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Interactions between Cyanidin 3-*O*-Glucoside and Furfural Derivatives and Their Impact on Food Color Changes

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The reaction between (+)-catechin and cyanidin 3-*O*-glucoside was investigated in the presence of furfural and 5-(hydroxymethyl)furfural using LC/DAD and LC/MS analysis, and the obtained results were compared with those recorded with malvidin 3-*O*-glucoside. The appearance of colorless and red and yellow compounds was observed showing that the two polyphenols competed in the condensation process with a predominant formation of the reddish adducts. The colored compounds formed in the case of cyanidin 3-*O*-glucoside seemed to be more stable than those formed when the reaction was conducted with malvidin 3-*O*-glucoside. The detection of these reddish and yellowish compounds constitutes a new support for the contribution of this kind of reaction in the color evolution of fruit-derived beverages. In addition, other unidentified compounds were also detected, showing the occurrence of other interaction pathways in addition to the polymerization process yielding oligomeric bridged derivatives and opening perspectives of further investigations of these model solutions.

KEYWORDS: (+)-Catechin; cyanidin 3-*O*-glucoside; furfural; 5-(hydroxymethyl)furfural; condensation; LC/DAD; LC/MS; browning; darkening

INTRODUCTION

Flavanols and anthocyanins are polyphenolic compounds found in many plants, fruits, and fruit-derived foods such as beverages and juices. They have attracted much attention in relation to their physiological activities, and their role has become an important issue in the relationship between health and human diet. In particular, the potential positive effects associated with consumption of fruit-derived foods are attributed to the presence of such natural compounds.

During conservation, phenolic compounds usually undergo progressive changes (*1*) that affect sensorial properties such as color, taste, and colloidal stability (*2*−*6*). Many studies have been reported of this complex phenomenon and the mechanisms involved in these transformations as well as the structures of the reaction products in model solution systems (*1*−*4*, *7*−*17*). In particular, the change of grape-derived food color, from a bright red to a reddish brown tint, has been investigated by many authors. Chemically, this has been attributed to the progressive formation of condensed pigments resulting from the reaction between free anthocyanins initially extracted from grapes and other phenolic compounds, particularly flavanols (*1*). Various mechanisms have been suggested to explain the formation of these pigments. Processes involving either direct condensation between flavanols and anthocyanins giving rise to xanthylium salts with a yellow brown hue (*1*, *4*, *18*) or reactions mediated by acetaldehyde with the formation of violet pigments (*1*−*4*, *7*−*9*, *11*, *19*) have been studied in model solution systems. It was also shown that other aldehydes such as glyoxylic acid, furfural, or 5-(hydroxymethyl)furfuraldehyde (HMF) can replace acetaldehyde in the latter mechanism (*14*, *17*, *20*), where, in addition to the reddish pigments, yellowish xanthylium salts were obtained. The presence of some of the obtained compounds has actually been observed in grape-derived foods (*14*, *21*−*24*).

Although the involvement and reactivity of malvidin 3-*O*-glucoside in these processes have been largely investigated and well documented, little was reported about other natural anthocyanins such as cyanidin 3-*O*-glucoside, which plays an important role in the color of different fruits. This work presents the results obtained when (+)-catechin was incubated with cyanidin 3-*O*-glucoside in the presence of furfural or HMF and offers information about the analysis and characterization of the compounds formed in such reactions using spectroscopic tools.

MATERIALS AND METHODS

Reagents. (+)-Catechin was purchased from Sigma (St. Louis, MO). Cyanidin 3-*O*-glucoside was purchased from Extrasynthèse (Genay, France). Furfural and HMF were obtained from Interchim (Montlucon, France) and Lancaster Synthesis (Strasbourg, France), respectively.

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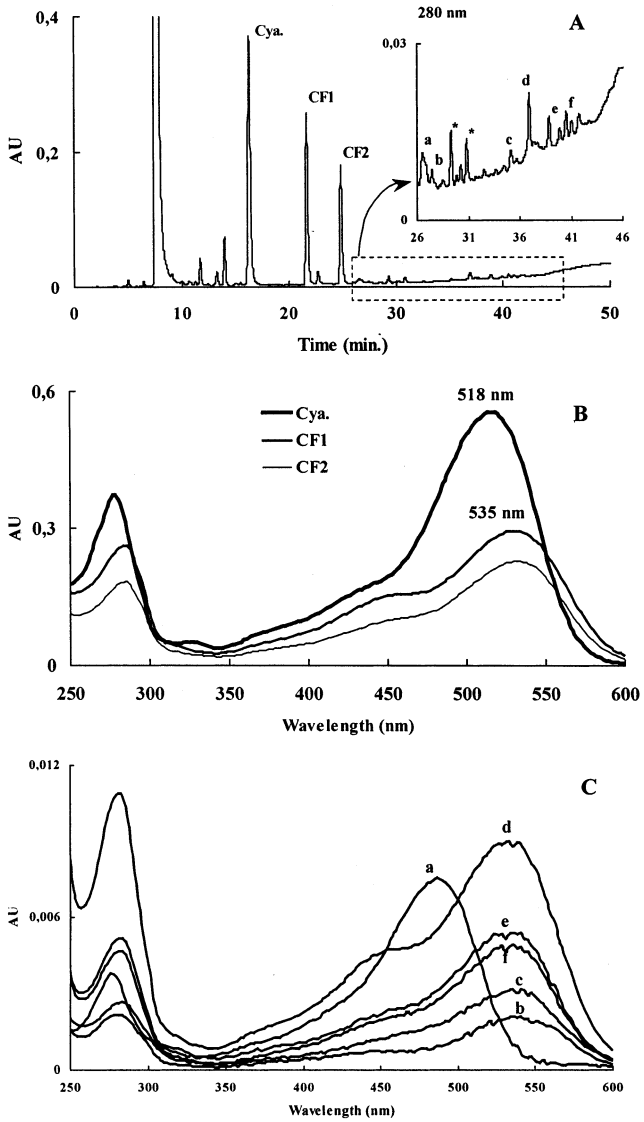

Figure 1. HPLC chromatogram of a mixture of (+)-catechin, cyanidin 3-*O*-glucoside, and furfural recorded at 280 nm (A). The UV–visible spectra of the major reddish and yellowish compounds in addition to that of cyanidin 3-*O*-glucoside are also presented (B, C). Peaks marked with an asterisk corresponded to colorless derivatives.

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. An equimolar mixture of (+)-catechin and cyanidin 3-*O*-glucoside (20 mM) was prepared in each obtained medium (0.5 mL), and 1 μL of furfural/HMF was then added. Each prepared solution was incubated in triplicates at room temperature and in the absence of light during a period of 1 month. Reactions were periodically monitored by liquid chromatography (LC) 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 the column.

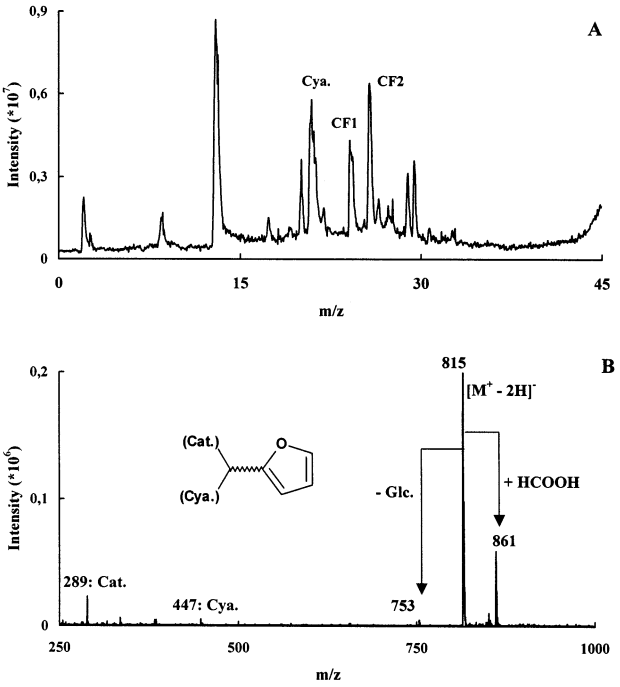

Figure 2. HPLC chromatograms of a mixture of (+)-catechin, cyanidin 3-*O*-glucoside, and furfural recorded with an electrospray mass detector (A). The mass spectrum of a colored dimer adduct is also presented (B).

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.

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 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.

Spectrophotometric Measurements. Spectrophotometric measurements were carried out using a GBC 111 UV−visible spectrophotometer fitted with a 10 mm path length quartz cell and equipped with GBC Scan Master manager software.

RESULTS AND DISCUSSION

Interaction between (+)-catechin and cyanidin 3-*O*-glucoside in the presence of furfural or HMF was investigated in an acidic model solution medium. The evolution of the reactants and of the newly formed compounds was monitored by LC/DAD and through LC/ESI-MS analysis. A general decrease in the concentrations of (+)-catechin and cyanidin 3-*O*-glucoside occurred in the mixture, concurrent with the formation of new colorless and colored products. When pH values were varied from 2.2 to 4.0, reaction rates decreased with the increase of pH values, owing to the higher availability of furfural of HMF carbocations at lower pH values as previously observed with either furfural derivatives, acetaldehyde, or glyoxylic acid (*10*, *11*, *17*, *25*).

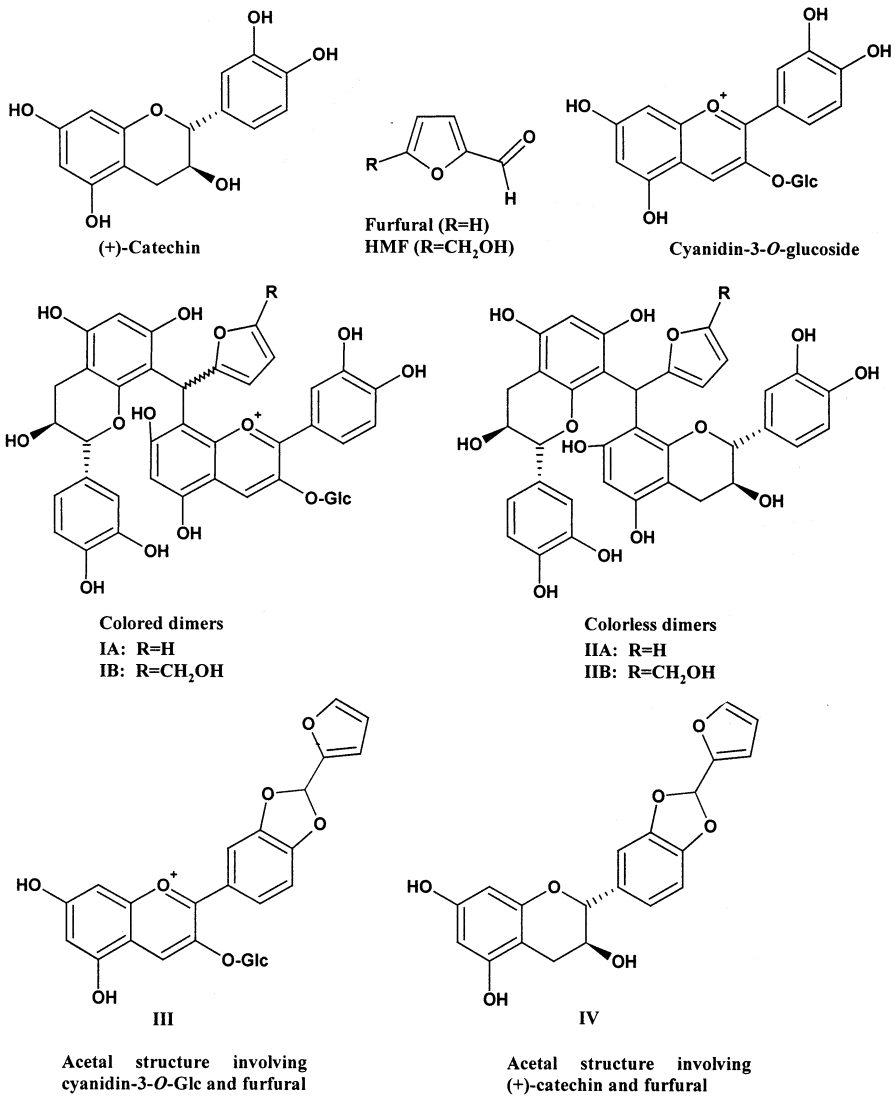

Figure 3. General structure of the dimeric colored (**IA** and **IB**) and colorless (**IIA** and **IIB**) derivatives obtained by interaction between (+)-catechin, cyanidin 3-*O*-glucoside, and furfural (R = H) or HMF (R = CH_2OH). Acetalic structures between the studied polyphenols and furfural are also presented (**III** and **IV**).

Color measurements of a model solution containing (+)-catechin, cyanidin 3-*O*-glucoside, and furfural showed that some browning reaction occurred. Thus, an increase of color in the brown region (420−450 nm) and in the violet region (540 nm) accompanied by a gradual loss of color in the red region (520 nm) was observed. This indicated that a reaction process involving the anthocyanin occurred in the mixture, which was supported by the color decrease observed around the maximum of cyanidin 3-*O*-glucoside.

Changes were monitored first by HPLC/DAD, and the formation of new compounds was observed as shown in the chromatographic profiles of **Figure 1A**. As shown in addition to the polyphenols initially introduced in the mixture, two major new colored compounds, labeled **CF1** and **CF2**, chromatographically distinguishable from cyanidin 3-*O*-glucoside were formed. The newly formed compounds were eluted later than the anthocyanin as those formed when malvidin 3-*O*-glucoside was incubated with (+)-catechin in the presence of acetaldehyde or furfural. Although **CF1** and **CF2** were present in all recorded chromatograms, compound **CF1**, which is the first adduct eluted after cyanidin 3-*O*-glucoside, was invariably present at the higher concentration. This differs from the results obtained in the case of malvidin 3-*O*-glucoside and (epi)catechin in the presence of acetaldehyde, furfural, or HMF, where the second adduct was prevalent (*11*, *17*).

The UV−visible spectra of compounds **CF1** and **CF2**, recorded between 250 and 600 nm, were similar to that of cyanidin 3-*O*-glucoside with a maximum absorbance in the red region (**Figure 1B**), indicating that the flavylium chromophore was still present in both pigments. Moreover, the spectra of compounds **CF1** and **CF2** showed an additional shoulder around 450 nm, and the wavelengths of their maximum absorbance in the visible range (535 nm) were significantly higher than that of cyanidin 3-*O*-glucoside (515 nm). This behavior was also observed in the case of the oligomers obtained when malvidin 3-*O*-glucoside and (epi)catechin were incubated with acetaldehyde, furfural, or HMF, giving rise to similar bridged pigments (*11*, *17*) where an equivalent batochromical shift (20 nm) was also observed. 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 (*9*). This bathochromic shift was also observed in mixtures containing blackberry juice anthocyanins in the presence of some aldehydic derivatives such as acetal-

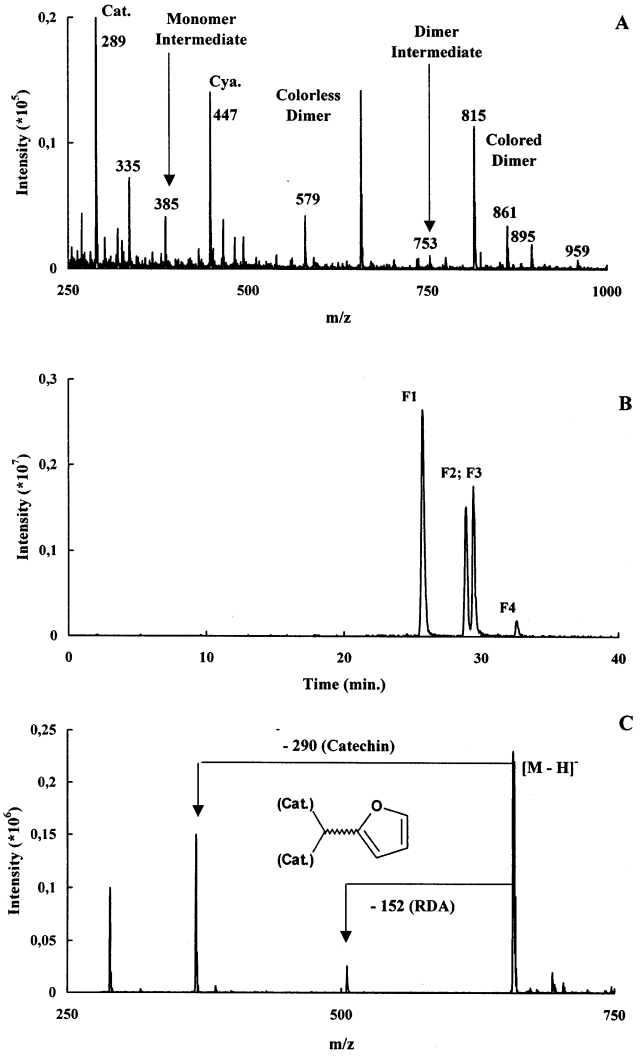

Figure 4. Mass spectrum corresponding to the entire TIC trace of a solution containing (+)-catechin, cyanidin 3-*O*-glucoside, and furfural showing the presence of various oligomeric compounds (A). An extracted ion current recorded at *m/z* 657 amu (B) and corresponding to the colorless dimers and their mass spectra are also presented (C).

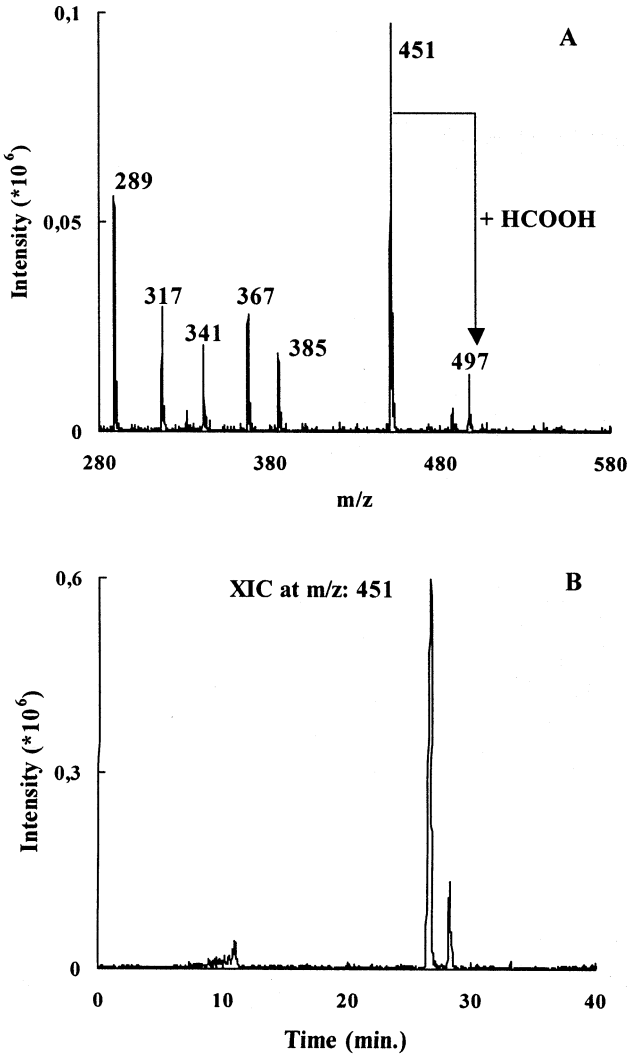

Figure 5. Mass spectrum (A) and its corresponding extracted ion current chromatogram recorded at *m/z* 451 amu (B) of the unidentified compounds detected in the mixture containing (+)-catechin, cyanidin 3-*O*-glucoside, and furfural.

dehyde, benzaldehyde, and formaldehyde (*26*). The authors reported that the highest shift was observed with formaldehyde.

In addition to the colored detected derivatives, other compounds were also detected as indicated in the chromatographic profile recorded at 280 nm between 26 and 46 min (**Figure 1A**). The UV−visible spectra of the detected compounds are shown in **Figure 1C**. Among these derivatives, compound **a** with a maximum around 490 nm may probably have a structure similar to that obtained from reaction involving malvidin 3-*O*-glucoside and acetaldehyde or furfural and resulting from a cycloaddition between the aldehyde and the anthocyanin (*21*, *27*, *28*). Compounds **b**−**f** showed absorption in the red region, meaning that their structures contained the flavylium moiety. The UV−visible spectra of these compounds shown in **Figure 1C** were similar to that of cyanidin 3-*O*-glucoside with a bathochromic shift of their visible absorption maxima, which were located at 535 nm for compounds **d**−**f** and at 540 nm for compounds **b** and **c**. The structures of these compounds may probably involve more than one unit of the flavanol or the anthocyanin moieties. This phenomenon was observed in the case of compounds involving malvidin 3-*O*-glucoside and acetaldehyde (*11*).

It must be noted that these compounds could also result from the condensation between flavanols and anthocyanins through the formation of ethyl-bridged derivatives induced by acetaldehyde as previously stated (*29*), where similar compounds with absorption maxima near 545 and 500 nm were detected by LC/DAD in a mixture containing malvidin 3-*O*-glucoside and monomeric or dimeric flavanols and in the absence of acetaldehyde. The production of acetaldehyde in hydroalcoholic solutions containing flavanols has been previously observed and can be attributed to the non-enzymatic oxidation of ethanol coupled to the autoxidation of the catechol ring of the flavanol unit (*30*). Its formation in the solution studied in this work could also explain the appearance of compounds with absorption maximum around 500 nm and resulting from a cycloaddition reaction between the anthocyanin and acetaldehyde.

LC/ESI-MS analysis of the mixture allowed us to obtain the chromatographic profile shown in **Figure 2A**, which represents the total ion current chromatogram (TIC) which showed that the two compounds named **CF1** and **CF2** were the major compounds formed in the mixture. The *m/z* values of both compounds detected at *m/z* 815 amu in the negative ion mode (**Figure 2B**) are in agreement with a molecular weight of 817 amu, which corresponds exactly to structures in which the

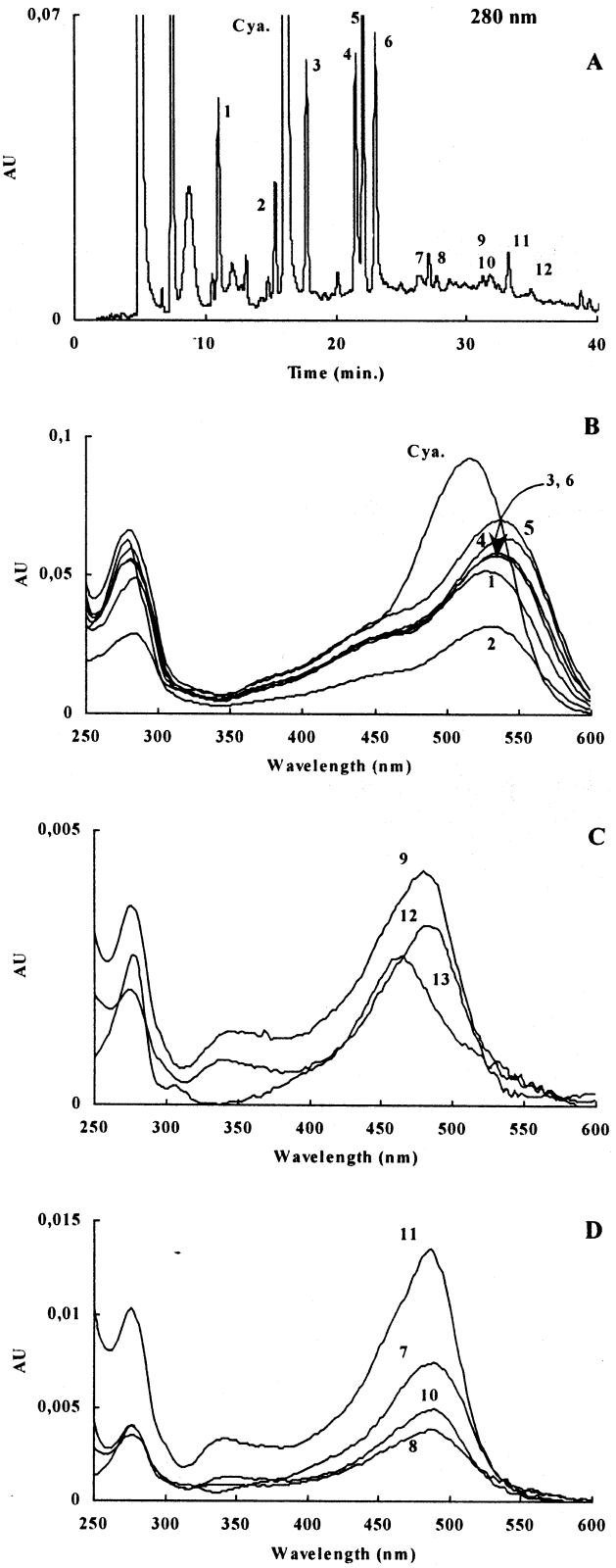

Figure 6. HPLC chromatograms of a mixture of (+)-catechin, cyanidin 3-*O*-glucoside, and HMF recorded at 280 nm showing residual polyphenols and the newly formed compounds (A). The UV–visible spectra of the major reddish and yellowish adducts are presented (B–D).

anthocyanin moiety was linked to the flavanol unit by a furfuryl bridge as shown in **Figure 3** (**IA**). In addition to the molecular ion located at 815 amu, the obtained mass spectrum showed, even with weak intensity, signals at *m*/*z* 753 amu corresponding to the loss of a glucose moiety (−162 amu) in addition to signals at *m*/*z* 289 and 447 amu corresponding to catechin and cyanidin ions.

An extracted ion current (XIC) chromatogram profile recorded at *m*/*z* 815 amu revealed the presence of the two dimer adducts **CF1** and **CF2**. It was also shown that the two dimers were stereoisomers formed through 8-8 linkage between the flavanol and the anthocyanin (*9*, *11*) and that only one A ring top of the anthocyanin can be involved in the polymerization process, whereas the two summits 6 and 8 are involved in the case of the flavanol (*11*).

In addition to these dimers, other oligomeric bridged compounds were also detected. Thus, the kinetics monitored by LC/MS allowed the detection of both homogeneous flavanol bridged compounds and those involving the flavanol and the anthocyanin (**Figure 4A**). The ion series detected at *m*/*z* 289, 385, 657, 753, and 815 could be attributed to catechin, a furfural adduct of catechin, a furfuryl-linked catechin dimer, a furfural adduct of dimer, and a colored dimer, respectively. In addition to each *m*/*z* value there were several ion peaks, suggesting that they could be regio- and/or stereoisomers owing to the existence of two reactive sites, namely, C-6 and C-8 of the flavanols and C-8 of the anthocyanin.

The signal ion observed at *m*/*z* 657 amu corresponded to a structure in which two catechin units are linked through a furfuryl bridge like that shown in **Figure 3** (**IIA**), which represents the 8-8 isomer. Due to the existence of two reactive site positions (namely, C-6 and C-8), the formation of four homogeneous dimers is then possible. This was observed in the extracted ion chromatogram recorded at *m*/*z* 657 amu as shown in **Figure 4B**. In addition to the molecular ion located at *m*/*z* 657 amu, the mass spectrum of these dimers (**Figure 4C**) recorded in the negative ion mode showed ion signals at *m*/*z* 505 and 367 amu corresponding, respectively, to the retro Diels−Alder fission and to the loss of one catechin unit. The detection of these dimers showed that the anthocyanin competed with the flavanol in the polymerization process.

As indicated above, **Figure 4A** also showed the presence of monomer and dimer intermediate adducts as their negative molecular ions observed at *m*/*z* 385 and 753 amu, respectively. Their detection 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 in the assay vial.

In addition to these compounds resulting from the polymerization processes involving furfural, (+)-catechin, and cyanidin 3-*O*-glucoside, the formation of other adducts was also observed. As an example an unidentified compound was detected at 26.5 min. Its mass spectrum presented in **Figure 5A** showed in particular signals at *m*/*z* 451 and 497 amu corresponding to the molecular ion and its stacking with a formic acid molecule, respectively. An extracted ion current chromatogram recorded at *m*/*z* 451 amu (**Figure 5B**) showed the presence of two compounds with a predominance of the first one. Its mass spectrum also showed the presence of a signal at *m*/*z* 289 amu, corresponding to a catechin unit and showing that this compound was a catechin-derived compound. Its molecular weight indicated that this may probably result from degradation and recombination of the first formed products and indicated the complex phenomenon that could take place in the studied model solutions.

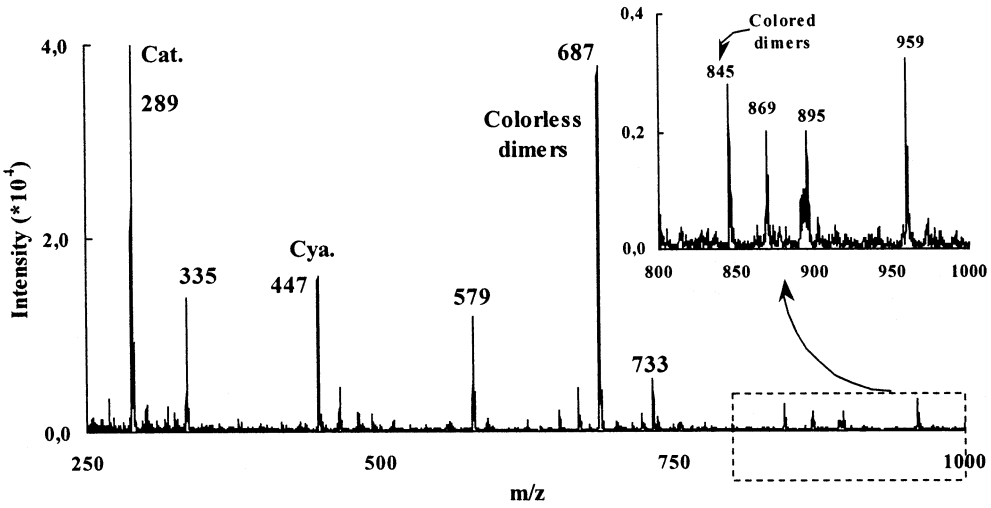

Figure 7. Mass spectrum corresponding to the entire TIC trace of a mixture of (+)-catechin, cyanidin 3-*O*-glucoside, and HMF recorded with an electrospray mass detector.

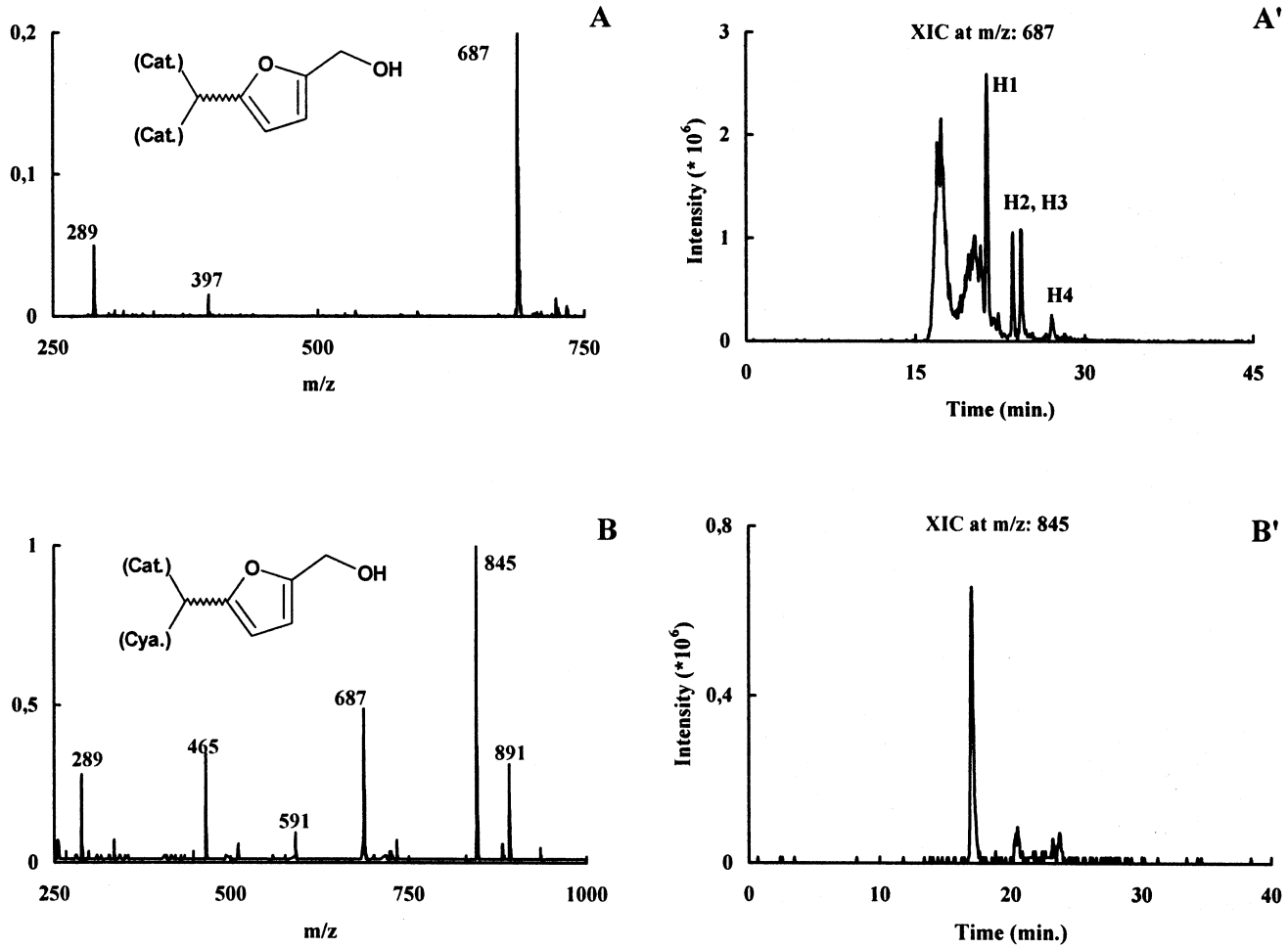

Figure 8. Mass spectra and their extracted ion current chromatograms of the colorless (A, A′) and the colored (B, B′) dimeric adducts detected in a mixture of (+)-catechin, cyanidin 3-*O*-glucoside, and HMF.

When furfural was replaced by HMF, similar compounds were obtained and reaction was faster with furfural. This difference in the reactivity of furfural and HMF was also observed when reactions were conducted with malvidin 3-*O*-glucoside and where reactions were faster with furfural (*17*). According to some previously reported results this may be due to the difference in reactivity of the two furfural derivatives as previously reported (*31*, *32*).

Reaction was first investigated by LC/DAD analysis, and the obtained results are summarized in the chromatographic profile recorded at 280 nm and shown in **Figure 6A**. The obtained results showed residual cyanidin 3-*O*-glucoside and the newly formed colored and colorless compounds. In the chromatographic profile, six major new red compounds marked **1**−**6** were detected in addition to cyanidin 3-*O*-glucoside, marked Cya. Among these colored compounds, two adducts were eluted

before the precursor anthocyanin, whereas the four others were eluted after cyanidin 3-*O*-glucoside. In such interactions involving flavanols, aldehydes, and anthocyanins, it was usually observed that the newly formed adducts were eluted later than their precursor anthocyanin (*11*, *17*).

The UV−visible spectra of these compounds in addition to that of cyanidin 3-*O*-glucoside are drawn in **Figure 6B**. The obtained results showed that the new pigments were characterized by spectra similar to that of their precursor anthocyanin, but their absorption maxima in the visible region were bathochromically shifted and located at 535 nm for compounds **1** and **2** and at around 540 nm for compounds **3**−**6**. In addition, their absorption maxima around 450 nm were more accentuated compared to that of cyanidin 3-*O*-glucoside. The fact that these pigments exhibited UV−visible absorbance spectra differing from their precursor by their higher maximum absorption wavelength in the visible range (535−540 nm in the acidic HPLC solvent) indicated that all of these compounds might be anthocyanin-derived pigments formed by condensation mechanisms, as reported in model studies (*2*, *4*, *21*).

It must be pointed out that, together with these red pigments, small amounts of other pigments showing absorbance maxima between 460 and 490 were also observed. These compounds marked from **7** to **13** are shown in the chromatogram profiles presented in **Figure 6A**. Compounds **9**, **12**, and **13** were characterized by the presence of an absorption maximum between 467 and 484 nm (**Figure 6C**), whereas the absorption maxima of compounds **7**, **8**, **10**, and **11** were located around 490 nm (**Figure 6D**). Compound **7** corresponded probably to xanthylium salts, which may or may not involve the anthocyanin, whereas the others with absorption around 490 nm and a shoulder near 350 nm may result from cycloaddition reaction between the anthocyanin and the aldehyde, giving rise to pigments as was shown with malvidin and where pigments with absorption around 500 nm were observed (*17*).

LC/ESI-MS analysis of a mixture containing (+)-catechin, cyanidin 3-*O*-glucoside, and HMF conducted in the negative ion mode allowed us to obtain the results shown in **Figure 7**, which represents the total mass spectrum of the mixture. The appearance of various signal ions pointed to the existence of an interaction between catechin and anthocyanin and HMF with the formation of new substances. The obtained mass spectrum showed in particular that condensation processes involving either catechin or catechin and cyanidin 3-*O*-glucoside occurred simultaneously in the mixture. This was demonstrated by detection of both colorless and colored dimers. The first ones involved two linked catechin units characterized by their molecular weight and detected as their negative molecular ion located at *m*/*z* 687 amu shown in their mass spectra (**Figure 8A**). This corresponds to a bridged dimeric structure consisting of two catechin units as shown in **Figure 3** (**IIB**). The second ones corresponded to the colored pigments involving both the flavanol and the anthocyanin and were detected at *m*/*z* 845 amu as shown by their mass spectra (**Figure 8B**). This corresponds exactly to a structure in which one (+)-catechin is linked through a hydroxymethylfurfuryl bridge to a cyanidin unit as shown in **Figure 4** (**IB**). These two kinds of compounds are shown in the extracted ion current chromatograms recorded, respectively, at *m*/*z* 687 and 845 amu (**Figure 8A′**,**B′**). The formation of both colorless and colored adducts demonstrated the competition between the flavanol and the anthocyanin in the polymerization process.

In addition to these compounds other derivatives were also detected. Among these xanthene and xanthylium salt derivatives

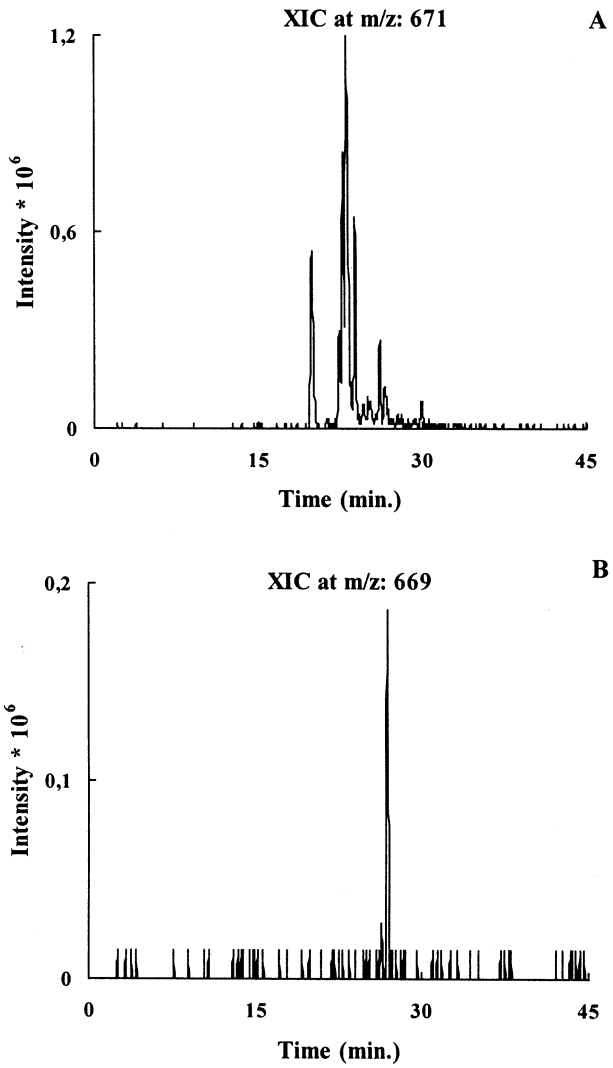

Figure 9. Extracted ion current chromatograms recorded at *m*/*z* 671 (A) and 669 amu (B) and corresponding, respectively, to the xanthene and xanthylium salts derivatives detected in a mixture of (+)-catechin, cyanidin 3-*O*-glucoside, and HMF.

were detected as their molecular ion by LC/ESI-MS conducted in the negative ion mode. Their signals located, respectively, at *m*/*z* 669 and 667 amu are in agreement with structure obtained by dehydration of the dimeric bridged derivatives giving rise to xanthene adducts, which by oxidation yield a xanthylium salt adducts as supported by the detection of yellowish compounds presenting absorption maxima around 460 nm and the extracted ion current chromatograms recorded at *m*/*z* 671 and 669 amu, corresponding to the xanthene and xanthylium salts, respectively (**Figure 9A**,**B**). It must be pointed out that the formation of these compounds was also observed when (+)-catechin was incubated with HMF and thus did not necessitate the presence of anthocyanin. It must also be noted that no xanthene adducts resulting from the colored dimeric derivative were detected. Thus, no compounds with a signal located at *m*/*z* 827 amu were observed in the total ion current chromatogram.

Finally, other compounds which may probably not result directly from the polymerization process involving the flavanol, the anthocyanin, and HMF were observed. The mass spectra of the detected compounds in addition to the extracted current chromatogram profiles recorded at their corresponding *m*/*z* value are summarized in **Figure 10**. Thus, a compound with a molecular weight of 576 amu was detected in the mixture

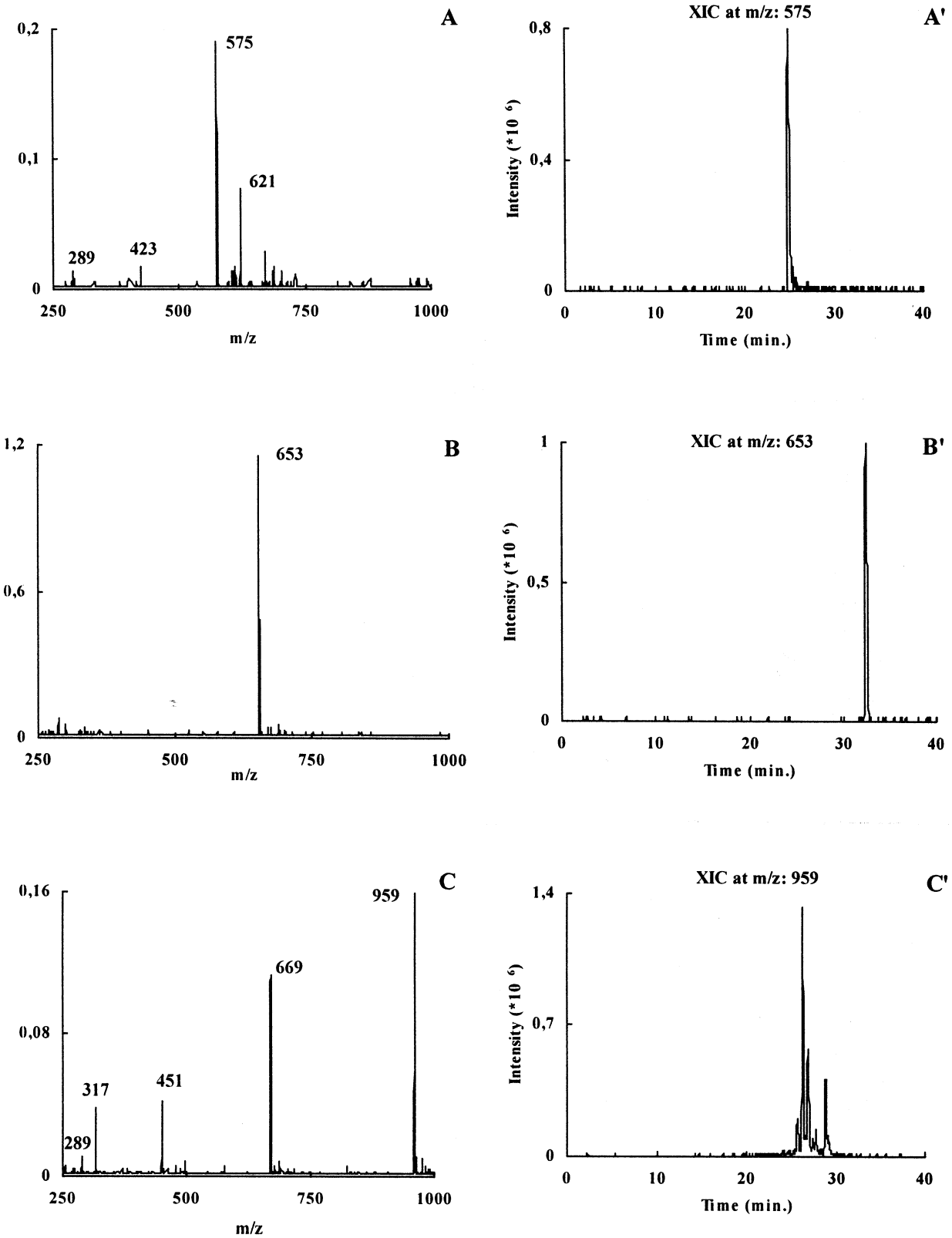

Figure 10. Mass spectra and their corresponding extracted ion current chromatograms of the unidentified derivatives detected in a solution containing (+)-catechin, cyanidin 3-*O*-glucoside, and HMF.

through LC/ESI-MS analysis conducted in the negative ion mode. Its mass spectrum (**Figure 10A**) presents, in addition to a signal located at *m*/*z* 575 amu corresponding to the molecular ion, signals at *m*/*z* 289, 415, and 423 amu and corresponding, respectively, to a catechin, a catechin HMF monomer adduct, and a retro Diels−Alder fission (−152 amu). This indicated that the corresponding adduct was a catechin-derived derivative. Only one peak was observed in the corresponding extracted chromatogram as is shown in **Figure 10A′**.

Another compound was detected at 32 min and presented a signal corresponding to the molecular ion at *m*/*z* 653 amu. The extracted ion chromatogram recorded at the same *m*/*z* value showed the presence of only one compound (**Figure 10B′**). Its mass spectrum drawn in **Figure 10B** also showed a signal at

m/*z* 289 amu, which let us suppose that it was a catechin-derived adduct. Finally, other compounds were detected in the extracted ion chromatogram recorded at *m*/*z* 959 amu (**Figure 10C′**). Their mass spectra showed, in addition to their molecular ion located at *m*/*z* 959 amu, signals at *m*/*z* 669, 451, 317, and 289 amu, indicating that these compounds may probably be catechin-derived adducts (**Figure 10C**).

From a mechanistic point of view, various condensation pathways between cyanidin and furfural involving either cyanidin keto-pseudobase, cyanidin cation, or cyanidin anhydrobase have been proposed (*26*, *33*). From a qualitative condensation test, these authors indicated that reaction of furfural was not possible with cyanidin 3-*O*-glucoside but only with its free aglycon. In this work, we showed that condensation between the glycoside and furfural or HMF was possible in the presence of (+)-catechin, and the formation of oligomeric bridged derivatives was demonstrated either by LC/DAD or by LC/ESI-MS analysis. Moreover, no compounds following the previously proposed mechanisms were detected by LC/ESI-MS analysis. Thus, no peaks were detected in the extracted ion current chromatograms recorded at *m*/*z* values corresponding to the compounds resulting from reaction between furfural or HMF and cyanidin keto-pseudobase, cyanidin cation, or cyanidin anhydrobase, respectively, as previously proposed (*26*, *33*).

Among the proposed mechanisms, that proposed by Debicki-Pospisil et al. (*26*), which concerned the reaction between cyanidin cation and furfural, corresponded in fact to an acetalization between the aldehydic group of furfural and the anthocyanin B ring dihydroxyl group, giving rise to a structure like that shown in **Figure 3** (**III**). As indicated above, the resulting compound was not detected in our study, either with the aglycon or with the glucoside. As the reaction involves the B ring dihydroxyl group, we tried to see if the reaction could be possible with (+)-catechin through its 3′,4′-dihydroxyl group, giving rise to the acetal compound shown in **Figure 3** (**IV**) with a molecular weight of 368 amu. An extracted ion current chromatogram recorded at *m*/*z* 367 amu showed a chromatographic profile similar to that recorded at *m*/*z* 657 amu (**Figure 4B**) with the presence of four adducts with a retention time similar to those of the dimeric compounds. This let us suppose that the obtained peaks corresponded in fact to **F1**, **F2**, **F3**, and **F4** adducts. The mass spectrum of one of them showed the same spectrum as those obtained for the dimeric derivatives with a signal ion of 657 amu corresponding to its molecular ion. This confirmed that the four peaks corresponded to the four dimeric adducts, which by loss of a catechin unit yielded the ions detected at *m*/*z* 367 amu. This demonstrated that the acetalization reaction between (+)-catechin and furfural did not occur in the analyzed mixture. This did not occur between the anthocyanin glucoside and furfural, showing thus the prevalent reactions occurring between flavanols and anthocyanins in the presence of aldehydic derivatives are the condensation processes yielding various bridged colored and colorless compounds.

CONCLUSION

On the basis of this investigation, it appears that furfural compounds play a major role in the polymerization process of flavanols and anthocyanins. Thus, various bridged oligomeric and polymeric adducts that finally precipitate were obtained. Such a reaction may contribute to the decrease of astringency and the change of color observed during the aging of grape-derived foods. The use of cyanidin 3-*O*-glucoside in this work instead of malvidin 3-*O*-glucoside showed that similar bridged compounds were obtained. Thus, the four colorless dimers in addition to colored adducts involving both the flavanol and the anthocyanin were obtained. However, in the case of cyanidin 3-*O*-glucoside the colored compounds seemed to be more stable and were obtained in sufficient amounts compared to those obtained when the reaction was conducted with malvidin 3-*O*-glucoside. This fact was observed with either furfural or HMF. Mechanistically speaking, the results presented here demonstrated clearly that the previously reported reaction pathways between furfural and cyanidin did not occur in the studied model solutions.

The formation of both flavanol−furfuryl and anthocyanin−furfuryl−flavanol adducts demonstrated the competitive action of flavanols and anthocyanins in the condensation process with a predominant formation of the colored ones. The detection of compounds exhibiting UV−visible spectra similar to those of xanthylium salts constitutes a new support for their contribution to color change and browning. Finally, the formation of other unidentified compounds in the studied model solutions was observed and demonstrated the complexity of such a mixture. This opened other perspectives for the investigation and exploration of such model solutions in order to understand the complex phenomenon that occurred during the maturation, storage, and aging of foods and fruit-derived foods and beverages where other polyphenolic compounds could react, such as (+)-catechin, and malvidin 3-*O*-glucoside or cyanidin 3-*O*-glucoside. 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 anthocyanin present.

LITERATURE CITED

(1) Somers, T. C. The polymeric nature of red pigments. *Phytochemistry* **1971**, *10*, 2175−2186.

(2) Timberlake, C. F.; Bridle, P. Interactions between anthocyanins phenolic compounds and acetaldehyde. *Am. J. Enol. Vitic.* **1976**, *27*, 97−105.

(3) Ribereau-Gayon, P.; Pontallier, P.; Glories, Y. Some interpretations of colour changes in young red wines during their conservation. *J. Sci. Food Agric.* **1983**, *34*, 505−516.

(4) Liao, H.; Cai, Y.; Haslam, E. Polyphenols Interaction. Anthocyanins: Copigmentation and colour change in red wines. *J. Sci. Food Agric.* **1992**, *59*, 299−305.

(5) Fulcrand, H.; Docco, T.; Es-Safi, N.; Cheynier, V.; Moutounet, M. Study of acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry. *J. Chromatog. A* **1996**, *752*, 85−91.

(6) Saucier, C.; Bourgeois, G.; Vitry, C.; Roux, D.; Glories, Y. Characterization of (+)-catechin-acetaldehyde polymers: a model for colloidal state of wine polyphenols. *J. Agric. Food Chem.* **1997**, *45*, 1045−4049.

(7) Haslam, E. In vini veritas: oligomeric procyanidins and the aging of red wines. *Phytochemistry* **1980**, *19*, 2577−2582.

(8) Bakker, J.; Picinelli, A.; Bridle, P. Model wine solutions: color and composition changes during aging. *Vitis* **1993**, *32*, 111−118.

(9) Escribano-Bailon, M. T.; Dangles, O.; Brouillard, R. Coupling reactions between flavylium ions and catechin. *Phytochemistry* **1996**, *41*, 1583−1592.

(10) Es-Safi, N.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Competition between (+)-catechin and (−)-epicatechin in acetaldehyde-induced polymerization of flavanols. *J. Agric. Food Chem.* **1999**, *47*, 2088−2095.

(11) Es-Safi, N.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-*O*-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47*, 2096−2102.

(12) Es-Safi, N.; Le Guernevé, C.; Labarbe, B.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Structure of a new xanthylium derivative. *Tetrahedron Lett*. **1999**, *40*, 5869−5872.
(13) Es-Safi, N.; Le Guernevé, C.; Cheynier, V.; Moutounet, M. New polyphenolic compounds with xanthylium skeletons formed through reaction between (+)-catechin and glyoxylic acid. *J. Agric. Food Chem*. **1999**, *47*, 5211−5217.
(14) Es-Safi, N.; Le Guernevé, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Xanthylium salts involved in wine color evolution. *Int. J. Food Sci. Technol*. **2000**, *35*, 63−74.
(15) Es-Safi, N.; Le Guernevé, C.; Cheynier, V.; Moutounet, M. New phenolic compounds obtained by evolution of (+)-catechin and glyoxylic acid in hydroalcoholic medium. *Tetrahedron Lett*. **2000**, *41*, 1917−1921.
(16) Es-Safi, N.; Le Guernevé, C.; Cheynier, V.; Moutounet, M. New polyphenolic compounds formed by evolution of (+)-catechin in hydro-alcoholic solution and their implication in color changes of grape-derived foods. *J. Agric. Food Chem*. **2000**, *48*, 4233−4240.
(17) Es-Safi, N.; Cheynier, V.; Moutounet, M. Study of the reaction between (+)-catechin and furfural derivatives in the presence of anthocyanins and their implication in food color change. *J. Agric. Food Chem*. **2000**, *48*, 5946−5954.
(18) Jurd, L.; Somers, T. C. The formation of xanthylium salts from proanthocyanidins. *Phytochemistry* **1970**, *9*, 419−427.
(19) Dallas, C.; Ricardo-Da-Silva, J. M.; Laureano, O. Products formed in model wine solutions involving anthocyanins, procyanidin B2, and acetaldehyde. *J. Agric. Food Chem.* **1996**, *44*, 2402−2407.
(20) Fulcrand, H.; Cheynier, V.; Oszmianski, J.; Moutounet, M. An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry* **1997**, *46*, 223−227.
(21) Cameira Dos Santos, P. J.; Brillouet, J. M.; Cheynier, V.; Moutounet, M. Detection and partial charaterisation of new anthocyanin-derived pigments in wine. *J. Sci. Food Agric*. **1996**, *70*, 204−208.
(22) Cheynier, V.; Fulcrand, H.; Sarni, P.; Moutounet, M. Application des techniques analytiques à l'étude des composés phénoliques et de leurs réactions au cours de la vinification. *Analusis* **1997**, *25*, M14−M21.
(23) Saucier, C.; Little, D.; Glories, Y. First evidence of acetaldehyde-flavanol condensation products in red wine. *Am. J. Enol. Vitic*. **1997**, *48*, 370−373.
(24) Mateus, N.; Silva, A. M. J.; Vercauteren, J.; De Freitas, V. Occurrence of anthocyanin-derived pigments in red wine. *J. Sci. Food Agric.* **2001**, *49*, 4836−4840.
(25) Garcia-Viguera, C.; Bridle, P.; Bakker, J. The effect of pH on the formation of coloured compounds in model solutions containing anthocyanins, catechin and acetaldehyde. *Vitis* **1994**, *33*, 37−40.
(26) Debicki-Pspisil, J.; Lovric, T.; Trinajstic, N.; Sabljic, A. Anthocyanin degradation in the presence of furfural and 5-hydroxymethylfurfural. *J. Food Sci*. **1983**, *48*, 411−416.
(27) Bakker, J.; Bridle, P.; Honda, T.; Kuwano, H.; Saito, N.; Terahara, N.; Timberlake, C. F. Isolation and identification of a new anthocyanin occurring in some red wines. *Phytochemistry* **1997**, *44*, 1375−1382.
(28) Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1998**, *47*, 1401.
(29) Francia-Aricha, E. M.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Effect of malvidin-3-monoglucoside on the browning of monomeric and dimeric flavanols. *Z. Lebensm. Unters. Forsch. A* **1998**, *207*, 223−228.
(30) Wildenradt, H. L.; Singleton, V. L. The production of acetaldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Vitic*. **1974**, *25*, 119−126.
(31) Burton, H. S.; McWeeny, D. J.; Biltchiffe, D. O. Non-enzymic browning: The role of unsaturated carbonyl compounds as intermediates and of SO_2 as an inhibitor of browning. *J. Sci. Food Agric*. **1963**, *14*, 911.
(32) Kanner, J.; Fishbein, J.; Shalom, P.; Harel, S.; Ben-Gara, I. Storage stability of orange juice concentrate packaged aseptically. *J. Food Sci*. **1982**, *47*, 429−431.
(33) Tinsley, I. J.; Bockian, A. H. Some effects of sugars on the breakdown of pelargonidin-3-glucoside in model systems at 90 °C. *Food Res.* **1960**, *25*, 161.

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