

## Article

# How the Management of pH during Winemaking Affects Acetaldehyde, Polymeric Pigments and Color Evolution of Red Wine

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**Abstract:** Due to climate change and the consequent rise in grape pH, there is often the necessity of acidifying musts or wines during winemaking. In this study, the effect of early (on musts, during fermentation) and late (on wines, after the end of the fermentation) acidification was evaluated. The experimental design consisted of the preparation of seven wines from the same batch of grapes fermented in a first tank at the original pH of 3.2 and two other tanks in which the pH was adjusted to 3.5 (3.5W) and 3.9 (3.9W). On the third day of fermentation, and one week after the end of the fermentation–maceration process, aliquots of both 3.5W and 3.9W were treated to lower pH to thus obtain four more wines. After one year of aging, wines treated so as to reach a 3.2 pH significantly differed from the control wine in terms of contents of acetaldehyde, tannins reactive towards proteins and polymeric pigments. Differences were more conspicuous when acidification was carried out after the end of the fermentation–maceration process. Data highlight that the timing of acidification has a significant effect on polymerization reactions typically occurring during wine aging.

**Keywords:** wine aging; polymeric pigments; acidification; acetaldehyde; must; red wine; acidification timing



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## 1. Introduction

Over the past years, wine production has been dramatically affected by climate changes. The main effects detected in grape composition and production are the increase in sugar content, the earlier shift of the ripening period and the higher degradation of organic acids during the late stages of ripening [1]. Acidity decrease is a serious concern for the wine industry. The level of titratable acidity of musts, along with changes in other parameters such as the concentration of potassium cations and pH, directly affects the quality of wines because when the values of these parameters fall outside their optimal ranges, they have detrimental effects on the microbial and sensorial quality of the finished wines. More specifically, wines with high pH values are more susceptible to microbial contamination [2], and sulfur anhydride to avoid microbial and oxidative risks is required at higher concentrations [3]. Therefore, acidity is a primary driver of important management decisions related to contamination risks [4]. Acidity and pH are determinants also for the sensorial properties of wines, although a direct correlation between pH and sensory attributes is not possible because of several factors, including the human physiology that determines the sourness perception, the importance of the buffering power and the whole gustative equilibrium during the wine tasting [5,6].

Several acidification practices, such as the addition of organic acids, the use of cation exchange resins and bipolar membrane electro dialysis, are permitted by the O.I.V. [7] as well as by specific country regulations. Across the European Community, acidification is

allowed only in certain winemaking regions (Regulation EU 1308, 2013). However, this practice can be carried out only if the initial acidity content of the wine is not increased by more than 1.5 g/L for musts and 2.5 g/L for wines (expressed in tartaric acid) (respectively 20.0 and 33.3 meq/L) (Regulation EU 1308, 2013).

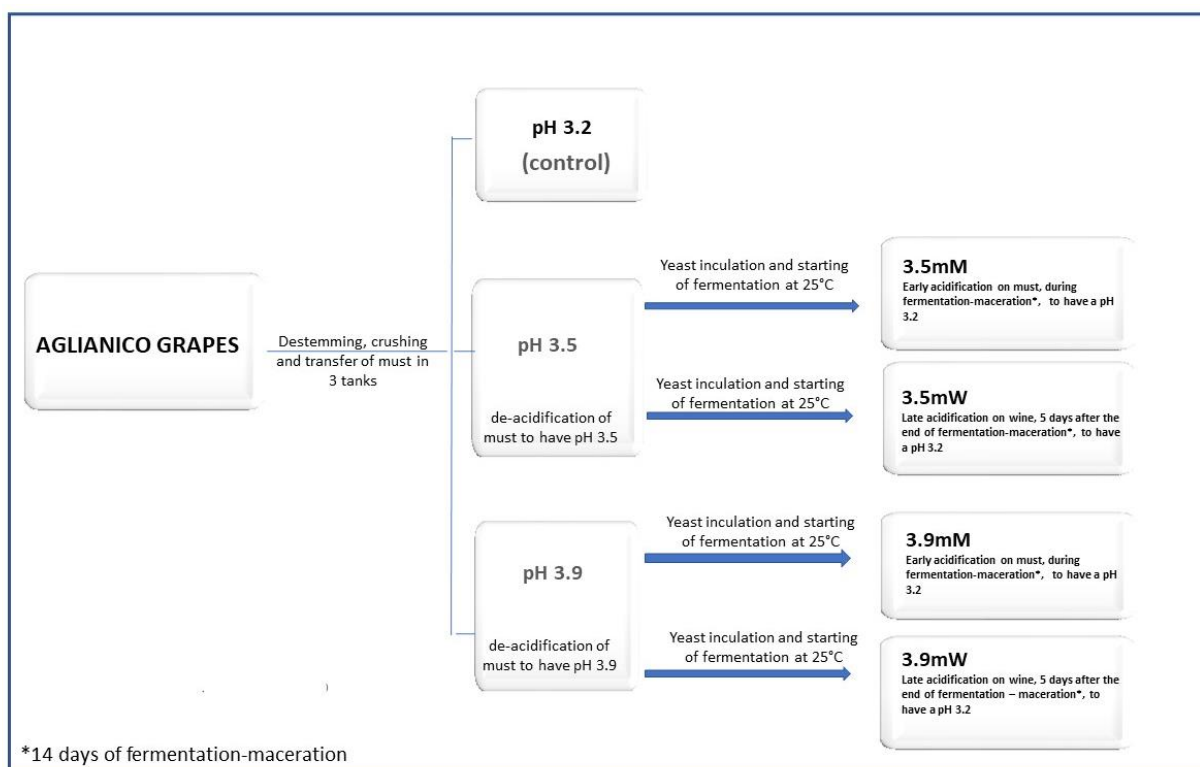
A crucial decision for winemakers is the timing of the acidification treatment, especially as to whether it would be better to acidify musts or wines. In the first case, the proliferation of spoiling lactic bacteria and other bacteria during alcoholic fermentation is prevented [2], but the levels of pH and acids in musts cannot be easily regulated because they could change in finished wines. In fact, during fermentation, great variations in pH and titratable acidity occur as a result of the activities of yeasts and bacteria [2] and of the precipitation equilibria linked to potassium bitartrate formation [3]. In addition, high levels of pH during fermentation could affect the metabolism of yeasts and the concentration of yeast-related compounds such as those involved in the formation of new pigments and of new polymeric tannic structures [8].

In this regard, the most important compound for wine quality is acetaldehyde, which is differently involved in both fermentation and aging. Besides being produced during fermentation through microbial metabolism, acetaldehyde can also be formed by oxidation reactions [9]. Acetaldehyde is a highly reactive compound, and in wines it undergoes several reactions, including those with sulfur dioxide, with flavonoids thus altering wine pigments, with polymeric tannins and with several other nucleophiles. Although reactions in which acetaldehyde is involved are of paramount importance for wine characteristics such as color and tannins [10], there are no studies yet about the effect of acidification management on acetaldehyde, pigments, color and tannins and on their evolution over time.

In previous studies, the impact of pH on the anthocyanin chemistry in model solution and real wine was investigated [11,12]. Herein, the effect of both early (on musts, during fermentation) and late (on wines 7 days after the end of the fermentation) acidification on the acetaldehyde content, wine phenolics and color was evaluated over a two-year aging.

## 2. Material and Methods

**Wines.** Aglianico grapes, characterized by a content of soluble solids of 22.6°Brix, were collected in September of 2018. The experimental design (Figure 1) consisted in the preparation of seven wines from the same batch of grapes fermented in a first tank at the original pH value of 3.2 (referred to as 3.2) and two other tanks in which the pH was adjusted to 3.5 (referred to as 3.5) and 3.7 (referred to as 3.7) with sodium hydroxide 10 N. On the third day of fermentation, and one week after the end of the fermentation–maceration process, some aliquots of both 3.5 and 3.7 were treated with hydrochloric acid (12 N) to reach a 3.2 pH, thus affording four more wines: LmM (low modified must), LmW (low modified wine), HmM (high modified must) and HmW (high modified wine). Fermentation took place at 25 °C after yeast inoculation (20 g/hL of FX10 Laffort Oenologie, France), and the cap was immersed twice a day. Maceration of the pomace lasted for 14 days. Successively, the must was pressed (about 8 bar) and the finished wine was obtained. After 2 months, wines were cold-stabilized at −6 °C for one week and filtered at 0.45 µm before bottle aging. At bottling, the total package oxygen (the sum of dissolved and headspace oxygen) measured by means of oxo-luminescence, using a Nomasense oxygen analyzer (Nomacor SA, Thimister Clermont, Belgium), was below 1 mg/L. Experimental wines were analyzed 5 days after the end of alcoholic fermentation (EAF), after 1 year of aging and after 2 years of aging. Data on wines are shown in the Supplementary Materials Tables S1–S3. All experiments were carried out in duplicate and two analytical replicates were performed.



**Figure 1.** Experimental plan. Aglianico grapes, variety used in the experiment; pH 3.2, control must and wine; pH 3.5, must originally at pH 3.2 but adjusted to pH 3.5; pH 3.9, must originally at pH 3.2 but adjusted to pH 3.9; LmM, must at pH 3.5 brought back to pH 3.2; LmW, wine at pH 3.5 brought back to pH 3.2; HmM, must at pH 3.9 brought back to pH 3.2; HmW, wine at pH 3.9 brought back to pH 3.2.

### 2.1. Base Analyses

Base parameters were measured according to the O.I.V. Compendium of International Methods of Wine and Must Analysis [13]. The following parameters were determined: alcoholic strength by volume (OIV-MA-AS312-01A), reducing sugars (OIV-MA-AS311-01A), total acidity (OIV-MA-AS313-01), pH (OIV-MA-AS313-15) and volatile acidity (OIV-MA-AS313-02),

### 2.2. CIELAB Coordinates and Color Intensity and Hue

The CIELAB parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) were measured using a Shimadzu UV-1800 model spectrophotometer (Kyoto, Japan) and determined with the Panorama software (Shimadzu, Milan, Italy) following the recommendations of the Commission Internationale de L'Eclairage (CIE). Color differences ( $\Delta E/ab$ ) were calculated as the Euclidean distance between two points in the 3D space defined by  $L^*$ ,  $a^*$  and  $b^*$ . All analyses were carried out in duplicate.

### 2.3. Spectrophotometric Analyses

Wine colorant intensity (CI), given by the sum of absorbances at 420 (yellow), 520 (red) and 620 nm (blue), and the hue (420/520 absorbance) were evaluated according to the Glories method [14]. The determination of the vanillin index (VAN) was carried out following the method described by Gambuti et al. [15]. Briefly, one test tube was prepared (wine diluted 1 to 10 with pure methanol), two microcentrifuge tubes were used and a first 1.5 mL microcentrifuge tube was prepared by dispensing 125  $\mu$ L of diluted wine and then adding 750  $\mu$ L of a vanillin solution (4% in methanol). After 5 min, the tube was placed in cold water (4 °C) and 375  $\mu$ L of concentrated hydrochloric acid was added. After a 15 min incubation in cold water, the mixture was placed at room temperature (20 °C) for

15 min; the absorbance was determined at 500 nm. For a second tube, the procedure was the same except that 750  $\mu$ L of pure methanol was used instead of the vanillin solution. The absorbance of 500 nm of this tube was considered blank. Concentrations were calculated as (+)-catechin (mg/L) using a calibration curve. The linearity range of the calibration curve was 2–250 mg/L. The slope and the intercept were as follows:  $0.02 < \Delta E < 0.05$  (slope: 277.26, intercept:  $-3.58$ );  $0.05 < \Delta < 0.18$  (slope: 250, intercept:  $-3.25$ );  $0.18 < \Delta < 0.83$  (slope: 314.23, intercept:  $-12.91$ ). Total anthocyanins, BSA tannins, total phenolics, small polymeric pigments (SPPs) and large polymeric pigments (LPPs) were determined by the Harbertson et al. assay [16]. In this assay, by combining protein precipitation using bovine serum albumin (BSA) (Sigma Life Science, Saint Louis, MO, USA) and bisulfite bleaching, two types of polymeric pigments in wines were determined: large polymeric pigments (LPPs) which precipitate with proteins and small polymeric pigments (SPPs) that do not precipitate. The chromatic characteristics were determined using a 7305 spectrophotometer (Jenway); 10 mm plastic cuvettes were used. All analyses were performed in duplicate.

#### 2.4. Chemicals

All solvents used in this study were of HPLC grade or higher. Glacial acetic acid, hydrochloric acid, methanol, acetonitrile, ethanol, sodium dodecyl sulfate (SDS), triethanolamine, iron chloride, vanillin, tartaric acid, formic acid, sulfuric acid, 2,4-dinitrophenylhydrazine, sodium hydroxide, bovine serum albumin (BSA), malvidin-3-monoglucoside and metabisulfite were purchased from J.T. Baker (Levanchimica, Bari, Italy). Water was purified using a Milli-Q purification system (MilliporeSigma, Burlington, MA, USA).

#### 2.5. High-Performance Liquid Chromatography Analyses of Anthocyanins

The separation of anthocyanins was carried out according to the directives of the Compendium of International Methods of Analysis of Wines and Musts of the O.I.V., Method OIV-MA-AS315-11 [13]. The analyses were performed using a Shimadzu LC10 ADVP HPLC apparatus (Shimadzu, Italy, Milan), consisting of an SCL-10AVP system controller, two LC-10ADVP pumps, an SPD-M 10 AVP diode array detector, a Shimadzu CTO-10ASvp column oven, a Shimadzu Sil-20AHT injection autosampler and a full Rheodyne model 7725 injection system (Rheodyne, Cotati, CA, USA) equipped with a 50 mL loop. A Waters Spherisorb column (250  $\times$  4.6 mm, 4 mm particle diameter) with precolumn was used. Fifty milliliters of wine or calibration standard was injected into the column. Detection was performed by monitoring absorbance signals at 518 nm. All samples were filtered through 0.45 mm Durapore membrane filters (Millipore, Ireland) in glass vials and immediately injected into the HPLC system. The HPLC eluents were as follows: solvent A: Milli-Q water (Sigma Aldrich)/Sigma Aldrich formic acid ( $\geq 95\%$ )/acetonitrile (Sigma Aldrich  $\geq 99.9\%$ ) (87:10:3 *v/v/v*); solvent B: water/formic acid/acetonitrile (40:10:50 *v/v/v*). A different gradient was used: zero-time conditions 94% A and 6% B; after 15 min the pumps were adjusted to 70% A and 30% B; at 30 min to 50% A and 50% B; at 35 min to 40% A and 60% B; and at 41 min, end of analysis, to 94% A and 6% B. After a 10 min equilibrium period, the next sample was injected. The calibration curve was obtained by injecting 5 solutions (in triplicate) containing increasing concentrations of malvidin-3-O-monoglucoside (Extrasynthese, Lyon, France). The calibration was characterized by a correlation coefficient ( $R^2$ ) = 0.996. The linearity range of the calibration curve was 2–200 mg/L. The precision of the method used was tested by six replicate analyses of a red wine sample containing 118.4 mg/L of total monomeric anthocyanins. The coefficient of variation was between 1.1% (for malvidin-3-O-monoglucoside) and 9.1% (for malvidin 3-(6II-coumaroyl)-glucoside) and demonstrated the good reproducibility of the HPLC analysis. The monomeric anthocyanin concentrations were expressed as mg/L of malvidin-3-O-monoglucoside.

### 2.6. High-Performance Liquid Chromatography Determination of Acetaldehyde

Total acetaldehyde (free + SO<sub>2</sub> bound) determination was conducted according to the method of Han and colleagues [17]. Briefly, aliquots of the wine sample (100 µL) were dispensed into a vial, followed by the addition of 20 µL of a freshly prepared 1.120 mg/L SO<sub>2</sub> solution prepared using a stock solution of K<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (2 g/L). Subsequently, 2 µL of 25% sulfuric acid (96% Carlo Erba, Milan, Italy) was added, followed by 140 µL of 2 g/L 2,4-dinitrophenylhydrazine reagent (Merck KGaA, Darmstadt, Germany). After mixing, the solution was allowed to react for 15 min at 65 °C in a laboratory oven and then promptly cooled to room temperature. The analysis of carbonyl hydrazones was carried out by HPLC with the same apparatus as that used for the chromatographic analyses of anthocyanins. A Waters Spherisorb column (250 × 4.6 mm, 4 mm particle diameter) was used for the separation. The chromatographic conditions were as follows: sample injection volume, 50 µL; flow rate, 0.75 mL/min; column temperature, 35 °C; mobile phase solvents, (A) 0.5% formic acid (Sigma Aldrich 95%) in Milli-Q water (Sigma Aldrich) and (B) acetonitrile (Sigma Aldrich 99.9%). The gradient elution protocol was as follows: 35% B to 60% B (t = 8 min), 60% B to 90% B (t = 13 min), 90% B to 95% B (t = 15 min, 2 min hold), 95% B to 35% B (t = 17 min, 4 min hold), total run time 21 min. The eluted peaks were compared with the derivatized acetaldehyde standard (≥99.5%, Sigma Chemistry, USA) obtained after reaction with 2,4-dinitrophenylhydrazine reagent (Sigma Chemistry, USA). The calibration curves were constructed by injecting 5 solutions (in triplicate) containing their respective standards covering the range of linearity 10–120 mg/L and were characterized by a correlation coefficient (R<sup>2</sup>) > 0.976. All analyses were conducted through two experimental replicates and two analytical replicates.

### 2.7. Statistical Analysis

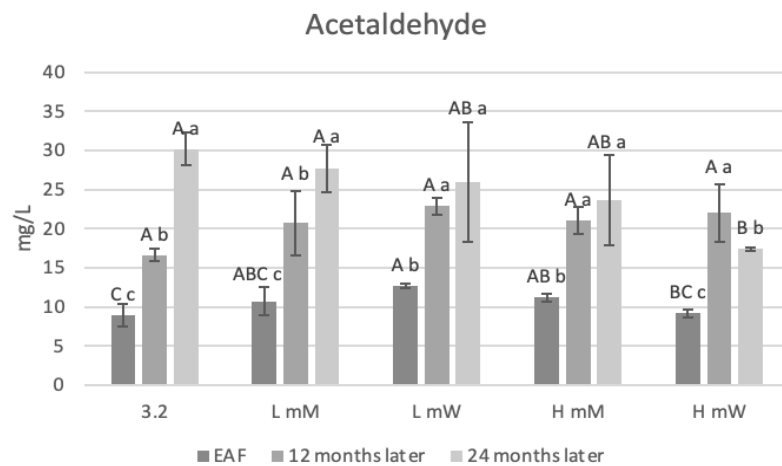
All analyses were performed by using XLSTAT (software Addinsoft, 2017.1, Paris, France). The effect of treatments was evaluated by the analysis of the variance (ANOVA) using the Tukey method for the significant differences procedure ( $p < 0.05$ ).

## 3. Results

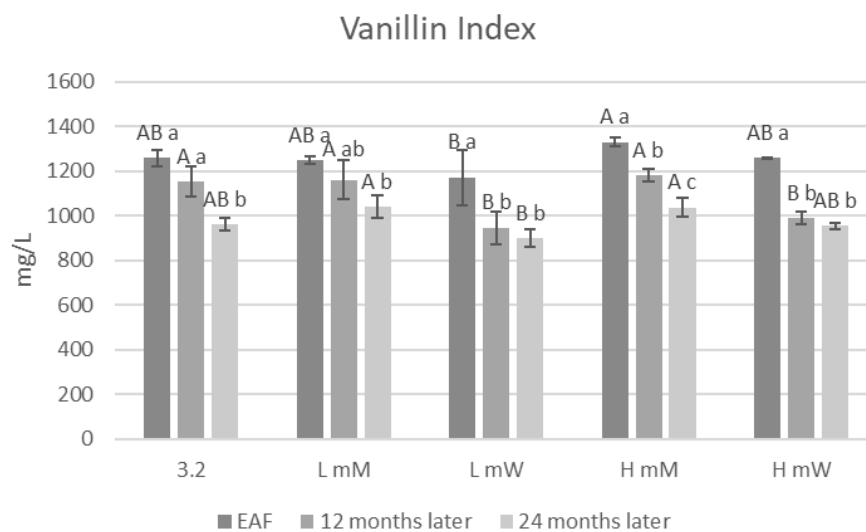
To understand the impact of the timing and level of the acidification on red wine evolution, four samples treated with two levels (low and high) of acid as musts (LmM and HmM) or wines (LmW and HmW) were analyzed just after the end of the alcoholic fermentation (EAF) and after 12 and 24 months of aging.

The evolution of acetaldehyde is shown in Figure 2. In control wine (pH 3.2), it increased over two years of aging. Conversely, after the first year of aging, a stabilization (LmM, LmW, HmM) or even a decrease (HmW) was observed. Acetaldehyde is a highly reactive molecule, and its variation trend detected in wines LmW, HmM and HmW could be ascribed to its quick consumption in reactions with flavans [18] that were more abundant in wines obtained through a maceration at higher pH values (Table S1) [12].

Data on the evolution of the vanillin index supported this hypothesis (Figure 3). The decrease in vanillin index in LmW, HmM and HmW was significant after one year of aging, while in 3.2 and LmM wines, a significant difference was observed only after two years of aging. Vanillin index can be considered an indirect, inverse measure of the oxidative polymerization of flavanols via methyl methine bridge, since acetaldehyde [19], like vanillin [20], reacts with the C8 and C6 positions of the flavanol A ring. As reported, part of these reactions could involve anthocyanins [10].

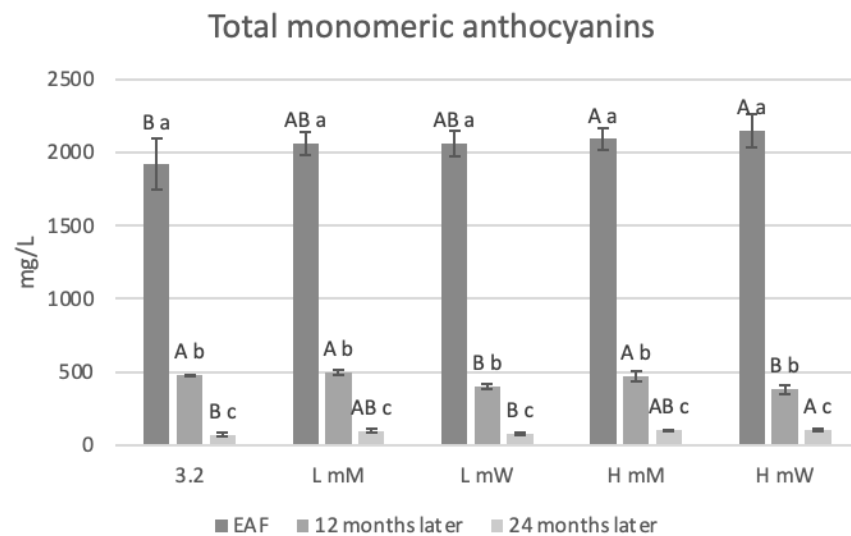


**Figure 2.** Evolution of acetaldehyde during aging of experimental wines: 3.2, control wine at pH 3.2; LmM, wine originally at pH 3.5 acidified during AF (alcoholic fermentation) to have pH 3.2; LmW, wine originally at pH 3.5 acidified seven days after AF to have pH 3.2; HmM, wine originally at pH 3.7 acidified during AF to have pH 3.2; HmW, wine originally at pH 3.7 acidified seven days after AF to have pH 3.2; EAF, end of alcoholic fermentation. Wine type at the same moment (A–C) and each wine through time (a–c) sharing the same letters are not significantly different.



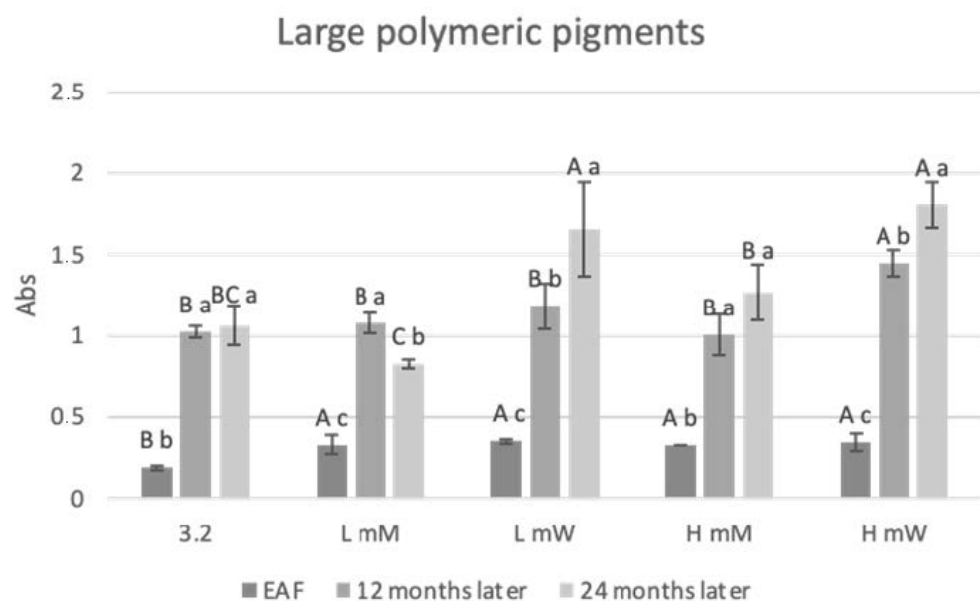
**Figure 3.** Evolution of vanillin index during aging of experimental wines. Codes assigned to wine samples are as in Figure 2. EAF: end of alcoholic fermentation. Wine type at the same moment (A,B) and each wine through time (a–c) sharing the same letters are not significantly different.

The content of total monomeric anthocyanins (Figure 4), determined by HPLC, corroborated the importance of choosing the acidification timing appropriately. Differences among samples were not significant at the end of the alcoholic fermentation but, after one year of aging, samples that had undergone late acidification showed lower contents of monomeric anthocyanins. This could be ascribed to the involvement of these native pigments in reactions of polymerization favored in late-acidified wines due to the higher concentration of flavanols [12].

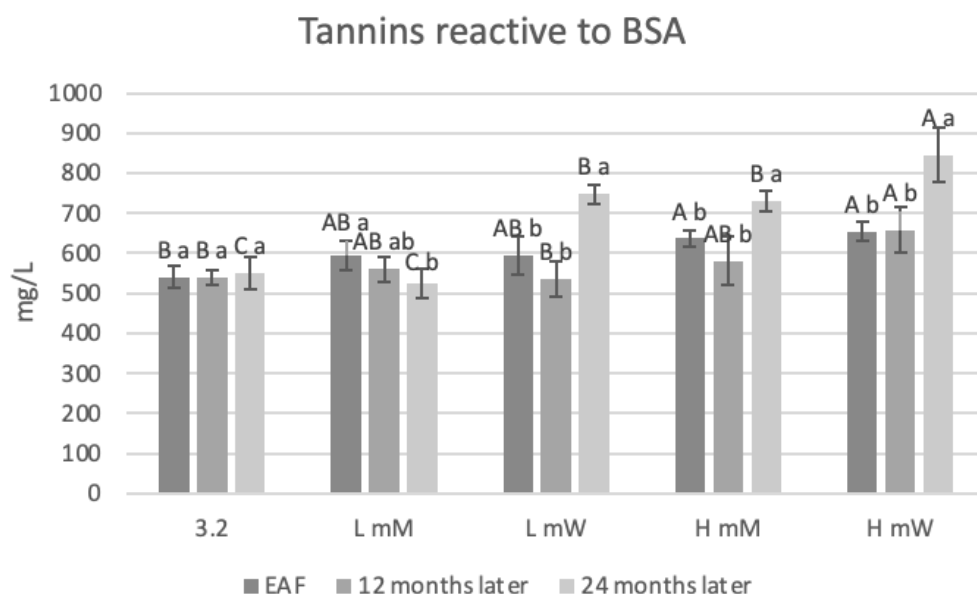


**Figure 4.** Evolution of total monomeric anthocyanins during aging of experimental wines. Codes assigned to wine samples are as in Figure 2. EAF: end of alcoholic fermentation. Wine type at the same moment (A,B) and each wine through time (a–c) sharing the same letters are not significantly different.

Data on large polymeric pigments (LPPs) (Figure 5) showed that the formation of polymeric structures was enhanced in wines obtained through the maceration of grapes at higher pH and that this effect is already significant after the end of alcoholic fermentation. These pigments are of great relevance as they contribute to the stability of the color intensity of wines over time. Experimental data showed that the later the acidification was performed, the higher the production of these long pigments occurred over time. BSA-reactive tannins (Figure 6), compounds with high molecular weight that also can be formed during wine production, oxidation and aging [21], are more abundant in wines obtained at higher pH values and then acidified.



**Figure 5.** Evolution of large polymeric pigments during aging of experimental wines. Codes assigned to wine samples are as in Figure 2. EAF: end of alcoholic fermentation. Wine type at the same moment (A–C) and each wine through time (a–c) sharing the same letters are not significantly different.



**Figure 6.** Evolution of tannins reactive to BSA during aging of experimental wines. Codes assigned to wine samples are as in Figure 2. EAF: end of alcoholic fermentation. Wine type at the same moment (A–C) and each wine through time (a,b) sharing the same letters are not significantly different.

As expected, changes in pigment composition even affected chromatic characteristics (Tables 1 and 2). The base color parameters CI and hue (Table 1) are obtained from absorbance values at 420, 520 and 620 nm [14]. At the end of the alcoholic fermentation, no significant differences in CI among wines were detected apart from a slightly higher value of hue in HmW. After one year of aging, with the exception of the 3.2 wine, CI increased for all samples, and wines acidified later (LmW and HmW) showed the highest values. The values of CI continued to rise over the second year of aging. This trend is common for these parameters during the aging of red wines and is related to reactions determining the production of new, more stable, pigments [21,22].

**Table 1.** Evolution of color parameters during aging of experimental wines. Absorbance at 420, 520 and 620 nm; colorant intensity (CI); and hue.

End of Alcoholic Fermentation																				
	3.2			L mM			L mW			H mM			H mW							
420 nm	1.88	±	0.03	A b	1.88	±	0.01	A b	2.03	±	0.11	A b	1.96	±	0.04	A b	2.02	±	0.09	A c
520 nm	3.54	±	0.11	A ab	3.48	±	0.06	A a	3.83	±	0.23	A a	3.60	±	0.11	A a	3.56	±	0.22	A b
620 nm	0.46	±	0.01	B b	0.47	±	0.01	B b	0.49	±	0.02	AB b	0.52	±	0.01	A a	0.48	±	0.03	B c
CI	5.88	±	0.15	A b	5.83	±	0.06	A b	6.34	±	0.36	A b	6.07	±	0.15	A a	6.06	±	0.30	A b
Hue	0.53	±	0.01	B c	0.54	±	0.01	B b	0.53	±	0.01	B b	0.54	±	0.01	B c	0.57	±	0.02	A b
1 year later																				
	3.2			L mM			L mW			H mM			H mW							
420 nm	1.90	±	0.05	C b	1.94	±	0.01	C b	2.18	±	0.07	B b	2.10	±	0.09	B ab	2.39	±	0.06	A b
520 nm	3.31	±	0.17	C b	3.55	±	0.13	BC a	3.84	±	0.23	B a	3.65	±	0.14	BC a	4.24	±	0.06	A a
620 nm	0.40	±	0.02	B b	0.42	±	0.01	B c	0.50	±	0.05	AB b	0.48	±	0.03	AB a	0.56	±	0.06	A b
CI	5.61	±	0.24	C b	5.91	±	0.13	C b	6.51	±	0.33	B b	6.23	±	0.26	BC a	7.18	±	0.18	A a
Hue	0.57	±	0.01	A b	0.55	±	0.02	A b	0.57	±	0.02	A ab	0.57	±	0.01	A b	0.56	±	0.01	A b



Table 1. Cont.

	2 years later														
	3.2		L mM		L mW		H mM		H mW						
420 nm	2.34	± 0.12	AB a	2.25	± 0.07	B a	2.56	± 0.14	A a	2.24	± 0.16	B a	2.54	± 0.06	A a
520 nm	3.79	± 0.17	BC a	3.71	± 0.09	C a	4.15	± 0.25	AB a	3.68	± 0.26	C a	4.37	± 0.09	A a
620 nm	0.65	± 0.07	A a	0.58	± 0.03	A a	0.69	± 0.11	A a	0.57	± 0.07	A a	0.65	± 0.02	A a
CI	6.78	± 0.35	B a	6.53	± 0.17	B a	7.40	± 0.38	A a	6.48	± 0.49	B a	7.56	± 0.14	A a
Hue	0.62	± 0.01	A a	0.61	± 0.00	A a	0.62	± 0.05	A a	0.61	± 0.00	A a	0.58	± 0.00	A a

Codes assigned to wine samples are as in Figure 2. Wine type at the same moment (A–C) and each wine through time (a–c) sharing the same letters are not significantly different.

Table 2. Evolution of color parameters during aging of experimental wines. CIELAB coordinates.

	1 year later														
	3.2		L mM		L mW		H mM		H mW						
L*	68.15	± 0.62	A a	67.33	± 1.26	AB a	65.93	± 0.61	BC a	67.38	± 0.43	AB a	65.25	± 0.47	C a
a*	19.33	± 0.80	C b	19.50	± 0.22	C b	21.23	± 1.23	AB a	20.23	± 0.39	BC a	22.40	± 0.18	A b
b*	7.55	± 0.31	BC b	7.68	± 0.31	BC b	8.20	± 0.39	B b	7.30	± 0.47	C b	9.73	± 0.32	A b
C*	20.75	± 0.66	C b	20.95	± 0.21	C b	22.80	± 1.01	B b	21.50	± 0.52	BC b	24.43	± 0.26	A b
h	21.40	± 1.50	AB b	21.50	± 0.88	AB b	21.23	± 2.07	AB b	19.85	± 0.83	B b	23.45	± 0.59	A b
	2 years later														
	3.2		L mM		L mW		H mM		H mW						
L*	65.58	± 0.90	AB b	66.03	± 0.55	AB a	64.80	± 1.18	B a	66.58	± 0.46	A b	64.45	± 0.06	B b
a*	21.00	± 0.18	B a	21.20	± 0.08	B a	22.68	± 0.78	A a	21.15	± 0.81	B a	23.63	± 0.15	A a
b*	9.60	± 0.35	B a	9.75	± 0.48	B a	10.53	± 0.67	B a	9.58	± 0.46	B a	11.65	± 0.17	A a
C*	23.10	± 0.00	C a	23.35	± 0.17	C a	24.98	± 0.44	B a	23.20	± 0.93	C a	26.38	± 0.21	A a
h	24.55	± 0.98	A a	24.70	± 1.07	A a	24.95	± 2.02	A a	24.30	± 0.34	A a	26.25	± 0.24	A a

Codes assigned to wine samples are as in Figure 2. Wine type at the same moment (A–C) and each wine through time (a–c) sharing the same letters are not significantly different.

The analysis of chromatic characteristics was completed with the determination of CIELAB variables in aged wines (Table 2).

After two years of aging, the color of wines was darker (lower L\*) and more colorful (larger C\*) than that observed after only one year of aging. Moreover, a loss of violet hue (increase in a\* and b\*) and a raise of tawny tonality (larger b\* and hue) were detected. Apart from the luminosity parameter L\*, for which there is no agreement in literature on its changes during wine evolution [23], the other trends are widely discussed in the literature and are typical of red wine evolution [24]. A different contribution of each class of anthocyanin derivatives (pinotins, flavanyl-pyranoanthocyanins, vitisin A, vitisin B, direct anthocyanin-flavan-3-ol condensation products, anthocyanin ethyl-linked flavan-3-ols) in various aging states is responsible for variation detected in CIELAB parameters, as recently shown in a study in which a regression model was used to evaluate the contribution of several anthocyanin derivatives to the chromatic attributes of the wines over time [23].

#### 4. Discussion

Red wine color is mainly determined by anthocyanins and their derivative pigments. The variation of these compounds in wine is a key factor in the evolution of wine color [25]. In this regard, wine pH is of critical importance as it affects all these compounds and their evolution. First, different pH levels cause anthocyanins to exist in different chemical species. At pH levels below 3, anthocyanins mainly exist as flavylium cations exhibiting intense red coloration. As the pH increases above 3, the flavylium cation equilibrates with two diastereomeric hemiacetals that are colorless. The pH level also affects reactions between anthocyanins and other wine compounds. Among others, reactions undergone by acetaldehyde with other flavonoids such as catechins lead to the formation of new pigments

displaying different chromatic characteristics [26]. pH is a crucial parameter governing such reactions, because a stronger acidic environment enhances the electrophilicity of the acetaldehyde carbonyl, thus causing it to be more susceptible to the attack of nucleophiles, including catechins.

In addition to all these considerations related to the chemical reactivity of pigments in wine, the effect of pH on the extraction of phenolic compounds during the first phases of winemaking should also be considered. In a previous work, it was shown that maceration of grapes at lower pH values favors the extraction of anthocyanins, while higher pH values determine a more massive extraction of flavanols [11]. Thus, the quality and quantity of pigments and flavonoids in initial wines change on the basis of the pH of the medium during the fermentation–maceration phase.

The outcome of our study confirmed the importance of the must pH, on account of the significant differences detected in terms of the evolution of monomeric anthocyanins, flavanols reactive towards vanillin and acetaldehyde among wines acidified either in the first phases of winemaking or after the fermentation–maceration. After one year of aging, a greater loss of monomeric anthocyanins occurred in wines in which the first phases of fermentation–maceration were conducted at higher pH (LmW and HmW). The greater decrease in vanillin index and the increase in LPPs and BSA-reactive tannins in LmW and HmW showed that reactions leading to the formation of new pigments and of polymeric structures are favored in wines acidified later. Significantly higher values of color intensity and a loss of a\* tint, typical of aged wines, were also detected in these wines.

Since during aging a more conspicuous formation of LPPs and BSA-reactive tannins (both generally constituted by structures ranging from trimers to octamers) with respect to short polymeric pigments is usually observed [21], it is reasonable to hypothesize that LmW and HmW are in a more advanced oxidative state than 3.2 wine.

## 5. Conclusions

Differences in the evolution of red wines based on the acidification timing applied during winemaking were for the first time analyzed. The observed differences in terms of acetaldehyde and polymeric phenolic evolution are likely correlated to the fact that maceration of grapes at lower pH favors the extraction of anthocyanins, while the increase in pH in this extractive phase determines a higher extraction of flavanols from grapes. This causes acetaldehyde to behave differently over time even if the pH value is the same (3.2) for all wines. High pH values during the first phases of winemaking favor the polymerization of phenolics over time, although pH is successively decreased at the same low value as 3.2. Results suggested that the acidification effect is stronger when pH is decreased after the end of fermentation.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/app12052555/s1>, Table S1. Main phenolics parameters, Total phenols, Total anthocyanins, Catechin and Epicatechin of wines at different pH levels analyzed after the end of alcoholic fermentation. Table S2. Base parameters, pH, titratable acidity (AT), free sulfure dioxide, total sulfure dioxide, residual sugar, volatile acidity, and alcohol of wines analyzed after the end of the alcoholic fermentation. 3.2, control wine at pH 3.2; LmM, wine originally at pH 3.5 acidified during AF to have pH 3.2; LmW, wine originally at pH 3.5 acidified just after AF to have pH 3.2; HmM, wine originally at pH 3.7 acidified during AF to have pH 3.2; HmW, wine originally at pH 3.7 acidified just after AF to have pH 3.2. Table S3. Base parameters, pH, titratable acidity (AT), free sulfure dioxide and total sulfure dioxide of wines analyzed after 24 months of aging. 3.2, control wine at pH 3.2; LmM, wine originally at pH 3.5 acidified during AF to have pH 3.2; LmW, wine originally at pH 3.5 acidified just after AF to have pH 3.2; HmM, wine originally at pH 3.7 acidified during AF to have pH 3.2; HmW, wine originally at pH 3.7 acidified just after AF to have pH 3.2.

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