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Laccase Catalysed Conjugation of Tea Polyphenols for Enhanced Antioxidant Properties

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Abstract : The oxidative enzymes specially laccase (benzenediol: oxygen oxidoreductase, E.C.1.10.3.2) from bacteria, fungi and plants have been playing an important role in green technologies due to their specific advantageous properties. Laccase from different sources and in different forms was used as a biocatalyst in many oxidation and conjugation reactions starting from phenol to hydrocarbons. Tea polyphenols and its derivatives attract the scientific community because of their potential use as antioxidants in food, pharmaceutical and cosmetic industries. Conjugate of polyphenols emerged as a novel materials which shows better stability and antioxidant properties in applied fields. The conjugation reaction of catechin with poly (allylamine) has been studied using free, immobilized and cross-linked enzyme crystals (CLEC) of laccase from Trametes versicolor with particular emphasis on the effect of pertinent variables and kinetic aspects of the reaction. The stability and antioxidant property of the conjugated product was improved as compared to the unconjugated tea polyphenols. The reaction was studied in 11 different solvents in order to deduce the solvent effect through an attempt to correlate the initial reaction rate with solvent properties such as hydrophobicity (logP), water solubility (logSw), electron pair acceptance (ETN) and donation abilities (DNN), polarisibility and dielectric constant which exhibit reasonable correlations. The study revealed, in general that polar solvents favour the initial reaction rate. The kinetics of the conjugation reaction conformed to the so-called Ping-Pong-Bi-Bi mechanism with catechin inhibition. The stability as well as activity of the CLEC was better than the free enzymes and immobilized laccase for practical application. In case of immobilized laccase system marginal diffusional limitation could be inferred from the experimental data. The kinetic parameters estimated by non-linear regression analysis were found to be KmPAA(mM) = 0.75, 1.8967 and Kmcat (mM) = 11.769, 15.1816 for free and immobilized laccase respectively. An attempt has been made to assess the activity of the laccase for the conjugation reaction in relation to other reactions such as dimerisation of ferulic acids and develop a protocol to enhance polyphenol antioxidant activity.

Keywords : laccase, catechin, conjugation reaction, antioxidant properties
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