Effect of Metal Cations on the Chemical Oxidation of Olive *o*-Diphenols in Model Systems

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The catalytic effect of different metal cations on the chemical oxidation of caffeic acid and hydroxytyrosol in model systems and at pH 7 and 30 °C was studied. The effect of the metals on the rate of consumption of the *o*-diphenols was in the order Co > Mn > Zn > Fe > Ca, Cu, Mg, Al. When the reactions were carried out in the presence of manganese, the consumption of *o*-diphenols and oxygen increased and darker solutions were obtained than in the absence of the cation. An increase in the rate of oxygen consumption by manganese in solution was also observed. The oxygen uptake after 100 h of reaction was around 2.0−2.5 mol/mol of *o*-diphenol when manganese was added and 1.5−1.8 mol if the cation was absent. These findings suggest that Mn could be used to catalyze the oxidation of olive *o*-diphenols during ripe olive processing.

Keywords: *Olive; o-diphenols; oxidation; metals*

INTRODUCTION

The oxidative browning of polyphenols in foods generally results in a loss of nutritional value and the appearance of undesirable brown colors. However, in some processed foods such as cherries, black tea, raisins, plums, chocolate, and ripe olives, these reactions are part of desirable changes essential to the product. Two types of phenolic browning reactions are involved. Enzymic oxidation is the most important reaction in fresh fruits and juices and during the first stages of vegetable processing, when polyphenol oxidase is present. In processed foods, with the enzyme removed or inactivated, nonenzymic autoxidation can still take place (Singleton, 1987).

Metal cations exert catalytic effects on the chemical oxidation of phenolic compounds (Ionescu et al., 1978; Shindo and Huang, 1984; Takizawa et al., 1985; Adrian et al., 1986), although few studies on these effects in foods are available (Chambionat, 1961; Coen et al., 1988). Likewise, metal cations may form colored complexes with phenolic compounds (Jurd and Geissman, 1956), particularly dark complexes with iron ions (Lattanzio et al., 1994).

The industrial procedure for the production of ripe olives consists of the successive treatment of fruits with dilute NaOH. During the intervals between the NaOH treatments, fruits are suspended in water through which air is bubbled. Throughout this operation olives darken progressively due to the oxidation of *o*-diphenols, hydroxytyrosol (3,4-dihydroxyphenylethanol) and caffeic acid (Brenes et al., 1992). Finally, different iron salts are added to fix the color formed (Brenes et al., 1995). However, the whole process lasts for 5−7 days and generates a high volume of wastewaters and a shiny dark color is not always obtained.

Caffeic acid in alkaline and acidic conditions has been used to model the oxidative reactions of this compound in foods (Cilliers and Singleton, 1989; Fulcrand et al., 1994). A study of the oxidation reactions of caffeic acid and hydroxytyrosol in solutions of the NaOH treatment of olives has also been reported (García et al., 1992).

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Using caffeic acid and hydroxytyrosol as a model, the purpose of this work was to study the effect of different metal cations on the oxidation of olive *o*-diphenols in accelerating these reactions and in the achievement of darker solutions, especially in alkaline conditions.

MATERIALS AND METHODS

Chemicals. Ferrous gluconate, manganese chloride, magnesium chloride, aluminum chloride, and cobalt chloride were purchased from Fluka (Buchs, Switzerland); cupric chloride, zinc chloride, and calcium chloride were obtained from Sigma (St. Louis, MO).

Caffeic acid was purchased from Fluka. Hydroxytyrosol was prepared by alkaline hydrolysis of oleuropein (Extrasynthese, Genay, France). Oleuropein (100 mg) was hydrolyzed in 20 mL of 6 M NaOH for 5 h at 30 °C under a N_2 atmosphere. The hydrolysate was adjusted to pH 3 with HCl and extracted with diethyl ether. The extracts were mixed with 10 mL of 0.1 M HCl, and the organic solvent was evaporated under vacuum. The aqueous solution was treated with 0.03 g of activated carbon (type GA, Industrias Kern, Barcelona, Spain) for 20 min and, after filtering, a colorless acidic solution containing the purified hydroxytyrosol was obtained.

Oxidation of Polyphenols. The experiments were carried out with 50 mL of solution containing 1 mM caffeic acid or hydroxytyrosol. This solution was also buffered at pH 7 with 20 mM bis-tris propane buffer (Sigma), and different metal ions from a concentrated solution were added to reach a concentration of 0.5 mM, except in the oxygen uptake experiments in which only 0.34 mM manganese was used. The mixture was incubated in a thermostatically controlled chamber at 30 °C and stirred on a magnetic stirrer.

Color of Liquids. Absorption spectra of solutions were measured using a Hewlett-Packard Model 8450 UV−vis spectrophotometer. Samples (200 μL) were withdrawn at regular intervals throughout the incubation period and mixed with 800 μL of a 80 mM bis-tris propane solution with the pH adjusted to 7.

The color of liquids was expressed either as absorbance at 420 nm (Cilliers and Singleton, 1989) or in terms of the CIE L^* a^* b^* parameters calculated from the absorption spectra (Gonnet, 1993).

HPLC Analyses. Samples (0.5 mL) taken at regular intervals throughout the incubation period were mixed with 5 μL of phosphoric acid, centrifuged at 13000*g*, and injected into the chromatograph. The HPLC apparatus was a Waters 600E (Millipore, Inc., Milford, MA) equipped with a diode array

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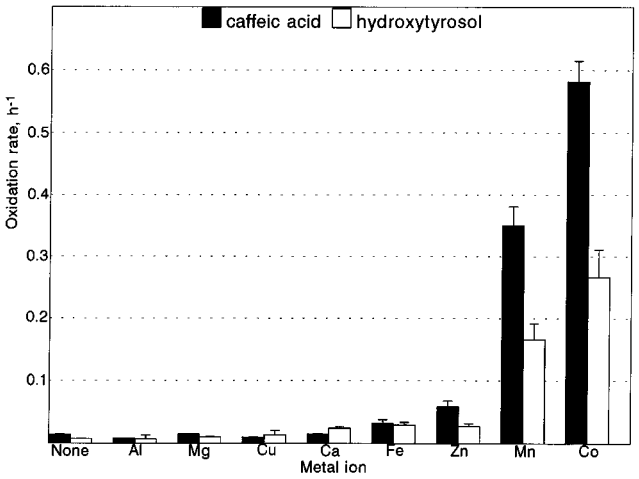

Figure 1. Effect of 0.5 mM concentrations of divalent and trivalent cations on the oxidation rate of caffeic acid and hydroxytyrosol (1 mM) in buffered solutions at pH 7 and 30 °C.

detector (Waters 994). The column was a reversed-phase Spherisorb ODS-2 (5 μm packing, 250 × 4 mm i.d.) column. The elution conditions were as follows: flow rate, 1 mL/min; solvent A, acetonitrile; solvent B, water with the pH adjusted to 2.5 with phosphoric acid. The mobile phase consisted initially of 5% of A and 95% of B. The concentration of the latter solvent was decreased to 75% in 15 min and then down to 20% in another 10 min. The column was re-equilibrated at the initial conditions after 15 min using the appropriate gradient. The wavelength selected for caffeic acid detection was 320 nm and 280 nm for hydroxytyrosol.

Oxygen Uptake. The experiments were carried out in sealed jars (250 mL) put in a thermostatic chamber at 30 °C. The consumption of oxygen during the oxidation reaction of caffeic acid or hydroxytyrosol was monitored with a Micro-Oxymax O_2/CO_2 respirometer (Columbus Instruments, Columbus, OH).

RESULTS AND DISCUSSION

The disappearance of caffeic acid and hydroxytyrosol was monitored by HPLC, and the data were fitted to first-order kinetics. Cilliers and Singleton (1989) demonstrated that caffeic acid oxidation followed first-order kinetics. The correlation coefficients of the fitted curves were always above 0.97 with five data points used in calculating the first-order rate constants.

The oxidation rate of caffeic acid was in general higher than that of hydroxytyrosol (Figure 1). Thus, when metal cations were not added to the reaction media, the oxidation rates of caffeic acid and hydroxytyrosol were 1400×10^{-5} and 722×10^{-5} h^{-1}, respectively. The oxidation rate of caffeic acid found in this work was higher than that reported by Cilliers and Singleton (1989), although these differences may be attributed to the different experimental conditions.

Of the metal cations tested, Co, Mn, Zn, and Fe were the most effective in accelerating the disappearance of *o*-diphenols in alkaline conditions. Aluminum, magnesium, copper, and calcium had a slight effect or no effect on the oxidation rate. A catalytic effect of copper ions on the oxidation of phenols has been reported, although in alcoholic solutions (Takizawa et al., 1985). Shindo and Huang (1984) demonstrated that the aluminum cation did not affect the darkening reactions of phenolic compounds during the formation of humic substances. In contrast, cobalt cations showed the highest catalytic effect on the oxidation rate of both caffeic acid and hydroxytyrosol. The effect of Co in ripe olive processing has been tested previously, although experimental data were scarce and health risks prevented use of this cation (Chambionat, 1961). To our knowledge, no data are available on the effect of Zn ions on the oxidation reactions of phenolic compounds in foods. This cation increased the oxidation rates of caffeic acid and hydroxytyrosol to 5900×10^{-5} and 2700×10^{-5} h^{-1}, respectively. Zinc ions could be used as catalysts in the oxidation reactions of phenolic compounds in foods since they are a permitted food additive. The addition of ferrous cations to the reaction media also accelerated the disappearance of *o*-diphenols, as was expected from the results of a previous study (Adrian et al., 1986), although in our case the oxidation rates of caffeic acid and hydroxytyrosol were rather similar. Iron is used in ripe olive processing, but its application is recommended only for color fixation and not for catalysis during the darkening step. At present, however, there is some controversy on the diverse and probably adverse effects of excess iron on health (McCord, 1994).

It can be observed in Figure 1 that manganese ions were quite effective in speeding up the oxidation rate of the phenolic compounds. The catalytic effect of manganese on the formation of humic acids has been thoroughly studied (Shindo and Huang, 1984; Pal et al., 1994), and this effect has been demonstrated to be greater than that of ferrous ions (Adrian et al., 1986). Manganese is considered as generally recognized as safe in the United States, and these results suggest that it may be possible to use it to accelerate the chemical oxidation of phenolic compounds in foods, particularly during the darkening step of ripe olive processing.

Figure 2 represents the UV−vis spectra of the caffeic acid solution before and after oxidation with and without Mn or Fe added to the reaction media. The buffer absorption was minimal above 250 nm. A decrease in the absorbance of the caffeic acid solutions was observed between 250 and 370 nm during the oxidation period, as was expected from the results of a previous study (Cilliers and Singleton, 1991). In contrast, the absorption in the 370−700-nm region increased as oxidation progressed, except when iron was present in the media. The absorbance vs wavelength profiles in the visible region of the oxidized caffeic acid solutions showed an asymptotic decline and, according to Shindo and Huang (1984), were not affected by the addition of manganese.

Cilliers and Singleton (1989) found a good correlation between the color produced at 420 nm and the caffeic acid consumed at all pH values and temperatures assayed. However, when metal cations are present in these phenolic solutions, colored complexes may be formed and the CIE L^* a^* b^* parameters are necessary to best characterize the color changes of the solutions. The addition of manganese to the nonoxidized caffeic acid media did not modify the color of it, but, if the cation was iron, the absorption spectra showed a shoulder peak around 600 nm, a peak rather similar to that reported for the catechol−Fe complex (Shindo and Huang, 1984).

Changes in the color of the solutions, with and without addition of manganese during oxidation, were rather similar. The lightness (L^*) diminished and the a^* and b^* parameters increased as the oxidation progressed. In contrast, the lightness (L^*) of the iron solutions increased with oxidation. This was related to the lighter visual color of the oxidized liquids.

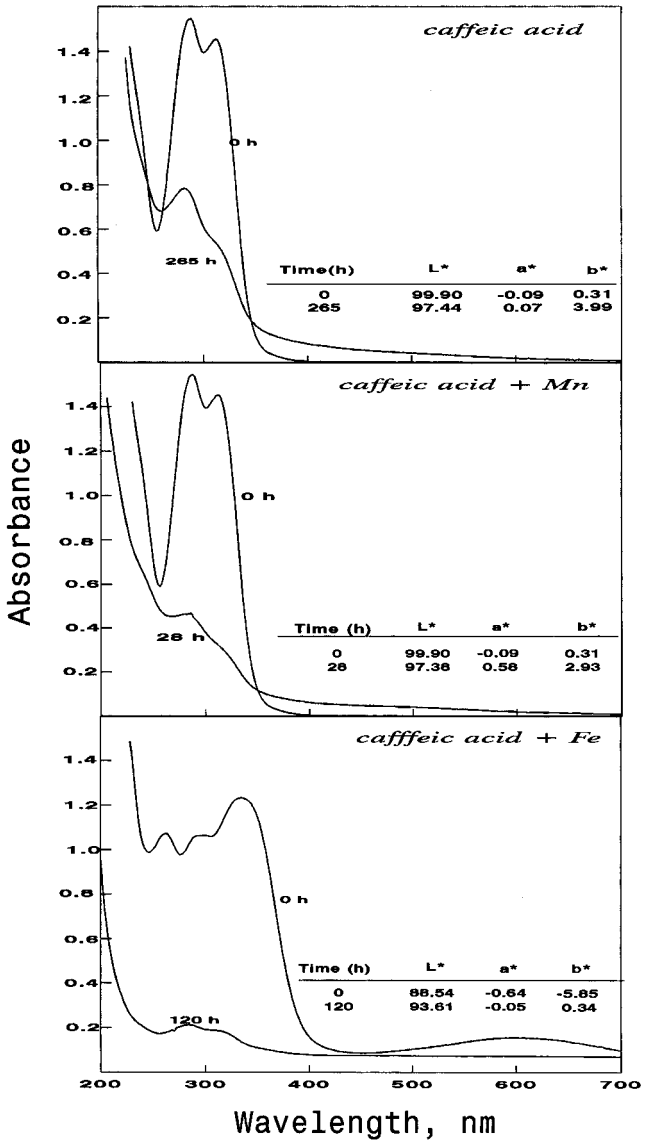

Figure 2. UV−visible spectra and CIE *L* a* b** parameters of caffeic acid before and after oxidation at pH 7 and 30 °C in the presence of Mn and Fe. Initial concentrations of caffeic acid and metal ions were 1 and 0.5 mM, respectively. Samples were measured with a 0.5-cm path length.

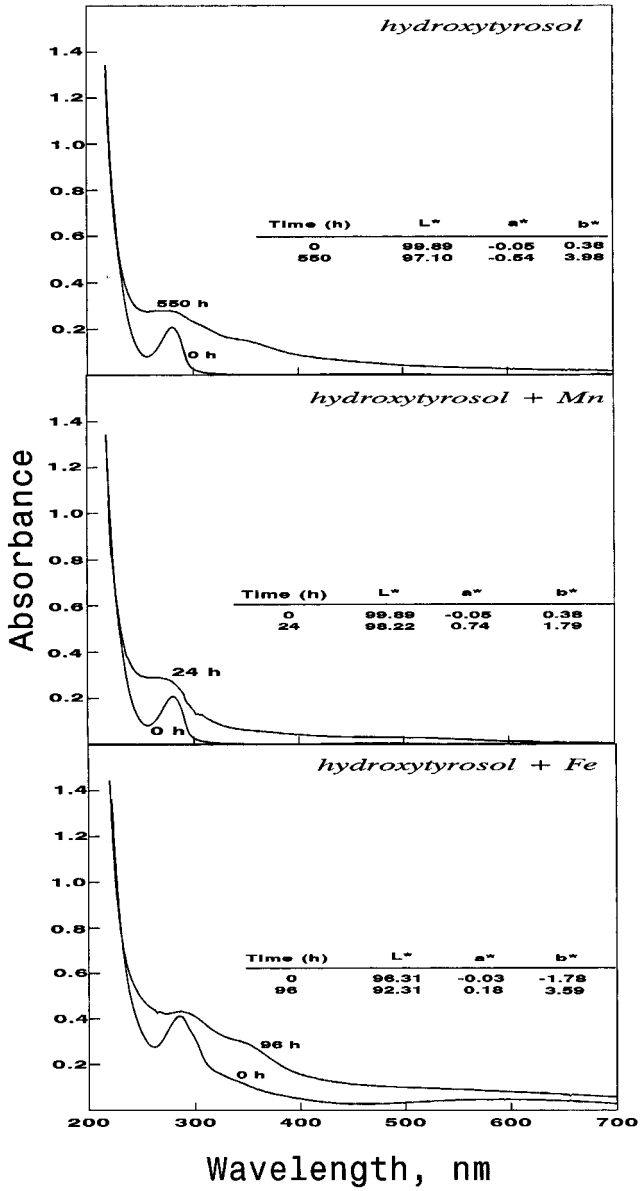

Figure 3. UV−visible spectra and CIE *L* a* b** parameters of hydroxytyrosol before and after oxidation at pH 7 and 30 °C in the presence of Mn and Fe. Initial concentrations of hydroxytyrosol and metal ions were 1 and 0.5 mM, respectively. Samples were measured with a 0.5-cm path length.

Each UV−vis spectrum of the hydroxytyrosol solutions (Figure 3) showed a sharp peak at 280 nm, as corresponds to this compound. This disappeared in all cases after oxidation. An increase in the absorbance of the solutions in the visible region was also observed after oxidation, even when iron was employed. Consequently, when no cation was added and when manganese or iron was present in the reaction media, the oxidation of hydroxytyrosol gave rise to a decrease in the lightness (*L**) and an increase in the *a** and *b** parameters of the solutions.

Figure 4 shows the change in the color, expressed as absorbance at 420 nm, of the caffeic acid and hydroxytyrosol solutions with and without added manganese during the oxidation process. The addition of manganese to the media not only accelerated the rate of formation of the color but also gave darker solutions, and the rate of formation of the color and the final values of absorbance were higher for caffeic acid than for hydroxytyrosol. Shindo and Huang (1992) also found a higher degree of darkening of the phenol oxidized solutions when manganese was present. They proposed that manganese ions act as Lewis acids by accepting electrons from the diphenols which are thus oxidized and subsequently polymerized.

A certain relationship between the rates of formation of the color and the consumption of *o*-diphenols can be observed in all cases from Figures 4 and Figure 5, as found by Cilliers and Singleton (1989) for caffeic acid oxidation in the absence of metal cations. These authors proposed that phenolate anions are involved in the formation of the color by reacting directly with triplet oxygen to form a semiquinone which then undergoes further reaction.

The consumption of oxygen during the oxidation process was also studied (Figure 6) and correlated with the rates of formation of color and consumption of *o*-diphenol. However, it seems that the consumption of oxygen continued long after *o*-diphenols ceased to be detected in the reaction media (Figure 5). Oxygen uptake increased gradually with time and after 100 h around 2.0−2.5 mol of O_2/mol of *o*-diphenol was con-

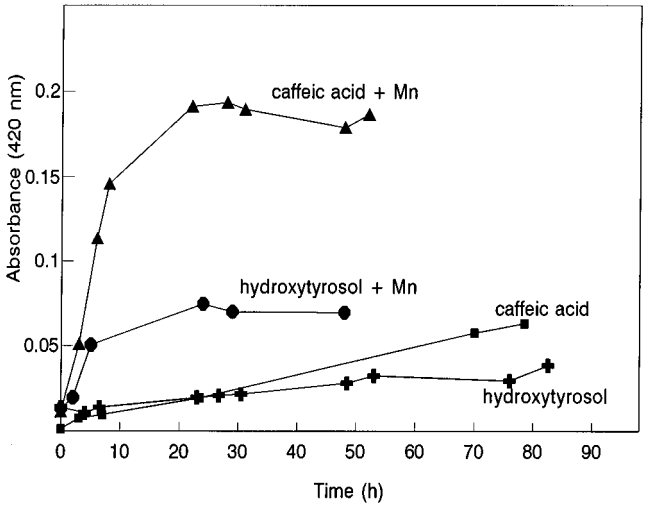

Figure 4. Influence of manganese (0.34 mM) on the color formation (absorbance at 420 nm) during the chemical oxidation of caffeic acid and hydroxytyrosol (1 mM) at pH 7 and 30 °C. Samples were measured with 1-cm path length.

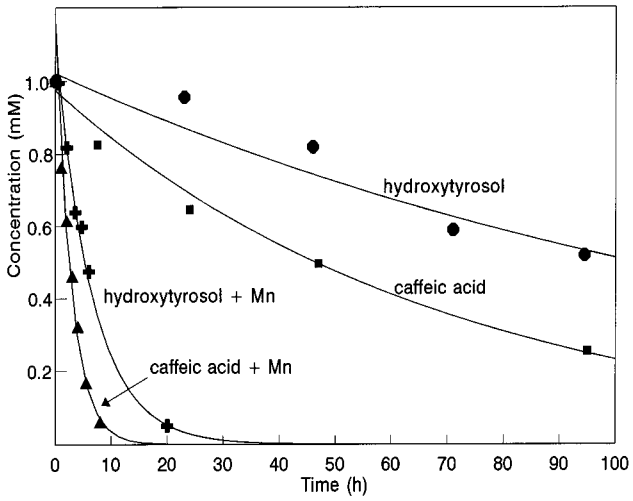

Figure 5. Influence of manganese (0.34 mM) on the consumption of caffeic acid and hydroxytyrosol during their chemical oxidation at pH 7 and 30 °C.

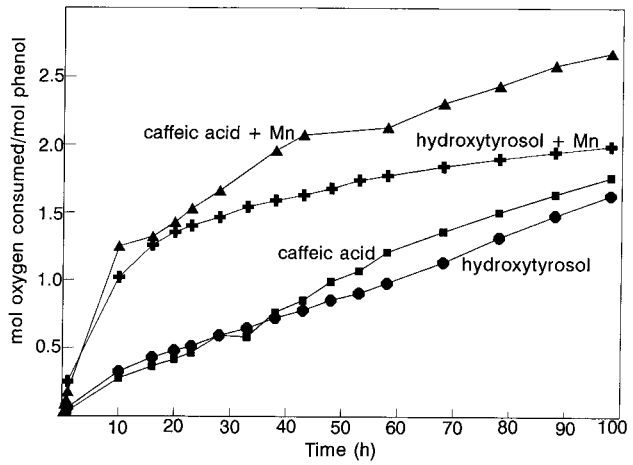

Figure 6. Oxygen uptake during the chemical oxidation of caffeic acid and hydroxytyrosol (1 mM) in the presence and absence of manganese (0.34 mM) at pH 7 and 30 °C.

sumed when manganese was present in the reaction media and around 1.5−1.8 mol of oxygen was consumed when the cation was not added. Adrian et al. (1986) also found a higher consumption of oxygen with manganese (1.5 mol of O_2/mol of catechol) than in the absence of the cation (1.0 mol of O_2/mol of catechol). The amount of oxygen consumed per mole of phenol has been demonstrated to depend on the phenol studied. Thus, in oxidation reactions carried out at pH 14 the moles of oxygen consumed per mole of phenol were 1.7 for caffeic acid, 2 for catechol, and 2.4 for gallic acid (Cilliers and Singleton, 1990). In our experiments caffeic acid consumed higher amounts of oxygen than did hydroxytyrosol both with and without manganese in the reaction media. However, it has also been reported that the uptake of O_2 during the oxidative reactions of phenolic compounds was only detected in reactions in which water served as solvent, no buffer was employed, and the level of manganese in the media was very low (Pal et al., 1994).

It has been reported that some metal cations may accelerate the oxidation reactions of *o*-diphenols involved in the black color formation of ripe olives. Manganese ions, in particular, not only accelerate the reactions but also give darker solutions. Thus, the use of this cation in ripe olive processing may prove to be attractive.

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