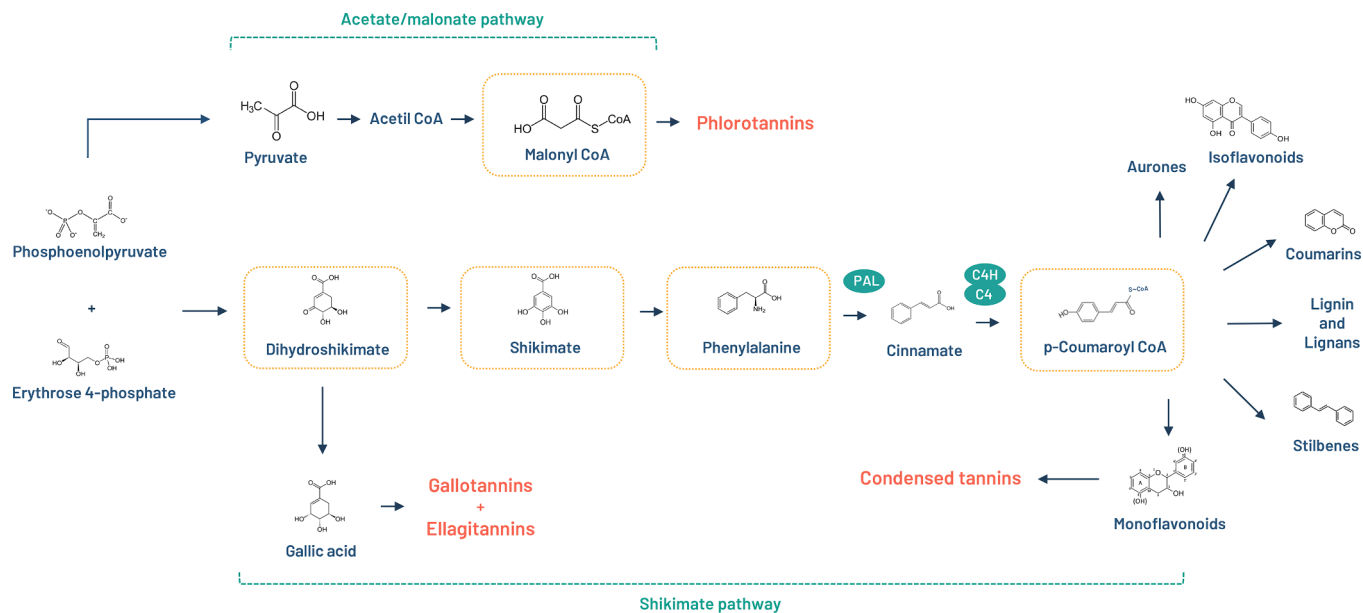


Fig. 4. Origins of tannin biosynthesis. The metabolites of the shikimate pathway are circled in yellow.

intermolecular oxidation processes of PGG, mediated by a laccase-type polyphenol oxidase. The oxidation of PGG to tellimagrandiin II and then to its dimer cornusin E, leads to the formation of the HHDP biaryl unit, which identifies the ellagitannins. The HHDP moiety released from the hydrolysis of ellagitannins has a spontaneous tendency to form ellagic acid, through lactonization (Montes-Ávila et al., 2017; Mora et al., 2022).

#### 4.2. Biosynthesis of condensed tannins

In the early stages of formation of proanthocyanidins, they share the same biosynthesis pathways with other flavonoids, which are generated by chalcone synthase (CHS), starting from p-coumaroyl-CoA (Mora et al., 2022). p-coumaroyl-CoA condensed with three molecules of malonyl-CoA generates a chalcone. The enzyme chalcone isomerase (CHI) converts the chalcone to (2S)-naringenin, recognised as precursor of different

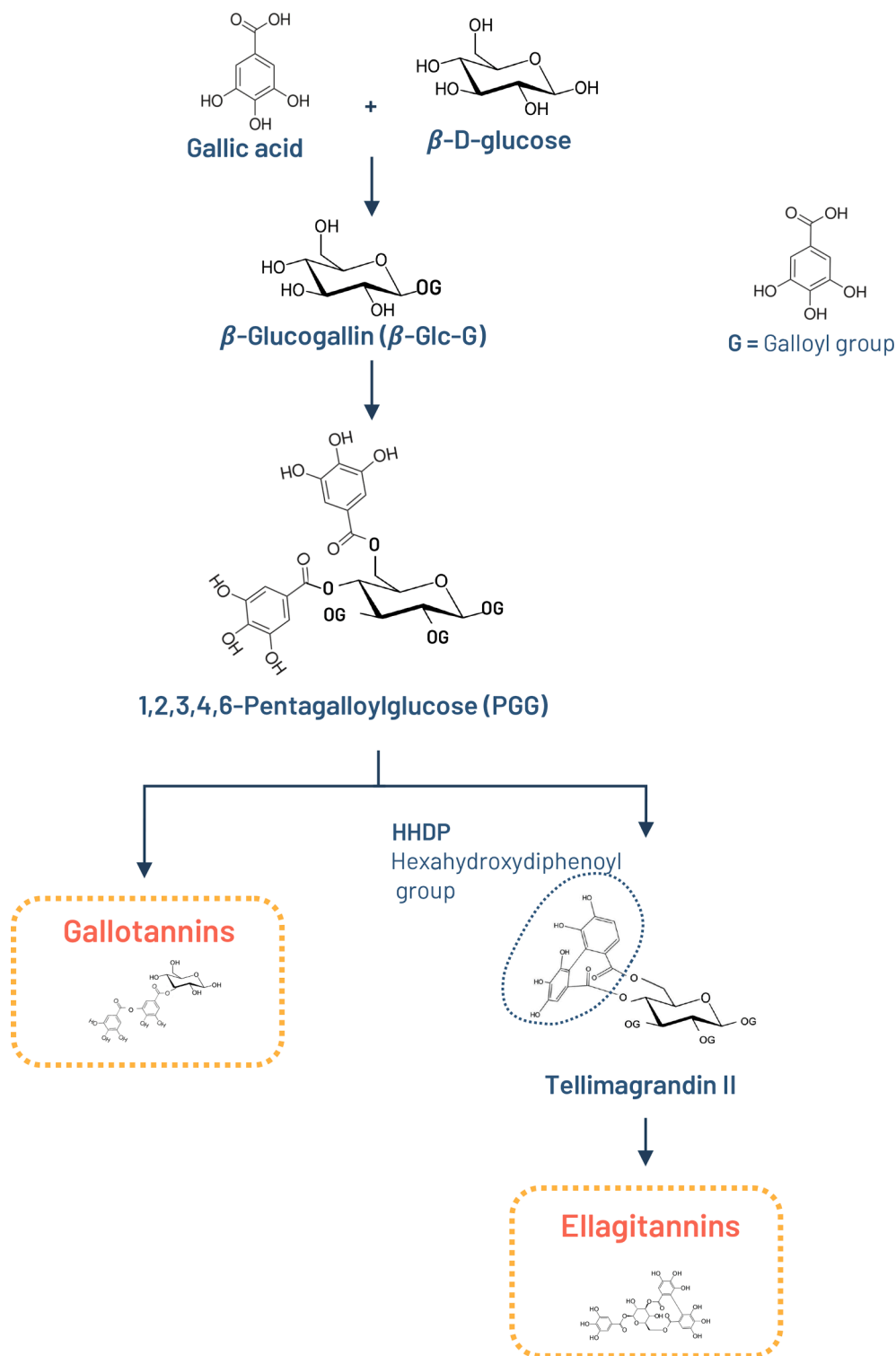


Fig. 5. Biosynthesis of hydrolysable tannins (Arbenz & Avérous, 2015).

types of flavonoids (i.e. flavonoids, isoflavonoids and flavanones). In order to generate proanthocyanidins, flavanone-3-hydroxylase (F3H) must act on naringenin to produce dihydrokaempferol. Depending on whether dihydrokaempferol is hydroxylated in the B-ring by flavonoid-3-hydroxylase or by flavonoid-3- $\beta$ -hydroxylase, it can generate dihydroquercetin or dihydromyricetin, respectively. The enzymes dihydroflavonol-4-reductase (DFR) and anthocyanidin synthase (ANS) transform the flavonoid precursors to anthocyanidins and proanthocyanidins (Montes-Ávila et al., 2017; Mora et al., 2022). Specific

enzymes for proanthocyanidins biosynthesis are the anthocyanidin reductase (ANR), which transforms the anthocyanidins to epi-flavan-3-ols (epicatechin, epiafzelechin and epigallocatechin); and the leucoanthocyanidin reductase (LAR), which transforms leucoanthocyanidins to flavan-3-ols (catechin, afzelechin and galocatechin). Epi-flavan-3-ols and flavan-3-ols are direct monomeric precursors of proanthocyanidins (Fig. 6).

So far it has not been possible to clarify the route of polymerization of flavan-3-ols. Generally, it is well accepted that the production of

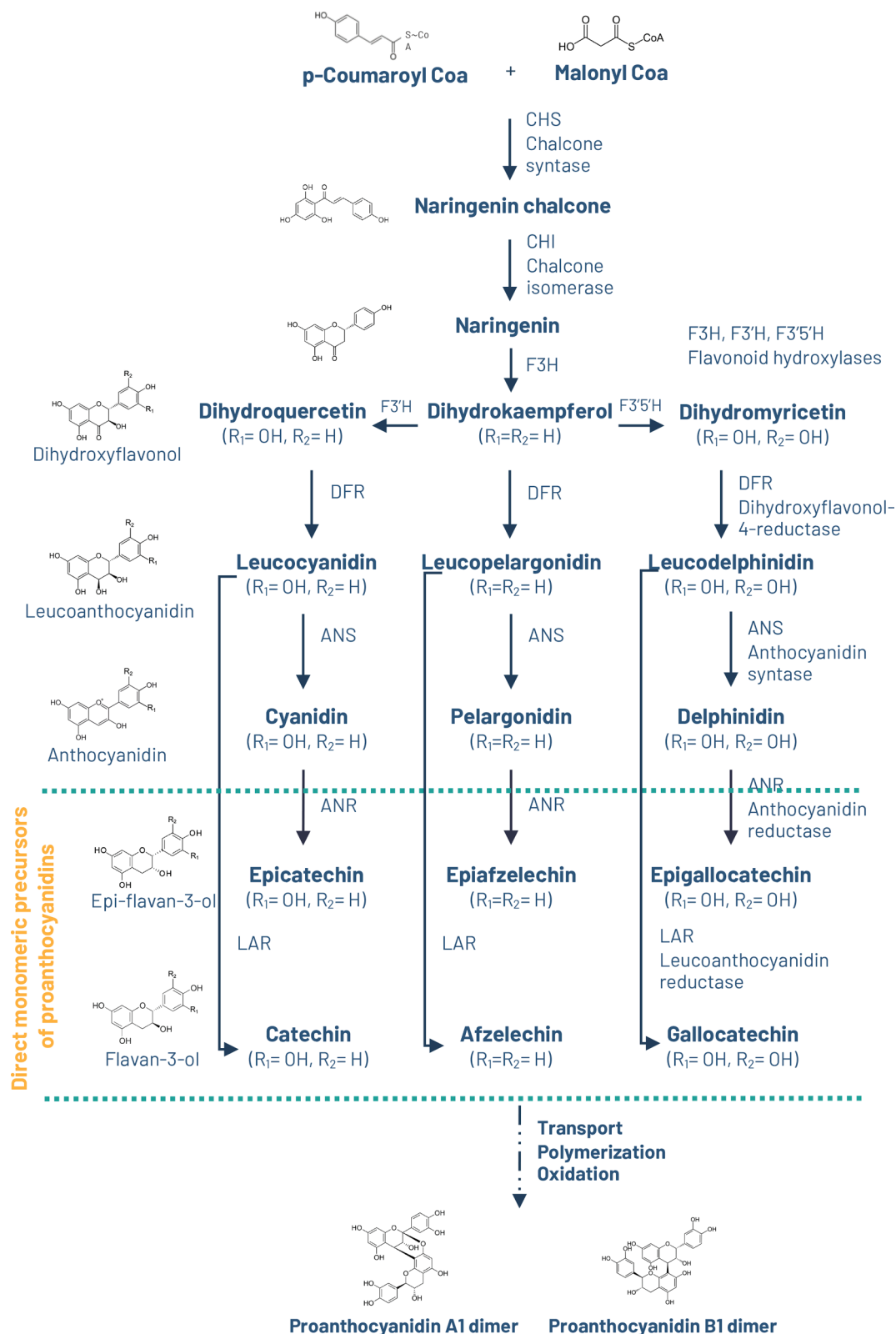


Fig. 6. Pathways of condensed tannin generation (Arbenz & Avérous, 2015).

monomers takes place in the cytosol and then they are gathered together with glycosylated flavan-3-ols in vesicles (tannosomes) and, through specific transporters, into vacuoles where they are polymerized, in an isolated compartment. Another very common transformation of monomers but still not fully understood is galloylation. Biosynthesis of proanthocyanidins is also regulated by numerous external factors such

as temperature, light and development stage of the plant, among others (Montes-Ávila et al., 2017).

#### 4.3. Biosynthesis of phlorotannins

There is still little information regarding the biosynthetic pathways



of the phlorotannins, in particular about several of the intermediate steps. It is thought that phloroglucinol monomers are biosynthesized via the acetate/malonate pathway, around the nucleus in the Golgi apparatus. The process starts with the initial conversion of two acetyl-CoA molecules to malonyl-CoA, in presence of carbon dioxide. Type III polyketide synthase leads then to cyclization and tautomerisation of malonyl-CoA, generating phloroglucinol (Emeline et al., 2021).

## 5. Extraction techniques

Tannins are widely present in nature and are a constituent part of our diet. However, to produce nutraceutical products rich in these phyto-complexes, it is necessary to extract them from the plant parts that are richest in them.

There are no universal standard extraction conditions for tannins and, in general, the procedures are optimized in accordance with the specific sample. There are many factors that could determine the generation of artifacts, so the choice of the right extraction process is crucial to avoid it. As cited before, tannins could form reversible complexes with other natural macromolecules (i.e. polysaccharides and proteins) during the normal physiological plant development or due to the extraction process. Other alterations that may arise during tannin extraction are related to the light-sensitivity of proanthocyanidins or the hydrolysis susceptibility of hydrolysable tannins. In view of this, the choice of the correct time and temperature of extraction, solvent polarities or sample/solvent ratio becomes crucial and determines the final composition and yield of the extracts (Das et al., 2020).

At the industrial level, there are various extraction methods, by solid-liquid extraction, that involve a more or less extensive use of solvents, but it must be borne in mind that only a few of these are permitted for use in foodstuffs (i.e. water or ethanol). This is why the extraction of tannins for food purposes (hence also for nutraceuticals) mainly involves the use of traditional methods such as maceration or decoction, with extensive use of water as a solvent.

Maceration is the most basic technique of extraction, consisting of placing suitably prepared plant material in a closed vessel together with the corresponding amount of solvent. Extraction takes place at first by osmosis and subsequently also by diffusion. Even though this technique has always been considered a popular choice thanks to its ease of application and low price, it presents the limitations of long times and relatively low yield.

Decoction further requires the use of high temperature (100 °C), which helps in pulling out tannins from the material together with the affinity of the solvent. Decoction is particularly effective for extracting condensed tannins. Similar to maceration, this method is easy and cheap to apply, and it is suitable for all production scales (Cuong et al., 2020).

The above-mentioned techniques, however, have serious issues in terms of the environment friendliness and sustainability, such as a very large waste of water, so greener techniques are being explored, which on the one hand reduce waste and on the other hand possibly increase the extraction yield (de Hoyos-Martínez et al., 2019).

The pressurized water extraction is a method used to reduce the handling time and the solvent consumption, by using high temperatures exceeding the boiling point and pressures above atmospheric pressure. The supercritical fluid extraction, instead, requires the use of fluids at their critical point of temperature and pressure, thus sharing features between liquids and gases.

Undoubtedly, among the methods that are being studied with most interest are microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). MAE combines traditional solvents with rapid microwave heating. The improvement of the extraction process is related to the rapid heating with the direct interaction of electromagnetic radiation of the solvent (which increases solubility), the material (which increases its porosity, thus its ability to be penetrated) or both. MAE resulted to be very effective for both tannins obtained from terrestrial plants and phlorotannins, reducing timing and increasing the

purity of the final product (Nisca et al., 2022; Zhang et al., 2018). The same is observed for UAE, which, alternatively, exploits the formation of microbubbles at frequencies over 20 kHz, resulting in mechanical vibrations that break up plant matrix tissues and facilitate solvent penetration, with a higher final yield (Dong et al., 2021; Ummat et al., 2020).

Higher extraction yields and amounts of tannins recovered make both MAE and UAE the best future perspectives for tannin extraction and some authors also showed a possible synergistic effect by using both methods simultaneously (de Hoyos-Martínez et al., 2019).

In recent years, more innovative extraction techniques are being introduced, relying on biotechnology to increase both the bioavailability and bioactivity of complex compounds, such as tannins. Spontaneous or forced fermentations with selected microorganisms have been introduced for greater control over a specific end product (Patrignani et al., 2020). This type of techniques derives from the widespread production of fermented foodstuffs, for which lactic acid bacteria were originally applied (Patrignani et al., 2020). Many of the studies conducted to date have focused on the reproduction of deglycosylation, deesterification, decarboxylation and demethylation reactions of phenolic compounds occurring in food (Septembre-Malaterre et al., 2018). However, just few *in vivo* studies have provided relevant information on the bioavailability and metabolism of polyphenols following fermentation, and most concern isoflavones (Hsiao et al., 2020; Piao & Eun, 2020).

As regards tannins, it could be interesting to apply this type of technique to obtain so-called 'postbiotic metabolites', which, in addition to having a higher bioavailability than the parent compounds, have very interesting biological effects at a systemic level (González-Sarrías et al., 2017).

Possible postbiotic metabolites include the urolithins studied in depth by the group of Tomás-Barberán (García-Villalba et al., 2020, 2022). Also of interest is exploiting the stimulation of short-chain fatty acid (SCFA) production by probiotic bacteria and other types of gut microbes, demonstrated in previous studies both *in vitro* and *in vivo* (Molino et al., 2018, 2021; Molino et al., 2022a). In fact, it is now recognised that tannins, similarly to complex carbohydrates, are able to interact with the human microbiota, producing a prebiotic effect (Gibson et al., 2017).

## 6. Tannin bioactivity is related to their chemistry

Tannins have been studied during years due to their beneficial properties including anti-inflammatory, antidiabetic, anti-hypercholesterolemic, and anticancer activity (Maugeri et al., 2022; Meng et al., 2021; Pizzi, 2019).

However, all these biological activities can be largely attributed to the chemical structure of tannins, which in turn determines their physical and chemical properties. The molecular weight also seems to play a role in their biological properties. For instance, some authors reported that for phlorotannins an increase in degree of polymerization results in a reduction in antioxidant activity, showing the strongest antioxidant activity with a degree of polymerization between 4 and 12, probably due to masking effects through the formation of hydrogen bonds (Hermund et al., 2018).

Tannins share with the other polyphenols a great content in hydroxyl groups (-OH), which confer to them a primarily antioxidant action. Moreover, the particularly distinguishing feature of tannins, their ability to complex with macromolecules (such as proteins and polysaccharides) and with divalent metals, endows them with complex biological activity. In fact, all these different types of molecules that can be bound represent possible molecular and cellular targets, such as membrane proteins, enzymes and pro-inflammatory signalling cascades.

### 6.1. Chemical structure and antioxidant activity

Tannins are good antioxidants thanks to the benzene ring skeleton and the phenolic hydroxyl group that can easily harbour the singlet

electron to stabilize free radical intermediates. Therefore, this direct antioxidant action consists of ensuring a balance by quick reduction–oxidation reactions between the pro-oxidant molecules and tannins themselves.

Tannins can exert their function primarily at cellular but also physiological levels, presenting themselves as promising agents for preventing or treating several pathophysiological conditions. An example is reported by several studies conducted on proanthocyanidin B2, which characterizes grape seed extract and, thanks to its antioxidant activity, determines important effects on cardiac well-being and inflammatory states (Gil-Cardoso et al., 2017; Tousson et al., 2018). In addition, the antioxidant effect could also determine cascading effects that may prevent the growth of cancer cells, both in the case of tannins produced by terrestrial plants and those produced by brown algae (Barbe et al., 2019; Shrestha et al., 2021).

This marked activity has received attention not only for applications to maintain human well-being, but also for a technological purpose to preserve products, preventing their degradation. In fact, an approach for preserving food products with natural substances to eliminate all chemically derived compounds is gaining momentum. Thus, in 2003 Beninger & Hosfield compared the antioxidant potential of tannins with that of a well-known chemical antioxidant, BHT (butylated hydroxytoluene). Among others, delphinidin and petunidin-3- glucoside showed an antioxidant activity comparable to 50% of the BHT (Beninger & Hosfield, 2003). A more recent study confirmed the same capacity for phlorotannins (Liu et al., 2017).

Apart from direct antioxidant capacity, much of the antioxidant activity of tannins is due to an indirect action. Indeed, these interesting bioactive molecules could act also as preventive antioxidants (co-antioxidant agents, inhibitors of oxidation catalysts) to maintain the right antioxidant/prooxidant balance. In the latter case, antioxidant action is carried out due to the tannin ability to complex with other molecules (i. e. metals or proteins), a topic that will be discussed in the following sections. Finally, hormetic processes, in which tannins can provide pro-oxidants capable of upregulating the expression of enzymes of fatty acid detoxification pathways, could exert an additional protective action (Franco et al., 2019).

## 6.2. Interaction with molecules

The popularity of the long tradition of tannin use in the tanning of hides, thus the precipitation of their proteins, has made this aspect a key element in their definition.

The field of nutrition and food sciences has mostly studied the interactions of tannins with proteins or metals, mainly due to their anti-nutritional potential (Chung et al., 1998). However, similarly to other polyphenols, these compounds can interact with many other types of molecules and, through scientific research, this ability could be exploited and find application in many fields such as the nutraceutical or even food technology industries, among others.

### 6.2.1. Tannin-Protein interactions

Tannins have a peculiar affinity for binding proteins, but the interactions are influenced by several factors. Protein binding can be covalent (as a result of the transition of the tannin molecule into a phenolic radical form or a quinone) or non-covalent (e.g. hydrogen and/or hydrophobic bonds). In the first case, the tannins should undergo oxidation to become quinones and semiquinones, which show high affinity for forming covalent bonds with protein subunits (amino acids) capable of donating electrons (e.g. lysine and cysteine) (Adrar et al., 2019). Recently, some authors reported that UVA-oxidized phlorotannins could find an application to improve myofibrillar protein gel quality (Wang et al., 2021). However, covalent bonds between tannins and proteins are still a matter of debate, while the function of non-covalent bonds is well recognised.

The principal driving forces implicated in non-covalent bonds are

hydrophobic interactions and hydrogen bonds, mainly hydrophobic weak interactions, which occur mostly at surface level (de Freitas & Nuno, 2012). The association between tannins and proteins occurs in a specific and selective way and has been summarized in three steps: (I) The generation of the first tannin-protein complexes is ensured by hydrogen bonds and hydrophobic interactions (i.e. van der Waals forces). The bonds can be generated at several sites in the protein, leading to condensation and subsequent generation of a spherical structure. (II) Protein-tannin complexes associate into larger structures with cross-links. (III) The combination of the large aggregates generates colloid-sized particles, possibly resulting in precipitation of the protein-tannin complexes (Molino et al., 2019).

The interaction of tannin with proteins is one of the main mechanisms of biological activity of tannins. It is known that this interaction is determined by both the structure of tannins and the structure of the protein. In the case of proteins, their size, charge, side chains, and conformation play a role, so that tannins interact worse with globular proteins. On the other hand, the tannins' molecular weight and degree of galloylation can influence their affinity for proteins, probably because tannin size determines the number of interaction sites. Nevertheless, it has to be taken into account that a large tannin structure can cause steric hindrance and impede access to binding sites, limiting solubility (Baxter et al., 1997). This aspect was recently investigated in more detail by Engström and co-workers (2022), who investigated the protein precipitation capacity of 20 different hydrolysable tannins, based on their structural and precipitate characteristics. The results showed that for some hydrolysable tannins total binding to proteins is never achieved, due to a certain solubilisation tendency of the tannins themselves, but also to a different affinity for proteins. This therefore confirms that tannin-protein interactions are highly specific depending on the precipitation capacity of the proteins and the stoichiometry of the precipitation reaction (Engström et al., 2022).

It is also important to underline that the flexibility of the tannin molecule plays an important role in the interaction with proteins, which explains the important biological activity of hydrolysable tannins against different bacterial toxins. For example, Olchowik-Grabarek et al. (2020) found that hydrolyxable tannins with galloyl group were more active against the hemolytic toxin of *Staphylococcus aureus* than those with valoneoyl group, due to the lower flexibility of such molecules. Shimamura et al. (2016) who found that those polyphenols with 1 or 2 hexahydroxydiphenoyl groups and/or a galloyl group were more active against the staphylococcal enterotoxin A (SEA) described similar results. Çin line with this, Maatsola et al. (2020) described that monomeric hydrolysable tannins containing three to four flexible galloyl groups have the highest inhibitory activity against *Streptococcus pneumoniae* haemolysin, followed by oligomers. In fact, in the case of oligomers, macrocyclic and C-glycosidic structures were weaker in their inhibition than the glucopyranose-based oligomers. Finally, in the case of condensed tannins, Lorenz et al. (2014) found that although the type of condensed tannin affect protein solubility, their degree of polymerization or proportion of cis–trans flavanol subunits could not explain precipitation capacities.

Protein-tannin interaction can also be limited by the interference of other molecules, such as polysaccharides (i.e., arabic gum, pectin, gellan, polygalacturonic acid, and xanthan), which could compete with proteins for binding tannins. This issue is not yet totally understood because it has been shown that the presence of polysaccharides, in particular in mannoproteins, can inhibit the evolution of tannin aggregate particle size but not their generation (Riou et al., 2002). Riou et al. (2002) proposed that the underlying mechanism is related to the generation of a ternary soluble complex among protein, tannin, and polysaccharide. However other authors hypothesized that tannins could be encapsulated by polysaccharides, thus limiting their aggregation with proteins (Riou et al., 2002).

In the food field, tannin-protein interactions have mostly been investigated for oenology, due to the fundamental role of tannins in

imparting key sensory characteristics to wine (such as astringency, roughness, dryness and bitterness) (S. Wang et al., 2023). In view of this, a large part of the studies on the interaction of these macromolecules has focused also on the mechanisms that occur with the first contact of tannins with proline-rich salivary proteins (PRP), in the mouth (Molino et al., 2019). However, innovative applications for this type of interaction have also begun to be found in recent years, such as in the case of emulsions in which covalent protein–tannin interactions are more desirable due to the greater stability that irreversible bonds can confer (Pham et al., 2019).

Binding with different types of proteins has many possible applications in nutraceuticals and several beneficial properties of tannins are related to this. Maugeri et al. (2022) report a series of interactions of tannins with various protein molecules, such as the inhibition of pro-oxidant enzymes such as NADPH oxidase of the NOX family, which is considered the major source of oxidative stress.

Interestingly, many of the mechanisms that lead to antioxidant activity are also closely linked to an anti-inflammatory effect and thus tannins can also be considered effective anti-inflammatory agents. Several authors have reported the ability of tannins to inhibit phospholipase A2 and the activities of Keap1 and NF- $\kappa$ B, proteins involved in the regulation of the inflammatory cascade (Adrar et al., 2019; Meng et al., 2021; Zhang et al., 2018).

Binding of enzymes and their subsequent inhibition also has important effects on the prevention of diseases. For example, several authors have reported neuroprotective effects by phlorotannins through the inhibition of enzymes of different kinds (such as glycogen synthase, kinase BACE1 and cholinesterases) and by binding neuronal receptors (Seong et al., 2019; Wang et al., 2018).

The impact on diabetes of tannin inhibition of enzymes involved in starch breakdown has also been studied. The hydrogen bonds and Van der Waals forces are key players in the interactions between tannin extracts (from both terrestrial plants and seaweeds) and  $\alpha$ -amylase and  $\alpha$ -glucosidase (Les et al., 2018; Meng et al., 2021; Sun et al., 2018).

Tannin antibacterial and antiviral effects are also related to tannin-protein interaction. In the first case, tannins showed bacteriostatic and bactericidal activity by inactivating the enzymes involved in bacterial cell wall synthesis, inhibiting ATPase enzymes in the cell membrane involved in bacterial growth, and inactivating several virulence factors (G. Dong et al., 2018; Farha et al., 2020; Olchowik-Grabarek et al., 2022).

Tannins derived from terrestrial plants resulted in a significant antiviral activity by inhibiting the expression of surface proteins, viz, hemagglutinin and neuraminidase (Adrar et al., 2019; Vilhelmova-Ilieva et al., 2020). Similar activities were shown by phlorotannins and, in particular, E. cava extract inhibited chymotrypsin-like cysteine proteinase, essential for viral replication (Park et al., 2013; Shrestha et al., 2021).

### 6.2.2. Tannin-Polysaccharide interactions

This type of interaction is well known and exploited, particularly in the beverage sector. Tannin-polysaccharide associations are spontaneous, quick and direct binding events and occur during the processing of vegetables and fruits. The complexation occurs in a very similar manner to that with proteins described above. Like for other polyphenols, non-covalent interactions, such as hydrogen bonds and hydrophobic effects are the key players in these interaction mechanisms. Additionally, charged groups of anthocyanins can also interact with those of polysaccharides through electrostatic interactions (Fernandes et al., 2020; Koh et al., 2020). The associations with different macromolecules can be distinguished by different kinetics and colloidal consequences. Ionic strength and a lower temperature can boost the interactions between tannins and polysaccharides, and high degree of polymerization corresponds to higher affinities (de Freitas & Nuno, 2012).

These types of interaction find a large application in oenology to

control astringency and clarification, thus improving the quality of wine in terms of roundness and sweetness (Graves & Sommer, 2021; Zhai et al., 2023).

Pear and apple have also been used as models given the large application of proanthocyanidins for clarification of fruit juices (Li et al., 2018).

Although the study of the anti-diabetic effect of tannins has focused in recent years on the ability of tannins to interact with proteins, thus with enzymes, some authors have previously also proposed a possible effect due to interaction directly with polysaccharides from the diet. Indeed, the regulation of the glycaemic state in diabetes could also depend on the reduction of the availability of glucose in the gastrointestinal tract, with a consequent delay in polysaccharide digestion (Serrano et al., 2009).

Tannin-polysaccharide combinations have also been studied for the production of nutraceutical products from a technological point of view. Indeed, there are many compounds that require protection in order to be administered and to arrive intact at the site of action. The microencapsulation technique is often used for this purpose and some authors proposed to use amidated pectin as a microencapsulation matrix. In particular, a study found that the interaction of tannins with pectin could enhance the structural properties of the beads themselves, achieving improved morphologies, smaller bead sizes and reduced extent of swelling (Molino et al., 2022b).

### 6.2.3. Tannin-Lipid interactions

Lipids are essential molecules involved in numerous biological processes, including energy storage, structural integrity, and cellular signaling. Understanding the interaction between tannins and lipids is crucial for comprehending their potential health benefits and implications in nutrition and medicine. In fact, the main interaction of tannins and cells are related with the physical interactions in the lipidic bilayer through hydrophobic interactions, electrostatic interactions, hydrogen bonding, and Van der Waals forces (Karonen, 2022). One common mode of interaction is the hydrophobic interaction between the hydrophobic regions of tannins and lipids (Furlan et al., 2015). Tannins, particularly those with a high degree of hydrophobicity, can insert themselves into lipid bilayers, leading to alterations in membrane properties (Virtanen et al., 2022). Another important mechanism of interaction is hydrogen bonding between tannins and lipids. Hydrogen bonding occurs between the hydroxyl groups of tannins and the polar head groups of lipids, leading to the formation of intermolecular bonds and affecting membrane fluidity and stability (Virtanen et al., 2021). In fact, a recent model of the interaction of hydrolyzable tannins and cell membrane lipids (Borisova et al., 2019) states that the molecules of hydrolyzable tannin bind electrostatically to the outer groups of the membrane protein responsible for the Ca<sup>2+</sup>-dependent chloride current and blocks it; in addition, some tannin molecules also penetrate into the hydrophobic region of the membrane, and when a particular concentration is reached, they form ion-conducting structures selective toward Cl<sup>-</sup>. All these interactions can have implications for various cellular processes, including membrane signaling, transport, and stability (Sekowski et al., 2016).

Moreover, tannins present the ability of inhibiting lipid peroxidation, which is a pathogenic signature of stressful conditions in cellular pro-oxidant states (Fraga-Corral et al., 2021). In this sense, proanthocyanidins help to restore the oxidative balance of the body by scavenging hydroxyl, superoxide and peroxy radicals and through the inhibition of the lipoxygenases (Smeriglio et al., 2017). In addition, lipid oxidation is a major concern in the food industry, leading to rancidity and loss of nutritional value. In this sense, tannins can also mitigate lipid oxidation by inhibiting the formation of reactive oxygen species and chelating metal ions that catalyze oxidation reactions (Zeng et al., 2019). This antioxidative action of tannins helps preserve the quality and stability of lipid-rich foods.

Tannins have been shown to influence lipid metabolism in several

ways. For example, tannins reduce lipid absorption in the gut through the formation of tannin-lipid complexes (Furlan et al., 2015), altering the physical properties of the lipids and potentially affecting their bioavailability and functionality. For example, the hypotriglyceridaemic effect of proanthocyanidins is related with the impairment of lipid availability (reduced absorption) in enterocytes, with an increased faecal excretion of cholesterol (Tebib et al., 1994). Additionally, tannins can modulate lipid metabolism by altering gene expression and enzymatic activity involved in lipogenesis, lipolysis, and cholesterol synthesis (Fang et al., 2022). These effects highlight the potential of tannins as therapeutic agents in managing metabolic disorders.

#### 6.2.4. Interaction of tannins with organic non-protein N compounds

The interest in studying the complexation between tannins and organic N compounds other than proteins arose following the discovery of tannic acid's ability to bind and precipitate choline, an amine and the precursor of the neurotransmitter acetylcholine (Kalina & Pease, 1977). Adamczyk et al. (2011) showed that tannins can react with a wide set of organic N compounds, including arginine, nitrogen bases, polyamines, chitin, and chitosan. Such reactions called for a change in the way of thinking about tannins: they can react with non-protein organic N compounds similarly to their reaction with proteins (Adamczyk et al., 2011).

As for tannin-protein interactions, reactions are influenced by the chemical structure of the molecules, and by the concentration and pH of the solution; and a greater ability to form multiple hydrogen bonds corresponds to a higher ease of forming complexes with tannins. For some compounds (i.e. polyamines, proteinaceous amino acids and nitrogen bases) a higher molecular mass and the presence of more amine groups determined a stronger reactivity towards tannins. For example, this is the case of arginine, the amino acid with the highest number of amine groups and almost the highest molecular mass (174 Da); and for spermine, the polyamine with the highest molecular mass (202 Da) and number of amine groups (Croteau et al., 2000), the two nitrogen bases with no amine groups present the lowest reactivity.

#### 6.2.5. Tannin-Metal ion interactions

Tannins are excellent chelators of metals, such as Fe(III), Al(III) and Cu(II), chelation being the main type of binding related to exchanges between phenolic hydroxyl groups and metal ions (Zeng et al., 2019).

The complexation with iron is the one that has been most studied of all. Each ferric ion can bind several tannin molecules simultaneously, being able to bind to at least three o-dihydroxyphenyl groups, and the generated lattice creates a precipitate. Once again, the chemical structure of the tannins has an influence with the interaction with the molecules. For example, a higher number of galloyl groups results in a higher iron-binding capacity by gallotannins. However, if these are characterised by a higher degree of galloylation, the interaction is hampered by steric hindrance and chelation efficiency is reduced (Engels et al., 2011).

Complexation of such metal ions by tannins has been largely studied in the past for its possible antinutrient consequences. This assumption has been related to the formation of stable tannin-Fe(III) complexes in the gut, which potentially inhibit the absorption of non-haem dietary iron through the gut barrier, after the intake of polyphenol-rich beverages such as wine or tea together with Fe(III) (Cook et al., 1995; Hurrell et al., 1999). Thus, some authors have reported that the reduction of the iron absorption could depend on the simultaneous ingestion of other foodstuffs such as complex composite meals (Cook et al., 1995), tea polyphenols (Disler et al., 1975), and ascorbic acid (Siegenberg et al., 1991).

However, the great concern about the antinutritional potential of tannins with regard to metal intake is being revised. Indeed, the extensive examination of interventional and epidemiological studies by Delimont et al. (2017) highlighted that most of the studies conducted in the past were obscured by several factors, such as lack of control of

concurrent antinutritional or iron-enhancing factors in diets, tannin-iron interaction in the food matrix as well as assessment of iron deficiency, rather than adequate iron stores. The authors concluded that long-term tannin consumption may impact iron status in a different manner, with the development of mechanisms of adaptation to tannins that could reduce the antinutritional effects over time. Indeed, this effect has emerged in general epidemiologic studies and long-term trials in which tannin consumption does not affect the iron status of individuals (Delimont et al., 2017).

Little is known about the influence of tannins and other polyphenols on the gut absorption of metal ions other than Fe(III), but this influence may also be relevant.

As far as aluminium is concerned, chelation by tannins is considered to be beneficial. There is an apparent link between metal dyshomeostasis and neurodegenerative diseases. In particular, the presence of Al (III) in drinking water has been often associated with an increase in Alzheimer disease risk (Molino et al., 2016; Santos-Buelga & Scalbert, 2000). This apparent correlation sparked the idea that metal chelation could be used therapeutically. Several publications reported that the intake of proanthocyanidins present in wine or tea were associated with a reduction of the risk of senile dementia (Cook et al., 1995; Hurrell et al., 1999). Similarly, in animal models of neurodegeneration various iron chelators showed neuroprotective effects (Mandel et al., 2011).

The influence of tea on Cu(II) absorption has been studied in rats (Greger & Lyle, 1988). Tea consumption caused an increase in copper absorption, in the plasma level of ceruloplasmin and in the copper retention in tissues, particularly in the liver. Finally, the low affinity of zinc for polyphenols at acidic and neutral pH is consistent with the lack of a significant effect of tea, wine and beer on its bioavailability in humans (Santos-Buelga & Scalbert, 2000).

In recent years, some authors have pointed out that tannins, in particular condensed tannins, could be used as potential modulators of metal absorption, to avoid exceeding healthy limits (Zhang et al., 2022). In the works of Zeng and collaborators, the effectiveness of precipitation of different metal ions by condensed tannins was compared and it decreased with the order of  $Fe^{2+} > Cu^{2+} > Zn^{2+}$  (Zeng et al., 2019a; Zeng et al., 2019b). Then, Zhang et al. (2022) further pointed out that trivalent metal ions exhibit higher affinities for condensed tannins than divalent ones (Zhang et al., 2022). Similar results were obtained from gallotannins, which could combine with metal ions but also further interact with protein to form tannin-metal-protein ternary complexes (Zhang et al., 2021).

## 7. Metabolic fate of tannins in humans.

In order to exploit the potential of tannins as nutraceuticals, it is also essential to understand their metabolic fate, which will ultimately influence their biological activities in the human body. Nowadays, the complete metabolic fate and bioavailability of tannins, or their metabolites, are not yet fully understood. Unlike monomeric phenols, which have been extensively studied in both animals and humans, polymeric tannins are a difficult matter of study due to their complexity and variability in chemical structure. Most consumed tannins can reach the colon almost intact, where the high molecular weight structures cannot be readily absorbed and must be further metabolised by the gut microbiota. While several studies have been conducted on the metabolism of ellagitannins and condensed tannins, phlorotannins are still unexplored. Only one study has been detected and provides no clear conclusions on the biotransformation of this class of tannins produced by brown algae (Corona et al., 2016).

### 7.1. Metabolism of hydrolysable tannins

Due to the low occurrence of gallotannins in food and their low intake, the metabolism of these compounds has been poorly investigated. After intake by healthy volunteers of gallotannin-rich fruits

several metabolites were identified in urine, such as methyl gallic acid, gallic acid sulphate, gallic acid glucuronide, galloylquinic acid, catechol sulphate, catechol glucuronide, pyrogallol sulphate, deoxypyrogallol sulphate, methyl pyrogallol sulphate and pyrogallol glucuronide (Barnes et al., 2016; Mosele, Macià, & Motilva, 2016).

Gallotannins are degraded through enzymatic activity during digestion and partly through metabolism by the gut microbiota. Comprehensive information on the metabolic pathways of gallotannins is not available, so Mosele et al. (2016) attempted to investigate the degradation steps of these phytocomplexes through human digestion and fermentation in vitro (Mosele, Macià, Romero, et al., 2016). Already under low-acid conditions, which simulate the gastrointestinal tract, hydrolysis causes a slight degradation of the original gallotannins depending on the level of gallic acid esterification.

After enzymatic digestion, higher molecular weight tannins from dodeca- to octa- O-galloyl glucoses are transformed to lower weight hepta- to tetra-O-galloyl glucoses with the additional production of free gallic acid, digallic acid and galloyl- shikimic acid. Finally, the action of the gut microbiota leads to gallic acid, protocatechuic acid and p-hydroxybenzoic acid production. Moreover, gallic acid can be further degraded to a simple catechol (Mosele, Macià, Romero, et al., 2016). Although little data is available in the literature, the information available so far suggests that mostly low molecular weight metabolites may be responsible for the biological activity of this class of tannins (Luca et al., 2020).

On the other hand, the metabolic fate of ellagitannins has been extensively documented. This class of tannins has never been detected in human plasma after intake of ellagitannin-rich foods (García-Muñoz & Vaillant, 2014; Tao et al., 2019). Among the causes that have been identified for this bioavailability impediment are the large size of the molecules and the possible interaction with proteins in saliva or in food (Mena et al., 2015). Ellagitannin degradation starts in the upper gastrointestinal tract and depends on several factors such as their chemical structure, their susceptibility to hydrolysis in the stomach and duodenum, as well as their potential interaction with the food matrix (García-Muñoz & Vaillant, 2014).

As most ellagitannins are sensitive to acidic and basic hydrolysis, they release the lactone ellagic acid in the stomach or small intestine, respectively. This molecule presents a low bioavailability probably due to several factors, i.e. its scarce water solubility, and its capacity to establish irreversible bindings with proteins, or to form poorly soluble complexes with calcium and magnesium ions. The small amounts of ellagic acid absorbed in the intestinal tract are found in plasma in conjugated form with methyl, sulfate, glucuronyl groups (Sallam et al., 2021).

At the same time, a subset of ellagitannins is resistant to enzymatic digestion, reaching almost intact the large intestine where they can potentially exert their biological activity and can be partially converted into ellagic acid by enzymes from the microbiota.

Ellagic acid can be further metabolised by the gut microbiota into urolithins (Uro, dibenzopyran-6-one metabolites) through reduction of one of the two lactone groups followed by decarboxylation and sequential dehydroxylation involving a subsequent reduction to tetrahydroxy (urolithin D), trihydroxy (urolithin C), dihydroxy (urolithin A and isourolithin A), and monohydroxy dibenzopyranones (urolithin B) (Aguilar-Zárate et al., 2017; Milala et al., 2017; X. Zhang et al., 2018). At present, just a few human bacterial strains, i.e. *Bifidobacterium pseudocatenulatum*, *Gordonibacter urolithinifaciens* and *Gordonibacter pamelaiae*, have been identified as responsible for the production of these metabolites (Beltrán et al., 2018; García-Villalba et al., 2022; Gaya et al., 2018). Based on the gut microbiota composition of each individual and thus the ability to produce urolithins, a classification into metabolotypes (A, B and O) was created. Even though the classification appeared to be consistent across multiple intervention studies, the factors determining the distribution of urolithin metabolotypes are not yet clear (García-Conesa et al., 2018).

As regards the metabolism of the other moieties of ellagitannins, not much is known (Piwowarski et al., 2016).

## 7.2. Metabolism of condensed tannins

Non-microbiota mediated metabolism of proanthocyanidins can be separated into two parts: the gastric and intestinal part. Some oligomeric and polymeric proanthocyanidins may already be subject to degradation into oligomers, monomers, and some further metabolites in the acidic gastric environment. Nevertheless, a definitive consensus on this has not yet been reached (Tao et al., 2019). The presence of food macronutrients may also affect the degradation of condensed tannins. For instance, formation of emulsions in the presence of fat could exert a protective effect for proanthocyanidins against degradation (Giltekin-Özğiven et al., 2016). In the presence of carbohydrates, the absorption rate of molecules such as catechin and epicatechin may be increased while that of epigallocatechin, epigallocatechin gallate and other oligomers may be reduced. The causes could be ascribed to a specific effect of carbohydrates on gastrointestinal physiology or an unidentified carbohydrate-flavanol transporter (Luca et al., 2020).

In the intestine, condensed tannins are absorbed in different parts based on their structure and molecular weight. Proanthocyanidin monomers can be readily absorbed in the proximal intestinal tract, while oligomer and polymer absorption rates decrease with increasing number of hydroxyl groups and molecular size (Pereira-Caro et al., 2018; Wu et al., 2017). The intestinal epithelium does not have specific receptors for condensed tannins, but it plays a significant role in their modification by inducing methylation and glucuronidation and increasing their bioavailability (Zumdzick et al., 2012). Once absorbed, proanthocyanidins and their metabolites can reach different organs and tissues through the circulatory system. Particularly in the liver, proanthocyanidins can be further modified by phase II enzymes (Luca et al., 2020).

Most condensed tannins (~85–90%) reach the large intestine, to be fermented by microbiota and transformed into metabolites and further derivatives, such as phenolic acids and valerolactones (de Moraes Barros et al., 2016; Mena et al., 2017).

An example of microbial metabolism of dimeric proanthocyanidins (dimer B1) was proposed by Tao et al. (2019). This metabolism involves a series of successive reactions with a cleavage of the interflavanic bond or C-ring, followed by oxidation of the A-ring, dihydroxylation and beta-oxidation. Finally, the monomers can be split into two molecules through a hydrolysis reaction. This may result in epigallocatechin (or epicatechin) and gallic acid. Other dimer transformations have been proposed by several authors, who also suggested other types of transformations such as the breaking of the interflavanic bond after cleavage of the C ring in the lower units or, alternatively, the oxidation of the A ring in the lower unit after cleavage of the C ring in the upper unit (Borges et al., 2018).

For condensed tannins with a degree of polymerisation higher than 3, information is scarce, but it appears that the gut microbiota plays a key role. Indeed, several studies found that after consumption of condensed tannins the presence of flavanols in the bloodstream due to possible acid hydrolysis in the stomach was minimal, while metabolites were measured in urine, indicating the importance of the catalytic activity of the microbiota. However, the pathways of oligomeric and polymeric proanthocyanidins still need to be elucidated (Wiese et al., 2015).

Regarding the bacteria involved in the degradation of condensed tannins, several taxa involved have been identified; they may also work in combination, acting simultaneously or sequentially. *Eubacterium oxidoreducens*, belonging to the Firmicutes, seems to be involved in the metabolism of oligomers and polymers, triggering the cleavage of the A-ring (Stoupi et al., 2010).

Other taxa found to be involved in primary degradation of condensed tannins are species belonging to *Eubacterium* (i.e. *cellulosolvens* and *ramulus*), *Bacteroides* (i.e. *uniformis*, *distasonis* and *ovatus*), and also

*Enterococcus casseliflavus* and *Lachnospiraceae* CG191 (Braune & Blaut, 2016). Subsequently, the hydrolysis products undergo ring-cleavage and decarboxylation, giving rise to metabolites such as hydroxyphenylpropionic acid and hydroxyphenylacetic acids (Braune et al., 2016; Braune & Blaut, 2016).

*Flavonifactor plautii* was instead identified as being responsible for the production of small metabolites such as 1-(3', 4'-dihydroxyphenyl)-3-(2'', 4'', 6''-trihydroxyphenyl)propan-2-ol to  $\delta$ -(3', 4'-dihydroxyphenyl)- $\gamma$ -valerolactone and  $\delta$ -(3', 4'-dihydroxyphenyl)- $\gamma$ -valeric acid (Mena et al., 2019). The probiotic bacteria *Streptococcus thermophilus* and *Lactobacillus casei-01* have been associated with the production of various types of phenolic acids, such as 3,4-hydroxyphenylacetic acid, 4-hydroxyphenylpropionic acid, m-coumaric acid and p-coumaric acid, through the metabolization of proanthocyanidins (S. Li et al., 2013).

Microbial metabolism of condensed tannins can also be influenced by the presence of other food components, such as  $\omega$ -3 polyunsaturated fatty acids (PUFAs), which have been shown to facilitate the microbial fermentation of proanthocyanidins (Molinar-Toribio et al., 2017).

## 8. Conclusions and future perspectives

According to available evidence, tannins, secondary metabolites produced by plants, exert numerous beneficial effects on human health and are gaining considerable scientific interest thanks to their promising potential in commercial applications as nutraceuticals.

However, despite much research on these promising compounds there are still several open questions. First of all, classification, which has not yet been fully established. Many authors today still do not take into account the category of phlorotannins, which are produced by brown algae instead of terrestrial plants. It is precisely these tannins that have been studied in recent years, revealing a great deal of potential for health benefits in humans, but there are still many remaining questions. Indeed, if the pathways of biosynthesis of terrestrial tannins are mostly clear, those of phlorotannins have not yet been fully elucidated.

The methods for extracting tannins from different sources are manifold and especially in recent years there has been an outbreak of increasingly refined techniques (i.e. MAE and UAE), mostly aimed at greater efficiency, environmental friendliness and standardisation. However, there are still many challenges for the exploitation of these technologies, which for the time being are mainly designed at laboratory level. In the near future, efforts should be made to achieve a scale-up at industrial scale, abandoning more obsolete currently used methods. Simultaneously, the composition of the new extracts obtained would have to be elucidated in order to understand how it could be exploited for multiple nutraceutical purposes.

At the same time, new biotechnologies could lead to the production of by-products with a much higher bioavailability than common extracts. However, for these technologies to be exploited to the fullest extent, the entire metabolization process of tannins, which is still not fully understood, would have to be clarified.

An open issue is that most of these new advances in tannin extraction could not match the requirements of the different food safety regulations. Nowadays, food legislation does not grow in step with technology, sometimes leading to the development and production of increasingly refined or concentrated products, which, however, cannot be marketed. An example of this is Europe, where Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients states that extracts not obtained by a classical extraction method are considered Novel Food. A future perspective that should not be underestimated is to work towards developing legislation in tandem with science.

The biological properties of tannins are strictly related to their chemical structure, especially their numerous phenolic ring structures together with the hydroxyl groups and the ability to bind other compounds. Given the variety of plants from which they can be produced, tannins can exhibit important structural diversity, but they all share

certain characteristics of composition and activity. Depending on the chemical conformation and the degree of polymerization there may be greater ease of bonding or, conversely, generation of steric hindrance. Thus, fully understanding the chemical properties and structure of tannins is crucial for the exploitation of their potential biological activity mechanism.

The importance of tannins' affinity for proteins is reflected in the wide range of applications they can find, ranging from modulating the sensory aspect of food to the possibility of interacting with bacteria and viruses. Similarly, the bond with other macromolecules has just as many outlets both in food technology and for potential biological effects in the human body. In this respect, it must be pointed out that many studies concerning the interactions of tannins with other molecules often date back several years, so outdated methods of investigation and limited knowledge may have led to inconclusive or sometimes ambiguous conclusions. For example, in the past, the tannin-metal interactions have always been associated only with a detrimental biological effect, whereas more recent studies have shown that these bonds are not always deleterious and above all can be exploited for nutraceutical purposes. On the other hand, the bonds with organic N compounds other than proteins have been studied to a limited extent, so the possible effects on human health have yet to be explored. Continuing to investigate the binding interactions between tannins and potential food sources is essential for subsequent dietary guidance on tannin intake or supplementation.

Finally, it cannot be overlooked that tannins as such do not have a high bioavailability, so, in order to make the best use of these phyto-complexes in the nutraceutical field, it is imperative to consider their metabolism, which is still not entirely clear, as well as the possible biological effects of the metabolites themselves. A goal for research over the next few years is therefore to bridge the gap between the biotransformation of tannins and the resulting potential mechanism of action, making it possible to identify localized molecular signatures that lead to the different biological effects.

In this sense, computational chemistry could represent an interesting tool for conducting studies of pharmacokinetics, toxicological profile, biological activity and their underlying mechanisms of action. Moreover, it could also provide great support in understanding how tannins might be metabolised by our organism (including the gut microbiota), particularly regarding phlorotannins on which evidence is still scarce (Meng et al., 2021).

## Author Contributions

SM designed the research and wrote the manuscript. MPF and JARH provided significant advice and critically edited the manuscript. JARH obtained funding and coordinated the Stance4Health project. All authors contributed to the article and approved the submitted version.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## References

- Adamczyk, B., Adamczyk, S., Smolander, A., & Kitunen, V. (2011). Tannic acid and Norway spruce condensed tannins can precipitate various organic nitrogen compounds. *Soil Biology and Biochemistry*, 43(3), 628–637. <https://doi.org/10.1016/j.soi.2010.11.034>
- Adrar, N. S., Madani, K., & Adrar, S. (2019). Impact of the inhibition of proteins activities and the chemical aspect of polyphenols-proteins interactions. *PharmaNutrition*, 7, Article 100142. <https://doi.org/10.1016/j.phanu.2019.100142>
- Aguilar-Zárate, P., Wong-Paz, J. E., Buenrostro-Figueroa, J. J., Ascacio, J. A., Contreras-Esquivel, J. C., & Aguilar, C. N. (2017). Ellagitannins: Bioavailability, Purification and Biotechnological Degradation. *Mini-Reviews in Medicinal Chemistry*, 17, 1–13.
- Arbenz, A., & Avérous, L. (2015). Chemical modification of tannins to elaborate aromatic biobased macromolecular architectures. *Green Chemistry*, 17(5), 2626–2646. <https://doi.org/10.1039/c5gc00282f>
- Das, A. K., Islam, M. N., Faruk, M. O., Ashaduzzaman, M., Dungani, R., Rosamah, E., Hartati, S., & Rumidatul, A. (2020). Hardwood Tannin: Sources, Utilizations, and Prospects. In A. Aires (Ed.), *Tannins - Structural Properties, Biological Properties and Current Knowledge* (pp. 1–18). IntechOpen. <https://doi.org/10.5772/intechopen.80170>
- Baba, A., Hoshino, T., Ogawa, S., & Takara, T. (2021). Improvement of glucose metabolism and safety of proanthocyanidins derived from acacia bark in healthy Japanese adults: A randomized, double-blind, placebo-controlled, parallel-group comparison trial. *Functional Foods in Health and Disease*, 11(9), 431–455. <https://doi.org/10.31989/FFHD.V11I9.822>
- Barbe, A., Ramé, C., Mellouk, N., Estienne, A., Bongrani, A., Brossaud, A., ... Dupont, J. (2019). Effects of Grape Seed Extract and Proanthocyanidin B2 on In Vitro Proliferation, Viability, Steroidogenesis, Oxidative Stress, and Cell Signaling in Human Granulosa Cells. *International Journal of Molecular Sciences*, 20(17), 4215. <https://doi.org/10.3390/IJMS20174215>
- Barnes, R. C., Krenek, K. A., Meibohm, B., Mertens-Talcott, S. U., & Talcott, S. T. (2016). Urinary metabolites from mango (*Mangifera indica* L. cv. Keitt) galloyl derivatives and in vitro hydrolysis of gallotannins in physiological conditions. *Molecular Nutrition and Food Research*, 60(3), 542–550. <https://doi.org/10.1002/mnfr.201500706>
- de Moraes Barros, H. R., Garcia-Villalba, R., Tomás-Barberán, F. A., & Genovese, M. I. (2016). Evaluation of the distribution and metabolism of polyphenols derived from cupuassu (*Theobroma grandiflorum*) in mice gastrointestinal tract by UPLC-ESI-QTOF. *Journal of Functional Foods*, 22, 477–489. <https://doi.org/10.1016/j.jff.2016.02.009>
- Baxter, N. J., Lilley, T. H., Haslam, E., & Williamson, M. P. (1997). Multiple Interactions between Polyphenols and a Salivary Proline-Rich Protein Repeat Result in Complexation and Precipitation. *Biochemistry*, 36, 5566–5577.
- Beltrán, D., Romo-Vaquero, M., Espín, J. C., Tomás-Barberán, F. A., & Selma, M. V. (2018). Ellagibacter isourolithinifaciens gen. nov., sp. nov., a new member of the family Eggerthellaceae, isolated from human gut. *International Journal of Systematic and Evolutionary Microbiology*, 68(5), 1707–1712. <https://doi.org/10.1099/ijsem.0.002735>
- Beninger, C. W., & Hosfield, G. L. (2003). Antioxidant Activity of Extracts, Condensed Tannin Fractions, and Pure Flavonoids from *Phaseolus vulgaris* L. Seed Coat Color Genotypes. *Journal of Agricultural and Food Chemistry*, 51(27), 7879–7883. <https://doi.org/10.1021/jf0304324>
- Borisova, M. P., Kataev, A. A., & Sivozhelezov, V. S. (2019). Action of tannin on cellular membranes: Novel insights from concerted studies on lipid bilayers and native cells. *Biochimica et Biophysica Acta - Biomembranes*, 1861(6), 1103–1111. <https://doi.org/10.1016/j.bbmem.2019.03.017>
- Borges, G., Ottaviani, J. I., van der Hoof, J. J., Schroeter, H., & Crozier, A. (2018). Absorption, metabolism, distribution and excretion of (–)-epicatechin: A review of recent findings. *Molecular Aspects of Medicine*, 61, 18–30. <https://doi.org/10.1016/j.mam.2017.11.002>
- Braune, A., & Blaut, M. (2016). Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes*, 7(3), 216–234. <https://doi.org/10.1080/19490976.2016.1158395>
- Braune, A., Engst, W., & Blaut, M. (2016). Identification and functional expression of genes encoding flavonoid O- and C-glycosidases in intestinal bacteria. *Environmental Microbiology*, 18(7), 2117–2129. <https://doi.org/10.1111/1462-2920.12864>
- Brillouet, J. M., Romieu, C., Schoefs, B., Solymosi, K., Cheynier, V., Fulcrand, H., ... Conéjéro, G. (2013). The tannosome is an organelle forming condensed tannins in the chlorophyllous organs of Tracheophyta. *Annals of Botany*, 112(6), 1003–1014. <https://doi.org/10.1093/aob/mct168>
- Catarino, M. D., Silva, A. M. S., & Cardoso, S. M. (2017). Fucaceae: A source of bioactive phlorotannins. *International Journal of Molecular Sciences*, 18(6), 1327. <https://doi.org/10.3390/ijms18061327>
- Chase, M. W., Christenhusz, M. J. M., Fay, M. F., Byng, J. W., Judd, W. S., Soltis, D. E., ... Weber, A. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, 181(1), 1–20. <https://doi.org/10.1111/boj.12385>
- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421–464. <https://doi.org/10.1080/10408699891274273>
- Cook, D., Reddy, B., & Hurrell, F. (1995). The effect absorption of red and white in humans on nonheme-iron. *The American Journal of Clinical Nutrition*, 800–804.
- Corona, G., Ji, Y., Aneboonlap, P., Hotchkiss, S., Gill, C., Yaqoob, P., ... Rowland, I. (2016). Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *British Journal of Nutrition*, 115(7), 1240–1253. <https://doi.org/10.1017/S0007114516000210>
- Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). Natural products (Secondary metabolites). In B. Buchanan, W. Gruissem, & R. Jones (Eds.), *Biochemistry & Molecular Biology of Plants* (pp. 1250–1318). American Society of Plant Physiologists.
- Cuong, D. X., Hoan, N. X., Dong, D. H., Thuy, L. T. M., Van, T. N., Thai, H. H., ... Xuan, D. (2020). Tannins: Extraction from Plants. In A. Aires (Ed.), *Tannins - Structural Properties, Biological Properties and Current Knowledge* (pp. 1–20). IntechOpen.
- Das, A. K., Islam, M. N., Faruk, M. O., Ashaduzzaman, M., & Dungani, R. (2020). Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany*, 135, 58–70. <https://doi.org/10.1016/j.sajb.2020.08.008>
- de Freitas, V., & Nuno, M. (2012). Protein/Polyphenol Interactions: Past and Present Contributions. Mechanisms of Astringency Perception. *Current Organic Chemistry*, 16(6), 724–746.
- de Hoyos-Martínez, P. L., Merle, J., Labidi, J., & Charrier - El Bouhtoury, F. (2019). Tannins extraction: A key point for their valorization and cleaner production. *Journal of Cleaner Production*, 206, 1138–1155. <https://doi.org/10.1016/j.jclepro.2018.09.243>
- Delimont, N. M., Haub, M. D., & Lindshield, B. L. (2017). The impact of tannin consumption on iron bioavailability and status: A narrative review. *Current Developments in Nutrition*, 1(2), 1–12. <https://doi.org/10.3945/cdn.116.000042>
- Disler, P. B., Lynch, S. R., Charlton, R. W., Torrance, J. D., Bothwell, T. H., Walker, R. B., & Mayet, F. (1975). The effect of tea on iron absorption. *Gut*, 16, 193–200.
- Dong, G., Liu, H., Yu, X., Zhang, X., Lu, H., Zhou, T., & Cao, J. (2018). Antimicrobial and anti-biofilm activity of tannic acid against *Staphylococcus aureus*. *Natural Product Research*, 32(18), 2225–2228. <https://doi.org/10.1080/14786419.2017.1366485>
- Dong, M., Hu, Y., Zhang, H., Lan, X., Ran, X., Li, Y., ... Han, S. (2021). Ultrasound-assisted extraction of bayberry tannin and optimization using response surface methodology. *BioResources*, 16(1), 1825–1841.
- Emeline, C. B., Ludovic, D., Laurent, V., Catherine, L., Kruse, I., Erwan, A. G., ... Philippe, P. (2021). Induction of Phlorotannins and Gene Expression in the Brown Macroalga *Fucus vesiculosus* in Response to the Herbivore *Littorina littorea*. *Marine Drugs*, 19(4), 185. <https://doi.org/10.3390/MD19040185/S1>
- Engels, C., Schieber, A., & Gänzle, M. G. (2011). Inhibitory spectra and modes of antimicrobial action of gallotannins from mango kernels (*Mangifera indica* L.). *Applied and Environmental Microbiology*, 77(7), 2215–2223. <https://doi.org/10.1128/AEM.02521-10>
- Engström, M. T., Virtanen, V., & Salminen, J.-P. (2022). Influence of the Hydrolyzable Tannin Structure on the Characteristics of Insoluble Hydrolyzable Tannin-Protein Complexes. *Journal of Agricultural and Food Chemistry*, 70, 13036–13048. <https://doi.org/10.1021/acs.jafc.2c01765>
- Fang, J., Zeng, L., He, Y., Liu, X., Zhang, T., & Wang, Q. (2022). Effects of Dietary Tannic Acid on Obesity and Gut Microbiota in C57BL/6J Mice Fed with High-Fat Diet. *Foods*, 11(21), 3325. <https://doi.org/10.3390/foods11213325>
- Farha, A. K., Yang, Q. Q., Kim, G., Li, H. B., Zhu, F., Liu, H. Y., ... Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience*, 38(September), Article 100751. <https://doi.org/10.1016/j.fbio.2020.100751>
- Fernandes, A., Oliveira, J., Fonseca, F., Ferreira-da-Silva, F., Mateus, N., Vincken, J. P., & de Freitas, V. (2020). Molecular binding between anthocyanins and pectic polysaccharides – Unveiling the role of pectic polysaccharides structure. *Food Hydrocolloids*, 102. <https://doi.org/10.1016/j.foodhyd.2019.105625>
- Fraga-Corral, M., Otero, P., Echave, J., Garcia-Oliveira, P., Carpena, M., Jarboui, A., ... Prieto, M. A. (2021). By-products of agri-food industry as tannin-rich sources: A review of tannins' biological activities and their potential for valorization. *Foods*, 10(1), 137. <https://doi.org/10.3390/foods10010137>
- Franco, R., Navarro, G., & Martínez-Pinilla, E. (2019). Hormetic and Mitochondria-Related Mechanisms of Antioxidant Action of Phytochemicals. *Antioxidants*, 8(373), 1–12. <https://doi.org/10.3390/antiox8090373>
- Furlan, C. M. C. M., Barbosa Motta, L., & Alves Cursino dos Santos, D. Y. (2011). Tannins: What do they represent in plant life? In G. K. Petridis (Ed.), *Tannins: Types, Foods Containing, and Nutrition* (Issue May 2014, pp. 1–13). 2010 Nova Science Publishers, Inc.
- Furlan, A. L., Jobin, M. L., Pianet, I., Dufourc, E. J., & Géan, J. (2015). Flavanol/lipid interaction: A novel molecular perspective in the description of wine astringency & bitterness and antioxidant action. *Tetrahedron*, 71(20), 3143–3147. <https://doi.org/10.1016/j.tet.2014.07.106>
- García-Conesa, M. T., Chambers, K., Combet, E., Pinto, P., García-Aloy, M., Andrés-Lacueva, C., ... González-Sarriás, A. (2018). Meta-analysis of the effects of foods and derived products containing ellagitannins and anthocyanins on cardiometabolic biomarkers: Analysis of factors influencing variability of the individual responses. *International Journal of Molecular Sciences*, 19(3), 694. <https://doi.org/10.3390/ijms19030694>
- García-Muñoz, C., & Vaillant, F. (2014). Metabolic Fate of Ellagitannins: Implications for Health, and Research Perspectives for Innovative Functional Foods. *Critical Reviews*

- in *Food Science and Nutrition*, 54(12), 1584–1598. <https://doi.org/10.1080/10408398.2011.644643>
- García-Villalba, R., Beltrán, D., Frutos, M. D., Selma, M. V., Espín, J. C., & Tomás-Barberán, F. A. (2020). Metabolism of different dietary phenolic compounds by the urolithin-producing human-gut bacteria: *Gordonibacter urolithinifaciens* and *Ellagibacter isourolithinifaciens*. *Food and Function*, 11(8), 7012–7022. <https://doi.org/10.1039/d0fo01649g>
- García-Villalba, R., Giménez-Bastida, J. A., Cortés-Martín, A., Ávila-Gálvez, M.Á., Tomás-Barberán, F. A., Selma, M. V., ... González-Sarrías, A. (2022). Urolithins: A Comprehensive Update on their Metabolism, Bioactivity, and Associated Gut Microbiota. *Molecular Nutrition and Food Research*, 66(21), Article e2101019. <https://doi.org/10.1002/mnfr.202101019>
- Gayá, P., Peiroten, Á., Medina, M., Ardévol, A., & Landete, J. M. (2018). *Bifidobacterium pseudocatenulatum* INIA P815: The first bacterium able to produce urolithins A and B from ellagic acid. *Journal of Functional Foods*, 45, 95–99. <https://doi.org/10.1016/j.jff.2018.03.040>
- Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., ... Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology and Hepatology*, 14(8), 491–502. <https://doi.org/10.1038/nrgastro.2017.75>
- Gil-Cardoso, K., Ginés, I., Pinet, M., Ardévol, A., Arola, L., Blay, M., & Terra, X. (2017). Chronic supplementation with dietary proanthocyanidins protects from diet-induced intestinal alterations in obese rats. *Molecular Nutrition & Food Research*, 61(8), 1601039. <https://doi.org/10.1002/mnfr.201601039>
- Giltekin-Özgenç, M., Berktaş, I., & Özelik, B. (2016). Change in stability of procyanidins, antioxidant capacity and in-vitro bioaccessibility during processing of cocoa powder from cocoa beans. *LWT - Food Science and Technology*, 72, 559–565. <https://doi.org/10.1016/j.lwt.2016.04.065>
- González-Sarrías, A., Espín, J. C., & Tomás-Barberán, F. A. (2017). Non-extractable polyphenols produce gut microbiota metabolites that persist in circulation and show anti-inflammatory and free radical-scavenging effects. *Trends in Food Science and Technology*, 69, 281–288. <https://doi.org/10.1016/j.tifs.2017.07.010>
- Graves, J., & Sommer, S. (2021). Polysaccharides influence the results of polymeric pigment analysis in red wines. *ACS Food Science & Technology*, 1(10), 1770–1775. <https://doi.org/10.1021/acfoodsctech.1c00106>
- Greger, J. L., & Lyle, B. J. (1988). Iron, Copper and Zinc Metabolism of Rats Fed Various Levels and Types of Tea. *The Journal of Nutrition*, 118(1).
- Gross, G. G. (1999). Biosynthesis, biodegradation, and cellular localization of hydrolyzable tannins. In U. Romeo, (Ed.), *Phytochemicals in Human Health Protection, Nutrition, and Plant Defense* (pp. 185–213). Kluwer Academic / Plenum.
- Harper, D. (2020). *Tannin*. Online Etymology Dictionary.
- Haslam, E. (2007). Vegetable tannins - Lessons of a phytochemical lifetime. *Phytochemistry*, 68(22–24), 2713–2721. <https://doi.org/10.1016/j.phytochem.2007.09.009>
- Hermund, D. B., Plaza, M., Turner, C., Jónsdóttir, R., Kristinsson, H. G., Jacobsen, C., & Nielsen, K. F. (2018). Structure dependent antioxidant capacity of phlorotannins from Icelandic *Fucus vesiculosus* by UHPLC-DAD-ECD-QTOFMS. *Food Chemistry*, 240, 904–909. <https://doi.org/10.1016/j.foodchem.2017.08.032>
- Hsiao, Y. H., Ho, C. T., & Pan, M. H. (2020). Bioavailability and health benefits of major isoflavone aglycones and their metabolites. *Journal of Functional Foods*, 74, Article 104164. <https://doi.org/10.1016/j.jff.2020.104164>
- Hurrell, R. F., Reddy, M., & Cook, J. D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition*, 81(4), 289–295. <https://doi.org/10.1017/s0007114599000537>
- Imbs, T. I., & Zvyagintseva, T. N. (2018). Phlorotannins are Polyphenolic Metabolites of Brown Algae. *Russian Journal of Marine Biology*, 44(4), 263–273. <https://doi.org/10.1134/S106307401804003X>
- Kalina, M., & Pease, D. C. (1977). The preservation of ultrastructure in saturated phosphatidyl cholines by tannic acid in model systems and type II pneumocytes. *Journal of Cell Biology*, 74(3), 726–741. <https://doi.org/10.1083/jcb.74.3.726>
- Karonen, M. (2022). Insights into Polyphenol-Lipid Interactions: Chemical Methods, Molecular Aspects and Their Effects on Membrane Structures. *Plants*, 11, 1809. <https://doi.org/10.3390/plants11141809>
- Khanbabaee, K., & van Ree, T. (2001). Tannins: Classification and definition. *Natural Product Reports*, 18(6), 641–649. <https://doi.org/10.1039/b101061l>
- Kiss, A. K., & Piwowarski, J. P. (2016). Ellagitannins, Gallotannins and their Metabolites-The Contribution to the Anti-Inflammatory Effect of Food Products and Medicinal Plants. *Current Medicinal Chemistry*, 25(37), 4946–4967. <https://doi.org/10.2174/0929867323666160919111559>
- Koh, J., Xu, Z., & Wicker, L. (2020). Binding kinetics of blueberry pectin-anthocyanins and stabilization by non-covalent interactions. *Food Hydrocolloids*, 99. <https://doi.org/10.1016/j.foodhyd.2019.105354>
- Kumar, L. R. G., Paul, P. T., Anas, K. K., Tejpal, C. S., Chatterjee, N. S., Anupama, T. K., ... Ravishanker, C. N. (2022). Phlorotannins-bioactivity and extraction perspectives. *Journal of Applied Phycology*, 34(4), 2173–2185. <https://doi.org/10.1007/s10811-022-02749-4>
- Les, F., Arbonés-Mainar, J. M., Valero, M. S., & López, V. (2018). Pomegranate polyphenols and urolithin A inhibit  $\alpha$ -glucosidase, dipeptidyl peptidase-4, lipase, triglyceride accumulation and adipogenesis related genes in 3T3-L1 adipocyte-like cells. *Journal of Ethnopharmacology*, 220, 67–74. <https://doi.org/10.1016/j.jep.2018.03.029>
- Li, S., Chen, L., Yang, T., Wu, Q., Lv, Z., Xie, B., & Sun, Z. (2013). Increasing antioxidant activity of procyanidin extracts from the pericarp of Litchi chinensis processing waste by two probiotic bacteria bioconversions. *Journal of Agricultural and Food Chemistry*, 61(10), 2506–2512. <https://doi.org/10.1021/jf305213e>
- Li, S., Wilkinson, K. L., & Bindon, K. A. (2018). Compositional variability in commercial tannin and mannoprotein products. *American Journal of Enology and Viticulture*, 69(2), 176–181.
- Li, Y., Zhu, L., Guo, C., Xue, M., Xia, F., Wang, Y., ... Yuan, C. (2021). Dietary Intake of Hydrolyzable Tannins and Condensed Tannins to Regulate Lipid Metabolism. *Mini-Reviews in Medicinal Chemistry*, 22(13), 1789–1802. <https://doi.org/10.2174/1389557522666211229112223>
- Liu, X., Yuan, W. Q., Sharma-Shivappa, R., & van Zanten, J. (2017). Antioxidant activity of phlorotannins from brown algae. *International Journal of Agricultural and Biological Engineering*, 10(6), 184–191. <https://doi.org/10.25165/J.IJABE.20171006.2854>
- Lorenz, M. M., Alkhafadji, L., Stringano, E., Nilsson, S., Mueller-Harvey, I., & Udén, P. (2014). Relationship between condensed tannin structures and their ability to precipitate feed proteins in the rumen. *Journal of the Science of Food and Agriculture*, 94(5), 963–968. <https://doi.org/10.1002/jsfa.6344>. Epub 2013 Sep 4 PMID: 23934572.
- Luca, S. V., Maccovei, I., Bujor, A., Miron, A., Skalicka-Woźniak, K., Aprotosoia, A. C., & Trifan, A. (2020). Bioactivity of dietary polyphenols: The role of metabolites. *Critical Reviews in Food Science and Nutrition*, 60(4), 626–659. <https://doi.org/10.1080/10408398.2018.1546669>
- Maatsola, S., Kurkinen, S., Engström, M. T., Nyholm, T. K. M., Pentikäinen, O., Salminen, J. P., & Haataja, S. (2020). Inhibition of Pneumolysin Cytotoxicity by Hydrolysable Tannins. *Antibiotics*, 9, 930. <https://doi.org/10.3390/antibiotics9120930>
- Maheswari, V., & Babu, P. A. S. (2022). Phlorotannin and its Derivatives, a Potential Antiviral Molecule from Brown Seaweeds, an Overview. *Russian Journal of Marine Biology*, 48(5), 309–324. <https://doi.org/10.1134/s1063074022050169>
- Mandel, S. A., Amit, T., Weinreb, O., & Youdim, M. B. H. (2011). Understanding the broad-spectrum neuroprotective action profile of green tea polyphenols in aging and neurodegenerative diseases. *Journal of Alzheimer's Disease*, 25(2), 187–208. <https://doi.org/10.3233/JAD-2011-101803>
- Maugeri, A., Lombardo, G. E., Cirmi, S., Süntar, I., Barreca, D., Laganà, G., & Navarra, M. (2022). Pharmacology and toxicology of tannins. *Archives of Toxicology*, 96, 1257–1277. <https://doi.org/10.1007/s00204-022-03250-0>
- Meikieham, N., Pizzi, A., & Stephanou, A. (1994). Induced accelerated autocondensation of polyflavonoid tannins for phenolic polycondensates I. 13C-NMR, 29Si-NMR, X-ray, and polarimetry studies and mechanism. *Journal of Applied Polymer Science*, 54(12), 1827–1845. <https://doi.org/10.1002/app.1994.070541206>
- Mena, P., Bresciani, L., Brindani, N., Ludwig, I. A., Pereira-Caro, G., Angelino, D., ... Del Rio, D. (2019). Phenyl- $\gamma$ -valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: Synthesis, analysis, bioavailability, and bioactivity. *Natural Product Reports*, 36(5), 714–752. <https://doi.org/10.1039/c8np00062j>
- Mena, P., Calani, L., Bruni, R., & Del Rio, D. (2015). Bioactivation of High-Molecular-Weight Polyphenols by the Gut Microbiome. In *Diet-Microbe Interactions in the Gut: Effects on Human Health and Disease* (pp. 73–101). Elsevier Inc.. <https://doi.org/10.1016/B978-0-12-407825-3.00006-X>
- Mena, P., González de Llano, D., Brindani, N., Esteban-Fernández, A., Curti, C., Moreno-Arribas, M. V., ... Bartolomé, B. (2017). 5-(3',4'-Dihydroxyphenyl)- $\gamma$ -valerolactone and its sulphate conjugates, representative circulating metabolites of flavan-3-ols, exhibit anti-adhesive activity against uropathogenic *Escherichia coli* in bladder epithelial cells. *Journal of Functional Foods*, 29, 275–280. <https://doi.org/10.1016/j.jff.2016.12.035>
- Meng, W., Mu, T., Sun, H., & Garcia-Vaquero, M. (2021). Phlorotannins: A review of extraction methods, structural characteristics, bioactivities, bioavailability, and future trends. *Algal Research*, 60. <https://doi.org/10.1016/j.algal.2021.102484>
- Milala, J., Kosmala, M., Karlińska, E., Juśkiewicz, J., Zduńczyk, Z., & Fotschki, B. (2017). Ellagitannins from Strawberries with Different Degrees of Polymerization Showed Different Metabolism through Gastrointestinal Tract of Rats. *Journal of Agricultural and Food Chemistry*, 65(49), 10738–10748. <https://doi.org/10.1021/acs.jafc.7b04120>
- Mittasch, J., Böttcher, C., Frolova, N., Bönn, M., & Milkowski, C. (2014). Identification of UGT84A13 as a candidate enzyme for the first committed step of gallotannin biosynthesis in pedunculate oak (*Quercus robur*). *Phytochemistry*, 99, 44–51. <https://doi.org/10.1016/j.phytochem.2013.11.023>
- Molinar-Toribio, E., Ramos-Romero, S., Fuguet, E., Taltavull, N., Méndez, L., Romeu, M., ... Pérez-Jiménez, J. (2017). Influence of omega-3 PUFAs on the metabolism of proanthocyanidins in rats. *Food Research International*, 97, 133–140. <https://doi.org/10.1016/j.foodres.2017.03.046>
- Molino, S., Casanova, N. A., Rufián Henares, J.Á., & Fernandez Miyakawa, M. E. (2019). Natural Tannin Wood Extracts as a Potential Food Ingredient in the Food Industry. *Journal of Agricultural and Food Chemistry*, 68(10), 2836–2848. <https://doi.org/10.1021/acs.jafc.9b00590>
- Molino, S., Dossena, M., Buonocore, D., Ferrari, F., Venturini, L., Ricevuti, G., & Verri, M. (2016). Polyphenols in dementia: From molecular basis to clinical trials. *Life Sciences*, 161, 69–77. <https://doi.org/10.1016/j.lfs.2016.07.021>
- Molino, S., Fernández-Miyakawa, M., Giovando, S., & Rufián-Henares, J. A. (2018). Study of antioxidant capacity and metabolism of quebracho and chestnut tannins through in vitro gastrointestinal digestion-fermentation. *Journal of Functional Foods*, 49, 188–195.
- Molino, S., Lerma-Aguilera, A., Jiménez-Hernández, N., Rufián-Henares, J. A., & Francino, M. P. (2022a). Evaluation of the Effects of a Short Supplementation with Tannins on the Gut Microbiota of Healthy Subjects. *Frontiers in Microbiology*, 13, 1–10. <https://doi.org/10.3389/fmicb.2022.848611>
- Molino, S., Lerma-Aguilera, A., Jiménez-Hernández, N., Gosalbes, M. J., Rufián-Henares, J.Á., & Francino, M. P. (2021). Enrichment of food with tannin extracts promotes healthy changes in the human gut microbiota. *Frontiers in Microbiology*, 12, 1–13. <https://doi.org/10.3389/fmicb.2021.625782>



- Molino, S., Rufián Henares, J.Á., & Gómez-Mascaraque, L. G. (2022b). Tannin-rich extracts improve the performance of amidated pectin as an alternative microencapsulation matrix to alginate. *Current Research in Food Science*, 5, 243–250. <https://doi.org/10.1016/j.crf.2022.01.014>
- Montes-Ávila, J., López-Angulo, G., & Delgado-Vargas, F. (2017). Tannins in fruits and vegetables: Chemistry and biological functions. In E.M. Yahia (Ed.), *Fruit and Vegetable Phytochemicals* (Vol. 1, pp. 221–268). <https://doi.org/10.1002/9781119158042.ch13>.
- Mora, J., Pott, D. M., Osorio, S., & Vallarino, J. G. (2022). Regulation of Plant Tannin Synthesis in Crop Species. *Frontiers in Genetics*, 13. <https://doi.org/10.3389/fgene.2022.870976>
- Mosele, J. I., Macià, A., & Motilva, M. J. (2016). Understanding of human metabolic pathways of different sub-classes of phenols from *Arbutus unedo* fruit after an acute intake. *Food and Function*, 7(3), 1700–1710. <https://doi.org/10.1039/c6fo00181e>
- Mosele, J. I., Macià, A., Romero, M. P., & Motilva, M. J. (2016). Stability and metabolism of *Arbutus unedo* bioactive compounds (phenolics and antioxidants) under in vitro digestion and colonic fermentation. *Food Chemistry*, 201, 120–130. <https://doi.org/10.1016/j.foodchem.2016.01.076>
- Nisca, A., Ștefănescu, R., Moldovan, C., Mocan, A., Mare, A. D., Ciurea, C. N., ... Tanase, C. (2022). Optimization of Microwave Assisted Extraction Conditions to Improve Phenolic Content and In Vitro Antioxidant and Anti-Microbial Activity in *Quercus cerris* Bark Extracts. *Plants*, 11(3). <https://doi.org/10.3390/plants11030240>
- Okuda, T., & Ito, H. (2011). Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. *Molecules*, 16(3), 2191–2217. <https://doi.org/10.3390/molecules16032191>
- Olchowik-Grabarek, E., Sekowski, S., Kwiatek, A., Placzkiewicz, J., Abdulladjanova, N., Shlyonsky, V., ... Zamaraeva, M. (2022). The Structural Changes in the Membranes of *Staphylococcus aureus* Caused by Hydrolysable Tannins Witness Their Antibacterial Activity. *Membranes*, 12(11), 1124. <https://doi.org/10.3390/MEMBRANES12111124>
- Olchowik-Grabarek, E., Sekowski, S., Bituicki, M., Dobrzynska, I., Shlyonsky, V., Ionov, M., ... Zamaraeva, M. (2020). Inhibition of interaction between *Staphylococcus aureus*  $\alpha$ -hemolysin and erythrocyte membrane by hydrolysable tannins: Structure-related activity study. *Scientific Reports*, 10, 11168. <https://doi.org/10.1038/s41598-020-68030-1>
- Park, J. Y., Kim, J. H., Kwon, J. M., Kwon, H. J., Jeong, H. J., Kim, Y. M., ... Ryu, Y. B. (2013). Dieckol, a SARS-CoV 3CLpro inhibitor, isolated from the edible brown algae *Ecklonia cava*. *Bioorganic & Medicinal Chemistry*, 21(13), 3730. <https://doi.org/10.1016/j.bmc.2013.04.026>
- Patrignani, F., D'Alessandro, M., Vannini, L., & Lanciotti, R. (2020). Use of functional microbial starters and probiotics to improve functional compound availability in fermented dairy products and beverages. *Sustainability of the Food System: Sovereignty, Waste, and Nutrients Bioavailability*, 167–180. <https://doi.org/10.1016/B978-0-12-818293-2.00009-4>
- Pereira-Caro, G., Ordóñez, J. L., Ludwig, I., Gaillet, S., Mena, P., Del Rio, D., ... Crozier, A. (2018). Development and validation of an UHPLC-HRMS protocol for the analysis of flavan-3-ol metabolites and catabolites in urine, plasma and feces of rats fed a red wine proanthocyanidin extract. *Food Chemistry*, 252, 49–60. <https://doi.org/10.1016/j.foodchem.2018.01.083>
- Pham, L. B., Wang, B., Zisu, B., & Adhikari, B. (2019). Complexation between flaxseed protein isolate and phenolic compounds: Effects on interfacial, emulsifying and antioxidant properties of emulsions. *Food Hydrocolloids*, 94, 20–29. <https://doi.org/10.1016/j.foodhyd.2019.03.007>
- Piao, Y. Z., & Eun, J. B. (2020). Physicochemical characteristics and isoflavones content during manufacture of short-time fermented soybean product (cheonggukjang). *Journal of Food Science and Technology*, 57(6), 2190–2197. <https://doi.org/10.1007/s13197-020-04255-2>
- Piwowski, J. P., Granica, S., Stefańska, J., & Kiss, A. K. (2016). Differences in Metabolism of Ellagitannins by Human Gut Microbiota ex Vivo Cultures. *Journal of Natural Products*, 79(12), 3022–3030. <https://doi.org/10.1021/acs.jnatprod.6b00602>
- Pizzi, A. (2019). Tannins: Prospectives and actual industrial applications. *Biomolecules*, 9(8), 344. <https://doi.org/10.3390/biom9080344>
- Ren, Y. Y., Zhang, X. R., Li, T. N., Zeng, Y. J., Wang, J., & Huang, Q. W. (2021). Galla Chinensis, a Traditional Chinese Medicine: Comprehensive review of botany, traditional uses, chemical composition, pharmacology and toxicology. *Journal of Ethnopharmacology*, 278, Article 114247. <https://doi.org/10.1016/j.jep.2021.114247>
- Riou, V., Vernhet, A., Doco, T., & Moutounet, M. (2002). Aggregation of grape seed tannins in model wine—effect of wine polysaccharides. *Food Hydrocolloids*, 16(1), 17–23.
- Sallam, I. E., Abdelwareth, A., Attia, H., Aziz, R. K., Homs, M. N., von Bergen, M., & Farag, M. A. (2021). Effect of gut microbiota biotransformation on dietary tannins and human health implications. *Microorganisms*, 9(5), 965. <https://doi.org/10.3390/microorganisms9050965>
- Salminen, J. P., & Karonen, M. (2011). Chemical ecology of tannins and other phenolics: We need a change in approach. *Functional Ecology*, 25(2), 325–338. <https://doi.org/10.1111/j.1365-2435.2010.01826.x>
- Santos-Buelga, C., & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds—nature, occurrence, dietary intake and effects on nutrition and health. *Journal of Agricultural and Food Chemistry*, 80(6), 109–1117. [https://doi.org/10.1002/\(SICI\)1097-0010\(20000515\)80:7<1094::AID-JSFA569>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1094::AID-JSFA569>3.0.CO;2-1)
- Sekowski, S., Ionov, M., Dubis, A., Mavlyanov, S., Bryszewska, M., & Zamaraeva, M. (2016). Biomolecular Interactions of Tannin Isolated from *Oenothera gigas* with Liposomes. *Journal of Membrane Biology*, 249, 171–179. <https://doi.org/10.1007/s00232-015-9858-x>
- Seong, S. H., Paudel, P., Choi, J.-W., Ahn, D. H., Nam, T.-J., Jung, H. A., & Choi, J. S. (2019). Probing multi-target action of phlorotannins as new monoamine oxidase inhibitors and dopaminergic receptor modulators with the potential for treatment of neuronal disorders. *Marine Drugs*, 17(377), 1–16. <https://doi.org/10.3390/md17060377>
- Sevnica. (2023a). Tannin extracts for vinification. Available at [http://www.tanin.si/2\\_oenology\\_vinification](http://www.tanin.si/2_oenology_vinification).
- Sevnica. (2023b). Tannins for animal breeding. Available at [http://www.tanin.si/2\\_animal\\_nutrition](http://www.tanin.si/2_animal_nutrition).
- Sevnica. (2023c). Tannin for better drinking water hygiene. Available at [http://www.tanin.si/2\\_oenology\\_spirits](http://www.tanin.si/2_oenology_spirits).
- Sevnica. (2023d). Tannin extracts for spirit production. Available at [http://www.tanin.si/2\\_animal\\_nutrition\\_desinfection](http://www.tanin.si/2_animal_nutrition_desinfection).
- Septembre-Malaterre, A., Remize, F., & Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Research International*, 104, 86–99. <https://doi.org/10.1016/j.foodres.2017.09.031>
- Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition and Food Research*, 53(2), 310–329. <https://doi.org/10.1002/mnfr.200900039>
- Shimamura, Y., Aoki, N., Sugiyama, Y., Tanaka, T., Murata, M., & Masuda, S. (2016). Plant-Derived Polyphenols Interact with *Staphylococcal* Enterotoxin A and Inhibit Toxin Activity. *PLoS ONE*, 11(6), e0157082. <https://doi.org/10.1371/journal.pone.0157082>
- Shrestha, S., Zhang, W., & Smid, S. D. (2021). Phlorotannins: A review on biosynthesis, chemistry and bioactivity. *Food Bioscience*, 39, Article 100832. <https://doi.org/10.1016/j.fbio.2020.100832>
- Siegenberg, D., Baynes, D. R., Bothwell, T. H., Mafarlane, B. J., Lamparelli, R. D., Car, N. G., ... Mayet, F. (1991). Ascorbic acid of polyphenols prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *American Society for Clinical Nutrition*, 1(53), 537–541.
- Sieniawska, E., & Baj, T. (2017). Tannins. In *Pharmacognosy* (pp. 199–232). Academic Press. <https://doi.org/10.1016/B978-0-12-802104-0.00010-X>
- Silvateam. (2023a). Tannins for wine production. Available at <https://www.silvateam.com/en/products-and-services/food-ingredients/tannins-wine-production.html>.
- Silvateam. (2023b). Tannins for beer. Available at <https://www.silvateam.com/en/products-and-services/food-ingredients/tannins-beer.html>.
- Silvateam. (2023c). Tannins and other natural extracts for animal nutrition. Available at <https://www.silvateam.com/en/products-and-services/animal-nutrition-natural-extracts-healthy-animals.html>.
- Silvateam. (2023d). Delivering nature's balance to human well-being. Available at <https://www.silvateam.com/en/products-and-services/delivering-nature-s-balance-human-well-being.html>.
- Smeriglio, A., Barreca, D., Bellocchio, E., & Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: Occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology*, 174(11), 1244–1262. <https://doi.org/10.1111/bph.13630>
- Stoupi, S., Williams, G., Drynan, J. W., Barron, D., & Clifford, M. N. (2010). Procyanidin B2 catabolism by human fecal microflora: Partial characterization of “dimeric” intermediates. *Archives of Biochemistry and Biophysics*, 501, 73–78. <https://doi.org/10.1016/j.abb.2010.02.009>
- Sun, L., Gidley, M. J., & Warren, F. J. (2018). Tea polyphenols enhance binding of porcine pancreatic  $\alpha$ -amylase with starch granules but reduce catalytic activity. *Food Chemistry*, 258, 164–173. <https://doi.org/10.1016/j.foodchem.2018.03.017>
- Tao, W., Zhang, Y., Shen, X., Cao, Y., Shi, J., Ye, X., & Chen, S. (2019). Rethinking the Mechanism of the Health Benefits of Proanthocyanidins: Absorption, Metabolism, and Interaction with Gut Microbiota. *Comprehensive Reviews in Food Science and Food Safety*, 18, 971–985. <https://doi.org/10.1111/1541-4337.12444>
- Tebib, K., Bitri, L., Besançon, P., & Rouanet, J. M. (1994). Polymeric grape seed tannins prevent plasma cholesterol changes in high-cholesterol-fed rats. *Food Chemistry*, 49(4), 403–406. [https://doi.org/10.1016/0308-8146\(94\)90012-4](https://doi.org/10.1016/0308-8146(94)90012-4)
- Tousson, E., Elgharabawy, R. M., & Elmasry, T. A. (2018). Grape Seed Proanthocyanidin Ameliorates Cardiac Toxicity Induced by Boldenone Undecylenate through Inhibition of NADPH Oxidase and Reduction in the Expression of NOX2 and NOX4. *Oxidative Medicine and Cellular Longevity*, 2018. <https://doi.org/10.1155/2018/9434385>
- Ummat, V., Tiwari, B. K., Jaiswal, A. K., Condon, K., Garcia-Vaquero, M., O'doherty, J., O'donnell, C., & Rajauria, G. (2020). Optimisation of Ultrasound Frequency, Extraction Time and Solvent for the Recovery of Polyphenols, Phlorotannins and Associated Antioxidant Activity from Brown Seaweeds. *Marine Drugs*, 18(250), 1–15. <https://doi.org/10.3390/md18050250>
- Vilhelmova-Ilieva, N., Galabov, S., Galabov, A. S., & Mileva, M. (2020). Tannins as Antiviral Agents. In A. Aires (Ed.), *Tannins - Structural Properties, Biological Properties and Current Knowledge* (pp. 1–13). IntechOpen. <https://doi.org/10.5772/intechopen.86490>
- Virtanen, V., Green, R. J., & Karonen, M. (2022). Interactions between Hydrolysable Tannins and Lipid Vesicles from *Escherichia coli* with Isothermal Titration Calorimetry. *Molecules*, 27, 3204. <https://doi.org/10.3390/molecules27103204>
- Virtanen, V., Rääkkönen, S., Puljula, E., & Karonen, M. (2021). Ellagitannin-Lipid Interaction by HR-MAS NMR Spectroscopy. *Molecules*, 26, 373. <https://doi.org/10.3390/molecules26020373>
- Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular Plant*, 3(1), 2–20. <https://doi.org/10.1093/mp/ssp106>

- Wang, C., Jiang, D., Sun, Y., Gu, Y., Ming, Y., Zheng, J., ... Qi, H. (2021). Synergistic effects of UVA irradiation and phlorotannin extracts of *Laminaria japonica* on properties of grass carp myofibrillar protein gel. *Journal of the Science of Food and Agriculture*, 101(7), 2659–2667. <https://doi.org/10.1002/JSFA.10890>
- Wang, J., Zheng, J., Huang, C., Zhao, J., Lin, J., Zhou, X., ... He, S. (2018). Eckmaxol, a Phlorotannin Extracted from *Ecklonia maxima*, Produces Anti- $\beta$ -amyloid Oligomer Neuroprotective Effects Possibly via Directly Acting on Glycogen Synthase Kinase 3 $\beta$ . *ACS Chemical Neuroscience*, 9(6), 1349–1356. <https://doi.org/10.1021/ACSCHENNEURO.7B00527>
- Wang, S., Olarte Mantilla, S. M., Smith, P. A., Stokes, J. R., & Smyth, H. E. (2023). Relationship between salivary lubrication and temporal sensory profiles of wine mouthfeel and astringency sub-qualities. *Food Hydrocolloids*, 135. <https://doi.org/10.1016/j.foodhyd.2022.108106>
- Wiese, S., Esatbeyoglu, T., Winterhalter, P., Kruse, H. P., Winkler, S., Bub, A., & Kulling, S. E. (2015). Comparative biokinetics and metabolism of pure monomeric, dimeric, and polymeric flavan-3-ols: A randomized cross-over study in humans. *Molecular Nutrition and Food Research*, 59(4), 610–621. <https://doi.org/10.1002/mnfr.201400422>
- Wu, T., Grootaert, C., Voorspoels, S., Jacobs, G., Pitart, J., Kamiloglu, S., ... Van Camp, J. (2017). Aronia (*Aronia melanocarpa*) phenolics bioavailability in a combined in vitro digestion/Caco-2 cell model is structure and colon region dependent. *Journal of Functional Foods*, 38, 128–139. <https://doi.org/10.1016/j.jff.2017.09.008>
- Zeng, X., Du, Z., Sheng, Z., & Jiang, W. (2019a). Characterization of the interactions between banana condensed tannins and biologically important metal ions (Cu<sup>2+</sup>, Zn<sup>2+</sup> and Fe<sup>2+</sup>). *Food Research International*, 123, 518–528. <https://doi.org/10.1016/j.foodres.2019.04.064>
- Zeng, X., Du, Z., Xu, Y., Sheng, Z., & Jiang, W. (2019b). Characterization of the interactions between apple condensed tannins and biologically important metal ions [Fe<sup>2+</sup> (3d6), Cu<sup>2+</sup> (3d9) and Zn<sup>2+</sup> (3d10)]. *LWT*, 114. <https://doi.org/10.1016/j.lwt.2019.108384>
- Zhai, H. Y., Li, S. Y., Zhao, X., Lan, Y. B., Zhang, X. K., Shi, Y., & Duan, C. Q. (2023). The compositional characteristics, influencing factors, effects on wine quality and relevant analytical methods of wine polysaccharides: A review. *Food Chemistry*, 403, Article 134467. <https://doi.org/10.1016/j.foodchem.2022.134467>
- Zhang, H., Zhang, L., Tang, L., Hu, X., & Xu, M. (2021). Effects of Metal Ions on the Precipitation of Penta-O-galloyl- $\beta$ -D-glucopyranose by Protein. *Journal of Agricultural and Food Chemistry*, 69(17), 5059–5066. [https://doi.org/10.1021/ACS.JAF.1C01185/ASSET/IMAGES/MEDIUM/JF1C01185\\_0006.GIF](https://doi.org/10.1021/ACS.JAF.1C01185/ASSET/IMAGES/MEDIUM/JF1C01185_0006.GIF)
- Zhang, L., Zhang, H., Tang, L., Hu, X., & Xu, M. (2022). Isolation, Characterization, Antioxidant Activity, Metal-Chelating Activity, and Protein-Precipitating Capacity of Condensed Tannins from Plum (*Prunus salicina*) Fruit. *Antioxidants*, 11(4), 714. <https://doi.org/10.3390/ANTIOX11040714/S1>
- Zhang, R., Yuen, A. K. L., Magnusson, M., Wright, J. T., de Nys, R., Masters, A. F., & Maschmeyer, T. (2018). A comparative assessment of the activity and structure of phlorotannins from the brown seaweed *Carpophyllum flexuosum*. *Algal Research*, 29, 130–141. <https://doi.org/10.1016/j.algal.2017.11.027>
- Zhang, X., Sandhu, A., Edirisinghe, I., & Burton-Freeman, B. (2018). An exploratory study of red raspberry (*Rubus idaeus* L.) (poly)phenols/metabolites in human biological samples. *Food and Function*, 9(2), 806–818. <https://doi.org/10.1039/c7fo00893g>
- Zumdick, S., Deters, A., & Hensel, A. (2012). In vitro intestinal transport of oligomeric procyanidins (DP 2 to 4) across monolayers of Caco-2 cells. *Fitoterapia*, 83(7), 1210–1217. <https://doi.org/10.1016/j.fitote.2012.06.013>