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Mild Photochemical Synthesis of the Antioxidant Hydroxytyrosol via Conversion of Tyrosol

SAMIA AZABOU,[†] WAHIBA NAJJAR,[‡] ABDELHAMID GHORBEL,[‡] AND SAMI SAYADI*,[†]

Laboratoire des BioProcédés, Centre de Biotechnologie de Sfax, BP "K", Tunisia, and Laboratoire de Chimie des Matériaux et Catalyse, Faculté des Sciences de Tunis, Tunisia

Hydroxytyrosol, a naturally occurred orthodiphenolic antioxidant molecule found in olive oil and olive mill wastewaters, was obtained from the wet hydrogen peroxide photocatalytic oxidation of its monophenolic precursor tyrosol. The liquid-phase oxidation of tyrosol to hydroxytyrosol was performed by use of an iron-containing heterogeneous catalyst (Al−Fe)PILC with the assistance of UV irradiation at 254 nm and at room temperature. The spectroscopic and HPLC data of the synthesized compound proved to coincide fully with those of a pure sample obtained by continuous countercurrent extraction. This reaction was found to be light-induced. The hydroxytyrosol synthesis reaction reached its maximum yield of 64.36% under the optimized operating conditions of 3.6 mM tyrosol, 0.5 g L^{-1} catalyst, and 10^{-2} M H_2O_2 with the assistance of UV light. Increasing the initial hydrogen peroxide concentration more than 10^{-2} M has a diminishing return on the reaction efficiency. Catalyst can be recuperated by means of filtration and then reused in a next run after regeneration since its activity did not significantly decrease (<10%). The reaction synthesis is operationally simple and could find application for industrial purposes.

KEYWORDS: Catalytic wet oxidation; hydrogen peroxide; heterogeneous catalyst; UV irradiation; tyrosol; hydroxytyrosol

INTRODUCTION

Hydroxytyrosol (3,4-dihydroxyphenylethanol), a naturally occurring orthodiphenolic antioxidant molecule found in olive oil and olive mill wastewaters, has been reported to exert several biological and pharmacological activities such as antibacterial activity (*1*), scavenging of free radicals (*2*, *3*), protection against oxidative DNA damage and LDL oxidation (*4*), prevention of platelet aggregation (*5*), and inhibition of 5- and 12-lipoxygenases (*6*, *7*). Despite the reported importance of hydroxytyrosol's biological properties, this molecule has been made available commercially only recently and for research purposes [approximate cost $1000/g (U.S.) (*8*)]. It is important that hydroxytyrosol can be synthesized easily and at competitive prices so that investigation of its biological properties, for instance as a preservative in foods, can more readily occur. There are several methods for the synthesis of hydroxytyrosol and recently several methods for the synthesis of this compound have been proposed.

Hydroxytyrosol could be recovered from olive mill wastewaters. For example, continuous countercurrent extraction (*9*) yielded 1 g of purified hydroxytyrosol from 1 L of olive mill wastewaters, whereas middle-pressure LC and preparative-scale TLC gave a yield of 91 mg L^{-1} (purity 80%) (*10*). Alternative synthesis procedures, usually utilizing 3,4-dihydroxyphenylacetic acid as precursor (*11*, *12*) or hydrolysis of oeluropein (*13*, *14*), have been reported. High amounts of hydroxytyrosol were obtained by different hydrothermal treatments of the two-phase olive waste or "Alperujo" (*15*). Enzymatic synthesis of hydroxytyrosol from its monophenolic precursor tyrosol with tyrosinase as biocatalyst was described by Espin et al. (*16*). Production of hydroxytyrosol via bacterial synthesis was investigated by Allouche et al. (*17*), Allouche and Sayadi (*18*), and Bouallagui and Sayadi (*19*).

Hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol possesses an extra hydroxy group in the ortho position (**Chart 1**). In this investigation, we have developed, for the first time, a mild and operationally simple method to produce high amounts of hydroxytyrosol from the chemoselective hydroxylation of tyrosol via the hydrogen peroxide photocatalytic oxidation process. This system is based on the generation of very reactive oxidizing free radicals, especially hydroxyl radicals ($HO^{\bullet}$, $HOO^{\bullet}$). Hydrogen peroxide yields two hydroxyl radicals after hemolytic scission. With the purposes of enhancing the decomposition of hydrogen peroxide toward hydroxyl radicals, either photochemical or catalytic activation is needed. Hydroxyl radicals can also be easily produced through a redox process in the presence of iron ions (homogeneous Fenton process) (*20*). However, the limited range of pH (3−5) in which the reaction takes place and the recovery

* Corresponding author: phone/fax 216 74 440 452; e-mail sami.sayadi@cbs.rnrt.tn.
[†] Centre de Biotechnologie de Sfax.
[‡] Faculté des Sciences de Tunis.

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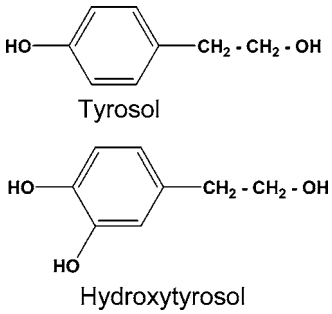
Chart 1. Chemical Formulas of Tyrosol and Hydroxytyrosol



of iron species are the major drawbacks of the homogeneous Fenton process. These drawbacks can be overcome in principle by use of heterogeneous solid Fenton-type catalysts such as transition metal-containing zeolites and pillared clays that have received considerable interest (*21*). Unlike the homogeneous systems, these solid catalysts could be recuperated by means of a simple separation operation and reused (*22*).

The novel chemical synthesis method developed in the present study, which could prove useful for laboratory applications, as well as for possible industrial exploitation, was deposited for a National INNORPI patent (*23*).

MATERIALS AND METHODS

Reagents. Tyrosol (4-hydroxyphenylethanol) was obtained from Sigma−Aldrich Chemie GmbH (Steinheim, Germany). Hydrogen peroxide (30% solution in water) was from CePharma (Tunisia). Hydroxytyrosol (3,4-dihydroxyphenylethanol) used as standard for chromatographic calibration was synthesized in our laboratory as described by Allouche et al. (*9*). Deionized water was used throughout the experiments and in the HPLC mobile phase. Pure HPLC solvents were used in all cases.

Catalyst Preparation. The clay used is a montmorillonite referenced KC_2 provided from CECA (France). Its general formula is $(Si_8O_{20}Al_{4-x}M_x)(OH)_{24}$ $CE_y \cdot nH_2O$, where CE are exchangeable cations. The composition of KC_2 is SiO_2, 57.34% (w/w); Al_2O_3, 16.59% (w/w); Fe_2O_3, 2.72% (w/w); CaO, 1.59% (w/w); MgO, 2.65% (w/w); MnO, 0.03% (w/w); Na_2O, 2.46% (w/w); K_2O, 1.11% (w/w); TiO_2, 0.27% (w/w); and P_2O_5, 0.14% (w/w). The intercalant solution was prepared by titration of an Al^{3+}/Fe^{3+} cationic solution with 0.2 mol L^{-1} NaOH. The cationic solution contained 0.18 and 0.02 mol L^{-1} $AlCl_3$ and $FeCl_3$, respectively. The NaOH solution was slowly added to the cationic solution at 70 °C until the OH/cation mole ratio was equal to 1.9. The intercalant solution was added to the clay suspension under stirring. The final (Al + Fe)/clay ratio was equal to 3.8 mol (kg of dry clay)$^{-1}$. After being aged for 24 h, the pillared clay precursor was washed until total elimination of chloride ions, dried at 60 °C, and finally calcined at 500 °C for 5 h.

Experimental Procedure. Catalytic wet hydrogen peroxide oxidation experiments with the assistance of UV irradiation at 254 nm were carried out with 100 mL aromatic solutions prepared with appropriate concentrations of tyrosol in deionized water. Solutions of tyrosol (pH 5.1) and 0.5 g L^{-1} heterogeneous catalyst, in an open Pyrex glass flask, were irradiated at room temperature (25 ± 2 °C) at a distance of 15 cm from the UV lamp under continuous magnetically stirring (250 rpm). Then 10^{-2} M H_2O_2 (30%) was added (zero time reaction). During the experiments, aliquots were withdrawn at regular intervals, with the purpose of monitoring the conversion of tyrosol into hydroxytyrosol, after being immediately centrifuged at 3500*g* for 10 min to completely remove catalyst particles.

HPLC Analysis. HPLC analysis used for monomeric phenols (hydroxytyrosol and tyrosol) was performed on a Shimadzu apparatus composed of an LC-10ATvp pump and an SPD-10Avp detector. The column was a C-18 (4.6 × 250 mm; Shimpack VP-ODS), and its temperature was maintained at 40 °C. The flow rate was 0.5 mL min^{-1}. The mobile phase used was 0.1% phosphoric acid in water (A) versus 70% acetonitrile in water (B) for a total running time of 50 min, and the following proportions of solvent B were used for the elution: 0−30 min, 20−50%; 30−35 min, 50%; and 35−50 min, 50−20%. The flow rate was 0.6 mL min^{-1}, and the injection volume was 20 μL (*24*). Identification and quantification of hydroxytyrosol were based on its spectrum, on its retention time in comparison with standard analyzed under the same conditions.

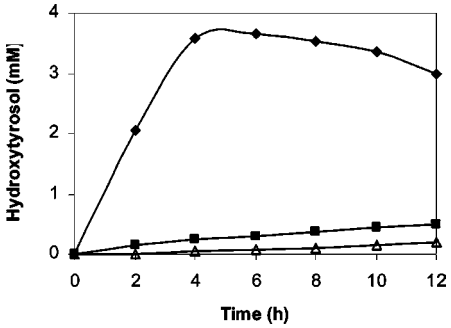


Figure 1. Hydroxytyrosol formation under different conditions: (◆) UV/H_2O_2/(Al–Fe)PILC; (■) UV/H_2O_2; (△) H_2O_2/(Al–Fe)PILC. Experimental conditions were as follows: 14.5 mM tyrosol, 10^{-2} M H_2O_2, 0.5 g L^{-1} (Al–Fe)PILC.

LC-MS Analysis. The LC-MS system used was a Waters apparatus composed of a 600 E pump, a Merck-Hitachi L-400 UV detector, and a Merck Lichrosphere 100 RP-18 column (4 × 250 mm). Positive-ion atmospheric pressure chemical ionization (APCI)-MS mode was obtained with a quadrupole ion trap instrument (Finnigan-MAT LCQ), as described previously (*25*). Identification of compounds by LC-MS analysis was carried out by comparing retention times and mass spectra of the unknown peaks to those of standards.

RESULTS AND DISCUSSION

Chemical Tyrosol Conversion: Preliminary Experiments. Aluminum-iron pillared clay [(Al−Fe)PILC] catalyst, in the presence of hydrogen peroxide with the assistance of UV irradiation at the wavelength 254 nm, catalyzed the ortho-hydroxylation of the monophenol tyrosol to form the *o*-diphenol hydroxytyrosol. This oxidation relies on the generation of the powerful oxidant hydroxyl radicals $^{\bullet}OH$ by the combination of the oxidant hydrogen peroxide and the redox process in the presence of iron ions and is generally schematized as follows (eqs 1 and 2):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HOO^{\bullet} \quad (2)$$

Blank controls (without catalyst) and dark controls (without UV irradiation) were also run, and no significant tyrosol conversion was observed (**Figure 1**). It should be noted that, in the absence of UV irradiation, tyrosol was not converted to hydroxytyrosol even with high hydrogen peroxide amounts. Moreover, tyrosol was not oxidized, by the homogeneous Fenton process (classic Fenton process) as it was reported by Antolovich et al. (*26*) and Khoufi et al. (*27*). The oxidation of tyrosol is found to be a light-induced reaction. The enhancement of tyrosol oxidation to form hydroxytyrosol is due to the increase in hydroxyl radical concentration upon photolysis of hydrogen peroxide (eq 3):

$$H_2O_2 \xrightarrow{h\nu} 2OH^{\bullet} \quad (3)$$

This reaction was monitored by HPLC. After 2 h and throughout the reaction course, an interesting observation was noted. The

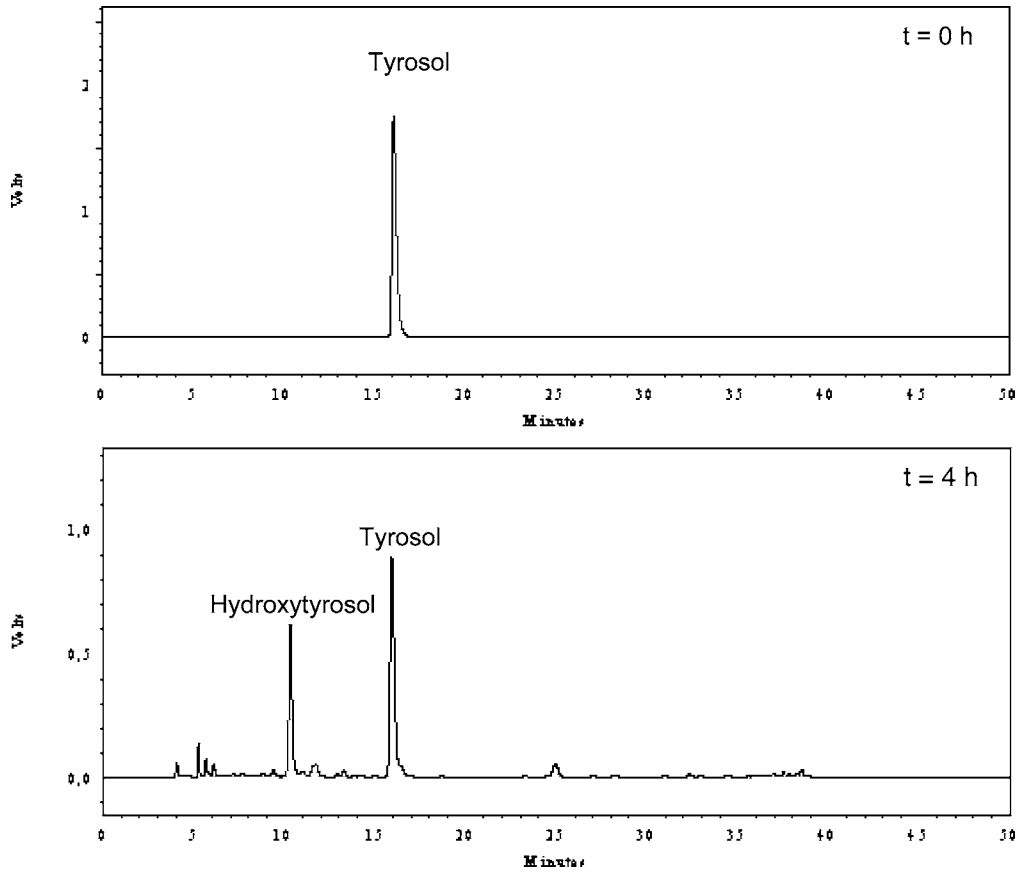


Figure 2. Detection by HPLC of hydroxytyrosol. Conditions were as follows: 14 mM tyrosol, 10^{-2} M H_2O_2, 0.5 g L^{-1} (Al–Fe)PILC, and UV light.

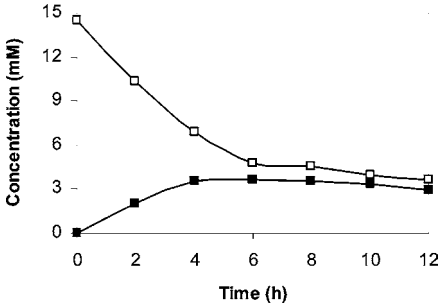


Figure 3. Time course of hydroxytyrosol formation (■) and tyrosol depletion (□). Experimental conditions were as follows: 14.5 mM tyrosol, 10^{-2} M H_2O_2, 0.5 g L^{-1} (Al–Fe)PILC, and UV light.

initial transparent color of the aromatic solution has been changed from colorless to yellow to brown and the intensity of the latter increased with time. **Figure 2** shows the evolution of the peaks during the reaction time. Tyrosol peak (retention time 15 min) decreased with the concomitant increase of hydroxytyrosol peak (retention time 9.8 min).

Figure 3 shows the time course of hydroxytyrosol formation and tyrosol depletion at an initial tyrosol concentration of 14.5 mM. During the first 6 h of reaction, accumulation of hydroxytyrosol was concomitant with the removal of tyrosol. The yield conversion reaction was around 28.7%. Nevertheless, after this time no further hydroxytyrosol accumulation was observed. Indeed, hydroxytyrosol concentration was found to decrease slowly, which could be due to the fact that this antioxidant is easily oxidized (*28*).

The identification of hydroxytyrosol was carried out by means of HPLC and LC-MS analysis with comparison with standard compounds. With regard to the HPLC analysis, a simple sharp peak of hydroxytyrosol was detected at retention time 9.8 min. With regard to LC-MS analysis, the spectrum of reaction solution after 4 h run, in comparison with zero time reaction, shows the appearance of new fragments at *m*/*z* 137 and 99, besides the fragment at *m*/*z* 121 (corresponding to tyrosol), that coincide fully with those of a pure hydroxytyrosol sample obtained by continuous countercurrent extraction (*14*).

Influence of Operating Parameters on Hydroxytyrosol Production. In order to improve hydroxytyrosol production, several main operating factors can be adjusted, mainly the concentrations of hydrogen peroxide, tyrosol, and catalyst.

Hydroxytyrosol production yields for the UV/H_2O_2/(Al−Fe)-PILC process are shown in **Figure 4** for different initial tyrosol concentrations within the range of 1.8−14.5 mM. The best hydroxytyrosol production yield (64.36%) was obtained with an initial tyrosol concentration of 3.6 mM. Decrease of hydroxytyrosol production yield for tyrosol concentrations higher than 3.6 mM could be explained by saturation of active catalytic sites. In fact, in the surface of (Al−Fe)PILC particles, the reaction occurs between the $OH^{\bullet}$ radicals generated at the active OH^- sites and the tyrosol molecules adsorbed on the surface of catalyst. When the initial tyrosol concentration is high, the number of these available active sites is decreased by the phenolic molecules, because of their competitive adsorption on the catalyst particles. But the intensity of light illumination period is constant and $OH^{\bullet}$ radicals formed on the surface of (Al−Fe)PILC are also constant. Thus, the reactive $OH^{\bullet}$ attacking the compound molecules decreases and simultaneously the conversion efficiency also decreases.

Tyrosol oxidation to form the antioxidant hydroxytyrosol has been studied at different initial hydrogen peroxide concentra-

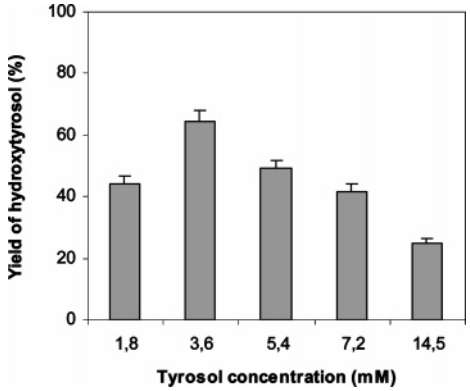


Figure 4. Hydroxytyrosol production yield in the presence of different initial tyrosol concentrations after 4 h reaction time. Experimental conditions were as follows: 10^{-2} M H_2O_2, 0.5 g L^{-1} (Al–Fe)PILC, and UV light.

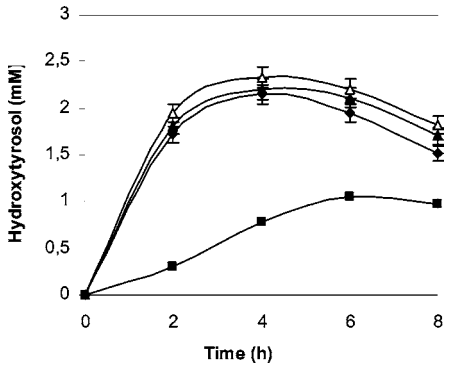


Figure 5. Time course of hydroxytyrosol formation with different initial H_2O_2 concentrations: (■) 4×10^{-4} M H_2O_2, (△) 10^{-2} M H_2O_2, (▲) 1.2×10^{-1} M H_2O_2, (◆) 2×10^{-1} M H_2O_2. Experimental conditions were as follows: 3.6 mM tyrosol, 0.5 g L^{-1} (Al–Fe)PILC, and UV light.

tions. **Figure 5** shows that as H_2O_2 concentration increases, the production of hydroxytyrosol is accelerated up to 10^{-2} M H_2O_2. This could be due to the fact that more hydroxyl radicals are formed as H_2O_2 concentration increases. A further increase of H_2O_2 concentration higher than 10^{-2} M decreases the hydroxytyrosol production. This finding could be explained by the fact that hydroxytyrosol production is enhanced by addition of H_2O_2 due to increase in the hydroxyl radical concentration. However, at low concentration, H_2O_2 cannot generate enough hydroxyl radicals and the oxidation rate of tyrosol is limited. At high H_2O_2 concentration H_2O_2 acts as a hydroxyl radical quencher (eqs 4 and 5), consequently lowering the hydroxyl radical concentration (*29*):

$$H_2O_2 + OH^\bullet \rightarrow HO_2^\bullet + H_2O \quad (4)$$

$$HO_2^\bullet + OH^\bullet \rightarrow H_2O + O_2 \quad (5)$$

Since $HO_2^\bullet$ is less reactive than $OH^\bullet$, increased amount of hydrogen peroxide has a diminishing return on the reaction rate. Therefore, it is important to highly optimize the applied dose of hydrogen peroxide to maximize the performance of the catalyst/H_2O_2/UV process and minimize the process cost.

Hydroxytyrosol production with different catalyst concentrations using (Al−Fe)PILC catalyst were conducted using the same operational conditions: room temperature 25 °C, 3.6 mM initial tyrosol concentration, and 10^{-2} M H_2O_2. The results are

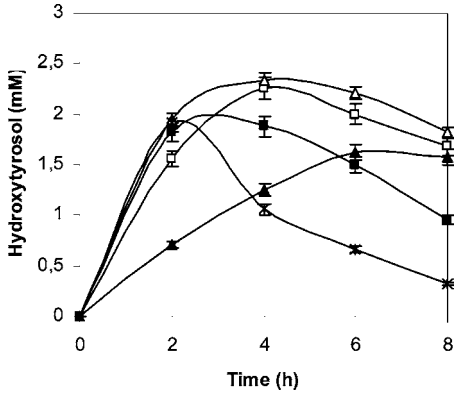


Figure 6. Influence of catalyst concentration on hydroxytyrosol formation: (▲) 0.1 g L^{-1}, (□) 0.25 g L^{-1}, (△) 0.5 g L^{-1}, (■) 1 g L^{-1}, (∗) 1.5 g L^{-1}. Experimental conditions were as follows: 3.6 mM tyrosol, 10^{-2} M H_2O_2, and UV light.

presented in **Figure 6**. The conversion efficiency increased with an increase in the amount of catalyst up to 0.5 g L^{-1}. This result indicates that no external mass transfer limitation occurs under our experimental conditions. When the amount of catalyst was further increased to 1.5 g L^{-1}, a decrease in the hydroxytyrosol concentration (after 2 h) could be seen. This observation leads to the premise that, for the given tyrosol concentration, there is an optimum concentration of (Al−Fe)PILC, which could be situated around 0.5 g L^{-1}.

The catalyst was used in three consecutive experiments, with the same catalyst load and the same above operational conditions. The catalyst after reaction was removed by filtration, washed with distilled water, and dried at 100 °C for 12 h. It is obvious that the activity decreased slowly (8%) during the successive runs. The poisoning of the active catalytic sites may be considered as the reason for this loss of activity. The oxidation of the organic species adsorbed on the catalyst active sites may be associated with the delays observed for short reaction times. When the catalyst was used after an intermediate calcination step, its catalytic activity was restored, indicating the absence of significant deactivation, due to the loss of iron.

The method proposed here for the synthesis of hydroxytyrosol via hydrogen peroxide photocatalytic oxidation has several advantages over other previously published methods. There is a chemical synthesis method that uses as precursor 3,4-dihydroxyphenylacetic acid, which is reduced with $LiAlH_4$ in tetrahydrofuran to give rise to hydroxytyrosol (*10*). This procedure has a high yield of 80−90% hydroxytyrosol formed. However, the process involves the use of a highly toxic reagent. The precursor tyrosol is cheaper than 3,4-dihydroxyphenylacetic acid. The alkaline hydrolysis of oleuropein to obtain hydroxytyrosol also involves the use of highly toxic reagents (*13*, *14*). A procedure for the preparation of hydroxytyrosol from tyrosol was investigated by Pezzella et al. (*30*). It involves oxidation of tyrosol with 2-iodoxybenzoic acid in methanol at −25 °C, followed by dithionite reduction to give a final yield of 30% after chromatographic fractionation. Additionally, biological methods using either whole-cell bacteria (*17*−*19*) or purified enzymes (*16*) to produce hydroxytyrosol were very interesting and attractive approaches since they are environmentally friendly and adaptable to a bioreactor for industrial purposes. However, isolation and purification of enzymes require expensive steps to be performed. Moreover, bioreactors that require sterile conditions and nutriments can have the disadvantage of high cost and low sustainability at large scale.

To our knowledge, this is the first study to demonstrate the ability to produce hydroxytyrosol by catalytic oxidation of tyrosol with the assistance of UV irradiation. Synthesis of hydroxytyrosol by this process is a very simple, mild, and fast method to obtain high amounts of hydroxytyrosol preliminarily purified. This method could be considered as an alternative cheaper and nonpolluting route for hydroxytyrosol production, a commercially unavailable compound with well-known biological properties that justify a potential commercial application. This chemical method is environmentally friendly since it does not use toxic reagents, taking into account that hydrogen peroxide is the most appealing oxidant agent since it does not form any harmful byproducts is a nontoxic and ecological reactant (*22*), and it will be degraded throughout the reaction run. Solid catalysts are not expensive and even they can be recuperated by means of a simple separation operation and then reused several cycles after regeneration (*22*). At the end of the reaction, solid catalyst can be removed by filtration and hydroxytyrosol could be purified by chromatography to obtain highly pure antioxidant in huge amounts. This process may prove useful not only for laboratory applications but also for potential industrial exploitation (i.e., for large-scale production).

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