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Crude peroxidase extract from onion: activity on o-diphenols and pentahydroxy chalcone oxidative cyclisation into aureusidin

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Abstract

The objective of this study was to investigate the enzymic activity of a crude onion peroxidase extract on *o*-diphenols and to optimise thereof the conditions for the oxidative cyclisation of pentahydroxy chalcone into the corresponding aurone (aureusidin); the reaction in question has already been observed in-house during previous studies. Protocatechuic acid (PA) was first used as the *o*-phenol substrate structure. The enzyme showed optimum activity at 30°C, pH 6, H₂O₂ concentration of 0.8mM and substrate concentration of 2mM. Using response surface methodology the enzymic reaction was further fine tuned in view of optimising the reaction conditions for the preparation of aureusidin from the precursor pentahydroxy chalcone, the point here being that the chalcone is still an *ortho* diphenol system, but much more subtle than PA. An experimental setup based on a 2³-full factorial, central composite design was implemented. The factors considered were pH, incubation time and temperature, and the model obtained produced a satisfactory fitting of the experimental data with regard to ($R^2 = 0.95$, $p = 0.0083$). The highest yield was theoretically predicted to be 308.65±43.802 expressed as the peak area, under optimal conditions (pH 5, 30°C and 5h). A scale up reaction that was carried out using the predicted optimal conditions led indeed to a high yield synthesis of aureusidin. However, an unexpected dimer of high regiospecificity (non-aromatic C sp²-sp² coupling) was also isolated, the yield of which was time-dependent. The conclusion is that indeed the crude onion POD enzyme extract may be used and further studied for the synthesis of fine chemicals, as a green and cheap alternative in the frame of organic synthetic chemistry.

Keywords: Aurones; chalcones; onion peroxidase