Chapter

Food Ellagitannins: Structure, Metabolomic Fate, and Biological Properties

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Abstract

Food sources of ellagitannins (ETs) are numerous, and dietary intake of these compounds is estimated up to 12 mg/day in some countries, even though ETs have been considered in the past as not bioavailable like other tannins and were mostly neglected by nutritionists. Nonetheless, new insights show that ETs are bioconverted by microbiota in the gut into metabolites called urolithins, which are bioavailable and can reach relatively high physiological concentration in the body up to 7 days after ingestion. According to the initial structure of ETs in the food source, the extent of bioconversion into urolithins may differ but all urolithins are susceptible to exert potential health benefits. Nonetheless, due to the intervention of microbiota, the production and excretion of urolithins are highly variable according to individuals, which have led to the classification of consumers into metabotype. According to metabotype, the potential health benefits of ellagitannins may differ among consumers. In in vitro, cellular and animal studies, numerous health benefits of ellagitannins and urolithins are reported mainly for the chemoprevention of hormone-dependent cancer and cardiovascular disease. Nonetheless, ellagitannins deserve closer attention from the scientific community to unravel more biological properties of this particular compound.

Keywords: ellagitannins, urolithins, microbiota, metabotype, chemoprevention

1. Introduction

Ellagitannins are food compounds that were quite neglected by nutrionists until last decade. As part of tannins, they had no good reputation and they were considered as antinutritional compounds. But new scientific insights have changed these perspectives, and ellagitannins now attract the attention of food scientists, nutritionists and consumers since the number of published papers on these compounds has considerably increased during the last decade. Ellagitannin is a hydrolyzable polymer contrary to the rest of the family of tannins and can be hydrolyzed to more simple monomers that can be eventually metabolized and that can become bioavailable with subsequent exposition of the body to these metabolites. For sure, if ellagitannins are widely present in nature, only few food sources are reported with relatively high content of this compound, and consequently exposition of consumers to food ellagitannins is relatively low, especially in the Western diet. But given

the health potential of ETs, ET-rich food now belongs to the select group of functional foods, and their consumption should be considerably enhanced in the future. Actually, given the main food source of ellagitannins such as berries and nuts, we can easily assume that the exposure to this compound and their metabolites was considerably higher in the hunter-gatherer diet than in modern time. Without presuming that an increase in ET intake would reduce significantly the impact of certain chronic diseases due to modern lifestyle, it is reasonable to argue that ETs have been part of our evolutionary history and they could potentially perform health care functions. New scientific insights presented in this chapter on the in vivo metabolisms of ellagitannins and the potential biological activities of generated metabolites tend to support this hypothesis. This review presents the main food source of ellagitannins, their general chemical structure, and how technologies and storage could eventually affect ellagitannin composition in processed foods. Then, we will review the metabolomic fate and the bioavailability of ellagitannins in humans, which is strongly related to the performance of intestinal microbiota, and finally, we will present a summary of the main biological activities, attributed to ETs and their derived metabolites.

2. Food occurrence of ellagitannins

Ellagitannins (ETs) are with gallotannins part of the hydrolyzable tannins and constitute the largest group among more than 500 hydrolyzable tannins characterized until now [1]. To date, more than 1000 natural ellagitannins have been identified in nature [2] but most of them are not preponderant in foods. The main ETs identified in foods (specially in fruits, nuts, and seeds) are punicalagin, sanguiin H6, lambertianin C, pedunculagin, vescalagin, castalagin, casuarictin and potentillin (seeds) [3].

Examples of concentration and ellagitannins identified in some foods are presented in **Table 1**. The occurrence of ETs in foods is restricted to a few fruits, such as berries of the genus *Rubus* (cloudberry, raspberry, blackberry, blueberry, and cranberry) and the genus *Fragaria* (strawberry), pomegranate, nuts (walnuts and almonds), seeds, and oak-aged wines [1, 6–8]. Recently, other ET food sources of

Food source	Ellagitannins	Content equ. EA (mg/100 g)
Blackberries (*Rubus* spp..)	Sanguiin H6, lambertianin D [7]	150–270 [3]
Strawberries (*Fragaria ananassa*)	Casuarictin, pedunculagin, sanguiin H6 [7, 8]	71–83 [3]
Cloudberries (*Rubus chamaemorus*)	Sanguiin H6, lambertianin C [7]	312 [3]
Raspberries (*Rubus idaeus*)	Sanguiin H6, sanguiin H10, lambertianin C [7, 9]	326 [10]
Pomegranate (*Punica granatum*)	Punicalagin [7]	58–177 [3]
Guava (*Psidium* friedrichsthalianum)	Pedunculagin, castalin, and vescalin [5]	63 [5]
Jabuticaba (*Myrciaria jaboticaba*)	Sanguiin H6-H10, lambertianin C [4]	900 [11]
Muscadine grapes (*Vitis rotundifolia*)	Sanguiin H5 [7]	3–91 [7]
Purple Grumixama cherry (*Eugenia brasiliensis*)	Pedunculagin, strictinin, castalagin, vescalagin [12]	16 [12]
Chestnuts (*Castanea sativa*)	Castalagin [7]	149 [13]
Pecans (*Carya illinoinensis*)	Pedunculagin [7]	316 [13]
Walnuts (*Juglans regia*)	Pedunculagin, casuarictin [14]	864 [13]

Table 1.
Main food source of ellagitannins.

local importance have been identified such as jabuticaba [4], guava, [5] and grumixama cherries [12]. It is interesting to note that according to a Brazilian research team, Jabuticaba berries from a particular variety cultivated in south Brazil have the highest registered ET content in fruits. Berries have almost three times more equivalent EA content than walnuts and pecans and at least 15 times more than other fruits and nuts [6]. In the berries from the genus *Rubus* and genus *Fragaria*, total equ. EA content represents the most important compounds with 50–88% of total phenolic. Also, ET content can be considerably affected by variety, ripeness, fruit parts, geographic origin, climate, season, cultural practices, and mineral nutrition [6].

ET daily intake is generally low and has been estimated around 5 mg/day for Western diets with major contributors being the red berries mainly strawberries, followed by raspberries and blackberries. Given the significant seasonality of the production of these fruits, the exposure to ellagitannins is very uneven during the year. In the Scandinavian countries, where the consumption of berries increases considerably in summer, daily intake can reach up to 12 mg/day [6, 9] with cloudberry, raspberry, rose hip, strawberry, and sea buckthorn being the main contributors with content from 1 to 330 mg/100 g (fresh weight basis).

3. Chemical structure of ellagitannins

ETs are esters of hexahydroxydiphenic acid (HHDP) and a polyol, usually glucose or quinic acid [3, 15, 16], that when they are hydrolyzed spontaneously suffer lactonization to form ellagic acid [10, 17] (**Figure 1**).

Chemical structure diversity among ETs is huge and is due to the possible variations in position, frequency, and stereochemistry of the HHDP units, galloylation extent, and/or anomeric stereochemistry of sugar moieties [17]. Thus, due to seemingly endless structural variations among ellagitannin, elucidating their native structure is often a challenge [17]. According to their chemical structure, ETs can readily undergo different chemical reactions such as transformation, isomerization and oligomerization, which finally determine overall physico-chemical properties, hydrolytic susceptibility, and finally biological activity in vivo [7].

The important structural diversity of ET structure is due to the different possible extent of galloylation and formation of aromatic C-glycosides, the number of intramolecular C-C coupling of galloyl groups and hydrolytic cleavage of galloyl-derived aromatic rings, the level of dehydrogenation, and oligomerization via oxidative C—O [2, 17]. According to the number of HHDP groups linked to sugar moiety, ETs can be classified into monomeric, oligomeric, and polymeric ellagitannins [18].

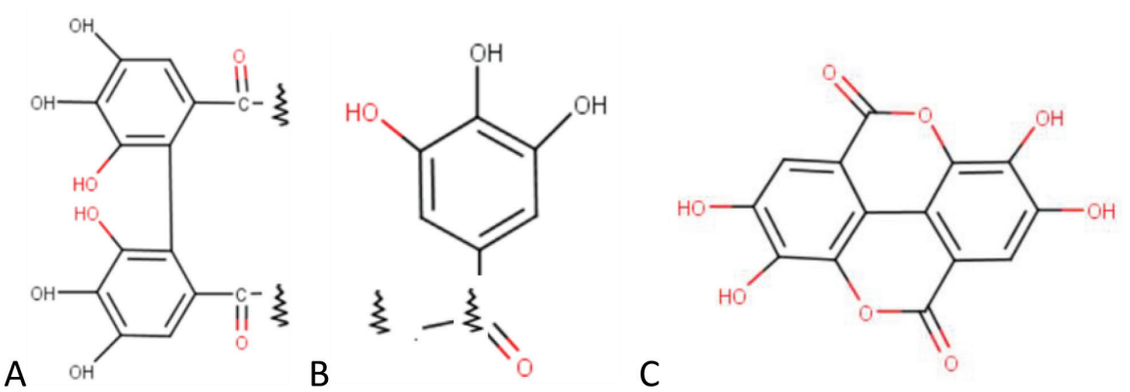

Figure 1.
Basic structures of ellagitannins: (A) HHDP acid (R radical); (B) galloyl unit (G radical); (C) ellagic acid.

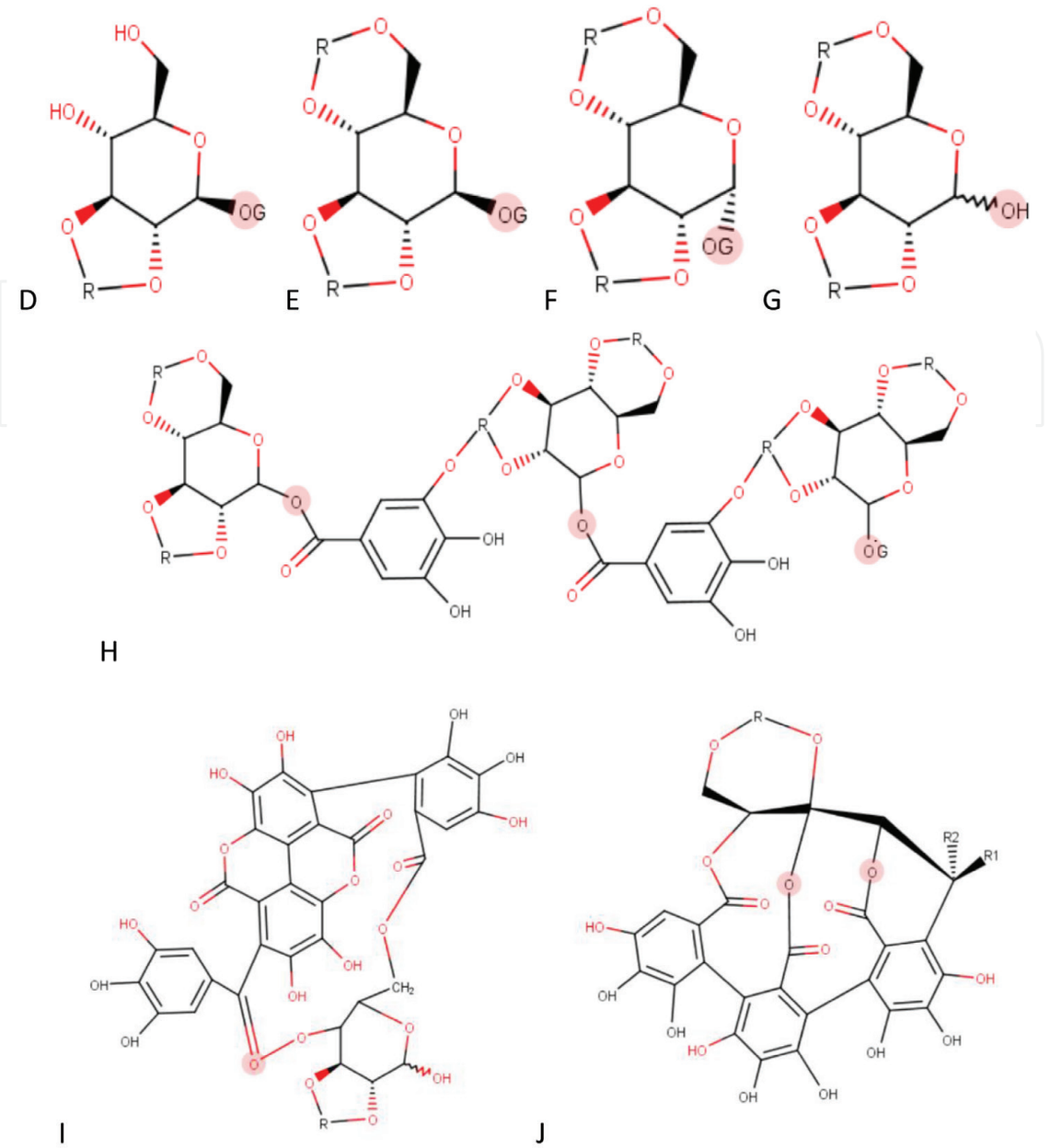

Figure 2.
Most common ellagitannins present in food: (D) sanguiin H5; (E) casuarictin; (F) potentillin; (G) pedunculagin; (H) lambertianin C; (I) punicalagin; (J) vescalagin R1: OH, R2:H o Castalagin R1:H, R2:OH.

ETs are generally hydrolyzable in acidic or basic solutions. Even though ETs are quite resistant to acid hydrolysis, neutral or slightly alkaline pH (from 7.0 to 7.3) are the best conditions for ET hydrolysis to occur [19]. During hydrolysis, the ester bounds in the polymer are cleaved and HHDP is released followed by a spontaneous lactonization of free HHDP unit into free ellagic acid or derivatives [20]. This reaction is mainly used for the detection and quantification of ellagitannins, as ET content in food samples is often expressed as ellagic acid equivalents (**Figure 2**). Acidic and basic hydrolysis of ETs can also occur during food processing, storage, and passage through the stomach and duodenum [10, 16, 21]. Although most ETs are hydrolyzable, further C—C coupling of polyphenolic residue with the polyol unit, such as in the case of vescalagin, can prevent hydrolysis [22]. In addition, ETs can undergo polymerization reactions during maturation of fruits or thermo-physical treatments, which can make them insoluble and eventually attached covalently to cell wall fragments [21]. Nonetheless, most often acidic or basic hydrolysis will

allow generating ellagic acid (EA) from ET even in mild conditions. As a consequence, EA, the dimeric derivative of gallic acid, is often spontaneously present in its free form in plants, next to ET [23].

3.1 Structural changes of ETs and EA during process and storage

Different studies have pointed out that there are important changes in ETs' composition during processing and storage with marked subsequent consequences on the bioavailability and bioactivity of these compounds [24, 25].

Mazur et al. [26] noticed a decrease of 7% of lambertianin C in red raspberry jam after 6 months of storage at 20°C in dark; meanwhile, ellagic acid derivatives and total phenolics increased by 47%. The reason was the spontaneous hydrolysis of ellagitannins to ellagic acid that may occur during storage. Authors showed also that according to the genotype of raspberry, losses of ET were more or less important. On the other hand, one ellagitannins, the sanguiin H6, remains remarkably stable during storage in this case [26]. Nonetheless, in another study, stored pasteurized blackberry juice showed after 6 months at 25°C a 46% loss in sanguiin H6/lambertianin A, 42% loss in lambertianin C, and 72% loss in lambertianin D. Like previously, the total amount of EA measured after hydrolysis registered only minimal changes, which evidenced the spontaneous depolymerization of ETs into EA during storage. At 5°C, half-life ($t_{1/2}$) of sanguiin H6 and lambertianin C was almost comparable (around 80 days), but when stored at 45°C, $t_{1/2}$ was four times higher for sanguiin than for lambertianin [25]. Sanguiin H6 is a dimer and lambertianin C a trimer, which could explain the higher stability of this compound during storage at relatively high temperature. At freezing temperature, no changes at all were observed over a 6-month period [24]. We can conclude that stability of ellagitannins during storage of processed foods depends on their chemical structure as well as the composition of the food matrix.

Between different processing alternatives evaluated by Hager et al. [24], canning, pureeing, and freezing had little effect on blackberry (cv. Apache) ellagitannins, but the removal of seeds in the press-cake generates a loss of up to 70%, and if juice is microfiltered, 12% more loss can be registered. In that case, some ellagitannins appear to be associated with cell fragments and with the mucilage that surrounds the seeds. In the case of Costa Rican guava, pressing appears to increase the content of some ETs. After pressing, an increase in pedunculagin isomer 1 of 25% was observed, while castalagin isomers presented a significant decrease (40%). Authors suggest that castalagin decrease may be linked to degradation and not to isomerization of the compounds, since no increase in vescalagin content was observed [5]. Milling appeared to enhance the content of pedunculagin isomer 1 and castalagin isomers by 34 and 31%, respectively. Actually, another study on the effects of mechanical and enzymatic pretreatments on the extraction of ellagitannins from blackberries showed that enzymatic treatment (pectinase and cellulase) combined with continuous pressing enhanced significantly ET content in the juice (from 437 to 982 mg ellagic acid equivalents/100 g (dry basis)) [27].

Critical steps on ET content during classical industrial processing of blackberry-based beverage in glass bottles were evaluated. Hot-filling that is characterized by long-term exposure of the beverage to high temperature was the operation that most degraded ellagitannins with losses of 80% in lambertianin C and 50% in sanguiin H6 in the final product. Again, sanguiin H6 showed a higher thermal stability than lambertianin. It was also observed that the intensity of thermal treatment during process affects stability of ETs during storage [25]. But, when dealing with equivalent total EA content after hydrolysis, no changes were observed. On the

other hand, in another food source, the ripe Costa Rican guava (*Psidium friedrichsthalianum* Nied), different pasteurization treatments (71.1°C for 4 s and 60°C for 8.2 min) were found to not affect ETs' final content. In this case, the composition in geraniin, vescalagin, and pedunculagin isomers remained basically constant during the process [5]. The analysis of these results shows that thermal stability at high temperature is also affected by the chemical structure of ET.

In other processing operations such as osmotic dehydration in 50–65°Bx sucrose solutions (30°C), in the case of blackberry, 80% of ellagitannins were retained after 1 h, while losses reached up to 45% after 3 h. The concentrations of the two main ellagitannins, lambertianin C and sanguiin H6, revealed similar patterns of variation. On the contrary, due to a much lower molecular weight, the loss of free ellagic acid reached up to 50% after 1 h of osmotic dehydration [23].

4. Metabolomic fate and bioavailability of ETs and EA

Many studies have proven that ETs are not bioavailable as such, and they have never been detected in human plasma after normal consumption of ET-rich foods [28, 29]. Ellagitannins are probably not bioavailable because of their size (above 634 Da for one of the simplest ellagitannins, the sanguiin H4 ($C_{27}H_{22}O_{18}$), up to 3740 Da for lambertianin D ($C_{164}H_{106}O_{104}$)). Their relatively high polarity and the presence of a C—C linkage could also explain this situation. During ingestion, ETs can also bind some proteins in saliva and cause astringency, and in this case, they may not be metabolized further [30]. Also, some ellagitannins are resistant to acid and basic hydrolysis in the GI tract and they can reach almost intact the large intestine [31]. However, for most ETs sensitive to acidic and basic hydrolysis in the stomach and the duodenum, respectively, they release free ellagic acid, which is at its turn poorly bioavailable. In the human digestive system, the bioavailability of EA derivatives depends on the part of gastrointestinal tract in which these compounds are released [7]. In stomach or small intestine, only low level of absorption could occur, and if EA can be detected in plasma and urine between 1 and 5 h after ingestion of dietary ETs, generally as methyl and dimethyl ethers or glucuronic acid conjugates, all these EA derivatives are always found at very low concentrations [29, 32, 33]. Low bioavailability of EA is probably due to low water solubility, and to its ability to bind irreversibly to cellular DNA and proteins, or to form poorly soluble complexes with calcium and magnesium ions which affects transcellular absorption [3, 33, 34].

The extent of the degradation of ETs in the upper GI tract depends on their chemical structure, the food matrix, and their susceptibility to acid/base hydrolysis in the stomach and duodenum [29]. Therefore, some ETs can reach the intestine where they can exert potential biological activity and eventually some can be partially converted into EA by enzymes from microbiota [35].

In the lower GI tract, released EAs can be partially metabolized by 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 step-by-step reduction to tetrahydroxy (urolithin D), trihydroxy (urolithin C), dihydroxy (urolithin A and isourolithin A), and monohydroxy dibenzopyranones (urolithin B) (**Figure 3**) [21, 35–37].

Urolithins appear to be the main plasma and urinary biomarker after consumption of ET-rich food (**Table 2**). Specifically, urolithin A and B and their phase II metabolites are the main metabolites in plasma and urine [10, 13] detected at micromolar concentration level. They can also be found at much higher concentration in some tissues or organs such as prostate gland and colon where they can accumulate [38, 39]. Persistence of urolithins in the body has been reported for long periods

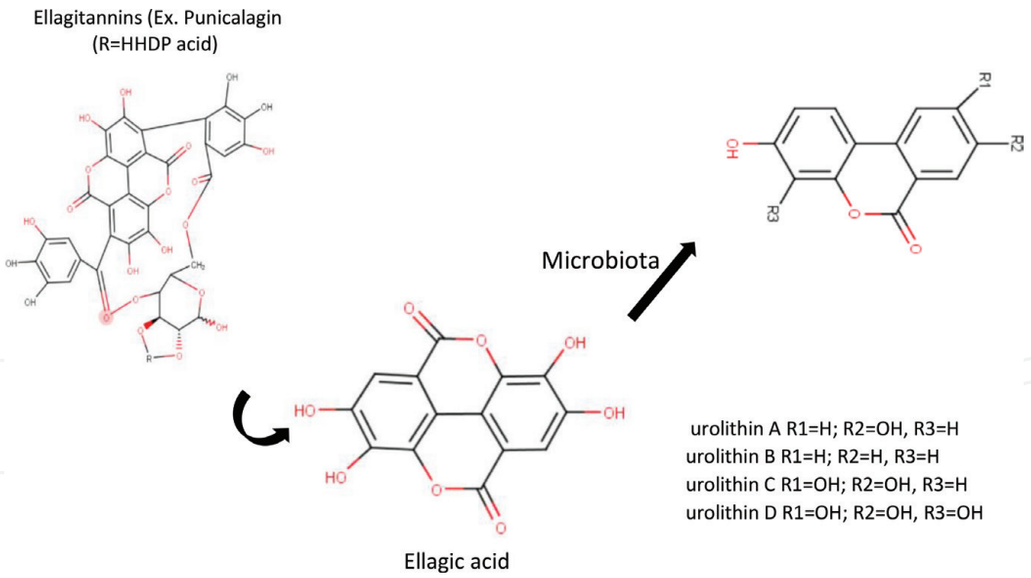

Figure 3.
Metabolism of ellagitannins in the GI tract.

after a single intake of ET-rich food. Urolithins in urine have been detected up to 7 days [58] after dietary ET intake, and this long persistence in the body is attributed to the involvement of microbiota and enterohepatic recirculation [9, 40–42].

Therefore, urolithins have been proposed to be responsible for biological activity of ETs [38] as they remain in the body at relatively low concentrations but during a long time with potential homeopathic-like effect. However, the concentration of urolithins in plasma, urine, and feces varies considerably between individuals [38, 46, 47]. Actually, the huge inter-individual variability of microbiota composition affects the production of urolithins, which is mediated by microbiota. During different studies, some individuals were labeled "low excreters" of urolithins after ingestion of ET-rich food, while other individuals were labeled as "high excreters of urolithins A or B." Therefore, recently, a stratification of individuals according to their urolithin excretion status in urine has been proposed. Three metabotypes were defined: metabotype A, which includes main excreters of urolithin A; metabotype B with main excreters of urolithin A and B; and metabotype 0 corresponding to low urolithin excreters [31]. This classification appeared to be consistent across multiple intervention studies, independent of the ET food source, and health status of participants [46]. The distribution of urolithin metabotypes in adult population varies probably according to geography, but in a Western adult population taking into consideration a large cohort of individuals, UM-A is the most abundant metabotype with 55% followed by UM-B (34%) and UM-0 (11%) [47]. Nonetheless, a recent study reported by Cortés-Marín and coworkers [47] showed for the first time in a Caucasian cohort (5–90 years, n = 839) that age could determine the individual's capacity to metabolize EA into urolithins A and B, even though the percentage of the population with low ability to excrete urolithins (metabotype 0) remains around 10% of the population whatever the age considered. The percentage of individual with metabotype A was higher in the case of children (80%) and decreased steadily after adolescence while metabotype B increased [47]. Authors also reported a significant association between increased physical activity and prevalence of UM-B especially between 5 and 18 years. On the other hand, no correlation of a specific metabotype with gender, body mass index, weight, health status, and diet was observed [47].

Nonetheless, most studies tend to show a strong persistence of metabotype status for adults even though recent research showed that individuals with UM-0 status have managed to become urolithin excreters of UM-A or UM-B after a long-term

Source ETs	Study design	Metabolites	Main observations
Red raspberry (300 g) single dose [9]	9 adults: 5 females and 4 males (22–44 years old) sampling: blood (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 24 h), urine (all until 48 h).	In urine, UA aglycone and phase II metabolites, In plasma: UA and UB and phase II derivatives dimethylellagic acid-O-gluc.	Urolithins A and B, present C_{max} in plasma in times >24 h, and in urine between 32 and 48 h after intake, showing high persistence in the body.
Pomegranate extract (1.8 g in 4 capsules) single dose [43]	10 men and 10 women (20–22 years old) sampling: blood (0, 0.5, 1, 2, 3, 4, 5, 6 and 24 h), urine 0, 24, 48 and 72 h.	In urine and plasma, UM-0: 2 people; UM-A: 16 people and UA were quantified after 24, 48, and 72 h); UM-B: 2 people (UA and UB).	EA bioavailability was affected by the presence of ellagitannin, pH, and protein as well as low pH. A higher free EA intake does not enhance bioavailability of EA but promotes urolithin production.
Strawberry extracts rich in: (1) free EA; (2) monomeric ETs, and (3) dimeric ETs (0.1% single intake) [37]	30 Wistar rats (10 each treatment), sampling: feces, urine and blood, intestinal digesta (2, 4, 7 days after intake); samples of digesta, stomach, small intestine, cecum.	In urine, plasma, and intestinal digesta: UA, UB, UA gluc, Nasutin A gluc, EA dimethyl-ether-gluc (DMEAG). Feces: Nasutin A and UA.	ETs and EA metabolites are found in gastrointestinal digesta, blood plasma, and urine, 2 days after intake. UA and DMEAG were founded in rats fed with free EA extract, Nasutin A and UA and Nasutin
Black raspberries (freeze-dried, 10% w/w, 6 weeks) [44]	12 male mice, sampling: blood and luminal, colon, liver, and prostate tissue.	Urolithin A (all mouse plasma, liver, prostate, colon, and luminal tissue), urolithin C (all tissues except prostate), urolithin B and urolithin D (only in luminal tissue).	Highest amount of UA was found in luminal tissue. UC was 45-fold more abundant in colon with respect to plasma. UB and UD were found in very low amounts with respect to UA. Luminal contents presented lower abundance of *Clostridium* with respect to *Barnesiella* in mice after intervention.
Pomegranate extract (PE) (1.8 g/day, 3 weeks) [45]	Simulator of the human intestinal microbial ecosystem TWIN-SHIME®, coupled to human colon adenocarcinoma cell line Caco-2 cells (HTB37), UM-A and UM-B were incubated.	Large intestine: IsoUA and UB, detected only in UM-B, UA in UM-A and UM-B.	Production of urolithin starts earlier and faster in UM-B than in UM-A. Chronic PE intake improves UA production in both metabotypes, showing similar profiles after 18 days. UA production was directly related with Gordonibacter abundance.

Table 2.
Some relevant studies on the metabolism of Ellagitannins-rich foods.

exposure (up to 6 months) to a high ET source, a pomegranate-concentrated extract [48]. Thus, it seems that microbiota ability to catalyze the production of urolithins could be influenced by long-term exposure to ET-rich food by promoting growth of the bacteria involved in the urolithin metabolism [40]. Nonetheless, it was not reported if the change of metabotype remains persistent after the end of the study, when diet comes back to normal.

Recently, an attempt to find a correlation between urolithin metabotypes and enterotypes of the human gut microbiome proposed by the Human Microbiome Project (HMP) [39] has been made by Romo-Vaqueroa and coworkers [49]

following a cohort of 249 healthy volunteers after walnut or pomegranate extract ingestion for 3 days. Results showed that urolithin metabotypes and enterotypes (enterotype 1 (preponderance of Bacteroides), enterotype 2 (preponderance of Prevotella), or enterotype 3 (preponderance of Ruminococcus)) were not coincident. Only for enterotype 2, UM-A was slightly higher than UM-B. Nonetheless, a higher diversity of microorganisms in UM-B individual with respect to UM-A, and even more with respect to UM-0, was found. Actually, urolithin B production requires a more complex enzymatic arsenal than urolithin A. It was observed that a higher relative importance of microorganisms from the Coriobacteriaceae family tends to be correlated with a higher preponderance of UM-B with respect to UM-A and even more with respect to UM-0. Two bacterial strains isolated from human microbiota, *Gordonibacter urolithinfaciens* and *Gordonibacter pamelaeae* from Eggerthellaceae family, which was previously considered as part of Coriobacteriaceae family, showed ability in vitro to transform EA into urolithin C [50]. Recently, a specific strain also isolated from human microbiota was able to further metabolize EA up to isourolithin-A and was named as a consequence *Ellagibacter isourolithinifaciens* [50, 51]. Nonetheless, until now, no other bacterial strains that could metabolize EA to urolithins B have been reported.

Another factor that can impact the rate of urolithin production in vivo is the food source and the chemical structure of ingested ETs. For example, more urolithins in prostate from patients who consumed walnuts rather than in patients who consumed pomegranate juice, even when the latter had a higher ET content, were found. [14]. Also, it appears that there is a kind of saturation of the metabolic pathways as the amount of urolithins excreted in vivo remains apparently independent of the quantity of ETs ingested. This was observed for different food sources of ETs consumed in normal quantities such as strawberries, raspberries, walnuts, and oak-aged red wine [52]. Probably, there exists a consumption threshold below which urolithin excretion cannot be detected.

At last, the bioavailability of ETs and EA could be affected by food processing. In a clinical study, 16 healthy volunteers consumed approximately the same quantity of ET but in different presentation: pomegranate juice (PJ), pomegranate polyphenol liquid extract (POMxl), and pomegranate polyphenol powder extract (POMxp). As a result, there were no statistical differences in the level of EA in plasma between the three interventions over a 6-h period. Only, POMxp presented a longer lag-time to reach the peak of maximum concentration compared to PJ and POMxl [53]. A similar study was performed with 20 healthy volunteers comparing pasteurized strawberry juice (80°C for 5 min) and the equivalent fresh fruits. In this case, processing did not affect the urinary excretion of urolithins. Although the amount of free EA was increased 2.5-fold during processing, no effect on the urinary excretion of urolithins was observed [40]. Actually, further researches are required for a more definitive assessment on the effect of processing on the production and excretion of urolithins.

5. Main biological activities of ETs and their metabolites

Ellagitannins, ellagic acid, and their metabolites have been reported to exhibit numerous beneficial effects on human health including anti-inflammatory, anticancer, antioxidant, prebiotic, and cardioprotective properties [21, 54]. However, in vitro studies with cells or in vivo studies with animals could give inconsistent or untranslatable information about bioactivity of these metabolites in humans. Abundant literature shows for example the impact of ETs and EA on cells from organs that are not part of the GI tract and the results are absolutely controversial and inconsistent with actual knowledge. The potential health effect of ETs and possibly EA can only be exerted

within the GI tract, as these compounds are poorly bioavailable. On the other hand, urolithin production is mediated by microbiota and studies on animals can hardly be extrapolated to humans, except in the case of germ-free animals used in human microbiota-related researches. Therefore, in this review, even though research studies are much more scarce, we will report results of biological activities of ETs and EA only related with the GI tract, and for urolithins, given the importance of microbiota in their metabolism, we will focus only on the results of clinical trials with humans.

5.1 Effect of ETs and EA in GI tract

In in vitro model of colon cancer, ellagic acid was found to have a significant antiproliferative effect inducing apoptosis of cancer Caco-2 cells via a mitochondrial pathway and without side effects on normal colon cells [55]. In another study in rats, EA showed anti-inflammatory properties by iNOS, COX-2, TNF-α, and IL-6 downregulation due to NF-κB repression. Authors conclude that EA may exert a chemopreventive effect on colon carcinogenesis [56]. Furthermore, ETs and EA have a high antioxidant activity (even higher than urolithins) and could be highly efficient to scavenge oxygen free radicals and eventually prevent inflammation and colon cancer [21]. At last, in nasopharyngeal carcinoma cell lines (NPC-BM1), EA has showed ability to downregulate Bcl-2 and DNA fragmentation, by increasing caspase-3 enzymatic activity, which reduces telomerase activity [57].

5.2 Effect of urolithins

Numerous studies have demonstrated the metabolism of EA into urolithins, in approximately 12–24 h, with persistence of urolithins up to 4–7 days in urine after dietary intervention [9, 41, 42, 58], and research interest has shifted to the potential effect of urolithins on health. Actually, urolithins may exert a much more consistent effect at the systemic level than EA with concentration in body fluids at least one order of magnitude higher in body fluids. Actually, EA has been reported in plasma at concentration around some nanomoles per liter, while urolithins have been reported at concentration level up to 80 μmol/l [58].

5.2.1 Anti-inflammatory effect

Inflammation is a primary defensive response against harmful factors that involved different mechanisms including the immune system cells [8]. The impact of urolithins on inflammatory processes has been well established on various in vivo and in vitro models [59–61]. In in vitro models, urolithin aglycones have been tested, while in vivo glucuronide conjugates of urolithins are the predominant metabolites present in plasma, tissues, and urine [62]. Urolithin aglycone is highly bioactive against inflammation, but some authors suggest that urolithin conjugate may be even more active. In a study, in which urolithin conjugates (iso-Uro-A-gluc, Uro-A-gluc, and Uro-B-gluc) were isolated from urine of a volunteer after ingestion of pomegranate juice (0.5 L/day), walnuts (30 g/day), hazelnuts (30 g/day), and fresh raspberries (200 g/day) for 5 days, the cleavage of glucuronides by endogenous β-glucuronidases released by human neutrophils was observed . β-Glucuronidase is an enzyme that is released from inflammatory cells and the lysosomes of necrotic cells, and high levels can be found in most solid tumors. Therefore, a wide number of structurally diverse glucuronide prodrugs have been designed with the aim of enhancing the selectivity of cancer chemotherapy. The results suggest that the selective activation of urolithin glucuronides by β-glucuronidase could locally increase the concentration of bioactive urolithin aglycones. More clinical trials are needed to

better understand the anti-inflammatory response attributed to urolithin [62] and the impact on the suppression of the immune responses, especially on inflammation-associated diseases, like cardiovascular diseases and cancer [63].

5.2.2 Chemoprevention of cancers

Several in vitro and in vivo (animal or human) studies have reported a protective effect of urolithins on prostate cancer [64–66]. However, recent clinical interventions have pointed out inconsistent results. A recent review compiles the data of clinical trial studies after consumption of pomegranate juice or extracts and discusses whether urolithin could inhibit or slow the growth of prostate cancer in patients. The authors reported a significant increase in urolithin A in prostate but a nonsignificant reduction in 8-hydroxy-2-deoxyguanosine, a marker of oxidation in cancer tissue, for neoadjuvant patients subjected to radical prostatectomy after pomegranate intake (1200 mg polyphenols/day) compared with placebo in a large trial (4 weeks, n = 33) [67]. Similar results with muscadine grape skin extract have evidenced no benefit on recurrent prostate cancer patients in spite of urolithin A increase [68]. Nonetheless, authors [67] have noted in a specific group of patients (named AA genotype), which has been previously associated with more hostile prostate cancer and more sensitivity to antioxidants, a significant increase in a prostate-specific antigen doubling times (PSADT), an antigen that is claimed to slow tumor growth.

After an acute intake of grumixama cherry juice (Brazilian) by healthy women (n = 10), the antiproliferative activity of urolithins against breast cancer cells (MDA-MB-231) was evaluated. The extracts of urine exhibited seven urolithins, mainly urolithin C and urolithin A. Those extracts obtained during 2–4 h after juice intake presented the highest inhibition of proliferation of MDA-MB 231 breast cancer cells. This inhibition was attributed to a significant G2/M cell cycle arrest (apoptosis) occurred in MDA-MB-231 cells, and was demonstrated by the increase in sub-G0/G1 populations. Additionally, the authors linked this inhibition to a possible synergy among anthocyanins and urolithins [12]. Then, the modulation of the positive-estrogen receptor in breast cancer cells (MCF7) is probably one of the potential actions of urolithin conjugates. On the other hand, in a study realized with breast cancer patients (n = 19) who consumed a mixed extract (493.4 mg phenolics/day) containing pomegranate, orange, lemon, olive, cocoa, grape seed extracts plus resveratrol, theobromine, and caffeine, it was shown that the main metabolites detected in breast tissues were urolithin-A-3-O-glucuronide. Nonetheless, no antiproliferative or estrogenic/antiestrogenic activities in MCF-7 breast cancer cells were reported [69].

Ellagitannin gut microbiota-derived metabolites have shown a wide range of colon anticancer effects both in cellular and animal studies [70–72]. However, the current clinical evidence that confirms their colorectal cancer (CRC) chemopreventive effect in humans is still very weak. A study evaluated the modification of microRNAs (miRs) expression, one CRC biomarker, in normal and malignant colonic tissues from CRC patients after pomegranate extract intake (900 mg/day before surgery). As a result, pomegranate consumption seems to moderate the modulation of various specific miRs in colon tissue, but there was no association between tissue urolithins and the detected miRs changes, which were attributed to a possibly critical surgery alteration in miRs levels that did not allow to discriminate between malignant and normal tissues [73]. Another more recent study in 35 patients with colorectal cancer (CRC), daily supplemented with pomegranate extracts, was conducted to evaluate the expression of various CRC-related genes in normal and cancerous colon tissues. Before (biopsies) and posterior (surgical samples) to pomegranate intake (5-35 days). Despite the consumption of pomegranate extract was significantly associated with a balancing effect in the expression

of genes regulated by the experimental protocol, these results were not associated with the individual metabotypes or the levels of urolithins and EA in the colon tissues. Consequently, the in vitro effects were not reproduced in vivo evidencing discrepancy between results [70]. In general, we can conclude that there is a lack of clinical interventions with ET-rich food in humans; besides, these kinds of studies are essential to corroborate the real effect of urolithins on cancer.

5.2.3 Reducing risks of cardiovascular disease (CVD)

The gut microbiota is frequently presented as a key factor in the evolution of obesity and cardiovascular disorders (CVD) [74]. One clinical study clustered urolithin metabotypes (UMs) of 18 healthy overweight/obese subjects with the aim of correlating metabotype status with CVD biomarkers after pomegranate extract consumption. In baseline and before UM clustering, the whole group exhibited mild dyslipidemia, and after clustering, only the serum lipid profile of UM-B individuals (n = 15) showed moderate risk values in total cholesterol, intermediate-LDL-cholesterol, as well as other serum lipids related to CVD risk. After ET intervention, only blood biomarkers of UM-B subjects were improved after pomegranate extract intake, reducing their CVD risk. Interestingly, a dose-dependent behavior was notable only in UM-B patients [48].

Another experiment comparing healthy patients with patients with metabolic syndrome (MetS), both consuming walnuts, showed that urolithin A only was inversely correlated with glycaemia in MetS individuals. Additionally, when MetS patients with UM-A were treated with statin, their lipid profile became similar to healthy individuals. This was not the case for individuals with UM-B [74].

Another study showed that the increasing relative importance within the microbiota of bacteria from the Coriobacteriaceae family such as *Olsenella, Senegalimassilia, and Slackia*, which characterized UM-B status, was positively correlated with blood cholesterol levels and normal BMI [49].

Endothelial dysfunction and inflammation are both usual events that occur in the development of atherosclerosis. The correlation between the plasma urolithin metabolites and improvement in endothelial function after red raspberry intake was reported. Endothelial function measured as flow media vasodilation (FMD) presented two peaks, first at 1–2 h after intake, linked with EA plasma peak concentration, and second peak at 24 h, associated with urolithin-3-glucuronide and urolithin-A-sulfate absorption peaks. Similar results were reported by other authors in cranberry and blueberry juice interventions [75], but it was shown that effect was the same when consuming 200 or 400 g of raspberries. Additional distinctive key factors in atherosclerosis development have been reported, as the capacity of monocytes to adhere to endothelial cells and the uptake and efflux of cholesterol by macrophages. In vitro, urolithins and EA were able to reduce adhesion of THP-1 monocytes to human umbilical vein endothelial cells and reduce secretion of sVCAM-1 and IL-6, a cellular adhesion molecule and a pro-inflammation cytokine, respectively. Also, urolithin C and EA were associated with decreased accumulation of cholesterol in THP-1–derived macrophages [76]. Attenuation of THP-1 also was reported in the presence of urolithin A in endothelial cells and also reduced considerably the expressions of ICAM-1 and MCP-1, an intercellular adhesion molecule and a monocyte chemotactic protein, respectively [77].

6. Concluding remarks

Ellagitannins are present in considerable amounts only in some specific food sources such as berries and nuts, but some tropical fruits deserve attention. Their

diverse structure can be modified during food processing resulting in free ET and EA derivatives, which are poorly bioavailable. After ingestion, most ETs are spontaneously converted into EA, which is poorly bioavailable and can be used as substrate by gut microbiota. The main products resulting from the action of gut microbiota on EA are urolithins. The main biomarkers in blood and urine of ET-rich food exposure are urolithins A and B. Nonetheless, there is an important interindividual variability in the excretion of urolithins, and this observation has led to the classification of the population in three metabotypes: the "low urolithin" excreters that represent approximately 10% of the population; the "urolithin A" excreters, the most important group with approximately 55% of individuals; and finally, the "urolithin A and B excreters" that represent around 35% of the population. The metabotype status appears to be quite persistent although it can change during life span and the constant exposure to ET-rich food appears to increase urolithin production. Microorganisms from the Coriobacteriaceae family were identified as urolithin producers and the relative importance of this family within the microbiota was apparently correlated with the metabotype. The stratification of individuals by their metabotype was essential to overcome inconsistencies during clinical trial, and it must be taken into account in all future intervention studies. The positive biological effects of ETs and EA at the level of the GI tract are consistent and reported by various authors. For urolithins, the panorama is more confused, and more long-term clinical intervention studies with human are required. Nonetheless, at the end of this review, the potential health effect of ET-rich foods is definitely promising and they deserve to be part of a healthy diet as functional foods.

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Conflict of interest

The authors confirm that there is no conflict of interest.

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