Impact of acidification at bottling by fumaric acid on red wine after 24 months

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Abstract. Global warming is directly linked to a lower concentration of organic acids in grape berries. Because of this lack of organic acids, wines tend to have higher pH levels and low titrable acidity. Many important factors are impacted, such as the chemical, microbiological and organoleptic equilibriums. It is common practice to acidify the wine in order to prevent these imbalances that can lead to wine defects and early spoilage. Tartaric acid (TA) is most commonly used by winemaker for wine acidification purposes. As a potential acidification candidate, fumaric acid (FA), authorized by the OIV in its member states for the inhibition of malolactic fermentation, could also be used since it has a better acidifying power than tartaric acid. Thus, the objective of the present study was to investigate the impact of the addition of FA at bottling in comparison to TA on white wine's quality. For this purpose, a sulfite-free Cabernet Sauvignon red wine was divided into two batches, one of which was sulfited at 80 mg/L. The two batches, sulfite-free and sulfited, were then redivided into five batches, one of which without any addition, two of the batches in which TA was added at concentrations of 1.25 and 2.5 g/L respectively, and two batches in which FA was added at concentrations of 1, and 2 g/L, respectively. Classical oenological parameters (pH, titratable acidity), color parameters (color intensity, CIELAB), total phenolic compounds (IPT, Folin), as well as total tannins, total anthocyanins and their composition (HPLC analysis) were analyzed. Sensory analyses were also performed on the wines in order to assess the organoleptic impact of FA addition.

1 Introduction

Global warming directly impacts the chemical composition of grape berries [1,2], particularly causing a decrease in organic acid levels, with malic acid being especially affected [3]. Additionally, higher temperatures have been linked to increased potassium (K^+) levels [4,5]. The combination of these factors results in higher pH levels in musts and wines [6,7], disrupting their physico-chemical balance [8]. Consequently, elevated pH levels in musts and wines can lead to microbiological issues, organoleptic changes like color alterations [9], and reduced aging capabilities. In this context, acidifying musts and wines is crucial to achieve optimal acidity levels.

In OIV member countries, must and wine acidification can be achieved by blending them with high-acidity counterparts, using membrane techniques, or chemically adding organic acids such as lactic acid, DL- or L(-)-malic acid, L(+)-tartaric acid, and citric acid (only for wines). In non-OIV member countries like the United States [10], fumaric acid (FA) can also be utilized for chemical acidification.

Fumaric acid, (E)-2-butenedioic acid, was first isolated from the plant *Fumaria officinalis*, which inspired its name. It is naturally produced by plants and many microorganisms as a key intermediate in the citrate cycle. Grape berries contain small amounts of FA, ranging from 0.07 to 10.69 mg/L [11-13]. As the least expensive food-grade acid and non-toxic, fumaric acid is widely used in the food industry as an antibacterial agent and acidulant [14]. The European Union Commission Regulations No 1129/2011 classifies it as a food additive other than colors and sweeteners with the E-number E297. It can be added to various food products, such as flavored fermented milk products, chewing gums, and flavored drinks, at concentrations up to 4000 mg/L or mg/kg depending on the product.

Numerous publications highlight fumaric acid's antibacterial properties. Its antibacterial effects have been demonstrated on non-heat processed vegetables [15] and in apple cider inoculated with *E. coli* [16]. Study found fumaric acid to be the most effective among lactic and acetic acids in inhibiting the growth of five pathogenic

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bacteria populations (at equivalent concentrations of 50 mM) on raw vegetables [17]. One primary effect of fumaric acid is likely its ability to lower pH levels, which limits bacterial development and growth [18]. Fumaric acid's antibacterial properties have also been explored in wines. Cofran and Meyer [19] revealed that fumaric acid concentrations above 0.36 g/L delayed malolactic fermentation, unlike tartaric and citric acids tested in the same study. This characteristic is valuable for high pH wines, such as those from warmer regions [20]. Morata *et al*. [21] demonstrated that fumaric acid, at concentrations between 300 and 900 mg. The sensory threshold of FA in red wine is around 1387 mg/L [22].

2 Materials and methods

2.1.1 Chemical impact of the addition of acid on wines - Acidification of wines

For the purpose of this work, a sulfite-free Cabernet Sauvignon red wine was divided into two batches, one of which was sulfited at 80 mg/L. The two batches, sulfite-free (NS) and sulfited (S), were then redivided into five batches, one of which without any addition (Control), two of the batches in which TA was added at concentrations of 1.25 and 2.5 g/L respectively, and two batches in which FA was added at concentrations of 1, and 2 g/L, respectively. All the modalities were then bottled and stored into a 15 °C room and analyzed after two years.

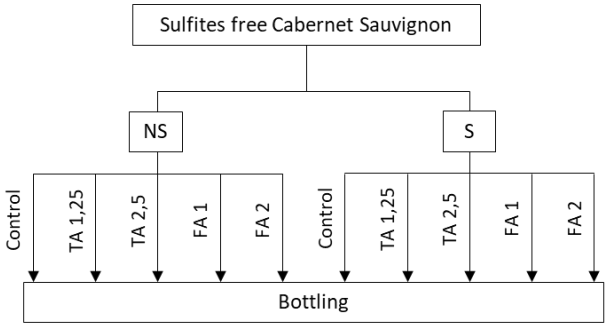

Figure 1. Experimental scheme of wine acidification.

2.1.2 Analysis of classic oenological parameters and titratable acidity

The pH, and titratable acidity (g/L H_2SO_4 eq.), were measured using a FOSS WineScan 79000 FTIR instrument (Foss, Nanterre, France).

2.1.3 Wine color analysis

CIELAB parameters [23], lightness (L^*), red-green coordinates (a^*, $-a^*$), yellow-blue coordinates (b^*, $-b^*$) were measured with a Konica Minolta CM-5 apparatus (Nieuwegein, Netherlands): to determine the colour difference between two wines, the delta E parameter was calculated according to the formula:

$$\Delta E=\sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$$

2.1.4 Spectrophotometric chromatic characteristics

Absorbances at 420 nm, 520 nm and 620 nm were measured in a 1 mm optical path cell using a Helios Alpha™ UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA). The spectrophotometric chromatic characteristics [24] were calculated as follows:

$$CI' = 10 * (A_{420nm} + A_{520nm} + A_{620nm});$$
$$Hue = A_{420nm} / A_{520nm.}$$

2.1.5 Analysis of total phenolic compounds in wines

Total polyphenol index (TPI)

The absorbance at 280 nm of the samples was measured in a quartz cell with an optical path of 1 cm and the TPI [25] calculated as follows, with DF as dilution factor:

$$TPI = DF * A_{280nm.}$$

Folin Ciocalteu Index (FCI)

According to Singleton and Rossi [26], 250 µL of Folin Ciocalteu reagent, 50 µL of 1/10 diluted red wine sample, 1 mL of 20% anhydrous Na_2CO_3 solution, and 3.7 mL of distilled water were added to a tube. After 30 min, the absorbance at 760 nm was measured in a 1 cm optical path cell against distilled water. Gallic acid was used as standard at concentrations ranging from 100 to 800 mg/L. FCI was expressed in mg of gallic acid equivalents per liter of must or wine.

Total tannins (TT)

Total tannins were determined according to Ribéreau Gayon and Stonestreet [27]. In two hydrolysis tubes, 1 ml of sample (diluted 50 times), 500 µL of water and 1.5 ml of hydrochloric acid 37% were added. The first tube was placed in an ice bath at 0 °C and the second one was placed in a water bath at 100 °C. After 30 min, absorbance of both tubes at 550 nm was measured in a 1 cm-path length cuvette and the difference of both absorbance (ΔA_{550nm}) was calculated. TT was determined by the following formula, expressed in g/L:

$$TT = DF * 0,3866 * \Delta A_{550nm},$$

Total anthocyanin concentration (TAC)

According to [27], a solution *A* containing 250 µL of sample, 250 µL of ethanol acidified to 0.1% HCl and 5 mL of 2% (v/v) HCl was prepared beforehand. To two tubes, 1 mL of solution *A* was added, then 400µL mL of distilled water in tube 1 and 400 µL of 15% potassium bisulphite in tube 2. After 20 min, the absorbance of both tubes was measured at 520 nm in a 1 cm path length cuvette. TAC was determined using malvidin-3-*O*-

glucoside as standard and was expressed in mg/L malvidin-3-*O*-glucoside equivalent.

2.1.6 HPLC analysis

Composition of proanthocyanidin monomers and dimers

Wines were filtered (0.45 µm) and 10 µL were then injected in a Vanquish HPLC system (ThermoFischer Scientific, Waltham, MA, USA) with a Thermo-Finnigan UV-Visible detector (UV-vis 200), a Vanquish autosampler and a Vanquish ternary pump coupled to a Chromeleon data system software. Separation was performed on a reverse-phase Lichrosphere 100-RP18 (250 mm x 2 mm, 5 µm; Merck, France) column [28]. The elution solvents were water-acidified with formic acid 0.5% (solvent A) and acetonitrile-acidified with formic acid 0.5% (solvent B) and the flow rate of 1 mL/min. Gradient was as follows: from 5% to 18% B in 30 min, 100% B for 1 min, 100% B for 7 min, from 100 to 5% B in 1 min, 5% B for 3 min. Eluting peaks were monitored by a UV-detector at 280 nm and a fluo-detector ($\lambda_{excitation}$ = 280 nm, $\lambda_{emission}$ = 320nm). Identification of catechin, epicatechin, B1, B2, B2 and B4 dimers was performed by comparison with injected external standards and previous results [29]. Catechin was used as external standard. The results were expressed as mg/L catechin equivalents.

Composition of anthocyanin

Red wines were first filtered (0.45 µm) and 20 µL were injected in a Thermo Scientific Accela (Thermo Fisher Scientific, Waltham, MA, USA) HPLC with an Accela 600 pump module and a UV-Visible diode array detector and Xcalibur Software [30]. The column used was a reverse-phase C18 Nucleosil (250 x 4.6 mm, 5 µm). Solvent A was water/formic acid (95:5, v/v) and solvent B was acetonitrile/formic acid (95:5, v/v). The mobile phase gradient with a flow rate of 1 mL/min was as follows: from 10% to 35% B in 25 min, 100% B at 35 min, 100% B from 35 to 40 min, 10% B at 41 min, and then 10% B for 4 min before the next injection. Eluting peaks were monitored at 520 nm. Peaks were identified by comparison with injected external standards and previous results [29]. Concentrations were expressed as mg/L malvidin 3-*O*-glucoside equivalents.

2.2 Sensory analysis

All sensory analysis sessions were carried out by judges recruited from the Oenology Department of the University of Bordeaux. The judges were selected on the basis of their interest, availability and experience in sensory analysis. All the sessions took place in a thermo-regulated room at 20°C with controlled hygrometry (ISO 8589:2007) in individual booths. For each test, 20 mL of solution were presented in glasses (NF V 09 110). The samples were presented randomly, numbered with 3-digit codes and in a balanced (profil, ranking test) manner. At least 18 judges were present during each sensory evaluation session.

2.3 Statistical analysis

All results of the chemical (performed in three replicates) and sensory analysis for all study media were statistically processed using RStudio software (Version 1.1.442 - © 2009-2018 RStudio, Inc.). The statistical analysis was performed separately between the sulfites free and sulfited modalities.

3 Results and Discussion

3.1 Chemical impact of the addition of fumaric acid in wines

3.1.1 Impact on pH and titrable acidity

The pH of 24 months old wines, as shown in Fig. 2, showed that sulfited wine with fumaric acid addition at 2 g/L had the lowest pH. On the contrary, for sulfites free wines, wine with fumaric acid addition was not significantly different than the unacidified control wine.

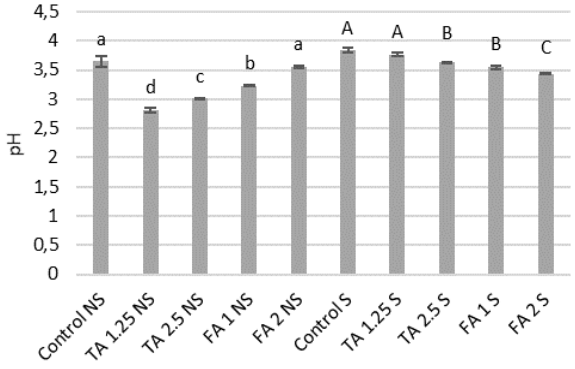

Figure 2. pH of 24 months old wines.

The total acidity analysis, as shown in Fig. 3, indicated no significant difference between the two wines with fumaric acid addition at 2 g/L, with or without sulfites. Fumaric acid addition at 2 g/L impact on total acidity was lower than tartaric acid addition at 2.5 g/L for both sulfites free and sulfited wines.

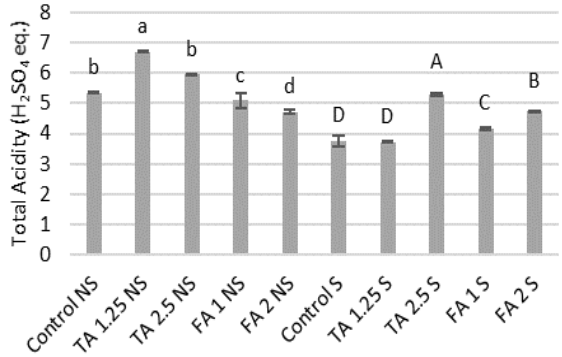

Figure. 3. Total acidity of 24 months old wines (H_2SO_4 eq.).

3.1.2 Impact on color

The impact of acid addition on wine color was studied and results are presented in Fig. 4 (CieLAB parameters on 24 months old wines) and Table 1 (color differences between wines based on CIELAB parameters).

For the sulfites free wines, wine with fumaric acid addition at 2 g/L was darker than wines with tartaric acid addition at both 1.25 g/L and 2.5 g/L. Both the control and fumaric acid at 2 g/L had a lower a* parameter than the other sulfites free wines. Wines with tartaric acid addition at both doses showed a higher b* parameter than the other sulfites free wines, with fumaric acid at 2 g/L having the lowest b* parameter.

As for the sulfited wines, wine with tartaric acid addition at 2.5 g/L was darker, had a lower a* parameter and a lower b* parameter than the other modalities.

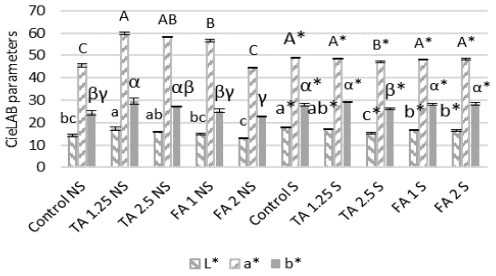

Figure 4. CieLAB parameters of 24 months old wines.

As seen in Table 1, for the sulfited wines, wine with tartaric acid addition at 2.5 g/L compared to the sulfited control wine, was the only wine that had a ΔE higher than 3. Wine with fumaric acid at 2 g/L was the closest to the sulfited control wine.

Table 1. Color difference (ΔE[1]).

	Control NS	**Control S**
TA 1.25 NS	15.3	11.0
TA 2.5 NS	13.1	9.5
FA 1 NS	11.2	8.7
FA 2 NS	2.4	8.2
TA 1.25 S	6.2	1.4
TA 2.5 S	2.5	3.5
FA 1 S	4.9	1.5
FA 2 S	5.2	1.3

[1] $\Delta E=\sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$ where L*, a* et b* are CIELAB parameters.
TA = Tartaric Acid, FA = Fumaric Acid, NS = No Sulfites, S = Sulfites.

3.1.3 Impact on phenolic compounds

For the total polyphenolic index, no significant differences were found (data not showed). Folin Ciocalteu Index (FCI), as shown in Fig. 5, indicated that the sulfited wine exhibited very slight differences in comparison to non-sulfited wines. For sulfites free wines, wines with acids had lower polyphenols contents compared to the sulfites free control. For the sulfited wines, results showed that there was a significant difference between the wine with tartaric addition at 2.5 g/L and fumaric acid addition at 2 g/L, with a higher concentration of tannins in the wine with fumaric acid at 2 g/L.

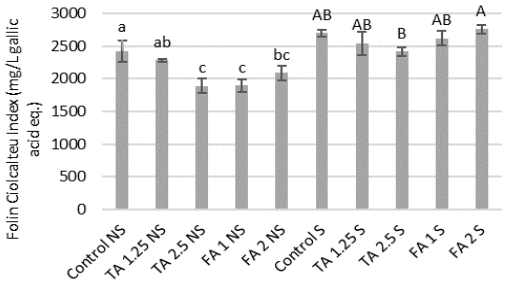

Figure 5. Folin Ciocalteu Index of 24 months old wines (mg/L of gallic acid eq.).

Sulfited wines showed no significant difference in total tannins concentration in between the modalities, as shown in Fig. 6. As for the sulfites free wines, a significant difference was seen between the wine with tartaric acid addition at 2.5 g/L and the wine with fumaric acid addition at 2 g/L. Wine with fumaric acid at 2 g/L had a 36% higher concentration in total tannins than wine with tartaric acid at 2.5 g/L.

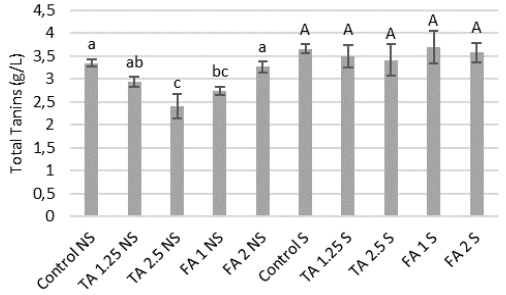

Figure 6. Total Tanins of 24 months old wines (g/L).

The analysis of proanthocyanidin monomers and dimers, as shown in Fig. 7, indicated that for sulfites free wines, tartaric and fumaric acids at 2.5 g/L and 2 g/L respectively had a significant higher concentration of proanthocanidin (monomers and dimers) than the tartaric and fumaric acids wines at half doses. Sulfites free wine with fumaric acid addition at 2 g/L was significantly higher in concentration than the sulfited free control wine. Concerning the sulfited wines, although slight significant differences were highlighted, wines exhibited concentrations of mono and dimers relatively similar.

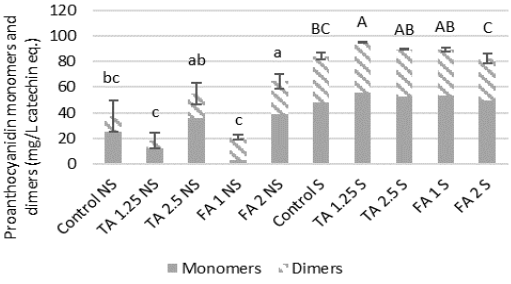

Figure 7. Proanthocyanidin monomers and dimers (mg/L catechin eq.).

Total anthocyanins concentration, as showed in Fig. 8, was not impacted by the addition of acids, no significant differences were found in between the sulfites free wines and in between the sulfited wines. A significant difference was observed between the sulfites free and sulfited wines, that can be explained by the addition of sulfites and their protection on color.

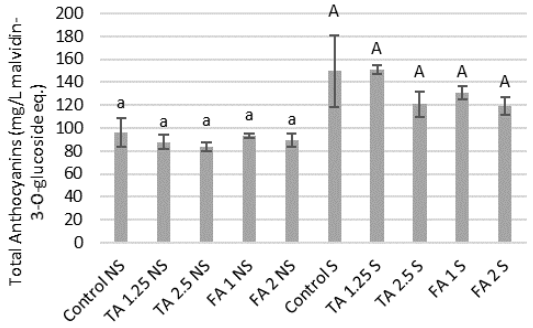

Figure 8. Total anthocyanins (mg/L malvidin 3-O-glucoside eq.).

The analysis of the composition of anthocyanins, as shown in Fig. 9, revealed a significant difference between all the modalities. All the acidified wines had a higher concentration of anthocyanins compared to their respective controls. For the sulfites free modalities, wine with fumaric acid at 2 g/L showed a significant higher concentration in anthocyanins than the other acidified wines. As for the sulfited wines, when compared in pair according to their doses, wines with fumaric acid were significantly higher than the wines with tartaric acid.

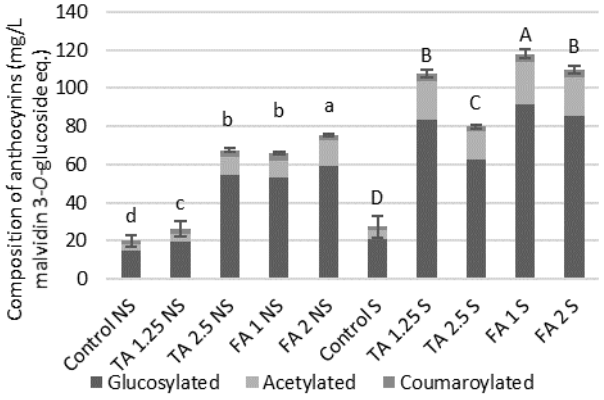

Figure 9. Composition of anthocyanins (mg/L malvidin 3-O-glucoside eq.).

3.2 Organoleptic impact

A sensory profile analysis was performed for six wines, both controls sulfites free and sulfited, and both tartaric and fumaric acid, at 2.5 g/L and 2 g/L respectively, sulfites free and sulfited. At least 14 trained judges were asked to rank the six wines from 0 to 9 on different parameters. Results showed that the sulfites free wines were perceived as having a higher color intensity than the sulfited wines. However, there was no significant difference between the six wines regarding the hue. Sulfites free wine with tartaric acid addition at 2.5 g/L was perceived as significantly more oxidized than the other wines. As for the perceived acidity, both wines with fumaric acid added at 2 g/L, sulfites free and sulfited, were perceived as higher than the rest of the modalities.

In the ranking test, no significant differences were perceived in between the sulfited wines. Sulfites wines were overall ranked higher than sulfites free wines, with the lowest ranking for sulfites free wine with tartaric acid addition at 2 g/L.

Table 2. Ranking Test.

	Ranking, *F*=25,23*
Control NS	61 ab
TA 2.5 NS	32 b
FA 2 NS	59 ab
Control S	85 a
TA 2.5 S	73 a
FA 2 S	68 a

*Significant ranking test when *F*>20,52 at 0,1%
TA = Tartaric Acid, FA = Fumaric Acid, NS = No Sulfites, S = Sulfites.

4 Conclusions

Overall, sulfited wine with fumaric addition at 2 g/L showed promising results. As for the impact on pH, sulfited wines with 2 g/L of fumaric acid had a lower pH than the other sulfited wines. When compared to both sulfites free and sulfited wines, the wines with fumaric acid at 2 g/L were closer in appearance and color than the rest of the modalities. The impact on polyphenolic compounds overall favorized the use of fumaric acid at 2 g/L. On the analysis of TPI, sulfited wine with fumaric acid at 2 g/L showed the highest value out of all the sulfited modalities. The same results were found in the Folin Ciocalteu Index, where sulfited wine with fumaric acid at 2 g/L had the highest concentration in all the sulfited wines. As for the analysis of the composition of anthocyanins, a clear difference was made between tartaric and fumaric addition. For both sulfites free and sulfited wines, when compared in pairs according to their doses, the wines with fumaric acid at 1 g/L and 2 g/L were significantly higher than the wines with tartaric acid addition at 1.25 g/L and 2.5 g/L.

Regarding the organoleptic impact of fumaric acid on red wine, the modalities with fumaric acid at 2 g/L, both sulfites free and sulfited, were perceived as having a higher acidity than the modalities with tartaric acid.

Overall, significant results were found on many wine properties, showing a clear difference between the impact of fumaric acid addition on red wine, compared to tartaric acid. The impact on polyphenolic compounds showed a potential protection of tannins and anthocyanins from fumaric acid addition, without damaging the sensory properties of the wine.

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