Effect of certain treatments to prevent or partially reverse the pinking phenomenon in susceptible white wines

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Abstract. Pinking is a phenomenon occurring in certain white wines produced under highly reductive conditions which occasionally develop a pink colouration when suddenly exposed to air. The pink colouration gives the impression of wine being stained with red grape pigments, but in fact the phenomenon is a transformation in the presence of oxygen of some specific phenolic compounds found in the susceptible varieties. In our experiments two white wines based on Sauvignon blanc and Chardonnay, respectively, were found to have a high potential toward pinking. This study evaluates the potential of certain treatments to prevent the development of pinking or to partially reverse it after occurrence. Treatments tested involved the addition of 20 to 40 mg/l ascorbic acid or 5 to 30 mg/l of catechinic tannin prior to bottling. Both types of treatments had the potential to prevent pinking, irrespective of dosage used, as long as a normal concentration of free SO_2 (above 0.8 mg/l molecular SO_2) was maintained in the wines. Other treatments tested for bottled wines already developing a pink shade were the exposure of the bottle to UV light or the keeping in complete darkness, respectively. In the absence of any other pinking preventive measure both treatments proved to have a certain effect upon reversing the phenomenon. The UV light treatment shows more potential to reverse pinking than darkness and it may work even better on bottles with lower UV light filtering power (in this experiment Antique green glass bottles, with 70–80% UV reduction effect, were used). Differences among the responses of varieties are also present, with Chardonnay being less responsive to antioxidants than Sauvignon blanc. The parameters determined for the evaluation of pinking level are: Pinking potential index (PPI), proanthocyanidins (PAC), co-pigmented anthocyanins (Cp), polymeric pigmented anthocyanins (P) and total pigments (TP), as well as the absorbtion at 420 nm (for browning), 500 and 520 nm (for pinking), 620 nm (for blue shades).

1. Introduction

Pinking is a phenomenon which describes the appearance of a pink colouration in certain white wines as a result of some chemical changes of their colourless phenolic compounds [1,2]. Most affected white wines are usually those produced under highly reductive conditions, the wines developing a pink colouration after a sharp exposure to air [1,3–5]. Redox potentials of musts and wines vary from a minimum +100 to +150 mV under anaerobic conditions, while under aerobic conditions may reach values of +500 to +550 mV [6]. During alcoholic fermentation redox potential is lowered to negative values, but at the end of the alcoholic fermentation, the redox potential increases from negative values to positive ones. Wines well protected from exposure to air reach a redox potential around +20 mV [6], but even higher values, up to +200 mV, are generally considered reductive conditions. Wines exposed to air may have a redox potential of about +400 mV or more, due to the absorption of oxygen [7].

Although the pinking phenomenon is often observed shortly after the completion of alcoholic fermentation or after bottling, sometimes it may occur even during the extraction of grape must [8–10]. Wines produced from white grape varieties such as Sauvignon blanc, Chardonnay, Riesling, Albarino, Garnacha blanc, Verdejo and Siria [6,11,12], have been found to be more sensitive to pinking, although this may not happen in successive vintage years. Recently, some evidences show that some compounds responsible to the pink colouration are actually anthocyanins (pigments typical for red wines) and their derivatives [11,12]. Measurable small amounts of anthocyanins, especially malvidin 3-O-glucoside were present in fresh grapes from white international varieties such as Chardonnay, Sauvignon blanc and Riesling. The concentrations of these pigments are 5000 to 60000 times lower compared to those in red grape cultivars and 10 to 100 times lower compared with rosé grape cultivars [12]. The same authors could not detect any pyranoanthocyanins in fresh grapes of white varieties, but they revealed for the first time the occurrence of this compound in fresh grapes of rosé and black skin varieties [12]. Pyranoanthocyanins are very stable, unbleachable derivatives of anthocyanins, which are considered to be formed only during red grape processing through the addition of compounds like pyruvate, acetaldehyde, hydroxycinnamates or vinyl-flavanols to the C4 and C5 hydroxyl positions of anthocyanins. The pyranoanthocyanins, especially Vitisin A, are pigments very stable in time and more resistant to oxidation compared to other anthocyanin derivatives,

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being found even in aged red wines of 20 or more years old [13]. However, these authors could not exclude the possibility that the pinking of other white grape variety wines may have a different origin [11].

Older studies on wine polyphenols suggested that the pinking phenomenon may occur due to the slow dehydration of leucoanthocyanidins (flavan-3,4-diols) to their corresponding flavenes (flav-3-en-3-ol) under highly reductive medium and then a quick oxidation to their corresponding coloured flavylium cations (cyanidin) upon the exposure to oxygen [14–16].

However, an alternative hypothesis of pinking phenomenon may be the slow acid catalysis cleavage of interflavan bonds of certain proanthocyanidins present in grape skins, to their corresponding carbocation intermediate, which, following an oxygen exposure, turn into flavylium cations. The proanthocyanidins derived from seeds may also be a source, after an acid hydrolysis of seeds. There is some evidence regarding skin proanthocyanidins indicating that they are not found only as aglycons, but sometimes also as glycosides, since glucose was detected in acid hydrolizate of skins [17].

Proanthocyanidins are soluble in ethyl alcohol, insoluble in water and slightly soluble in hydroalcoholic mediums such as wine. Thus, we can assume that co-pigmentation in white wines may be a possible mechanism for stabilization of resulted anthocyanidins, if glycosylated flavonols are present to act as cofactors. Some authors suggest, however, that co-pigmentation is less significant in rosé and probably not significant in white wines because of low to very low anthocyanin concentrations [16, 18]. The minimum requirements for the occurrence of co-pigmentation are not fully understood and the formation of these complexes in certain circumstances may involve other factors. At very low concentrations of anthocyanin (about 35 µM), a high ratio of co-pigments to anthocyanin did not produce the co-pigmentation effect unless metallic ions were added [19, 20]. Many studies shows the importance of the ratio of cofactor to pigment and the nature of cofactors, with increasing the ratio and concentrations leading to a more intense colour [18]. In white wines we expect to have an increased ratio of cofactors to pigments because of the very low to non-detectable concentrations of red pigments in white grape varieties. For example, in seven Spanish white wines with total flavonol concentration varying from 2 to 7 mg/l, with an average 4.29 mg/l [21], the minimum concentration of anthocyanins needed for pink colour to become apparent was 0.3 mg/l [11].

Addition of catechinic tannins in wines susceptible to pinking could prevent the formation of pink colour because of their properties to get oxidized faster or to alter the chemical equilibrium between polyphenols involved.

To prevent the phenomenon, a test for pinking susceptibility or pinking potential of white wines was proposed [5, 22], being especially recommended to those wineries where the phenomenon previously occurred. The wines susceptible to develop pinking phenomenon can easily be identified by the colour change toward pink, which starts to be visible when the measured pinking potential is greater than 5 [22]. In accordance to AWRI guidance, in normal winery conditions, the threshold for post-fermentation susceptibility to pinking can be considered greater than 15 [22]. In such cases, the preventive treatment suggested is the addition of ascorbic acid added before bottling [23]. An alternative is to reduce the concentrations or remove entirely the precursors from wines by means of fining treatments [2]. The ascorbic acid acts as nucleophile and possibly works through the attack the electrophile compounds responsible for pinking [24, 25]. The co-pigmentation phenomenon may slow the process, but the overall effect of ascorbic acid is still present [26].

For wines affected by pinking or susceptible to pinking, the recommended fining treatments use products able to remove the pigments, but without causing overfining, such as PVPP, potassium caseinate and possible combinations of these with bentonite [2, 14]. Activated charcoal, although very effective, also removes other compounds too, important for the aroma or the general quality of wines [14].

Nevertheless, when the pinking phenomenon occurred in bottled wines, a partial reversal may be possible through the exposure of affected wines to direct sunlight or by keeping in a controlled environment, such as under UV light [22]. The exposure of wines to UV light affects the anthocyanin content by promoting a photochemical degradation [27], which involves an excited state of flavylium cation as an intermediate product [28]. Further, the final degradation products are derived through the hydrolysis of flavylium cations which lead to smaller colourless molecules, similar to the case of thermal degradation [28, 29].

The presence of ascorbic acid during UV light treatment seems to have a protective effect on anthocyanin content, while a reverse phenomenon was observed on thermal treatment, leading to an increased rate of degradation [27]. From the degradation of ring A generally results 2,4,6-trihydroxybenzaldehyde and from ring B the 4-hydroxy-3,5-dimethoxybenzoic acid from malvidin, 3,4-dihydroxybenzoic acid from cyanidin, 3,4,5-trihydroxybenzoic acid from delphinidin and 4-hydroxybenzoic acid from pelargonidin [28, 29].

2. Material and methods

2.1. Winemaking

The wines evaluated in this study were obtained in cellar conditions in volumes of 50 hl from Sauvignon blanc and Chardonnay grape varieties cultivated in Stefanesti-Arges. Both varieties were harvested late, in September, on 24th and 28th respectively.

Antioxidant protection of grapes during the processing was ensured with a dose of 60 mg/kg SO_2 with potassium metabisulfite (BASF) and 1 g/q catechinic tannin (Ti Premium, Enologica Vason). Vinification of both Sauvignon blanc and Chardonnay grapes involved a 6 hour maceration at 12 °C, using 2 g/q Zimaskin pectolitic enzyme (Enologica Vason). The free-run must was clarified at 12 °C for 24 hours by gravitational settling using an additional 1 g/hl pectolitic enzyme, Zimaclar pH3 (Enologica Vason). The limpidity of the resulted musts was determined by measuring turbidity, which was of 150 NTU for Sauvignon blanc must and 130 NTU for Chardonnay. During all steps of grape and must processing food grade CO_2 was employed to ensure protection. The free-run must of Sauvignon blanc had the following parameters: 22.5%

Brix; total titratable acidity of 97.98 meq/l; pH 3.20 and 90 mg/l YAN, while the free run must of Chardonnay had 21.2% Brix; total titratable acidity of 115.44 meq/l; pH 3.28 and 157 mg/l YAN. The clarified must of Sauvignon blanc was co-inoculated with a dose of 20 g/hl dry yeasts consisting of a blend of VIN7 and QA23 (1:1), while the clarified must of Chardonnay was co-inoculated with 20 g/hl dry yeasts Lalvin CY 3079 and 1 g/hl lactic acid bacteria Lalvin 31 to perform the alcoholic and malolactic fermentation concomitantly. Yeasts and bacteria were provided by Lallemand. Both musts were supplemented with nutrients for alcoholic fermentation in accordance to their YAN levels. Sauvignon blanc was supplemented with 40 g/hl Fermaid O (organic nutrient from Lallemand) and 20 g/hl V Activ Premium (from Enologica Vason), while Chardonnay must was supplemented with only 20 g/hl Fermaid O and 5 g/hl V Activ Premium. The temperatures of both vats were maintained to about 18 °C.

After the alcoholic and malolactic fermentations, both wines were racked from lees, sulphited with 70 mg/l SO_2 and treated with 60 g/hl Na-bentonite for removal of excess protein. After complete sedimentation of bentonite, both wines were racked again, cold stabilised using cold temperatures of winter and then filtered using medium (1–3 µm) and then fine (0.2–0.45 µm) cellulose pads. Usual concentrations of the free sulphur dioxide were maintained during the production close to 0.8 mg/l molecular SO_2.

2.2. Pinking susceptibility testing

The samples prepared to assess the protective potential of ascorbic acid and catechinic tannin against pinking are presented in Table 1.

The susceptibility of developing pinking was determined by using the Pinking potential index (PPI). The determination of PPI is based on the measurement of optical densities difference at 500 nm of a 10 mm optical path control sample against a sample treated with hydrogen peroxide and incubated 24 hours at 25 °C. Specifically, 12 ml of sulphited wines (80 ppm), treated or not with various antioxidant doses, were mixed with 3 ml 0.3% solution of H_2O_2 to induce pinking in susceptible wines. The results were calculated versus control samples prepared similarly, without the addition of hydrogen peroxide. The absorbance difference (ΔDO500) is measured and then multiplied with 100 [5,30], IP = ΔDO500 × 100. The wines susceptible to pinking can easily be identified by the colour change toward pink, which starts to be visible at an IP > 5. In accordance to AWRI guidance [22] in normal winery conditions, the threshold for post-fermentation susceptibility to pinking can be considered at IP > 15.

Differences in sample colour were also calculated for the measurements of absorbance at 420, 520, 620 nm.

To assess the proanthocyanidins (PAC) the butanol-HCl assay was used, as described and modified by Vermerris and Nicholson [31]. The anthocyanidins resulted after depolymerasation and oxidation of proanthocyanidins are spectrophotometrically measured at 550 nm and expressed as mg/l cyanidin.

2.3. Reversibility of pinking testing

In order to assess the ***reversibility of pinking***, the susceptible wines from tanks were bottled immediately after filtration, using a classical gravitational filler without addition of any inert gases and devoid of partial vacuum in bottle. The bottles used for storage were Antique green with an UV light filtration of 80%.

After two weeks, the pinking phenomenon occurred in both wines. At this point, the samples from tanks (without pinking) and the wines in bottles (with pinking) were analysed. From each varietal wine, three bottles were introduced in a closed chamber and exposed to UV light (36W fluorescent light) at room temperature (about 20 °C), while another three bottles were kept in dark conditions at the same temperature.

The treatments for pinking reversibility assessment and observations are summarized in Table 2.

Table 1. Doses of antioxidant added before testing the pinking susceptibility.

Wine sample codification		Applied treatments before testing the pinking susceptibility	
Sauvignon blanc	**Chardonnay**	**Ascorbic acid, mg/l**	**Catechinic tannin, mg/l**
SB_A00T00	CH_A00T00	**0**	**0**
SB_A20T00	CH_A20T00	**20**	**0**
SB_A30T00	CH_A30T00	**30**	**0**
SB_A40T00	CH_A40T00	**40**	**0**
SB_A00T05	CH_A00T05	**0**	**5**
SB_A00T10	CH_A00T10	**0**	**10**
SB_A00T15	CH_A00T15	**0**	**15**
SB_A00T20	CH_A00T20	**0**	**20**
SB_A00T25	CH_A00T25	**0**	**25**
SB_A00T30	CH_A00T30	**0**	**30**
SB_A20T05	CH_A20T05	**20**	**5**
SB_A20T10	CH_A20T10	**20**	**10**
SB_A20T15	CH_A20T15	**20**	**15**
SB_A20T20	CH_A20T20	**20**	**20**
SB_A30T05	CH_A30T05	**30**	**5**
SB_A30T10	CH_A30T10	**30**	**10**
SB_A30T15	CH_A30T15	**30**	**15**
SB_A30T20	CH_A30T20	**30**	**20**
SB_A40T05	CH_A40T05	**40**	**5**
SB_A40T10	CH_A40T10	**40**	**10**
SB_A40T15	CH_A40T15	**40**	**15**
SB_A40T20	CH_A40T20	**40**	**20**

2.4. Wine analysis

The wines were analysed right before bottling by assessing the standard physico-chemical parameters in accordance to the OIV methods [32], including the alcohol content, titratable acidity (TA), volatile acidity (VA), pH, sugar content, total extract and dry extract.

To assess the formation of anthocyanin-based pigments in the stored wine after 2 weeks all the samples (with 3 repetitions each) were analysed and the following parameters determined by spectrophotometric method [33–35], with the results expressed in absorbance units (AU): monomeric coloured anthocyanins (A), co-pigmented anthocyanins (Cp), polymeric pigmented

Table 2. Applied treatments for testing the reversibility of the pinking phenomenon.

Wine sample codification		Applied treatments to test pinking reversibility	
Sauvignon blanc	**Chardonnay**	**Storage recipient**	**Observations and treatments during storage**
SB_tank	CH_tank	Tank	Wine without visible pink shades
SB_bottle	CH_bottle	Bottle	Wine with fully developed pinking phenomenon at about 2 weeks after bottling
SB_UVlight	CH_UVlight	Bottle	Wine with fully developed pinking at about 2 weeks after bottling, and then exposed for **1 month at UV light**
SB_dark	CH_dark	Bottle	Wine with fully developed pinking at about 2 weeks after bottling, and then **kept for 1 month in complete darkness**

anthocyanins (P), colourless anthocyanins (Cl), total coloured pigments (TCP), total coloured and uncoloured pigments (TP).

Also, the PPI and PAC were also determined as described above.

All the spectrophotometric determinations were conventionally referred to the optical path of 10 mm and performed in quartz or glass cuvettes, depending on the method used, with a UV–VIS double beam Specord 250 spectrophotometer from Analytik Jena AG, running the software WinAspect version 2.2.7.

The statistical analyses used were ANOVA and Tukey test with Origin 10.0 software.

3. Results and discussions

Wines used in this research were analysed before performing any test and the results are presented in Table 3. To ensure normal antioxidant protection the levels of total sulfur dioxide were adjusted to about 80 mg/l.

3.1. Treatments with antioxidants to prevent pinking

After treatment with combinations of antioxidants, the optical densities at 365 nm (UV-light for flavonols), 420 nm (browning), 500 nm (pinking), 520 nm (red) and 620 nm (violet/blue) were measured. To determine the colour changes that would take place during oxidation, the wines were also challenged with hydrogen peroxide, and same optical densities were determined as well. The results for both varieties are included in Table 4.

Also, for the determination of pinking susceptibility, based on determination at 500 nm with and without addition of H_2O_2 the PPI was calculated and susceptibility considered at a value larger than 15. The PPI results for all the wines, treated with various dosages of antioxidants, are presented in Fig. 1.

3.1.1. Treatments with ascorbic acid (0, 20, 30, 40 mg/l)

Based on our analyses Sauvignon blanc and Chardonnay displayed several differences in colour change and pinking susceptibility. Thus, the observations were presented for each variety separately.

Sauvignon blanc – ascorbic acid dose influence

As compared to control wines, the absorbance at 365 nm and 420 nm (browning) is not influenced by the ascorbic acid. Although the values are comparable with the control, and correlation coefficients with the dosage is very low ($R^2 = 0.23$ and $R^2 = 0.07$, respectively) Tukey test shows some significant differences.

The absorbance at 500 nm (pinking) and 520 nm increases slightly with the addition of 20 mg/l ascorbic acid, but decreases slightly with 30 and 40 mg/l. Tukey test shows significant differences and the correlation coefficients are $R^2 = 0.35$ and $R^2 = 0.32$, respectively.

Ascorbic acid treatments do not influence much the violet shades (620 nm) although the values have the tendency to decrease ($R^2 = 0.41$). Tukey test finds here too some significant differences.

Sauvignon blanc – ascorbic acid H_2O_2 (expected evolution in the presence of O_2)

In the presence of ascorbic acid and oxygen the absorbance at 365 nm slightly decreases for all the used dosages of ascorbic acid ($R^2 = 0.75$), as opposed with the case when not H_2O_2 challenged.

The absorbance at 420 nm (browning) decreases by about 35% for all the used dosages of ascorbic acid ($R^2 = 0.74$), as opposed with the case of not H_2O_2 challenged, confirming the observation that ascorbic acid actually confers protection against browning during wine storage.

The absorbance at 500 nm (pinking) decreases by about 70% as compared to a control also challenged with H_2O_2, for all the ascorbic acid doses applied. At 520 nm this parameter decreases by about 80%. In both cases $R^2 = 0.76$.

Ascorbic acid treatments also influence the violet shades (620 nm), decreasing the values by 90% as compared to a control also challenged with H_2O_2 ($R^2 = 0.75$).

Thus, all the colour paramenters are decreased irrespective of the ascorbic acid dosage, any dose conferring protection against oxidation and pinking during storage.

Chardonnay – ascorbic acid dose influence

For Chardonnay, the values recorded at 365 nm remain also unchanged for all the used dosages of ascorbic acid ($R^2 = 0.02$, although Tukey test is showing significant differences).

The browning (420 nm) decreases by about 3% in direct correlation with the used dosage of ascorbic acid

Table 3. Physico-chemical parameters of bulk wines.

Parameter	Sauvignon blanc	Chardonnay	Method [32]
Alcoholic strength, % vol.	13.35	13.15	OIV-MA-AS312-01A
Total titratable acidity, meq/l	77.84	87.44	OIV-MA-AS313-01
Volatile acidity, meq/l	8.16	5.50	OIV-MA-AS313-02
pH	3.26	3.42	OIV-MA-AS313-01
Free sulfur dioxide, ppm	40.30	34.75	OIV-MA-AS323-04A
Total sulfur dioxide, ppm	78.00	81.90	OIV-MA-AS323-04A
Molecular sulfur dioxide, ppm	1.51	0.90	–
Non-reducing dry extract, g/l	17.40	20.10	OIV-MA-AS2-03B
Total dry extract, g/l	19.80	21.70	OIV-MA-AS2-03B
Reducing sugars, g/l	2.40	1.60	OIV-MA-AS311-01A
Density, g/cm^3	0.99040	0.99160	OIV-MA-AS2-01A
Total polyphenol index (TPI), AU	10.3	12.6	Spectrophotometrical
Pinking potential index	23.9	33.7	Iland, 2004

($R^2 = 0.94$ and significant differences confirmed with Tukey test).

The pinking (500 nm) decreases slightly, by about 9–14%, in a dose dependent manner ($R^2 = 0.95$), with the addition of 20 to 40 mg/l ascorbic acid. Regarding the values recorded at 520 nm, absorbance is also decreasing ($R^2 = 0.57$), 40 mg/l inducing a slight decrease of about 5% (significant difference shown by Tukey test).

For this variety, ascorbic acid treatments reduce the violet shade (620 nm) by 30% ($R^2 = 0.86$).

Chardonnay – ascorbic acid H_2O_2 (expected evolution in the presence of O_2)

Challenged with hydrogen peroxide, this wine records at 365 nm a slight increase (0.5%) for 20 mg/l and a slight decrease (0.5%) for 30 and 40 mg/l ascorbic acid. The linear correlation with the dose is low, $R^2 = 0.31$.

The values at 420 nm (browning) decrease (by about 3%) for all the used dosages of ascorbic acid ($R^2 = 0.86$ and Tukey test is showing significant differences).

For pinking, the values recorded at 500 nm are significantly decreasing (by about 50%) with the addition of 20 to 40 mg/l ascorbic acid ($R^2 = 0.85$). As compared to the case of the samples not challenged with H_2O_2, the decrease is more evident, proving that ascorbic acid protects against pinking during the wine storage.

Same behaviour as for 500 nm is also recorded for absorbance at 520 nm ($R^2 = 0.84$). In the wines not challenged with H_2O_2 the effect of ascorbic acid was not yet evident, but it is now, when conditions of oxidative evolution are simulated.

Ascorbic acid treatments reduce the blue shade too (620 nm), by 40% ($R^2 = 0.84$), more than in wines not challenged with H_2O_2.

Thus, in case of exposure to oxidative conditions, all the colour paramenters could be decreased with the addition of ascorbic acid, the protection against oxidation and pinking during storage being dose-dependent.

3.1.2. Treatments with catechinic tannin (0, 5, 10, 15, 20, 25, 30 mg/l)

Sauvignon blanc – catechinic tannin dose influence

As compared to the treatment with ascorbic acid, the doses of tannin used led to different specific effects.

The absorbance determined at 365 nm, which is correlated with the flavonoid content in wines, is progressively increasing, with a correlation coefficient of $R^2 = 0.93$.

In the case of browning (420 nm) the values of absorbance increased slightly and linearly with the dose ($R^2 = 0.88$), due to the color of the tannin itself.

Regarding the absorbance at 500 nm (pinking) and 520 nm (red) no major influence was observed, due to the low values recorded for all samples (the slight increase in colour is due to the colour of the tannin added, $R^2 = 0.63$ and $R^2 = 0.51$, respectively).

The behaviour at 620 nm is unrelated to the dose ($R^2 = 0.08$), all values for the violet colour parameter being low.

Sauvignon blanc – catechinic tannin H_2O_2 (expected evolution in the presence of O_2)

When challenged with H_2O_2, the wines have a similar tendency as observed in the absence of H_2O_2 but the correlation of the values with the absorbance is not as good anymore, confirming that aside of the added tannin colour, with oxidation, other mechanisms also contribute to changing in colour.

The absorbance at 365 nm has a slight increasing tendency with the tannin dose, but correlation with the dose is low ($R^2 = 0.13$).

The values for 420 nm (browning) decrease as compared to control, the most protective dosages being 5–20 mg/l, followed by 25–30 mg/l, same as in the absence of H_2O_2, thus the browning progresses independently from the presence of tannin (no linear correlation, $R^2 = 0.14$).

Absorbance at 500 nm (pinking) records a relevant drop, all the treated samples retaining only approximately 40% from the value determined in control, irrespective of the tannin dosage ($R^2 = 0.33$). Here too, the most protective doses are 5–20 mg/l.

The red colour shade (520 nm) also decreases very much, the samples treated with 5–25 mg/ having values of about 30% of those determined for control. The slightly higher value observed for the dose of 30 mg/l suggests lower protection than 5–25 mg/l, but is actually due to the colour of the tannin. As with addition of tannin the values drop to the same level as compared tot control, there is no linear correlation with the dose, $R^2 = 0.35$.

Table 4. The effect of treatments with catechinic tannin and ascorbic acid on the optical densities measured at selected wavelengths important for the colour evaluation of Sauvignon blanc and Chardonnay wines.

Tannin	Ascorbic acid	Sauvignon blanc – absorbance values*									
		Not challenged with H_2O_2					Challenged with H_2O_2				
		365 nm	420 nm	500 nm	520 nm	620 nm	365 nm	420 nm	500 nm	520 nm	620 nm
T0	A00	4.8117	0.3282	0.0923	0.0657	0.0143	5.0472	0.4930	0.3563	0.3188	0.0815
	A20	4.9453	0.3445	0.0947	0.0677	0.0150	4.8000	0.3205	0.0970	0.0665	0.0073
	A30	4.8298	0.3302	0.0898	0.0638	0.0128	4.7918	0.3147	0.0925	0.0622	0.0042
	A40	4.9142	0.3353	0.0890	0.0628	0.0122	4.8057	0.3272	0.1007	0.0690	0.0087
T5	A00	4.9370	0.3528	0.1052	0.0775	0.0227	4.8723	0.3607	0.1360	0.1050	0.0243
	A20	4.8688	0.3298	0.0868	0.0608	0.0098	4.8242	0.3265	0.1033	0.0712	0.0067
	A30	4.8368	0.3237	0.0827	0.0543	0.0030	4.8388	0.3297	0.1003	0.0688	0.0078
	A40	4.9912	0.3498	0.0953	0.0655	0.0110	4.8383	0.3390	0.1083	0.0757	0.0118
T10	A00	4.9503	0.3497	0.0978	0.0693	0.0135	4.8875	0.3550	0.1347	0.1030	0.0180
	A20	4.9987	0.3567	0.0997	0.0710	0.0145	4.8725	0.3500	0.1130	0.0802	0.0148
	A30	5.0655	0.3693	0.1037	0.0725	0.0157	4.9205	0.3623	0.1207	0.0852	0.0162
	A40	4.9140	0.3522	0.1000	0.0703	0.0153	4.9165	0.3562	0.1127	0.0790	0.0143
T15	A00	5.0210	0.3538	0.0968	0.0678	0.0110	4.9072	0.3593	0.1312	0.1000	0.0212
	A20	4.9048	0.3517	0.1025	0.0748	0.0207	4.9425	0.3623	0.1225	0.0878	0.0150
	A30	5.1297	0.3817	0.1108	0.0790	0.0200	4.9503	0.3692	0.1282	0.0918	0.0147
	A40	4.9127	0.3585	0.1027	0.0722	0.0155	4.9447	0.3705	0.1193	0.0830	0.0128
T20	A00	5.0190	0.3650	0.1070	0.0775	0.0195	4.9467	0.3677	0.1297	0.0973	0.0217
	A20	4.9113	0.3495	0.0983	0.0705	0.0148	5.0047	0.3853	0.1330	0.0960	0.0185
	A30	5.1620	0.3860	0.1128	0.0805	0.0200	5.0460	0.3923	0.1315	0.0950	0.0202
	A40	4.9377	0.3600	0.1007	0.0697	0.0123	4.9913	0.3827	0.1232	0.0878	0.0195
T25	A00	5.0918	0.3798	0.1132	0.0828	0.0215	5.0472	0.3818	0.1398	0.1063	0.0223
T30	A00	5.1240	0.3775	0.1105	0.0802	0.0187	4.8000	0.3967	0.1468	0.1123	0.0265
		Chardonnay– absorbance values*									
T0	A00	6.0632	0.4398	0.1758	0.1482	0.0693	6.2160	0.6430	0.4667	0.3952	0.1005
	A20	6.0833	0.4298	0.1612	0.1487	0.0482	6.2478	0.5070	0.2542	0.2352	0.0658
	A30	6.0653	0.4277	0.1572	0.1453	0.0475	6.1630	0.4688	0.2117	0.1965	0.0585
	A40	6.0695	0.4210	0.1527	0.1407	0.0440	6.1818	0.4795	0.2222	0.2062	0.0603
T5	A00	6.0872	0.4253	0.1618	0.1432	0.0500	6.2482	0.5082	0.2490	0.2295	0.0650
	A20	6.0828	0.4195	0.1527	0.1413	0.0418	6.2925	0.5225	0.2657	0.2462	0.0708
	A30	6.1002	0.4257	0.1533	0.1415	0.0418	6.2550	0.5122	0.2462	0.2292	0.0780
	A40	6.0897	0.4175	0.1493	0.1385	0.0405	6.3262	0.5433	0.2857	0.2640	0.0772
T10	A00	6.1008	0.4355	0.1650	0.1422	0.0488	6.3075	0.5387	0.2757	0.2548	0.0875
	A20	6.1318	0.4392	0.1638	0.1513	0.0478	6.2788	0.4962	0.2275	0.2097	0.0622
	A30	6.1248	0.4323	0.1535	0.1415	0.0425	6.3218	0.5265	0.2545	0.2342	0.0685
	A40	6.1012	0.4240	0.1535	0.1425	0.0427	6.2993	0.5247	0.2555	0.2345	0.0700
T15	A00	6.1585	0.4402	0.1647	0.1468	0.0465	6.3277	0.5417	0.2698	0.2482	0.0797
	A20	6.1710	0.4465	0.1652	0.1520	0.0480	6.2368	0.4857	0.2128	0.1967	0.0623
	A30	6.2212	0.4502	0.1635	0.1498	0.0452	6.4252	0.5677	0.2878	0.2631	0.0785
	A40	6.1542	0.4398	0.1628	0.1505	0.0495	6.3950	0.5773	0.3103	0.2860	0.0858
T20	A00	6.2143	0.4560	0.1732	0.1565	0.0515	6.3547	0.5255	0.2323	0.2120	0.0652
	A20	6.2300	0.4673	0.1788	0.1645	0.0562	6.3947	0.5553	0.2745	0.2520	0.0762
	A30	6.1992	0.4470	0.1637	0.1508	0.0458	6.4063	0.5830	0.3048	0.2802	0.0855
	A40	6.1903	0.4487	0.1683	0.1560	0.0523	6.5000	0.6073	0.3342	0.3080	0.0922
T25	A00	6.2398	0.4672	0.1772	0.1603	0.0515	6.2592	0.4940	0.2093	0.1933	0.0642
T30	A00	6.3430	0.4957	0.1915	0.1732	0.0577	6.4358	0.5570	0.2652	0.2422	0.0735

* The values in the table are averages of 3 measurements. For simplicity, the SD values were not included, but the correlations were calculated with all the data.

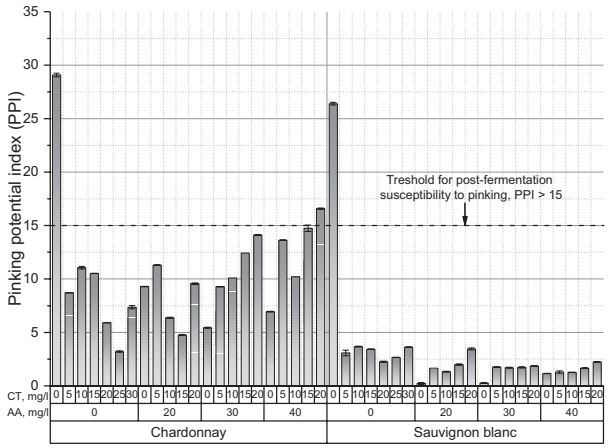

Figure 1. Catechinic tannin and ascorbic acid efficiency for reduction of pinking in Chardonnay band Sauvignon wines (CT = addition of catechinic tannin; AA – addition of ascorbic acid).

The behaviour at 620 nm is the same as in the case of 520 nm, the violet shades decreasing by 30% with any of the tannin treatments, $R^2 = 0.32$.

Chardonnay – catechinic tannin dose influence

At 365 nm, where usually flavonols are detected, the absorbance values are linearly increasing with the dosage of the tannin used (correlation coefficient $R^2 = 0.94$).

The values at 420 nm are slightly decreasing for 5–15 mg/l tannin, but increase from 20 mg/l, as the colour of tannin influences the measurement for browning. The overall colour is dependent on the tannin added ($R^2 = 0.77$ when control is taken into account, or $R^2 = 0.93$ when only tannin-treated samples are included).

For pinking, measured at 500 nm, the results surprisingly show that it is not influenced by the tannin treatment, doses of 5–25 mg/l inducing only non significant differences as compared to control samples. However, treatment with 30 mg/l increases this value by about 9%, most likely due to the tannin color. The influence of tannin colour is overall very low, but the slight increased values for absorbance at 500 nm are very well correlated with the dose ($R^2 = 0.86$).

The red colour shade (520 nm) has the same trend as the pinking at 500 nm.

With the addition of tannin, at any dose, the violet shades (620 nm) drop by about 30%. From the dose of 30 mg/l the effect of the tannin colour is also present, but not significant.

Chardonnay – catechinic tannin H_2O_2 (expected evolution in the presence of O_2)

When challenged with H_2O_2, the wines have similar behavior at 365 nm, the absorbance values slightly increasing progressively with the dosage of the tannin used ($R^2 = 0.98$, after removing the outlier at 25 mg/l).

The browning (420 nm) drops by about 15% for the doses of 5–25 mg/l, then, the colour of tannin influences the results starting 30 mg/l. The challenge with H_2O_2 shows more clear that tannins tends to protect a little from browning in the long run.

Regarding the determinations at 500 nm (pinking) and 520 nm (red) it was observed that, challenged with H_2O_2, the absorbance is reduced by all doses of tannin by about 50% as compared to control also challenged with H_2O_2. Again, from 30 mg/l the colour of tannin interferes with the results.

At 620 nm, the violet shades kept their tendency to decrease by about 30% with all tannin dosages.

3.1.3. Combined treatments with ascorbic acid and catechinic tannin. PPI index

For both varieties, Sauvignon blanc and Chardonnay, the colour parameters are influenced by tannin and ascorbic acid, added either independently or in combination.

Two-way ANOVA test shows that the interaction of both antioxidants induces significant differences in colour parameters (absorbance at 365, 420, 500, 520 and 620 nm), all treatments leading to more or less improvement as compared to no treatment (control).

As added tannin has also its own influence on colour of wine, the combined effect of ascorbic acid and tannin is not discussed for each parameter, but only for the PPI index. The interaction of these two factors has also a statistically significant effect on the reduction of the PPI. The influence of both additives, added either independently or in combination, can be seen in Fig. 1.

As it can be easily seen, Sauvignon blanc responds better to the protection with antioxidants, while in Chardonnay, although the effect is similar, the reduction of the pinking susceptibility is smaller at the same tannin and ascorbic acid doses.

In Sauvignon blanc, the highest protection effect (lowest PPI) is obtained with independent doses of 20 and 30 mg/l ascorbic acid (PPI around 0.25), followed by 40 mg/l ascorbic acid (PPI around 1.20). The addition of tannin is also lowering the PPI, but not as much as the ascorbic acid, values being in the range of 2.30–3.70). Anyway, as compared to the not treated sample which reaches a PPI of 26.4, any of the treatments can be considered effective.

For Chardonnay, the effect of ascorbic acid is not as important as in Sauvignon, doses of 20–40 mg/l ensuring only a reduction from PPI = 29 to a PPI in the range 5.5–9.3, which is not sufficient to guarantee protection from pinking, since a PPI larger than 5 means that the pinking shadow is already apparent. Tannin as well is not conferring sufficient protection, as the PPI of wines treated with several doses of tannin varies in the range of 5.9–11.1. The combination of 15 mg/l tannin and 20 mg/l ascorbic acid seems to be protective, being the only one leading in Chardonnay to a PPI lower than 5. Some other combinations can however be even less protective than the addition of single antioxidants. For example the combination 20 mg/l tannin and 40 mg/l ascorbic acid leads to a PPI of 16.6, while 20 mg/l tannin or 40 mg/l ascorbic acid alone lead to a much lower PPIs, of 5.92 and 6.95, respectively.

3.2. Treatment attempts during storage to reverse pinking

Same wines were also subjected to treatments with UV light or darkness, after bottling, to asses the

Table 5. Optical densities of wines and ANOVA – Tukey Test HSD, $p < 0.05$.

Wine samples (buffered (pH=3.6)	*Spectrophotometric analyses of colour (10 mm cuvette)		
	Abs. 420 nm, AU	Abs. 520 nm, AU	Abs. 620 nm, AU
	Sauvignon blanc		
SB_tank	0.6722[a]	0.1508[a]	0.0310[a]
SB_bottle	0.7977[b]	0.4138[d]	0.0863[b]
SB_UVlight	0.8438[c]	0.3113[b]	0.0837[b]
SB_dark	0.8400[c]	0.3405[c]	0.0872[b]
	Chardonnay		
CH_tank	0.8162[a]	0.2147[a]	0.0647[a]
CH_bottle	0.8167[a]	0.3355[d]	0.1088[c]
CH_UVlight	0.8700[b]	0.3100[c]	0.0905[b]
CH_dark	0.8665[b]	0.2665[b]	0.0892[b]

* results expressed as absorbance units.

possibility of pinking reduction or reversibility. Based on spectrophotometric measurements, several parameters were determined and reported in absorbance units (color parameters in Table 5, various pigments in Table 6), as well as proanthocyanidin expressed in cyanidin equivalents (Table 7).

Sauvignon blanc response to reverse pinking

Following bottling, browning (420 nm) is progressing, irrespective of the UV light or darkness treatment. One month after bottling browning increased by 18% in non treated wines and even more in the treated ones, by another 5%.

The red shades (520 nm) are appearing in all susceptible wines bottled in non-reductive conditions. Thus, 1 month after bottling, due to bottling shock, the wines almost tripled their values as compared to those still in tank. These values were however reduced by about 25% and 17% with a UV light and darkness treatments, respectively. These treatments help, but they do not sufficiently decrease the shade of pink in the wines. The values of treated wines are still double as compared to the wine in tank.

At 620 nm the values are increasing 3 times after bottling and are not reduced irrespective of the UV light/darkness treatment (Tukey test shows no significant differences among the wines in bottles, treated or not).

– PAC content (proantocyanins determined at 550 nm) is significantly different for the samples kept in tank, where the proantocyanins are in larger quantities (not affected by oxidation), while for the wines in bottles this concentration decreases, but most likely due to transformation in coloured compounds.

Co-pigmented anthocyanins (Cp) are increasing substantially (almost 2.8 times) following the bottling. These are the pigments which are the most responding to UV light treatment, their values falling after the treatment to about 66% from the level of the wines in bottle and to about 81% from the level of the wine kept in tank (which is not yet showing pinking).

Polymeric anthocyanins (P) are increasing following bottling by 1.7 times, being slightly reduced by darkness treatment.

Total pigments (TP) are also increasing 2.3 times following bottling. They are reduced by both UV light and darkness treatment (about 22% reduction as compared to bottles not treated), but the final value is still 1.7 times over that of the wine kept in tank.

Free antocyanins were not detected, while the values for flavones and total polyphenolic index had no relevant fluctuations.

Chardonnay response to reverse pinking

In Chardonnay too, the values at 420 nm which describe the browning are rising in time, but especially when the bottled wine is subjected to UV light or darkness treatment. In one month, the browning increases insignificantly in untreated bottles, but 1.1 times in those treated with UV light or kept in darkness.

The pinking (500–520 nm) increases at bottling by 1.6 times and is only slightly reduced by UV light treatment (8%) or darkness (20%), the values remaining well above those of the wines kept in tank.

The blue shade (620 nm) is progressing with storage time too, irrespective of the treatment. After one month the bottled wines have 1.7 times higher values than the control in tank, treatments with UV light or darkness being able to reduce them by a mere 7 and 8% respectively, the final values being still 1.4 times above those of the wine in tank.

Thus, while any of the treatments are able to reduce the values of the colour parameters correlated with browning and pinking, they are not able to sufficiently reduce them to levels observed in unbottled wines kept in reductive conditions.

PAC content (proantocyanins, Table 7) is also significantly different for the samples of Chardonnay kept in tank. After bottling the PAC content falls by about 30% and is not influenced by any of the treatments in a sufficient way.

Co-pigmented anthocyanins (Cp) are increasing 1.4 times after bottling. These pigments in Chardonnay are equally responding to UV light and darkness treatment, their values decreasing after the treatment to about 60% for the UV light treatment and to only 83% for the darkness treatment, compared to the level of the wines in bottle. Interestingly, the level of the co-pigmented anthocyanins in wines treated with UV light is lower even than in wine kept in tank (which is not yet showing pinking). This shows that Chardonnay may respond better than Sauvignon blanc to treatments with the UV light.

Polymeric anthocyanins (P) are increasing following bottling 1.5 times, being only very little decreased by either UV light or darkness treatment, showing as well that Chardonnay is difficult to treat after pinking.

Total pigments (TP) are also increasing 1.5 times following bottling. They are reduced by the UV light treatment to 71% and by darkness to 85% from the levels in bottles, remaining still above the levels in tank, comparing to which they are 1.1 and 1.3 times higher, respectively.

Free antocyanins were not detected.

Table 6. Spectrophotometric analyses of wine pigments and ANOVA – Tukey Test HSD, $p < 0.05$.

Wine samples (buffered pH = 3.6)	*Spectrophotometric analyses of wine pigments (10 mm cuvette)							
	A	Cp, AU	P, AU	Cl, AU	TCP, AU	TP, AU	F, AU	TPI, AU
	Sauvignon blanc							
SB_tank	n.d.	0.16^{c}	0.12^{d}	0.02	0.28^{c}	0.30^{c}	0.82^{d}	10.32^{a}
SB_bottle	n.d.	0.44^{a}	0.20^{c}	n.d.	0.64^{a}	0.64^{a}	0.93^{a}	10.08^{d}
SB_UVlight	n.d.	0.29^{b}	0.20^{b}	n.d.	0.49^{b}	0.49^{b}	0.86^{c}	10.15^{c}
SB_dark	n.d.	0.29^{b}	0.22^{a}	n.d.	0.50^{b}	0.50^{b}	0.89^{b}	10.20^{b}
	Chardonnay							
CH_tank	n.d.	0.24^{c}	0.15^{c}	n.d.	0.38^{d}	0.38^{d}	1.10^{c}	12.62^{b}
CH_bottle	n.d.	0.35^{a}	0.22^{a}	n.d.	0.57^{a}	0.57^{a}	1.22^{a}	12.47^{c}
CH_UVlight	n.d.	0.21^{d}	0.21^{b}	n.d.	0.41^{c}	0.41^{c}	1.20^{b}	12.45^{d}
CH_dark	n.d.	0.29^{b}	0.20^{b}	n.d.	0.49^{b}	0.49^{b}	1.20^{b}	12.70^{a}

* Results expressed as absorbance units, were: A – monomeric anthocyanins; Cp – copigmented anthocyanins; P – polymeric pigments; Cl – colourless anthocyanins; TCP – total coloured pigments; TP – total pigments; F – flavone cofactors; TPI – total polyphenol index.

Table 7. Proanthocyanidin analyses of wine samples and ANOVA – Tukey Test HSD, $p < 0.05$.

Wine samples	Proanthocyanidins, expressed as mg/l cyanidin equivalents	
	Sauvignon blanc	Chardonnay
Tank	33.46^{a}	46.46^{a}
Bottle	26.55^{c}	31.32^{c}
UV light	23.79^{d}	31.03^{d}
Dark	26.80^{b}	32.64^{b}

* Results expressed as mg/l cyanidin equivalents.

4. Conclusions

The pinking phenomenon in wines appears as a result of oxidation and may involve several mechanisms, which lead to changes in colour, not only toward pinking, but also toward browning.

Preventing measures can be applied for susceptible wines, but if they are not sufficiently well kept under control and oxidation occurs, limited options are available for reversing pinking.

For prevention, ascorbic acid addition works well for both Chardonnay and Sauvignon, while addition of tannin may be useful only in Sauvignon.

The ascorbic acid is conferring protection against oxidation and pinking during storage, any dose tested being protective for Sauvignon blanc, while in Chardonnay the effect is dose-dependent, which means that higher doses are needed for better results.

Catechinic tannin also confers protection from oxidation, but brings also some of its own colour into wines, so the dose used should be lower than 30 mg/l because beyond that its colour clearly begins to have an effect of the wine colour. Up to 30 mg/l, especially in Chardonnay, the benefic effect is demonstrated to be dose-dependent.

The effect of antioxidants was very well expressed by the Pinking Potential (PPI) index. The effect was more clear in Chardonnay than in Sauvignon, but this is also due to the fact that the former was much more susceptible to pinking than the Sauvignon.

After pinking occurs, treatments of the bottled wines with the UV light and storage in complete darkness may partially reverse the pinking, but not sufficiently to reach the level before bottling, when the wine was in a reductive state. None of the treatments proved better than the other or sufficiently useful to be applied consistently. Thus, preventing oxidation is the still the best approach to protect susceptible white wines from turning pink.

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