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Effect of Phenolic Acids on Human Saliva: Evaluation by Diffusion and Precipitation Assays on Cellulose Membranes

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Abstract : Phenolic compounds are secondary metabolites present in some foods, such as wine. Polyphenols comprise two main groups: flavonoids (anthocyanins, flavanols, and flavonols) and non-flavonoids (stilbenes and phenolic acids). Phenolic acids are low molecular weight non flavonoid compounds that are usually grouped into benzoic (gallic, vanillinic and protocatechuic acids) and cinnamic acids (ferulic, p-coumaric and caffeic acids). Likewise, tannic acid is an important polyphenol constituted mainly by gallic acid. Phenolic compounds are responsible for important properties in foods and drinks, such as color, aroma, bitterness, and astringency. Astringency is a drying, roughing, and sometimes puckering sensation that is experienced on the various oral surfaces during or immediately after tasting foods. Astringency perception has been associated with interactions between flavanols present in some foods and salivary proteins. Despite the quantitative relevance of phenolic acids in food and beverages, there is no information about its effect on salivary proteins and consequently on the sensation of astringency. The objective of this study was assessed the interaction of several phenolic acids (gallic, vanillinic, protocatechuic, ferulic, p-coumaric and caffeic acids) with saliva. Tannic acid was used as control. Thus, solutions of each phenolic acids (5 mg/mL) were mixed with human saliva (1:1 v/v). After incubation for 5 min at room temperature, 15-µL aliquots of the mixtures were dotted on a cellulose membrane and allowed to diffuse. The dry membrane was fixed in 50 g/L trichloroacetic acid, rinsed in 800 mL/L ethanol and stained for protein with Coomassie blue for 20 min, destained with several rinses of 73 g/L acetic acid and dried under a heat lamp. Both diffusion area and stain intensity of the protein spots were semiqualitative estimates for protein-tannin interaction (diffusion test). The rest of the whole saliva-phenol solution mixtures of the diffusion assay were centrifuged and fifteen-µL aliquots of each supernatant were dotted on a cellulose membrane, allowed to diffuse and processed for protein staining, as indicated above. In this latter assay, reduced protein staining was taken as indicative of protein precipitation (precipitation test). The diffusion of the salivary protein was restricted by the presence of each phenolic acids (anti-diffusive effect), while tannic acid did not alter diffusion of the salivary protein. By contrast, phenolic acids did not provoke precipitation of the salivary protein, while tannic acid produced precipitation of salivary proteins. In addition, binary mixtures (mixtures of two components) of various phenolic acids with gallic acid provoked a restriction of saliva. Similar effect was observed by the corresponding individual phenolic acids. Contrary, binary mixtures of phenolic acid with tannic acid, as well tannic acid alone, did not affect the diffusion of the saliva but they provoked an evident precipitation. In summary, phenolic acids showed a relevant interaction with the salivary proteins, thus suggesting that these wine compounds can also contribute to the sensation of astringency.

Keywords : astringency, polyphenols, tannins, tannin-protein interaction
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