Prooxidative activity of polyphenols induces protein oxidation and protein-polyphenol adduct formation in model food systems

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Polyphenols are naturally present in most plant-based foods, including fruits, vegetables, tea, coffee, wine, beer, and chocolate drinks. Plant extracts rich in polyphenols are also used as food additives or supplements due to their well-known biological activities, particularly their antioxidant properties [1]. Polyphenols scavenge reactive oxygen species (ROS) and chelate transition metal ions, thereby acting as antioxidants. However, a less explored aspect is the formation of by-products during these antioxidant pathways. The autoxidation of polyphenols while exhibiting antioxidant activity generates hydrogen peroxide (H2O2) and o-quinones, which act as ROS and electrophiles, respectively, further leading to protein oxidation and protein-polyphenol adduct formation.

This study aimed to investigate the relationship between polyphenol autoxidation and protein-polyphenol interactions in generating H2O2, inducing protein oxidation, and forming protein-polyphenol adducts. The results showed that polyphenols, when heated in millimolar concentrations, generated micromolar levels of H2O2, with the concentration depending on reaction time, temperature, and pH. The presence of cysteine, methionine, and milk protein isolates in the reaction system effectively scavenged H2O2 with 35-100% efficiency. Mechanistic analysis of protein oxidation revealed that cysteine and methionine underwent oxidation via a two-electron mechanism. LC-Orbitrap MS/MS analysis allowed quantification of over 10 different protein oxidation products including kynurenine and dioxyindolylalanine diastereomers ranging from nanomolar to micromolar range. In addition, more than 170 distinct protein-polyphenol adducts, comprising of Michael addition products and Schiff bases were identified by deconvoluting LC-MS chromatograms.