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Evaluation of Differential Interaction between Flavanols and Saliva Proteins by Diffusion and Precipitation Assays on Cellulose Membranes

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Abstract : Astringency is a drying, roughing, and sometimes puckering sensation that is experienced on the various oral surfaces during or immediately after tasting foods. This sensation has been closely related to the interaction and precipitation between salivary proteins and polyphenols, specifically flavanols or proanthocyanidins. In addition, the type and concentration of proanthocyanidin influences significantly the intensity of the astringency and consequently the protein/proanthocyanidin interaction. However, most of the studies are based on the interaction between saliva and highly complex polyphenols, without considering the effect of monomeric proanthoancyanidins present in different foods. The aim of this study was to evaluate the effect of different monomeric proanthocyanidins on the diffusion and precipitation of salivary proteins. Thus, solutions of catechin, epicatechin, epigallocatechin and gallocatechin (0, 2.0, 4.0, 6.0, 8.0 and 10 mg/mL) were mixed with human saliva (1: 1 v/v). After incubation for 5 min at room temperature, 15 µL aliquots of each mix were dotted on a cellulose membrane and allowed to dry spontaneously at room temperature. The membrane was fixed, rinsed and stained for proteins with Coomassie blue. After exhaustive washing in 7% acetic acid, the membrane was rinsed once in distilled water 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 15-µL aliquots from each of the supernatants were dotted on a cellulose membrane. The membrane was processed for protein staining as indicated above. The blue-stained area of protein distribution corresponding to each of the extract dilution-saliva mixtures was quantified by Image J 1.45 software. Each of the assays was performed at least three times. Initially, salivary proteins display a biphasic distribution on cellulose membranes, that is, when aliquots of saliva are placed on absorbing cellulose membranes, and free diffusion of saliva is allowed to occur, a non-diffusible protein fraction becomes surrounded by highly diffusible salivary proteins. In effect, once diffusion has ended, a protein-binding dye shows an intense blue-stained roughly circular area close to the spotting site (non-diffusible fraction) (NDF) which becomes surrounded by a weaker blue-stained outer band (diffusible fraction) (DF). Likewise, the diffusion test showed that epicatechin caused the complete disappearance of DF from saliva with 2 mg/mL. Also, epigallocatechin and gallocatechin caused a similar effect with 4 mg/mL, while catechin generated the same effect at 8 mg/mL. In the precipitation test, the use of epicatechin and gallocatechin generated evident precipitates at the bottom of the Eppendorf tubes. In summary, the flavanol type differentially affects the diffusion and precipitation of saliva, which would affect the sensation of astringency perceived by consumers.

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