Iron-Catalyzed Oxidation of (+)-Catechin in Model Systems

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Autoxidation of (+)-catechin in wine-like model solutions containing 0−20 mg L^{-1} of ferrous iron was monitored by HPLC with diode array detection. The rate of catechin oxidation and the extent of browning increased with the iron level. Some of the oxidation products were formed in larger amounts at higher iron concentrations. These included colorless compounds and yellow pigments with absorbance maxima between 440 and 460 nm. The iron-catalyzed reactions leading to these compounds competed with iron-independent reactions generating different products which are presumably identical to those formed in enzymically oxidized catechin solutions.

Keywords: *(+)-Catechin; autoxidation; ferrous ions; CLHP; browning*

INTRODUCTION

Oxidative browning of phenolic compounds is a major cause of quality degradation in fruit-derived foods and beverages. The first step is the enzymatic oxidation of phenols to the corresponding *o*-quinones, which takes place in the early stages of processing, *i.e.*, as soon as the cells are damaged by crushing or cutting. The role of hydroxycinnamates such as chlorogenic (caffeoylquinic) and caffeoyltartaric acids, the major substrates for polyphenol oxidases, in the initiation of enzymatic browning is well documented. In particular, grape must browning was shown to be largely related to the must hydroxycinnamic acid content (Cheynier *et al*., 1990) but significantly enhanced by addition of flavanols (Rigaud *et al*., 1991). On the other hand, wine browning susceptibility is highly correlated with the concentration of flavanols (Simpson, 1982; Cheynier *et al*., 1989). Conversion of flavanols to brown pigments in wine is non enzymic and may proceed via two different reaction mechanisms. The first one involves acid-catalyzed C−C bond-breaking and bond-making reactions characteristic of condensed tannin chemistry (Haslam, 1980). The second is autoxidation, which occurs very fast in alkaline media but also takes place under acid conditions such as those prevailing in wine, especially if metal catalysts are present (Cilliers and Singleton, 1989).

Oxidation of (+)-catechin has been achieved enzymically by using polyphenoloxidase (Goodenough *et al*., 1983; Oszmianski and Lee, 1990; Rouet-Mayer *et al*., 1990; Guyot, 1994; Guyot *et al*., 1995) or peroxidase (Weinges *et al*., 1969; Weinges, 1971) and chemically (Young *et al*., 1987; Jensen and Pedersen, 1983). Complex mixtures including yellow pigments and colorless C−C or C−O−C linked dimers were obtained in most studies (Weinges *et al*., 1969; Weinges, 1971; Young *et al*., 1987; Guyot, 1994) whereas autocondensation of catechin in alkaline environment yielded catechinic acid derivatives (Jensen and Pedersen, 1983). However, catechinic acid rearrangement was shown to be impeded by trace amounts of various additives, such as silica, boric acid, aluminium trichloride (Meikleham *et al*., 1994), and zinc(2+) acetate (Pizzi and Tekely, 1995). Under these conditions, heterocycle opening occurs more readily but the C2 reactive site thus formed is blocked, so that condensation with the A-ring of another flavonoid unit take place rather than intramolecular bond rotations leading to catechinic acid rearrangement. As well, catechinic acid rearrangement does not seem to occur at pH values below 8 (Meikleham *et al*., 1994).

Thus, the nature and relative amounts of the products formed appear highly dependent on the reaction medium. They may be influenced by the type of reaction (one-electron oxidation, yielding a semiquinone radical, or two-electron oxidation, yielding the *o*-quinone) but also, as mentionned above, by the presence of additives or the pH of the solution.

Whereas enzymatic oxidation of catechin has been thoroughly studied, investigations of its nonenzymic reactions have been restricted to alkaline pHs, in spite of their occurrence and important consequences in acidic media like wine. Nevertheless, the reaction mechanisms prevailing respectively at pH values above 4 and below 4 may be different. In particular, polyphenoloxidase-catalyzed oxidation of catechin yielded mostly dimers linked by a C−O interflavanic linkage at pH values up to 4, meaning that radical coupling was prevailing in acidic media, whereas catechin dimers linked by biphenyl bonds, formed by nucleophilic addition of the 1,4 Michael type, were the predominant products generated at higher pH values (Guyot, 1994).

The purpose of the present work was to study the autoxidation of catechin under the conditions encountered in wines and to compare the products thus formed with those obtained by enzymatic oxidation in mustlike model systems. This was achieved in pH 3.7 hydroalcoholic solutions with iron as a catalyst. The latter was provided as Fe^{2+}, at levels varying between 1 and 20 mg L^{-1} since wine usually contains 2 to 5 mg L^{-1} and sometimes up to 20−30 mg L^{-1} of ferrous ions (Navarre, 1991).

MATERIALS AND METHODS

Materials. Standard (+)-catechin was obtained from Sigma Chemical Co (St. Louis, MO). Prior to use, it was purified by using a high pressure preparative chromatographic system including two gradient pumps (M306, head pump 200WTi, Gilson), an injection pump (M306, head pump 10SC, Gilson), a hydraulic compression column (500 × 40 mm, ISA Jobin-Yvon), and a UV detector (M875-UV, Jasco) set at 280 nm. The column was filled with 500 mL of Fractogel TSK HW-40F (Merck, Darmstadt, Germany) under 7 bars at ambient temperature. Elution was carried out isocratically with

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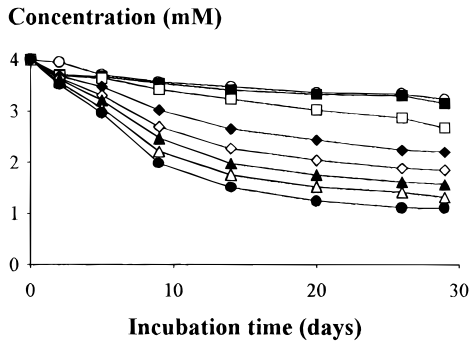

Figure 1. Influence of iron (Fe^{2+}) concentration on the rate of (+)-catechin degradation in winelike model solutions. Iron concentration: 0 (○), 1 (■), 2 (□), 5 (◆), 8 (◇), 10 (▲), 15 (△), 20 (●) mg L^{-1} provided as iron sulfate.

methanol at 20 mL min^{-1} and the catechin peak was collected, taken to dryness by rotary evaporation, and freeze-dried. Potassium hydrogen tartrate, benzoïc acid, and ferrous sulfate were purchased from Prolabo (Paris, France).

Incubation Conditions. (+)-Catechin (4 mM) and benzoic acid (2 mM), used as an internal standard for quantification purposes, were dissolved in aqueous potassium hydrogen tartrate (0.02 M, pH 3.7) containing ethanol (20%, v). The rather high ethanol concentration was chosen in order to avoid microbial development during storage of the solutions. Ferrous sulfate was added so as to give final iron concentrations of 1, 2, 5, 8, 10, 15, and 20 mg/L. Twenty-five milliliter aliquots of these solutions were kept in 25-mL vials closed by rubber caps and allowed to react at room temperature. Samples (40 μL each) were taken through the rubber caps after 0, 2, 5, 9, 14, 20, 26, and 29 days and injected directly onto the HPLC system.

Enzymatic oxidation was carried out by using grape polyphenoloxidase extract as described by Guyot *et al.* (1995), but with the hydrogen tartrate buffer at pH 3.7.

HPLC Analyses. The HPLC apparatus was a Hewlett-Packard HP 1050 system equipped with a diode array detector.The column was reversed-phase SFCC Spherisorb ODS2 (5-μm packing) (250 × 4 mm) protected with a guard column of the same material (Merck, Darmstadt, Germany).

Elution conditions were as follows: flow rate, 1 mL min^{-1}; oven temperature, 30 °C; solvent A, 2% formic acid in water; solvent B, acetonitrile/water/formic acid (80/18/2, v/v/v); linear gradients from 5 to 30% B in 50 min and from 30 to 50% in 10 min, followed by washing and reconditioning of the column. The elution was monitored simultaneously at 280, 400, and 430 nm and UV−visible spectra were recorded from 250 to 600 nm.

Calibrations were performed for (+)-catechin by injections of known dilutions. Quantitations of (+)-catechin and oxidation products were based on peak areas at 280 or 430 nm.

Browning Measurements. Browning of the solutions was measured as the increase of absorbance at 430 nm using a Uvikon 810 spectrophotometer (Kontron Instruments, Milano, Italy), with 0.1-cm path length. Catechin-free solutions at each iron concentration were prepared and stored like the catechin solutions and used as references to take into account eventual color changes due to iron oxidation.

All analyses were performed in duplicate.

RESULTS AND DISCUSSION

Autoxidation of (+)-catechin in winelike solutions containing 0−20 mg L^{-1} ferrous ions, provided as iron sulfate, was monitored by HPLC. Although some catechin losses were observed in the iron-free solutions, the degradation rate increased with increasing amounts of iron, over the 1-month period studied (Figure 1). Catechin losses after 29 days of incubation, browning of the solutions, measured as the increase of absorbance at 430 nm, and iron concentration were highly correlated together ($p < 0.001$, $n = 8$).

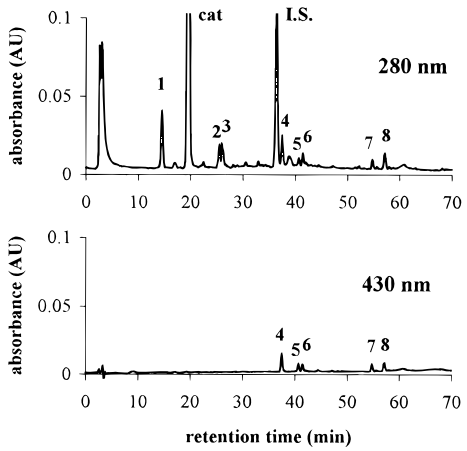

Figure 2. Chromatograms at 280 and 430 nm of a (+)-catechin solution after 29 days incubation in winelike model solution containing 20 mg L^{-1} ferrous ions. cat, (+)-catechin; I.S., benzoic acid, used as internal standard; 1−8, degradation products.

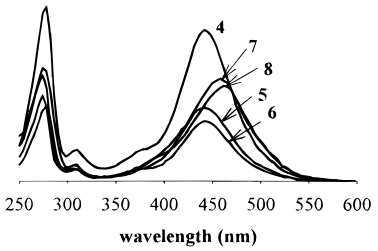

Figure 3. UV−visible spectra of degradation products 4−8.

Numerous reaction products, including both colorless compounds and material absorbing at 430 nm, were detected on all chromatograms. As an example, chromatographic profiles at 280 and 430 nm of a catechin solution incubated for 29 days with 20 mg L^{-1} iron are shown in Figure 2. Most of the products formed eluted later than (+)-catechin, suggesting that they were less polar and/or larger molecules, as observed earlier in enzymically oxidized catechin solutions (Oszmianski and Lee, 1990; Guyot *et al.*, 1995). The pigments (peaks 4−8) exhibited similar UV−visible spectra (Figure 3) with absorbance maxima at 280 and 440 nm (peaks 4−6) or 460 nm (peaks 7, 8). The UV−visible spectra of all colorless compounds were the same as those of (epi)catechin and procyanidins, with a maximum absorbance near 280 nm, and are therefore not shown. This suggests that the original structure was retained in colorless autoxidation products, like in all C−C and C−O linked dimers characterized in enzymically oxidized catechin solutions (Guyot, 1994).

Although coelutions of different compounds are possible, diode array analysis of each peak detected on the different HPLC profiles suggested that the same major products were formed in the presence of iron at all concentrations. Larger amounts of all these products were formed as the concentration of ferrous ions in the solution increased. Yellow products accumulated gradually throughout the incubation period (*e.g.*, peaks 4 and 8, Figure 4B,C) although the concentration of peak 8 seemed to level off after 29 days at the highest iron concentrations tested (15 and 20 mg L^{-1}). The peak areas of colorless products reached a maximum and then decreased within the first month of incubation, in solutions containing 5 mg L^{-1} iron and above (*e.g.*, peak

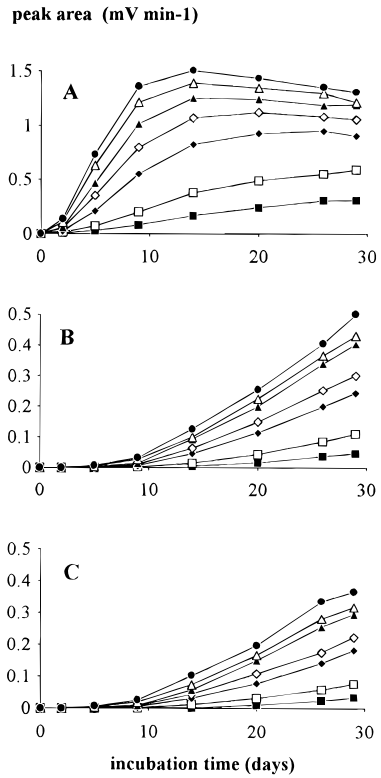

Figure 4. Influence of iron (Fe^{2+}) on the concentration of catechin degradation products 1 (A), 4 (B) and 8 (C) in winelike model solutions. Iron concentration: 1 (■), 2 (□), 5 (◆), 8 (◇), 10 (▲), 15 (△), 20 (●) mg L^{-1} provided as iron sulfate. Concentration of catechin degradation products are expressed as HPLC peak areas at 280 nm (1) or 430 nm (4 and 8).

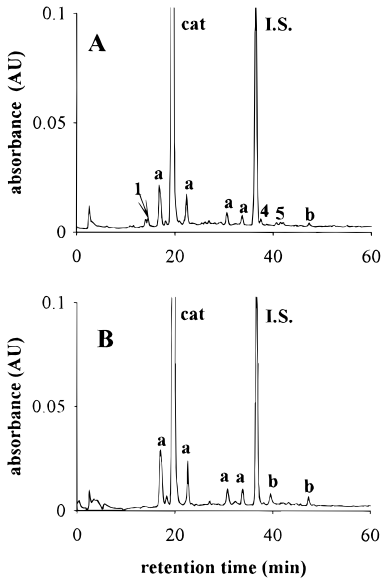

Figure 5. Chromatograms at 280 nm of (+)-catechin incubated for 3 months in iron-free winelike model solution (A) and enzymically oxidized for 1 h (B). a, b: colorless (a) and yellow (b) oxidation products formed by iron-independent reactions.

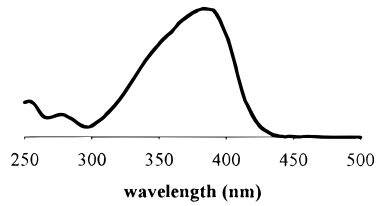

Figure 6. UV−visible spectrum of the major pigment formed by autoxidation of (+)-catechin in the iron-free solution (Figure 5A,b).

1, Figure 4A). This maximum was both higher and reached earlier as the concentration of iron increased, suggesting that colorless compounds were intermediate products in the iron-induced degradation reactions.

Note that the role of colorless products as intermediates in the reaction pathways leading to brown pigments has been demonstrated earlier in enzymatically oxidized catechin solutions (Guyot, 1994). As well , the colorless C2−C8 linked dimer formed after opening of the heterocyclic ring in the autoxidation of catechin has been shown to proceed to yellow para alkyl quinone pigments (Meikleham *et al.*, 1994).

After 1 month of incubation, the iron-free catechin solution contained a few degradation products, none of which corresponded to the major products formed in the presence of iron, suggesting that different reactions were taking place. However, analysis of the solution after 3 months of incubation (Figure 5A) showed the presence of small amounts of the major peaks formed in iron-containing solutions along with the products detected after 1 month. This suggests that formation of the former did not require the presence of iron but was accelerated by it, although contamination of the solution by trace amounts of iron or other catalyzing material cannot be ruled out. Besides, all these compounds, which included colorless compounds (denoted a) and a yellow pigment (denoted b), were present in small amounts in the iron-containing solutions (Figure 2). Thus it seems that reactions facilitated by the presence of iron and iron-independent reactions competed in all solutions, with the former prevailing at higher iron concentrations. Absorbance maximum of the major pigment formed by iron-independent reactions (Figure 5A,b) was at 385 nm (Figure 6) and thus much lower than those of pigments resulting from iron-dependent reactions (λ_{max} 440−460 nm; Figure 3). This suggests that it hardly contributed to browning and may explain why autoxidative degradation of catechin induced larger discoloration at higher iron concentrations.

In fact, the chromatographic profiles of the iron-free autoxidized solution resembled those obtained previously for polyphenoloxidase-catalyzed catechin oxidation (Guyot *et al.*, 1995). Comparison of the chromatographic profiles at 280 nm of the iron-free autoxidized solution after 3 months of incubation (Figure 5A) and of a catechin solution oxidized enzymically for 1 h (Figure 5B) shows that the products specific to iron-catalyzed oxidation were different from enzymatic oxidation products, whereas the products predominating in the iron-free autoxidized solution coeluted with them. The UV−visible spectrum (λ_{max} 385 nm, Figure 6) of the compound eluting at 47.3 min (Figure 5A,b) is characteristic enough to allow us to postulate that it is identical to one of the major pigments generated by enzymatic oxidation (Guyot, 1994; Guyot *et al.*, 1995) coeluting with it (Figure 5B). The structure of this pigment, corresponding to dehydrodicatechin A described earlier by Weinges *et al.* (1971), has been definitely established by MS and NMR spectroscopy by Guyot (1994). It derives from the degradation of a C6′−C8-linked catechin dimer (dehydrodicatechin B4) formed by nucleophilic attack of the C8 carbon of one catechin unit by the *o*-quinone resulting from enzymatic oxidation of the B-ring of another catechin unit.

Thus it seems that some catechin *o*-quinones—and probably also some semiquinone radicals giving rise to

the C−O linked dimers characterized earlier (Guyot, 1994), which were prevailing at lower pH values—are produced by autoxidation. However, in the presence of iron, other mechanisms yielding totally different products either directly from catechin or from the products of (semi)quinone reactions occur at a faster rate so that they become predominent.

As well, some iron-mediated oxidation of (+)catechin may occur in musts but at a very low rate if compared to polyphenoloxidase-catalyzed oxidation. Therefore, it is likely that oxidation of (+)-catechin in wine, which usually contain a few mg L^{-1} metal ions (iron, copper) (Navarre, 1991), and in musts yields different products, possibly exhibiting different organoleptic properties. In particular, yellow pigments resulting from enzymatic oxidation or from autoxidation in the absence of metal ions and those formed by iron-catalyzed autoxidation showed different UV−visible spectra, with absorbance maxima at 385−412 nm and 440−460 nm, respectively. Although the catechin concentration used in this study was of course much higher than that normally found in wines, the well-known effect of flavanols on the browning susceptibility of white wines may be related to the formation of the latter products exhibiting characteristic intense yellow color. This should be investigated further.

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