



Cutinase from *Fusarium oxysporum* catalyzes the acylation of tyrosol in an aqueous medium: Optimization and thermodynamic study of the reaction



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ABSTRACT

Recently, tyrosol has gained attention as a result of its many pharmacological properties and due to the fact that it can be isolated from cheap and abundant resources. Lipophilic tyrosyl esters, which are scarce in nature, have proven in certain cases to acquire improved biological activity compared to tyrosol itself, increasing their potential use in the food and cosmeceutical industries. The enzymatic approach for the synthesis of such esters has prevailed, as it is “green”, compared to chemical practices. We hereby report the enzymatic synthesis of tyrosyl esters of various aliphatic fatty acids performed by a recombinant cutinase from *Fusarium oxysporum* (FoCut5a). The reaction system used consists of an aqueous phase saturated with the corresponding fatty-acid vinyl ester, which played the role of the acyl donor. We also proceeded to the study of several parameters on the yield of the tyrosyl butyrate ester synthesis. The maximum yield achieved was 60.7% after 4 h at 20 °C, in pH 7.0, with initial tyrosol concentration of 12.5 mM and using 5 μg FoCut5a mL⁻¹ reaction as catalyst. The optimum reaction conditions can be considered mild, highlighting the environmentally friendly nature of this reaction, along with the fact that there are not any harmful reagents involved. Additionally, the use of two thermodynamic models, Conductor-like Screening Model for Real Solvents (COSMO-RS) and UNIFAC Functional-group Activity Coefficients (UNIFAC), were employed for the prediction of reactants' and products' solubilities and their distribution in the reaction biphasic system, aiming to correlate the reaction yields with these important thermodynamic quantities and understand the ability of this enzymatic reaction in synthesizing tyrosyl esters.

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1. Introduction

Tyrosol is a phenolic compound primarily occurring in olive oil [1], as well as olive leaf extracts [2] and olive-mill wastewaters [3]. During the past decade many pharmacological properties of tyrosol have been unveiled. Being an antioxidant [4], tyrosol is considered to be cardioprotective, inhibiting LDL-oxidation, thus preventing coronary heart disease [5], inducing survival and longevity proteins, which act as myocardial protection against ischemia related stress [6] and showing antithrombotic activity during platelet

aggregation [7,8]. Some researchers have further proved that tyrosol acts as a neuroprotective agent, preventing Alzheimer's [9] and Parkinson's diseases [10] and also as a preventing agent of tumoral diseases [4,11]. Lipophilization of natural antioxidants is a familiar practice (see review from Figueroa-Espinoza et al. [12]) aiming to broaden their applications in oil-based food processing and cosmetics. Acylating tyrosol with a lipophilic moiety makes it an amphiphilic molecule, which could accumulate at oil-water or oil-air interfaces where oxidation is considered to occur, increasing the oil protection. Synthesis of phenolic derivatives can be achieved either chemically or enzymatically. Chemical procedures involve reagents, such as *N,N'*-dicyclohexylcarbodiimide, tetrahydrofuran and dichloromethane [13], which may prove harmful for the human health and are not environmentally friendly. Using enzymes as catalysts has been demonstrated as a “green”, safe and efficient

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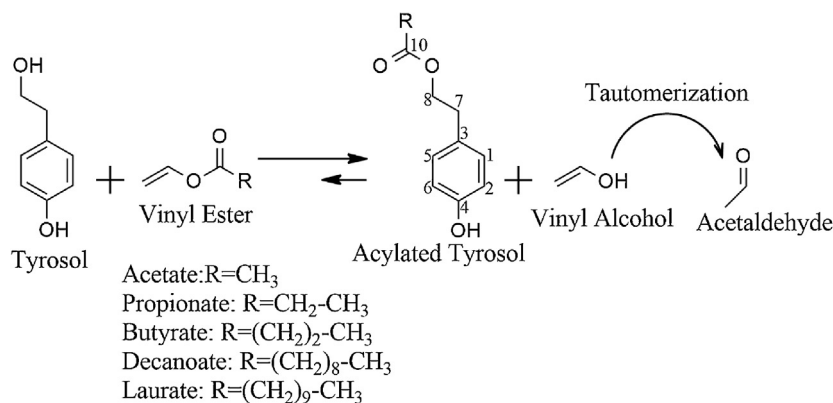


Fig. 1. Schematic depiction of the transesterification reaction of tyrosol with various vinyl esters differing in their carbon-chain length.

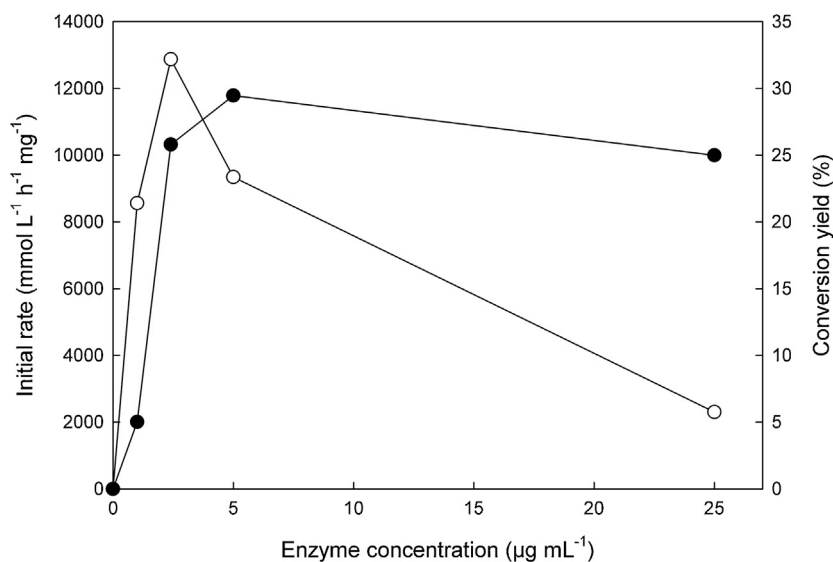


Fig. 2. Effect of the concentration of added enzyme on conversion yield (●) and initial rate (○). Reactions were performed in phosphate-citrate pH 7 buffer, with 50 mM tyrosol for 4 h at 30 °C.

alternative for food, pharmaceutical and cosmeceutical applications.

So far, there has been a plethora of reports for synthesis reactions performed by hydrolytic enzymes. The water content in such kind of reactions is a very important parameter. On one hand, water molecules bound on the enzyme ensure its catalytic properties [14], while the use of miscible organic solvents causes the loss of the enzyme's essential surface water molecules affecting its catalytic activity [15]. On the other hand, the excess of water triggers the enzyme's hydrolytic activity at the expense of its synthetic activity [16]. This type of reaction has also been carried out in supercritical carbon dioxide [17–19] and in micro- [20] and mini- [21] emulsion systems with 2% (v/v) and 50 or 80% (w/w) water content respectively. Furthermore, some reactions of this sort have been reported to occur in diphasic systems: water-heptane [22] or water-oil [23].

Cutinases are small members of the serine-hydrolase family that have shown to hydrolyze esters, preferably with short-length chains (C₂ or C₄ being the optimum for them) [24]. Their active site is accessible to the solvent, in contrary to the one of lipases [25], therefore there is no need for interfacial activation. Cutinases, except from the wide range of substrates that can hydrolyze, they are also capable of catalyzing esterification and transesterification reactions. *Fusarium solani pisi* and *Burkholderia cepacia* cutinases have been shown to synthesize flavor compounds such as short-chain alkyl esters [21,26,27] in isooctane or miniemulsion systems.

The esterification of various natural phenolic acids with aliphatic alcohols has been performed by a commercial *F. solani* cutinase, but with low conversion yields [28]. Furthermore, esterification and transesterification of various phenolic acids or phenolic acid esters with *n*-butanol or *n*-propanol, have been carried out in hexane-miniemulsion systems, by different feruloyl esterases, with good yields [29–31].

In this study, the enzymatic synthesis of alkyl esters of tyrosol was carried out in an aqueous system under mild reaction conditions, increasing the lipophilicity of tyrosol for its potential use in cosmeceutical or oil-based foods. Their synthesis, so far, has been performed with either chemical synthesis [32] or enzymatically with the use of lipases [33]. In the present reaction system, the aqueous phase is saturated with the acyl donor (vinyl ester) and the transesterification reaction is catalyzed by FoCut5a, a cutinase from *Fusarium oxysporum*. The synthesis of five tyrosyl esters was performed and the effect of several parameters including temperature, pH, enzyme loading, and substrate concentration on the transesterification reaction of tyrosol with vinyl butyrate was studied. To increase the understanding of the reaction system, the use of activity coefficient models was carried out, such as the quantum chemistry based Conductor-like Screening Model for Real Solvents (COSMO-RS) that is the thermodynamic tool for the prediction of reagents/products solubility or the group contribution UNiQuac Functional-group Activity Coefficients (UNIFAC) model.

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