beverages

MDPI

Article

Oxidative Evolution of Different Model Rosé Wines Affected by Distinct Anthocyanin and Tannin Contents

Federico Baris *, Antonio Castro Marin and Fabio Chinnici *

Department of Agricultural and Food Sciences, University of Bologna, 40126 Bologna, Italy; antonio.castromarin2@unibo.it

* Correspondence: federico.baris2@unibo.it (F.B.); fabio.chinnici@unibo.it (F.C.)

Abstract: The quality of rosé wines significantly depends on their phenolic composition, particularly tannins, anthocyanins, and their derivatives, which determine the perceived color of these products and their color evolution throughout the storage and shelf-life periods. This study investigated the impact of phenolic content on the oxidation and color evolution of five different model rosé wines obtained by blending a fixed amount of grape tannins with varying concentrations of oenocyanin to modulate their respective ratio and color intensity. The solutions were monitored for color and pigment changes promoted by oxidation in a Fenton-like environment. The findings revealed a potential correlation between the initial phenolic concentration and the different degrees of oxidation within each solution, resulting in significant variations in CIELAB data. Overall, all solutions exhibited a substantial decrease in redness, with losses ranging from 23% in the darkest solution to 43% in the lightest one compared to their initial levels. Additionally, their color profiles shifted toward yellow hues, up to triple the original value, indicating the degradation of the pigments responsible for the characteristic rosé color. Greater amounts of anthocyanins preserved higher Fe(II) concentrations over time, suggesting the antioxidant role of these compounds. The whole dataset also permitted the evaluation of the different oxidation susceptibilities of individual anthocyanins, among which derived pigments, such as vitisins, proved to be notably more stable than native pigments, particularly delphinidin and petunidin.

Keywords: color of wine; rosé wine; color measurement; color evolution; tannins; anthocyanin; oxidative behavior

Citation: Baris, F.; Castro Marin, A.; Chinnici, F. Oxidative Evolution of Different Model Rosé Wines Affected by Distinct Anthocyanin and Tannin Contents. *Beverages* **2024**, *10*, 43. https://doi.org/10.3390/beverages10020043

Academic Editor: Stamatina Kallithraka

Received: 12 May 2024
Revised: 24 May 2024
Accepted: 29 May 2024
Published: 4 June 2024

1. Introduction

Over the last decade, the consumption of rosé wines around the world has seen a constant increase. Italian rosé is extremely popular abroad, but it is struggling to find space in Italy, and its national consumption has decreased by about 40% compared to the global level over the last 10 years [1]. The positive trend in international preferences could offer producers an opportunity to improve the communication and attractiveness of rosé wine to the domestic market. To an even greater extent than white and red wines, the color of rosé plays a key role in defining the perceived quality of the product, which can range from pale rosé nuances to orange ones, as in wines whose color is referred to as onion skin tones [2]. The phenolic class of anthocyanins and the resulting pigments are primarily responsible for the color of red and rosé wines [3]. Other phenolics involved in color expression are hydroxycinnamic acids, which are abundant in grape pulp and skins, and flavanols, which include monomers like catechins, oligomers, and polymers (called proanthocyanidins, or condensed tannins) located in seeds, skins and, in smaller amounts, pulp [4]. Due to differences in location and solubility, phenolic acids and, to a lesser extent, anthocyanins are readily extracted from the grape tissues into the must, while flavanols are better extracted with longer macerations in the presence of alcohol [5]. In rosé wine, being produced with a very short maceration stage, only limited quantities of

anthocyanins are extracted from the grape skins to the must. However, the scarcity of stable anthocyanin–phenolic complexes and the predominance of monomeric anthocyanins often result in reduced color stability [6]. During bulk wine storage or the bottle shelf-life, in fact, color changes may take place, mainly driven by chemical oxidation boosted by unwanted oxygen exposure during winemaking or due to the gradual entry of oxygen through the closure [7]. The quality of these products could therefore progressively decrease with time once they have been bottled, thus determining the overall shelf-life of the product.

In red wines particularly, during storage, anthocyanins are subjected to a variety of reactions and condensations, leading to the formation of anthocyanin derivatives. The color and stability of these derivatives vary from compound to compound, but some of them may play a crucial role in stabilizing the color of wines [8]. For instance, the addition of carbonylic compounds, such as pyruvic acid or acetaldehyde, to the carbon at position 4 and the hydroxyl group at position 5 of the anthocyanin molecule produces a new pyran ring, giving rise to the so-called pyranoanthocyanins [9] characterized by orange nuances [10,11]. Also, anthocyanins can react with other wine phenolics via acetaldehyde bridging, generating adducts with maximum visible absorption at >540 nm and imparting a bluish nuance to wine [12]. Due to the compositional specificity of rosé wines, the expression and stability of their color may depend on distinct factors, including the grape variety, the maceration length, and the molar ratio between anthocyanins and tannins (extracted from the berries or added during vinification). In addition, dissolved oxygen and reactions involving the above-mentioned phenolics can play a significant role in color evolution [13]. High amounts of anthocyanins can lead to drastic color losses because of oxidation; an excess of polyphenols can also negatively affect the aroma of wines due to the possible formation of quinones that can oxidize volatile compounds and varietal aromas such as thiols, giving rise to aromatic losses [14]. In a great number of these oxidative reactions, the so-called Fenton pathway, where the redox cycling of dissolved iron generates radical species such as hydroxy or ethyl-hydroxy radicals after the transient formation of H_2O_2, plays a key role [15]. In red wines, there is some evidence, however, that specific anthocyanin/tannin ratios may facilitate the formation of stable pigments, which can modulate the kinetics involved in the oxidative cascade [16,17]. As a matter of fact, both the color (e.g., the anthocyanin content) and phenolic contents of light to dark rosé wines vary widely [2,18].

Despite these variabilities, information on the effects of distinct anthocyanin and tannin contents on the color evolution of rosé wine is still lacking in the literature. This study aimed, hence, to investigate the possible impact of rosé wine anthocyanin and phenolic contents on the oxidative color evolution occurring during bottle storage. Accordingly, color and pigment changes in model rosé wines with five different anthocyanin/tannin levels were monitored over time after exposure to a Fenton environment. For each solution, CIELAB and spectrophotometric parameters, together with the evolution of native and derived pigments, were evaluated.

2. Materials and Methods

2.1. Reagents

Oenocyanin and grape tannin solutions were kindly provided by Caviro Extra S.p.A. (Faenza, Italy). Ascorbic acid, (+)-tartaric acid, catechin (+), Fe(II) sulfate heptahydrate, sodium acetate, and potassium chloride were obtained from Sigma-Aldrich (Milano, Italy). HPLC-grade acetonitrile, acetic acid, formic acid, 37% HCl, 97% ethanol, methanol for HPLC, Folin–Ciocalteu phenol reagent (FC), vanillin (VAN), sodium carbonate, ethylenediaminetetraacetic acid (EDTA), 30% hydrogen peroxide, and 96% sulfuric acid were obtained from Merck (Darmstadt, Germany). Water was of MilliQ quality. Ferrozine was purchased from Fluka (Charlotte, NC, USA).

2.2. Samples

Model rosé wines were obtained by blending grape tannin solutions and oenocyanin at different concentrations. The chemical characterization of these two starting materials is shown in Table S1. The target phenolic and color ranges were established from bibliographic data [2,19] to cover the actual variability reported in rosé wines for those parameters. To this aim, two stock solutions of tannins and oenocyanin were appropriately diluted with a model wine (4 g/L tartaric acid, 10% ethanol, pH 3.2) previously stirred for 8 h in open air to reach oxygen saturation at room temperature. The amount of tannins was kept constant for all samples (which contained about 800-fold-diluted raw tannin solution), while oenocyanin was varied to reach the target rosé color and to modulate the relative anthocyanin-to-tannin ratio. Five solutions (1 L each) with distinct color densities were obtained, e.g., VD (Very Dark), D (Dark), I (Intermediate), L (Light), and VL (Very Light) rosé wines. To the air-saturated model wines, 5 mg/L of Fe(II) as iron sulfate heptahydrate was then added. For each trial and each sampling time, 40 mL of the solution was poured into 50 mL Schott bottles (in triplicate), and 100 µmol/L of H2O2 was added to start the Fenton reaction. The bottles were kept at 20 °C in a dark place for 20 days to avoid the influence of temperature and light. Samples were analyzed after 24 h (T24), 48 h (T48), 168 h (T168), and 20 days (T480). Solutions analyzed at the beginning of the storage time are indicated as T0.

2.3. Phenolic and Tannin Contents, Total Anthocyanins, Color, and CIELAB Coordinates of Model Rosé Wines

The color of the solutions was followed by means of spectrophotometric absorbances at 420 nm, 520 nm, and 620 nm and their derived indexes color density (CD) and dA% (the percentage of red produced by the flavylium cation) according to Glories [20] and Negueruela et al. [21]. The CIELAB coordinates L* for lightness, a* for red/green nuances and b* yellow/blue ones, H* for the hue, and C* for chroma, together with ΔE* (overall colorimetric difference between two wines), were also obtained according to OIV [22] by using a Jasco V 730 spectrophotometer and Spectra manager software suite (Jasco, Cremella, Italy). The Folin–Ciocalteu method [23] was used for the determination of the total polyphenols index (TPI), which is expressed as mg/L of gallic equivalents (GAE). Vanillin-reactive flavanols (VRF) were analyzed according to Margheri and Falcieri [24]. To this aim, a vanillin reagent consisting of a 1 g/L vanillin solution in 70% sulfuric acid was prepared. Hence, 1 mL of ethanol was added to 2 mL of diluted wine (1:10), together with 2 mL of vanillin reagent. The developed color was read at 500 nm after 20 min against a blank obtained by substituting the vanillin reagent with 70% sulfuric acid.

Total anthocyanins were measured according to Giusti et al. [25]. Two buffer solutions were prepared, the first composed of potassium chloride (0.025 M), adjusted to pH 1 with HCl, and the second one with sodium acetate (0.4 M) and adjusted to pH 4.5 with the same acid. Each sample was separately diluted with each buffer for proper measurement. After 15 min, the absorbance of each dilution was measured at 520 nm and at 700 nm (to correct for haze) against a cuvette filled with distilled water. The results, expressed as mg/L of malvidin-equivalent (MVDE), were made using the molar absorption coefficient of malvidin (ε = 28,000 $M^{-1} \times cm^{-1}$) and considering the dilution factor.

The polymer contribution to the color was measured according to Boulton [26] after adjusting the samples to pH 3.6 with 1 M NaOH. Specifically, 160 µL of a 5% SO_2 solution was added to 2 mL of each sample and measured at 520 nm (A SO_2). Additionally, 20 µL of a 10% acetaldehyde solution was added to 2 mL of each sample and measured at 520 nm after 45 min (A acet). The polymer contribution to the overall color (PC) was obtained by using the following ratio: A SO_2/A acet, both expressed as percentages. All of the previous analyses were carried out in duplicate.

2.4. Determination of Fe(II) and Total Fe in Model Rosé Wines

The procedure proposed by Nguyen and Waterhouse was followed [27]. For Fe(II) determination, 1 mL of wine was transferred into a tube containing 10 µL of ferrozine solution (3.5% in H_2O) and immediately vortexed before the addition of 1.5 mL of EDTA (0.005% in model wine). Absorbance was measured at 562 nm after 1 min. For the determination of total iron, in a separate tube, EDTA in the analytical procedure was replaced with 1.5 mL of ascorbic acid solution (0.1% in model wine), and measurements were taken after absorbance stabilized following the complete reduction of Fe(III) to Fe(II) (up to 30 min). All absorbance measurements were taken against a blank (2.5 mL of model wine, 10 µL of ferrozine solution). These absorbances were used to calculate Fe(II) and total iron concentrations from a calibration curve obtained with standard Fe(II) solutions in the 0.1–8 mg/L concentration range. Fe(III) amounts were calculated as the difference between Fe(II) and total iron values. Readings were taken in duplicate.

2.5. Identification and Quantification of Anthocyanins in Model Rosé Wines

Anthocyanins were analyzed according to a previous method [28] by using an HPLC instrument equipped with a Jasco PU-2089 quaternary gradient pump, a Jasco AS-2057 Plus Intelligent Sampler autosampler, and a Jasco UV/Vis MD-910 PDA detector (Jasco, Tokyo, Japan). The column was a Synergi 4 µm Hydro-RP 80A 250 × 3.0 mm i.d. (Phenomenex, Torrance, CA, USA). Elution program: initial conditions: 85% Solvent A (formic acid 10% in bidistilled water)/15% Solvent B (formic acid–acetonitrile–bidistilled water 10:45:45); 17 min 70% A/30% B; 45 min 27% A/73% B; 48 min 0% A/100% B; 52 min 0% A/0% B/100% C (acetonitrile 100%); 55 min initial conditions 85% A/15% B. Identification was based on the retention times and UV spectra of individual pigments, as reported by Chinnici et al. [28]. Quantification was carried out by means of a calibration curve of a pure standard of malvidin-3-O-glucoside. All the analyses were carried out in duplicate.

2.6. Statistical Treatment of Data

Chemical data were subjected to one-way ANOVA analysis together with Tukey (HSD) post hoc comparison test to identify significant differences within populations by using the XLSTAT 2016 software package (Addinsoft, Paris, France).

3. Results and Discussion

3.1. Initial Color of Model Rosé Wines and Its Oxidative Evolution during Storage

The initial color and phenolic content of the five model rosé wines prepared as previously indicated are reported in Table 1. The CIELAB and color parameters of the solutions adequately represent the range of variability in rosé wines and are in line with literature data. As a comparison, for instance, in 268 commercial rosé wines from 21 different countries, Leborgne et al. [2] found color densities spanning from 0.15 to 2.07. In those wines, L* was between 57.6 and 97.3, while a* and b* indexes were, respectively, from 0.88 to 50.9 and from 5.02 to 31.59. In addition, total anthocyanins varied from 10.6 to 32.2 mg/L. The same authors grouped all the wines into light (mean CD = 0.88), medium (mean CD = 1.16), and dark (mean CD = 1.50) rosé wines. Pérez-Magariño and González-San Jose [29] showed that the H* parameter ranged from 30° to 50° in 126 rosé wines from different Spanish production zones. Phenolics have been found to span from 90 mg/L in weakly tannic rosé wines up to 1 g/L in rosé wines high in tannins [19,30]. Table 1 also reports the dA% index, which was studied by Negueruela et al. [21] in red wines, representing the percentage of red color due to the flavylium forms of free and combined anthocyanins. It has been suggested that, in tawny red wines, this index would be less than 40%, while ruby-colored wines show dA% between 60% and 80% [20]. In our samples, the polymeric pigment contribution to color was initially between 55.16% (in VL solutions) and 41.95% (in VD model wines), certainly due to the high level of constitutive polymers coming from oenocyanin (Table S1). The same analytical method [26] could have provided information on the extent of copigmentation of those solutions, but as expected, the 20-fold dilutions needed for

this determination drove spectrophotometric readings to nil in the lightest rosé solutions, which impeded measurements (in VD and D model wines, however, copigmentation was estimated to contribute about 7% to the total color).

Table 1. Color and phenolic parameters of the distinct model rosé wines at T0. The data are given as means ± standard deviations. The color simulation of the samples shown in the legend row, based on CIELAB parameters, was obtained by using the software https://convertingcolors.com (accessed on 20 May 2024). H* = hue; CD = color density; dA% = contribution of flavylium ions to color; TPI = total phenols (mg/L GAE); VRF = vanillin-reactive flavanols (mg/L CE); TA = total anthocyanin (mg/L MVDE); PC% = polymer contribution to red color. Sample codes: VD T0 = Very Dark; D T0 = Dark; I T0 = Intermediate; L T0 = Light; VL T0 = Very Light.

	VD T0	D T0	I T0	L T0	VL T0
L*	60.2 ± 0.2	75.7 ± 0.2	85.5 ± 0.1	91.2 ± 0.1	93.9 ± 0.1
a*	40.07 ± 0.16	24.45 ± 0.20	14.11 ± 0.10	7.05 ± 0.07	3.64 ± 0.05
b*	4.22 ± 0.08	4.58 ± 0.05	5.37 ± 0.07	6.03 ± 0.06	6.29 ± 0.03
dA%	53.94 ± 0.03	53.20 ± 0.10	44.57 ± 0.03	29.59 ± 0.17	11.13 ± 0.73
C*	40.29 ± 0.12	24.78 ± 0.18	15.16 ± 0.09	9.23 ± 0.08	7.33 ± 0.03
H*	6.01 ± 0.09	10.60 ± 0.04	20.82 ± 0.11	40.56 ± 0.04	59.93 ± 0.25
CD	1.50 ± 0.00	0.89 ± 0.00	0.53 ± 0.00	0.36 ± 0.00	0.27 ± 0.00
TPI	749 ± 2.03	637 ± 5.35	567 ± 0.55	555 ± 4.37	486 ± 10.15
VRF	457 ± 5.70	362 ± 3.98	310 ± 3.33	252 ± 3.33	245 ± 0.81
TA	13.42 ± 0.29	6.41 ± 0.14	2.94 ± 0.01	1.47 ± 0.04	0.67 ± 0.03
PC%	41.95 ± 0.24	45.05 ± 0.24	45.14 ± 0.33	53.55 ± 0.00	55.16 ± 0.48

During the experiment, the solutions underwent distinct changes depending on their initial phenolic content, especially regarding CIELAB data. In Table 2, the changes in the color and phenolic parameters of model wines at T480 are shown as percentage variations with respect to T0. In general, all of the samples lost some of the red nuance (diminution in a*) and greatly augmented their yellowness (increase in b*), which were expected consequences of oxidative browning and free anthocyanin loss. It is worth noting, however, that in the darker rosé solutions, redness decreased to a significantly lower extent with respect to L and VL samples. The yellow tint rose by similar absolute values in all the samples (ranging between 13.46 and 14.50 units without a clear trend), which made the percentage variation of the solutions increase from the lightest to the darkest solutions (Table 2).

After 20 days of storage, our studies also showed an increasing diminution in dA%, ranging from the smallest decrease in the darkest solutions up to the complete loss in the lightest ones. Furthermore, these results were accompanied by parallel dramatic and significant changes in chroma (C*) and hues (H*), augmented by 6% to 201% and 40% to 412%, respectively, but with opposite trends. In fact, while the darkest-colored solution (VD) exhibited the strongest augmentation in hue, the lightest one (VL) showed the highest increase in chroma, making the color of the latter samples significantly more saturated than they were at the initial starting point. At the same time, VD samples acquired yellow tones, which were not present at T0. The total polyphenol index (TPI) and vanillin-reactive flavanols (VRF), on the other hand, decreased by about 10–15% and 15–19%, respectively, in all the samples and in a very similar way, independently of their initial amount.

Table 2. Changes in color and phenolic parameters (as percentage variation with respect to T0) of distinct model rosé wines after 20 days (T480) of forced oxidation. The data are given as means ± standard deviations. The color simulation of the samples shown in the legend row, based on CIELAB parameters, was obtained by using the software https://convertingcolors.com (accessed on 20 May 2024). dA% = contribution of flavylium ions to color; ΔE* = total color difference; TPI = total phenols (mg/L GAE); VRF = vanillin-reactive flavanols (mg/L CE); TA = total anthocyanins (mg/L MVDE); PC% = polymer contribution to red color. Sample codes: VD T480 = Very Dark; D T480 = Dark; I T480 = Intermediate; L T480 = Light; VL T480 = Very Light. In the same row, different letters indicate significant differences at $p < 0.05$ (n = 6).

	VD T480	D T480	I T480	L T480	VL T480
L*	11.8 ± 0.3 a	4.3 ± 0.3 b	1.3 ± 0.2 c	−1.3 ± 0.5 d	−2.2 ± 0.6 d
a*	23.05 ± 0.69 a	−22.59 ± 1.33 a	−25.38 ± 1.89 a	−35.43 ± 1.22 b	−42.97 ± 1.33 c
b*	336.18 ± 1.28 a	313.12 ± 0.87 b	257.11 ± 2.06 c	228.24 ± 1.11 d	215.59 ± 1.34 e
dA%	−26.53 ± 2.83 a	−50.94 ± 2.96 ab	−73.46 ± 2.89 ab	−100.00 ± 0.00 b	−100.00 ± 0.00 b
C*	6.28 ± 0.27 d	8.32 ± 0.46 d	12.70 ± 0.50 c	112.22 ± 1.08 b	201.14 ± 1.45 a
H*	412.95 ± 5.60 a	324.12 ± 5.75 b	194.02 ± 4.38 c	89.99 ± 0.64 d	40.21 ± 0.31 e
ΔE*	18.35 ± 0.15 a	15.72 ± 0.09 b	14.30 ± 0.03 c	14.05 ± 0.02 cd	13.80 ± 0.03 d
CD	22.30 ± 5.68 c	31.47 ± 8.33 c	68.96 ± 13.88 b	77.9 ± 14.43 b	121.95 ± 11.62 a
TPI	−10.80 ± 3.86 a	−13.59 ± 3.46 a	−15.30 ± 1.05 a	−15.44 ± 0.76 a	−15.83 ± 1.42 a
VRF	−15.69 ± 0.96 a	−19.38 ± 1.43 a	−18.30 ± 0.67 a	−13.46 ± 1.57 a	−16.97 ± 3.43 a
TA	−51.67 ± 2.81 a	−50.57 ± 3.36 a	−50.63 ± 4.45 a	−41.20 ± 1.86 ab	−35.26 ± 3.37 b
PC%	26.43 ± 0.57 a	19.34 ± 0.47 b	18.65 ± 0.78 b	8.91 ± 0.18 c	7.05 ± 0.70 c

The total anthocyanin parameter (TA) decreased by about 41–51% for four out of the five solutions, and only VL solutions had a significantly smaller decline when compared to T0 (−35%). These results are in accordance with other studies [17], where the gradual decrease in pigments during wine aging contributed to an increase in lightness and a loss of redness. Anthocyanins may be involved in the creation of new pigments with different light absorption properties, which can also contribute to the color change. Apparently, in fact, highly colored rosé wines (VD, D, and I) lost red color to a lesser extent when compared to L and VL samples, despite the higher percentage diminution in total anthocyanins (TA) (Table 2). In these solutions, the increased contribution of polymeric anthocyanins (PC%) at T480, on the one hand, and the complete disappearance of anthocyanins in the flavylium form in L and VL model wines, on the other hand, could have played a substantial role. Indeed, in red wines, previous research stressed the role of oxygen in favoring the formation of anthocyanin/tannin polymers during the storage period, with a consequent increase in color densities and contextual anthocyanin diminution [31]. The richness of the initial anthocyanin content of VD, D, and I solutions may certainly have favored the prevalence of these pigmented polymers and derivatives in the global color of the samples. The timewise changes in the a* and b* CIELAB parameters' absolute values for each solution are presented in Figure 1 to illustrate the relative trends over time. This figure suggests that the darkest solutions had the greatest overall variation, as also confirmed by ΔE* data (Table 2), which sums up the changes in the L*, a*, and b* parameters of each solution throughout its storage.

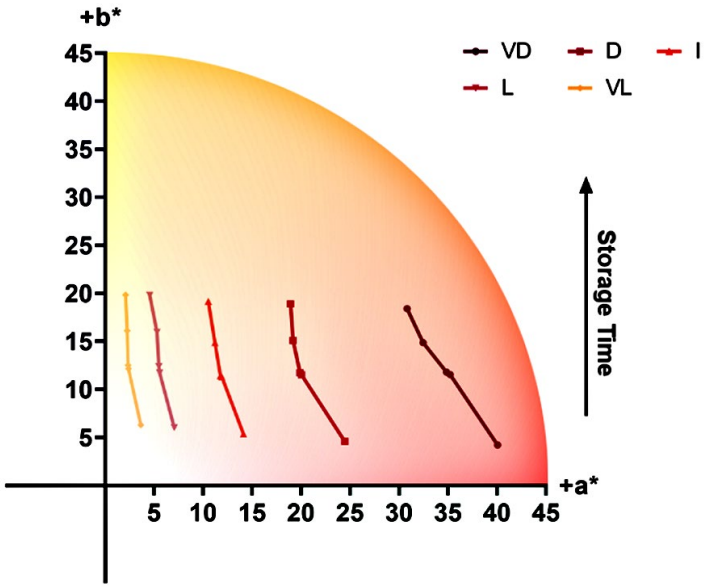

Figure 1. A representation of the evolution of the CIELAB parameters of the different solutions over time. The arrow labeled "Storage Time" indicates the direction of their color evolution over four sampling points from T0 to T480. The CIELAB color space reported in the figure does not represent the actual color at each given value and is reported for illustrative purposes only. Sample codes: VD = Very Dark; D = Dark; I = Intermediate; L = Light; VL = Very Light.

3.2. Iron Speciation

It has been suggested that, in wines, the redox status of transition metals, particularly iron, may provide clues to the overall oxidative pressure due to the matrix [32]. Iron, in fact, can overcome the initial energy barrier associated with the electron transfer among oxygen and phenolics by redox cycling between its two oxidation states, namely, Fe(II) and Fe(III) [15]. These phenomena are characterized by initial rapid iron oxidation until a stable equilibrium is reached between the reduced (Fe(II)) and oxidized (Fe(III)) forms, which ultimately depend on the dissolved oxygen levels and wine composition (i.e., phenolics and antioxidant molecules). In white wines, for instance, this plateau may be reached after more than 40 h [32], while shorter times to plateau (3 h) were reported for red wines subjected to air saturation [27]. To our knowledge, no data on Fe redox kinetics have been reported for rosé wines. As shown in Figure 2, in the samples under investigation, an equilibrium was reached after rapid oxidation occurred during the first few days. However, the higher the total phenolic content of wines, the more abundant the Fe(II) species present at equilibrium, suggesting significant differences in oxidative strength depending on the phenolic richness of the samples. In this study, the model wines were all prepared with the same starting model wine and identical amounts of tannins. They only differed in anthocyanin levels, so it can be assumed that such results depended on these compounds and the inherent reactions promoted by them. These results represent, to our knowledge, one of the first clear pieces of evidence that anthocyanins can increase the Fe(II)/Fe(III) ratio. In addition, for VD wines, the equilibrium was reached somewhat earlier (after 48 h) than other solutions, further confirming the role of phenols in regulating the redox status of iron in wines [27,32].

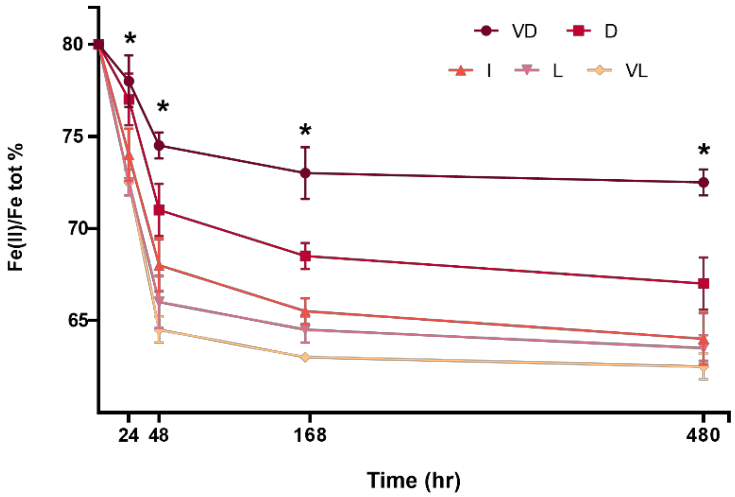

Figure 2. The evolution of the Fe(II)/Fe(tot) percentage in the solutions over time. (*) indicates, for each sampling point, significant differences at $p \leq 0.05$ (n = 6). Sample codes: VD = Very Dark; D = Dark; I = Intermediate; L = Light; VL = Very Light.

3.3. Evolution of Monomeric Anthocyanins and Derived Pigments

To investigate the overall stability of anthocyanins against oxidation, they were quantified by HPLC and grouped into six main families, including both native compounds (glucosides, acetylglucosides, and coumaroylglucosides) and their derivatives (vitisins, vinylphenol, and ethyl-bridged adducts). Table S2 details the compounds included in each family. Figure 3 shows the residual amount of each chemical class after 20 days of simulated oxidation (as a mean percentage of the entire sample set, independently of the anthocyanin/tannin ratio). Table S3 provides the significant differences among the families at each sampling point during the storage of the solutions. All of these classes proved to be oxidized in the long term, but the derived pigments were significantly more stable than monomeric anthocyanins. In fact, while the latter degraded by up to about 75%, vitisins and the other derivatives only lost, on average, 30–45% of their initial content. Pyranoanthocyanins' (vitisin A, vitisin B, and vinyl adducts) stability has been claimed to be due to the presence of pyran rings at positions 4 and 5 of the anthocyanin skeleton, which enhances their resistance to nucleophilic attacks [33,34]. In wines, vitisins' slow degradation may result from their contextual formation during aging due to the interaction between pyruvic acid and key pigments, such as malvidin-3-glucoside and malvidin-3-acetylglucoside [11].

Given that our samples were made from model solutions lacking pyruvic acid, our results suggest that their higher stability may instead be due to stronger steric hindrance preventing an attack by water molecules or nucleophilic compounds [35]. Regarding ethyl-bridged adducts, their number during wine oxidation could depend on the dynamic balance between degradation and formation over time. In fact, the progressive generation of acetaldehyde from ethanol oxidation leads to the progressive synthesis of this kind of compound [36]. These mentioned mechanisms are key points in wine color stabilization and justify the prevalence of these derived pigments in red wines aged in the presence of oxygen [11]. As far as monomeric anthocyanins are concerned, despite scientific evidence that acylation may contribute to the stabilization of glycosylated anthocyanins against oxidation [37], our data did not show significant variation in the Glc/(Acglc + Coumglc) ratio during the storage time, which can also be somewhat inferred from Figure 3.

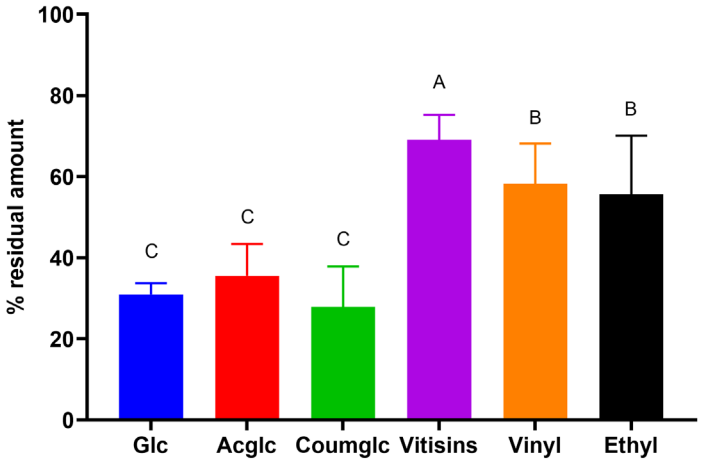

Figure 3. Residual amounts (as percentages) of the different anthocyanin families after 20 days. For each mean value, the entire sample set was considered independently of their initial anthocyanin/tannin ratio. Glc = glucosides; Acglc = acetylglucosides; Coumglc = coumaroylglucosides; Vinyl = vinylphenol adducts; Ethyl = ethyl-bridged adducts. Different letters indicate significant differences at $p \leq 0.05$ (n = 30).

The dataset has also been useful in highlighting the oxidative behavior of individual anthocyanins. The evolving contents of some of the most representative of them are depicted in Figure 4. Among the 23 anthocyanins identified, Dp-3-glc and Pt-3-glc degraded the most and the fastest three weeks after the first sampling to less than 10% and 13%, respectively, compared to their initial amounts. This may be due to the presence of an o-diphenolic group in the B-ring of these molecules, which has been established to be particularly prone to both enzymic and chemical oxidation [38]. The replacement of one -OH by hydrogen or a methoxy moiety, as in Mv-3-glc and Pn-3-glc, significantly improves the molecule's stability to oxidation, as, in fact, we found in our samples (Figure 4 and Table S2), in accordance with previous investigations [38,39], apart from Cy-3-glc. In those studies, in fact, despite the presence of an o-diphenolic group in its B-ring, Cy-3-glc turned out to be the most stable pigment.

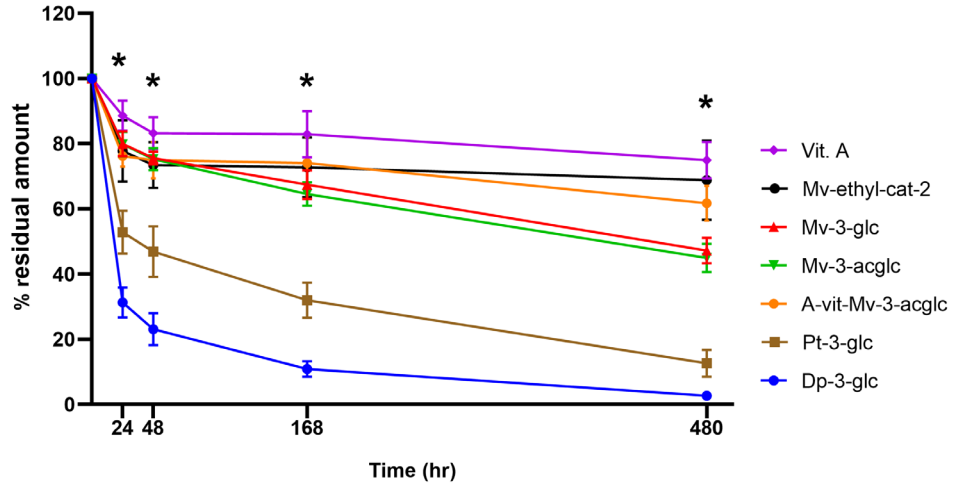

Figure 4. The evolution of residual amounts of some specific anthocyanins (as percentages) over a period of 20 days. For each mean value, the entire sample set was considered independently of the initial anthocyanin/tannin ratio. Vit. A = vitisin A; Mv = malvidin; Pt = petunidin; Dp = delphinidin; Glc = glucosides; Acglc = acetylglucosides; Ethyl = ethyl-bridged adducts. (*) indicates, for each sampling point, significant differences at $p \leq 0.05$ (n = 30).

Our data, indeed, suggest that it was clearly more stable than the other *o*-diphenolic anthocyanins but declined faster than Mv-3-glc and Pn-3-glc, as already found in grape must by Cheynier et al. [40]. As previously mentioned for Figure 3, pigment derivatives, particularly vitisin A and one of the four isomers of Mv-ethyl-bridge adducts, were the least sensitive to oxidation, in agreement with other research [11,33,36].

For each individual solution, the data shown in Table S2 also allow for the evaluation of the potential impact of the initial phenolic content on the oxidation kinetics. Due to the high dilution of oenocyanin needed for the preparation of the VL solution, it was not possible to quantify the vinylphenol and ethyl-bridged adducts in that sample (Table S2). The different families exhibited similar behavior in the solutions over time. Vitisins and vinyl-adduct families were hardly influenced by the initial amount of phenols in the solutions, except for the unexpectedly low stability of Mv-3-glc-4-vinyl-(epi)cat in the L sample at the end of storage. Considering glucoside, coumaroylglucoside, and ethyl-adduct families, the solutions containing the highest concentrations of phenols (VD and D) tended to better preserve these pigments. The evolution of acetylated anthocyanins may suggest the opposite behavior, in which VL samples emerged as the most stable to oxidation for each sampling point. This is indeed due to the fact that, in VL samples, this family was only represented by the most stable acetylated anthocyanin (Mv-3-acglc) because of the negligible contents of the other compounds within this class.

4. Conclusions

The results of these studies suggest that differences in the anthocyanin/tannin ratio play a role in the color stability of rosé model wines during storage. Although all rosé model wines were susceptible to oxidation, the ones with higher initial anthocyanin content were demonstrated to better preserve their color. CIELAB data highlighted that, in the darkest solutions, there was a minor loss in redness and a simultaneous increase in the polymer contribution to the overall color. For all the solutions, the augmentation of the yellow nuances appeared to be related to their initial phenolic contents, while no significant differences in the TPI and VRF changes were observed among the distinct solutions. The anthocyanin content was linked to a higher final Fe(II)/Fe(tot) ratio, which may be one of the mechanisms by which these compounds modulate the oxidative phenomena of rosé wines. Independently of the initial phenolic content, individual pigments degraded at different rates, with vitisins exhibiting greater stability than monomeric anthocyanins. Di/tri-hydroxylated anthocyanins, such as Dp-3-glc and Pt-3-glc, were found to be the least stable pigments, suggesting that the choice of grape varieties could influence the oxidative evolution of the resulting rosé wines. In light of these results, the current market trend toward paler rosé wines may drive the sale of products with more drastic color changes during their storage and shelf-life.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/beverages10020043/s1: Table S1: Color and phenolic parameters (±SD) of oenocyanin and tannin solutions used to prepare model rosé wines; Table S2: Evolution of single anthocyanins (as percentages with respect to T0) over time in different samples; Table S3: Overall mean residual amounts (as percentages) of different anthocyanin families at each sampling point.

Author Contributions: Conceptualization, F.C. and F.B.; methodology, F.B.; validation, F.C., F.B. and A.C.M.; investigation, F.B.; resources, F.C.; data curation, F.B.; writing—original draft preparation, F.B.; writing—review and editing, F.C.; supervision, F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available at https://amsacta.unibo.it/id/eprint/7705 (accessed on 20 May 2024).

Acknowledgments: The authors acknowledge the support of Caviro Extra S.p.a. for generously providing the stock solutions used in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Iazzi, A.; Scorrano, P.; Rosato, P.; Grandhi, B. Millennial Generation Preferences for Rosé Wine: An Exploratory Study of the Italian and French Markets. *Br. Food J.* **2020**, *122*, 2443–2461. [CrossRef]
2. Leborgne, C.; Lambert, M.; Ducasse, M.A.; Meudec, E.; Verbaere, A.; Sommerer, N.; Boulet, J.C.; Masson, G.; Mouret, J.R.; Cheynier, V. Elucidating the Color of Rosé Wines Using Polyphenol-Targeted Metabolomics. *Molecules* **2022**, *27*, 1359. [CrossRef]
3. Brouillard, R.; Chassaing, S.; Fougerousse, A. Why Are Grape/Fresh Wine Anthocyanins so Simple and Why Is It That Red Wine Color Lasts so Long? *Phytochemistry* **2003**, *64*, 1179–1186. [CrossRef] [PubMed]
4. Mané, C.; Souquet, J.M.; Ollé, D.; Verriés, C.; Véran, F.; Mazerolles, G.; Cheynier, V.; Fulcrand, H. Optimization of Simultaneous Flavanol, Phenolic Acid, and Anthocyanin Extraction from Grapes Using an Experimental Design: Application to the Characterization of Champagne Grape Varieties. *J. Agric. Food Chem.* **2007**, *55*, 7224–7233. [CrossRef]
5. Fulcrand, H.; Atanasova, V.; Salas, E.; Cheynier, V. The Fate of Anthocyanins in Wine: Are There Determining Factors? *ACS Symp. Ser.* **2004**, *886*, 68–88. [CrossRef]
6. Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. *Handbook of Enology, Volume 1: The Microbiology of Wine and Vinifications*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2006; ISBN 0-470-01034-7.
7. Skouroumounis, G.K.; Kwiatkowski, M.J.; Francis, I.L.; Oakey, H.; Capone, D.L.; Duncan, B.; Sefton, M.A.; Waters, E.J. The Impact of Closure Type and Storage Conditions on the Composition, Colour and Flavour Properties of a Riesling and a Wooded Chardonnay Wine during Five Years' Storage. *Aust. J. Grape Wine Res.* **2005**, *11*, 369–377. [CrossRef]
8. He, F.; Liang, N.N.; Mu, L.; Pan, Q.H.; Wang, J.; Reeves, M.J.; Duan, C.Q. Anthocyanins and Their Variation in Red Wines I. Monomeric Anthocyanins and Their Color Expression. *Molecules* **2012**, *17*, 1571–1601. [CrossRef]
9. Cheynier, V.; Prieur, C.; Guyot, S.; Rigaud, J.; Moutounet, M. The Structures of Tannins in Grapes and Wines and Their Interactions with Proteins. *ACS Symp. Ser.* **1997**, *661*, 81–93. [CrossRef]
10. Rivas-Gonzalo, J.C.; Santos-Buelga, C. Understanding the Colour of Red Wines: From Anthocyanins to Complex Pigments. In *Polyphenols, Wine and Health*; Springer: Dordrecht, The Netherlands, 2001; pp. 99–121. [CrossRef]
11. Alcalde-Eon, C.; Escribano-Bailón, M.T.; Santos-Buelga, C.; Rivas-Gonzalo, J.C. Changes in the Detailed Pigment Composition of Red Wine during Maturity and Ageing: A Comprehensive Study. *Anal. Chim. Acta* **2006**, *563*, 238–254. [CrossRef]
12. Mateus, N.; Silva, A.M.S.; Rivas-Gonzalo, J.C.; Santos-Buelga, C.; De Freitas, V. A New Class of Blue Anthocyanin-Derived Pigments Isolated from Red Wines. *J. Agric. Food Chem.* **2003**, *51*, 1919–1923. [CrossRef]
13. Wirth, J.; Caillé, S.; Souquet, J.M.; Samson, A.; Dieval, J.B.; Vidal, S.; Fulcrand, H.; Cheynier, V. Impact of Post-Bottling Oxygen Exposure on the Sensory Characteristics and Phenolic Composition of Grenache Rosé Wines. *Food Chem.* **2012**, *132*, 1861–1871. [CrossRef]
14. Gil, M.; Louazil, P.; Iturmendi, N.; Moine, V.; Cheynier, V.; Saucier, C. Effect of Polyvinylpolypyrrolidone Treatment on Rosés Wines during Fermentation: Impact on Color, Polyphenols and Thiol Aromas. *Food Chem.* **2019**, *295*, 493–498. [CrossRef]
15. Danilewicz, J.C. Review of Reaction Mechanisms of Oxygen and Proposed Intermediate Reduction Products in Wine: Central Role of Iron and Copper. *Am. J. Enol. Vitic.* **2003**, *54*, 73–85. [CrossRef]
16. Garrido-Bañuelos, G.G.; Buica, A.; Sharp, E.; de Villiers, A.; du Toit, W.J. The Impact of Different Tannin to Anthocyanin Ratios and of Oxygen on the Phenolic Polymerisation Over Time in a Wine-like Solution. *S. Afr. J. Enol. Vitic.* **2019**, *40*, 1. [CrossRef]
17. Gambuti, A.; Picariello, L.; Rinaldi, A.; Moio, L. Evolution of Sangiovese Wines with Varied Tannin and Anthocyanin Ratios during Oxidative Aging. *Front. Chem.* **2018**, *6*, 63. [CrossRef] [PubMed]
18. Salinas, M.R.; Garijo, J.; Pardo, F.; Zalacain, A.; Alonso, G.L. Color, Polyphenol, and Aroma Compounds in Rose Wines after Prefermentative Maceration and Enzymatic Treatments. *Am. J. Enol. Vitic.* **2003**, *54*, 195–202. [CrossRef]
19. Kelebek, H.; Canbas, A.; Selli, S. HPLC-DAD-MS Analysis of Anthocyanins in Rose Wine Made from Cv. Öküzgözü Grapes, and Effect of Maceration Time on Anthocyanin Content. *Chromatographia* **2007**, *66*, 207–212. [CrossRef]
20. Glories, Y. La Couleur Des Vins Rouges. 2e Partie: Mesure, Origine et Interprétation. *OENO One* **1984**, *18*, 253–271. [CrossRef]
21. Negueruela, A.I.; Ech, J.F.; Pi, M.M. A Study of Correlation Between Enological Colorimetric Indexes and CIE Colorimetric Parameters in Red Wines. *Am. J. Enol. Vitic.* **1995**, *46*, 353–356. [CrossRef]
22. OIV. Compendium of International Analysis of Methods—OIV Chromatic Characteristics Determination of Chromatic Characteristics According to CIELab (Resolution Oeno 1/2006). Available online: https://www.oiv.int/public/medias/2478/oiv-ma-as2-11.pdf (accessed on 20 May 2024).
23. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
24. Margheri, G.; Falcieri, E. Importanza Dell'evoluzione Delle Sostanza Polifenoliche Nei Vini Rossi Di Qualità Durante l'invecchiamento. *Vini D'italia* **1972**, *14*, 501–511.
25. Giusti, M.M.; Wrolstad, R.E. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, *1*, F1.2.1–F1.2.13. [CrossRef]
26. Boulton, R. The Copigmentation of Anthocyanins and Its Role in the Color of Red Wine: A Critical Review. *Am. J. Enol. Vitic.* **2001**, *52*, 67–87. [CrossRef]

27. Nguyen, T.H.; Waterhouse, A.L. A Production-Accessible Method: Spectrophotometric Iron Speciation in Wine Using Ferrozine and Ethylenediaminetetraacetic Acid. *J. Agric. Food Chem.* **2019**, *67*, 680–687. [CrossRef] [PubMed]
28. Chinnici, F.; Sonni, F.; Natali, N.; Galassi, S.; Riponi, C. Colour Features and Pigment Composition of Italian Carbonic Macerated Red Wines. *Food Chem.* **2009**, *113*, 651–657. [CrossRef]
29. Pérez-Magariño, S.; José, M.L.G.-S. Prediction of Red and Rosé Wine CIELab Parameters from Simple Absorbance Measurements. *J. Sci. Food Agric.* **2002**, *82*, 1319–1324. [CrossRef]
30. Suriano, S.; Basile, T.; Tarricone, L.; Di Gennaro, D.; Tamborra, P. Effects of Skin Maceration Time on the Phenolic and Sensory Characteristics of Bombin o Nero Rosé Wines. *Ital. J. Agron.* **2015**, *10*, 21–29. [CrossRef]
31. Ribéreau-Gayon, P.; Pontallier, P.; Glories, Y. Some Interpretations of Colour Changes in Young Red Wines during Their Conservation. *J. Sci. Food Agric.* **1983**, *34*, 505–516. [CrossRef]
32. Danilewicz, J.C. Fe(II):Fe(III) Ratio and Redox Status of White Wines. *Am. J. Enol. Vitic.* **2016**, *67*, 44. [CrossRef]
33. Dipalmo, T.; Crupi, P.; Pati, S.; Clodoveo, M.L.; Di Luccia, A. Studying the Evolution of Anthocyanin-Derived Pigments in a Typical Red Wine of Southern Italy to Assess Its Resistance to Aging. *LWT* **2016**, *71*, 1–9. [CrossRef]
34. Marquez, A.; Serratosa, M.P.; Merida, J. Pyranoanthocyanin Derived Pigments in Wine: Structure and Formation during Winemaking. *J. Chem.* **2013**, *2013*, 713028. [CrossRef]
35. Mazza, G.; Brouillard, R. Color Stability and Structural Transformations of Cyanidin,5-Diglucoside and Four 3-Deoxyanthocyanins in Aqueous Solutions. *J. Agric. Food Chem.* **1987**, *35*, 422–426. [CrossRef]
36. Boido, E.; Alcalde-Eon, C.; Carrau, F.; Dellacassa, E.; Rivas-Gonzalo, J.C. Aging Effect on the Pigment Composition and Color of Vitis Vinifera L. Cv. Tannat Wines. Contribution of the Main Pigment Families to Wine Color. *J. Agric. Food Chem.* **2006**, *54*, 6692–6704. [CrossRef] [PubMed]
37. Zhao, C.-L.; Yu, Y.-Q.; Chen, Z.-J.; Wen, G.-S.; Wei, F.-G.; Zheng, Q.; Wang, C.-D.; Xiao, X.-L. Stability-Increasing Effects of Anthocyanin Glycosyl Acylation. *Food Chem.* **2017**, *214*, 119–128. [CrossRef] [PubMed]
38. Zhao, X.; He, F.; Zhang, X.-K.; Shi, Y.; Duan, C.-Q. Impact of Three Phenolic Copigments on the Stability and Color Evolution of Five Basic Anthocyanins in Model Wine System. *Food Chem.* **2022**, *375*, 131670. [CrossRef] [PubMed]
39. Cabita, L.; Fossen, T.; Andersen, Ø.M. Colour and Stability of the Six Common Anthocyanidin 3-Glucosides in Aqueous Solutions. *Food Chem.* **2000**, *68*, 101–107. [CrossRef]
40. Cheynier, V.; Souquet, J.-M.; Kontek, A.; Moutounet, M. Anthocyanin Degradation in Oxidising Grape Musts. *J. Sci. Food Agric.* **1994**, *66*, 283–288. [CrossRef]