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Role of Aldehydic Derivatives in the Condensation of Phenolic Compounds with Emphasis on the Sensorial Properties of Fruit-Derived Foods

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The reactions between (epi)catechin, mavidin 3-*O*-glucoside, and some aldehydes were investigated by LC/DAD and LC/ESI-MS analysis. The obtained results showed that the acetaldehyde-mediated condensation occurred more generally and glyoxylic acid, furfural, and 5-(hydroxymethyl)furfural (HMF) react in the same way in the first stages of the reactions. In terms of reactivity, reactions were faster with acetaldehyde than with glyoxylic acid, furfural, or HMF, where the reactions were slower. In the case of acetaldehyde, the obtained purple derivatives were more predominant and stable than the colorless adducts and no xanthylium salt was detected. Interactions involving glyoxylic acid yield purple adducts, which were obtained in small amount compared to the colorless ones. The latter were shown to proceed to more polymerized and yellowish derivatives. Finally, in the case of furfural and HMF, purple compounds involving flavanol and anthocyanin units were detected, and colorless compounds were shown to be predominant and to yield yellowish xanthylium salts.

KEYWORDS: (+)-Catechin; (−)-epicatechin; malvidin 3-*O*-glucoside; phenolic compounds; aldehyde; acetaldehyde; glyoxylic acid; furfural; 5-(hydroxymethyl)furfural; HMF; LC/DAD; LC/MS; browning; darkening

INTRODUCTION

Phenolic compounds have attracted considerable interest because of their ubiquitous occurrence within the plant kingdom and numerous important properties. In particular, they are major fruit-derived food constituents, responsible for some of their organoleptic features. They are also receiving increasing attention as natural antioxidants and potential health-promoting agents. Their eventual health effects are attracting considerable interest in the international scientific community.

Polyphenols show a great diversity of structures from rather simple molecules (monomers and oligomers) to polymers. These phenolic compounds are highly unstable and are rapidly transformed into various reaction products when plant cells are damaged and also during food processing. Indeed, phenolic compounds are highly reactive species due to the acidic character of their hydroxyl groups and the nucleophilic properties of the phenolic rings that undergo various types of reactions in the course of food processing. Among these reactions, oxidation processes and addition reactions, leading to various adducts and eventually tannin-like polymeric compounds, are particularly important (*1*−*13*). These reactions start as soon as plant cells are damaged or broken (i.e., at crushing or pressing) and continue throughout processing and aging. They lead to a great diversity of products, thus adding to the complexity of food phenolic composition. The new compounds formed often show specific organoleptic properties, distinct from those of their precursors. Therefore, a better understanding of their structures and properties and the mechanisms generating them appears to be essential to predict and control food quality.

Major progress achieved in food technology over the past decade concerns, on the one hand, the structural determination of phenolic compounds and, on the other hand, the elucidation of reaction mechanisms modifying polyphenols in the course of food processing and storage. Among the reactions involved in the change of color or astringency during aging or storage of fruit-derived foods, the interaction referred to as anthocyanin−tannin condensation has been studied by several authors. Within this reaction, two mechanisms have been postulated: direct nucleophilic addition of flavanols onto the electrophilic anthocyanin, generating orange xanthylium salts, and aldehyde-mediated condensation, yielding purple pigments. The interaction of phenolic compounds with aldehydic reactants plays a major role during the storage of fruits and fruit-derived foods (*3*−*12*, *14*, *15*).

Fruit-derived foods are complex mixtures able to undergo many different reactions. The full characterization of these reactions has not been achieved because of difficulties involved in extracting and separating the newly formed compounds

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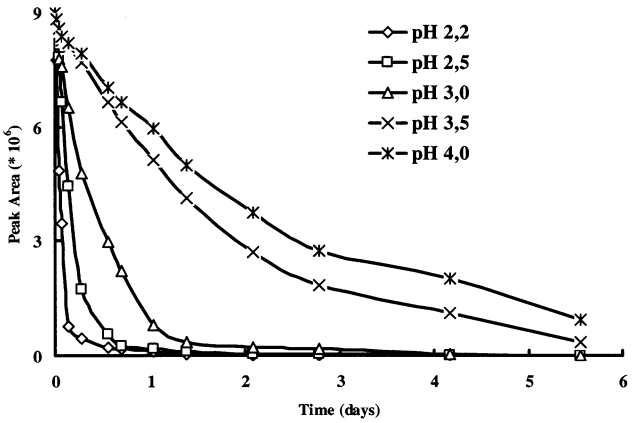

Figure 1. Evolution of (+)-catechin with time and pH when incubated with acetaldehyde in a model solution system.

directly from food products. Model solutions mimicking food products constitute a simplified medium for their exploration, allowing the detection of the newly formed compounds, their isolation, and their structure elucidation. Whereas the color of a new fruit-derived product is due to its initial chemical composition, the subsequent color changes during aging involve generally condensation of phenolic compounds. Direct condensation between anthocyanins and flavanols is very slow. However, rapid polymerization between anthocyanin and catechins or tannins occurs in the presence of aldehyde derivatives with increased color intensity and stability, but further reactions with polymerized flavanols lead to instability, precipitation, and decreased color (*4*, *16*−*21*).

The present paper summarizes and gathers our recent findings about the reactions between phenolic compounds and some aldehydes [acetaldehyde, glyoxylic acid, furfuraldehyde, and 5-(hydroxymethyl)furfuraldehyde] as well as the structures of the newly formed compounds. A comparative study on the reactivity of these aldehydes on phenolic compounds is presented. Finally, the implication of these reactions and these new products on the sensorial properties of fruit-derived foods is also discussed.

MATERIALS AND METHODS

Reagents. Deionized water was purified with 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). (+)-Catechin and (−)-epicatechin were purchased from Sigma (St. Louis, MO). Malvidin 3-*O*-glucoside was purchased from Extrasynthèse (Genay, France). Acetaldehyde was obtained from Merck (Darmstadt, Germany). Glyoxylic acid was obtained from Aldrich (Paris, France). Furfural and 5-(hydroxymethyl)furfural (HMF) were obtained from Interchim (Montculon, France) and Lancaster Synthesis (Strasbourg, France), 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 pHmeter (Illkirch, France). (+)-Catechin or (−)-epicatechin (20 mM) was prepared in triplicate in each obtained medium (0.5 mL). The aldehydic derivatives [acetaldehyde, glyoxylic acid, furfuraldehyde, and 5-(hydroxymethyl)furfuraldehyde] were added to give a final concentration of 20 mM.

Solutions containing malvidin 3-*O*-glucoside or cyanidin 3-*O*-glucoside and (+)-catechin or (−)-epicatechin were prepared in the same way. The prepared solutions were incubated in triplicate at 20 °C and in absence of light, and reactions were periodically monitored by liquid chromatography (LC) coupled with a diode array detector

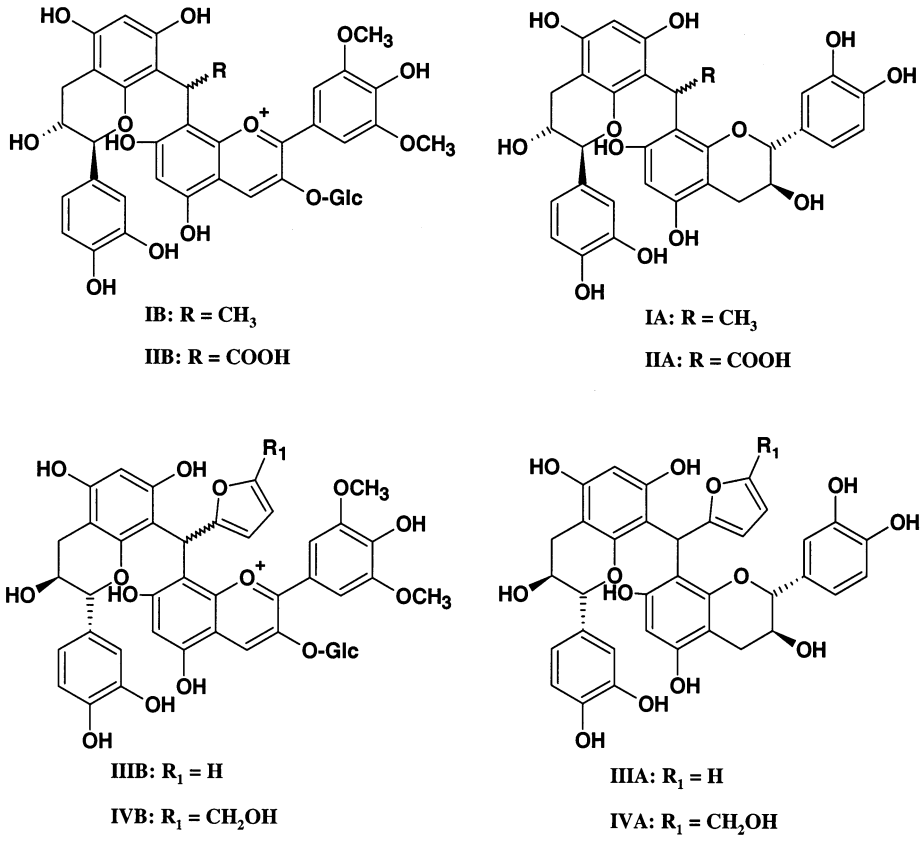

Figure 2. General structures of the dimeric colorless (**IA**, **IIA**, **IIIA**, and **IVA**) and colored (**IB**, **IIB**, **IIIB**, and **IVB**) derivatives obtained by interaction involving flavanols, anthocyanins, and acetaldehyde (R = CH_3), glyoxylic acid (R = COOH), furfural (R_1 = H), and HMF (R_1 = CH_2OH).

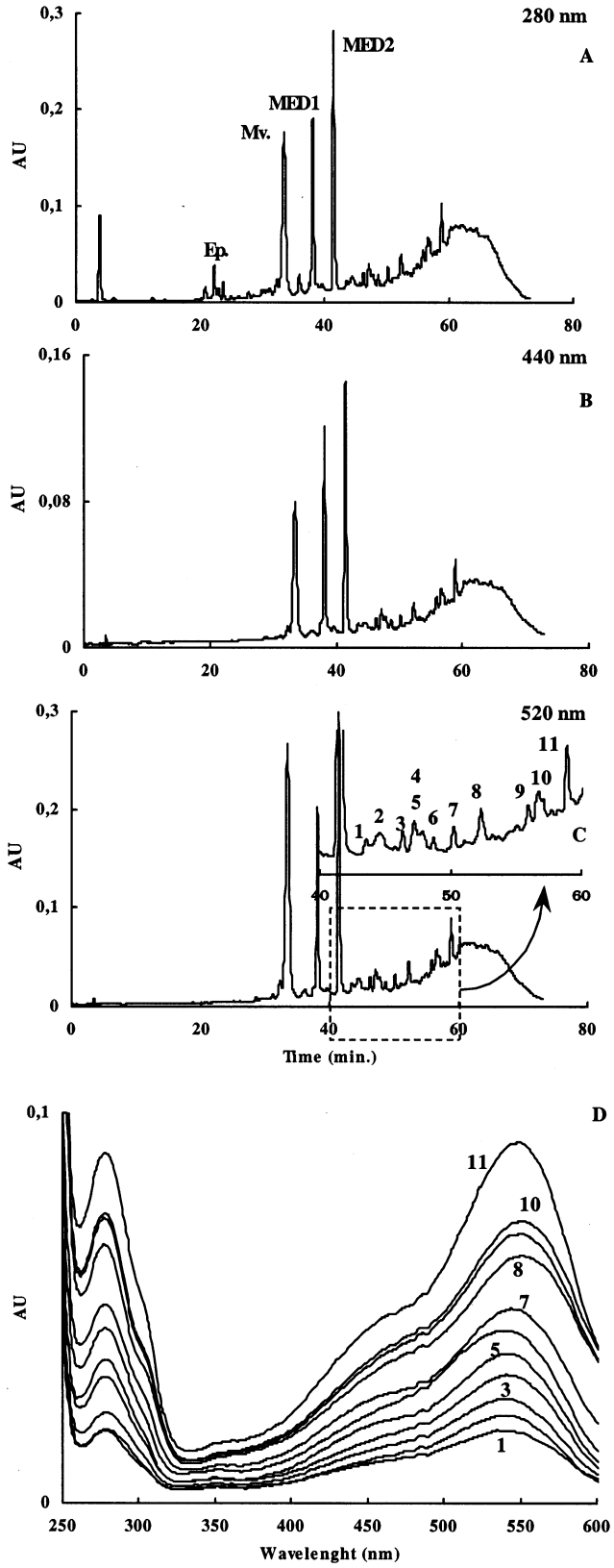

Figure 3. HPLC chromatograms of a mixture of (+)-catechin, malvidin 3-*O*-glucoside, and acetaldehyde recorded at 280 (A), 440 (B), and 520 nm (C) showing the formation of more polymerized colored derivatives. MED1 and MED2 represent the two formed colored dimers. The spectra drawn in panel D show the bathochromic shift observed in the visible region for more polymerized compounds.

(DAD) and with an electrospray mass spectrometry (ESI-MS) detector during a period varying from 6 days for acetaldehyde to 3 months for furfural and HMF depending on the reaction rate, which was found to be influenced by the used aldehyde. Quantification of residual flavanols and of all colorless compounds was achieved on the basis of peak areas at 280 nm, using (+)-catechin or (−)-epicatechin as standard. Quantification of residual anthocyanin and of all purple compounds was achieved on the basis of peak areas at 520 nm, using malvidin 3-*O*-glucoside as standard. Identification of the formed compounds was achieved on the basis of their UV−visible and MS spectroscopy and by comparison with standards.

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 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 positive or negative ion mode. Ion spray voltage/orifice voltages were selected at −4 kV/−70 V and +5 kV/+60 V, respectively. 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 narrowbore 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), 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.

Absorption Spectra. Spectrophotometric measurements and UV−visible spectra were recorded with a 111 GBC spectrophotometer fitted with a quartz cell and equipped with GBC Scan Master software.

RESULTS AND DISCUSSION

Reactions Involving Acetaldehyde. Among the aldehydic derivatives, acetaldehyde has been found in many foods and beverages including fruits, vegetables, bread, dairy products, meats, and alcoholic or nonalcoholic beverages (*22*−*24*). It is widely used in such artificial fruit flavors as apple, apricot, banana, and peach and is commonly found in fruits such as citrus fruits because it is an intermediate product in the respiration of higher plants. It is also formed by oxidation of ethanol or by decarboxylation of pyruvic acid during fermentation of grapes (*25*).

The reactions of (+)-catechin and (−)-epicatechin in the presence of acetaldehyde were studied in model solution systems. When incubated separately with acetaldehyde and at pH values varying from 2.2 to 4.0, (+)-catechin or (−)-epicatechin was transformed to various compounds initially absent in the mixtures; reactions were faster with (−)-epicatechin than with (+)-catechin. HPLC-DAD analysis showed that the formed compounds were characterized by UV−visible spectra

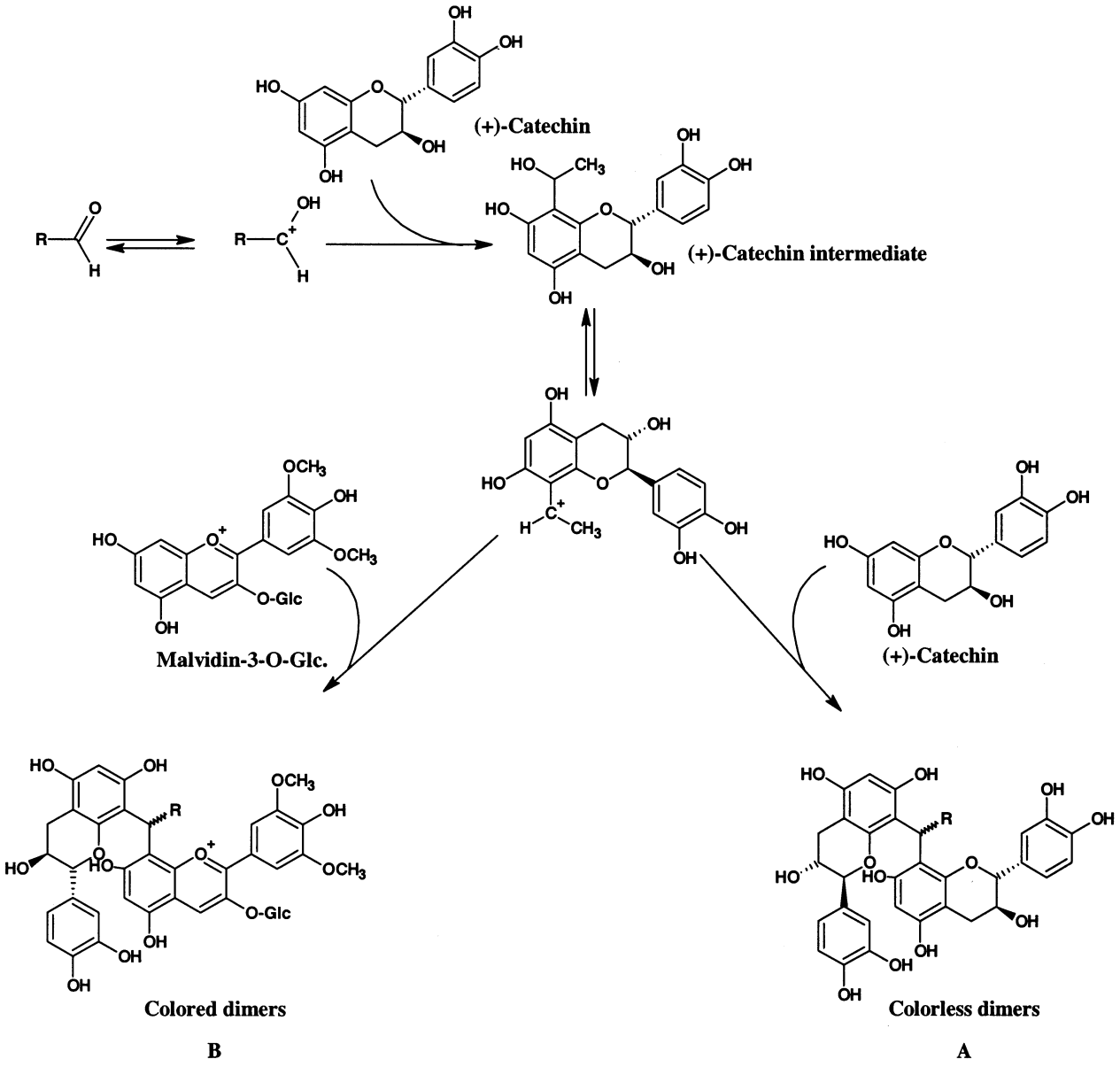

Figure 4. General mechanism of the aldehyde-induced polymerization of flavanols and anthocyanins with formation of colorless (A) and colored compounds (B).

similar to that of their precursor with absorption maxima at 280 nm showing that the original flavanic skeleton was retained. Besides, reaction kinetics slowed when the pH increased, as can be observed in **Figure 1**, in agreement with the literature data (*26*−*28*).

ESI-MS analysis of solutions containing (+)-catechin or (−)-epicatechin and acetaldehyde conducted in the negative ion mode allowed detection of many peaks corresponding to various oligomers with several mass peaks corresponding to the doubly charged ions of larger molecular weight polymers as previously reported by us (*18*). These compounds consisted of flavanol units linked with ethyl bridges as shown in **Figure 2** (**IA**). Detection of the ethanol−flavanol adduct (Cat.−CH_2OH−CH_3) by HPLC coupled to ion spray mass spectrometry (ESI-MS) as the negative ion at *m*/*z* 333 amu allowed us to demonstrate that the reaction starts with protonation of acetaldehyde in acidic medium followed by nucleophilic addition of the flavanol (C-6 or C-8 of the A-ring) on the resulting carbocation, as previously postulated by Timberlake and Bridle (*4*) and confirmed later by our group (*18*, *19*). The ethanol adduct then loses a water molecule to give a new carbocation intermediate, which is in turn attacked by another flavanol unit to yield an ethyl-linked dimer adduct as shown in **Figure 2** (**IA**). The presence of intermediate ethanol adducts of dimer and trimer species confirmed that polymerization continued following the same mechanism.

In mixtures containing both flavanols and acetaldehyde, new compounds besides the homogeneous bridged derivatives were detected. These compounds were concluded to be heterogeneous oligomers consisting of (+)-catechin and (−)-epicatechin linked with an ethyl bridge. In this case, (−)-epicatechin decreased more rapidly than (+)-catechin, showing that the reaction was faster with the former. This was also observed in solutions containing the two flavanols and the (+)-catechin−ethanol intermediate. Under these conditions, the homogeneous (+)-catechin bridged dimers and heterogeneous dimers were obtained by the action of the intermediate on (+)-catechin and (−)-epicatechin, respectively. In addition, the homogeneous

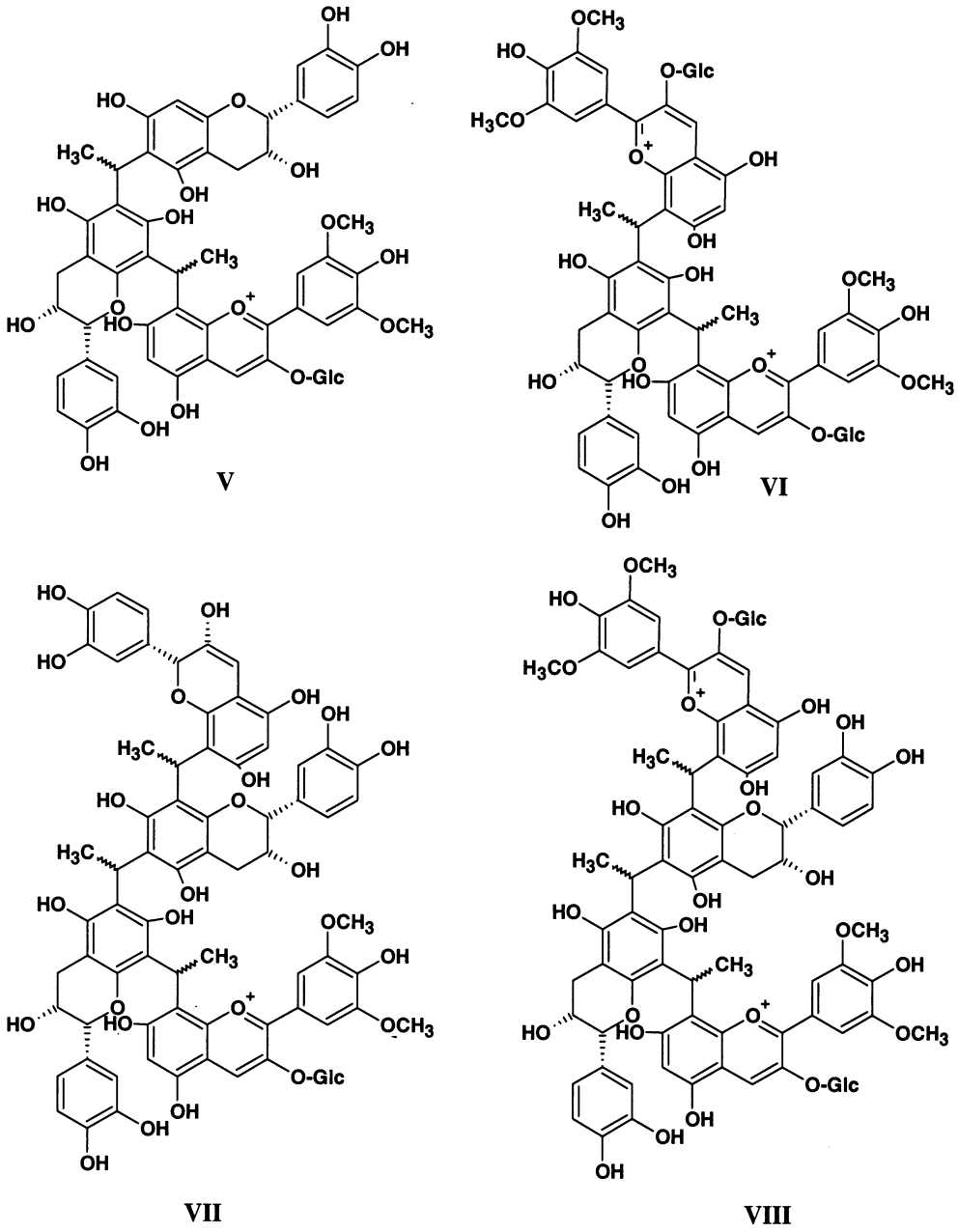

Figure 5. General structures of the trimeric (**V** and **VI**) and tetrameric (**VII** and **VIII**) colored derivatives detected in mixtures containing (–)-epicatechin, malvidin 3-*O*-glucoside, and acetaldehyde.

(−)-epicatechin ethyl-bridged dimers were also detected, showing that ethyl linkages underwent depolymerization and recombination reactions.

In a mixture containing malvidin 3-*O*-glucoside, (−)-epicatechin, and acetaldehyde, the two reactions compete and the formation of adducts containing only homogeneous ethyl-bridged flavanols or involving (−)-epicatechin and the anthocyanin was observed. Monitoring the evolution of the reaction indicated that the homogeneous bridged derivatives were formed first and then transformed to colored derivatives, which seemed to be more stable (**Figure 3A**). The latter were characterized by absorption maxima bathochromically shifted (around 540 nm) compared to that of malvidin 3-*O*-glucoside (525 nm). This bathochromic shift was more accentuated for more polymerized products as shown in **Figure 3D**, where absorption maxima attained 555 nm for compounds eluted in the end of the chromatogram.

HPLC-ESI-MS of the mixture conducted in the positive ion mode showed the presence of both colorless ethyl-linked flavanol oligomers and colored flavanol−anthocyanin adducts, meaning that competition took place between both species in the addition process. Thus, both colorless (**IA**) and colored (**IB**) dimers were detected as their molecular ion in the positive ion mode at *m*/*z* 607 and 809 amu, respectively. From a mechanistic point of view, the reaction proceeds in the same way in the first stage, giving the monomer ethanol intermediate as shown in **Figure 4** (R = CH_3). This intermediate then loses a water molecule to give a new carbocation, which is in turn attacked either by another flavanol unit to yield an ethyl-linked dimer or by an anthocyanin to give a flavanol−ethyl−anthocyanin adduct. The fact that only the flavanol unit could be attacked by the first ethanal carbocation was supported by the absence of a malvidin 3-*O*-glucoside intermediate. Thus, no signals was detected at *m*/*z* 537 amu corresponding to a methyl carbinol

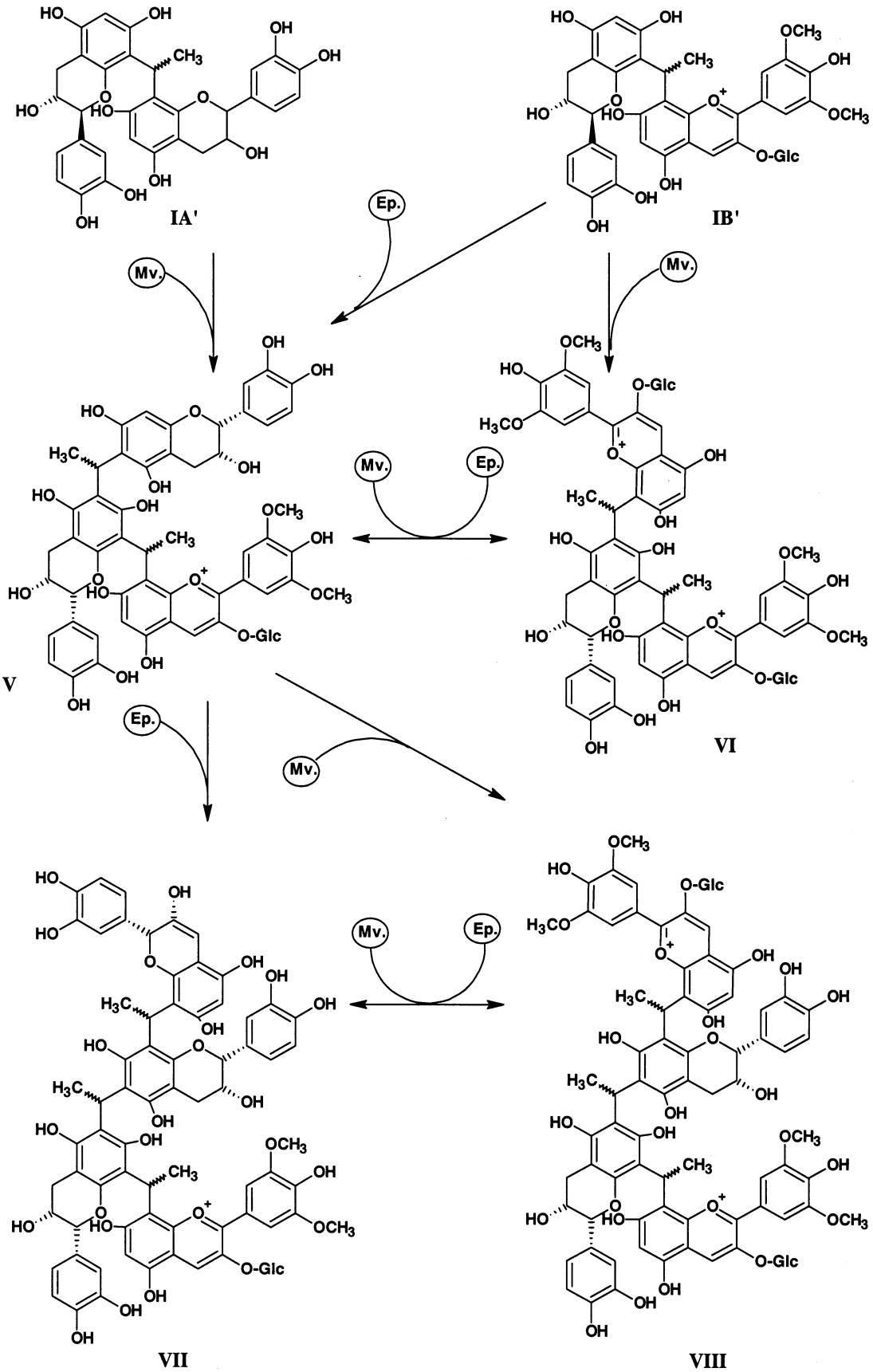

Figure 6. Reaction pathways showing the formation of the trimeric (**V** and **VI**) and tetrameric (**V** and **VI**) colored adducts from colorless (**IA′**) and colored (**IB′**) dimers.

group fixed on an anthocyanin unit, and the only detected monomer intermediate was that of epicatechin with a molecular ion observed at *m/z* 335 amu. In this reaction, both the flavanol and the anthocyanin act as nucleophiles, whereas the protonated form of the aldehyde is the electrophilic species. The resulting ethyl-linked pigments are relatively stable, probably protected

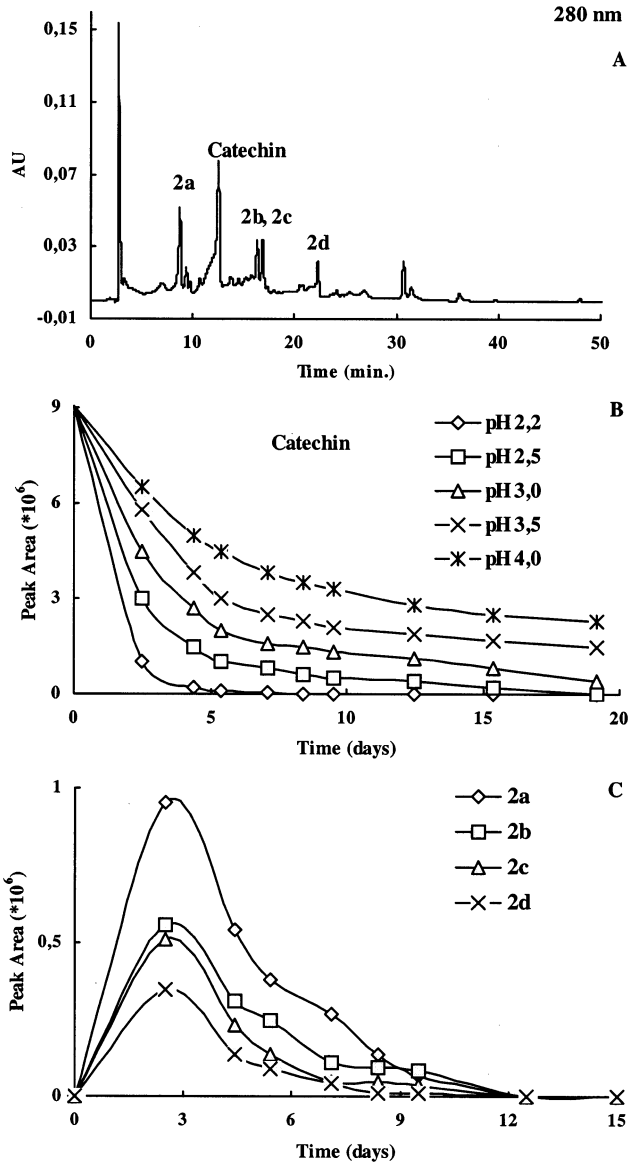

Figure 7. HPLC chromatogram of a mixture of (+)-catechin and glyoxylic acid recorded at 280 nm (A) showing residual flavanol and new formed compounds (**2a**–**d** are the four colorless dimers). Evolution of (+)-catechin with time and pH (B) and evolution of the four dimeric derivatives with time during reaction are also presented (C).

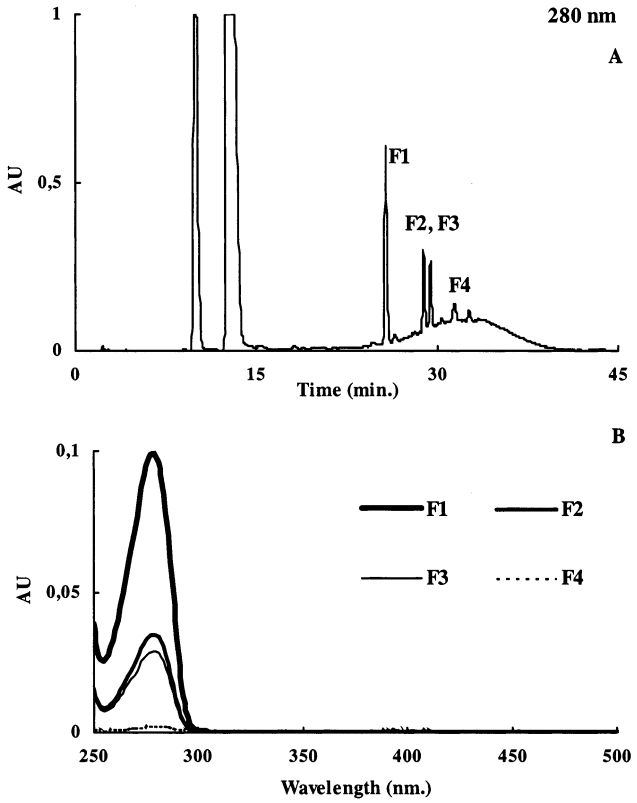

Figure 8. HPLC chromatograms of a mixture of (+)-catechin and furfural recorded at 280 nm (A). Compounds **F1**–**4** are the four formed colorless adducts. The UV–visible spectra of the four major derivatives are also presented (B).

from the hydration reaction by self-association phenomenon, leading to non-covalent dimers associated by a sandwich-type stacking (*29*).

The acetaldehyde-induced condensation proceeds more easily without the anthocyanins. In fact, pigments were shown to react through a single position (only the C-8 top), whereas flavanols were able to react twice, through the C-8 and C-6 positions. In other words, this means that anthocyanins are terminal units ending off the polycondensation reaction, whereas flavanols are propagation units maintaining the polymerization process. Under acidic conditions, the ethyl bond connecting two flavanol units is more labile than those connecting an anthocyanin to a flavanol. This was supported by thiolysis, by which colored dimers were shown to be more resistant than the colorless ones (*19*).

When the reaction was allowed to proceed, more polymerized adducts were detected by LC/ESI-MS analysis conducted in the positive ion mode. Thus, signals corresponding to one flavanol and one anthocyanin (*m*/*z* 809 amu), two flavanols and one anthocyanin (*m*/*z* 1125 amu), one flavanol and two anthocyanins (*m*/*z* 664 amu), three flavanols and one anthocyanins (*m*/*z* 1441 amu), and two flavanols and two anthocyanins (*m*/*z* 822 amu) were detected. The structures corresponding to the trimeric (**V** and **VI**) and tetrameric (**VII** and **VIII**) colored adducts are shown in **Figure 5**. It seems from the detected oligomeric derivatives that the reaction stops when both chain ends are occupied by an anthocyanin moiety. Thus, no adducts with more than two anthocyanin moieties were detected. In other words, this means that the anthocyanin played the role of ending chain, whereas the flavanol allowed the formation of more polymerized colorless and colored adducts as indicated above.

The formation of the trimeric (**V** and **VI**) and tetrameric (**VII** and **VIII**) colored derivatives detected in a mixture containing (−)-epicatechin, malvidin 3-*O*-glucoside, and acetaldehyde could be envisaged either from the colorless (**IA′**) or the colored (**IB′**) dimers as shown in **Figure 6**. Whereas the colorless dimer (**IA′**) could yield a colored trimer (**V**) by linking an anthocyanin moiety through an ethyl bridge, both colored trimers (**V** and **VI**) could be obtained from the colored dimer (**IB′**) by involvement of the flavanol or the anthocyanin moieties. As the linkage between the ethyl bridge and the anthocyanin or the flavanol moieties is sensitive to acidic conditions, where they were shown to be cleaved (*19*), each colored trimer could be converted to the other by a depolymerization and recombination process. Each conversion is accompanied by a loss of a flavanol unit and a reaction of the obtained intermediate with an anthocyanin moiety and vice versa. As may be expected, the formation of compounds involving more anthocyanins units is prevalent because the linkage between (−)-epicatechin and the bridge is more sensitive to acid conditions than that between

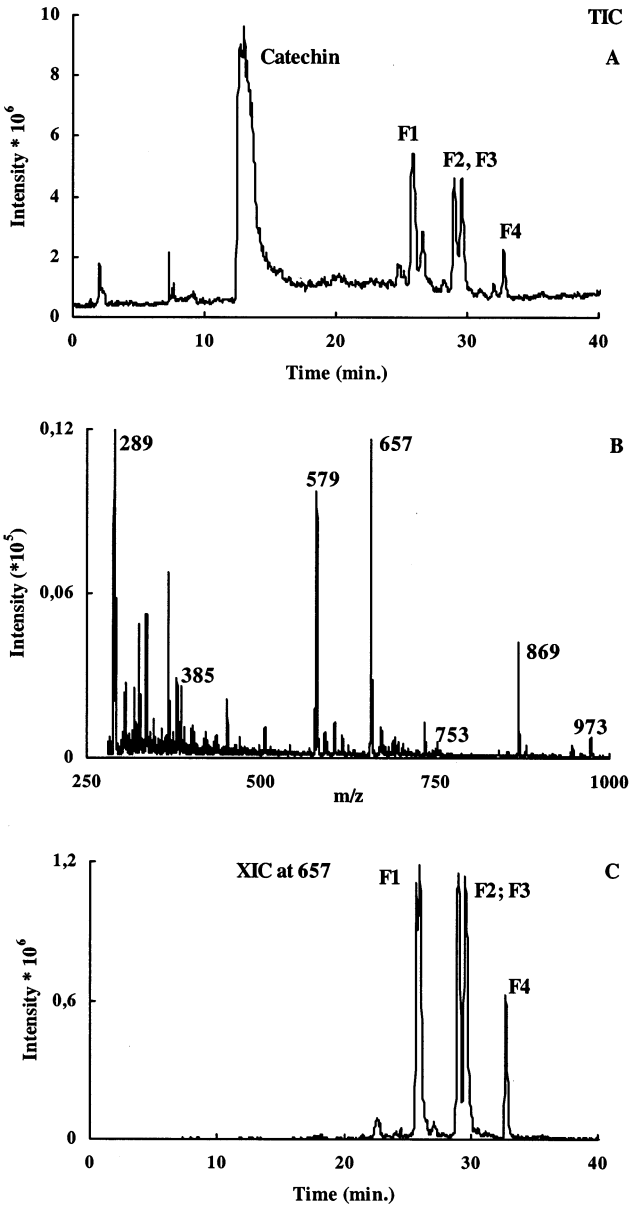

Figure 9. HPLC chromatograms of a mixture of (+)-catechin and furfural recorded with an electrospray mass detector (A). The mass spectrum of the infused reaction (B) and the extracted ion current recorded at *m/z* 657 amu and corresponding to the colorless dimers are also presented (C).

the ethyl group and malvidin 3-*O*-glucoside (*19*). This was demonstrated on the chromatographic profile of a more evolved solution summarized in **Figure 3C**, which clearly showed that the detected peaks absorbed in the visible region, meaning that they contained the flavylium units.

As indicated above, the anthocyanin moiety plays the role of ending chain in the polymerization process, which stops when both chain ends were occupied by an anthocyanin unit. The trimer (**VI**), which presents two malvidin 3-*O*-glucoside units as chain ends, could not conduct directly to tetramers because only one summit of the anthocyanin moiety could be involved in the polymerization process, whereas the trimer (**V**) containing two flavanols units could. Thus, two tetramer derivatives (**VII** and **VIII**) could be obtained by linking an anthocyanin or a flavanol moiety through an ethyl bridge. The depolymerization and recombination processes observed above between the two trimers could also occur between the formed tetramers with a prevalence of that involving two anthocyanin moieties. The polymerization process could obviously continue from the tetramer involving only one anthocyanin unit, giving pentamers and so on. The formation of more polymerized derivatives was recently shown in a model solution system containing (+)-catechin, malvidin 3-*O*-glucoside, and acetaldehyde, where a pentamer containing four flavanol units and an anthocyanin moiety was detected by LC/ESI-MS analysis (*30*). Each obtained oligomer or polymer could involve at most two anthocyanin moieties, whereas more flavanol units could be involved. This explains the fact that the flavanol peak area decreased more rapidly compared to that of the anthocyanin as observed in the chromatographic profile recorded at 280 nm at the end of the reaction (**Figure 3A**). This fact showed that during food storage and aging, acetaldehyde could strongly contribute to the formation of oligomeric colored pigments by bridging flavanol and anthocyanin units and then participate in the stability of the food color.

In addition to monomer flavanols, the acetaldehyde-induced condensation was also shown to occur between procyanidins and anthocyanins, where similar bathochromic shifts were observed on the UV−visible spectra of the mixtures (*31*, *32*). Some of the bridged oligomeric colorless and colored compounds formed from condensation between (+)-catechin, malvidin 3-malvidin-3-*O*-glucoside, and acetaldehyde were detected in grape-derived beverages (*33*, *34*). Nevertheless, other anthocyanins are expected to behave similarly. This suggests that a great diversity of products can be generated during food processing, maturation, and storage. Their levels depend obviously on the nature and relative amount of flavanols and anthocyanins present.

In food-processing technology, practices that increases acetaldehyde formation, such as aeration, may promote polymerization of anthocyanins and catechins or tannins, thereby improving color intensity and stability. Indeed, addition of exogenous acetaldehyde, or that produced by a coupled autoxidation of ethanol and phenolic compounds (*35*), provokes copolymerization of anthocyanins and catechins or tannins and then enhances color intensity. From a sensory point of view, this polymerization is sometimes desirable, because it reduces the tannins level and, hence, alleviates astringency and bitterness (*7*, *36*). However, it can cause turbidity and deposits, presumably due to precipitation of larger molecular anthocyanin−tannin complexes (*4*, *7*, *19*) or formation and precipitation of catechin−acetaldehyde colloidal polymers (*18*, *20*, *21*). Thus, acetaldehyde is known to have an impact on haze formation in beer through reaction with catechins and preformed complex phenolics (*37*). An appreciable presence of acetaldehyde in the free state (>5 mg/L) can destabilize a red wine by inducing a phenolic haze and eventual deposition of condensed pigments, which occurs very rapidly at higher levels of acetaldehyde (*38*).

Reactions Involving Glyoxylic Acid. Another example involving the interaction between phenolic compounds and aldehydic derivatives is the evolution of (+)-catechin in iron-catalyzed medium containing tartaric acid, which is transformed by oxidation to glyoxylic acid (CHO−COOH) (*39*, *40*). This prompted us to study the role that glyoxylic acid could play as an aldehydic compound in the polymerization of phenolic compounds. It may be noted that this compound is a carboxylic acid found in green fruits, seedlings, and young leaves and is the starting point for the glyoxylate cycle.

The reaction between (+)-catechin and glyoxylic acid was conducted in hydroalcoholic medium, and bridged derivatives analogous to those obtained with acetaldehyde were obtained.

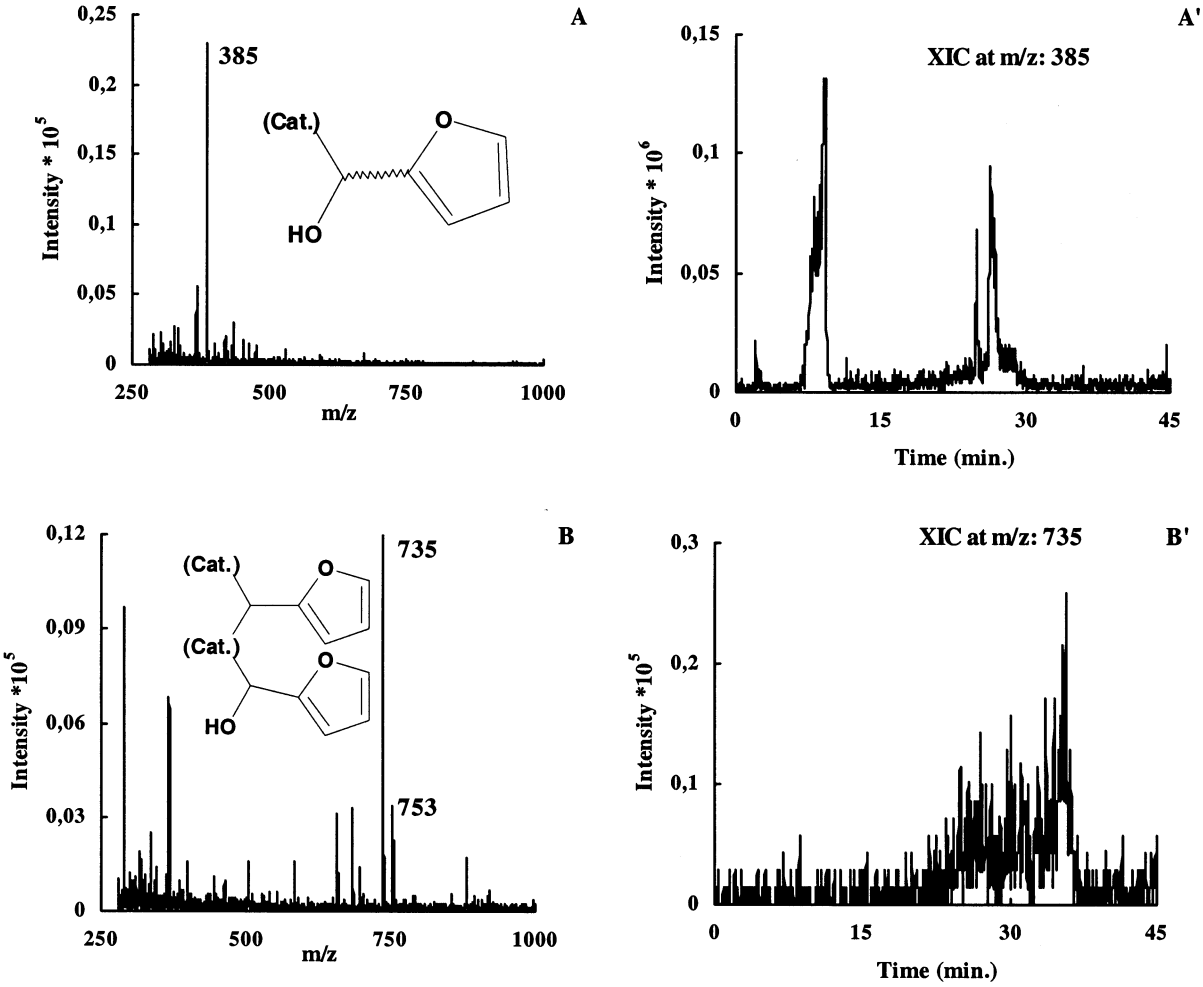

Figure 10. Mass spectra of the monomer (A) and dimer (B) intermediates and the corresponding extracted ion current chromatograms (A′, B′) detected in a mixture of (+)-catechin and furfural.

LC/MS analysis of the incubated solution, conducted in the negative ion mode, showed an *m*/*z* signal located at 635 amu for the four major compounds **2a**−**d** shown in the chromatographic profile drawn in **Figure 7A**. This indicated that they consisted of two (+)-catechin units bridged by a carboxymethine group (**Figure 2**, **IIA**) as previously reported (*40*, *41*). These experiments showed that the formation of these dimers proceeds via a mechanism similar to acetaldehyde-induced polymerization, in which acetaldehyde is replaced by glyoxylic acid, indicating that glyoxylic acid reacts in the same way in the very first steps. However, various compounds with absorptions between 440 and 460 nm were also detected in the case of glyoxylic acid and were not observed with acetaldehyde.

To evaluate the effect of pH changes on the composition of the incubated solutions, the condensation of (+)-catechin with glyoxylic acid was monitored by HPLC in the pH range from 2.2 to 4.0. Quantification of all products was achieved on the basis of peak areas at 280 nm, using (+)-catechin as standard. The concentration of residual (+)-catechin is presented in **Figure 7B**. (+)-Catechin concentration showed a gradual decrease at all pH values, owing to the higher availability of glyoxylic acid carbocation at lower pH values. This phenomenon was also observed in the condensation of (+)-catechin with acetaldehyde in the presence or absence of malvidin 3-*O*-glucoside (*19*, *27*, *28*) and is in agreement with the postulated mechanism (*4*, *18*).

Variations in relative amounts of the four dimers formed during incubation of the mixture are reported in **Figure 7C**. The four compounds accumulated gradually at the beginning of the reaction and then decreased, probably as more polymerized products were formed. The figure also shows that among the four formed dimers, compound **2a** (8-8 isomer) was the one formed at higher concentration, followed by compounds **2b** and **2c** (8-6 and 6-8 isomers), which were formed at similar amount, and finally compound **2d** (6-6 isomer), which was formed at lower amount. All of these dimers were shown to evolve easily to yellowish xanthylium salt derivatives with absorption maxima located between 440 and 460 nm (*42*−*44*). In addition to these yellowish compounds other derivatives with absorption maxima around 300 nm were also detected. These compounds were shown to be mono- and biformylated catechin derivatives (*45*).

When malvidin 3-*O*-glucoside was incubated in the presence of (+)-catechin and glyoxylic acid, both colorless and colored compounds, initially absent in the mixture, were obtained, meaning that the anthocyanin and the flavanol competed in the interaction. LC/DAD and ESI-MS analyses of the sample conducted in the positive ion mode enabled the detection of colorless along with the colored dimers, which were detected as their negative ion at *m*/*z* 839 amu. However, it seems that the process involving only flavanols and leading to colorless bridged compounds was largely predominant compared to the reaction involving the anthocyanin. Thus, the areas of the two peaks corresponding to the colored dimers consisted of (+)-catechin and malvidin 3-*O*-glucoside linked through a carboxymethine bridge (**Figure 2**, **IIB**) and were small compared to those corresponding to the colorless ones (**Figure 2**, **IIA**).

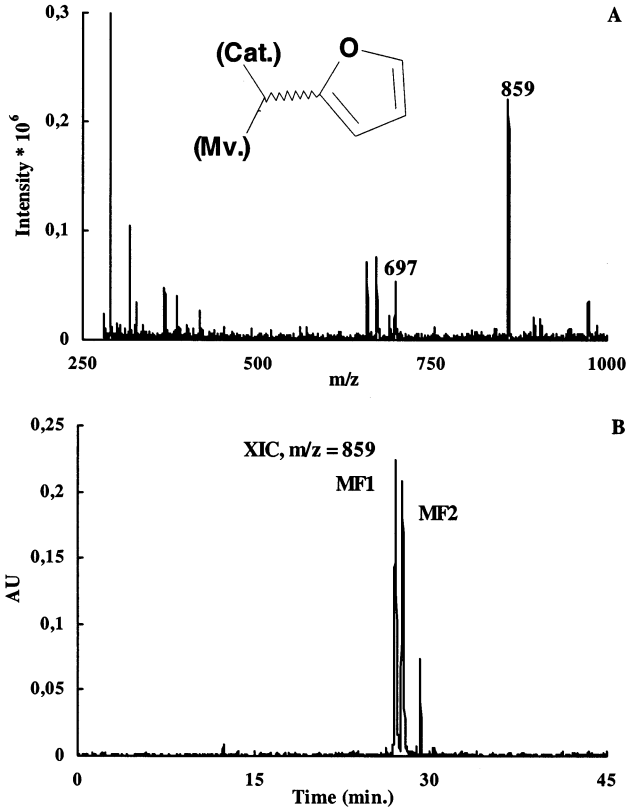

Figure 11. Mass spectrum (A) and the corresponding extracted ion current chromatogram (B) recorded at *m/z* 859 amu showing the two colored dimers **MF1** and **MF2** detected in a mixture of (+)-catechin, malvidin 3-*O*-glucoside, and furfural.

In addition to these dimers, which present an absorption maxima around 545 nm as their analogous catechin−malvidin ethyl-linked derivatives, other compounds showing similar UV−visible spectra with a maximum at 500 nm were also observed. These compounds result probably from a cycloaddition reaction involving malvidin 3-*O*-glucoside. Similar malvidin-derived pigments were detected in the case of acetaldehyde, and analogous adducts involving malvidin 3-*O*-glucoside, procyanidin B3, and acetaldehyde were also detected in a model solution system (*46*). As the reaction was allowed to proceed, successive condensations led to numerous oligomers and polymers, which were eluted as a large unresolved bump in the end of the chromatogram.

These results indicated that polyphenols can react with acetaldehyde and glyoxylic acid to form various oligomeric colorless and colored bridged adducts. This demonstrates the effect of these aldehydic derivatives on the polymerization reactions and their influence on color change and astringency and, by the way, their participation in browning reactions observed in food-processing technology.

Reactions Involving Furfural. 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. These compounds were also shown to give rise to components such as furfural and its derivatives, which were reported to be responsible for undesirable flavor in juices (*47*).

The formation of furfural compounds and the increase of their concentration during storage of fruit-derived juices are well documented and are considered as indications of quality deterioration and a degree of damage to the product caused by excessive heat during processing or subsequent storage (*48*, *49*).

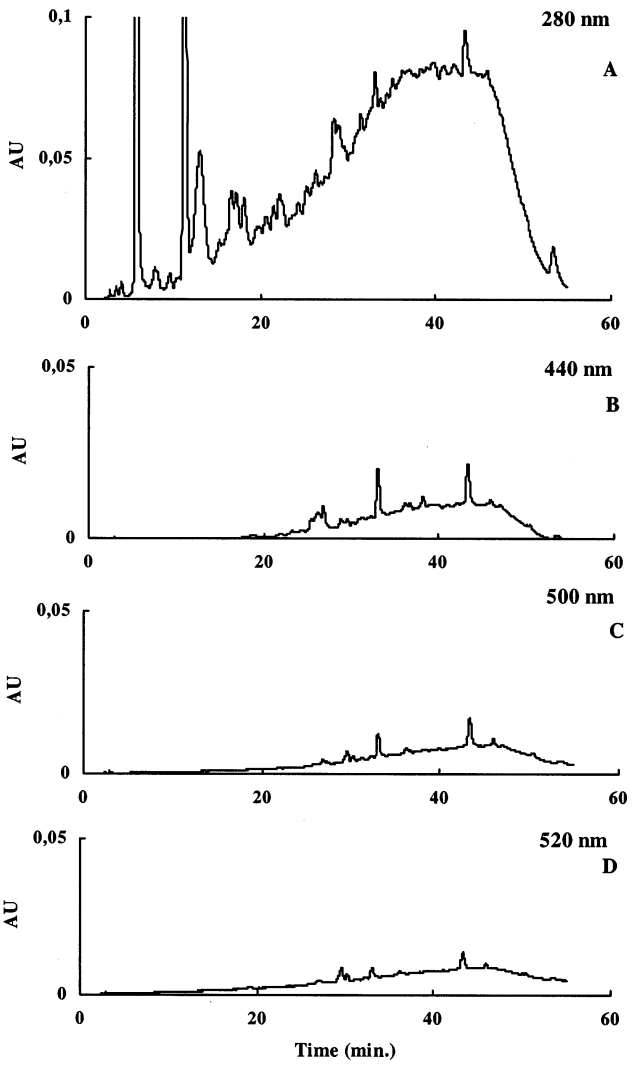

Figure 12. HPLC chromatograms of a mixture of (+)-catechin, malvidin 3-*O*-glucoside, and furfural recorded at 280 (A), 440 (B), 500 (C), and 520 nm (D) showing the formation of more polymerized colorless and colored compounds.

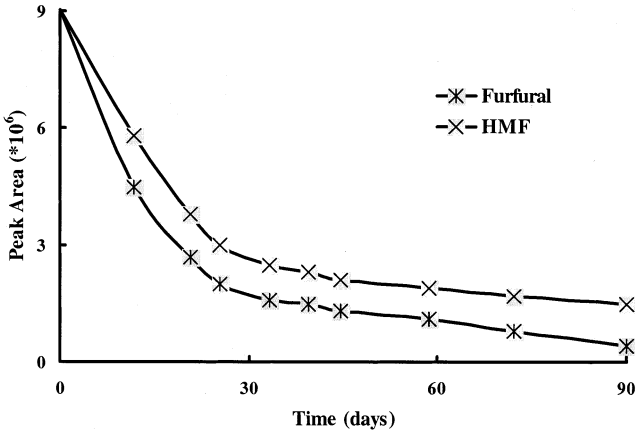

Figure 13. Comparison of (+)-catechin evolution when incubated with furfural or HMF at pH 2.2.

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 (*49*, *54*).

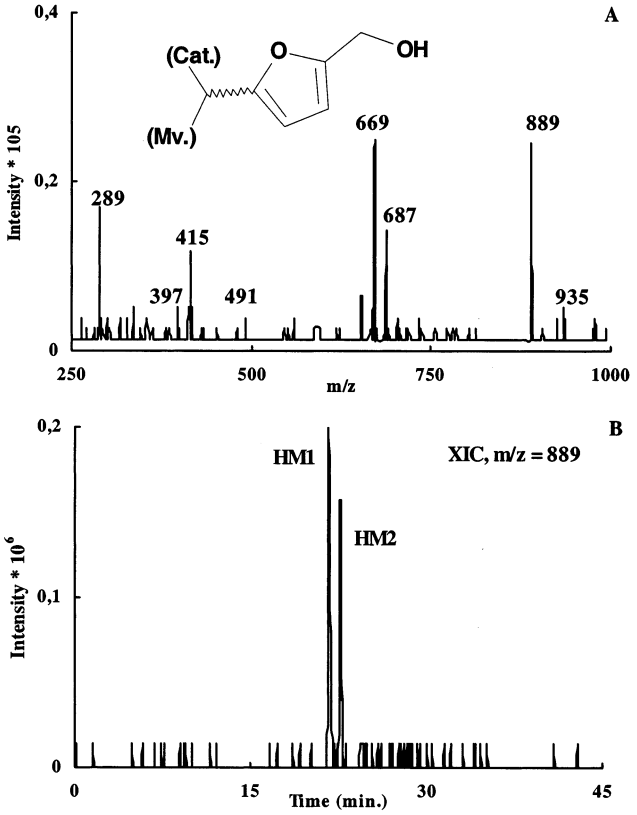

Figure 14. Mass spectrum (A) and corresponding extracted ion current chromatograms (B) recorded at *m/z* 889 amu showing the two colored dimers **HM1** and **HM2** detected in a mixture of (+)-catechin, malvidin 3-*O*-glucoside, and HMF.

When (+)-catechin was incubated with furfural, the mixture underwent a progressive darkening, suggesting that a reaction occurs and a possible contribution of such interactions to fruit-derived foods darkening observed during storage (*49*). Monitoring the evolution of the mixture by HPLC/DAD analysis showed the appearance of new peaks in addition to those of the reactants (**Figure 8A**). Among these compounds four major compounds with absorption maxima at 280 nm were observed. The UV−visible spectra of the major formed compounds recorded between 250 and 500 nm were similar to that of (+)-catechin with a maximum absorbance near 280 nm (**Figure 8B**), suggesting thus that the original flavanol structure was retained (*55*).

The total ion current chromatogram recorded when the reaction was monitored by LC-MS indicated in particular the presence of the four adducts **F1**−**4** (**Figure 9A**). The mass spectrum obtained in the negative ion mode when the reaction was infused through a syringe allowed the detection of both oligomers and intermediate ions (**Figure 9B**). Among the detected compounds catechin ion (*m/z* 289 amu), monomer intermediate ion (*m/z* 385 amu), dimer ion (*m/z* 657 amu), and dimer intermediate ion (*m/z* 753 amu) can be observed in **Figure 9B**. In addition to each *m/z* value there were several peaks, suggesting that they could be regio- and/or stereoisomers. Thus, four dimeric adducts along with intermediate adducts in each solution were detected by LC/ESI-MS analysis. This was confirmed on the extracted ion current (XIC) chromatogram profile recorded at *m/z* 657 amu (**Figure 9C**), where the presence of four dimeric adducts can be observed. Mass spectrometric analysis revealed thus that compounds **F1**−**4** all had a molecular weight of 658 and corresponded to a structure in which two (+)-catechin units are linked by a furfuryl bridge as shown in **Figure 2** (**IIIA**).

In addition to these dimers, other oligomeric bridged compounds were also detected. Thus, the formation of intermediate furfuryl adducts of monomer (*m/z* 385 amu) and dimer derivatives (*m/z* 753 amu) was observed as shown in the extracted mass chromatographic profiles recorded, respectively, at *m/z* 385 and 753 amu (**Figure 10**). 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, other compounds presenting UV−visible spectra similar to those of xanthylium salts with absorption maximum located around 440 nm were also observed either through LC/DAD or through LC/ESI-MS analysis.

When malvidin 3-*O*-glucoside was added to the mixture, oligomeric colorless and colored pigments involving both (+)-catechin and anthocyanin moieties were detected, showing that the two polyphenols competed in the condensation process as was observed in the case of acetaldehyde and glyoxylic acid. Among the obtained colored pigment adducts, two colored compounds were observed. Their UV−visible spectra were similar to that of malvidin 3-malvidin-3-*O*-glucoside but 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.

Upon LC-MS analysis conducted in the negative ion mode, a molecular ion at *m/z* 859 amu was observed for both pigments (**Figure 11A**). This is consistent with one flavylium moiety linked to one (+)-catechin moiety through a furfuryl bridge (**Figure 2**, **IIIB**), in agreement with the previously postulated mechanism concerning acetaldehyde (*4*, *18*, *19*). An extracted ion current chromatogram recorded at *m/z* 859 amu (**Figure 11B**) showed the presence of the two colored adducts **MF1** and **MF2**, confirming thus the results obtained through LC/DAD analysis.

As may be expected, the evolution of the reaction allowed successive condensations yielding numerous oligomers and polymers. These were eluted as a large unresolved bump in the end of the chromatogram obtained through LC/DAD analysis. Analysis of the sample enabled the detection of colorless oligomers along with the colored adducts, as shown in the chromatographic profiles recorded at 280, 440, 500, and 520 nm (**Figure 12**). As shown, the chromatogram recorded at 280 nm (**Figure 12A**) showed residual (+)-catechin and furfural in addition to a bump consisting probably of more polymerized bridged derivatives. The chromatographic profile clearly shows that the polymeric fraction of the analyzed solution predominantly consisted of colorless adducts detected at 280 nm as opposed to yellowish or purple ones detected at 440 (**Figure 12A**) or 520 nm (**Figure 12B**), respectively. This fact showed that the bridged oligomers obtained are not rapidly transformed, yielding purple colored compounds. This was not observed in the case of acetladehyde, where the peaks recorded at 280 and 520 nm showed equivalent intensities as shown in **Figure 3A**,**C** in the case of (−)-epicatechin and malvidin 3-*O*-glucoside.

The fact that, in the case of furfural, the disappearance of malvidin 3-*O*-glucoside was not accompanied by an equivalent formation of bridged colored compounds showed that the anthocyanin should probably suffer a degradation reaction in

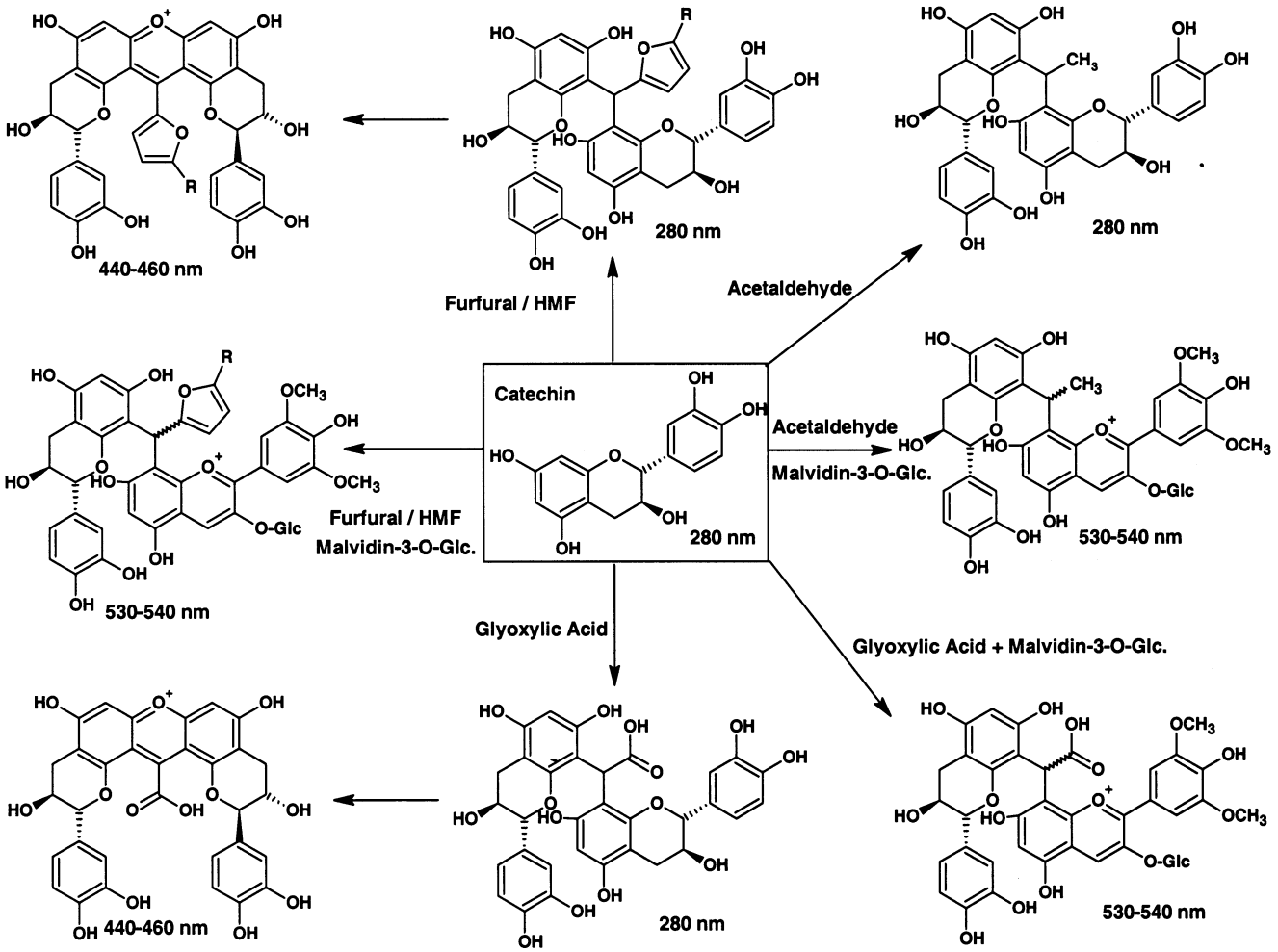

Figure 15. Phenolic reactions with aldehydes leading to colorless and colored compounds. Absorption maxima of the formed compounds are also indicated.

addition to the condensation process involving (+)-catechin and furfural. This fact also showed that the contribution of furfural in color stabilization through anthocyanin condensation is negligible compared to its participation in flavanol polymerization. Its more pronounced influence in the condensation of (+)-catechin suggests that furfural could play a role in taste transformation such as astringency decrease during storage or aging of foods.

Reactions Involving HMF. When (+)-catechin was incubated in the presence of HMF, reaction between the two reactants occurred with formation of analogous oligomeric compounds, which were detected upon LC/DAD analysis. Comparison of the results obtained between the two furfural derivatives showed that reaction was faster with furfural that with HMF as shown in **Figure 13**, which clearly showed that, with time, (+)-catechin decreased more rapidly with furfural than with HMF. This may be due to the difference in reactivity of both molecules as previously indicated (*56*, *57*).

Mass spectrometric analysis conducted in the negative ion mode revealed the presence of four compounds with ion signals at *m*/*z* 687 amu and corresponding to colorless dimers (**Figure 2**, **IVA**). Intermediate adducts of monomer (*m*/*z* 415 amu) and dimer (*m*/*z* 783 amu) were also observed, and the formation of more polymerized compounds was also observed in the case of HMF by the appearance of a brown precipitate in the vial. Other compounds with absorption maxima around 440 nm, corresponding probably to xanthylium salts, were also observed. 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 and was also confirmed on the ion-extracted mass chromatogram recorded at *m*/*z* 667 amu. The detection of their corresponding xanthene derivatives at *m*/*z* 669 amu supports the proposed structures.

In the presence of HMF, malvidin 3-*O*-glucoside and (+)-catechin underwent a polymerization reaction as revealed either through LC/DAD or through LC/ESI-MS analysis, and detection of colorless (λ_{max} = 280 nm) and colored compounds (λ_{max} = 545 nm) initially absent in the mixture was observed. The UV−visible spectra of these colored compounds were similar to that of malvidin 3-*O*-glucoside with a bathochromic shift of their visible absorption maxima, which were located around 545 nm.

LC/ESI-MS analysis showed the presence of four dimeric colorless (**Figure 2**, **IVA**) and two colored dimers (**Figure 2**, **IVB**) adducts as their molecular ions located at *m*/*z* 687 and 889 amu, respectively. An extracted ion chromatogram recorded at *m*/*z* 889 amu showed the presence of two dimeric adducts **HM1** and **HM2** consisting of (+)-catechin and malvidin 3-*O*-glucoside linked through an HMF bridge as shown on the obtained extracted mass chromatogram (**Figure 14**).

CONCLUSION

Fruit-derived foods are generally complex mixtures able to undergo, during storage, many different reactions that lead to

the formation of brown polymers. Whereas the color of a new fruit-derived product is due to its high chemical content, the subsequent color changes during aging involve generally condensation of phenolic compounds. Direct condensation between anthocyanins and flavanols is very slow. However, rapid polymerization between anthocyanin and catechins or tannins occurs in the presence of aldehyde derivatives, with increased color intensity and stability, but further reaction with polymerized catechin and tannins leads to instability, precipitation, and decreased color. A number of competing reactions involving polyphenols and aldehydes were shown to occur during food processing, maturation, storage, and aging. The results presented in this paper and summarized in **Figure 15** showed the major role played by aldehydic compounds in the condensation of flavanols giving various colorless and colored polyphenolic compounds. The formation of such compounds in a model solution system indicates their probable contribution to the color transformation of fruit-derived foods.

The acetaldehyde-mediated condensation was found to occur more generally, and glyoxylic acid, furfural, and HMF react in the same way in the first stage of the reactions. Thus, similar colorless (**IA**, **IIA**, **IIIA**, and **IVA**) and colored (**IB**, **IIB**, **IIIB**, and **IVB**) derivatives were shown to be formed with the four studied aldehydes. However, some of the dimers formed in the case of glyoxylic acid, furfural, and HMF evolved to yellow pigments, corresponding to xanthylium salts, whereas this was not observed in the case of acetaldehyde.

Comparison of the results obtained with the studied aldehydic derivatives showed that in terms of reactivity, reactions were faster with acetaldehyde where purple derivatives were more stable than the colorless adducts. The obtained results also showed that in the case of acetaldehyde no compound with a UV−visible spectrum similar to that of a xanthylium salt was detected either through LC/DAD or through LC/ESI-MS analysis. Interactions involving glyoxylic acid were slower than those observed in the case of acetaldehyde, as shown by comparison of **Figures 1** and **7C**. Colorless adducts were predominant compared to the purple formed adducts and were shown to proceed easily to more polymerized compounds and to give various yellowish derivatives. Purple compounds were detected with small peak areas compared to those obtained in the case of acetaldehyde. In the case of furfural and HMF, reaction was faster with the former but proceeded more slowly than with acetaldehyde or glyoxylic acid. Purple compounds involving flavanol and anthocyanin units were also detected, and colorless compounds were shown to be predominant. Finally, yellowish compounds with xanthylium salt skeletons were also detected either by LC/DAD or by LC/ESI-MS analysis. Their formation was confirmed by detection of their corresponding xanthene derivatives.

Comparison of the studied reactions also showed that they might not contribute similarly in the change of sensorial properties of foods during storage and aging. Thus, acetaldehyde seemed to contribute more to anthocyanin color stability by polymerization with flavanols, whereas glyoxylic acid, furfural, and HMF induced reactions in which the involvement of flavanols was predominant, giving colorless and yellowish compounds. Their contribution seemed to be more accentuated in browning and astringency decrease than in color stability.

The reactions described in this work were conducted in a model solution system and did not obviously describe what really occurs during the storage and aging of food systems, which have different pH values and complex polyphenolic composition and thus showed different evolutions and transformations. It seemed that the formation of the products described in this paper proceeded very quickly compared to those probably formed in fruit-derived foods, which may be relatively slow during the conservation and aging. Nevertheless, it is expected that such experiments may at least enable elucidation of the simpler polymerization products such as those slowly formed during maturation and storage. The role of these interactions in grape-derived foods color evolution may be increased by the involvement of other polyphenolic compounds. In particular, proanthocyanidins, which are the main grape flavanol constituents, could also be involved in such reactions, giving xanthylium salt derivatives. This may increase the importance of such reactions in the color evolution of grape-derived foods.

Although the reactions described above have been shown to modify the properties of polyphenols, they are not sufficient to explain the variations observed in the organoleptic properties (taste and color) among the various types of fruit-derived foods. As well, the relationships existing between structure and taste (e.g., astringency and bitterness) of proanthocyanidins and proanthocyanidin-derived molecules remain to be established. Answers to these questions will require the use of different complementary approaches, including chemical, sensory, and statistical analysis.

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