

Effect of ellagitannins, ellagic acid and volatile compounds from oak wood on the (+)-catechin, procyanidin B1 and malvidin-3-glucoside content of model wines

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Abstract

Background and Aims: During ageing in oak barrels, wine undergoes changes because of the release of polyphenols and other molecules from wood. The aim of this study was to evaluate the influence of some oak wood-derived volatile compounds, ellagic acid and oak wood extracts on the levels of (+)-catechin, procyanidin B1 and malvidin-3-glucoside.

Methods and Results: Phenolics and the oak wood derived volatile compounds studied were quantified by HPLC and by GC, respectively. Additionally, the new compounds formed in the solutions were characterised by their spectral properties. Ellagic acid and/or oak wood extracts slowed the decline in the levels of (+)-catechin and procyanidin B1. In contrast, the decrease in malvidin-3-glucoside was more pronounced in the presence of ellagic acid and oak wood chip extracts. Furfural slowed (+)-catechin degradation, while breakdown of malvidin-3-glucoside was slightly more pronounced in the presence of guaiacol, furfural, vanillin and eugenol. (+)-Catechin, procyanidin B1 and malvidin-3-glucoside did not significantly affect the rate of the degradation of ellagitannins during the storage time studied. Finally, new HPLC peaks were detected in the solutions containing (+)-catechin and ellagic acid, as well as with malvidin-3-glucoside with ellagic acid and oak wood extract.

Conclusions: Malvidin 3-glucoside and (+)-catechin and procyanidin B1 presented distinct behaviours during time in the presence of volatile and non-volatile compounds from oak wood.

Significance of the Study: This work points out the importance of oak wood components in the degradation of anthocyanins and tannins, as well as the reactions that occur during the ageing of red wine.

Abbreviations

ANOVA analysis of variance; **B1ME**, **B2ME** and **C1MO** novel products formed in solutions containing malvidin 3-glucose (M) and ellagic acid (E) or oak wood extract (O);

GC gas chromatography; **HPLC** high performance liquid chromatography;

λ_{\max} maximum absorption wavelength; **NHTP** 2,3,5-nonahydroxyterphenoyl

Keywords: (+)-catechin, ellagic acid, ellagitannins, malvidin-3-glucoside, model wine, oak wood, procyanidin B1, volatile compounds

Introduction

Proanthocyanidins and anthocyanins are of undoubted importance in enology because they contribute to the sensory properties of wine, and they have an important role in the process of maturation and ageing of red wines. During wine ageing, these highly reactive species undergo several reactions, such as polymerisation, inter-

actions with polysaccharides, proteins and other substances, and can be oxidised, leading to the formation of a great diversity of products and a substantial modification of the properties of the wine.

During ageing in oak barrels, the composition of both red and white wines undergoes changes because of the addition of phenolic compounds and other molecules

extracted from the wood. Such compounds include lignins, hydrolysable and condensed tannins, gallic acid, ellagic acid, aromatic carboxylic acids, and various aldehydes. Quinn and Singleton (1985) report that ellagitannins and ellagic acid accounted for 10% of the total phenolics in Riesling wines treated with oak wood chips. These compounds are oak-derived components that occur in especially high levels in barrels made from European oak (Masson et al. 1995, De Simon et al. 2006, Prida and Puech 2006, Jordão et al. 2007).

Ellagitannins can be hydrolysed and are soluble in water/ethanol solutions (Jordão et al. 2005), as well as wines and spirits (Moutounet et al. 1989, Viriot et al. 1993). It has been suggested that ellagitannins may be involved in the oxidation mechanisms of red and white wines (Pontallier et al. 1982, Moutounet et al. 1989). Vivas and Glories (1996) showed that ellagitannins have an important role in wine oxidation process, quickly absorbing the dissolved oxygen and facilitating the hydroperoxidation of wine constituents. Thus, ellagitannins (from wood barrels, wood chips or as oenological tannins) have a protective effect against phenolic oxidation during the ageing process of wines (Guerra et al. 1996, Vivas and Glories 1996, Obradovic 2006, Roure and Anderson 2006). More precisely, oak wood ellagitannins also affect proanthocyanidin condensation rates (Vivas and Glories 1993) and reductions in anthocyanin content (Jordão et al. 2006).

Oak also contains a high level of volatile compounds that have a great impact on aroma of wood-matured wines (Ribéreau-Gayon et al. 2006). Studies carried out on the contributions of oak to the olfactory characteristics of wine have shown that they are influenced mainly by compounds such as furfural, guaiacol, whisky lactone and vanillin (Chatonnet et al. 1999, Pollnitz et al. 1999, Arapitsas et al. 2004). Some investigators have reported that the phenolic fraction can affect the composition and the volatility of some aroma compounds (Dufour and Bayonove 1999, Escalona et al. 2002).

Among the new compounds formed during red wine ageing, it is well known that anthocyanins form co-pigment complexes, especially with other flavonoids (Asen et al. 1972, Dallas et al. 1996b, Remy et al. 2000, Boulton 2001, Salas et al. 2004). Several studies have shown the structural diversity of anthocyanin-derived pigments in red grapes and wines (Mateus et al. 2001, 2002a,b). Among the detected anthocyanin-derived pigments were anthocyanins linked to a (+)-catechin unit via an ethyl linkage, pigments in which anthocyanins are linked to a (+)-catechin unit or a procyanidin dimer via a vinyl linkage and pigments in which anthocyanins are linked to a 4-vinylphenol group. In addition, there are reports of the formation of some anthocyanin-derived pigments in red wines and grape pomace that are more stable than the original anthocyanins (Bakker and Timberlake 1997, Fulcrand et al. 1998). Recently, a new class of blue anthocyanin-derived pigments, named portisins, were isolated from Port wines. Portisins arise from an acetaldehyde-mediated reaction between anthocyanin-pyruvic acid adducts and flavan-3-ols (Mateus et al. 2003).

The environment evolving inside oak barrels during the maturation of wines also provides conditions for further reactions, such as oxidation, hydrolysis and polymerisation, involving wood compounds and wine phenolics. There are recent reports on the formation of several oligomeric pigments resulting from reactions between malvidin-3-glucoside and (+)-catechin mediated by oak-derived furfural, methyl-furfural and vanillin (Freitas et al. 2004a, Pissarra et al. 2005, Sousa et al. 2007). In addition, under acidic conditions, new catechin-derived pigments, called oaklins, are produced from the reaction of flavan-3-ols with sinapaldehyde or coniferaldehyde (Freitas et al. 2004b, Pissarra et al. 2005, Sousa et al. 2005). Oaklins have been detected in commercial red wines aged in oak barrels, and it has been reported that they contribute to changes in the colour and astringency of wines during ageing (Sousa et al. 2005). Based on experiments with model wine solutions at pH 3.5, Nonier et al. (2006) reported a complete structural study of the formation of condensed dimers from catechin and oak wood furfuraldehyde.

With regard to non-volatile wood constituents, Lefevre et al. (2004) reported a condensation reaction between C-glycosidic ellagitannins and malvidin-3-glucoside. The UV-visible analysis of the adduct thus formed indicated a bathochromic shift of 20 nm with a concomitant threefold hyperchromic shift compared with the initial flavylum species. Additionally, other investigators reported a condensation reaction between the ellagitannin vescalagin, (+)-catechin and (-)-epicatechin (Quideau et al. 2003). As a result of these reactions, new compounds called acutissimins were formed. Acutissimins are inhibitors of human DNA topoisomerase II and are 250-fold more potent *in vitro* than etoposide, a clinical anti-cancer drug. Quideau et al. (2005) showed an important aspect of the chemistry of oak-derived NHTP-bearing C-glycosidic ellagitannins. These natural products are extracted by the wine solution during ageing in barrels and have the capability to combine covalently by means of substitution reactions with a variety of grape-derived nucleophilic species, such as ethanol, flavanols, anthocyanins and thiols. Recently, Saucier et al. (2006) estimated the levels of four flavano-ellagitannins and another newly identified wine polyphenol, β -1-O-ethylvescalagin, in Bordeaux red wine aged for 18 months in oak barrels. These five ellagitannin derivatives are derived from the nucleophilic substitution reaction of vescalagin with grape flavan-3-ols (+)-catechin and (-)-epicatechin or ethanol.

Despite these studies, the potential influence of some oak wood volatile and non-volatile compounds on the evolution of individual procyanidins and anthocyanins during red wine maturation has not been examined in detail, especially in regard to the formation of new compounds and the evolution of each procyanidin and anthocyanin. Thus, the aim of the current study was to use model wine solutions to evaluate the influence of furfural, eugenol, guaiacol, vanillin, ellagic acid, ellagitannins and oak wood extracts (*Quercus pyrenaica* Willd.) on the changes in the levels of (+)-catechin, procyanidin B1 and malvidin-3-glucoside.

Materials and methods

Samples

(+)-Catechin, procyanidin B1 and ellagic acid were purchased from Aldrich (Paris, France), Extrasynthèse (Genay, France) and Fluka–Biochemika (Buchs, Switzerland), respectively. Malvidin-3-glucoside chloride was obtained from Polyphenols Laboratories SA (Sandnes, Norway), while furfural was purchased from Fluka–Biochemika (furfural), while Merck – Darmstadt (Germany) supplied vanillin, guaiacol and eugenol. Oak wood chips were obtained from *Quercus pyrenaica* (Portuguese oak wood, from Gerês region) with medium grain (3.0 to 3.5 mm) and medium toasting (20 min 160–170°C on the wood surface). In order to reproduce extraction conditions similar to those in wine, the oak wood chip samples used in this study (20 g/L) were placed in 500 mL of model alcohol solution (pH 3.5, 12% alcohol content and 2 g/L of tartaric acid) for 15 days at 20 ± 2°C in the dark and stirred daily. At the end of this maceration, the extract was filtered through glass wool prior to being used in the study.

Model wine solutions

(+)-Catechin, procyanidin B1, malvidin-3-glucoside, ellagic acid, furfural, eugenol, guaiacol and vanillin were dissolved separately in 12% (v/v) ethanol solutions containing 2 g/L of tartaric acid and adjusted to pH 3.5. The compounds were used in amounts and combinations detailed in Table 1. These concentrations (Table 1) equate with the levels of (+)-catechin, procyanidin B1, malvidin-3-glucoside and ellagic acid that occur in red wine during ageing in barrels. Thus, 20 different experimental mixtures (in 10 mL test tubes) were prepared in duplicate and filtered (0.45 µm). Each experimental tube containing the mixtures were shaken daily and kept in darkness at 20 ± 2°C. At each sampling point during the 64-day incubation period, duplicate aliquots of about 0.5 mL were taken from each test tube for analysis.

HPLC analysis of malvidin-3-glucoside, (+)-catechin and procyanidin B1

A Perkin Elmer HPLC system (Norwalk, Connecticut, USA) was used for analysis of malvidin-3-glucoside, (+)-catechin and procyanidin B1 in the model wine solutions. It was equipped with a 410-LC pump, a solvent programmer (Model 420), a manual injector (Rheodyne 7125-A) fitted with a 20-µL loop and a Konik absorbance monitor linked to a Konichrom data station (Konik Instruments, Barcelona, Spain). The column (250 × 4.6 mm, particle size 5 µm) was a C₁₈ LiChrospher® 100 (Merck) protected by a guard column of the same material.

For malvidin-3-glucoside HPLC analysis, a method described by Dallas et al. (1996a) was followed. The solvents used were: solvent A (40% formic acid), solvent B (pure acetonitrile) and solvent C (double distilled water). Initial conditions were 25% of solvent A, 10% of solvent B and 65% of solvent C, followed by a linear gradient from 10 to 30% of solvent B, and 65 to 45% of solvent C for 40 min at a flow rate of 1 mL/min and detection at 520 nm.

The (+)-catechin and procyanidin B1 were also analysed by HPLC using the method of Dallas et al. (1996a). For (+)-catechin, the solvents used were: solvent A (2.5% acetic acid), solvent B (80% acetonitrile + 20% solvent A) and solvent C (double distilled water), while for procyanidin B1, the solvents used were: solvent A (10% acetic acid) and solvent B (double distilled water). For (+)-catechin, the linear gradient used 93% of solvent A and 7% of solvent B for 26.1 min, followed by 88% of solvent A and 12% of solvent B for 90 s. The flow rate was 0.9 mL/min with detection at 280 nm. For procyanidin B1, the linear gradient was run with 10% of solvent A and 90% of solvent B to 70% of solvent A and 30% of solvent B for 45 min, followed by another linear step of 90% of solvent A and 10% of solvent B for 25 min, after which it remained constant for 12 min. The flow rate used for procyanidin B1 was 1.0 mL/minutes and detection was at 280 nm.

Table 1. Different experimental model wine solutions prepared, and used in this study.†

1. (+)-Catechin	2. (+)-Catechin + Ellagic acid	16. Vanillin
	3. (+)-Catechin + Oak wood extract	17. Furfural
	4. (+)-Catechin + Vanillin	18. Guaiacol
	5. (+)-Catechin + Furfural	19. Ellagic acid
	6. (+)-Catechin + Guaiacol	20. Oak wood extract
7. Procyanidin B1	8. Procyanidin B1 + Ellagic acid	
	9. Procyanidin B1 + Oak wood extract	
10. Malvidin-3-glucoside	11. Malvidin-3-glucoside + Ellagic acid	
	12. Malvidin-3-glucoside + Oak wood extract	
	13. Malvidin-3-glucoside + Furfural	
	14. Malvidin-3-glucoside + Guaiacol + Eugenol	
	15. Malvidin-3-glucoside + Vanillin	

†All experiments were prepared in duplicate. Final concentrations of 40 mg/L for (+)-catechin, 50 mg/L for procyanidin B1, malvidin-3-glucoside and ellagic acid, 10 mg/L for vanillin, 15 mg/L for furfural and 5 mg/L for guaiacol and eugenol, and 20 g/L for oak wood extract.

HPLC analysis of ellagitannins and ellagic acid

The method used was described by Viriot et al. (1994) and used a Konic 500B pump with a 7161-LC Rheodyne injection valve and a UV-Vis model 206 PHD diode array detector at 280 nm for ellagitannins and 370 nm for ellagic acid. Data was processed with a Konikron data system. The column was as detailed above.

For ellagitannins a linear gradient was used: solvent A (1% aqueous phosphoric acid) and solvent B (methanol), and a gradient of 0–10% of solvent B in 30 min at a flow rate of 1 mL/min. Ellagic acid was analysed using the following linear gradient: 0–90% of solvent B in 30 min at a flow rate of 1 mL/min.

The chromatographic peaks of ellagitannins were identified according to reference data previously described by Viriot et al. (1994). Ellagic acid was identified and quantified based on comparison of retention times and calibration with an authentic standard.

Gas chromatography analysis

Model wine solutions containing the volatile compounds vanillin, eugenol, furfural or guaiacol were analysed by GC. 2 µL samples were injected (air 100 KPa and hydrogen 50 KPa) into a Carlo Erba (Rodano-Mi, Italy) 8000 gas chromatograph with a flame ionisation detector (FID), a capillary Carbowax 20M column (0.25 mm × 0.25 µm × 60 m, Bellefonte, Philadelphia, USA) and an injector in the splitless mode (50:1) at 250°C. The column temperature programme was from 70 to 230°C at 3°C/min followed isothermally for 20 min at 230°C followed by programming from 230 to 240°C at 5°C/min, after which the temperature was maintained at 240°C for 30 min.

Statistical analysis

In order to study the potential effects of ellagitannins, ellagic acid, volatile compounds and oak wood extract on (+)-catechin, procyanidin B1 and malvidin-3-glucoside levels, an analysis of variance and comparison of treatment means (ANOVA, one-way) was performed using SPSS software program version 11.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Changes of (+)-catechin, procyanidin B1 and malvidin-3-glucoside

In Figure 1, changes in the levels of (+)-catechin and procyanidin B1 in model wine solutions containing oak wood extract and ellagic acid over a 64-day period are shown. In all solutions, there was general decrease of (+)-catechin and procyanidin B1 that occurred most rapidly during the first 16 days of the incubation period and then slowed down and eventually stabilised.

The presence of oak wood extract and pure ellagic acid clearly affected the (+)-catechin and procyanidin B1 levels (Figure 1). Thus, under our experimental conditions, the amounts of (+)-catechin and procyanidin B1 decreased less in model wine solutions containing oak wood extract or ellagic acid. This difference was more evident in model wine solutions containing (+)-catechin

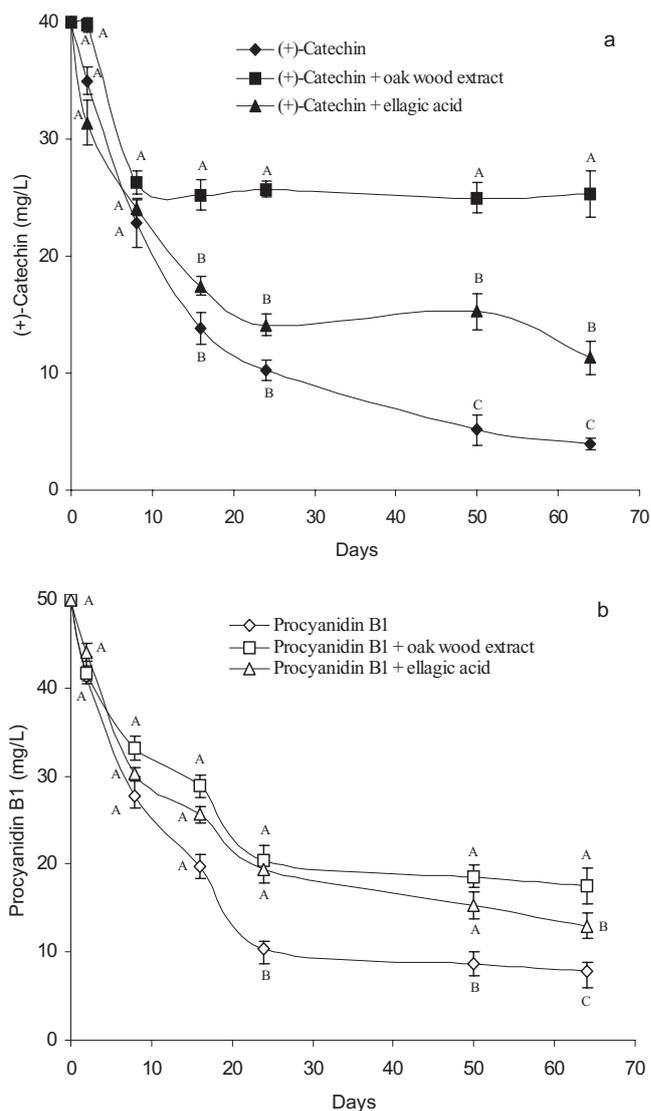


Figure 1. Influence of oak wood extract and ellagic acid on the (+)-catechin (a) and procyanidin B1 (b) content of model wine solutions over a 64-day period. Data points derived for given day showing the same letter are not significantly different ($P < 0.05$). Mean values ($n = 4$).

and oak wood extract. Thus, after 64 days, the content of (+)-catechin alone in solution or together with oak wood extract or pure ellagic acid was 3.9, 25.3 and 11.3 mg/L, respectively. Final values for procyanidin B1 following incubation with and without either oak wood extract or ellagic acid were 7.9, 17.5 and 13.0 mg/L, respectively.

Oak wood-derived volatile compounds such as furfural, vanillin, eugenol and guaiacol that are transferred to wine matured in barrels were examined for their influence on the (+)-catechin content (Figure 2). A general decrease in (+)-catechin was observed in all the model wine solutions studied. This was more evident in the first 16 days of our study. The decrease, however, was less substantial in solutions containing (+)-catechin and furfural.

The influence of furfural, vanillin, eugenol and guaiacol on malvidin-3-glucoside is also illustrated in Figure 2. The anthocyanin declined more slowly than (+)-catechin, especially during the initial 24 days of

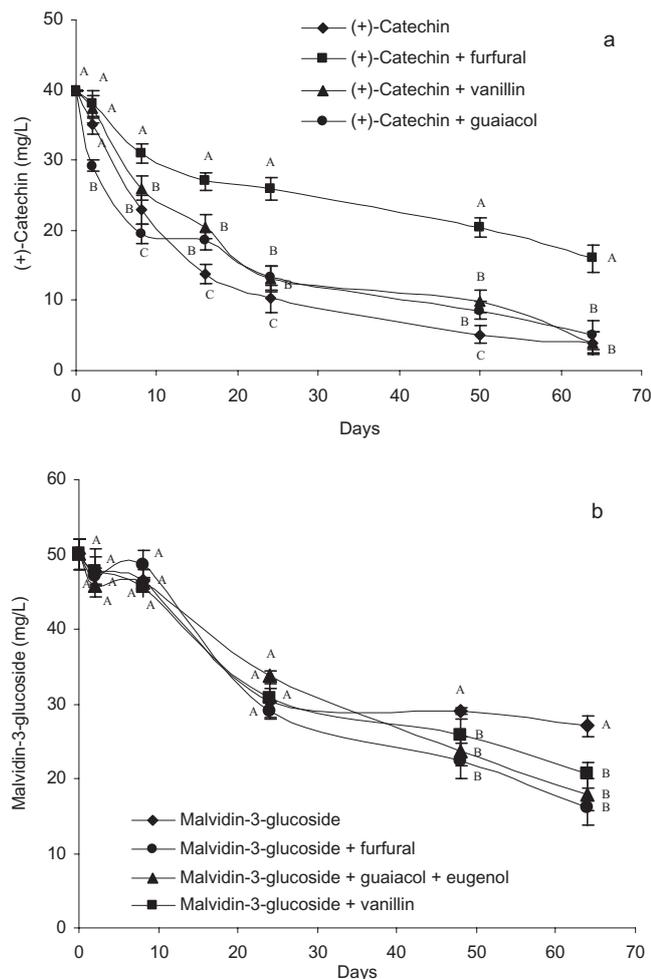


Figure 2. Influence of furfural, guaiacol, eugenol and vanillin on the (+)-catechin (a) and malvidin-3-glucoside (b) content of model wine solutions over a 64-day period. Data points derived for given day showing the same letter are not significantly different ($P < 0.05$). Mean values ($n = 4$).

storage. After 64 days, when incubated alone, the malvidin-3-glucoside content was 27.0 mg/L, and this fell to 16.2 mg/L in the presence of furfural and 17.8 mg/L when incubated with eugenol and guaiacol.

The influence of ellagic acid and oak chips on the malvidin-3-glucoside content of the model wine solutions is presented in Figure 3. There was a decrease in malvidin-3-glucoside levels, especially during the first 16 days of storage, and this occurred more rapidly in the presence of ellagic acid and the oak wood extract. After 64 days, when incubated alone, the malvidin-3-glucoside content was 30.0 mg/L, and this fell to 21.0 mg/L in the presence of the oak extract and 19.0 mg/L when incubated with ellagic acid. For ellagic acid, there was an initial rapid decline, which continued at a more pronounced rate in the presence of malvidin-3-glucoside (Figure 3).

Changes in the levels of ellagitannins, ellagic acid and volatile compounds

The ellagitannins content of the model wine solutions containing the oak wood extract and mixtures of oak

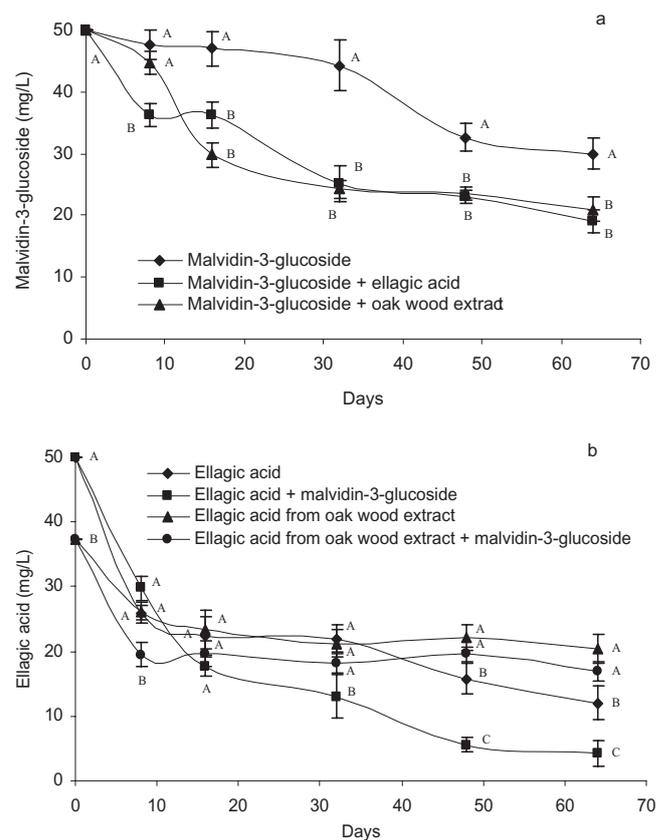


Figure 3. The effect of an oak extract on the malvidin-3-glucoside (a) and ellagic acid (b) content of model wine solutions over a 64-day period. Data points derived for given day showing the same letter are not significantly different ($P < 0.05$). Mean values ($n = 4$).

wood extract, (+)-catechin, procyanidin B1 and malvidin-3-glucoside are presented in Table 2. In all cases, the concentration of vescalagin, castalagin, grandinin, roburin E and D and ellagic acid decreased progressively during the 64 day storage period. The decreases were similar in all the model wine solutions studied and in most cases independent of the presence or absence of (+)-catechin, procyanidin B1 and malvidin-3-glucoside. However, ellagic acid shows a decrease more evident in the presence of malvidin-3-glucoside (Figure 3).

Changes in the levels of furfural, guaiacol, eugenol and vanillin during storage are presented in Table 3. All are characterised by a continuous steep decline throughout the 64-day storage period with no significant influence of either (+)-catechin and malvidin-3-glucoside.

New products formed and UV-Vis spectra properties

Figure 4 shows a typical chromatographic profile recorded at 280 nm in model wine solution containing (+)-catechin and ellagic acid and the newly formed product. The new peak formed had a retention time of 9.4 min and like (+)-catechin had a λ_{\max} at 280 nm. In a model system containing malvidin-3-glucoside and ellagic acid, HPLC revealed two new peaks, labelled B1ME and B2ME, that were detected after 8-day storage (Figure 5). These peaks had retention times of 9.1 and

Table 2. Changes of ellagittannins† and ellagic acid‡ from oak wood in model wine solutions containing (+)-catechin, procyanidin B1 or malvidin-3-glucoside.

Compounds	Model wine solutions (days)																			
	0			2			8			24			64							
	Oak wood extract	Oak wood extract + Cat.	Oak wood extract + BI	Oak wood extract	Oak wood extract + Cat.	Oak wood extract + BI	Oak wood extract	Oak wood extract + Malv.	Oak wood extract + BI	Oak wood extract	Oak wood extract + Cat.	Oak wood extract + BI	Oak wood extract	Oak wood extract + Malv.	Oak wood extract + BI	Oak wood extract + Malv.				
Vescalagin	53.2 ^A ± 0.2	53.2 ^A ± 0.4	53.1 ^A ± 0.6	56.3 ^A ± 0.9	49.8 ^A ± 0.5	50.6 ^A ± 2.3	51.7 ^A ± 2.1	49.1 ^A ± 3.9	43.2 ^B ± 1.0	44.5 ^B ± 2.5	47.9 ^A ± 1.4	35.4 ^C ± 2.3	38.9 ^B ± 2.1	35.4 ^C ± 2.3	31.6 ^C ± 1.9	34.5 ^C ± 2.5	25.2 ^D ± 1.3	22.3 ^D ± 2.3	21.8 ^D ± 3.2	20.3 ^D ± 1.9
Castalagin	60.0 ^A ± 0.4	60.1 ^A ± 0.5	60.3 ^A ± 0.7	62.3 ^A ± 0.5	55.2 ^B ± 0.9	54.7 ^B ± 1.2	52.5 ^B ± 1.6	58.8 ^A ± 3.4	43.2 ^C ± 1.0	44.5 ^C ± 2.5	47.9 ^B ± 1.4	41.7 ^C ± 2.5	28.3 ^D ± 1.3	33.7 ^D ± 1.9	31.8 ^D ± 1.9	32.7 ^C ± 1.5	25.6 ^D ± 3.2	20.9 ^E ± 1.6	22.2 ^E ± 2.3	27.8 ^E ± 1.3
Grandinin	25.1 ^A ± 0.4	25.0 ^A ± 0.3	25.0 ^A ± 0.4	24.0 ^A ± 0.2	21.1 ^B ± 1.3	20.1 ^B ± 0.6	22.8 ^B ± 0.6	19.6 ^B ± 1.9	18.3 ^B ± 0.5	17.2 ^B ± 1.6	19.6 ^B ± 2.4	15.7 ^B ± 1.0	16.1 ^B ± 0.7	15.0 ^B ± 2.1	16.4 ^B ± 1.5	17.2 ^B ± 3.5	14.9 ^B ± 1.2	11.7 ^C ± 2.0	8.2 ^C ± 0.8	12.3 ^C ± 1.5
Roburim D	13.4 ^A ± 0.9	13.2 ^A ± 1.0	13.7 ^A ± 0.5	9.23 ^A ± 1.2	10.4 ^A ± 0.2	9.3 ^A ± 0.3	11.9 ^A ± 0.4	4.9 ^B ± 0.4	9.3 ^A ± 0.2	8.7 ^A ± 0.4	10.5 ^A ± 0.8	8.7 ^A ± 0.4	8.9 ^A ± 0.4	9.1 ^A ± 0.3	10.7 ^A ± 0.7	6.4 ^B ± 0.5	5.8 ^B ± 0.3	6.9 ^B ± 0.3	6.2 ^B ± 0.7	3.8 ^B ± 0.4
Roburim E	21.7 ^A ± 1.2	20.9 ^A ± 1.2	21.0 ^A ± 0.3	23.2 ^A ± 0.6	18.2 ^A ± 0.7	15.4 ^B ± 1.2	17.4 ^A ± 1.2	17.4 ^A ± 1.9	12.1 ^C ± 1.7	13.4 ^C ± 1.8	12.9 ^C ± 1.1	11.6 ^C ± 2.0	9.5 ^D ± 1.0	8.4 ^D ± 0.5	9.9 ^D ± 0.3	9.2 ^D ± 1.2	8.0 ^D ± 0.5	7.7 ^D ± 0.7	10.4 ^D ± 1.3	8.7 ^D ± 0.9
Ellagic acid	53.3 ^A ± 0.7	52.1 ^A ± 0.8	53.2 ^A ± 0.2	ND	50.7 ^A ± 2.0	43.2 ^B ± 3.2	45.9 ^B ± 0.5	ND	31.9 ^B ± 2.2	37.6 ^B ± 2.7	39.9 ^B ± 1.7	ND	34.5 ^B ± 3.2	29.8 ^C ± 1.8	33.7 ^B ± 2.5	ND	25.1 ^C ± 3.0	24.3 ^C ± 4.1	26.9 ^C ± 2.5	ND

(±) Standard deviation; means (n = 4) followed by the same letter for each line are not significantly different (P < 0.05).

†Medium values in mg/L of ellagic acid equivalents.

‡Medium values in mg/L of ellagic acid. BI, procyanidin B1; Cat., (+)-catechin; Malv., malvidin-3-glucoside; ND, not determined.

Table 3. Changes of furfural, guaiacol, eugenol and vanillin† in model wine solutions containing (+)-catechin or malvidin-3-glucoside.

Compounds	Model wine solutions (days)														
	0			2			8			24			64		
	Volatile compound alone	Volatile compound + Cat.	Volatile compound + Malv.	Volatile compound alone	Volatile compound + Cat.	Volatile compound + Malv.	Volatile compound alone	Volatile compound + Cat.	Volatile compound + Malv.	Volatile compound alone	Volatile compound + Cat.	Volatile compound + Malv.	Volatile compound alone	Volatile compound + Cat.	Volatile compound + Malv.
Guaiacol	5.0 ^A ± 0.00	5.0 ^A ± 0.00	5.0 ^A ± 0.01	4.96 ^A ± 0.12	4.29 ^A ± 0.23	4.79 ^A ± 0.03	4.01 ^A ± 0.23	4.20 ^A ± 0.33	4.10 ^A ± 0.21	3.64 ^A ± 0.14	3.50 ^A ± 0.23	3.40 ^A ± 0.28	2.71 ^B ± 0.30	2.63 ^B ± 0.23	2.53 ^B ± 0.29
Furfural	15.0 ^A ± 0.01	15.0 ^A ± 0.00	14.99 ^A ± 0.01	14.96 ^A ± 0.10	14.90 ^A ± 0.23	14.20 ^A ± 0.43	14.43 ^A ± 0.12	14.29 ^A ± 0.13	14.09 ^A ± 0.23	11.96 ^A ± 0.52	12.24 ^A ± 0.44	12.24 ^A ± 0.44	7.05 ^B ± 0.62	6.89 ^B ± 0.79	6.89 ^B ± 0.79
Vanillin	10.0 ^A ± 0.02	10.0 ^A ± 0.00	10.0 ^A ± 0.00	9.97 ^A ± 0.02	9.80 ^A ± 0.11	9.80 ^A ± 0.11	9.12 ^A ± 0.10	9.50 ^A ± 0.33	9.10 ^A ± 0.23	7.46 ^A ± 0.30	7.90 ^A ± 0.17	7.10 ^A ± 0.57	5.36 ^B ± 0.12	4.90 ^B ± 0.43	4.70 ^B ± 0.63
Eugenol	5.0 ^A ± 0.00	ND	5.0 ^A ± 0.00	4.66 ^A ± 0.12	ND	4.19 ^A ± 0.33	3.21 ^A ± 0.27	ND	3.10 ^A ± 0.51	3.14 ^A ± 0.57	ND	3.30 ^A ± 0.68	3.11 ^A ± 0.40	ND	2.93 ^B ± 0.59

(±) Standard deviation; means (n = 4) followed by the same letter for each line are not significantly different (P < 0.05).

†Medium values in mg/L. Cat., (+)-catechin; Malv., malvidin-3-glucoside; ND = not determined.

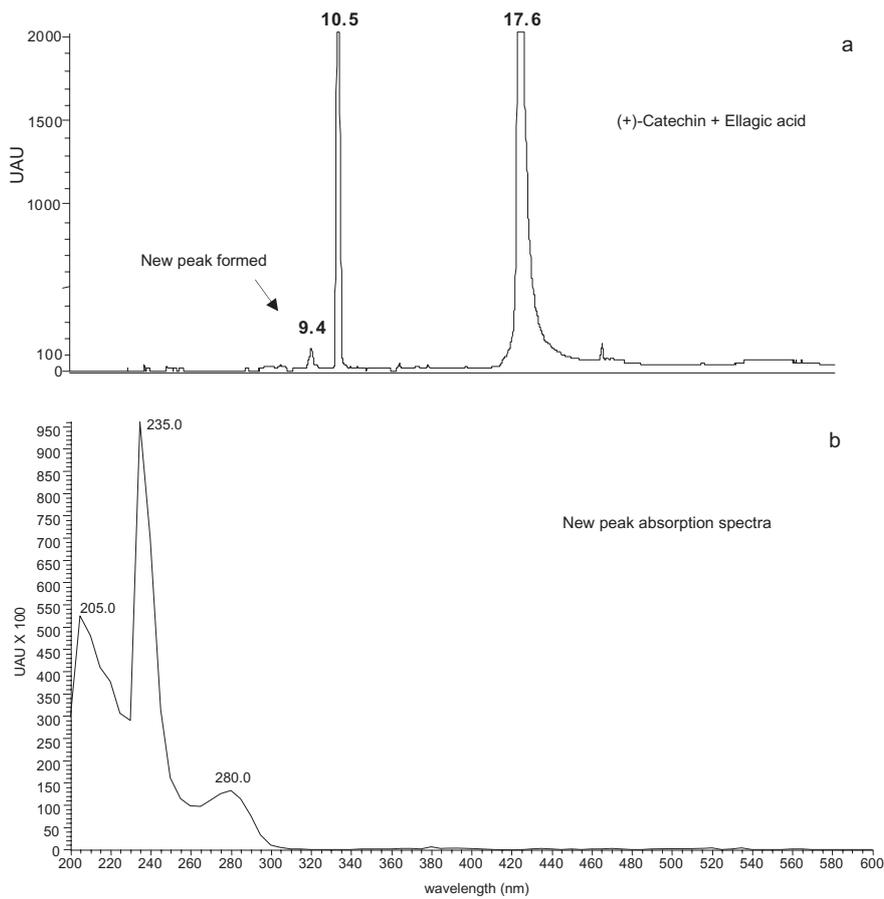


Figure 4. Reverse-phase HPLC-(280 nm) traces obtained in model wine solution containing (+)-catechin and ellagic acid (a) and the absorption spectra of the new peak formed (b).

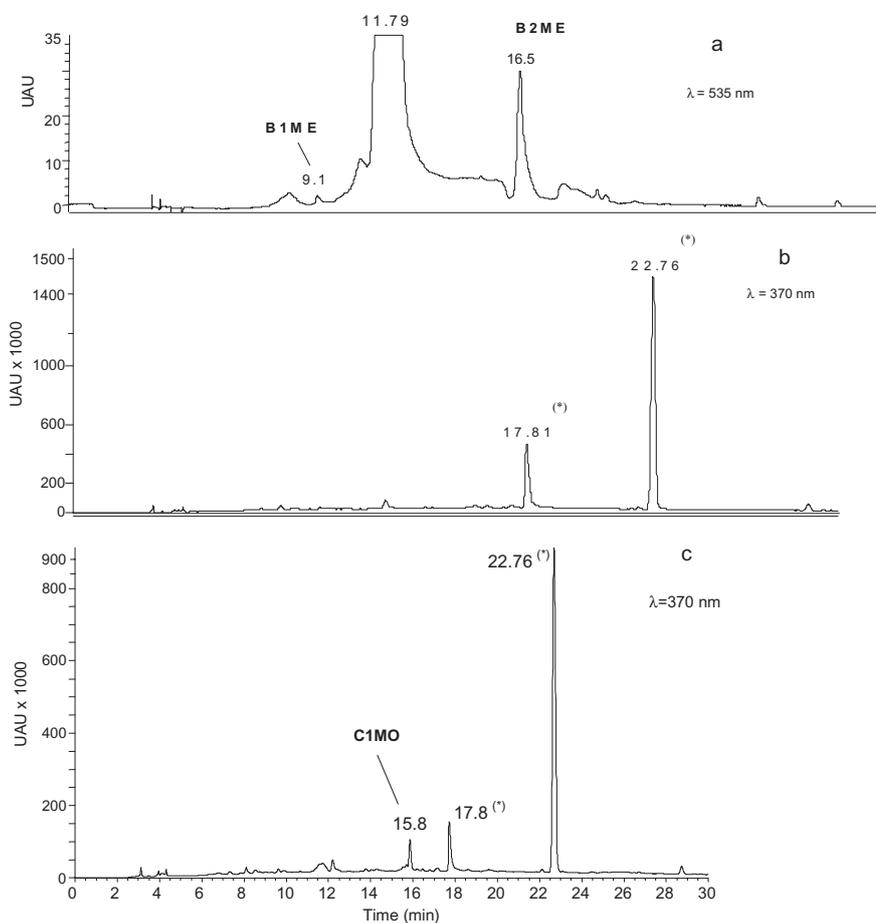


Figure 5. Reverse-phase HPLC chromatograms obtained in different model wine solutions. Model wine solution containing malvidin-3-glucoside and ellagic acid (a) and the new peaks formed (labelled B1ME and B2ME); model wine solution containing oak wood extract alone (b); model wine solution containing malvidin-3-glucoside and oak wood extract (c) and the new product formed (labelled C1MO). (*) Unknown compounds.

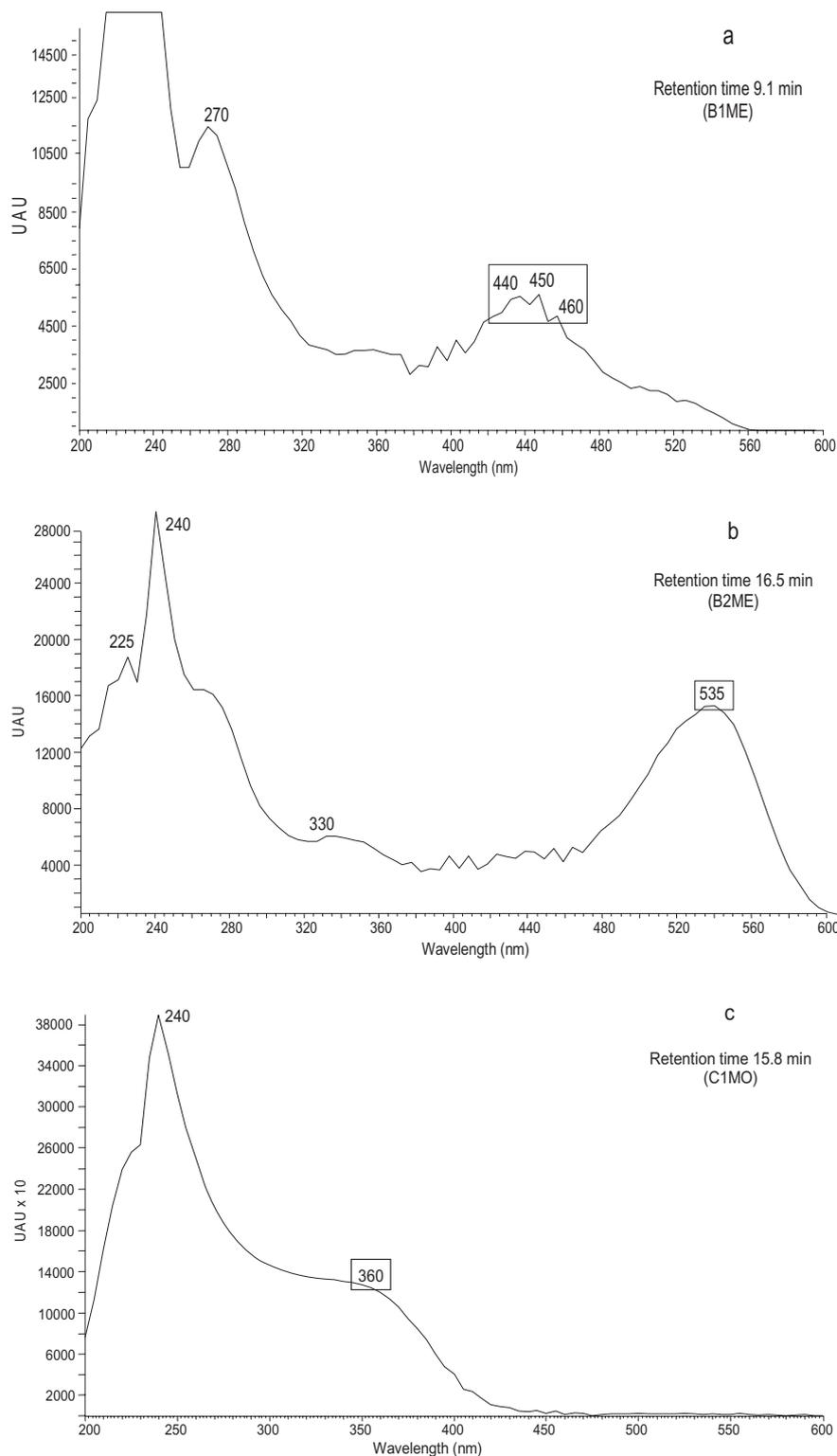


Figure 6. Absorption spectra of HPLC peaks, formed in model wine solutions. Product formed in a model wine solution containing malvidin-3-glucoside and ellagic acid (a and b); product formed in model wine solution containing malvidin-3-glucoside and oak wood extract (c).

16.5 min, respectively. A further new peak, CIMO, with a retention time of 15.8 min, was detected after 8-day storage in the presence of malvidin-3-glucoside and oak wood extract (Figure 5).

The absorbance spectra of the peaks B1ME and B2ME, with λ_{max} values at 440–450 and 535 nm, respectively, are shown in Figure 6. The C1MO peak had a λ_{max} at around 360 nm (Figure 6).

Discussion and conclusions

Changes of wine phenolic compounds and wood constituents in model solutions

It has been reported that the levels of phenolic compounds, including (+)-catechin, procyanidin B1 and malvidin-3-glucoside, fall during storage and ageing of red wine (Dallas et al. 1996a,b, 2003). This was confirmed in the current study using model wine solutions in

which (+)-catechin and procyanidin B1 content were monitored during storage in the presence and absence of an oak wood extract and ellagic acid. However, the results obtained also indicate that ellagic acid, and especially oak wood extract components, may reduce the decrease of both (+)-catechin and procyanidin B1. The potential effect of the oak wood extract was more pronounced for (+)-catechin than for procyanidin B1. At same time, a new product with an absorbance spectrum similar to that of (+)-catechin was formed and was detected in the solution containing both ellagic acid and (+)-catechin. This component was initially detected after 8-days storage and slowly increased thereafter. It is likely to be a (+)-catechin derived product, but it was present in very low amounts, so it was not feasible to isolate and purify it.

According to Sousa et al. (2007), some volatile compounds mediate reactions that lead to the formation of new pigments, which provide improved protection against the nucleophilic attack of water and the action of bisulphite action compared with malvidin-3-glucoside. There are data indicating that vanillin and guaiacol do not affect the degradation of (+)-catechin, while the presence of furfural may slow down the rate of breakdown. According to previous studies (Es-Safi and Cheynier 2004, Freitas et al. 2004a, Pissarra et al. 2005, Sousa et al. 2007), an aldehyde reacts with flavan-3-ols in a similar manner to its reaction with (+)-catechin, inducing their degradation and resulting in the formation of new pigments.

The levels of furfural, guaiacol, eugenol and vanillin in the model wine solutions declined continually through the 64-day incubation. Ellagic acid and oak-derived components, including possibly ellagitannins, enhanced the rate of malvidin-3-glucoside decrease, which was less pronounced in the case of the volatile aldehydes investigated. It appears probable that some oak wood components, including ellagic acid, have an impact on anthocyanin content and consequently increase the decrease of these pigments. The results obtained accord with the data obtained for red wine (De Coninck et al. 2006) and model solutions (Jordão et al. 2006). These authors reported that the content of malvidin-3-glucoside decreased faster in the presence of both oak wood extract and oak wood chips. Consequently, a decrease of red colour using a^* CIELAB coordinate was also observed.

In the solutions containing a mixture of malvidin-3-glucoside and ellagic acid, the new HPLC peaks B1ME and B2ME were detected. These peaks increased slightly during the 64-day storage. In addition, a new peak-designated C1MO was detected in solutions containing malvidin-3-glucoside and oak wood extract. This peak had a shoulder at 360 nm, which is near to the 370 nm λ_{\max} of ellagic acid. The λ_{\max} of B1ME was 440–450 nm, and that of B2ME was 535 nm, both of which are different to the absorbance maxima of the original malvidin-3-glucoside (520 nm) and ellagic acid. The three new compounds were present in only trace quantities, so it was not a practical proposition to attempt their isolation and characterisation.

Recently, Lopes et al. (2007) identified a new compound resulting from the oxidative degradation of malvidin-3-glucoside under acid conditions in model wine solutions stored at 25 and 90°C. The compound was identified as 8- β -D-glucopyranosyl-2,4-dihydroxy-6-oxo-cyclohexa-2,4-dienyl acetic acid, which results from nucleophilic attack of malvidin-3-glucoside by hydrogen peroxide through a Baeyer–Villiger oxidation followed by other oxidations. Dueñas et al. (2006) conducted several experiments with flavan-3-ol monomers and identified xanthylum chromophore formation with a λ_{\max} at 438 nm, which is very similar to that of B1ME.

The results presented in the current report show that the presence of (+)-catechin, procyanidin B1 or even malvidin-3-glucoside in the model system did not markedly affect the content of individual ellagitannins. However, for some individual ellagitannins, a slight reduction in content was more evident in solutions containing oak wood extract and malvidin-3-glucoside or (+)-catechin or procyanidin B1. Arguably, the decrease in ellagitannin content during the 64-day incubations could be because of their oxidation by dissolved oxygen, although no new HPLC peaks were detected as a consequence of ellagitannin degradation.

Similar results were observed with ellagic acid, both from oak wood extract and when it was added as a pure compound to the different model wine solutions.

The present work points out the importance of oak wood components in the degradation of anthocyanins and tannins, as well as in reactions that occur during the ageing of red wine. It is also possible that the new products formed could participate in the changes that we usually observed in red wine matured in oak barrels. In fact, wood extracts are themselves progressively ceded to the wine and then interact with the various compounds of importance to wine. However, a longer storage time, spectral, structural molecular and colour change analysis result will be needed as part of further research in order to provide a better knowledge of the new products formed.

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