Research Paper

Oxidation of oleuropein: Electron paramagnetic resonance and spectrophotometric studies

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The autoxidation at alkaline pH and enzymatic oxidation by mushroom tyrosinase of oleuropein, the dominant biophenol present in the fruits and leaves of *Olea europea*, was followed by both electron paramagnetic resonance (EPR) and absorption spectroscopy. For comparison, the same oxidation processes were applied to 4-methylcatechol, a simple polyphenol present in olive mill wastewaters. EPR spectra of stable *o*-semiquinone radicals produced during autoxidation at pH 12 and short-lived *o*-semiquinone free radicals produced during autoxidation at pH 9.0 or tyrosinase action and stabilized by chelation with a diamagnetic metal ion (Mg^{2+}) were recorded for both polyphenols, and the corresponding hyperfine splitting constants were determined. The UV-Vis spectral characteristics of the oxidation of polyphenols were highly dependent on the type of polyphenol, oxidant type and the pH of the reaction. The kinetic behavior of tyrosinase in the presence of oleuropein and 4-methylcatechol was followed by recording spectral changes at 400 nm (absorption maximum) over time. The tysosinase activity with oleuropein showed a pronounced pH optimum at pH 6.5 and a minor one around pH 8. From the data analysis of the initial rate at pH 6.5, the kinetic parameters $K_m = 0.34 \pm 0.03$ mM and $V_{max} = (0.029 \pm 0.002)$ ΔA_{400} min^{-1} were determined for oleuropein.

1 Introduction

Olive oil is a product with major importance for the Mediterranean diet and the economy of many countries. Olives and virgin olive oil contain phenolic compounds, which are the most important vegetable antioxidants in these traditional foods. Phenolic compounds in the leaves and fruits of the olive plant are important factors to consider in order to evaluate virgin olive oil quality, because they are partly responsible for its autoxidation stability and organoleptic characteristics due to their high antioxidant activity [1, 2]. Among the phenolic components, the secoiridoid oleuropein glucoside is the principal biophenol commonly found in *Olea europaea* fruits and leaves, while it is also present in virgin olive oil, together with

its respective aglycone and the decarboxylated dialdehyde derivative [3]. The aldehydic form of oleuropein seems to be responsible for the bitterness of virgin olive oil [4].

Plant phenols are highly reactive molecules undergoing a variety of degradative non-enzymatic (autoxidation) and enzymatic oxidation reactions, as substrates for a number of oxidoreductases. These oxidative browning processes play a significant role both in the loss of nutritional value and the appearance of undesirable brown colors in plant-derived foods, as well as in human health because of the toxicity of the degradation products of polyphenols [1, 5–8]. Thus, oxidation of catechols, either by chemical or by enzymatic means, leads to reactive free radicals (*o*-semiquinones, superoxide) and quinones; both oxidation products may contribute to the toxicity of catechols through their capacity to react with nucleophilic groups [9]. The *o*-semiquinone radicals in oxidized *o*-hydroxy phenols are known to be stabilized in the presence of diamagnetic ions such as Zn^{2+}, Cd^{2+} or Mg^{2+}. This stabilization enables the characterization of these transient free radicals involved in first oxidation steps of catechols,

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by EPR spectroscopy [10]. Recently, the pro-oxidant behavior of oleuropein in the presence of iron was characterized by spin-trapping EPR methodology [11].

The presence of oxidizing enzyme activities, such as lipoxygenase (LOX) and polyphenol oxidase (tyrosinase), has been reported in Greek virgin olive oil samples [12, 13] and olive fruit [14–16]. Tyrosinase (polyphenol oxidase, EC 1.14.18.1) is a copper-containing tetrameric enzyme widespread in animals, plants and microorganisms [17]. The enzyme catalyzes the oxygenation of phenols to catechols by oxygen (so-called phenolase activity) as well as the dehydrogenation of catechols to the corresponding *o*-quinones (catecholase activity) [18]. These latter products are unstable in aqueous solution and undergo further non-enzymatic processes to form melanin-like stable compounds [19]. Tyrosinase exhibits also a substrate inhibition effect (suicide inactivation) when acting on *o*-diphenols [20, 21].

The aim of this study was to investigate and compare the oxidation of two polyphenols with different molecular structures, oleuropein, the dominant biophenol present in the fruits and leaves of *Olea europea* [3], and 4-methylcatechol, a simple polyphenol present in olive mill wastewaters [22], by various oxidation processes and the concomitant production of polyphenol free radicals by electron paramagnetic resonance (EPR) spectrometry, and then to correlate the results obtained using the EPR spin stabilization technique with kinetic studies by classical optical spectroscopy. Oxidations were performed either by autoxidation at alkaline pH values where the oxidation rate is high [5] in the presence of NaOH (which is used for debittering of olives [23] at pH 9.0) or by a commercial polyphenol oxidase (tyrosinase), which can mimic the action of the polyphenoloxidase present in olives and olive oil [12].

2 Materials and methods

2.1 Materials

Tyrosinase from mushroom and 4-methylcatechol were purchased from Sigma (St. Louis, MO, USA). Oleuropein was from Extrasynthèse (Genay, France). All other chemicals were of the highest purity available.

2.2 Methods

2.2.1 EPR studies

Measurements of the EPR studies were carried out at constant room temperature (25 °C), using a Bruker ER 200D spectrometer operating at the X-Band. Reaction samples were contained in an aqueous WG-813-Q Wilmad (Buena, NJ) Suprasil flat cell at room temperature. Typical instrument settings were: center field, 3460 G; scan range, 50; gain, 20,000; time constant, 500 ms; modulation amplitude, 0.5 G; phase, 90°; frequency, 9.80 GHz. Data collection was per-

formed using the computerized program DAT-200 (Data Acquisition Program, University of Lubeck, Germany) and analyzed with the Graphic Evaluation Program (GEP) version 1.2 for personal computer. Simulations of the experimental spectra were conducted with the simulation program WINSIM (National Institute of Environment and Health Sciences).

2.2.1.1 Autoxidation of polyphenols

For the autoxidation experiments at pH 12 or 9.0, the polyphenols (3 or 0.3 mM, respectively) were dissolved in a solution of sodium hydroxide (90 mM) or 50 mM Tris-HCl buffer pH 9.0 containing 0.5 M Mg^{2+} (anhydrous magnesium chloride), respectively, in a final volume of 1 mL at 25 °C [9]. After 2 min, the reaction mixture was transferred to the EPR cell and the spectra were recorded at room temperature.

2.2.1.2 Enzymatic oxidation of polyphenols

Semiquinone radicals were generated by tyrosinase-catalyzed oxidation of 4-methylcatechol or oleuropein (20 mM) in Tris-HCl buffer, 50 mM, pH 7.0, containing Mg^{2+} (0.5 M) [9]. Enzymatic oxidation of polyphenols was started by adding 50 μg/mL tyrosinase, and the EPR spectra were recorded as described above.

2.2.2 Spectrophotometric studies

2.2.2.1 Kinetics of autoxidation

Kinetics studies of the autoxidation processes of oleuropein and 4-methylcatechol were also carried out using absorption spectrophotometry on a Cary 1E Varian spectrophotometer. The temperature was controlled at 25 °C. The assay conditions were as described above except that the polyphenols were present at a concentration of 1 mM.

2.2.2.2 Kinetics and optimum pH for tyrosinase activity

Tyrosinase activity was determined using a spectrophotometric method based on the initial rate of increase in absorbance at 400 nm [24]. The assay conditions were as described for the EPR studies, except that the addition of Mg^{2+} was omitted. The reaction started by adding 13 μg of tyrosinase in 1 mL of reaction mixture, and the kinetics of the reaction was followed at 400 nm. Reference samples contained all the components except the enzyme. The kinetic parameters for oleuropein were determined at 50 mM phosphate buffer pH 6.5 and polyphenol concentrations from 0.03 to 1 mM. The optimum pH for tyrosinase activity was determined at pH values of 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0, respectively, using 50 mM buffer solution (acetate, pH 5.5;

phosphate, pH 6.0–6.5; Tris-HCl, pH 7.0–9.0). Oleuropein and 4-methylcatechol were present at 0.25 mM.

3 Results and discussion

3.1 EPR studies

3.1.1 Autoxidation of polyphenols

The autoxidation of oleuropein and 4-methylcatechol at alkaline pH was followed by EPR, and the corresponding spectra of the produced *o*-semiquinone radicals were recorded. Figures 1 and 2 show both experimental and simulated EPR spectra of the stable *o*-semiquinone radicals produced by oleuropein (Fig. 1A, B) and 4-methylcatechol (Fig. 2A, B) during autoxidation at pH 12, respectively. As can be observed, in both cases well-resolved EPR spectra consisting of 12 peaks were obtained. In the case of oleuropein (Fig. 1) the EPR spectrum consists of three groups of four peaks whereas in the case of 4-methylcatechol the EPR spectrum consists of four groups of triplets. Simulation of the experimental spectra enables the determination of the hyperfine splitting constants of the stable *o*-semiquinone radicals. The hyperfine splitting constants of the simulated EPR spectra are shown in Table 1. In the case of oleuropein, computer-simulated spectra showed two large hyperfine splittings indicative of the interaction of the unpaired electron with the two equivalent side chain methylene protons ($\alpha^H_{CH_2} = 3.39$ G, $\alpha^H_{CH_2} = 3.36$ G) and also three smaller splittings ($\alpha^H_3 = 0.38$ G, $\alpha^H_5 = 0.54$ G, $\alpha^H_6 = 0.46$ G) from the inequivalent protons of the aromatic ring, C-3, C-5, and C-6. In the case of 4-methylcatechol, the computer-simulated spectra showed three large hyperfine splittings attributable to the three equivalent side chain methylene protons ($\alpha^H_{CH_3} = 5.08$ G, $\alpha^H_{CH_3} = 5.02$ G, $\alpha^H_{CH_3} = 5.02$ G) and three smaller splittings due to the inequivalent aromatic protons ($\alpha^H_3 = 0.10$ G, $\alpha^H_5 = 0.95$ G, $\alpha^H_6 = 0.90$ G). Experimental results concerning autoxidation of 4-methylcatechol and the identification of the produced primary radicals are in good agreement with previous findings [25].
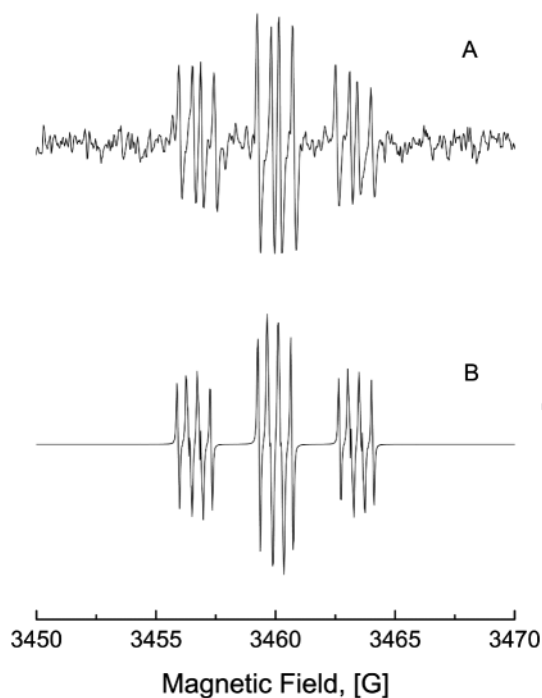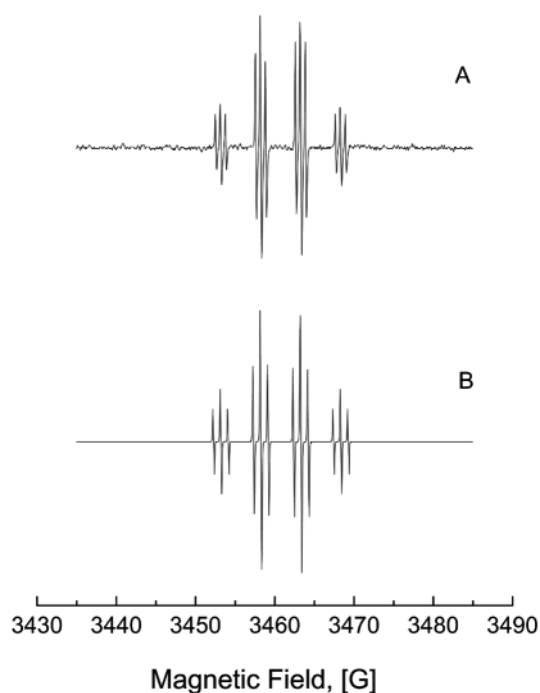

Figure 1. (A) Experimental and (B) computer-simulated EPR spectra of *o*-semiquinone stable radical produced by oleuropein (3 mM) autoxidation in NaOH (90 mM), pH 12.
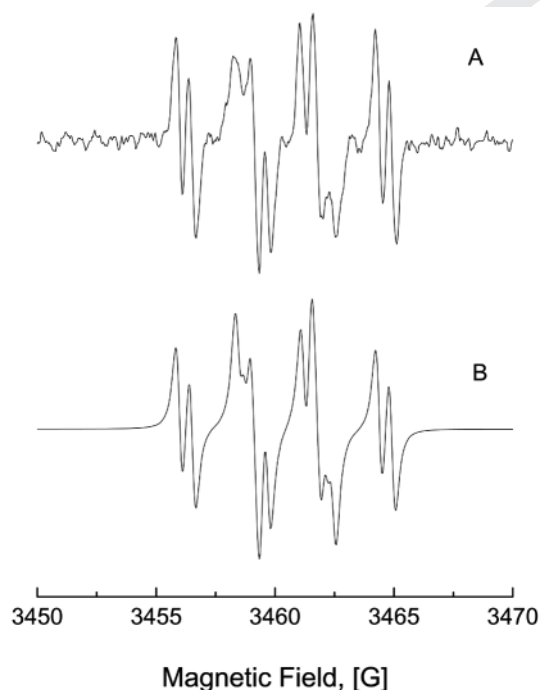
Figure 3 shows both experimental and simulated EPR spectra of the metal-complexed *o*-semiquinone radicals produced from the autoxidation of oleuropein at pH 9.0 in the presence of Mg^{2+}. In the absence of the stabilizing metal cation, no EPR spectrum was detected. The uncomplexed *o*-semiquinone radicals from the autoxidation of oleuropein and other polyphenols were not observed at physiological pH values because of their rapid rate of dimerization, disproportionation or comproportionation [26]. Transient species involved in the first oxidation steps of polyphenols can be stabilized in the presence of diamagnetic metal ions, such as Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+} and La^{2+}, and then characterized by

Table 1. Hyperfine splitting constants, α, of the *o*-semiquinone radicals produced from the oxidation of oleuropein and 4-methylcatechol as calculated from the simulated EPR spectra.

Oxidation system	Parent compound	Hyperfine splitting constants [G]				
		α^H_{CH3}	α^H_{CH2}	α^H_3	α^H_5	α^H_6
Autoxidation						
pH 12	oleuropein		3.39, 3.36	0.38	0.54	0.46
	4-methylcatechol	5.08, 5.02, 5.02		0.10	0.95	0.90
pH 9, Mg^{2+}	oleuropein		3.16, 2.48	0.10	2.75	0.54
Enzymatic oxidation						
Tyrosinase pH 7, Mg^{2+}	oleuropein		3.44, 3.05	0.67	3.20	0.73
	4-methylcatechol	4.80, 4.70, 4.65		0.45	4.00	0.77

Figure 2. (A) Experimental and (B) computer-simulated EPR spectra of *o*-semiquinone stable radical produced by 4-methyl-catechol (3 mM) autoxidation in NaOH (90 mM), pH 12.

Figure 3. (A) Experimental and (B) computer-simulated EPR spectra of Mg^{2+}-*o*-semiquinone radical complex produced by oleuropein (0.3 mM) autoxidation in 50 mM Tris-HCl buffer pH 9.0 containing 0.5 M Mg^{2+}.

EPR spectroscopy. In the present study, the use of Mg^{2+} was preferred for the complexation of the semiquinone radicals because of the increased kinetic stability of the radical ions and the absence of additional hyperfine coupling to the metal in the EPR spectrum of the complex [27].

The EPR parameters of the Mg^{2+}-complexed *o*-semi-quinone radicals produced from the autoxidation of oleuro-pein at pH 9.0 were obtained from computer simulations of the experimental spectra. The computer-simulated spectra showed two large hyperfine splittings from the side chain methylene protons ($\alpha^H_{CH_2} = 3.16$ G, $\alpha^H_{CH_2} = 2.48$ G), a large hyperfine splitting from the proton at C-5 in the aromatic ring ($\alpha^H_5 = 2.75$ G), and two smaller splittings from the aromatic protons at C-3 and C-6 ($\alpha^H_3 = 0.10$ G, $\alpha^H_6 = 0.54$ G). It should be recognized that the two methylene protons are magnetically inequivalent because of their attachment to a chiral center. The observed differences between the EPR spectrum of the *o*-semiquinones at pH 12 and the spectrum of the metal-complexed *o*-semiquinones at pH 9.0 can be attrib-uted to the redistribution of spin density due to the com-plexation of the *o*-semiquinone with the metal cation. Similar results were reported when DOPA semiquinones were inves-tigated and their EPR spectra were analyzed [28].

When the autoxidation of 4-methylcatechol at pH 9.0 in the presence of Mg^{2+} was considered, the intensity of the EPR spectrum was very low and consequently computer simula-tion analysis was not possible.

3.1.2 Enzymatic oxidation of polyphenols

The tyrosinase-catalyzed oxidation of oleuropein and 4-methylcatechol at pH 7.0 in the presence of Mg^{2+} was fol-lowed by electron paramagnetic resonance spectroscopy and the resulting EPR spectra were recorded. Addition of Mg^{2+} was necessary in order to stabilize the resulting unstable semiquinone radicals. Figures 4 and 5 show both experi-mental and simulated EPR spectra of the Mg^{2+}-*o*-semi-quinone radical complexes produced by oleuropein (Fig. 4A, B) and 4-methylcatechol (Fig. 5A, B), respectively. As it can be seen from Fig. 4, the EPR spectrum of the metal-complexed *o*-semiquinone radical of oleuropein is character-ized by four groups of triplets and is rather similar to the EPR spectrum detected when oleuropein was autoxidized at pH 9. Table 1 shows the hyperfine splitting constants calculated from the corresponding simulated EPR spectra. A consider-able increase of the hyperfine splitting constant α^H_5 was observed ($\alpha^H_5 = 3.20$ G) and was attributed to the complexa-tion of the semiquinone radical and the consequent redis-tribution of spin density.

Figure 5 shows the EPR spectrum of the metal-complexed *o*-semiquinone radical of 4-methylcatechol when oxidized by tyrosinase at pH 7.0, in the presence of Mg^{2+}. Five main groups characterize the spectrum, which is typical of the metal-complexed *o*-semiquinone radicals of catechols [9]. The computer simulation of the experimental EPR spectrum
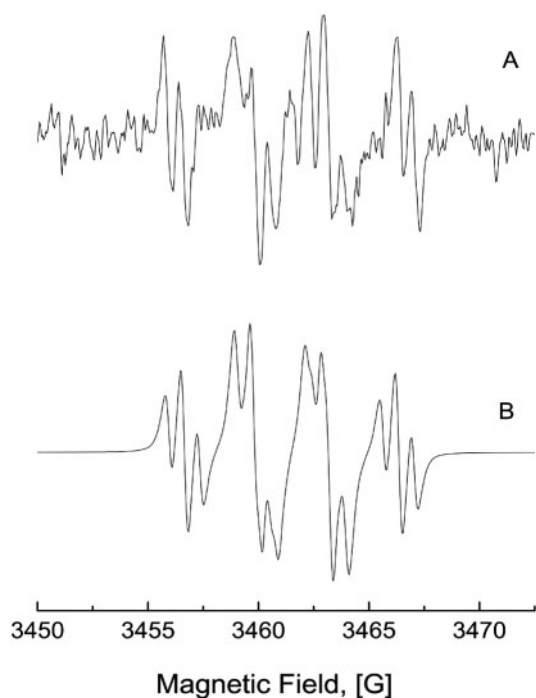
Figure 4. (A) Experimental and (B) computer-simulated EPR spectra of Mg^{2+}-*o*-semiquinone radical complex produced by the enzymatic oxidation of oleuropein (20 mM) by tyrosinase (50 μg/mL) in 50 mM Tris-HCl buffer pH 7.0 containing 0.5 M Mg^{2+}.
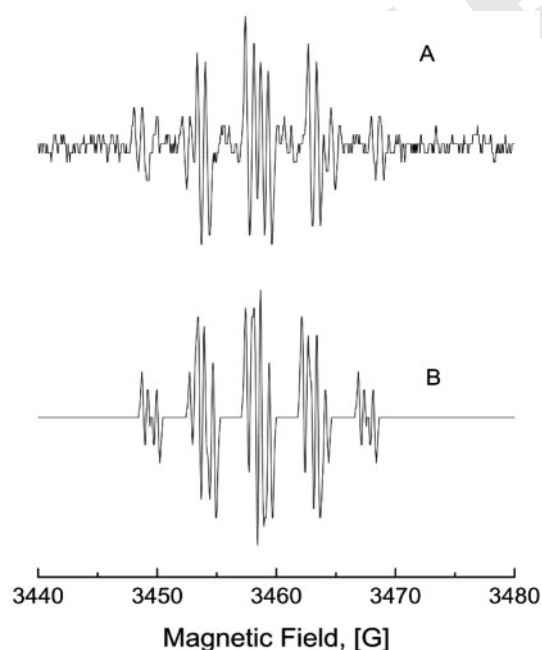
Figure 5. (A) Experimental and (B) computer-simulated EPR spectra of Mg^{2+}-*o*-semiquinone radical complex produced by the enzymatic oxidation of 4-methylcatechol (20 mM) by tyrosinase (50 μg/mL) in 50 mM Tris-HCl buffer pH 7.0 containing 0.5 M Mg^{2+}.

showed major splittings attributable to the equivalent protons of the side chain methyl group ($\alpha^H_{CH_3}$ = 4.80 G, $\alpha^H_{CH_3}$ = 4.70 G, $\alpha^H_{CH_3}$ = 4.65 G) and the proton at C-5 in the aromatic ring (α^H_5 = 4.00 G) and smaller splittings from the aromatic protons at C-3 and C-6 (α^H_3 = 0.45 G, α^H_6 = 0.77 G) (Table 1).

From the EPR measurements mentioned above, we can conclude that the oxidation of *o*-hydroxy phenols such as oleuropein and 4-methyl catechol proceeds through the formation of semiquinone radicals. Autoxidation under strongly alkaline conditions gave stable *o*-semiquinone radicals detectable even in the absence of stabilizing metal ions. On the contrary, at lower pH values, complexation of semiquinone free radicals with diamagnetic metal ions such as Mg^{2+} was necessary in order to detect and characterize the radicals. When enzymatic oxidation of *o*-hydroxy diphenols was considered at neutral pH, the addition of Mg^{2+} was also essential to stabilize and measure free radical species. In both cases, complexation of the free radicals was experimentally verified through the considerable increase of the α^H_5 splitting constants attributed to the redistribution of spin density in the presence of metal ions.

3.2 Spectrophotometric studies

3.2.1 Kinetics of autoxidation

The autoxidation of two polyphenols, namely oleuropein and 4-methylcatechol, at alkaline pH was followed by absorption spectrophotometry. The spectral characteristics of the oxidation of polyphenols were highly dependent on the type of polyphenol, the oxidant type and the pH of the reaction.

As it was shown in the previous part of this work, autoxidation of oleuropein and 4-methylcatechol at alkaline pH proceeds through the formation of *o*-semiquinone radicals. Figure 6A, B shows the autoxidation process of oleuropein and 4-methylcatechol, respectively, at pH 12. In the case of oleuropein (Fig. 6A), the rapid formation of an intermediate compound at 385 nm was observed immediately after the addition of the polyphenol to the reaction system. Then, this intermediate compound slowly transformed to a second product. This transition was indicated by a depletion of the absorption spectra with time, the appearance of an absorption shoulder at 439 nm and also the appearance of two isosbestic points, at 370 and 406 nm, respectively. When autoxidation of 4-methylcatechol was considered (Fig. 6B), an initial broad peak at around 470 nm was observed after 25 s. After 5 min, two absorption peaks were recorded, a main peak at 439 nm and a second, smaller one at 415 nm. The intensity of both peaks increased with time. Thus, from the above spectral characteristics, we conclude that the mechanism of oleuropein autoxidation is different from that of 4-methylcatechol since the kinetics of the autoxidation process and the absorption characteristics of the autoxidation products are different for the two polyphenols. These results are in good agreement with
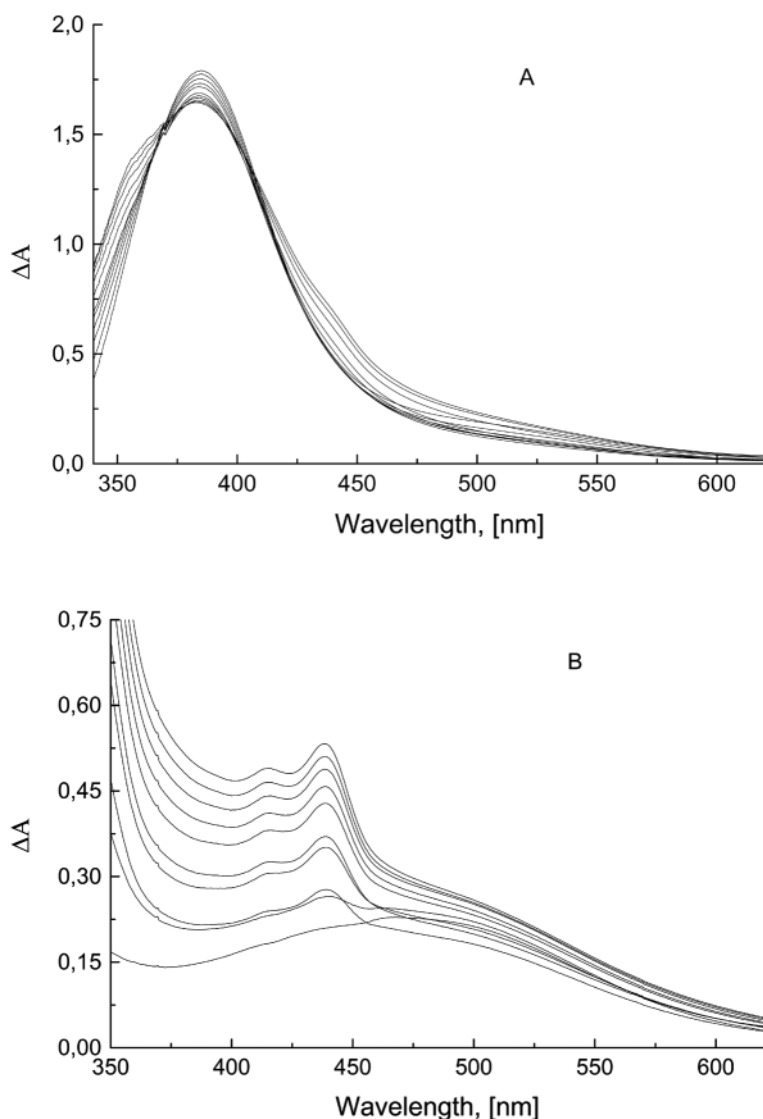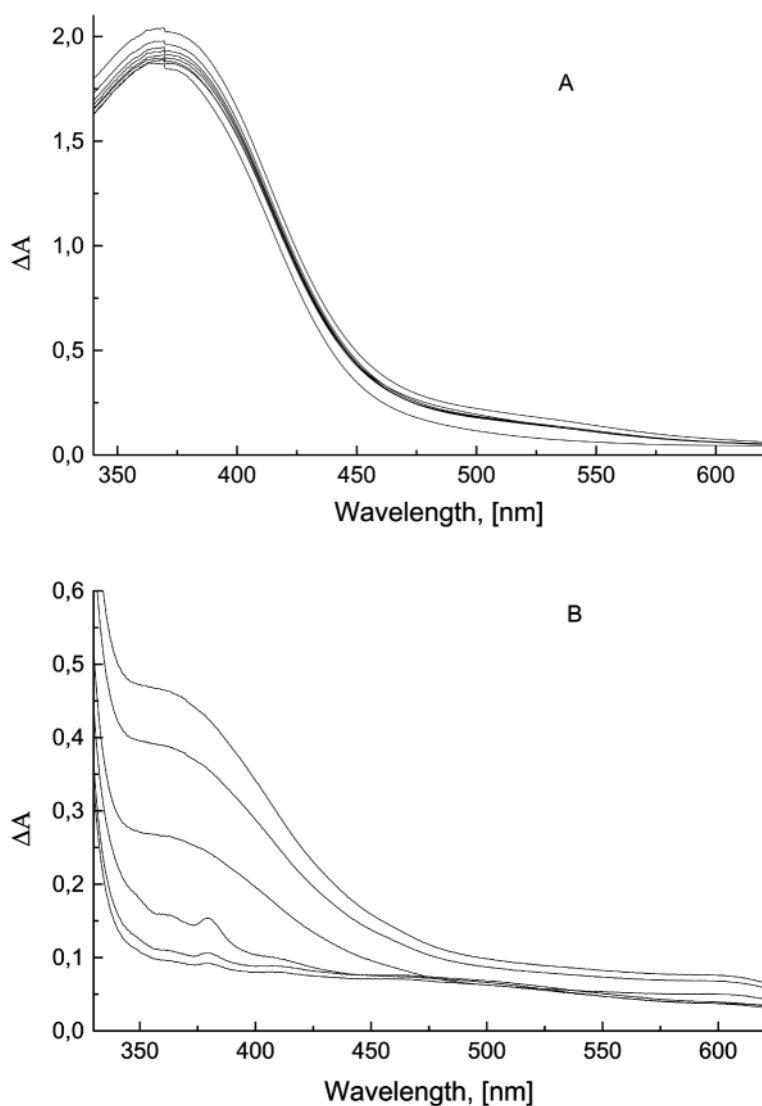
Figure 6. Kinetics of (A) oleuropein and (B) 4-methyl-catechol autoxidation at pH 12. Experimental conditions were: 1 mM oleuropein, 1 mM 4-methylcatechol, 90 mM NaOH (pH 12); temperature 25 °C. Reaction times from bottom to top: 25 s, 5, 10, 15, 20, 25, 30, 35, 40 and 45 min.

the results obtained from the EPR spectroscopic study. In the case of oleuropein, the EPR spectra recorded were characterized by a lower signal-to-noise ratio in comparison to that of 4-methylcatechol (Figs. 1, 2) probably due to the quick decomposition of the semiquinone radical in the case of oleuropein. However, we cannot exclude the possibility that the lower signal-to-noise ratio may also be due to the lower number of radicals produced in the case of oleuropein.

The kinetics of the autoxidation of oleuropein and 4-methylcatechol at pH 9.0, in the presence of Mg^{2+}, can be seen in Fig. 7A and B, respectively. In the case of oleuropein (Fig. 7A), a main peak at 375 nm was observed immediately after the addition of the polyphenol to the reaction medium. It is remarkable that during the time period in which autoxidation of oleuropein was followed (45 min) no decomposition of the initial product was observed. In the case of 4-methylca-

techol (Fig. 7B), an absorption peak at 380 nm appeared immediately after the initiation of the oxidation reaction. During the first 5 min of the reaction, the absorption was decreased while later an absorption shoulder around 360 nm appeared and a broad peak at 570–640 nm was gradually formed.

From the spectrophotometric recordings mentioned above, we assume that, at pH 9.0, oleuropein and 4-methyl-catechol exhibit different behaviors during the autoxidation process. Autoxidation of oleuropein is characterized by the formation of an almost stable oxidation product, while in the case of 4-methylcatechol the autoxidation process involves the slow transformation of an initial oxidation product to other compounds. These spectrophotometric results are in good agreement with the results obtained from the EPR spectroscopic study. In the case of oleuropein autoxidation at pH 9,

Figure 7. Kinetics of (A) oleuropein and (B) 4-methyl-catechol autoxidation at pH 9.0. Experimental conditions were: 1 mM oleuropein, 1 mM 4-methylcatechol, 50 mM Tris-HCl buffer solution (pH 9.0) containing 0.5 M Mg^{2+}; temperature 25 °C. Reaction times from bottom to top: 20 s, 1.5, 3, 4, 5, 7, 10, 15, 25, 35 and 45 min in the case of oleuropein and 20 s, 1.5, 5, 25, 35 and 45 min in the case of 4-methylcatechol.

well-defined EPR spectra were recorded and characterized by computer simulation (Fig. 3), while in the case of 4-methyl-catechol the intensity of the EPR spectrum was very low, probably due to the fast decomposition of the initially formed radicals to other compounds.

3.2.2 Kinetics and optimum pH for tyrosinase activity

The kinetics of the enzymatic oxidation of oleuropein by mushroom tyrosinase was investigated over the pH range of 5.5–9.0. Studies on oxidation of oleuropein by tyrosinase showed that the reaction was accompanied by the formation of an absorption peak at 400 nm in the case of pH 5.5–7.5, and at 420 nm for pH values higher than 8.0. Also a broad absorption peak at about 520 nm could be observed, which is more intense at neutral and alkaline pH. The optimum pH of

oleuropein oxidation by tyrosinase was 6.5, although a second activity maximum was observed at alkaline pH. When the same experiments were carried out for 4-methylcatechol, the maximum enzymatic activity was at pH 4.5 while a second activity maximum was observed at pH 7. These results concerning the pH activity profile of the enzymatic oxidation of 4-methylcatechol are in good agreement with previous findings reporting pH optima around pH 4.5 or 7.0, or both [16].

The kinetics of oleuropein oxidation induced by mushroom tyrosinase followed a Michaelis-Menten pattern. Relative kinetic constants were determined as $K_m = 0.34 \pm 0.03$ mM and $V_{max} = (0.029 \pm 0.002) \ \Delta\alpha_{400}$ min^{-1}. These results are in good agreement with the literature where it was shown that the K_m value of mushroom tyrosinase for other phenolic compounds used as substrates, namely DL-DOPA, dopamine and 4-methylcatechol, were of the same
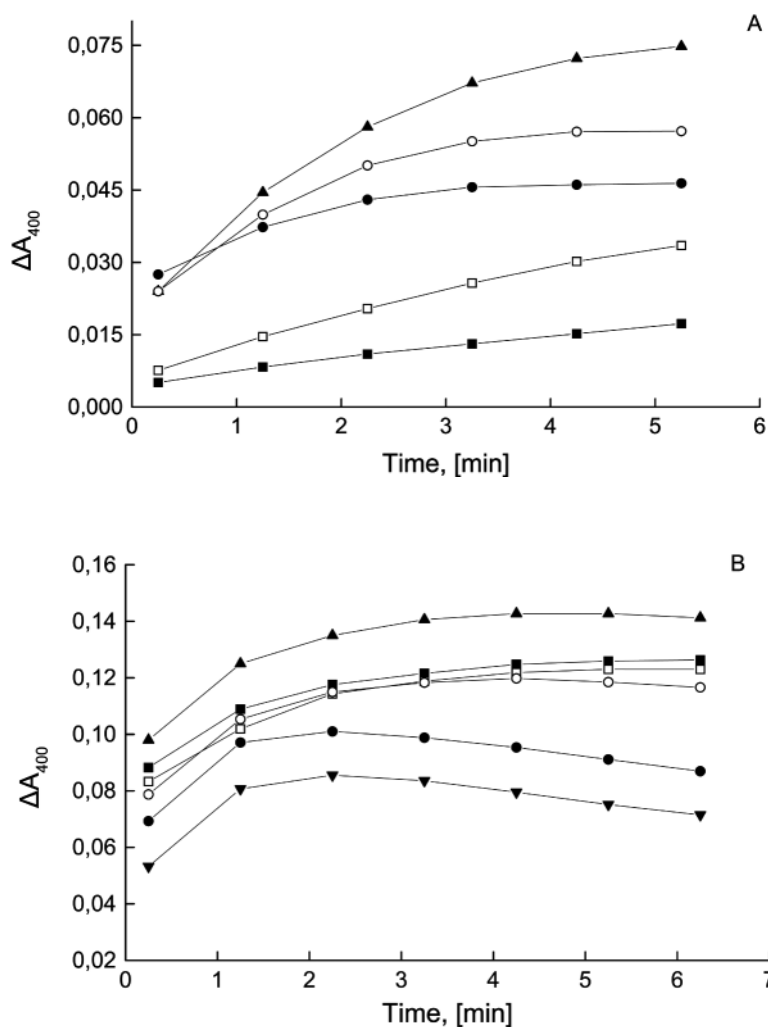
Figure 8. Evolution of oleuropein (A) and 4-methylcatechol (B) oxidation products catalyzed by mushroom tyrosinase at different substrate concentrations. Experimental conditions were: tyrosinase 13 μg/mL, oleuropein: (■) 0.03 mM, (□) 0.09 mM, (▲) 0.5 mM, (○) 2 mM, (●) 5 mM; and 4-methylcatechol: (■) 0.5 mM, (▲) 2 mM, (○) 5 mM, (●) 10 mM, (▼) 15 mM; phosphate buffer 50 mM, pH 6.5, 25 °C.

order of magnitude [29]. Figure 8A shows the evolution of oleuropein oxidation products at different oleuropein concentrations. It can be observed that at oleuropein concentrations higher than 0.5 mM, the enzymatic reaction, followed at 400 nm, reached a plateau after a few minutes. The same behavior was observed when the enzymatic oxidation of 4-methylcatechol was considered (Fig. 8B). In this case, the reaction rapidly leveled off when the concentration of 4-methylcatechol was higher than 5 mM. From the above results, we can suggest that the rapid decrease of the enzyme-induced absorption changes at 400 nm may be due to either a suicide inactivation reaction [20, 21] or the transformation of the initially formed product(s).

In conclusion, we have characterized the autoxidation and tyrosinase-catalyzed oxidation processes of oleuropein and the simple polyphenol 4-methylcatechol by EPR and UV-Vis spectroscopy. The two spectroscopic methods applied in this study approached the oxidation processes of the two catechols

in a complementary way. EPR spectroscopy enabled the detection and characterization of transient free radicals formed during the first steps of the oxidation procedures, while UV-Vis spectroscopy has been used to record spectral changes of chromophoric compounds produced when the polyphenols were either chemically or enzymatically oxidized.

We have successfully employed the spin stabilization technique to detect and analyze the reactive *o*-semiquinone radicals of oleuropein and to compare them with those of 4-methylcatechol. Semiquinone radicals derived from the *o*-dihydroxy phenolics studied are extremely unstable and rapidly convert to other compounds. Both EPR and absorption spectroscopy data showed that the mechanism of the transformation of the initially produced radicals to other oxidation products differs for the two polyphenols studied. In addition, for the same polyphenol, the mechanism of oxidation and the type of the generated products is highly depended on the oxidant type and the pH of the reaction.

Conflict of interest statement

The authors have declared no conflict of interest.

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