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MINI-REVIEW

Microbial production of ellagic acid and biodegradation of ellagitannins

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Abstract In the last years, tannin biodegradation has been the subject of a lot of studies due to its commercial importance and scientific relevance. Tannins are molecules of low biodegradation and represent the main chemical group of natural anti-microbials occurring in the plants. Among the different kinds of tannins, ellagitannins represent the group less studied manly due to their diversity and chemical complexity. The general outline of this work includes information on tannins, their classification and properties, biodegradation, ellagic acid production, and potential applications. In addition, it describes molecular, catalytic, and functional information. Special attention has been focused on the biodegradation of ellagitannins describing the possible role of microbial enzymes in the production of ellagic acid.

Keywords Ellagitannins · Ellagic acid · Biodegradation

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Introduction

Tannins are natural compounds widely distributed in the plant kingdom. These compounds are present in roots, leaves, fruits and seeds. The function of tannins is the defense system of plants against microbial and animal attacks due to their astringent capacity and the ability to form complexes with proteins and polysaccharides (Swain and Bate-Smith 1962). Tannins are a complex family of polyphenolic compounds, water-soluble, with molecular weights between 500 and 3,000 Da (Lekha and Lonsane 1997). However, these molecular weight values change when they are polymerized to form condensed or hydrolysable tannins reaching values close to 40,000 Da (Spencer et al. 1988; Khanbabaee and van Ree 2001). Tannins are secondary metabolites of plants (Bhat et al. 1998). It is generally accepted that considering their sugar content, polymerization and esterification degrees they are divided in three groups: condensed tannins, hydrolysable tannins and complex tannins. However, recently a further breakdown into four groups was proposed: condensed tannins, complex tannins, gallotannins, and ellagitannins (Khanbabaee and van Ree 2001).

Condensed tannins are polymers of flavan-3 ol or flavan-3,4-diol that do not contain sugar residues (Lekha and Lonsane 1997). They are known as polymeric proanthocianidins and leucoanthocyanidins based on flavan units, mainly catechin or epicatechin. A typical condensed tannin can be represented by the dimmer of proanthocyanidins linked to flavan units. To date, condensed tannins of high molecular weight have not been recovered and purified. Oligomers and polymers of low molecular weight have however been characterized. Their carbon–carbon linkages are not susceptible to formation of news linkages after their hydrolysis (Garro et al. 1997).

Complex tannins are those compounds resulting from the linking of catechins or epicatechins with gallic or ellagic acids due to reactions catalyzed by light, heat, and oxygen. A typical mixed tannin is catechin gallate, containing hydrolysable and condensed moieties.

Gallotannins are hydrolysable tannins formed from galloyl units linked to a glucosidic core (Khanbabaee and van Ree 2001). Gallotannins are polyphenols susceptible to hydrolysis by enzymes (tannase activity), acids or alkalis (Huang et al. 2005). A typical gallotannin is tannic acid, molecule formed by a central glucose moiety and five (pantagalloyl glucose) to nine (nanogalloyl glucose) galloyl residues (Belmares-Cerda et al. 2004). It is important to note that to be considered as hydrolysable, a tannin molecule must have at least three galloyl radicals esterified with a polyol (Sánchez-Alvarado 2001).

Ellagitannins contain a group hexahydroxydyphenic acid (HHDP), which after hydrolysis is dehydrated followed by spontaneous lactonization forming ellagic acid (Fig. 1). Monophenols derived from tannins are an important group of molecules with interesting biological activities, among which are gallic, ellagic, quinic, caffeic, and ferulic acids.

Physiological properties of tannins

Tannins are located in vacuoles of intact plant cells, and are released upon attack by diverse microorganisms, including viruses, bacteria, and fungi, thereby avoiding potential infection of plant tissues (Field and Lettinga 1992a; Silva et al. 1997). In addition, the astringent properties of tannins stop the infestation of insects (Goldstein and Swain 1965). In addition, tannins offer protection against ruminants due to the formation of complexes between plant tannins and animal proteins such as hydroxyproline-rich proteins. Formation of such complexes results in a bitter and disagreeable sensation which deters potential predators (Edelmann and Lendi 2002). Molecules present in the plants that are susceptible to microbial degradation, such as proteins and polysaccharides, have evolved to become highly resistant to such degradation when linked to tannins (Betnoit et al. 1968; Aguilar and Gutierrez-Sanchez 2001).

Tannins act as growth inhibitors towards many microorganisms including bacteria, yeasts, and fungi. They are therefore recalcitrant to enzyme degradation by most microorganisms (Field and Lettinga 1992b; Silva et al. 1997). Condensed tannins are more resistant to microbial attack than hydrolysable tannins and are more toxic for foodborne pathogens (Aguilera-Carbo et al. 2005). Scalbert (1991) reported that tannins retard the decomposition of solid organic material through inhibition of degrading enzymes of attacking microorganisms. When tannins are complexed with microbial proteins or polysaccharides, the interactions formed are often irreversible, and this characteristic confers bactericide and bacteriostatic properties. However, some microorganisms tolerate the presence of tannins and/or use these compounds as carbon source (Knudson 1913; Deschamps et al. 1983; Field and Lettinga 1992b; Cruz-Hernández et al. 2005, 2006). That ability is generated by the production of a tannin-degrading enzyme or tannase, produced mainly by microorganisms of the genus *Aspergillus* and *Penicillium* (Belmares-Cerda et al. 2004; Aguilar et al. 2007a, b).

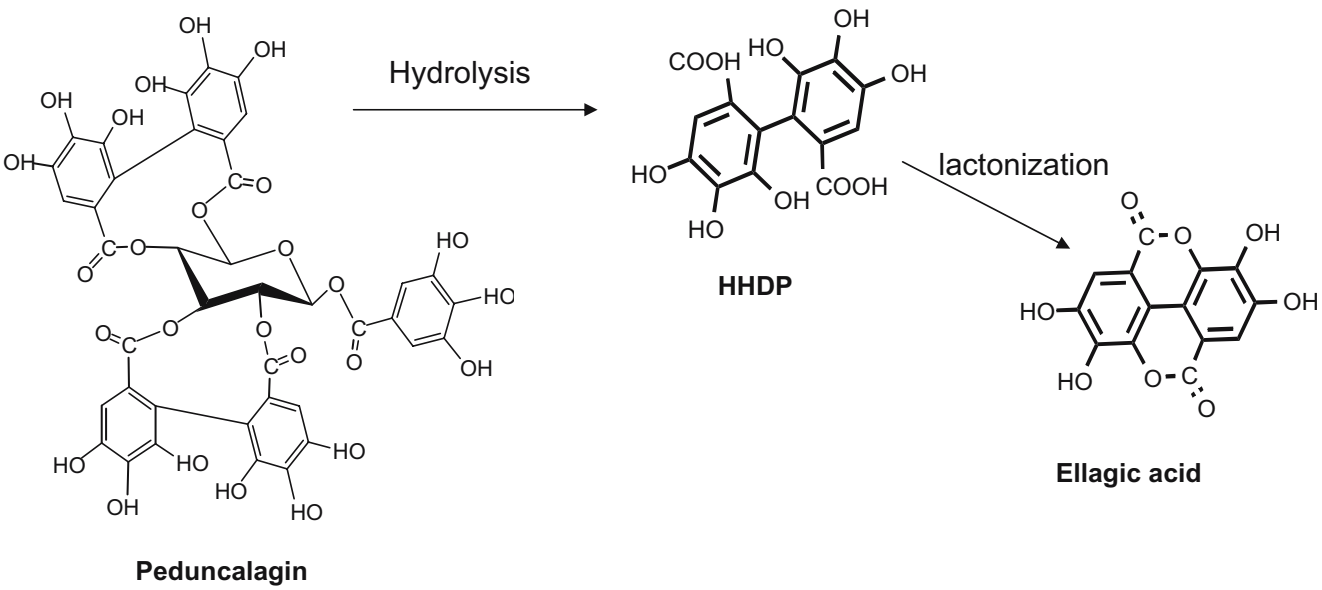

Fig. 1 Scheme of enzymatic hydrolysis of an ellagitannin to produce ellagic acid by a microbial conversion

Ellagitannins biosynthesis

The compound widely accepted as the major precursor of ellagitannins in plants is 1,2,3,4,6,-penta-*O*-galloyl-*b*-D-glucose (PPG), (Niemetz et al. 2001). The metabolic pathway for production of these compounds has been elucidated. The oxidation of PGG is carried out enzymatically by a polyphenoloxydase (laccase) catalyzing the formation of hexahydroxydiphenic acid (HHDP) *in situ* (Niemetz and Gross 2003, 2005).

Enzyme studies have shown that the biosynthesis of 1,2,3,4,6-pentagalloyl-β-*d*-glucose, the common and immediate precursor of the two sub-classes of hydrolyzable tannins (gallotannins and ellagitannins), involves a series of highly position-specific galloyltransferase reactions which depend on β-glucogallin (1-*O*-galloyl-β-*d*-glucose) as principal acyl donor. This mechanism applies also to the subsequent addition of galloyl groups to pentagalloylglucose, yielding complex gallotannins characterized by meta-digalloyl residues (Hoffmann and Gross 1990).

Studies with cell-free extracts from sumac (*Rhus typhina*) leaves revealed the existence of several isoenzymes that catalyzed the in vitro acylation of pentagalloylglucose. Among these, three galloyltransferases (A, B, and C) were isolated and separated according to their different molecular weights. Galloyltransferase C has been purified to apparent homogeneity and consisted of four identical subunits converting pentagalloylglucose to the gallotannins and ellagitannins (Niemetz and Gross 2003). Galloyltransferases A and B were found to preferentially form polygalloylglucoses. It is thus evident that galloyltransferases A and B promote substitution at positions 2 and 4 of the pentagalloylglucose core, while transferase C is specific for the acylation of position 3.

Ellagitannins and ellagic acid

Ellagitannins and ellagic acid have been studied mainly for their positive effect on human health and for their physiological properties such as anti-tumoural (Feldman et al. 1999; Ito et al. 1999; Wang et al. 1999; Talcott et al. 2003), anti-peroxidating (Okuda et al. 1993), anti-viral (Uchiumi et al. 2003; Ruibal et al. 2003; Notka et al. 2004), anti-oxidant (Tan et al. 1991; Da Porto et al. 2000; Gil et al. 2000; Anderson et al. 2001; Vattem and Shetty 2003; Fukuda et al. 2003; Olsson et al. 2004), anti-foodborn pathogens (Aguilera-Carbo et al. 2005), and the anti-mutagenic (Tatsuo et al. 1999) activities. Furthermore, ellagitannins are considered as activators of glucose transport (Hayashi et al. 2002), and chelating agents of metals. Industrially, ellagitannins and ellagic acid are used in creams, eau de toilette and other cosmetic products which have a clearing effect of the skin (Patent CPE: 88109207.6). However, in the food industry these compounds have not been approached.

Ellagitannins can be classified according to the number of HHDP groups present in the molecule (Quideau and Feldman 1996) as well as the different monomeric structures that then make up the oligomers and polymers (Fig. 2).

Ellagic acid is a dilactone of the hexahydroxydephenic acid with a molecular weight of 338.2 g/mol. It is highly thermostable due to the four rings of the molecule (Fig. 1), which represent lipophilic dominance, and the four phenolic groups and the two lactones representing the hydrophilic zone (Bala et al. 2006). These properties of ellagic acid result in high water insolubility. However, it is soluble in acidified methanol (Lei 2002), ethanol (Shi et al. 2005), and dimethyl sulfoxide (Bala et al. 2006).

Monomeric ellagitannins are comprised of a sole HHDP group linked to a glucosidic core. Carbon–carbon oxidative linkage between galloyl groups generates HHDP transforming PGG into a monomeric ellagitannin (Haslam and Cai 1994). Generally, the linked carbons in the galloyl groups are in positions C2–C3, and C4–C6 of the glucose molecule. Furthemore, the linked carbons can correspond to carbons presents in galloyl radicals located in C1–C6, C3–C6 and C2–C4 of the glucose core. These different bonding capacities confer structural complexity to monomeric ellagitannins. In addition, complexity reaches higher levels of galloylation and stereochemistry of the HHDP groups (Haslam and Cai 1994; Quideau and Feldman 1997; Lei 2002). Notable ellagitannins present in plants are 4,6-HHDP and 2,3-HHDP. Typical 4,6-HHDP monomeric ellagitannins are the tellimagradin II, Strictin, and Tellimagradin I (Fig. 2); typical 2,3-HHDP monomeric ellagitannins are the pterocaryanin C, sanguine H5 and H4, Mixtures of 4,6-HHDP and 2,3-HHDP ellagitannins are casuarictin, potentillin, and pedunculagin (Lei 2002). Other significant groups of monomeric ellagitannins are formed by acyclic aromatic glucosides represented by vascalagin and castalagin, which are characterized by the presence of a flavogalloyl group with three linked galloyl groups (Quinn and Singleton 1985; Virot et al. 1994).

Monomeric ellagitannins can polymerized to form oligomeric and polymeric ellagitannins. Polymerization is the result of the oxidative linkage C–O between galloyl groups and HHDP with galloyl groups (Quideau and Feldman 1996; Zhang et al. 2001). Typical oligomeric ellagitannins are vascalagin and/or castalagin: roburin A and Roburin D (Quideau and Feldman 1996, 1997; Zhang et al. 2001; Lei 2002).

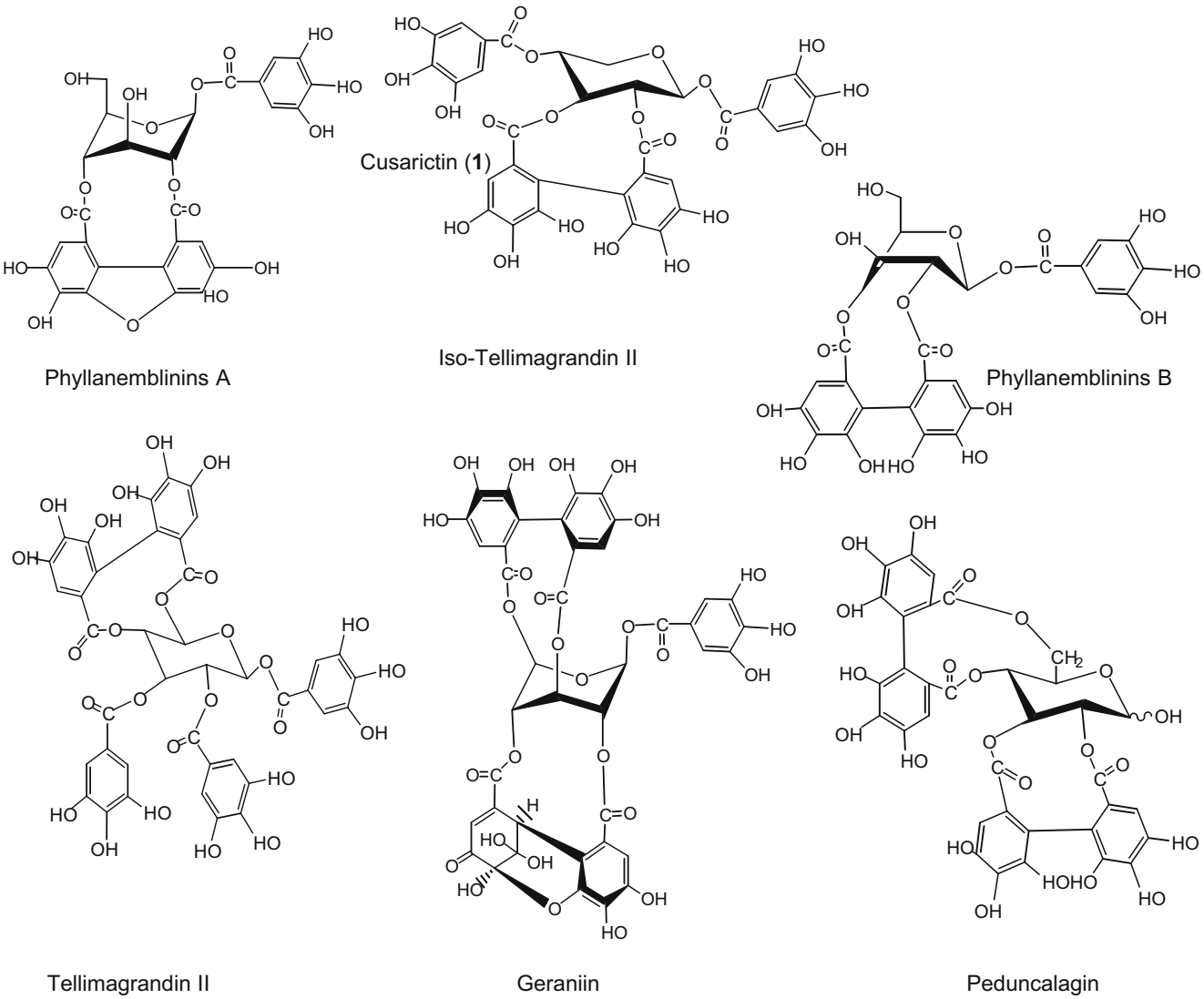

Fig. 2 Typical monomeric and dimeric ellagitannins

Sources of ellagitannins

There are more than 500 different reported structures of ellagitannins (Feldman et al. 1999). Table 1 presents some typical ellagitannins and their natural sources. Shrubby trees are excellent sources of ellagitannins. El-Toumy et al. (2001) have reported several ellagitannins obtained and characterized from *Punica granatum* among which are: ellagic acid-4-*o*-α-L-rhamnopyranose, 6-*o*-galloyl-(α/β)-D-glucopyranose, 6-*o*-galloyl-2,3-*S*-hexahydroxyphenoyl-(α/β)-D-glucopyranose, coralagin, 3,3′/-di-*o*-methyl ellagic acid, 3′/-*o*-methyl-3,4-methylendioxy ellagic acid. El-Toumy and Rauwald (2002) reported the punicacortein D, punicalin, punicalagin, 2-*O*-galloylpunicalin and two new ellagitannins, diellagic acid rhamnosyl (1–4) glucopyranose and 5-galloylpunicacortein D.

Ellagitannins and ellagic acid are consumed constantly in fruit, seeds, and in the foods or beverages based on fruit juices and jams, etc. (Clifford and Scalbert 2000). High contents of ellagitannins and ellagic acid have been reported in strawberry, cranberry, blueberry, and blackberry (Constantin 1997; Kähkönen et al. 2001; Lei et al. 2001; Mullen et al. 2002; Wada and Ou 2002; Vattem and Shetty 2003; Määttä-Riihinen et al. 2004; Kaponen et al. 2007). In addition, their presence has been reported in pecans, nuts, some roots, and pomegranates (Table 2).

Extraction, characterization, and quantification

It is important to apply the best techniques for extraction of ellagitannins and ellagic acid. The key step of the extraction process is the selection of the solvents and their mixtures. Better results have been found using methanol/water or acetone/water. Lei (2002) reported a comparison of those mixtures and concluded that the mixture of acetone/water in

Table 1 Sources of main ellagitannins

Ellagitannins	Fuente vegetal	References
Vascalagin, castalgin and valonea	*Quercus* sp.	Lei et al. 2001, Huang et al. 2005
Nobotanins G, K, P,Q, R,S and T; brediatin B, nobotanin A, B, F, β-glucogallin, pedunculagin,, 4-6-(*S*)-HHDP-glucopyranose, malabathrin D, pterocaryanin C, (1,4,6-*O*-trigaloyl-2,3-*O*-HHDP-β-D-glucose), Casuarictin (1-*O*-galloyl-2,3/4,6-*O*-bisHHDP-β-D-glucose)	*Monochaetum multiflorum*	Isaza et al. 2004
Nobotanins O and P, Stachyurin, Casuarinin, Madinillin B, Nobotanins A, B, D, F, G, J and M, Pedunculagin, Causarictin	*Tibouchina multiflora*	Yoshida et al. 1999
Thonningianins A and B	*Thonningia sanguinea*	Ohtani et al. 2000, Mullen et al. 2003
Ellagic acid -4-arabinose, Sanguiin H-6, Sanguiin H-10, Lambertianin C, ellagic acid-4-acethylxylosa, ellagic acid-4-acethylarabinose	*Rubus sp.*	
Jolkinnin, Geraniin, Corilagin, Carpinusin, Putranjivain, Helioscopinin B, Helioscopinin A	*Euphorbia jolkinii*	Lee et al. 2004, Chen et al. 1999,
Cuphiins D_1 and D_2, Woodfordin C, Miryccitrin, Tellimagrandin II, Oenothein B	*Cuphea hyssopifolia*	Zhang et al. 2001
Phyllanemblinins A, B and F, Corilagin, 1-(β),2,3,6-tetra-*O*-galloylglucose, Chebulanin, chebulagic acid, Elaeo-carpusin, punicafolin, tercatain, mallonin, Putranjivain A	*Phyllanthus emblica*	
Ellagic acid 4-*O*-α-l-rhamnopyranose, 6-*O*-galoil-(α/β)-d-glucopyranose, 6-*O*-galoyl-2,3-*S*-haxahydroxyphenoyl-(α/β)-d-glucopyranose, coralagin, 3,3′/-di-*O*-methyl ellagic acid, 3′/-*O*-methyl-3,4-methylendioxy ellagic acid. punicacortein D, punicalin, punicalagin, 2-*O*-galloylpunicalin, diellagic acid rhamnosyl (1–4) glucopyranose y 5-galloylpunicacortein D	*Punica granatum*	El-Toumy et al. 2001, El-Toumy and Rauwald 2002, Machado et al. 2001, Seeram et al. 2005

The key biosynthesis way is the 1,2,3,4,6,-penta-*O*-galloyl-β-D-glucose formation.

Table 2 Ellagitannins-rich plants of economical importance used as foods

Family	Latín name	Common name
Anacardiaceae	*Anacardium occidentale*	Cashew
	Pistacio vera	Pistachio nut
	Mangifera indica	Mango
Betulaceae	*Corylus avellana*	Hazelnut
Ebenaceae	*Diospyros* kaki	Persimmon
Fagaceae	*Castanea* sativa	Chestnut
Juglandaceae	*Juglans* regia	Nut
Myrtaceae	*Psidium* guajava	Guava tree
	Eugenia caryophyllata	Clove
	Pimenta officinalis	Green pepper
Punicaseae	*Punica granatum*	Pomegranate
Rosaceae	*Prunus domestica*	Plum
	Prunus armeniaca	apricot
	Prunus persica	Peach
	Prunus avium	Wild cherry
	Fragaria spp	Strawberry
	Rubus idaeus	Raspberry
	Rubus fruticosus	Blacberry bush
	Ribes nigrum	Blackcurrant
	Ribes rubrum	Redcurrant
	Ribes grossularia	Wild currant
Theaceae	*Camelia sinenis*	Te
Vitaceae	*Vitis vinifera*	Grape
	Vitis rotundifolia	wine grape (moscatel)

Source: Clifford and Scalbert (2000)

a 7:3 ratio was the best condition where the highest yields were reached. It is important to use a temperature of 60°C and avoid light during the extraction process with constant agitation. The amount of dried sample recommended is a gram per 10 ml of solvent.

After 12 or 24 h of extraction, samples should be filtered to remove contaminants such as fibers, pigments, and other compounds. S and then solvent is then removed by evaporation. The obtained aqueous extract is frozen and lyophilized to produce the dry sample, which is considered as total polyphenols. From this sample fractionation of different tannins is carried out depending on the techniques and equipment available. For this reason, it is important to review the methods to quantify ellagitannins.

First methodologies used for ellagitannin quantification were based on the capacity of tannins to form complexes with proteins; however, these methods are very unspecific. Spectrophotometric assays were developed generally using as the key reactants potassium iodate, pyridine, sodium nitrate, and methanolysis (Wilson and Hagerman 1990; Lei et al. 2001; Hartzfel et al. 2002).

Chromatographic methods used to quantify ellagitannins and ellagic acid include high performance liquid chromatography (HPLC) thin layer chromatography (TLC) and electrophoresis chromatography (EC). The last one shows low reproducibility. However, it is easy to

carry out and enables purification of ellagitannins (Ferreres et al. 1994; Andrade et al. 1997). In the case of TLC, it has not been considered a quantification techniques. Nevertheless, the presence or absence of some particular compounds can be carried out (Lei 2002; Machado et al. 2002).

HPLC is the most adequate (but most expensive) tool for separation and quantification of ellagitannins. Several methodologies have been reported (Okuda et al. 1986; Scalbert et al. 1990; Bianco et al. 1998; Häkkinen et al. 1998; Doussot et al. 2000; Lei et al. 2001; Lee and Talcott 2002). Exact and meticulous HPLC analysis has a critical point and several advantages. It is important to consider the kind or sample, its nature and solubility. Most techniques have focused on the run conditions on HPLC with deficiencies in the extraction conditions and on sample pre-treatment.

The best way to define the qualitative composition of ellagitannins is using mass spectrophotometry (MS^n; Mullen et al. 2003; Määttä et al. 2003) and nuclear magnetic resonance (NMR; Lee and Talcott 2005), tools which have given most of the detailed insight on ellagitannins reported until today.

Biodegradation of ellagitannins and ellagic acid production

Nowadays, numerous studies have been carried out on gallotannin biodegradation and have gained great success in further utilization (Bhat et al. 1998). Some of the industrial applications of these findings are in the production of tannase, the biotransformation of tannic acid to gallic acid or pyrogallol, and detannification of food and fodder. Although ellagitannins have the typical C–C bound, which is more difficult to degrade than gallotannins, concerted efforts are still in progress to improve ellagitannin degradation and utilization (Li et al. 2006). In recent years, more attention is mainly focused on intestinal microflora biodegradation of tannins especially ellagitannins that can contribute to the definition of their bioavailability for both human beings and ruminants (Goel et al. 2005). Furthermore, there have been endeavors to utilize the tannin-degrading activity of different fungi for ellagitannin-rich biomass, which will facilitate application of tannin-degrading enzymes in strategies for improving industrial and livestock production. Table 3 presents a list of microorganisms and ellagitannin-rich materials used to produce ellagic acid.

Due to the complicated structures of complex tannins and condensed tannins, their biodegradation is much more difficult with little work carried out (Bhat et al. 1998). Therefore, major emphasis on the mechanisms of gallotannin and ellagitannin biodegradation is needed for an overall understanding of the biodegradation of complex tannins and condensed tannins. Biodegradation of tannins is in an incipient stage and further studies have to be carried out to exploit the potential of various tannins for largescale applications in food, fodder, medicine, and tannery effluent treatment (Li et al. 2006).

Several studies have reported the biodegradation of tannins (gallotannins) to produce the antioxidant gallic acid (Aguilar et al. 2007a, b). This bioprocess includes the use of microbial cultures to induce the biosynthesis of the tannin acyl hydrolase (EC 3.1.1.20) generally referred to as tannase, an esterase which hydrolyses ester bonds present in tannins (Aguilar and Gutierrez-Sanchez 2001). However, in the particular case of ellagitannins, the information is

Table 3 Microorganisms and ellagitannins-rich materials used for ellagic acid production

Microorganism	Culture system	Sources of Ellagitannins	References
Lentinus edodes	SSF	Cranberry pomace	Zheng and Shetty 2000, Vattem and Shetty 2003
Rhizopus oligosporus	SSF	Cranberry pomace	Vattem and Shetty 2002
Aspergillus niger/Candida utilis	Co-culture/SmF	Fruit shell of Quercus aegilops (valonea)	Shi et al. 2005
Aspergillus SHL 6	SmF	Fruit shell of Quercus aegilops (valonea)	Huang et al. 2005
Aspergillus niger GH1	SSF	Pomegranate husk	Robledo-Olivo et al. 2006, Aguilera-Carbo et al. 2007
Aspergillus oryzae	SmF	Acorn fringe	Huang et al. 2007a
Aspergillus oryzae/Trichoderma reesei	SmF	Acorn cups	Huang et al. 2007b, c
Aspergillus niger PSH	SSF	Leaves of creosote bush (*Larrea tridentata*) and tar bush (*Fluorencia cernua*)	Ventura et al. 2007
Aspergillus niger GH1	SSF	Leaves of creosote bush (*Larrea tridentata*)	Aguilar et al. 2007a

scarce and confusing (Scalbert et al. 1990; Vivas et al. 2004), mainly due to their chemical complexity and diversity of kinds of ellagitannins.

Saavedra et al. (2005) reported that the production of ellagic acid has not been explored due to its high production cost and the great amount of by-products generated as a result of ellagitannin biodegradation, which results in serious problems related to recovery and purification of ellagic acid.

Until now, the study of ellagitannin degradation using biological methods (enzymatic or microbial) is emerging as a promising and fascinating topic (Huang et al. 2005, 2007a, b, c, d; Robledo-Olivo et al. 2006; Aguilera-Carbo et al. 2007). Published works on this topic have focused on ellagic acid production mention of the enzymes involved. However, it is known that the selective hydrolysis of galloyl groups of the ellagitannin phylianmblinin is catalyzed by tannase (Zhang et al. 2001). Yoshida et al. (1999) reported the production of nobotannin K from complex ellagitannins through catalysis of tannase; however, the biochemical mechanism was not clearly explained.

Figure 3 shows a summarized ellagitannin biodegradation pathway which highlights the lack of information regarding the first enzymes involved in the primary stages of hydrolysis of ellagitannins and also about the conditions of lactonization of ellagic acid. Until today, both points have not been clearly described. The rest of the biodegradation pathway has been well studied and described (Bhat et al. 1998; Li et al. 2006).

Vattem and Shetty (2002, 2003) reported on ellagic acid production from cranberry pomace fermented by a solid state culture using *Lentinus edodes*, attributing the catalysis to the enzyme β-glucosidase, Huang et al. (2005) presented valonea tannin hydrolase as responsible for the biodegradation of valonea tannins. Nevertheless, this enzyme is itself a tannin acyl hydrolase.

Li et al. (2006) erroneously reported in their review that Vaquero et al. (2004) published that the tannin acyl hydrolase produced by species of *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Pediococcus* hydrolysed gallotannins, ellagitannins, and condensed tannins present in muscadine

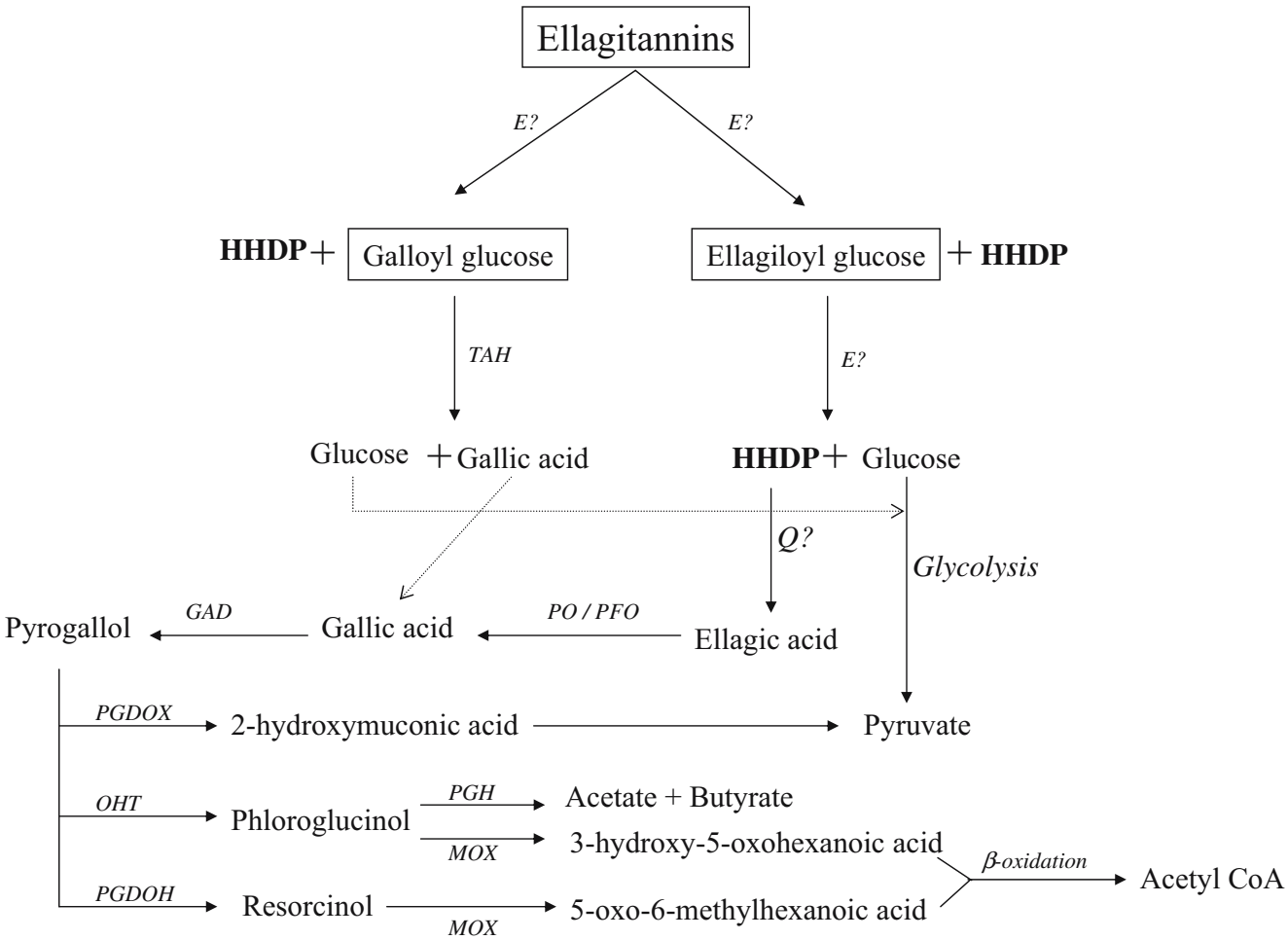

Fig. 3 Scheme of biodegradation of ellagitannins. *E?*, unknown enzymes; *TAH*, tannin acyl hydrolase; *PO*, peroxidase; *PFO*, polyphenoloxidase; *GAD*, gallic acid descarboxylase; *PGDOX*, pyrogalloldioxigenase; *OHT*, hydroxyltransferase; *PGDOH*, pyrogalloldeshydroxylase, *PGH*, phloroglucinol hydrolase, *MOX*, monoxygenase, *Q?*, the lactonization is spontaneous or enzymatically calatyzed

grapes. Actually, Vaquero et al. (2004) only considered tannase production by lactobacilli without evaluation of ellagic acid released from ellagitannins.

With regards to the chemical nature of ellagitannins, glucosidase activity reported by Vattem and Shetty (2003) and Ramirez-Coronel et al. (2003) could be associated with ellagitannin biodegradation. To corroborate such a statement, Lee and Talcott (2005), incubated ellagitannins from muscadine grape seeds with commercial tannase and α-glucosidase. They found no activity with tannase, while α-glucosidase showed high reactivity to ellagitannins. However, these reports are not enough to know in a precise manner the biodegradation of ellagitannins.

Aguilera-Carbo et al. (2007) reported on the ellagic acid production by fungal solid state culture using polyurethane foam (PUF) as support and an aqueous extract obtained from pomegranate husk (*Punica granatum*) as carbon and energy source. In that study *Aspergillus niger* GH1 was able to consume ellagitannins during the first 36 h of culture with a maximum ellagic acid concentration reached at 48 h. This study demonstrated the feasibility of ellagic acid production through biotechnology. The authors attributed ellagitannin biodegradation to a new tannase, which is probably different from tannin acyl hydrolase. This hypothesis was recently supported by Huang et al. (2007a, d) in their study of individual and interactive effects of physicochemical parameters in ellagitannin acyl hydrolase activity and ellagic acid production by *Aspergillus oryzae* using ellagitannins from acorn fringe of oak as substrate and during the evaluation of effect of ellagitannin acyl hydrolase, xylanase, and cellulase on ellagic acid accumulation from cup extract of valonea acorns.

The most attractive results in microbial ellagic acid production have been reported by the group of Huang in China using a co-culture of *Aspergillus oryzae* and *Trichoderma reesei* employing as source of ellagic acid acorn cups extract containing up to 62% ellagitannins (Huang et al. 2007b, d).

In relation to the use of enzymatic treatments to produce ellagic acid, Huang et al. (2007c) reported that it is possible to reach better results with the use of combinations of ellagitannin acyl hydrolase, β-glucosidase and polyphenol oxidase or ellagitannin acyl hydrolase, cellulase, and xylanase from acorn fringe.

All reported works have focused on the evaluation of ellagic acid released considering aspects of process operation, recovery and optimization. However, physiological, biochemical, calatyltic, and molecular aspects have not been considered seriously. In addition, a study to confirm the difference between tannin acyl hydrolase and ellagitannin acyl hydrolase is still necessary to demonstrate the catalytic differences of both enzymes and to understand the biodegradation processes of gallotannins and ellagitannins.

To say that ellagitannins and ellagic acid are compounds of importance is an understatement in light of their many implications in the pharmaceutical, cosmetic, environmental, food, nutraceutical, and beverage industries. However, it is necessary to develop new processes and to study new sources for their production. Moreover, it is necessary to identify and characterize key enzymes involved in the hydrolysis HHDP group of ellagitannins.

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