Study of the Reactions between (+)-Catechin and Furfural Derivatives in the Presence or Absence of Anthocyanins and Their Implication in Food Color Change

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(+)-Catechin was separately incubated with furfural or with 5-(hydroxymethyl)furfural, and the formation of new oligomeric bridged compounds having flavanol units linked by furfuryl or 5-hydroxymethylfurfuryl groups was observed. LC/ESI-MS analyses detected four dimeric adducts along with intermediate adducts in each solution, and reaction was faster with furfural than with hydroxymethylfurfural. In addition, new compounds exhibiting the same UV−visible spectra as xanthylium salts with absorption maxima around 440 nm were also detected. When malvidin 3-*O*-glucoside or cyanidin 3-*O*-glucoside was added to the mixtures, new oligomeric colorless and colored pigments involving both (+)-catechin and anthocyanin moieties were detected, showing thus that the two polyphenols competed in the condensation process. Among the obtained colored pigment adducts, two dimeric compounds in which the flavanol was bridged to the anthocyanin were observed. Their UV−visible spectra were similar to the spectrum of malvidin 3-*O*-glucoside, but their maximum in the visible region was bathochromically shifted.

Keywords: *(+)-Catechin; malvidin 3-O-glucoside; furfural; 5-(hydroxymethyl)furfural; HMF; condensation; xanthylium salts; LC/DAD; LC/MS; darkening; browning; color change*

INTRODUCTION

Fruit-derived foods are generally complex mixtures able to undergo during production, maturation, or storage many different changes resulting from complex transformations involving both enzymatic and nonenzymatic reactions. These transformations generally result in browning, discoloration, or darkening, and this reactivity raises an important economic question. Brown pigments are formed in the advanced stages of browning reactions, which generally occur slowly in food systems (Labuza and Saltmarch, 1981) and can be the major causes of changes in color, flavor, functional properties, and nutritional value (Nemr et al., 1988; Van Boekel and Berg, 1993).

Browning reactions resulting from either enzymatic or nonenzymatic polyphenol oxidation were shown to take part in a complex polymerization process which led to the formation of various bridged colorless and colored pigments, indicating their influence on color change and deastringency (Pierpoint, 1966; Timberlake and Bridle, 1976; Singleton et al., 1985; Cheynier et al., 1990, 1995; Fulcrand et al., 1996; Es-Safi et al., 1999a−d, 2000a−c). In addition to polyphenols, other compounds such as carbohydrates can participate in the alterations observed during heat processing or storage of foodstuffs rich in reducing sugars, particularly at improper temperatures. Among these alterations, the Maillard reaction is considered to be one of the main interactions responsible of browning. This reaction was also shown to give rise to components such as furfural and its derivatives, which were reported to be responsible for undesirable flavors in juices (Tatum et al., 1975).

The formation of furfural compounds and the increase of their concentration during storage of fruit-derived juices is well documented and is considered to be an indication of quality deterioration and a degree of damage to the product caused by excessive heat during processing or subsequent storage (Maraulja et al., 1973; Lee and Nagy, 1988). 5-(Hydroxymethyl)furfural (HMF) and furfural are the main furfural compounds used to evaluate nonenzymatic browning in foods and have been used as indicators of the Maillard reaction in numerous foodstuffs such as juices, honey citrus products, infant milk, and breakfast cereals (Jeuring and Kuppers, 1980; Lee and Nagy, 1988; Vinas et al., 1992; Garcia-Villanova et al., 1993; Lo Coco et al., 1994; Albala-Hurtado et al., 1997).

Although HMF is not present in fresh grapes (Fuleki and Pelayo, 1993), it is thought that the conditions used during the processing of grape-derived foods give rise to the formation of furfural and HMF (Pollard and Timberlake, 1971). Rossi and Pompei (1987) indicated that the amounts of phenolics and HMF are parameters used to establish law specifications of rectified concentrated grape must. Wucherpfennig and Burkhardt (1983) found 26 mg of HMF/L in freshly bottled red grape juice, which increased to 40 mg/L after storage at 35 °C for 7 weeks. Kern (1964) found that grapes had a greater capacity to form HMF than other fruits and detected 110 and 94 mg of HMF/L in freshly bottled red and white grape juices, respectively.

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It appears thus that the occurrence of furfural derivatives in fruit-derived foods is well documented. However, the literature on their interaction with polyphenolic compounds and the structures of the subsequently formed derivatives is very limited. The majority of the reported works were focused only on the influence of furfural and HMF on anthocyanin degradation. Meschter (1953), Markakis et al. (1957), Tinsley and Boockian (1960), and Daravingas and Cain (1968) found that furfural and HMF accelerate the degradation of anthocyanins, even at low HMF concentration, whereas Casoli and Dall'Agilio (1967) and Calvi and Francis (1978) found that low HMF concentrations, which typically occur in fruit products during processing and storage, could not have an effect on the pigment degradation.

In a relatively recent work, the influence of furfural and HMF on the degradation of cyanidin 3-*O*-glucoside was investigated in a model solution system, and the acceleration of the pigment degradation was observed (Debicki et al., 1983). Some hypotheses on the structure of the formed compounds were reported, but the authors were unable to identify the respective formed compounds. Furfural was also supposed to be related to darkening, but its role in the formation of such colored complexes is still not clear, as previously indicated (Lee and Nagy, 1988).

The aspect of the interactions between polyphenols and furfural derivatives remains thus an open research area of good interest. The purpose of this study was to investigate the interactions involving (+)-catechin, furfural, and HMF in the presence or absence of anthocyanins in model solution systems.

MATERIALS AND METHODS

Reagents. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA) prior to use. Acetonitrile was purchased from BDH (Poole, U.K.). Methanol, formic acid, and acetic acid were obtained from Prolabo (Fontenay S/Bois, France). Malvidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside were purchased from Extrasynthèse. Furfural and HMF were obtained from Interchim and Lancaster Synthesis, respectively.

Reactions. An acidic solution was prepared with 17 μL of acetic acid and 50 μL of ethanol in 433 μL of water, giving a pH value of 2.2. Various pH values ranging from 2.2 to 4.0 were obtained by the addition of 1 M sodium hydroxide to the medium described above and were adjusted using a 93313 Bioblock pH meter. (+)-Catechin (20 mM) was prepared in each obtained medium (0.5 mL), and 1 μL of furfural/HMF was then added. Solutions containing malvidin 3-*O*-glucoside or cyanidin 3-*O*-glucoside and (+)-catechin were prepared in the same way. The prepared solutions were incubated at room temperature and in the absence of light, and reactions were monitored by liquid chromatography coupled with a diode array detector (DAD) and with an electrospray mass spectrometry (ESI-MS) detector.

Analytical HPLC/DAD analyses. HPLC/DAD analyses were performed by means of a Waters 2690 separation module system including a solvent and a sample management system, a Waters 996 photodiode array detector, and Millenium 32 chromatography manager software. UV−visible spectra were recorded from 250 to 600 nm. The column was a reversed-phase Lichrospher 100-RP18 (5 μm packing, 250 × 4 mm i.d.) protected with a guard column of the same material. Elution conditions were as follows: 1 mL/min flow rate; temperature, 30 °C; solvent A, water/formic acid (98:2, v/v); solvent B, acetonitrile/water/formic acid (80:18:2, v/v); elution from 5 to 30% B in 40 min, from 30 to 40% B in 10 min, and from 40 to 100% B in 5 min, followed by washing and re-equilibrating of the column.

MS Apparatus and LC/ESI-MS Analyses. MS measurements were performed on a Sciex API I Plus simple quadruple mass spectrometer equipped with an electrospray ionization source. The mass spectrometer was operated in negative-ion mode. Ion spray voltage was selected at −4 kV and orifice voltage at −70 V. For direct injection, the solution was introduced into the electrospray source at a constant flow rate of 5 μL/min with a medical syringe infusion pump in combination with a 100 μL syringe.

HPLC separations were carried out on a narrow-bore reversed-phase column with an ABI 140 B solvent delivery system (Applied Biosystems, Weiterstadt, Germany). The column was connected with the ion spray interface via a fused-silica capillary (length, 100 cm; 100 μm i.d.). The reaction mixture was injected with a rotary valve (Rheodyne model 8125) fitted with a 20 μL sample loop. The separation was achieved on a Lichrospher 100-RP18 column (5 μm packing, 250 × 4 mm i.d., Merck, Darmstadt, Germany), with a flow rate of 280 μL/min. The elution was done with solvents A and B used in HPLC/DAD analysis and the conditions adapted as follows: isocratic 10% B in 4 min, linear gradient from 10 to 15% B in 11 min, from 15 to 50% B in 25 min, and from 50 to 100% B in 5 min, followed by washing and reconditioning of the column. The absorbance at 280 nm was monitored by an ABI 785A programmable absorbance detector and by a Waters 990 DAD linked to 990 system manager software.

RESULTS AND DISCUSSION

To investigate reactions between flavanols and furfural derivatives, (+)-catechin was separately incubated with furfural or HMF at pH 2.2. The evolution of the reactants and of the newly formed compounds was monitored by HPLC with diode array detection, and their molecular weights were determined by electrospray mass spectrometry. In both cases, a general decrease in the concentration of (+)-catechin and the appearance of numerous products initially absent in the mixture were observed. This was also observed when reactions were conducted at pH values ranging from 2.2 to 4.0, but the reaction rate increased with the decrease of pH, owing to the higher availability of furfural or HMF carbocations at lower pH values as previously observed with acetaldehyde (Es-Safi et al., 1999a).

When (+)-catechin was incubated with furfural, the mixture underwent a progressive darkening, and at the end of the assay the solution became completely black, suggesting thus a possible contribution of such reaction in fruit-derived foods darkening observed during storage as previously stated (Lee and Nagy, 1988). It was indeed supposed that darkening parallels the increase of furfural and HMF during the storage of grape-derived juices. Their contribution to juice flavor or taste did not appear to be important but was thought to be related to the darkening of juice. However, their role in the formation of colored complexes was not clear (Lee and Nagy, 1988). Our results confirm thus these hypotheses and gave new support to the involvement of furfural in these color transformation.

Upon HPLC/DAD analysis, the presence of various new compounds initially absent in the solution was observed, indicating thus that a reaction between (+)-catechin and furfural occurred. Among the formed compounds, the UV traces recorded at 280 nm revealed the presence of four major peaks in addition to those of (+)-catechin and furfural (Figure 1). The newly formed compounds were eluted later than their precursors, indicating therefore that they were less polar molecules. The UV−visible spectra of the formed compounds, recorded between 250 and 600 nm, were similar to the

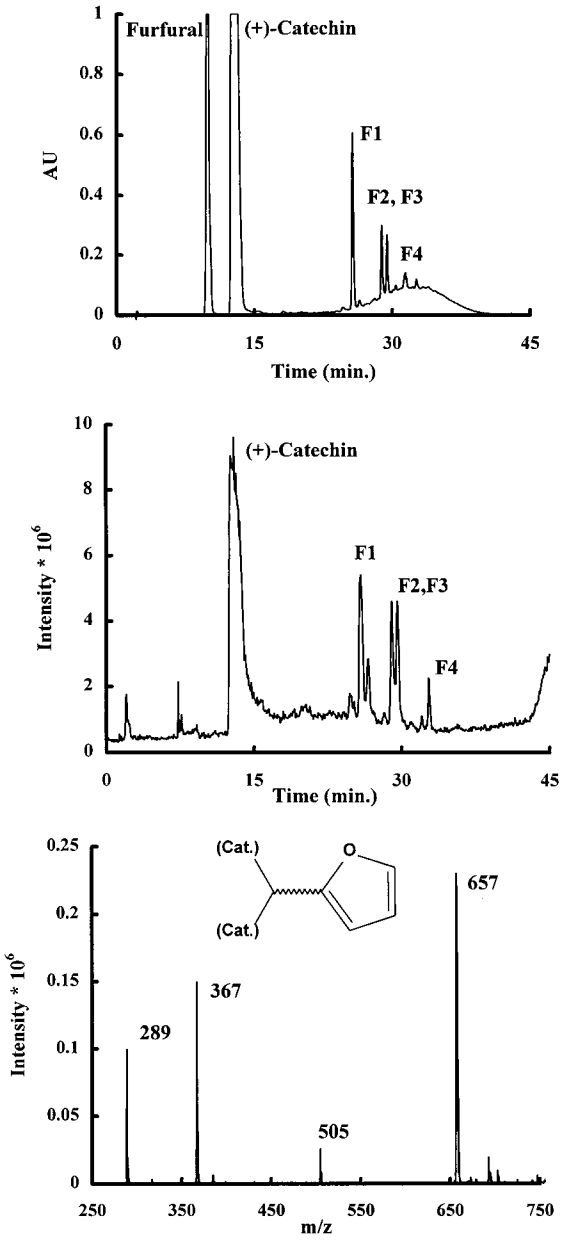

Figure 1. HPLC chromatograms measured with a UV detector at 280 nm (top) and with an electrospray mass detector (middle) of a mixture of (+)-catechin and furfural. The mass spectrum of a dimer adduct is also presented (bottom).

spectrum of (+)-catechin with a maximum absorbance near 280 nm, suggesting thus that the original flavanol structure was retained.

The first two peaks were, respectively, furfural and (+)-catechin, and the four others (F1, F2, F3, and F4) were potentially condensed products of (+)-catechin and furfural. LC/MS analysis, conducted in the negative ion mode, allowed molecular weight determination of the major peaks formed through this reaction. The obtained results indicated that these products were oligomeric derivatives consisting of (+)-catechin units bridged by furfuryl groups formed according to the mechanism previously postulated by Timberlake and Bridle (1976) in the case of acetaldehyde. The total ion current (TIC) chromatogram profile shown in Figure 1 indicated the formation of mainly dimer adducts along with the monomer ion peak. The *m/z* values of compounds F1, F2, F3, and F4 detected at *m/z* 657 (Figure 1) showed that the constitutive units were linked by furfuryl

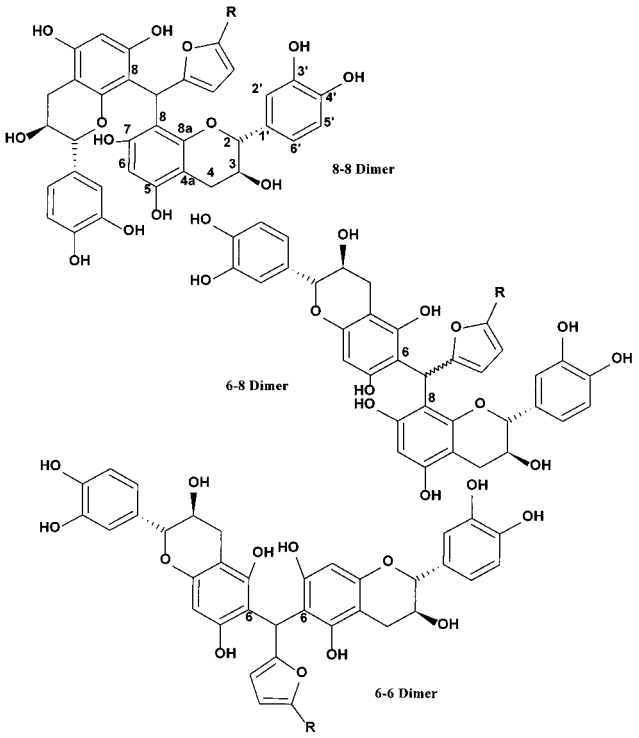

Figure 2. General structures of (+)-catechin−furfuryl (R = H) and (+)-catechin−hydroxymethylfurfuryl (R = CH_2OH) dimers.

bridges, demonstrating the role of furfural in the polymerization process.

An extracted ion current (XIC) chromatogram profile recorded at *m/z* 657 revealed the presence of the four dimeric adducts. Mass spectrometric analysis revealed thus that compounds F1, F2, F3, and F4 all had molecular weights of 658 and corresponded to a structure in which two (+)-catechin units are linked by a furfuryl bridge as shown in Figure 2. The formation of the four dimers is in agreement with the fact that flavanols could be linked through C6 or C8, yielding four isomers with C6−C6, C8−C8, and C6−C8 (*R* and *S*) bonds, taking into account the presence of an asymmetric carbon for the C6−C8 isomers (Figure 2).

In addition to these dimers, other oligomeric bridged compounds were also detected. Among them, intermediate furfuryl adducts of monomer (*m/z* 385) and dimer derivatives (*m/z* 753) were observed. The detection of these intermediate adducts demonstrated the role of furfural in the polymerization process and suggested the formation of more polymerized compounds. This was confirmed by the appearance of a bump in the end of the chromatographic profile and showed that the reaction evolved to more polymerized compounds which finally precipitate as a black solid in the assay vial.

In addition to these bridged oligomeric compounds, new adducts exhibiting UV−visible spectra similar to those of xanthylium salt derivatives with absorption maxima around 440 nm were also detected. Their molecular ions (*m/z* 637) fit well with products derived from the first formed dimers by dehydration and oxidation processes, giving thus xanthylium salts with a structure like that shown in Figure 3and obtained according to the mechanism recently demonstrated in the case of glyoxylic acid (Es-Safi et al., 1999c,d, 2000a,c), as confirmed by the detection of their corresponding xanthene derivatives at *m/z* 639.

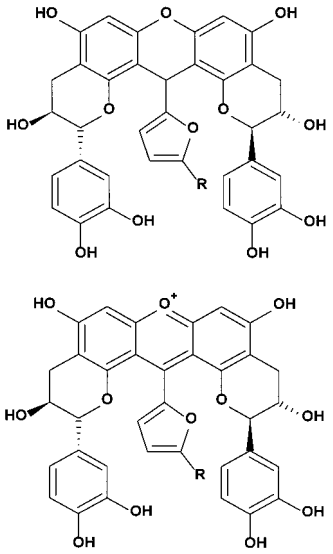

Figure 3. Structures of the xanthene (top) and xanthylium salts (bottom) derivatives obtained from (+)-catechin−furfuryl (R = H) and (+)-catechin−hydroxymethylfurfuryl (R = CH_2-OH) dimers.

Because the polymerization mechanism was demonstrated with furfural, a comparison was done with HMF, a furfural derivative that is accumulated like furfural during storage in fruit-derived foods (Kanner et al., 1982; Lee and Nagy, 1988). Analogous products were detected, but the reaction was faster in the former. This may be due to the difference in reactivity of the two furfural derivatives as previously reported (Burton et al., 1963; Kanner et al., 1982).

HPLC/DAD analysis of the mixture showed the presence of four major additional peaks (H1, H2, H3, and H4) in addition to (+)-catechin and HMF (Figure 4). Mass spectrometric analysis revealed that the four compounds gave all $[M - H]^-$ ion signals at *m/z* 687 as shown on the obtained mass spectrum (Figure 4). Their molecular weight (688 amu) corresponds exactly to a structure in which two (+)-catechin units are linked by a hydroxymethylfurfuryl similar to those shown in Figure 2. In addition, intermediate adducts of monomer (*m/z* 415) and dimer (*m/z* 783) were also observed and demonstrate the role of HMF in the flavanol polymerization mechanism. The formation of more polymerized compounds was also observed in the case of HMF by the appearance of a brown precipitate in the vial.

The ability of these oligomeric bridged derivatives to yield xanthylium salts was also observed in this case by either LC/DAD or LC/ESI-MS analysis. Thus, the presence of compounds with absorption around 440 nm was observed in the chromatogram recorded at 440 nm, and their UV−visible spectra were similar to those of xanthylium salts as shown in Figure 5. Their presence was also confirmed by LC/ESI-MS analysis with detection of compounds showing mass spectra with $[M^+ - 2H]^-$ ion signals at *m/z* 667 amu (Figure 6) and by the mass chromatogram recorded at *m/z* 667. This molecular weight corresponds exactly to a structure obtained from dimeric flavanol−hydroxymethylfurfuryl adducts, by loss of a water molecule followed by an oxidation process (Figure 3). The detection of their corresponding xanthene derivatives at *m/z* 669 (Figure 6) supports this pathway. In addition to xanthylium salts, the presence of other compounds exhibiting UV−visible spectra with absorption maxima around 400 nm were also observed (Figure 5).

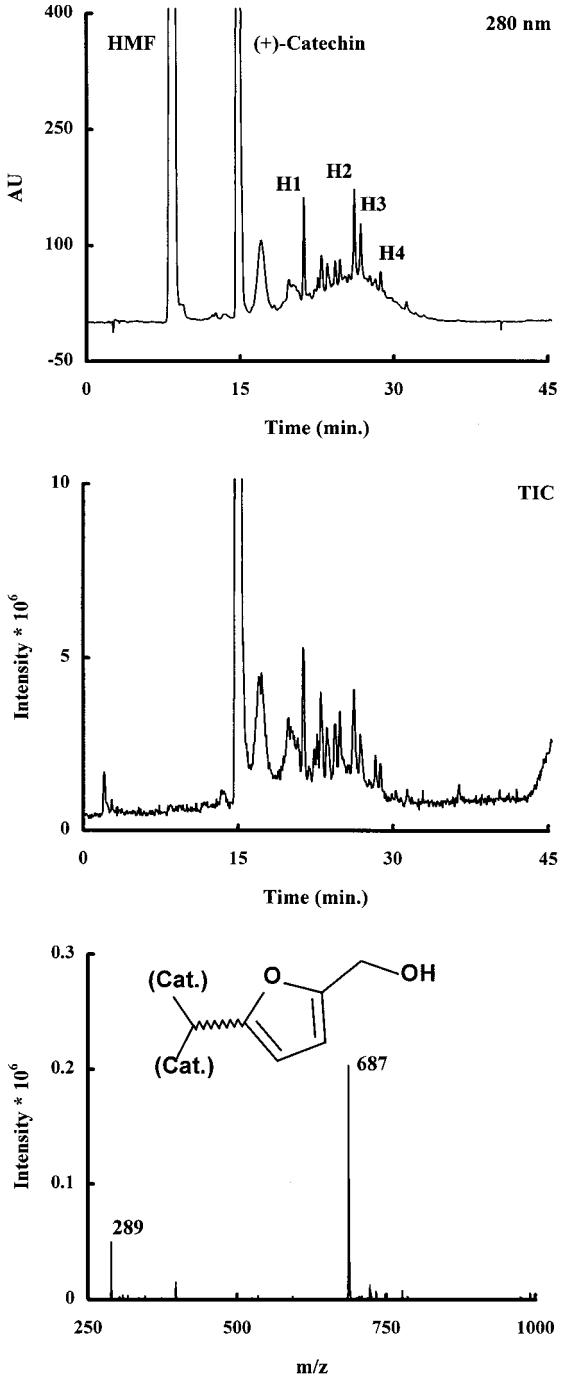

Figure 4. HPLC chromatograms measured with a UV detector at 280 nm (top) and with an electrospray mass detector (middle) of a mixture of (+)-catechin and HMF. The mass spectrum of a dimer adduct is also presented (bottom).

After demonstration of the role of furfural and HMF in the flavanol polymerization process, the evolution of such model solutions was investigated in the presence of anthocyanins. Thus, reactions between (+)-catechin, malvidin 3-*O*-glucoside or cyanidin 3-*O*-glucoside, and furfural or HMF were investigated. A general decrease in the concentrations of (+)-catechin and anthocyanin was observed along with the appearance of oligomeric products in which the flavanol was linked either to another flavanol or to an anthocyanin unit by a furfuryl bridge.

Figure 7 represents typical HPLC chromatograms, recorded at 280 and 520 nm, showing residual (+)-catechin, malvidin 3-*O*-glucoside, and the newly formed

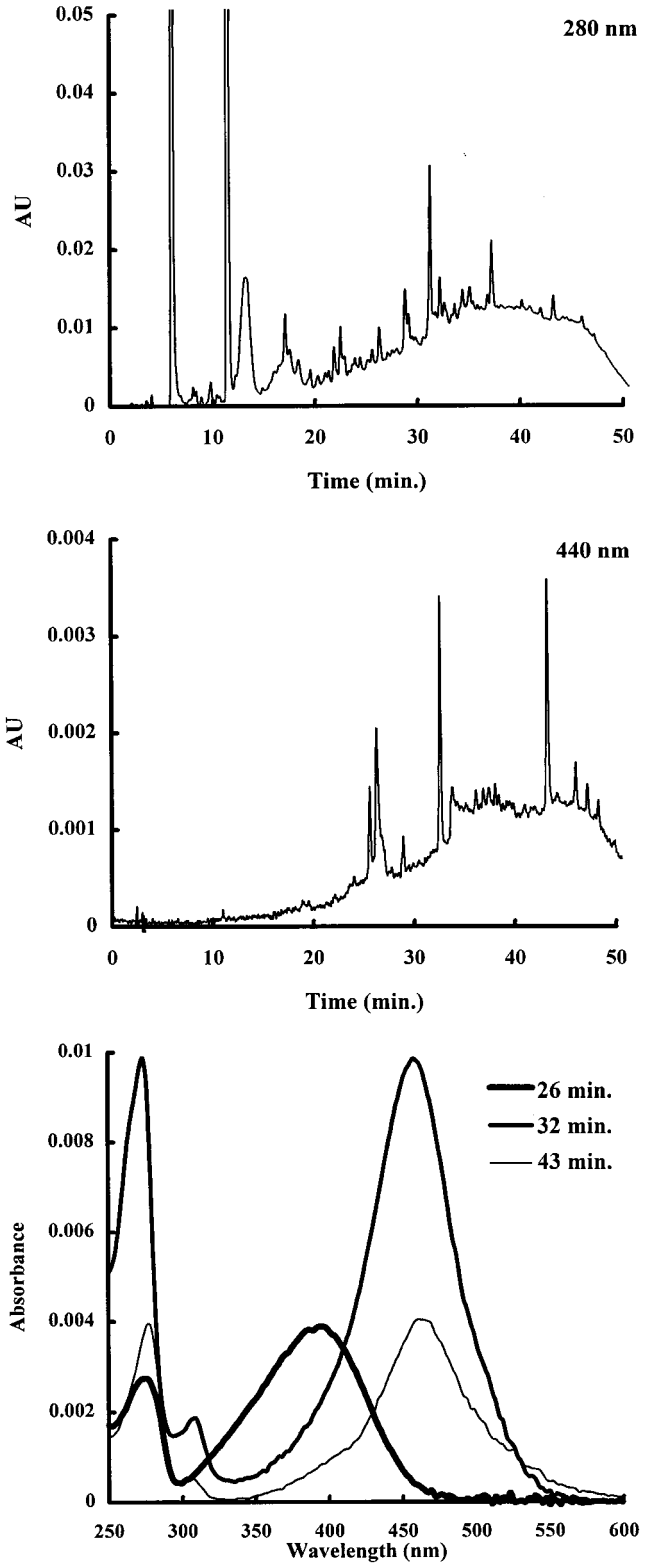

Figure 5. HPLC chromatograms recorded at 280 nm (top) and 440 nm (middle) of a mixture of (+)-catechin and HMF showing residual reagents and newly formed compounds. The UV−visible spectra of some of the obtained compounds are also presented (bottom).

compounds, which were eluted later than flavanol. In the HPLC chromatogram recorded at 520 nm, the presence of residual malvidin 3-*O*-glucoside and of new colored substances was observed. Two major compounds (MF1 and MF2) were detected corresponding to two new condensed pigments. The UV−visible spectra of these

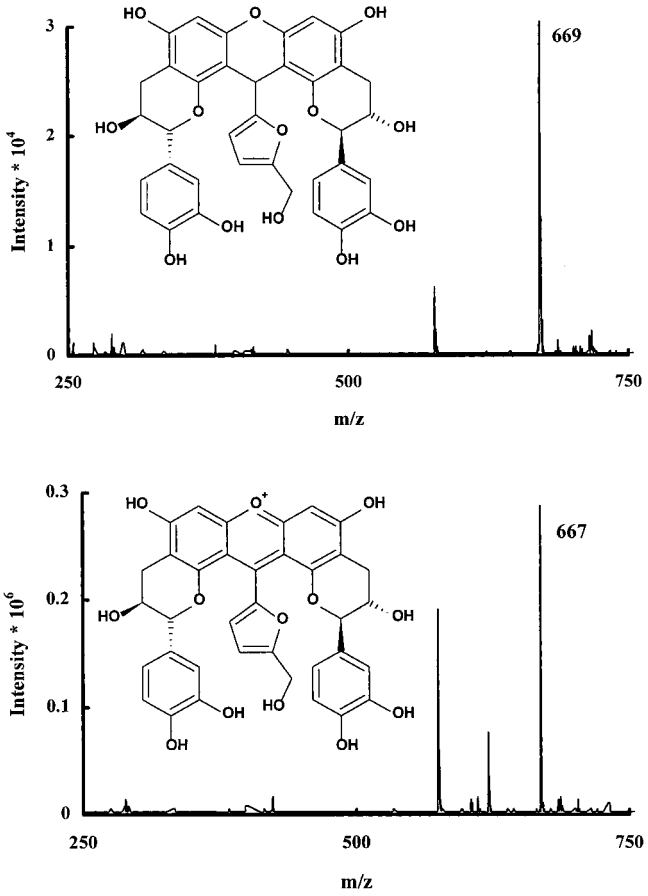

Figure 6. Mass spectra of the xanthene (*m*/*z* 669) and xanthylium salt (*m*/*z* 667) adducts detected in a mixture of (+)-catechin and HMF.

products were similar to the spectrum of malvidin 3-*O*-glucoside, indicating that the flavylium chromophore was still present in both pigments. Moreover, the spectra of compounds MF1 and MF2 showed an additional shoulder around 450 nm, and the wavelengths of their maximum absorbance in the visible range (545 nm) were significantly higher than that of malvidin 3-*O*-glucoside (525 nm). This behavior was also observed in the case of the oligomers obtained when malvidin 3-*O*-glucoside and (+)-catechin were incubated with acetaldehyde, giving rise to similar bridged pigments (Es-Safi et al., 1999b). This fact is probably due to some inter- or intramolecular copigmentation effect as described in the case of the colored ethyl-bridged compounds obtained through interaction between (+)-catechin, acetaldehyde, and a synthetic flavylium pigment (Escribano-Bailon et al., 1996).

Upon LC/MS analysis, a molecular ion at *m*/*z* 859 was observed for both pigments as shown in Figure 7. This is consistent with one flavylium moiety linked to one (+)-catechin moiety through a furfuryl bridge (Figure 8). An ion extracted current chromatogram recorded at *m*/*z* 859 revealed the presence of the two colored adducts, confirming thus the results obtained through LC/DAD analysis.

It must also be noted that no monomer intermediate adduct or dimeric compound consisting only of malvidin 3-*O*-glucoside units was detected. This showed that the anthocyanin moiety was not attacked by the first carbocation formed by protonation of the aldehyde. In contrast, both (+)-catechin intermediate monomer and dimer were detected, showing thus that the first car-

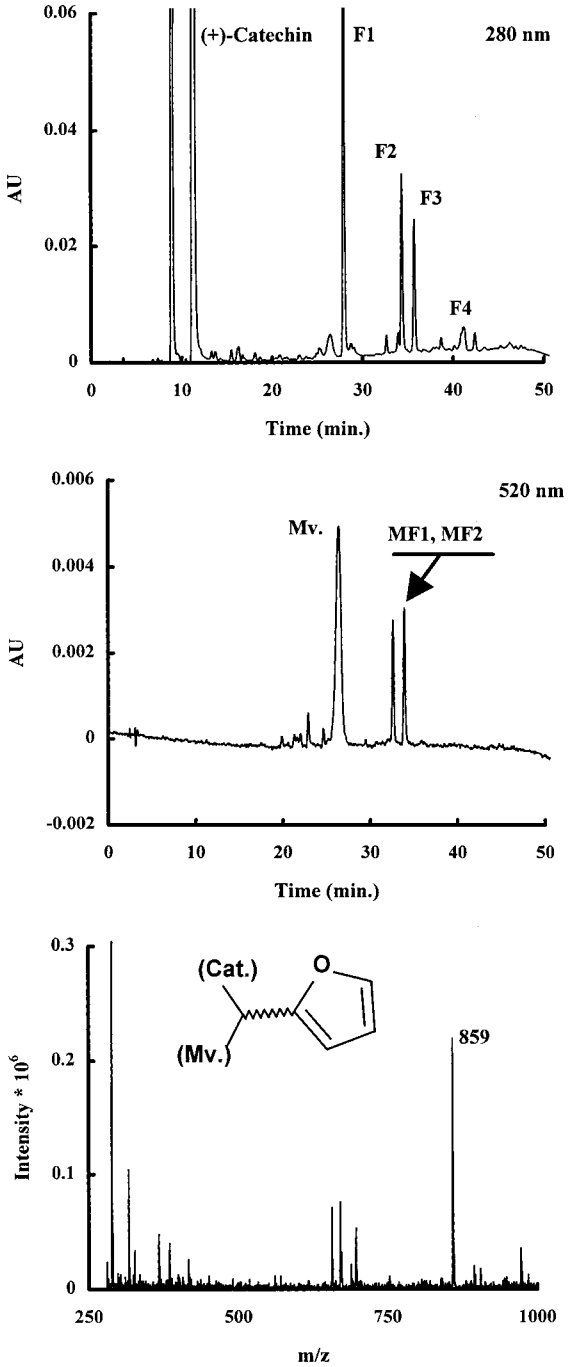

Figure 7. HPLC chromatograms recorded at 280 nm (top) and 520 nm (middle) of a mixture of (+)-catechin, malvidin 3-*O*-glucoside, and furfural. The mass spectrum of a malvidin 3-*O*-glucoside−furfuryl−catechin adduct showing its signal ions $[M^+ - 2H]^-$ at $m/z = 859$ is also presented (bottom).

bocation attacks the flavanol, which after further loss of water molecule attacks either another flavanol or an anthocyanin, giving, respectively, a flavanol bridged dimer or an anthocyanin−flavanol bridged adduct as was observed with acetaldehyde (Es-Safi et al., 1999b).

In addition to anthocyanin−flavanol bridged derivatives, homogeneous flavanol bridged adducts were also detected, showing that the anthocyanin competed with the flavanol in the condensation process. The chromatographic profiles recorded during the reaction evolution showed that the homogeneous flavanol dimers were always predominant compared to those involving both (+)-catechin and malvidin 3-*O*-glucoside. This was not

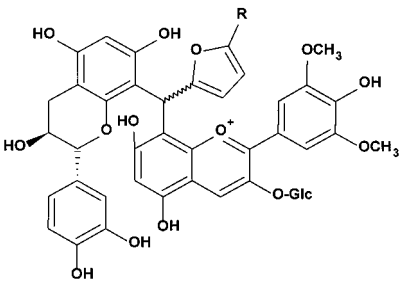

Figure 8. Structure of the dimer derivatives involving malvidin 3-*O*-glucoside, (+)-catechin, and furfural (R = H) or HMF (R = CH_2OH).

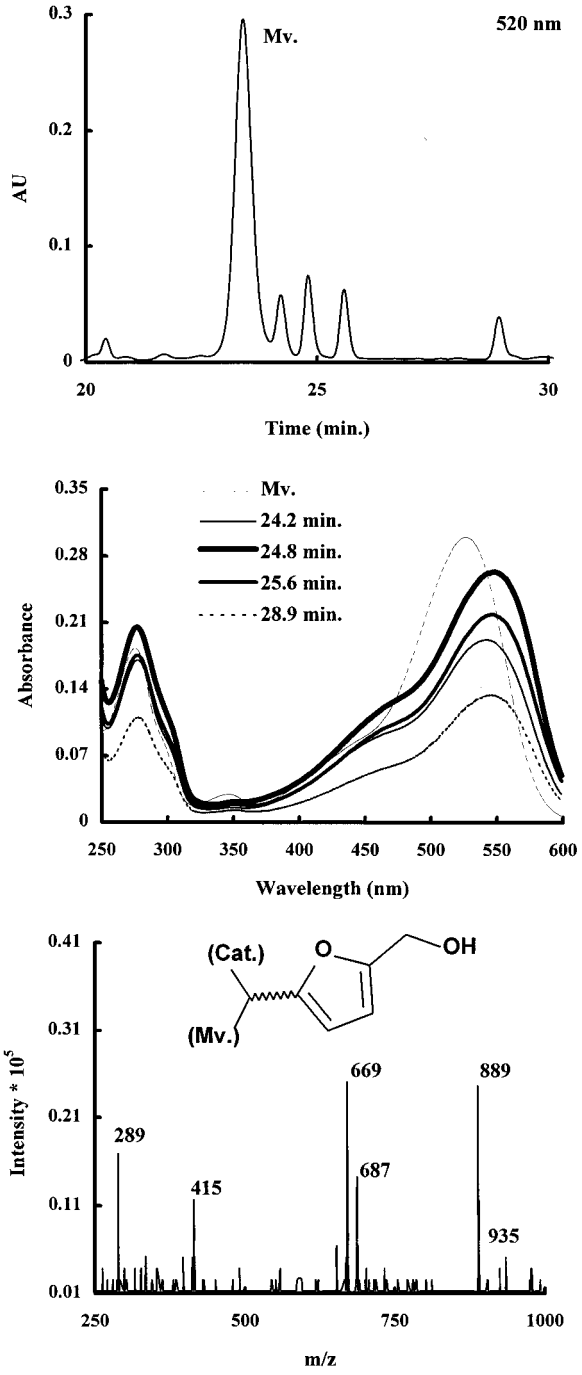

Figure 9. HPLC chromatogram recorded at 520 nm (top) of a mixture of malvidin 3-*O*-glucoside, (+)-catechin, and HMF showing residual reagents and newly formed colored compounds. UV−visible spectra of the major formed pigments (middle) and a mass spectrum of a dimer pigment (bottom) are also presented.

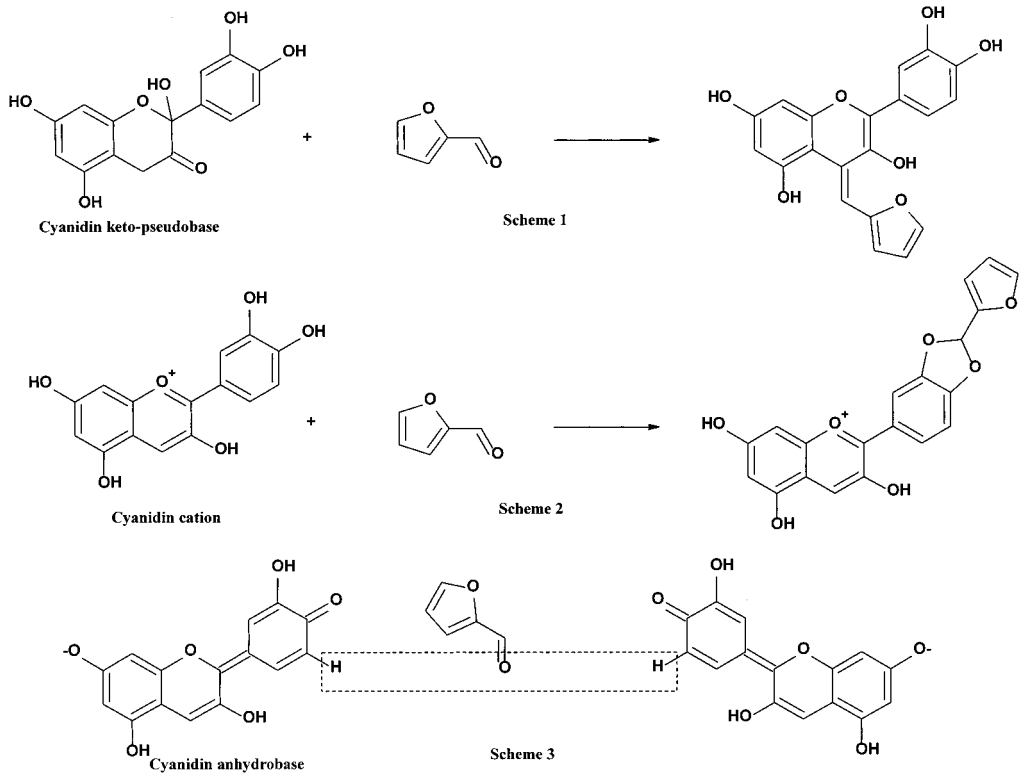

Figure 10. Condensation reactions previously reported between cyanidin and furfural.

the case when the reaction was investigated in the presence of acetaldehyde and when the homogeneous flavanol dimers were predominant in the beginning of the reaction and disappeared rapidly in detriment to those involving both polyphenols (Es-Safi et al., 1999b). The fact that the anthocyanin decreased more rapidly than (+)-catechin, in addition to the fact that this decrease was not accompanied by an increase of the colored bridged pigments, showed that the anthocyanin should suffer another reaction in addition to the polymerization process involving (+)-catechin and furfural. This was confirmed by the detection of compounds exhibiting UV−visible spectra with absorption maximum at 500 nm and which were similar to those obtained from reaction between acetaldehyde and malvidin 3-*O*-glucoside and resulting from a cycloaddition reaction between the aldehyde and the anthocyanin (Bakker and Timberlake, 1997).

When furfural was replaced by HMF, the formation of analogous oligomeric compounds was observed. Upon LC/DAD analysis the formation of new compounds initially absent in the mixture and eluted after the anthocyanin was observed in the chromatographic profile recorded at 280 nm. Among these, four of them eluting between 20 and 30 min showed absorption at 520 nm, meaning that their structures contained the flavylium moiety (Figure 9). The UV−visible spectra of these four compounds shown in Figure 9 were similar to the spectrum of malvidin 3-*O*-glucoside with a bathochromic shift of their visible absorption maxima, which were located around 545 nm.

Besides, LC/ESI-MS analysis conducted in the negative ion mode allowed detection of two dimer adducts, with ion peaks corresponding to furfuryl linked compounds containing one anthocyanin and one catechin unit (*m*/*z* 889, Figure 9). This was also confirmed in the extracted chromatogram recorded at *m*/*z* 889, by which the two dimer pigments were observed.

As indicated, in food processing, along with degradation of anthocyanins, degradation products of carbohydrates such as furfural and HMF are also formed (Shenoy, 1993). These compounds were reported to react with anthocyanins to give dark brownish reaction products. Various condensation pathways between cyanidin and furfural have been proposed. Among the proposed mechanisms, Tinsley and Boockian (1960) proposed a reaction between cyanidin keto-pseudobase and furfural, which was supposed to yield the compound shown in Scheme 1 of Figure 10. The interaction between furfural or HMF and cyanidin 3-*O*-glucoside was also investigated, and the degradation of the pigment was shown to be accelerated (Debicki-Pospisil et al., 1983). The authors proposed reaction between furfural and the two hydroxyl groups of the anthocyanin B ring (Scheme 2, Figure 10). They also proposed the reaction of two molecules of cyanidin anhydrobase with furfural (Scheme 3, Figure 10). From a qualitative condensation test, these authors reported that cyanidin 3-*O*-glucoside could enter the reaction with furfural only if it had been previously hydrolyzed at position 3, that is, that the reaction was possible only with the free aglycon cyanidin. However, in this work we were able to show by LC/ESI-MS analysis that malvidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside underwent condensation reaction with either furfural or 5-(hydroxymethyl)furfural, giving rise to various oligomeric bridged compounds. No compound following the previously proposed mechanisms was detected by LC/ESI-MS analysis in our study either with malvidin 3-*O*-glucoside or with cyanidin 3-*O*-glucoside. When the latter was incubated with furfural or HMF, the formation of compounds similar to those observed with malvidin 3-*O*-glucoside was detected either by LC/DAD or by LC/ESI-MS analysis.

On the basis of this investigation, it appears that furfural compounds play a major role in the flavanol

polymerization process. In the presence or absence of anthocyanins, various bridged oligomeric and polymeric adducts that finally precipitate were obtained. Such reactions may contribute to the decrease of astringency and the change of color observed during the aging of grape-derived foods. In addition, the fact that the incubated solutions became black with time showed that such reaction contributes to the darkening of food and supports the hypothesis previously supposed. Our study gave thus new information on the formation and the structure of such colored complexes.

The competitive action of flavanols and anthocyanins in the condensation process was demonstrated by the obtention of both flavanol−furfuryl and anthocyanin−furfuryl−flavanol adducts. The detection of compounds exhibiting UV−visible spectra similar to those of xanthylium salts constitutes a new support for their contribution in color change and browning.

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