Formation of Flavanol-aldehyde Adducts in Barrel-aged White Wine – Possible Contribution of These Products to Colour

N. Vivas1*, M.F. Bourden Nonier1, C. Absalon2, V. Lizama Abad1, F. Jamet1, N. Vivas de Gaulejac1, C. Vitry3 and É. Fouquet4

(1) Demptos coopersage posted to ISM UMR No. 5255/CESAMO, Université Bordeaux 1, 351 cours de la libération, F-33405 Talence, France
(2) ISM UMR No. 5255/CESAMO, Université Bordeaux 1, 351 cours de la libération, F-33405 Talence, France
(3) Departamento de Tecnología de alimentos, Universidad Politécnica de Valencia, Camino de vera, s/n 46022 Valencia, Spain
(4) ISM UMR No. 5255/COO, Université Bordeaux 1, 351 cours de la libération, F-33405 Talence, France

Submitted for publication: July 2008
Accepted for publication: September 2008
Key words: wine; aldehydes; colour; LC-ES/MS; HPLC-DAD

This paper describes the formation and diversity of new compounds resulting from the polymerisation of furanic and phenolic flavanol-aldehydes with HPLC-DAD and LC-ES/MS analysis. Polymerisation, resulting from nucleophilic reactions, formed dimers, trimers, soluble and insoluble polymers. Reactions in hydroalcoholic solution with pure aldehydes (phenolic and furanolic) and flavans (catechin) were studied. The study was repeated with different aldehydes in white wine. This research focused particularly on the colour properties of the released products and their potential impact on the colour of white wine. Some products were purified and isolated; these were mainly catechin-furfuraldehyde, catechin-methyl-5-furfuraldehyde, catechin-hydroxymethyl-furfuraldehyde, catechin-vanillin, and catechin-syringaldehyde dimers. The most powerful coloured products resulted from furanolic aldehydes. Over the course of the experiment, the reaction produced dimers, trimers and oligomers. After 50 to 60 days, the colour of the solution was mainly due to soluble polymeric forms. In addition, the role of SO2 generally used during vinification and ageing, was studied. The influence of SO2 on the kinetics of the reaction was limited.

The high reactivity of flavans and related compounds such as proanthocyanidins is well known (Cheynier, 2005). All such reactions affect wine, polyphenol stability, tannin palate texture (e.g. astringency) and wine quality (Haslam, 1980; Vivar-Quitina, 2002; Salas et al., 2003). Both oxygen and oxidation represent important parameters for the evolution of wine composition during winemaking, ageing and bottled conservation (Vivas & Glories, 1996; Atanasova et al., 2002; Vivas et al., 2005). Colour provides the first contact with a wine in a tasting session and has a strong influence on the remainder of the tasting (Morrot et al., 2001). In red wines, colour characteristics and evolution are often studied, especially by direct linkage with the polymerisation of anthocyanin-tannin complexes (Salas et al., 2004) or with the addition of aldehydes such as acetaldehyde (Fulcrand et al., 1996), glyoxalic acid, pyruvic acid, furfuraldehyde and hydroxymethyl-furfuraldehyde (Fulcrand et al., 1998; Es-Safi et al., 2002), vinyl phenols (Hakansson et al., 2003) or various aldehydes from spirits (Pissara et al., 2004). Some of the products formed display different colours than the originals. The new pigments significantly affect the wine’s colour and stability (Freitas & Mateus, 2006). Due to the lower concentration of proanthocyanidins, flavanol-aldehyde reactions have been studied less in the relation to white wines.

All research that is specifically concerned with white wine has been carried out for the purpose of elucidating the molecular origin of the phenomenon. No chemical change in colour occurred during the evolution of white wine except for the oxidation of tannins (Simpson, 1982). Some studies only focused on the observation of colour by spectrophotometric methods used to directly measure absorbance at specific wavelengths (in general 400 to 440 nm) or after calculation, e.g. using the CIE L*, a*, b* system (Gomez-Miguez et al., 2006; Recamales et al., 2006). Given the very low level of polyphenols in white wine (Vivas et al., 2003), it is generally accepted that the origin of the colour is assigned mainly to tannins and partly to flavonols (quercetin, etc.) (Ribéreau-Gayon et al., 2000). Yet, in both white and red wines, all the chemical (presence of flavans and tannins) and physicochemical (low pH) conditions are present to initiate nucleophilic additions of different aldehydes to flavanol-flavanol or proanthocyanidin-proanthocyanidin complexes (Es-Safi et al., 2002; Laurie & Waterhouse, 2006). These reactions produced numerous new structures with a greater variety of colour (yellow, orange, brown), some of them very similar to the diverse colours of white wine.

The composition and quality of some white wines fermented and aged in new oak barrels were altered, particularly in flavour and colour (Towel & Waterhouse, 1996; Herjavec et al., 2007). This suggests the possibility of a condensation reaction with flavonols, proanthocyanidins and some aldehydes from the oak. These reactions could affect the colour of the white wine in contact with the oak, and change the visual feel and wine evaluation. In fact, in the case of white wine, there are enough proanthocyanidins (10 to 100 mg/L) and flavonols (5 to 20 mg/L) (Cheynier et al., 1998; Vivas et al., 2003) to produce, via this reaction, sufficient coloured products to contribute to the evolution of the colour.

In this paper, we describe both the effects of proanthocyanidin, flavonoid and aldehyde interactions on the colour of model solutions, and the actual impact and potential influence of nucleophilic additions of furanic and phenolic aldehydes from oak wood on the change in the initial colour of wine. To mimic these reactions during the ageing process, we followed the reactions for...
Flavanol-Aldehyde Adducts in Barrel-Aged Wine

a maximum of 60 days, mainly because the main transformations take place during the first two or three months, corresponding to the maximum free aldehyde content in the wine. To increase the reaction, we conducted all the experiments at 20°C, because at the normal temperature of the cellar (13 to 17°C) the kinetics of the reaction were very low.

MATERIALS AND METHODS

Reagents

Deionised water was purified using a Milli-Q system (Millipore, Bedford, MA) prior to use. Acetonitrile, methanol, ethanol and formic acid were obtained from Prolabo (Pessac, France). Sodium hydroxide was purchased from Aldrich (Saint-Quentin, France). (+)-Catechin, tartaric acid, furfuraldehyde, hydroxymethylfurfuraldehyde (HMF) and methyl-5-furfuraldehyde were purchased from Aldrich (Saint-Quentin, France). Syringaldehyde and vanillin were obtained from Aldrich (Saint-Quentin, France). The SO₂ in NaHSO₃ form came from Prolabo (Fontenay-sous-Bois, France). The Enzymatic Kit for acetaldehyde determination was obtained from Boehringer (Ingelheim, France).

Condensation reactions (flavanol-aldehyde adducts)

The wine-testing solution (HAS) used was 12% ethanol (EtOH) buffered to pH 3.5 (5 g/L tartaric acid, 1 N NaOH at pH 3.5). Two pH values from 3.0 to 3.5 were obtained by adding 1 N NaOH to the medium described above, and adjusted using a pH-meter. The aim was to drive the reactions to produce pigments; for that, (+)-catechin and aldehydes were incubated in wine-like solution at the more appropriate ratio of polyphenol/aldehyde concentration. (+)-Catechin (1.65 mM/L) was prepared with each of the resulting solutions (50 mL). This concentration, corresponding to 0.5 g/L, was 10 times more compared to the average concentration of procyanidins in white wines. The aldehyde derivatives (furfuraldehyde, hydroxymethyl-furfuraldehyde, methyl-5-furfuraldehyde, vanillin, syringaldehyde) were added at a concentration of 3.14 mM/L. The ratio 1.65/3.14 was a good balance for rapid synthesis of nucleophilic adducted in accordance to our previous work (Nonier et al., 2007).

The prepared solutions were stored with a small headspace under nitrogen in order to avoid oxidation. They were incubated at

![Diagram of compounds](image_url)
Evolution over time of a synthetic solution of furfuraldehyde and catechin, as measured by HPLC. Tentative identification of the main products was performed by ESI-MS. Experimental conditions: aqueous-alcohol solution (12% vol EtOH, 5 g/L tartaric acid adjusted to pH 3.5 with NaOH N), temp 20°C, in the dark. The solution contained 1.65 mM/L of catechin and 3.14 mM/L of furfuraldehyde (or another aldehyde).

20°C in a dark room. The reactions were monitored periodically by liquid chromatography (LC), coupled with UV detection, and electrospray ionisation mass spectrometry (ESI-MS) detection for periods that varied according to the nature of the aldehyde. Quantification of residual (+)-catechin and of all the compounds formed was achieved using peak areas at 280 nm. The products formed, particularly oligomers and polymers, were identified by UV-visible, high-resolution MS and LC-MS3 analysis (Nonier Bourden et al., 2008a). In additions, we used low pressure chromatography to purify each main compound produced with all the selected aldehydes and determined the structure by homo- and hetero-2D NMR of 1H, 13C, using the same procedures as Nonier et al. (2006a) for furfuraldehyde. We obtained nine different standards for HPLC calibration (Fig. 1): three dimers of furfuraldehyde (6-6, 8-8, 6-8), two dimers of hydroxymethyl-furfuraldehyde (8-8, 6-8), two dimers of methyl-5-furfuraldehyde (8-8, 6-8), one dimer of vanillin (8-8) and one dimer of syringaldehyde (8-8). The same experiments were repeated with SO2 to study its influence on the reactivity of the solutions. An amount of 30 mg/L of free NaHSO3 was added to each solution, and the SO2 concentration was checked by the classical iodometric method (OIV, 2002).

The samples were prepared and subsequently analysed in triplicate. The results that are presented were the average of triplicates. The averages present a variation of less than 10%.

**HPLC/UV-visible analysis**

HPLC/UV-visible analyses were performed using a Waters separation module system, a Waters UV-visible detector, and Milenium 32 Chromatography Manager software. UV-visible spectra were recorded at both 280 nm and 440 nm. The column was a reverse-phase Intercim C18 (10 μm packing, 250 x 4.7 mm i.d.), protected by a guard column of the same material. Solvent A was water:formic acid (98:2, v/v) and solvent B acetonitrile:water:formic acid (80:18:2, v/v). The column was left at ambient
Flavanol-Aldehyde Adducts in Barrel-Aged Wine

0.12

Hydroxymethyl-furfuraldehyde 0.12 97 Furfuraldehyde

0.10

54

CD

0.08

0.08

C

0

0.06

0.06

0.04 0.04

0.02 0.02

15

0

350 400 450 500 550 600 350 400 450 500 550 600

Wavelength (nm)

Wavelength (nm)

FIGURE 3
Visible spectra for some aldehyde-catechin solutions. For experimental conditions, see Fig. 2.

temperature. The elution program was performed at a constant flow of 1 mL/min, with a gradient of 5 to 30% of Solvent B in 40 min, and then rising to 40% of Solvent B in the next 10 min, and finally to 100% of Solvent B in the subsequent 5 min. This was followed by washing and re-equilibrating the column over a period of 15 min. The injection volume was 20 L.

LC/ESI-MS analysis
MS measurements were performed on a LCQ ThermoTM instrument with an electrospray ionisation source, in negative-ion mode, with a spray voltage of 4.5 kV and a capillary temperature of 275°C. The LC was the same as in the HPLC/UV-visible section. The conditions for LC were identical to those described above.

Absorption spectra
Spectrophotometric measurements (at 420 nm and 440 nm) were performed using an Anthelie SecomamTM spectrophotometer, and UV-visible spectra were recorded using a spectrophotometer fitted with a quartz cell (1 cm). The wine-like samples were diluted in hydroalcoholic solution (HAS) (1/20) to adjust the optical density to lower than 1.5, and then their spectra were measured. Solutions of pure dimers, oligomers and polymers of catechin-aldehyde adducts (at 1% w/v) were prepared in an aqueous-alcohol solution (12% vol, ethanol:water) adjusted to different pH levels, and absorbance at 420 nm was measured. The prepared solutions were stored in the dark and under nitrogen in order to avoid oxidation during measurements. Each sample was filtered prior to the UV-visible and absorbance measurements.

RESULTS
Flavanol-aldehyde reactions
In an acidic medium, the flavanols with the aldehydes easily produced several nucleophilic adducts, as shown in Fig. 2 for the furfuraldehyde example. For the first 10 days of the analysis, the reaction led to a dimeric form, then to trimeric and oligomeric forms and, finally, after one month, to a polymeric form. For each of the aldehydes, the colour of the solution evolved over the course of the experiment, mainly in the range of 400 to 450 nm (Fig. 3). Vanillin and syringaldehyde produced a slight increase in colour intensity in comparison to furanic aldehydes. In particular, furfuraldehyde and hydroxymethyl-furfuraldehyde quickly produced a higher level of coloured matter or products with high colouring power. The maximum absorbance level after 54 days was 0.11 and 0.08 for hydroxymethyl-furfuraldehyde and furfuraldehyde respectively. The colour of the solutions, estimated

Flavanol-Aldehyde Adducts in Barrel-Aged Wine

Hydroxymethyl-furfuraldehyde

Furfuraldehyde

Vanillin

Syringaldehyde

FIGURE 4
Evolution of the main chromatographic peak during reaction between aldehydes and catechin. Numbers 1 to 6 mark the order of appearance of the peaks. For experimental conditions, see Fig. 2. Curves 1-4: dimers; Curves 5-6: trimers. For vanillin we do not have curve 2, as for other aldehydes.

The colour levels of the main products were measured after purification and compared to those of catechin and quercetin. Quercetin was chosen because it is generally admitted that flavonoids and particularly quercetin can affect the colour of white wines. The yellow colour of the wine-like solution with 1% (w/v)
of each compound, as measured at 420 nm, displayed a different colour intensity (Table 1). All the catechin-aldehyde adducts displayed a higher colour intensity than catechin. Dimers formed with furfuraldehyde and syringaldehyde exhibited a colour almost identical to that of quercetin at pH 3.5 (E<sub>420</sub> value: 2.6, 2.8, and 3.7 respectively), the average pH of white wines; and the catechin-vanillin dimer was the least coloured product. The dimer with methyl-5-furfuraldehyde was the most powerfully colouring product (E<sub>420</sub>: 14.2). A dimer adduct, produced from two procyanidins-B3 (catechin-catechin) and one furfuraldehyde displayed an interesting value (E<sub>420</sub>: 3.2). Oligomers (a mix of trimers and tetramers) showed a colouring intensity of above 10 (E<sub>420</sub>: 10.4, 12.5).

However, during the reaction, dimers and trimers were quickly replaced by the high molecular weight form of the adduct. As shown in Fig. 6, the colour of the solution after 54 days at 440 nm was due to oligomers and soluble polymers, characterised by a late retention time and visualized at 280 nm. All of these compounds were less polar than the dimers and were non-ionisable under electrospray conditions (ES<sup>-</sup>, ES<sup>+</sup>). Consequently, further purification of these compounds is required, as is an NMR investigation to elucidate their exact structure. This work is in progress.

Nevertheless, this form of coloured matter shows specific spectra and interesting absorbance properties in the visible range (Fig. 7). The maximum intensity is located between 420 and
Furfuraldehyde

Hydroxymethyl-furfuraldehyde

Methyl-5-furfuraldehyde

Vanillin

Syringaldehyde

455 nm in the yellow region. Syringaldehyde adducts show an additional shoulder around 500 to 520 nm in the red region. The large majority of compounds that were formed exhibit a light and bright yellow colour (λ\text{max}: 400 to 430 nm) and a deep brown-yellow colour (λ\text{max}: 430 to 460 nm).

Influence of SO₂

The bisulphite ion is generally reactive with aldehydes, in particular acetaldehyde, forming a sulphite-aldehyde combination (Blouin, 1966). In the case of furanic and phenolic aldehydes, a similar reaction can occur to limit the formation of catechin-aldehyde adducts. The effects of the oenological level of SO₂ in one furanic aldehyde (e.g. hydroxymethyl-furfuraldehyde) and one phenolic aldehyde (e.g. vanillin) were compared for a period of 80 days (Fig. 8). In the case of wine-like solutions and white wine, SO₂ at 30 mg/L (free SO₂) slowed the kinetics of the reaction, but only by 5 to 10%, and its effect was limited. In addition, SO₂ produced some other structures, including catechin aldehyde SO₂ structures which also have a yellow colour (Nonier Bourden et al., 2008b).

Experiments on white wines

A young white wine that had undergone a complete winemaking process without wood contact and that was clear coloured was selected. It was divided into six different samples, to each of which was added one of the tested aldehydes. The concentrations were adjusted for each aldehyde to represent 10 times more than the average concentration in wine aged with wood contact and in accordance with the proportion of each of the aldehydes in wine (Chatonnet et al., 1992). During the reaction (at room temperature in the dark), the colour was followed using the L*, a*, b* system.

TABLE 1
Colorimetric properties of catechin, quercetin and different catechin-aldehyde products.
(Measure of E\textsuperscript{420nm} \* in hydroalcoholic solution)

<table>
<thead>
<tr>
<th></th>
<th>pH 2.0</th>
<th>pH 3.5</th>
<th>pH 4.0</th>
<th>pH 5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>0.72</td>
<td>0.36</td>
<td>0.61</td>
<td>0.25</td>
</tr>
<tr>
<td>Quercetin</td>
<td>3.28</td>
<td>3.74</td>
<td>5.38</td>
<td>6.87</td>
</tr>
<tr>
<td>Catechin-furfuraldehyde dimer</td>
<td>2.35</td>
<td>2.60</td>
<td>2.90</td>
<td>2.90</td>
</tr>
<tr>
<td>Catechin-methyl-5-furfuraldehyde dimer</td>
<td>14.35</td>
<td>14.22</td>
<td>14.74</td>
<td>13.64</td>
</tr>
<tr>
<td>Catechin-vanillin dimer</td>
<td>1.35</td>
<td>1.35</td>
<td>1.60</td>
<td>1.10</td>
</tr>
<tr>
<td>Catechin-syringaldehyde dimer</td>
<td>3.28</td>
<td>2.84</td>
<td>2.69</td>
<td>2.65</td>
</tr>
<tr>
<td>Procyanidin B3-furfuraldehyde</td>
<td>–</td>
<td>3.20</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catechin-furfuraldehyde oligomers</td>
<td>9.85</td>
<td>10.45</td>
<td>11.00</td>
<td>10.80</td>
</tr>
<tr>
<td>Catechin-hydroxymethyl-furfuraldehyde oligomers</td>
<td>–</td>
<td>12.50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catechin-vanillin oligomers</td>
<td>2.31</td>
<td>2.94</td>
<td>3.16</td>
<td>2.26</td>
</tr>
</tbody>
</table>

*O.D. at 420 nm of 10 g/L of each compounds (12% vol. ethanol, pH adjusted by 1N NaOH)
Spectrophotometric measures were performed after appropriate dilution
E\textsuperscript{420} index was corrected from dilution level

FIGURE 7
Visible spectra of the main coloured compounds identified in Fig. 6.
FIGURE 8
Impact of SO₂ in a wine-like solution (see Fig. 2) and in a white wine (produced without wood contact) on the reaction rate, as estimated by the residual aldehyde content in the solution, measured by HPLC. Free SO₂ concentration was adjusted to 30 mg/L. No significant quantity of acetaldehyde was formed (checked by enzymatic method).

FIGURE 9
a*, b* representation of the colour (in the La*b* system) of a white wine before (0) and after 56 days of reaction (t) with different aldehydes. T: control; F: furfuraldehyde (40 mg/L); M5F: methyl-5-furfuraldehyde (6 mg/L); HMF: hydroxymethyl-furfuraldehyde (22 mg/L); V: vanillin (16 mg/L); S: syringaldehyde (12 mg/L).

FIGURE 10
Extract at 54 to 61 min of white wine chromatogram at 440 nm with different aldehydes in comparison to controlled wine after 56 days of reaction. (furfuraldehyde, 40 mg/L; hydroxymethyl-furfuraldehyde, 22 mg/L; syringaldehyde, 12 mg/L).


White wine + hydroxymethyl-furfuraldehyde
White wine + furfuraldehyde
White wine + syringaldehyde
Control white wine
As shown in Fig. 9, the results were collected at the beginning and again at the end of the experiment (56 days later). A variation in the b* parameter was detected with respect to the control sample. Important differences were noted in the furfuraldehyde, methyl-5-furfuraldehyde, vanillin and syringaldehyde samples.

After 56 days, the chromatogram revealed oligomeric and polymeric forms with high absorbance in the 400 to 455 nm range, as previously reported for wine-like solutions (Fig. 10). Therefore, the reaction took place in the wine rather than in the synthetic solution, and resulted in a similar form with a specific colour.

**DISCUSSION**

In low-pH media, aldehydes can react with flavan-3-ols and proanthocyanidins. The resulting compounds influence the colour of solutions, mainly the yellow. These reactions are particularly well known in the case of red wines. During the ageing process we observed a polymerisation reaction with acetaldehyde in particular (Haslam, 1980; Fulcrand et al., 1996; Es-Safi et al., 1999). The resulting products have an effect on the colour of the solution, with varying impact depending on the polymeric degree and pH (Es-Safi et al., 2000). Acetaldehydes are produced after oxidation or by yeast during alcoholic fermentation (Cheynier, 2005).

Some wines were aged in new barrels and, after some time, they exhibited different colours, aromas and taste than did the same wine aged in stainless steel or concrete tanks (Boïdard et al., 1989; Sefton et al., 1989; Cerdan-Garde & Ancín-Azpilicueta, 2006; Herjavec et al., 2007). This might be due to the extraction of wood components such as polyphenols and flavours, in addition to oxidative reactions. Furfuraldehyde, vanillin and syringaldehyde turned out to be the main aldehydes in wood (Puech, 1966). Their concentration was limited in fresh wood (less than 1 mg), but increased significantly up to several milligram during toasting in cooperage production (Cadahia et al., 2003). The source of production was the pyrolytic reactions of polysaccharides and lignins (Nonier et al., 2006b). Dependent upon the initial concentrations of reaction precursors, and by analysing the colouring power of the released products, it was possible to find enough polymeric components to modify the colour of the wine, even if SO₂ slightly limited the mechanism to around 5 to 10% in comparison with the control solution without SO₂. On the other hand, SO₂ had a limited impact on the extraction and final concentration of furanic and phenolic aldehydes in wines aged in new oak barrels (Ancin et al., 2004).

Ageing on yeast lees is an important technique in the ageing process of white wine, and it is particularly important in barrel ageing. In this case, the fining effect of lees can affect the level of total aldehydes by reducing aldehydes to their corresponding alcohol forms (Marsal et al., 1988). Finally, these two last parameters probably influence a large part of the aldehyde-flavanol reactions. Further investigation into this is needed.

**LITERATURE CITED**


