Probing *Escherichia coli* biofactories for the discovery of novel hydroxytyrosol derivatives

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Hydroxytyrosol (HT) is the most characteristic phenyl alcohol of olive tree (*Olea europaea*) products and by-products. HT has been recognized as being of particular interest due to its impressive biological and pharmacological properties[1]. Low yield and high cost are the most frequent barriers to its isolation and/or chemical synthesis. However, the production of HT using biotechnological approaches represents an attractive alternative strategy. Based on previous collaborative work[2], metabolically engineered *Escherichia coli* biofactories utilizing L-tyrosine or L-DOPA as precursors, has been established leading to equimolar production of HT. In a continuation study, the development of an efficient methodology for the isolation of HT, as well as co-produced metabolites throughout the biosynthesis process arise as next study-points. Thus, the scope of the present work was biaxal; on the one hand the development of a novel methodology for the isolation of HT from alternative sources and on the other hand the isolation of HT metabolites. The unambiguous identification of compounds will assist significantly to the verification of the engineered pathway, as well as to the discovery of novel HT derivatives with possible improved biological profile. In this context, a simple, robust, and effective experimental procedure that includes an ACE extraction hyphenated at-line with a stepwise gradient CPC fractionation, followed by a prep-HPLC-DAD further purification step was designed and implemented. The proposed procedure resulted in the isolation of 20 compounds belonging to phenolic acids, phenylalcohols, diketopiperazines, hydroxybenzaldehydes, phenolic esters and nitrobenzenes. Their structure elucidation carried out using HPLC-DAD, LC–HRMS/MS and 1D & 2D NMR techniques. The proposed workflow is the first reported method describing the direct isolation of pure metabolites from *E. coli* biofactories and introduces a novel approach to obtain HT metabolites from different matrices.

Keywords: *Escherichia coli*, engineered metabolic pathway, hydroxytyrosol metabolites, L-DOPA, preparative isolation, ACE, CPC

References

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2. Trantas, E. *et al. PLoS One* **14**, 1–23 (2019).

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