

# The origin of pinking phenomena in white wines: An update

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**Abstract.** Pinking is the term used to describe the appearance of a salmon-red blush color that may appear in bottled white wines, produced solely from white grape varieties. It is perceived as an undesirable phenomenon for both, wine consumers and winemakers. Although with seasonal and regional variations, the pinking has been observed worldwide, with predominance in white wines produced from *Vitis vinifera* L. grape varieties. The pinking origin of Síría white grapes has been studied in detail and it has been shown that the origin of the pinking phenomenon in white wines from *Vitis vinifera* L. Síría grape variety are anthocyanins, mainly malvidin-3-*O*-glucoside. The minimum amount of anthocyanins needed for the visualization of the wine pink color was 0.3 mg/L. Further studies in other white monovarietal wines that occasionally suffer from this defect, like white wine from Malvasia Fina grape variety, Loureiro grape variety, Sauvignon Blanc grape variety and Albariño grape variety, have shown that this wines produced from this grape varieties also show low amounts of anthocyanins, mainly malvidin-3-*O*-glucoside. These results show that the presence of low but visible detectable anthocyanins as the origin of the pinking phenomena is also observed in other white grape varieties besides that of Síría.

## 1. Introduction

Pinking is observed as an undesirable phenomenon in white wines for both, wine consumers and industry, due to the appearance of a pink-red blush in white wines made only from white grapes [1,2]. Although with seasonal and regional differences, the pinking has been detected worldwide, with prevalence in white wines made from *Vitis vinifera* L. white grape varieties such as Chardonnay, Chenin Blanc, Crouchen, Muscat Gordo Blanco, Palomino, Riesling, Sauvignon Blanc, Semillon, Sultana, and Thompson Seedless [1,3]. Pinking is predominantly detected when white wines are processed under reducing conditions [1–4]. The pinking phenomenon is generally perceived in white wines after bottling and storage, or after alcoholic fermentation, although occasionally it happens as soon as the grape must is extracted [5–7]. Pinking is supposed to be due to oxidative modifications in white wines when exposed to oxygen [1,7], although the compounds responsible for the pinking phenomena were until recently unknown [8]. There are several preventive or curative enological treatments for the pinking visual defect, such as the application of polyvinylpyrrolidone (PVPP), PVPP associated with bentonite, or by increasing the redox

potential by application of ascorbic acid in pre-bottling step [9]. However, these treatments increase winemaking costs and can impact on the wine sensory profile [10]. Consequently, the objective of this work was to better understand the pinking phenomenon, namely its origin, and the technological factors that influenced the presence of the pinking visual defect in white wines, using Síría, a white grape variety. The detection of pinking compounds was expanded to other white wines obtained exclusively from white grape varieties like Malvasia Fina, Loureiro, Sauvignon Blanc and Albariño.

## 2. Material and methods

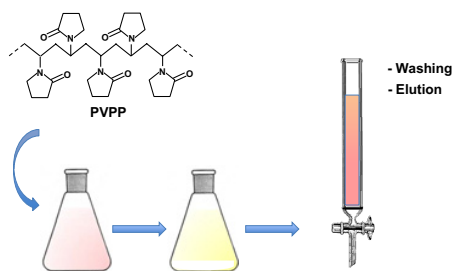
### 2.1. Grape and wine samples

White grapes (Fig. 1a) and white wines (Fig. 1b) from *Vitis vinifera* L., Síría grape variety, used in this study were from the designation of origin Beira Interior, sub-region Castelo Rodrigo, in Portugal, and provided by the Producers Union “Adega Cooperativa de Figueira de Castelo Rodrigo” (ACFCR). Winemaking was carried out at the ACFCR. The white wine from Malvasia Fina grape variety was from the Douro Region, the white wines from Loureiro grape variety and Sauvignon Blanc grape variety were from the Vinhos Verdes Region and the white wine from Albariño grape variety from Galicia in Spain.

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**Figure 1.** a) White grapes and b) white wines with the pinking visual defect from *Vitis vinifera* L., Síria grape variety.



**Figure 2.** Isolation of the compounds responsible for the pinking visual defect by PVPP column chromatography.

## 2.2. Visible absorption spectra and wine chromatic characteristics

The visible absorption spectra were acquired in a 4 cm path length glass cell from 380 to 780 nm. The spectra were converted into a 1 cm path length and the chromatic characteristics of the wines were calculated according to the CIElab method described by OIV [11]. All analyses were performed in duplicate.

## 2.3. Isolation of the pinking compounds from Síria white wines by PVPP column chromatography

To a white wine presenting pinking, PVPP (0.8 g/L) were applied and the suspension was filtered and washed with water (100 mL) followed by ethanol 95% (100 mL). The PVPP, showing a pink color, was loaded on an empty SPE cartridge with frits and eluted sequentially with 3 mL of: 1) acetonitrile, 2) acetone, 3) aqueous solution of 1% HCl, 4) ethanol, and 5) 0.1 M NH<sub>3</sub> in ethanol (Fig. 2). Each eluent was separately collected, the solvent was removed by centrifugal evaporation, and the solution was reconstituted with 0.2 mL of a methanol:water (1:1 v/v) solution before analysis by HPLC. All analyses were performed in duplicate.

## 2.4. Isolation of pinking compounds by reversed phase solid phase extraction

The pinking compounds present in the samples were purified and concentrated by reversed phase (C-18) solid phase extraction. Briefly, 1 g of C-18 SPE column was conditioned by applying 4 times 5 mL methanol and 4 times 5 mL of a 0.1 M HCl solution. Then, the samples were applied: 1) 35 mL of grape must resulting from pressing Síria white grape varieties adjusted to pH 1 with 3 M HCl, or 2) 100 mL of white wines from Síria grape

variety adjusted to pH 1 with 3 M HCl. After application of the sample, the column was washed with 4 times 5 mL of the 0.1 M HCl solution and the compounds retained were eluted with 4 times 5 mL of methanol. The fractions were pooled and the methanol was removed by rotary-evaporation at 35 °C. The dried residue was dissolved in 2 mL of a methanol:water (1:1 v/v) solution and analyzed by HPLC. All analyses were performed in duplicate.

## 2.5. HPLC with photodiode array detector

The phenolic compounds present in grape must and wine water:methanol extract was performed by reversed phase (C-18) HPLC (Ultimate 300) equipped with a PD-100 UV-vis diode array detector. Separation was performed by gradient elution on an ACE 5 C-18 column (5 µm; 250 mm × 4.6 mm). Conditions of HPLC analysis were as follows: solvent A – 95:5 water/formic acid (v/v) and solvent B – methanol. A linear gradient analysis was used as follows: 5% solvent B during 2 min, increase to 80% solvent B over 68 min and then isocratic for 8 min, decreasing to 5% solvent B over 2 min, and isocratic for 5 min. Injected volume was 100 µL, the flow rate was 1.0 mL/min, and the column temperature was 35 °C. The eluent was continuously monitored from 250 to 600 nm with a photodiode array detector. All analyses were performed in duplicate. Anthocyanins identity were made by injection of pure standards in the case of cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside and malvidin-3-*O*-glucoside and comparison of their retention times and UV-Vis spectra. Other anthocyanins were identified by comparison of the elution order and UV-Vis spectra reported in the literature. Additionally, all anthocyanin-3-*O*-glucosides were confirmed by tandem mass spectrometry.

## 2.6. Total monomeric anthocyanins

Total monomeric anthocyanins were determined by the pH differential method AOAC Official Method 2005.02 with slight adjustment [12]. Due to the low concentration of anthocyanins in white wines with pinking, the absorbance at 520 and 700 nm was measured using a 10 cm path length glass cell and expressed as milligrams of malvidin-3-*O*-glucoside per liter (molar extinction coefficient of 28000 L/cm/mol and MW of 493.4 g/mol). All analyses were performed in duplicate.

## 3. Results and discussion

### 3.1. Isolation and characterization by RP-HPLC-DAD of the compounds related to the pinking

The visible absorption spectra of a Síria white wine and the same white wine where pinking was induced by standing in a glass during 24 h (Fig. 3) showed that the color of the wine changed from a clear pale yellow ( $L^*=99.3$ ;  $a^*=-0.471$ ;  $b^*=3.288$ ) to a pale salmon ( $L^*=98.4$ ;  $a^*=0.392$ ;  $b^*=4.004$ ), with a clear increase in the absorbance in the visible region ( $\Delta E^*=1.39$ ). When subtracting the initial absorbance to the absorbance after 24 h, it was clearly observed a peak in the visible region between 440 nm and 630 nm, with a maximum around 500–520 nm. This behavior is the pinking phenomenon



**Figure 3.** Appearance of a Síría white wine (left) and the same white wine where pinking was induced by standing in a glass in contact with air during 24 h (right).

described in the literature, where the presence of the pink-salmon color is induced by oxidation of the white wines [1,2,6].

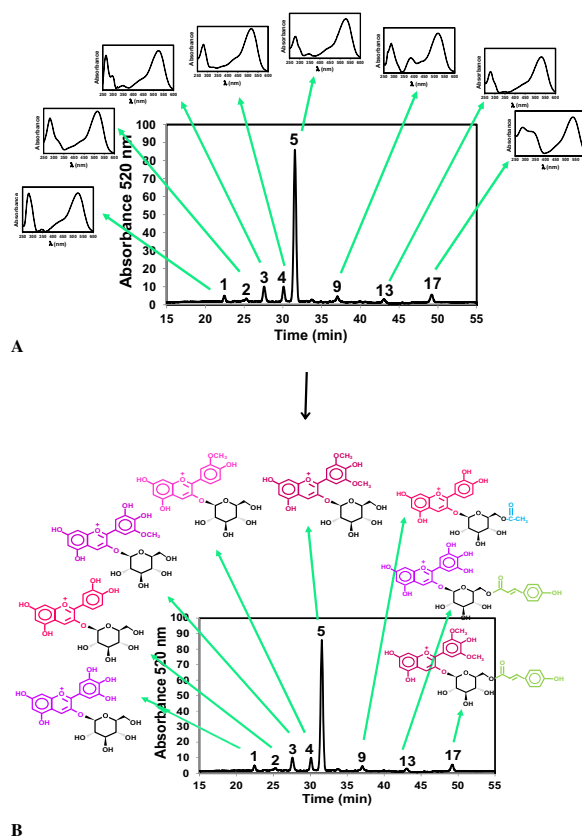
In the wineries when the pinking visual defect is perceived previously to wine bottling, currently PVPP addition is used for the removal of the compounds responsible for this visual defect. Therefore, this approach was also used in this work to remove the compounds responsible for the white wines pinking visual defect. After application of PVPP to the wines, the PVPP becomes pink and the white wine recovered a pale yellow color. The PVPP-retained pink compounds, and when the compounds were eluted from the column, the pink color disappeared. As shown in Fig. 4 the eluted compounds from the PVPP were analyzed by HPLC-DAD yielding a maximum of 18 peaks all with absorbance in the visible region, with maxima in the 500–540 nm range, which correspond to the wavelength of the pinking compounds [1,2].

The UV-visible absorption spectra of the main compounds show that 10 of the 18 identified peaks recovered from the white wine with pinking, representing 86% of the total area, have clear anthocyanin-like absorption spectra. Peaks at retention times 21.58 min, 24.27 min and 30.68 min were coincident with the retention times and UV-spectra of pure standards of delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside.

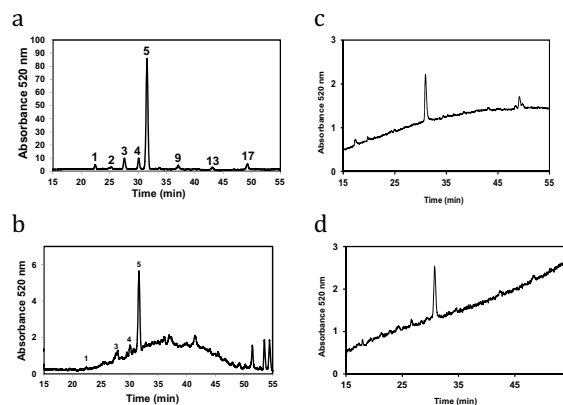
Figure 5a) shows a RP-HPLC chromatogram of the extracts obtained by RP-SPE of white wine from Síría grape variety and Fig. 5b) the RP-SPE extracts of the same white wine analyzed one year after the first analysis. These chromatograms showed that the anthocyanins are not stable during bottle storage. It is observed a loss of monomeric anthocyanins during wine storage due to their polymerization. Also, other white wines made from Malvasia Fina grape varieties (Fig. 5c), from the Douro region and Albariño grape variety from Galicia in Spain were analyzed (Fig. 5d) after a 25-fold concentration by vacuum evaporation, allowing the clear identification of the presence of anthocyanins. These results are in accordance to Arapitsas et al. [13] that detected and quantified anthocyanins in other white grape varieties (Chardonnay, Sauvignon Blanc and Riesling).

The concentration of total monomeric anthocyanins measured by the differential pH method was 0.3 mg of anthocyanins/L.

These results suggested that the main compounds present in Síría white wine with pinking should be anthocyanins and that this amount of monomeric



**Figure 4.** Anthocyanin chromatograms and the UV-visible absorption spectra (A), anthocyanin molecular structure (B) responsible for the pinking visual defect.

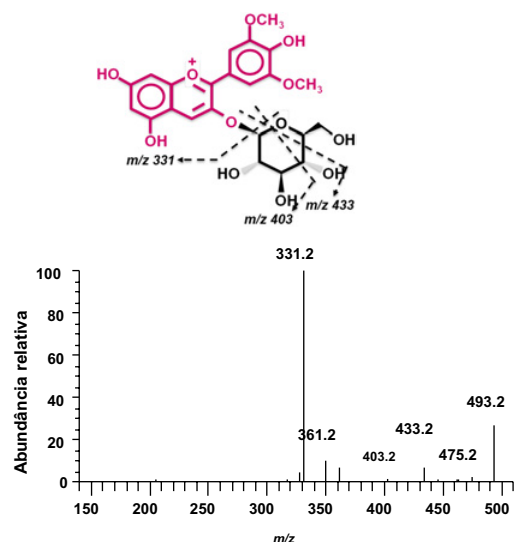


**Figure 5.** RP-HPLC chromatograms of the extracts obtained by a) RP-SPE extracts of white wine from Síría grape variety b) RP-SPE extracts of white wine from Síría grape variety, analyzed one year after the first analysis c) white wine from Loureiro grape variety d) white wine from Malvasia Fina grape variety.

anthocyanins is enough to have the pink color in the white wine, as further confirmed by tandem mass spectrometry.

### 3.2. Confirmation of the chemical nature of the pinking related compounds by tandem mass spectrometry

Ions at *m/z* 449, 463, 465, 479, and 493 were observed by ESI-MS and attributed to the  $M^+$  ions anthocyanin-3-*O*-glucosides (cyanidin, peonidin, delphinidin, petunidin and malvidin, respectively, Fig. 4 B). All attributions



**Figure 6.** ESI Tandem mass spectrum of the  $M^+$  ion of malvidin-3-*O*-glucoside, at  $m/z$  403.2.

were confirmed by tandem mass spectrometry (Fig. 6 for malvidin-3-*O*-glucoside as an example).

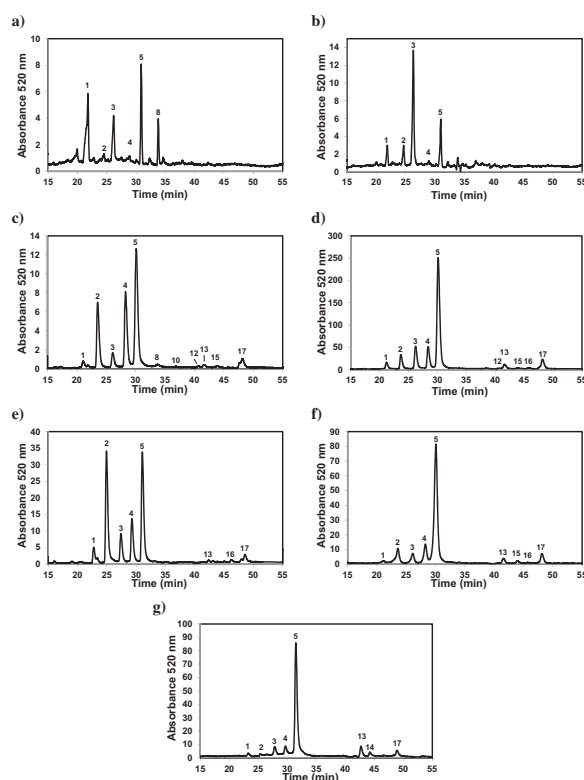
### 3.3. Distribution of anthocyanins in Síria white grapes pulp and skin and the effect of pressing on the white wine pinking anthocyanin profile

To better understand the origin of the anthocyanins in the Síria white grape variety all winemaking steps were followed. The analysis showed that the anthocyanins were located both in the grape pulp and skin without conferring a visible color to the grape berry (Fig. 7a and 7b, respectively). Throughout the grape pressing process, the anthocyanin profile and concentration on the grape must sampled changed at different pressures showing that the highest amounts of anthocyanins were extracted at pressure stage 2 (Fig. 7d). White wine produced from these grape variety contained anthocyanins even after PVPP application (Fig. 7g).

The Síria grape must present evidently a pink color (Fig. 8), and the color changed with the pH variation, being more intense at pH 1.39 and disappeared entirely at pH 4.01 (Fig. 9). At pH 4.01 no pink color could be detected, showing that the anthocyanins were the only compounds with pink color present in the grape must.

In fact, when this white wine was exposed to oxygen, the presence of pinking was detected from the top to the bottom of the bottle, attributed to the reduction of free sulfur dioxide due to the contact to oxygen (Fig. 10).

However, the pinking severity observed in each year in the sub-region Castelo Rodrigo white wine from Síria grape variety was not the same, possibly due to the influence of weather conditions. It was shown that it is negatively related with the increase of the average temperature of the first 10-days of October, the final period of Síria grape varieties maturation (Fig. 11), suggesting that higher temperatures occurring during this period decreased the pinking compounds responsible for the wine coloration.



**Figure 7.** RP-HPLC chromatograms of the extracts obtained from a) Síria grape berry pulp; b) Síria grape berry skin; c) Síria grape must; d) Síria grapes must obtained until 2 kPa pressure; e) Síria grape must obtained until 4 kPa pressure; f) Síria grape must obtained until 6 kPa pressure; g) White wine after vinification including PVPP application. 1 - delphinidin-3-*O*-glucoside; 2 - cyanidin-3-*O*-glucoside; 3 - petunidin-3-*O*-glucoside; 4 - peonidin-3-*O*-glucoside; 5 - malvidin-3-*O*-glucoside; 8 - delphinidin-3-*O*-(6-*O*-acetyl)-glucoside; 10 - petunidin-3-*O*-(6-*O*-acetyl)-glucoside; 12 - malvidin-3-*O*-(6-*O*-acetyl)-glucoside; 13 - delphinidin-3-*O*-(6-*O*-coumaroyl)-glucoside; 14 - cyanidin-3-*O*-(6-*O*-coumaroyl)-glucoside; 15 - petunidin-3-*O*-(6-*O*-coumaroyl)-glucoside; 16 - peonidin-3-*O*-(6-*O*-coumaroyl)-glucoside; 17 - malvidin-3-*O*-(6-*O*-coumaroyl)-glucoside.



**Figure 8.** Grape must recovered during pressing of Síria white grapes variety from Figueira de Castelo Rodrigo vineyards at 2 bar.

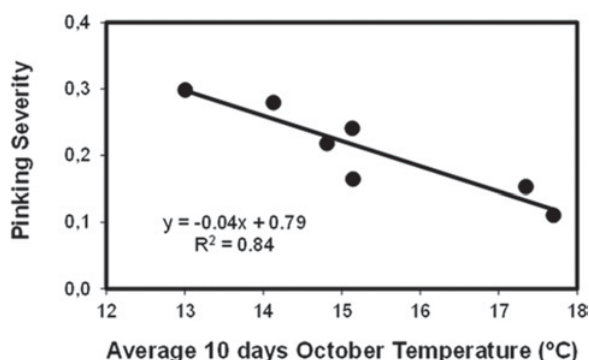


**Figure 9.** Effect of pH (1.39; 3.02 and 4.01) on the color of the must of Síria white grapes variety from Figueira de Castelo Rodrigo vineyards at 2 bar.





**Figure 10.** Wine bottle showing the pinking color from the top to the bottom when exposed to oxygen.



**Figure 11.** Average mean temperature of first 10-days October versus pinking severity of white wines in the sub-region Castelo Rodrigo, Portugal (2005–2011).

#### 4. Conclusions

It has been found that the compounds responsible for the appearance of pinking in white wines from Sória grape variety are anthocyanins, namely malvidin-3-*O*-glucoside present in small concentrations (~ 0.3 mg/L) in white wines produced under reducing conditions. The presence of anthocyanins in wines from white grape varieties were also found in monovarietal wine from white grape varieties like Malvasia Fina, Loureiro, Sauvignon Blanc and Albariño, in these varieties the pinking phenomena is

due to the presence of small amount of anthocyanins but high enough to be detected by the naked eye in the bottled wines.

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