Interactions between Salivary Proteins and Dietary Polyphenols: Potential Consequences on Gastrointestinal Digestive Events

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ABSTRACT: The present review documents the current knowledge and hypotheses on how polyphenols−saliva interactions may modulate the bioaccessibility or bioavailability of nutrients and highlights research prospects in the field. After an updated description of the different classes of dietary polyphenols and their modifications by food processing or digestion, an overview of interactions between salivary proteins and polyphenols (with an emphasis on tannins) is provided. *In vitro* studies show that the solubility of salivary protein−tannin complexes in gastric conditions depends on the degree of tannin polymerization, while complexes are partly solubilized by bile salts. Salivary proteins−polyphenols interactions may affect digestive processes. For example, polyphenols can bind to and inhibit salivary amylase, with downstream consequences on starch digestion. Some salivary proteins (PRPs) prevent tannin-induced reduced protein digestibility, probably through binding tannins before they interact with digestive proteases. Salivary proteins may also act as scavenger molecules to limit the intestinal uptake of tannins.

KEYWORDS: *digestion, saliva, polyphenols, tannins, proline-rich proteins, mucins*

INTRODUCTION

The health benefits of dietary polyphenols are tremendously well-documented, for example regarding cardiovascular health,[1] metabolic diseases,[2] or cognitive decline.[3] When it comes to digestion, the impact of polyphenols is described mainly in relation to inhibition of enzymes[4,5] or modulation of gut microbiota.[6] Another aspect of polyphenols that is extensively studied is their contribution to the sensory properties of food: bitterness and astringency. For the latter, a key mechanism at the origin of this sensation resides in the ability of polyphenols to aggregate proteins including those forming the mucosal pellicle. Such binding may initiate changes in the friction forces at the surface of the oral mucosa and/or intracellular signaling leading to the release of a neurotransmitter.[7] This mechanism is modulated by the presence in saliva of proteins presenting a high affinity for tannins. In fact, when polyphenols enter the digestive tract, it is expected that part of them will be in the form of salivary proteins−polyphenols complexes.

In parallel, the impact of oral physiology (mastication and salivation) on digestion is gaining interest. For example, *in vitro* digestion was applied to boli produced by *in vivo* mastication of protein-fortified sponge cakes[8] or to boli produced by *in vitro* simulated normal or deficient mastication of meat products[9] or pasta.[10] The impact of saliva on digestion has also been described, focusing on its enzymatic content,[11] antioxidant properties,[12] or the effect it may have on emulsions.[13]

The present article aims at bringing these different fields closer together. This narrative review does not intend to summarize the plethoric literature on the effect of polyphenols on digestion but is focused on the polyphenols−saliva interactions and how these may modulate the bioaccessibility or bioavailability of polyphenols themselves or of other nutrients. For that purpose, the review provides an updated description of the different classes of polyphenols and how they are modified by either food processing or digestion. A second part will deal with salivary proteins, especially their fate during digestion, their interactions with polyphenols, and how the resulting complexes may be affected by digestion. This will provide the necessary background to then consider the impact of such interactions (binding, complexation, and/or precipitation) on digestive processes. Some hypotheses have been tested experimentally and published, and they are reported in this article. We also report articles suggesting an impact of polyphenols−salivary proteins interactions, even when this was not tested specifically. Finally, we will also formulate novel hypotheses based on the current knowledge.

DIFFERENT CLASSES OF DIETARY POLYPHENOLS

Polyphenols are very widespread secondary metabolites mainly found in higher plants and in some brown algae. They constitute a vast family of compounds with very diverse molecular structures. However, the term "polyphenol" is often used inappropriately in the literature, in particular by mistakenly considering as polyphenols compounds having in their structure only a single phenolic nucleus. Thus, a comprehensive definition for polyphenols was recommen-

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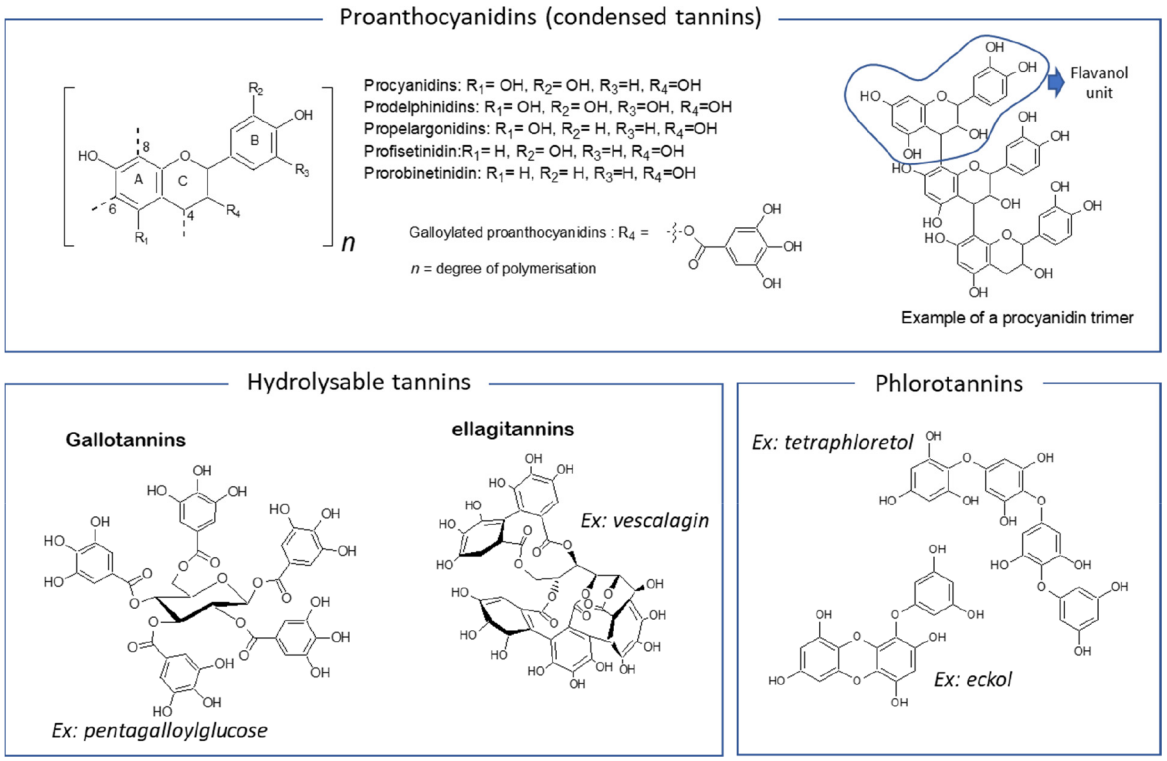

Figure 1. Three classes of vegetable tannins

ded:[14] The term "polyphenol" should be used to define plant secondary metabolites derived exclusively from the shikimate-derived phenylpropanoid and/or the polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression. Thus, very widespread simple phenolics such as hydroxybenzoic or hydroxycinnamic acids must not be considered as "true polyphenols".

With regard to this definition and based on biosynthetic pathways, several categories of polyphenols are distinguished. A special mention is made for flavonoids, of which there are more than 8000 different polyphenolic structures which are subdivided into a large series of subclasses (anthocyanins, flavanols, flavonols, flavones, etc.) according to the oxidation state and chemical functionalization scheme of the heterocycle.[15] Stilbenes (for instance, resveratrol) and lignans are two other less widespread polyphenol classes for which numerous papers related to their biological properties have been published.[16,17]

The other method of polyphenol categorization refers to a specific physicochemical feature of certain polyphenols—their tanning property—that refers to the ability of some polyphenols to associate to proteins. Obviously, this property is of particular importance in the context of the present review focusing on polyphenol interactions with salivary proteins. Historically, the term "vegetable tannin" refers to a plant extract used for the conversion of animal skin into leather. Still today, the generally accepted definition for a "vegetable tannin" is the one first proposed by Theodore White, a chemist in the leather industry,[18] and later completed by Swain and Bate-Smith.[19] Vegetable tannins are water-soluble phenolic compounds having a molecular weight between 500 and 3000 Da and having the ability to precipitate alkaloids, gelatin, and other proteins. Nevertheless, the upper value of molecular weight (3000 Da) must be now reconsidered since several recent studies have demonstrated that much higher molecular weight tannins are water-soluble and in general have even stronger capacity to precipitate proteins.[20,21]

Tannins are classified into three categories as depicted in Figure 1. First, condensed tannins (proanthocyanidins) are flavanol dimers, oligomers, and polymers. They are divided into subclasses (procyanidins, prodelphinidins, and profisetinidins) depending on the nature of their flavanol constitutive units ((epi)catechin, (epi)gallocatechin, and fisetinidol, respectively). In some cases, a part of the flavanol units are esterified by gallic acid, thus forming galloylated proanthocyanidins. They are found in many edible plant materials, including fruits (apples, grapes, plums, red fruits, ...), some legumes (beans and lentils), nuts (almonds, hazelnuts, pecans, ...), grains (barley, sorgho, ...), and processed food (fruit juices, cocoa, wine, and ciders).[22] Second, hydrolyzable tannins (gallo- and ellagitannins) are mainly gallic or ellagic acid polyesters of a polyol moiety, which is generally glucose. Simply considering ellagitannins, near to 1000 different hydrolyzable tannin structures have been identified.[23] They are less widespread in comparison to condensed tannins; however, they are found in diverse sources, such as strawberries, raspberries, pomegranates, mangos, pecans, walnuts, and wines, in particular those aged in oak barrels.[14,22] Third, phlorotannins[24] are oligomers and polymers composed mainly of phloroglucinol units linked together by biaryl or/and diaryl ether bonds and are essentially found in brown algae.

Food Processing: Oxidation and Structural Changes of Polyphenols. During food processing, a part of native polyphenols is involved in biochemical and chemical reactions, undergoing significant changes in their molecular structure and, thus, generating new molecules that can be described as "secondary polyphenols".[25] Those newly formed products

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contribute to the organoleptic and nutritional properties of foods, as do native polyphenols. For instance, they contribute to the color and the flavor of various plant-based beverages such as tea, coffee, juices, ciders, and wines.[25,26]

Among reactions involving polyphenols, oxidation is the one occurring the most frequently during plant raw material transformation.[27] From a chemical point of view, oxidation of polyphenols can be summarized by the ability of the phenolic nucleus to lose an electron or a hydrogen atom leading to the formation of reactive species. As a consequence, oxidation of polyphenols is also clearly the main reaction route which leads to the formation of many "secondary polyphenols" which can be described as "oxidation products".

The oxidation of catechol or pyrogallol groups present in the structures of phenolic compounds (tannins, flavonoids, hydroxycinnamic and hydroxybenzoic acids, etc.) leads to the formation of very reactive intermediate species such as quinones and semiquinones. Note that depending on the situation, these species can be either chemically generated in low concentrations but over long periods, as for example in the case of wine aging, or rapidly produced in large quantities under enzymatic catalysis by polyphenoloxidases and peroxidases during a precise step of the transformation process. For black tea, for example, this occurs during the bruising and rolling operations,[28] while for apple juice or cider, this is observed during the crushing and pressing of the fruits.[29] The kinetic aspects of these oxidation steps strongly depend on environmental conditions such as the availability of oxygen, the pH, and the presence of transition metals capable of catalyzing oxidation. Thus, in a weakly acidic environment (which is the case with most plant foods), the autoxidation of phenolic compounds is all the more favored as the pH is close to neutrality.

Once formed, quinones and semiquinones then evolve rapidly according to various reaction pathways: (i) electron transfer reactions generating new quinone or radical species, (ii) intramolecular additions and rearrangements, and (iii) intermolecular additions with other phenolic or nonphenolic compounds. Finally, depending on the plant matrix considered (the nature, concentrations, and proportions of the different categories of polyphenols and other constituents present) and environmental conditions imposed by the transformation processes (pH, availability of oxygen, etc.), this multiplicity of reaction pathways logically leads to the generation of numerous oxidation products exhibiting structures whose molecular architectures are sometimes very different from those of their native phenolic precursors. Although our objective is not here to make an exhaustive review of these different structures of oxidation products, we can, however, cite a few relevant examples. Thus, the theaflavins from black teas with their specific benzotropolone nucleus are generated by oxidative coupling between epicatechin and epigallocatechins.[30] Interestingly, B-type procyanidins can be converted into A-type either enzymatically[31] or chemically[32] as a result of an intramolecular oxidative coupling reaction. Intra- and intermolecular autoxidative coupling of condensed tannins in wine was characterized in model solutions showing that intermolecular coupling was highly favored when tannins are more concentrated.[33] Still in the field of wine chemistry, the coupling of anthocyanins with catechins and condensed tannins (proanthocyanidin oligomers) often proceeds through oxidative mechanisms leading to the formation of pyranoanthocyanin derivatives that contribute to the color change during red wine aging.[34]

In the case of apple condensed tannins, it has been shown that the structural modifications generated by oxidation (essentially additional intramolecular linkages) significantly modify some of their physicochemical properties, such as their solubility, their rigidity, and their solvation properties in water−ethanol mixtures.[35] Although little knowledge is currently available on this issue, it is expected that these structural modifications also affect the ability of tannins to interact with proteins, including salivary proteins. Recently, in relation to what could be the perception of astringency in juices made from some cider apple varieties, a series of dehydrodimers with original structures resulting from oxidative coupling of caffeoylquinic acid (i.e., chlorogenic acid) were studied for their ability to complex saliva proteins in model solutions.[36] Contrarily to what is generally expected in the case of tannin−salivary protein interactions, those dehydrodimers presented a low interaction with proline-rich proteins (PRPs) but revealed a specific interaction with statherins, P−B peptide, and cystatins. Obviously, this specific example does not allow establishment of general rules regarding the impact of oxidation of polyphenols on their ability to interact with salivary proteins, and further studies regarding catechins and condensed and hydrolyzable tannins are needed to better understand the structure−activity relationships that characterize those interactions.

Modification during Digestion. The stability and metabolization of polyphenols in the digestive tract depend on the class to which they belong and also vary mainly according to the gastrointestinal compartment considered. Polyphenols are relatively stable in an *in vitro* simulated gastric environment (acidic pH in the presence of pepsin) while they are much more degraded under the neutral pH conditions of the intestine, in the presence of pancreatin and bile salts, which are favorable to autoxidation reactions.[37−39] For instance, catechins were significantly degraded under simulated digestion, and some of them, in particular those containing a pyrogallol group, were partly converted into dimers by autoxidative coupling.[38,40] Interestingly, those dimers were analogues of theasinensins, a class of polyphenols also found in mildly fermented tea.[38] Furthermore, regarding glycosylated flavonoids, the small intestine is favorable to some deglycosylation reactions. Indeed, human small intestine epithelial cells produce β-glucosidase enzymes able to hydrolyze quercetin glycosides into free quercetin, which is much more bioavailable.[41]

However, considering the numerous studies published in this field, it seems that one of the most important biotransformations of dietary polyphenols occurs as a result of the biological action of the gut microbiota.[42,43] Thus, flavonols,[44] catechins,[45] and proanthocyanidins (i.e., condensed tannins)[46,47] are largely converted into a series of small molecules corresponding to phenolic acids, with most of them being benzoic, phenylacetic, or phenyl propionic acids and their hydroxylated derivatives. In the case of catechins[48] and proanthocyanidins,[49] a series of hydroxylated phenyl valeric acids are also produced. Interestingly, those metabolites are produced even from nonextractable polyphenols.[42] When they are highly polymerized, procyanidins are poorly metabolized.[46] Regarding ellagitannins, they are partly metabolized into urolitin,[50] a specific tricyclic metabolite that exhibits phytoestrogenic activities.[51]

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SALIVARY PROTEINS

Whole saliva is defined as the biological fluid present in the mouth. The reader is directed to excellent reviews for further information on saliva functions and composition.[11,52,53] One should note, however, that saliva flow and composition are highly variable between subjects and depend on many factors, such as age,[54,55] diet,[56,57] medication and/or pathologies,[58] or sensory stimulation during food intake.[59,60] In any case, apart from water, saliva is composed mainly of proteins. In fact, it contains thousands of different proteins and peptides. These are either secreted by the salivary glands or originate from the crevicular fluid (an exudate of plasma reaching the oral cavity through the gingival crevices), epithelial cells, oral microbiota, or food remnants. The estimation of the proportions of the different proteins suggests that the most abundant proteins are those secreted by salivary glands (mucins, α-amylase, cystatins, histatins, statherin, and PRPs) together with immunoglobulins and albumin.[61,62] PRPs are of special interest for this review because of their high affinity for tannins. They represent up to 70% of proteins secreted by the parotid glands and 20−30% of proteins in whole saliva.[63] Fragments of PRPs also represent a large part of the adult saliva peptidome[64] and the vast majority of oligopeptides identified in human infant saliva.[54] The PRPs family is characterized by a high heterogeneity: the products of six genes are found in saliva as a complex mixture of entire, truncated, phosphorylated, glycosylated, and dimerized proteoforms.[65]

Fate of Salivary Proteins along the Digestive Tract. Except during the sleeping period, where salivary flow is virtually null,[66] saliva is constantly secreted and thus swallowed. It is estimated that an adult swallows around 0.6−1 L of saliva per day.[60,67] There are large variations in the reported protein concentration in whole saliva, which may depend on the assay used or the sampling conditions. Mean values are, for example, 0.6 mg·mL^{-1}[68] in unstimulated saliva of individuals (n = 200) included in an observational cohort or 1.06 mg·mL^{-1} (n = 19),[69] 1.77 mg·mL^{-1} (n = 30),[70] or 2.2 mg·mL^{-1} (n = 13)[71] in unstimulated saliva of healthy individuals. In any case, this implies that the total amount of protein originating from saliva would not exceed 2 g per day, well below the daily dietary protein intake.

Like any protein entering the digestive tract, it is expected that salivary proteins will be rapidly degraded by proteolytic enzymes. A study unexpectedly reported that secreted carbonic anhydrase 6 was present in mouse stools, with an apparent molecular weight on electrophoretic gels suggesting that it was intact or at least little degraded,[72] but such a result would need confirmation. The particular case of proline-rich proteins (PRPs) also deserves some attention. Their unique composition in amino acids renders them particularly resistant to digestive hydrolysis. Indeed, the presence of proline residues after basic amino acids (Lys-Pro and Arg-Pro) precludes their cleavage by trypsin. For example, after a protein-free meal, oligopeptides in the mass range 500−5000 kDa were sought in mini-pig jejunal aspirates: 23 endogenous peptides were identified, among which 20 corresponded to PRP fragments of 8 to 22 amino acids (personal data).

Salivary Proteins Interacting Preferentially with Polyphenols. The presence of proteins which are effective precipitators of tannins has been reported in the saliva of some mammals. These proteins are called tannin-binding salivary proteins (TBSPs) and include mainly PRPs and histatins. Their presence in saliva is thought to be related to diet, as they are abundant in species consuming high amounts of tannins, such as herbivores, lagomorphs, rodents, and omnivores, while they are absent in the saliva of mammals weakly exposed to tannin.[73−75] Hypotheses explaining the presence of TBSPs in mammalian saliva postulate that these proteins participate in the maintenance of oral homeostasis, counteract the effect of tannins, and/or trigger the sensation of astringency, which signals a high tannin concentration in food. In rats, PRP secretion is induced by a tannin-rich diet.[76] This secretion precludes the antinutritional effect of tannins[76] and improves the consumption of solutions containing tannins,[77] suggesting that their presence reduces the intensity of unpleasant sensations (bitterness and/or astringency) induced by tannin. In agreement with these observations, PRPs protect the oral mucosal pellicle from aggregation by tannins.[78] Therefore, rather that inducing astringency, TBSPs can be considered as counterdefenses inherited from natural selection, which modulate astringency intensity by scavenging tannins.

PRPs have a higher capacity to protect proteins from aggregation by tannins than other salivary proteins.[76] This capacity has been attributed to their intrinsically disordered conformation[79] and to their tandem repeat sequences composed of proline clusters surrounded by flexible amino acids.[80] The rigid proline clusters provide stable binding sites to initiate the interaction with tannins,[81,82] while the flexibility of surrounding amino acids allows structural rearrangement,[80,83] stabilizing the interaction through the establishment of additional hydrogen bonds between the peptide chain and tannins.[84] This structural rearrangement may explain the higher affinity of full PRPs compared to proline-rich peptides.[85] The several tandem repeat sequences also provide several binding sites, allowing several stoichiometries of interaction.[79] Once soluble noncovalent complexes are formed, bound tannins, which are described as multidentate ligands, may bridge tannin·PRP complexes, leading to the formation of aggregates.[81] A number of at least three tannins per protein is required to form aggregates. PRP−tannin aggregates grow with tannin concentration up to their precipitation.[81] Beside PRPs, histatins have been reported to precipitate tannin more effectively than acidic PRPs (aPRPs) at pH 7.4, while acidic PRPs show a greater ability at pH 3.0. This difference may result from different mechanisms of interactions. Nevertheless, both histatins and aPRPs efficiently protected salivary amylase from tannin inhibition.[86] Interaction of tannin with histatins has been reported to involved $\pi-\pi$ stacking between the ring of aromatic amino acids and the phenolic ring of tannins.[87] Other works have evidenced that aPRP, histatins, and statherins are more prone to be precipitated by tannins than basic PRP (bPRP) and glycosylated PRP (gPRP).[88] For bPRP, this effect could be due to Coulombic repulsion between the bPRP−tannin soluble complexes decreasing the formation of aggregates, as bPRPs are highly charged at the acidic pH used in this study. Regarding gPRP, the presence of glycosylation has been reported to increase the solubility of the gPRP−tannin supramolecular edifices compared to nonglycosylated PRPs.[89] The presence of sugar chains may reduce the aggregation due to steric hindrance. Investigations on the affinity of TBSPs for tannins have revealed that bPRP and gPRP have a higher affinity for tannins than aPRP,[90] illustrating that a difference should be made between the TBSPs' affinity for tannin and their propensity to be precipitated. The same study highlighted the influence of the

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tannin structure on TBSP−tannin affinity, with changes of the ranking of TBSP affinity as a function of tannin structure. Another study also reported a higher affinity of bPRPs for flavan-3-ol than aPRPs.[91]

Mucins, which play an important role in the lubrification of the oral cavity, are other salivary proteins prone to interact with tannins. The interaction between the mucin MUC5B and tannins is followed by a structural reorganization of the mucin network[92] up to the formation of aggregates. MUC5B is the main salivary protein composing the mucosal pellicle, which is a thin layer of salivary proteins anchoring at the surface of the oral cells via interactions with the transmembrane mucin MUC1.[93] The aggregation of the mucosal pellicle by tannins leads to an increase of the friction forces at the surface of the oral mucosa.[78] This phenomenon is thought to be involved in the sensation of astringency and may induce the cleavage of the transmembrane MUC1, which is an external sensor of epithelial cells.[7]

Regarding the impact of the phenolic structure on tannin−protein interactions, the affinity of proanthocyanidins for proteins increases with the number of constitutive units and their level of galloylation.[94] The presence of a third hydroxyl group on the B-ring has also been reported to increase the affinity of tannins for proteins.[95] Indeed, the number of hydroxyl groups increases the stability of the tannin-protein interaction,[84] via the establishment of hydrogen bonds, while their positions are also important.[96] The establishment of interactions also seems to depend of the polyphenol spatial configuration.[97] For example, molecules with flavan-3-ol monomers linked via C4−C6 linkages have a higher affinity for proteins than their isomers with C4−C8 linkages.[94,98] Moreover, *cis*-2,3-flavan-3-ols have a slightly higher affinity for proteins than *trans*-2,3-flavan-3-ols.[96]

Persistence of Salivary Proteins−Polyphenols Complexes along the Digestive Tract. In order to investigate preabsorption events in the gastrointestinal tract, polyphenols are sometimes subjected to *in vitro* digestion, which includes an oral phase.[99] However, the most widely used and recognized protocols (e.g., ref 100) recommend the use of artificial saliva, which typically contains only amylase. Artificial saliva's protein composition is, therefore, far less complex than true saliva. The question of the resistance of complexes (specifically PRPs−tannins complexes) to digestion has, nevertheless, been addressed using *in vitro* approaches. Thus, insoluble complexes formed from basic PRPs and condensed tannins remained stable in conditions mimicking those in the stomach.[101] Similar observations were reported for histatin−tannin aggregates, with most aggregates remaining insoluble under *in vitro* conditions mimicking those of the stomach and of the small intestine without bile salts.[102] For both types of aggregates, the presence of bile salts in intestinal conditions partly solubilized the insoluble complexes. Whether tannins remained in the form of soluble complexes or were released was not investigated. More recently, it was shown that SP−tannins complexes with a low degree of polymerization were disrupted (or at least dissolved) by gastric digestion while complexes made of salivary proteins and tannin tetramers and above were significantly less solubilized, which the authors interpreted as being more resistant to gastric digestion.[103] Finally, an *in vivo* study showed a 3- to 4-fold increase of fecal excretion of a hydrolyzable tannin (pentagalloyl glucose) in PRPs-secreting rats compared to rats which were not secreting PRPs. The authors, therefore, proposed that some PRP−tannins complexes may be excreted in feces.[104]

IMPACT OF PP−SPS INTERACTIONS ON DIGESTIVE PROCESSES: EXPERIMENTAL EVIDENCE AND HYPOTHESES

Digestive Enzymes' Activities. *Sugars' Metabolizing Enzymes.* Some studies report interactions of polyphenols with salivary amylase. Tea, but not tea depleted in tannins, was shown to reduce amylase activity in saliva,[105] and isolated human salivary amylase was inhibited by a gallotannin.[106] A recent study reported that some flavonoids (ECG and EGCG) bind very rapidly, within seconds, to human salivary amylase.[107] In the earlier articles, the relevance of amylase inhibition was described in relation to the development of caries, but the downstream consequences on digestion were not envisaged. This is probably the case because salivary amylase was traditionally not considered as a direct contributor to starch digestion except within the oral cavity. However, it was evidenced that the kinetics of the gastric pH decrease was compatible with preservation of salivary amylase activity for at least 20 min.[108] In *in vitro* dynamic conditions, 80% of starch was released (under solubilized or hydrolyzed forms) during the first 20 min of gastric digestion in the presence of salivary amylase, vs approximately 10% when saliva was replaced by water. Taken together, these articles suggest that inhibition of salivary amylase by tannins may have an impact on starch digestion in the gastrointestinal tract. Pancreatic amylase or intestinal α-glucosidases are also inhibited by polyphenols or phenolic extracts from various plant species,[109−111] which is rather considered as an opportunity to control glucose uptake and, therefore, manage type 2 diabetes in humans.[112] However, we found no report of the impact of tannin-binding salivary proteins (TBSPs) on the activities of such gastrointestinal enzymes. In contrast, it was shown that the brush border enzyme lactase was inhibited by EGCG but also that binding of EGCG to PRPs could alleviate this inhibition.[113]

Lipases. Similarly to sugar metabolizing enzymes, the general trend described is a reduction of pancreatic lipase activity by polyphenols.[114,115] Gastric lipase has received less attention, but at least one study reports its inhibition by myricitrin-5-methyl ether, a star anise flavonoid.[116] Again, the literature does not describe the impact of the formation of salivary proteins−PP complexes on lipase activity.

Proteases. Due to the importance of protein metabolism on muscle mass, much research has focused on the impact of dietary polyphenols (particularly condensed tannins) on protein digestibility in animals.[117,118] The overall consensus is that diets rich in tannins have a negative impact on weight, but the underlying mechanisms (systemic or digestive) are not fully understood. Focusing on protein digestion, *in vitro* studies on gastric or gastrointestinal proteolysis sometimes differ in their conclusions,[119−121] even within the same study, depending on the protein substrate.[122] In fact, tannins may have different consequences on protein digestibility, as summarized by Butler.[117] On one hand, tannins may denature dietary protein substrates and, thus, facilitate the action of proteases. Contrarily, binding of tannins to protein substrates may hinder accessibility of proteases to the cleavage sites. Finally, and more generally for all hydrolytic enzymes, binding of tannins to the enzymes themselves could either modify the tridimensional structure of their catalytic sites (allosteric inhibition) or preclude access to the catalytic site by steric hindrance

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(competitive inhibition) and, thus, directly inhibit their activity. For example, phenolic extracts from various plant species can drastically inhibit the activity of isolated digestive enzymes, for example, the proteolytic enzymes pepsin, trypsin, and chymotrypsin.[123,124] The activity of the brush border enzyme aminopeptidase N is also inhibited by some flavonoids of grape seeds.[99] In this context, salivary proteins (particularly PRPs) have long been considered as major actors in the protection against the detrimental impact of tannins through binding of those molecules before they can interact with proteolytic digestive enzymes. This hypothesis is partly supported by animal studies: in rodents, a tannin-rich diet induces weight loss attributed to reduced food digestibility, which is continuous in hamsters but reversible after 3 days in rats and mice. In parallel, feeding on tannins induces in rats and mice a dramatic increase in salivary proline-rich proteins (PRPs) within 3 days,[76] while this is not observed in hamsters.[125] Another study on rats demonstrated that diet supplementation in propanolol, which can suppress PRP production in saliva, aggravated the adverse effect of tannins on growth.[126] However, this study and others[104] suggested that the reduced apparent nitrogen digestibility induced by dietary tannins, and calculated using the nitrogen content in feces, may not be due to reduced digestibility of dietary protein but rather to increased nitrogen excretion related to higher secretion of PRPs. Shimada[75] even proposed that tannins should not be regarded as inhibitors of digestion but rather as toxins with systemic impacts on growth and metabolism. In fact, it is plausible that both effects exist, depending on the type and structure of tannins.

Oxidation of Nutrients. It is admitted that the gastric environment is suitable for oxidation of dietary nutrients, particularly lipid peroxidation. It was demonstrated that, on average (pooling salivas of 7 volunteers), saliva could limit lipid peroxidation of turkey meat in simulated gastric fluid and that this effect was greatly enhanced in the presence of red wine polyphenols.[12] Unfortunately, the impact of polyphenols alone was not evaluated in this study, so it is unknown whether the effect is of an additive or synergetic type. Depending on their structure, polyphenols may chelate Fe^{2+} ions, increasing their oxidation to Fe^{3+} ions in the presence of oxygen. This mechanism decreases the quantity of Fe^{2+} that can participate in the Fenton reaction and, thus, precludes the production of hydroxyl radicals.[127] This antioxidant activity of polyphenols may be limited by the presence of TBSP, which may compete with Fe^{2+} for the binding to polyphenols.[128] However, and in contrast to this hypothesis, a study evaluated the oxidant scavenging activities (OSAs) of various polyphenols and polyphenol-containing beverages and noted a very large intensification of the OSA in the presence of human saliva.[129] The authors suggested that salivary proteins help solubilize lipophilic polyphenols and, thereby, increase their availability. Whether this translates into limitation of oxidation in the gastric compartment is not described.

Physical Modification of the Gastric and Intestinal Mucus. The structure of the mucus layer, particularly in the small intestine, can modulate the rate of nutrient absorption. For example, dietary fibers (alginate) can combine with the intestinal mucus and delay the transport of lipid digestion products.[130] Various polyphenols can interact with oral or gastrointestinal mucins. For example, the oral mucins MUC5B and MUC7 aggregate in the presence of EGCG.[92] Binding of EGCG to purified porcine gastric mucin was characterized and indicated a multilayer binding process.[131] Cross-linking of the GI purified mucins MUC5A and MUC2 by tea-derived galloylated polyphenols was also demonstrated.[132] Using a cell-based model of oral epithelium lined by a mucosal pellicle,[93] it was shown that the presence of bPRPs greatly moderated the impact of EGCG on the formation of MUC5B aggregates.[78] In contrast, such a formal demonstration of the impact of salivary proteins in the gastrointestinal tract has to our knowledge not been performed. Nonetheless, some observations are in line with this hypothesis. For example, in pigs fed a diet rich in shredded acorns, the cardiac gland region of the stomach became dark-brownish, and fluorescent microscopy revealed that tannins bonded to the gastric mucin, but no tissue damage was observed. This relative innocuity of acorn tannins was accompanied by an accumulation of endogenous proline in the stomach. Although the origin of this proline was not precisely determined, the authors highlighted the possibility that it may correspond to salivary PRPs.[133] Therefore, PP−SP interactions would not prevent binding to gastric mucins but would limit the disruption of the mucin layer. If similar protective phenomena occur toward intestinal mucins, this would most likely preserve the physical structure of the mucus layer and, in turn, the diffusion of nutrients and the rates of nutrient absorption.

Transport of Polyphenols across the Intestinal Epithelial Barrier. Some studies have focused on the transport of polyphenols across the intestinal epithelium, in most cases using cell culture methods. However, studies often use polyphenols in their original form and not as complexes with dietary or salivary proteins. An interesting study investigated whether a basic PRP (IB4) modulated such transport in Caco-2 cells.[134] It was found that the presence of IB4 reduced the transport of a hydrolyzable tannin (pentagalloyl glucose) in both the apical to basolateral and basolateral to apical directions and that this was concomitant with the formation of insoluble tannins−IB4 complexes. The study was further extended to another salivary protein (histatin 5) and to two polyphenols.[135] The presence of histatin 5 considerably reduced the transport of pentagalloyl glucose across cells but hardly affected the transport of quercetin. The authors, therefore, concluded that some salivary proteins may act as scavenger molecules as far as in the intestine, to limit the uptake of tannins. Another article documented that the cytotoxic effect of EGCG was higher on intestinal HT29 (non-mucin-secreting) than on HT29-MTX-E12 (mucin-secreting) intestinal cells and overall much higher than the cytotoxicity of EC, which has lower ability to bind proteins. The results suggested a binding of EGCG to the mucus which prevented absorption of the noxious polyphenol. The presence of β-caseins further reduced the EGCG cytotoxicity, and the authors pointed out that β-casein may be considered as a simple model to replicate the effect of salivary PRPs due to common characteristics such as richness in proline residues.[136] Therefore, by analogy and as in the previously quoted articles, PRP−EGCG interactions would represent a protective mechanism to limit tannin bioavailability.

Modifications of the Chyme Physical Properties. One of the functions of saliva is to produce a food bolus that is lubricated and cohesive and, thus, safe to swallow. Saliva can also induce in-mouth emulsification of oil or fat[137] or flocculation of emulsions.[138] A recent study documented the impact of proteins modeling salivary constituents (α-amylase, mucin, or a combination of both) on the particle size of peanut

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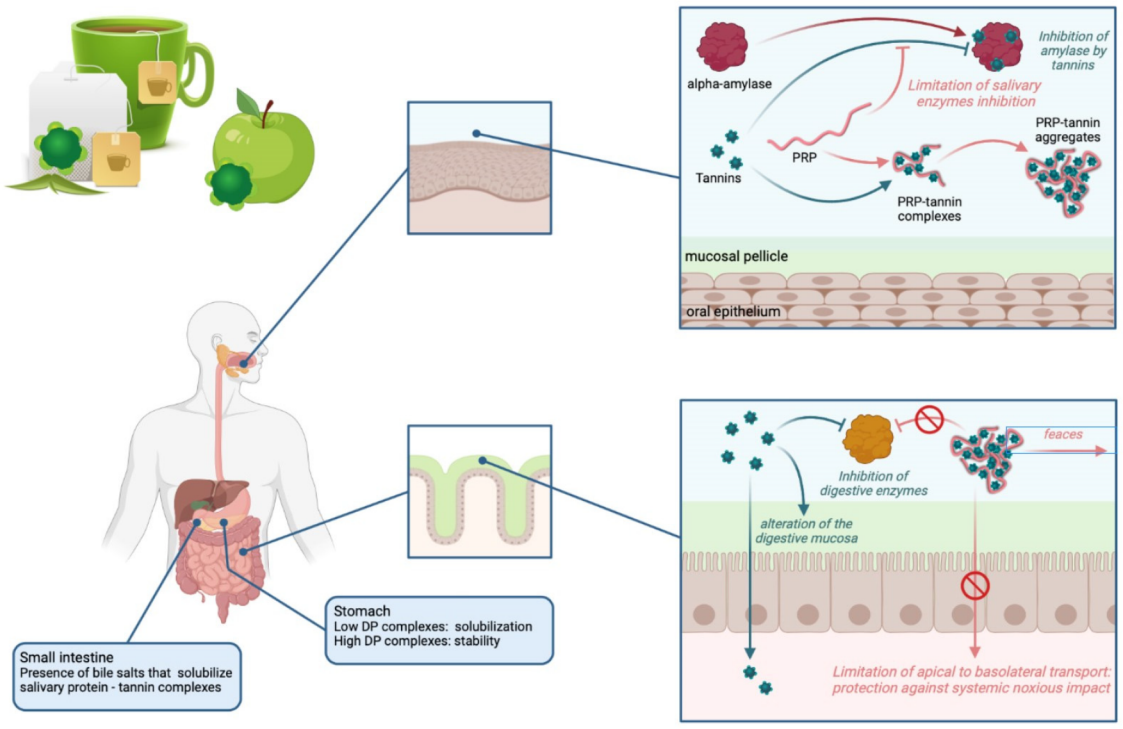

Figure 2. Schematic view of some consequences of salivary proteins−tannins complexation along the digestive tract. Created with BioRender.com.

olesome emulsions after *in vitro* oral and gastrointestinal digestion. Salivary constituents had no impact on the particle size distribution after the model oral phase but induced flocculation in gastric conditions, particularly in the presence of mucins.[13] Given the high affinity of polyphenols for mucins as described above, it would be of interest to determine whether this effect is modified by the presence of polyphenols.

RESEARCH PROSPECTS

This rapid review of the literature highlighted that research on the consequences of polyphenols on digestive processes is active, and so is work on polyphenols−salivary proteins interactions. The impact of saliva on digestive processes is also an emerging field. However, only a limited number of studies have combined those topics. The few experimental works on the interactions salivary proteins−polyphenols (and even more specifically salivary proteins−tannins) all point at beneficial consequences on digestive events: such interactions would protect against the inhibition of digestive enzymes (amylase, lactase, and proteolytic enzymes), would limit the alteration of the gastric and intestinal mucosal integrity, and would reduce intestinal absorption of potentially toxic tannins (Figure 2).

Other potential consequences regarding oxidation of nutrients or the physical properties of the chyme in the gastric compartment (with, in turn, impact the digestibility or digestion kinetics) are only hypothetical and deserve further attention.

In any case, the studies focusing on the matter should carefully consider the ratio of the different interactors; that is, what is the proportion of polyphenols or tannins bound by salivary proteins, and how do these saliva proteins compete with proteins of the food matrix or with other host-derived proteins (in particular mucins of the gastrointestinal lining)? To our knowledge such data are not available in conditions mimicking digestion of a mixed meal containing polyphenols. One should also consider that salivary proteins−tannins complexes formed in the oral cavity will be modified downstream during digestion. Studies on absorptive events (in particular, those using cellular models) could, therefore, take advantage of the now well-recognized *in vitro* digestion models to prepare the materials that may be applied to cells. It is thanks to those improvements that the combined effects of polyphenol and salivation on digestion will be adequately characterized. Such results would be of interest when formulating nutritional recommendations on tannin-containing foods, particularly for specific populations affected by hyposalivation due to pathologies, medical treatments, or aging.

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