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ORIGINAL RESEARCH ARTICLE

Solubility, acidifying power and sensory properties of fumaric acid in water, hydro-alcoholic solutions, musts and wines compared to tartaric, malic, lactic and citric acids

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ABSTRACT

The lower content of organic acids in grape berries due to global warming, and therefore high pHs, is forcing winegrowers to acidify must or wine to preserve their microbiological stability and their physico-chemical equilibrium. Acidification is essential to avoid damageable consequences on the colour and the sensory quality of the wines. Of the possible modes of acidification, chemical acidification is still the most common one used by OIV country members. It consists of adding lactic, malic, tartaric or citric acids to the musts and wines. Fumaric acid, with its high acidifying power and availability on the market, could be an interesting alternative at lower cost than other acids. Very few studies describing the effects of the addition of this acid on the chemical and organoleptic quality of musts and wines have been published. The present study therefore investigated the impacts of fumaric acid - namely its solubility, acidifying power, and impacts on colour and phenolic compounds - on musts and wines in comparison to other acids. Sensory analyses were also carried out to evaluate the perception threshold of each acid in the wines and compare how the acids are perceived. Except for its low solubility, fumaric acid seems to be a good candidate for an economic alternative to wine acidification. It had the highest acidifying power and slightly affected wine chemistry and organoleptic qualities. Further studies are needed to determine the appropriate step during which this acid should be added during winemaking.

KEYWORDS: fumaric acid, solubility, acidifying power, musts, wines, sensory analysis, perception threshold

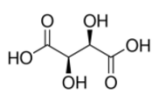
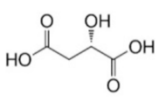
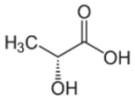
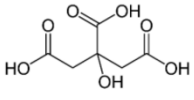
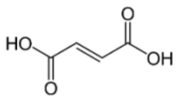
INTRODUCTION

Global warming has direct consequences on the chemical composition of the grape berry (Barnuud *et al.*, 2014; Keller, 2010), leading in particular to lower level of organic acids. Malic acid is particularly affected (Mira de Orduña, 2010; Neethling *et al.*, 2012). Moreover, higher levels of potassium (K^+) have also been reported at higher temperatures (Coombe, 1986; Hale, 1981). The combination of these factors induce more elevated pHs in musts and wines (Boulton, 1980; Duchêne *et al.*, 2020) which disturb their physico-chemical equilibrium (Baduca Campeanu *et al.*, 2012; Leeuwen and Destrac-Irvine, 2017). Thus, higher pHs in musts and wines generate microbiological disorders and organoleptic alterations, such as colour changes (Ugliano, 2009), and lead to poor aging capacity (Mira de Orduña, 2010). In this context, the acidification of musts and wines is essential for adjusting acidity to an optimal level. The country members of the International Organisation of Vine and Wine (OIV) can process acidification in musts and wines by blending them with respective musts and wines with elevated acidity, by using membrane techniques, or chemically by adding organic acids like lactic acids, DL- or L(-)-malic acid, L(+)-tartaric acid and citric acid (only for wines). Outside the OIV country members, like in the United States (*Electronic Code of Federal Regulations (ECFR)*, s. d.), fumaric acid (FA) can also be used for chemical acidification.

Fumaric acid, (*E*)-2-butenedioic acid, was isolated for the first time from the plant *Fumaria officinalis* which gave it its name. It is naturally produced by plants, as well as by many microorganisms, and is a key intermediate in the citrate cycle. In grape berries, small amounts of FA, of 0.07-10.69 mg/L, have been found (Eyduran *et al.*, 2015; Romero *et al.*, 1993; Sensoy, 2015). Known as the least expensive of the food-grade acids and being nontoxic, fumaric acid is mostly used in the food industry as an antibacterial agent and acidulant (Das *et al.*, 2016). In the European Union Commission Regulations No 1129/2011, it is classified as a food additive other than colours and sweeteners with the E-number E297. It can be added, for example, to flavoured fermented milk products, chewing gum and flavoured drinks at concentrations of up to 4000 mg/L or mg/kg, depending on the food product.

A lot of publications describe the antibacterial properties of fumaric acid. The antibacterial effect of fumaric acid has also been demonstrated by Lu *et al.* (2011) on none-heat processed vegetables, by Comes and Beelman (2002) in apple cider inoculated with *E.Coli,* and by Podolak *et al.* (1996) on vacuum-packaged ground beef. Moreover, Ohsone *et al.* (1999) have shown that fumaric acid is the most effective of the lactic and acetic acids for inhibiting the development of five pathogenic bacteria populations (at equivalent concentrations of 50 mM) on raw vegetables. One of the main effects of fumaric acid is probably the lowering of the pH, thus limiting bacterial development and growth (Gurtler and Mai, 2014). The antibacterial properties of fumaric acid have also been investigated in wines. Already in 1970, Cofran and Meyer (1970) showed that the addition of fumaric acid at concentrations higher than 0.36 g/L retarded malolactic fermentation, in contrast to the tartaric and citric acids tested in the same study. This ability for fumaric acid to inhibit/delay the growth of lactic acid bacteria is of interest for high pH wines, such as wines from warm regions (Ough and Kunkee, 1974; Pilone *et al.*, 1974; Rankine, 1977). More recently, Morata *et al.* (2019) showed that, at concentrations of between 300 and 900 mg/L, fumaric acid was able to inhibit malolactic fermentation, and could stop the process for almost two months when malolactic fermentation was running at 600 mg/L, even with high lactic acid bacteria populations. Recently, the OIV adopted a new resolution, OIV-OENO 581A-2021, authorising the addition of between 300 and 600 mg/L fumaric acid to wines precisely for inhibiting malolactic fermentation.

TABLE 1. Main characteristics and structures of organic acids used as acidity correctors.

	Number of C	Number of carboxylic acid groups	Number of double bonds	pKas at 20°C	M_w g/mol	Concentration in mM for 1 g/L	Concentration in mN for 1 g/L	Factors to convert into tartaric acid g/L equivalent based on the ratios of their	
								M_w	N
Tartaric acid	4	2	0	2.98-4.34[1]	150.087	6.663	13.326	1.000	1.000
Malic acid	4	2	0	3.40-5.05[2]	134.087	7.458	14.916	0.893	0.893
Lactic acid	3	1	0	3.81[2]	90.080	11.101	11.101	0.600	1.200
Citric acid	6	3	0	3.09-4.39-5.74[2]	192.124	5.205	15.615	1.280	0.853
Fumaric acid	4	2	1	3.03-4.44[3]	116.070	8.615	17.230	0.773	0.773
									
Tartaric acid									
									
Malic acid									
									
Lactic acid									
									
Citric acid									
									
Fumaric acid									

[1] Sakurai *et al.* (2005). [2] Ribéreau-Gayon *et al.* (2012). [3] Zhang *et al.* (2013).

Besides its antibacterial properties, fumaric acid has interesting physicochemical properties for the food industry, such as its very high acidifying power. It is one of the strongest food acidulants with reduced cost, meaning that a smaller amount is required to acidify the product in comparison with other organic acids. This acid contains two acid carbonyl groups and it is the only one among other acidity correctors to have a double bond (Table 1).

Due to its low molecular weight and its pKa1 value = 3.03, FA has more buffering capacity than other food acids at a pH of around 3.0 (Das *et al.*, 2016), which means it can limit changes in pH. One of the weak points of FA is its low solubility compared to other acids. In water, at temperatures of around 25°C, an FA solubility of 6.6-8.1 g/L has been reported (Dang *et al.*, 2009; Das *et al.*, 2016; Felthouse *et al.*, 2000), compared to other acids in the same conditions: 1447 g/L for citric acid, 1395 g/L for malic acid and 1395.8 g/L for tartaric acid (Apelblat and Manzurola, 1987). At the same temperature, a solubility of 5.8 g/L of fumaric acid in 12.59 % ethanol has been found by Yang *et al.* (2013). Unfortunately, no data are available for the solubility of FA in musts and wines.

In addition to its antimicrobial and acidifying properties, FA can impact flavours (Yang *et al.*, 2011). It is known to have a fruity-like taste (Goldberg and Rokem, 2009) and a persistent, long-lasting sourness due to its hydrophobic nature (Das *et al.*, 2016). In a sensory evaluation of acids in water by free-choice profiling, Rubico and McDaniel (1992) showed that FA and TA are perceived as more astringent than other food acidulants, and that FA is sourer than MA and CA. Fumaric acid taste has also been studied in wines. Buechsenstein and Ough (1979) compared three acids as wine acidulants and concluded that their relative sourness was fumaric > DL -malic > citric on a pound-weight basis, in an order of lowest to highest molecular weight. More recently, Morata *et al.* (2019) showed that 600 mg/L FA in red wine seemed to improve the perception of wine in terms of acidity and body. In white wine, the detection thresholds of FA has been determined by Ough (1963); FA was reported to have the lowest threshold of 1 g/L compared to tartaric, citric and adipic acids (thresholds of 1.3, 1.8 and 1.3 g/L respectively). More knowledge on thresholds in wines is needed to better deal with the addition of FA in wines and to control its impact.

Thanks to its low cost, its high acidifying power and the possibility that FA could help to reduce the amounts of SO_2 used during winemaking due to its anti-microbial properties, the OIV is now considering this acid as a possible alternative for its country members. The impact of fumaric acid addition on microbial pathogens in food products, as well as on lactic bacteria in wines, has been well documented; however, very few studies describing the effects of the addition of this acid on the physicochemical, chemical and organoleptic quality of musts and wines have been published. Thus, in this study, the solubility and the acidifying power of fumaric acid in model solutions, musts and wines were compared to other acids already authorised by the OIV. Moreover, the effect on oenological and chemical parameters of adding this acid to a red wine and a white wine was then studied. Finally, the detection thresholds of fumaric acid for model solutions and wines were determined and compared to other acids. Triangle tests were also carried out to highlight the sensory differences between acids.

MATERIALS AND METHODS

1. Experimental materials

1.1. Chemicals

Water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). Fumaric, L- (+)- tartaric, DL- malic, citric, lactic and hydrochloric (37%) acids of analytical grade, as well as food grade, were purchased from Sigma Aldrich (Saint Louis, USA), like sodium hydroxide. Ethanol and acetonitrile of high-performance liquid chromatography (HPLC) grade were purchased from VWR International (Pessac, France). (+)-catechin, malvidine-3-*O*-glucoside chloride, gallic acid, Folin–Ciocalteu reagent (2N) and sodium carbonate were purchased from Merck (Darmstadt, Germany). Sodium bisulfite solution (40%) and formic acid (98%) from Fisher Chemical (Illkirch, France).

1.2. Musts and wines

Musts. 25 kg of black and white grapes, Cabernet-Sauvignon (CS) and Sémillon (Sem) respectively, were harvested in October 2018 in the experimental plots of the Vitadapt project (Villenave d´Ornon, France). The grapes were pressed using a hydraulic press. The obtained musts were sulfited according to the state of health of the grapes (3 g/hL for white and 5 g/hL for red grapes) using a Bisulfite18 solution at 183 ± 3 g/L of SO_2 (Laffort, Bordeaux, France), and were then stored at -20 °C before analysis.

Wines. The analyses were carried out on white wine from Sauvignon blanc (SB, alcohol % = 12.5) cultivar and red wines from Cabernet-Sauvignon (CS, alcohol % = 12.8) and Merlot (alcohol % = 13.7) cultivars sold commercially (Leclerc brand) and packaged in a bag in box.

2. Analysis of the properties of fumaric acid

2.1. Solubility

The measurement of the solubility of fumaric acid, in comparison with other organic acids in powder form (tartaric, malic and citric), was carried out in several different media: mQ water, 10 % hydro-alcoholic solutions at 12 % and 16 % ethanol (v/v), CS and Sem musts, and CS, Merlot and SB wines. For each analysis, an excess of acid was added to 10 ml of the corresponding solution, which was then stirred for at least 16 hours. After that, the solution was filtered through a glass funnel filter with sintered glass disc (porosity n°3) with a paper filter (1.6 µm, Whatman) under vacuum. The retentate was then dried at 50 °C until it reached a constant mass. Finally, the excess dried acid was weighed. Solubility was calculated as the difference between the mass of acid added and the mass of dried acid residue divided by the final volume of the saturated solution (g/L).

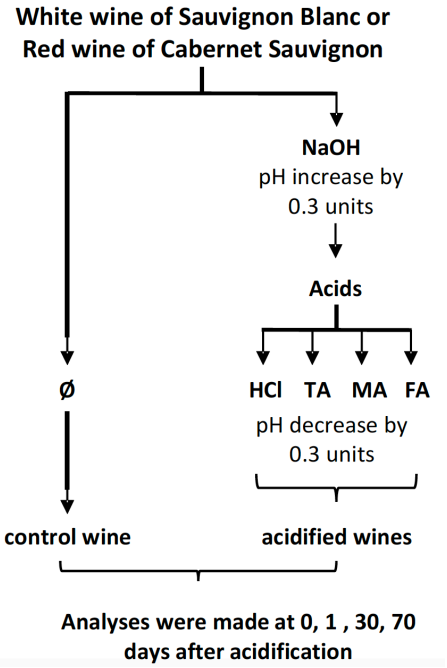


FIGURE 1. Experimental diagram of wine acidification.

2.2. Decrease in pH of model solutions, musts and wines

The amount of acids (mg/L) needed to lower the pH by 0.1-0.2-0.3-0.4-0.5 units were determined for fumaric, tartaric, malic, citric, and lactic acids in Sem- and CS-musts (at their initial pH) and CS-, Merlot- and SB-wines (with pH adjusted to 4 using NaOH).

3. Chemical impact of adding acid to wines

3.1. Acidification of wines

CS- and SB-wines were each divided into two batches (1/5-4/5); one batch was used as the control wine and the other one for acidification with the different acids. Before acidification, NaOH was added to the second batch of white and red wines to decrease the pH by 0.3 units. The resulting wines with higher pH were then divided into four sub-batches and the different acids, fumaric, tartaric, malic and hydrochloric acids, were finally added to lower the pH by 0.3 units to the initial pH of white and red control wines (Figure 1). Thus, all the wines from each cultivar, control and acidified wines had the same pH. This precaution avoids the possible effects of pH differences on the variables studied (such as colour). To perform the triplicate analyses at 0, 1, 30 and 70 days after the addition of acid to both white and red wines, aliquots of each treatment were placed in twelve tubes of 50 mL closed with a screw cap and parafilm.

3.2. Analysis of classic oenological parameters and titratable acidity

In the control wines (without added acid) at T = 0, pH, titratable acidity (TitAc, g/L tartaric acid eq.), volatile acidity (VA, g/L H_2SO_4 eq.), total sugars (g/L), density (kg/m^3), alcohol (ABV, % vol), and organic acids (g/L) were measured using a FOSS WineScan 79000 FTIR instrument (Foss, Nanterre, France).

For the study of acid impact on wine quality, titratable acidity was assessed by titrating the different samples with 0.1M sodium hydroxide. 0.1M NaOH was added to the wines until a pH of 11 was reached. For each volume of added 0.1M NaOH, pH was measured using a Mettler Toledo pH-meter (Viroflay, France). The volume of 0.1M NaOH added to obtain a pH of 7 (equivalence) was finally evaluated by plotting the pH versus the added volume of 0.1M NaOH. Titratable acidity was expressed in g/L tartaric acid equivalent.

3.3. Wine colour analysis

3.3.1. CIELAB parameters

CIELAB parameters (CIE, 1986), 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 using the formula: $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$

3.3.2. 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) for red wine. Only the 420 nm absorbance was measured for white wine. The spectrophotometric chromatic characteristics (Glories, 1984) were calculated as follows:

$CI' = 10 * (A420\text{ nm} + A520\text{ nm} + A620\text{ nm})$;

$Hue = A\,420\text{ nm}/A\,520\text{ nm}$;

$\%\text{ yellow} = (10 * 100 * A420\text{ nm})/CI'$;

$\%\text{ red} = (10 * 100 * A520\text{ nm})/CI'$;

$\%\text{ blue} = (10 * 100 * A620\text{ nm})/CI'$.

3.4. Analysis of total phenolic compounds in wines

3.4.1. 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 (Ribéreau-Gayon *et al.*, 2012) calculated as follows: $TPI = DF * A280\text{ nm}$ with DF as the dilution factor.

3.4.2. Folin Ciocalteu Index (FCI)

According to Singleton and Rossi (1965), 250 µL of Folin Ciocalteu reagent, 50 µL of 1/10 diluted (red wine) or undiluted (white 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.

3.4.3. Total tannins (TT)

Total tannins were determined according to Ribéreau-Gayon and Stonestreet (1966). In two hydrolysis tubes, 1 ml of sample (red wine diluted 50 times or undiluted white wine), 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 in a water bath at 100 °C. After 30 min, the absorbance of both tubes at 550 nm was measured in a 1 cm-path length cuvette and the difference between both absorbances ($\Delta A_{550\ nm}$) was calculated. TT was determined according to Ribéreau-Gayon *et al.* (2012) using the following formula $TT = DF \times 0.3866 \times \Delta A_{550\,nm}$ and expressed in g/L.

3.4.4. Total anthocyanin concentration (TAC)

TAC was determined only for the samples of Cabernet-Sauvignon wine. According to (Ribéreau-Gayon and Stonestreet (1965), 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 400 µL 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 the standard and was expressed in mg/L malvidin-3-*O*-glucoside equivalent.

3.5. HPLC Analyses

3.5.1. 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 according to González-Centeno *et al.* (2012). 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. The 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, and 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). The identification of catechin, epicatechin, B1, B2, B2 and B4 dimers was performed by comparison with injected external standards and previous results (Chira, 2009). Catechin was used as the external standard. The results were expressed as mg/L catechin equivalents.

3.5.2. Composition of anthocyanins in the red wine

Red wines were first filtered (0.45 µm) and 20 µL were injected into 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 according to González-Centeno *et al*. (2017). 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 (Chira *et al.*, 2009). Concentrations were expressed as mg/L malvidin 3-*O*-glucoside equivalents.

4. Sensory analyses

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 black glasses (NF V 09 110). The samples were presented randomly, numbered with 3-digit codes and in a balanced (thresholds and triangular tests) manner. At least 20 judges were present during each sensory evaluation session. All products used in these sessions were Food chemical codex (FCC) or Food Grade (FG) listed.

4.1. Perception thresholds

The taste detection thresholds of the different organic acids were evaluated according to the norm ISO 13301: 2002 in four different media: water, hydro-alcoholic solution with 12 % (v/v) ethanol, CS-wine and SB-wine. The 3-AFC method was used (3 forced choice alternatives). During the sessions, the judges were given several sets of three glasses, two without acid and one supplemented with stepwise-increasing concentrations (factor 2) of the acid to be evaluated (fumaric, tartaric, malic, citric or lactic acid). The judges were asked to taste the samples in a defined order. The judges had to indicate which glass they thought was different, even if they were not sure of their answer. The choice of the acid concentration range was made beforehand in the laboratory and based on preliminary tests in the corresponding medium. The detection thresholds (mg/L) were evaluated by the Best Estimate Threshold method (BET) (ISO 13301 : 2002) and by the Maximum Likelihood method according to Tempere *et al.* (2011) ; for the latter method, R-software was used for graphic resolution and sigmoidal regression.

Thresholds in wines calculated for fumaric and citric acids were considered as absolute thresholds, as these acids were minor acids in wine. The tartaric acid threshold was a difference threshold, this acid being present in the tested wines before addition. For malic acid and lactic acid, the type of threshold depended on whether the wine had undergone malolactic fermentation. Absolute threshold corresponds to the smallest added concentration of acid to elicit a sensation by an individual, whereas the difference threshold is the minimal difference in acid concentration required to be noticeable by an individual (Orellana-Escobedo *et al*., 2012).

For each wine, Weber ratio of an acid was calculated as its difference threshold divided by its initial concentration.

4.2. Triangular tests

Several triangular tests (ISO 4120 : 2004) were carried out on the SB- and CS-wines in order to determine significant

TABLE 2. Solubility of fumaric, tartaric, malic and citric acids at 25 °C in water, musts, hydroalcoholic solutions and wines.

Acids	Water	Sem must	CS must	12 % Ethanol	16 % Ethanol	SB-wine	CS-wine	Merlot wine
Citric acid	960.0a ± 27.5	1082.7a ± 53.6	1106.3a ± 64.9	1048.3a ± 13.3	1098.3a ± 5.5	1079.7a ± 28.9	1056.7a ± 17.4	1102.3a ± 15.4
Malic acid	845.0b ± 9.7	870.7b ± 15.5	868.3b ± 45.1	918.6b ± 15.0	875.7b ± 18.6	1047.0a ± 47.7	932.3b ± 16.5	978.7b ± 21.4
Tartaric acid	786.0c ± 19.1	749.3c ±16.2	740.7c ± 40.0	922.3b ± 8.1	890.7b ± 5.7	1049.3a ± 35.3	922.7b ± 7.4	992.3b ± 17.2
Fumaric acid	5.3d ± 0.2	4.7d ± 0.1	4.7d ± 0.1	6.4c ± 0.1	7.1c ± 0.1	14.9b ± 0.3	9.3c ± 0.1	14.9c ± 0.2

CS = Cabernet-Sauvignon, Sem = Sémillon, SB = Sauvignon blanc.
Different letters show significant difference between acids with $\alpha = 0.05$ for each medium.

taste differences between the control wine and the wines with added tartaric, malic, lactic, citric and fumaric acids, as well as to compare the wines with added fumaric acid with those with the other added acids. The pH of the acidified wines was first increased by 0.3 units using FG NaOH, and then it was returned to their initial pH (pH of the control wine) using the different acids. During the sessions, the judges were asked to taste the samples in the defined order and to indicate which one they thought was different, even if the judges were not sure of their answer.

5. Statistical analyses

All the results of the chemical (performed in three replicates) and sensory analyses for all study media were statistically processed using RStudio software (Version 1.1.442 - © 2009-2018 RStudio, Inc.).

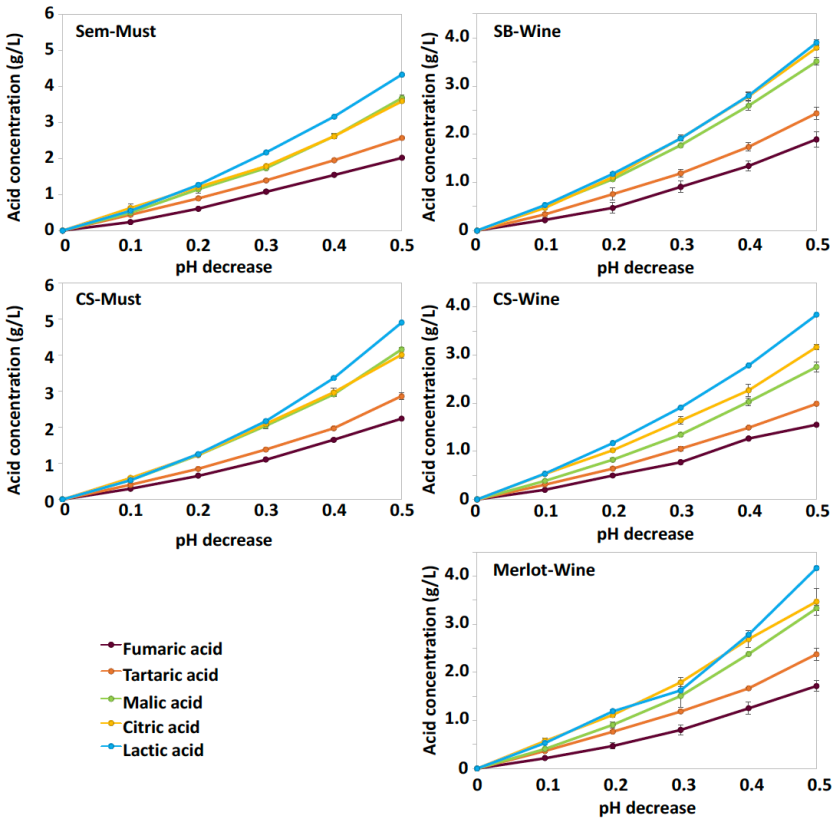
RESULTS AND DISCUSSION

1. Physicochemical properties of fumaric acid

1.1. Solubility

Solubility tests for fumaric acid were performed on water and hydro-alcoholic solutions (12 % and 16 %), and on white and red musts and wines of CS, SB and Merlot. The resulting solubilities were compared with those of tartaric, malic and citric acids. The results are shown in Table 2.

The results show drastic significant differences between the solubility of fumaric acid and that of the other tested organic acids, regardless of the studied media. Indeed, in aqueous media (water and musts), the solubility of fumaric acid (5.3 ± 0.2 and 4.7 ± 0.1 g/L respectively) was ~150 to 230 times lower than for the other acids, citric acid being the most soluble. The solubility of fumaric acid in musts was



Acid concentration (g/L) to lower the pH by 0.1 units					
Acids	Sem- must	CS-must	SB-wine	CS-wine	Merlot-wine
Fumaric acid	0.24a[1]± 0.02	0.29a± 0.01	0.22a± 0.04	0.20a± 0.03	0.22a± 0.02
Tartaric acid	0.44b± 0.01	0.40b± 0.03	0.34b± 0.03	0.31b± 0.02	0.36b± 0.03
Malic acid	0.49bc± 0.04	0.53c± 0.02	0.51c± 0.02	0.39c± 0.04	0.41b± 0.03
Citric acid	0.63c± 0.11	0.59d± 0.03	0.47c± 0.04	0.53d± 0.03	0.57c± 0.07
Lactic acid	0.46b± 0.01	0.44b± 0.01	0.44c± 0.01	0.45c± 0.00	0.44b± 0.01

FIGURE 2. Added acid concentration (g/L) to decrease pH by 0.1 to 0.5 units in white and red musts and wines.

CS = Cabernet-Sauvignon, Sem = Sémillon, SB = Sauvignon blanc.
Different letters show significant difference between acids with α = 0.05 for each medium.

TABLE 3. Oenological characteristics of SB- and CS-wines.

Oenological parameters	SB-wine	CS-wine
Alcohol (% v/v)	12.53 ± 0.01	12.75 ± 0.03
Density (kg/m^3)	989.2 ± 0.1	991.3 ± 0.1
Sugars (g/L)	1.63 ± 0.15	6.50 ± 0.10
pH	3.34 ± 0.01	3.61 ± 0.01
Titratable acidity (g/L tartaric acid eq.)	6.54 ± 0.01	4.76 ± 0.01
Volatile acidity (g/L H_2SO_4 eq.)	0.28 ± 0.01	0.44 ± 0.01
Tartaric acid (g/L)	3.73 ± 0.01	2.80 ± 0.01
Malic acid (g/L)	1.71 ± 0.01	-
Lactic acid (g/L)	0.06 ± 0.01	1.20 ± 0.02

CS = Cabernet-Sauvignon, SB = Sauvignon blanc.

slightly lower than in water, which can be partly explained by the presence of a large quantity of sugars (Richert, 1930). The fumaric acid solubility evaluated here was of the same order as those published by Das *et al*. (2016) (6.3 g/L) and Felthouse *et al*. (2000) (7.0 g/L).

In 12 % and 16 % hydro-alcoholic solutions, fumaric acid solubilities were found to be 6.4 ± 0.1 g/L and 7.1 ± 0.1 g/L respectively. Significantly higher fumaric acid solubility values were observed with increasing alcoholic degree (p-value = 3.3*10^{-5}). Compared to other acids, fumaric acid was 120 to 165 less soluble in 12-16 % hydro-alcoholic solutions. This ratio was further reduced in wines (fumaric acid ~ 65 to 115 times less soluble). Thus, the solubility of fumaric acid reached 14.9 ± 0.3 g/L in SB- and Merlot-wines; *i.e.* about 3 times more than in water. There were also differences in solubility depending on the wine (9.3 ± 0.1 g/L for CS-wine and 14.9 ± 0.3 g/L for SB- and Merlot-wines), which did not seem to be related to the type of wine (red or white).

Due to its hydrophobic nature, the solubility of fumaric acid decreases with increasing solvent polarity, which explains its very limited solubility in water (Yang *et al.*, 2013). In this experiment, it should be noted that the time to solubilise 1 g/L fumaric acid in water and in the 12 % hydro-alcoholic solution were estimated to occur about 20 and 10 minutes respectively after addition while stirring, whereas that of the other acids was almost instantaneous. The difficulty to dissolve fumaric acid in aqueous solutions is known and constitutes the main obstacle to its use. However, the solubility values obtained in this study significantly exceeded the authorised quantity of 3 g/L for acidification in wine in the USA (Smith and Hong-Shum, 2008). This acid could therefore be used in the same way as other acids in winemaking conditions.

1. 2. pH lowering

Figure 2 shows the amount of acids (fumaric, tartaric, malic, citric and lactic) that need to be added to the musts and wines to decrease the pH from 0.1 to 0.5 units.

The amounts of fumaric acid and tartaric acid necessary for the same pH decrease were systematically lower than for other acids. These acids therefore had more acidifying power in musts and wines than the others. In contrast, according to the plot profiles of Figure 2, lactic acid was found to be the weakest acid, as it required the highest amount to be added to the musts and wines to lower the pH to the same target level as the other acids. Thus, the strengths of the studied acids was classified as follows: FA > TA > CA ≈ MA > LA in musts and FA > TA > MA > CA > LA in red wines and FA > TA > MA > CA ≈ LA in SB-wine.

Furthermore, in order to lower the pH of musts and wines by 0.1 units (Table in Figure 2), significantly less fumaric acid (30 %) was needed than tartaric acid. Thus, in addition to the fact that fumaric acid is the cheapest and most readily available acid on the market, it also has the highest acidifying power. Indeed, the results obtained here clearly show that fumaric acid is the most effective in reducing the pH of musts and wines, regardless of the type of wine. Such low quantities of added fumaric acid for decreasing pH represent a considerable financial gain for a wine cellar. The only negative point is its low solubility, but it is however higher than the doses needed to acidify must or wines. A negative aspect of tartaric acid is its possible precipitation during storage, unlike fumaric acid with which this does not occur.

Besides its high acidifying potential, fumaric acid is also known to have antimicrobial activity; it can inhibit bacterial development (Comes & Beelman, 2002; Lu *et al.*, 2011; Morata *et al.,* 2019; Ohsone *et al.*, 1999; Pilone *et al.*, 1974). These properties could allow the use of SO_2 to be reduced at the vatting when FA is added to the must, protecting the must from bacterial growth or blocking a non-desired malolactic fermentation. They are of particular interest in white wines, especially in Cognac, which must be sulfite free with a high acidity. However, it should be taken into account that when added before alcoholic fermentation or in the presence of residual living yeasts, fumaric acid is converted into malic acid by yeasts via the citric acid cycle (Bressler *et al*., 2002; Peleg *et al*., 1990; Pines *et al*., 1996).

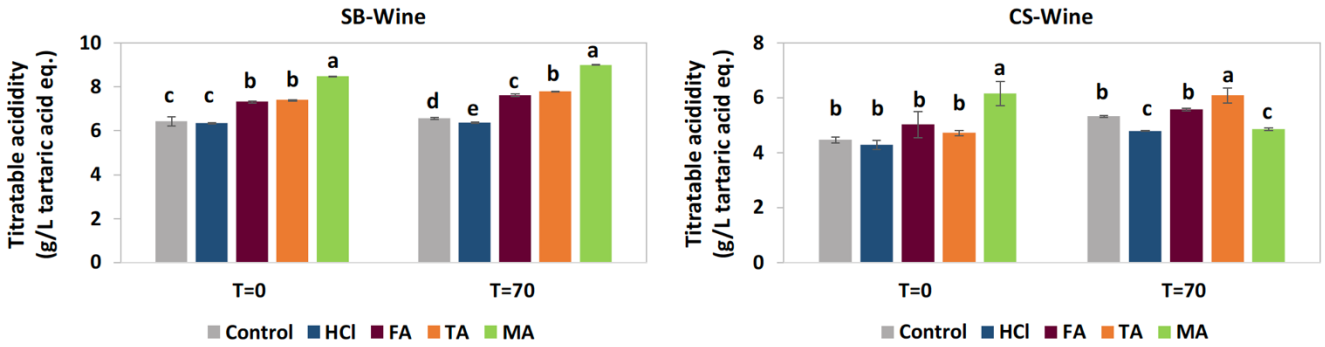


FIGURE 3. Titratable acidity (g/L tartaric acid eq.) just after acid addition (T = 0) and 70 days later (T = 70) in Sauvignon blanc and Cabernet-Sauvignon wines.

SB = Sauvignon blanc, CS = Cabernet-Sauvignon, FA = Fumaric acid, TA = Tartaric acid, MA = Malic acid.
Different letters show significant difference between acids with α = 0.05 for each time point.

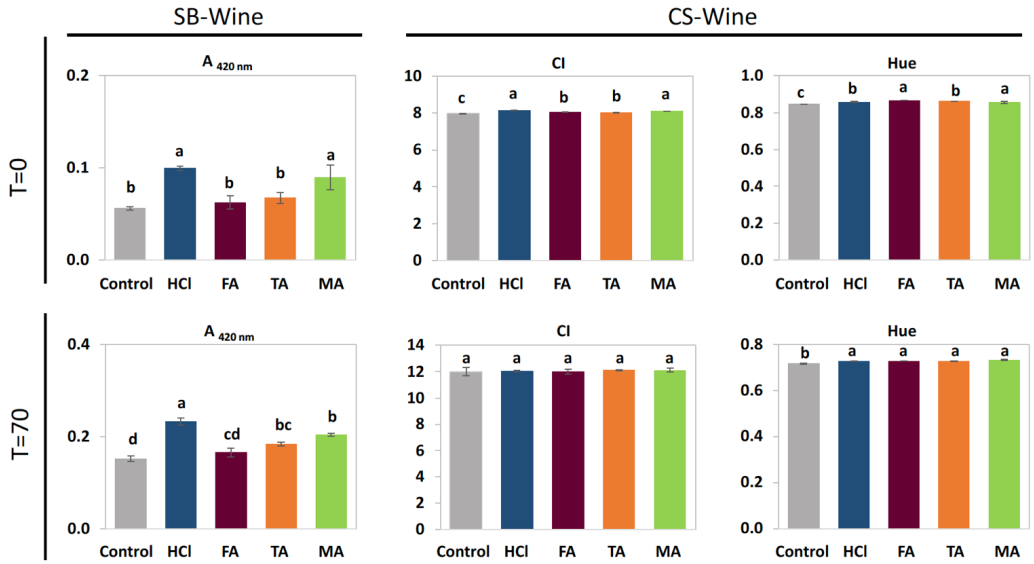


FIGURE 4. Chromatic spectrophotometric parameters just after acid addition (T = 0) and 70 days later (T = 70) in Sauvignon blanc and Cabernet-Sauvignon wines.

SB = Sauvignon blanc, CS = Cabernet-Sauvignon, FA = Fumaric acid, TA = Tartaric acid, MA = Malic acid.
Different letters show significant difference between acids with α = 0.05 for each parameter.

2. Chemical impact of adding fumaric acid to wines

The chemical impact of the three acids with the strongest acidifying power, namely fumaric, tartaric and malic acids, was assessed in a white SB-wine and in a red CS-wine. The oenological characteristics of SB- and CS-wines are shown in Table 3.

2.1. Impact on titratable acidity

Except for the control samples, NaOH was first added to increase the pH of wines by 0.3 units and fumaric, tartaric, malic or hydrochloric acids were then added to recover the initial pH of the wines. Thus, the pHs of treated white and red wines were equal to the corresponding control wines. Figure 3 shows the titratable acidity measured in SB- and CS -wines just after the addition of the acids (T = 0) and 70 days later (T = 70).

In the case of SB-wine, a significant effect of acid addition was shown just after the addition of acids and also 70 days later. The addition of fumaric, tartaric and malic acids led to an increase in titratable acidity, malic acid always being the one with the highest titratable acidity. In contrast, hydrochloric acid did not impact titratable acidity. These results persisted after 70 days. In the case of CS-wine, the results were different between T = 0 and T = 70. Just after the addition of acids, only malic acid had an impact on titratable acidity, leading to a higher value. After 70 days, titratable acidy evolved. The titratable acidity of the HCl- and malic acid-samples were lower than in the control wine, and the tartaric acid sample was higher, whereas fumaric acid did not show any significant differences compared to the control wine. Briefly, regarding both type of wines, the titratable acidity of wine with added fumaric acid was close to that of wine with added tartaric acid.

2.2. Impact on colour

The impact of acid addition on wine colour was studied and results are shown in Figure 4 (spectrophotometric parameters) and Table 4 (colour differences between wines based on CIELAB parameters).

In the case of white wine, the impact of acid addition on the yellow colour ($A_{420\ nm}$) depended on the acid. Fumaric and tartaric acid did not have a significant impact compared to the control wine. In contrast, the addition of HCl and malic acid significantly increased the intensity of the yellow colour. After 70 days, the HCl acidified wine stayed the most yellow and fumaric acidified wine was the most similar to the control, meaning that the addition of fumaric acid did not affect the yellow hue. In general, it was noted that, 70 days after the addition of the acids, the intensity of yellow colour was twice as high in all the samples. The colour difference between wines (ΔE) calculated according to CIELAB parameters (Table 4) were all less than 1, indicating that human eye was not able to perceive the nuances. Indeed, according to García-Marino *et al.* (2010), values of ΔE greater than or equal to 3 means that the difference between both wines was perceptible to the naked eye.

Regarding red wine, the colour intensities (CI) and the hues were all around 8 and 0.8 respectively just after the addition of acids. After 70 days, CIs increased to 12 and hues slightly decreased to 0.7. Some significant differences were found just after the treatment, acid addition leading to an increase of CI and hue. However, after 70 days, the differences in colour intensity disappeared. A slight difference between the acidified wines and the control persisted for the hue. Regarding the ΔE parameter, values were all under 3. However, the comparison between control and acidified wines at T = 0 gave values higher than 2, showing a slight impact of acid on wine colour just after the addition. After 70 days, these differences faded, especially between the control and the FA-acidified wine (ΔE = 0.12), meaning that addition of FA did not impact the colour of the red wine at the same pH.

2.3. Impact on phenolic compounds

The impact of acid addition on phenolic compounds was studied in white and red wines just after the acids were added (Table 5).

The addition of fumaric acid to white and red wines significantly increased the total phenolic compounds index (TPI) at T = 0: by 40 % and 6 % respectively. In white wine, this effect of fumaric acid addition was even more pronounced since the TPI value in white wine was lower than in red wine. This difference persisted in the white wine after 70 days of storage. These higher TPI values in both wines with added fumaric acid can possibly be explained by the absorption spectrum of this compound. Indeed, fumaric acid shows an absorption maximum at 208 nm, which decreases with increasing wavelength, but is not zero at 280 nm in contrast to the other added acids (results not shown). The amounts of fumaric acid added to lower the pH by 0.3 units were sufficient to explain this difference. While the difference was the same for red and white wine (difference of ~ 3-4 between TPI of the control wine and TPI of FA-acidified wine), it was much more visible in white wine, because the TPI of white wine control was much lower than that of red wine.

In both wines, Folin Ciocalteu Index (FCI) and total tannins (TT) were not affected by acids just after addition and after 70 days of storage. Regarding mono- and dimeric flavan-3-ols in white and red wines, the concentrations of monomers and dimers were quite similar just after addition and after 70 days of storage, although slight significant differences were detected due to low standard deviations.

TABLE 4. Colour difference (ΔE[1]) between control and acidified white and red wines.

	SB-Wine		CS-Wine	
	D = 0	D = 70	D = 0	D = 70
Control-FA	0.17	0.25	2.93	0.12
Control-TA	0.11	0.25	2.15	1.64
Control-MA	0.10	0.30	2.07	1.58
Control-HCl	0.03	0.10	2.40	1.30
FA-TA	0.06	0.47	0.79	1.52
FA-MA	0.08	0.52	0.86	1.47
FA-HCl	0.15	0.32	0.54	1.19
TA-MA	0.02	0.05	0.11	0.07
TA-HCl	0.09	0.16	0.27	0.34
MA-HCl	0.07	0.21	0.33	0.30

[1] $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$ where L*, a* and b* are CIELAB parameters.

SB = Sauvignon blanc, CS = Cabernet-Sauvignon, D = Day, FA = Fumaric acid, TA = Tartaric acid, MA = Malic acid.

TABLE 5. Phenolic compounds in SB and CS wines the day of acid addition (T = 0) and 70 days after acid addition (T = 70) (Part 1/3).

SB-Wine																					
Time	T = 0										T = 70										
Treatment	Control		FA		TA		MA		HCl		Control		FA		TA		MA		HCl		
	Total phenolics																				
IPT	7.5b[1]	± 0.2	10.5a	± 0.2	7.5b	± 0.0	7.3b	± 0.1	7.6b	± 0.1	6.6b	± 0.1	8.1a	± 0.2	7.0b	± 0.1	6.8b	± 0.2	6.8b	± 0.3	
IFC (mg/L GA eq.)	257.4	± 10.8	245.5	± 1.8	244.6	± 6.0	242.5	± 1.5	245.5	± 5.2	193.3	± 6.4	184.9	± 6.6	193.7	± 6.9	191.1	± 7.4	196.4	± 13.5	
TT (g/L)	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.2	± 0.0	0.2	± 0.0	0.2	± 0.0	0.2	± 0.0	0.2	± 0.0	
	Monomers and dimers flavan-3-ols (mg/L)																				
B3	0.5b	± 0.01	0.6ab	± 0.06	0.6a	± 0.40	0.6ab	± 0.07	0.5ab	± 0.01	nd		nd		nd		nd		nd		
B1	1.5bc	± 0.05	1.6a	± 0.02	1.6ab	± 0.04	1.4c	± 0.07	1.5bc	± 0.02	3.1c	± 0.1	3.2bc	± 0.1	3.4a	± 0.0	3.3abc	± 0.0	3.4ab	± 0.1	
Cat	3.0	± 0.02	3.0	± 0.02	3.0	± 0.05	3.0	± 0.03	3.0	± 0.04	1.8a	± 0.0	1.7ab	± 0.0	1.5c	± 0.0	1.6bc	± 0.1	1.7ab	± 0.1	
B4	0.1	± 0.00	0.1	± 0.01	0.1	± 0.01	0.1	± 0.03	0.1	± 0.01	nd		nd		nd		nd		nd		
B2	0.1	± 0.00	0.1	± 0.01	0.1	± 0.00	0.1	± 0.00	0.1	± 0.01	nd		nd		nd		nd		nd		
Epicat	0.7	± 0.01	0.7	± 0.04	0.7	± 0.01	0.7	± 0.02	0.7	± 0.02	0.7	± 0.0	0.7	± 0.0	0.7	± 0.0	0.7	± 0.0	0.7	± 0.0	
Total monomers	3.7	± 0.0	3.7	± 0.0	3.6	± 0.1	3.6	± 0.01	3.7	± 0.04	2.5a	± 0.1	2.4bc	± 0.0	2.2a	± 0.1	2.3bc	± 0.1	2.5ab	± 0.1	
Total dimers	2.1b	± 0.0	2.4a	± 0.1	2.4a	± 0.1	2.2ab	± 0.14	2.2ab	± 0.05	3.1c	± 0.1	3.2bc	± 0.1	3.4a	± 0.1	3.3abc	± 0.0	3.4ab	± 0.1	

SB = Sauvignon blanc, CS = Cabernet-Sauvignon, FA = Fumaric acid, TA = Tartaric acid, MA = Malic acid.

[1] Different letters show significant difference between acids with $\alpha = 0.05$.

TABLE 5. Phenolic compounds in SB and CS wines the day of acid addition (T = 0) and 70 days after acid addition (T = 70) (Part 2/3).

CS-Wine																					
Time	T=0										T=70										
Treatment	Control		FA		TA		MA		HCl		Control		FA		TA		MA		HCl		
								Total phenolics													
IPT	55.5b	± 0.8	59.1a	± 1.6	55.7b	± 0.7	55.0b	± 1.4	56.0b	± 0.2	49.8b	± 0.8	51.3b	± 0.8	51.0b	± 0.8	49.3b	± 1.1	58.0a	± 1.0	
IFC (mg/L GA eq.)	2556.9	± 54.1	2592.2	± 53.1	2552.6	± 21.5	2577.8	± 4.7	2543.5	± 17.1	2295.5	± 35.3	2209.7	± 34.0	2274.1	± 25.1	2241.9	± 17.0	2206.3	± 81.0	
TT (g/L)	3.1	± 0.2	3.2	± 0.2	3.3	± 0.1	3.3	± 0.0	3.2	± 0.1	5.2	± 0.8	4.9	± 0.4	4.4	± 0.3	4.8	± 0.9	5.5	± 0.2	
CAT (mg/L mal-3-*O*-glu. eq.)	280.9	± 11.4	273.9	± 7.8	269.7	± 3.5	286.2	± 21.2	268.4	± 11.3	135.1	± 2.2	142.7	± 15.8	139.8	± 16.5	141.6	± 12.8	142.3	± 8.8	
								Monomers and dimers flavan-3-ols (mg/L)													
B3 dimer	16.8b	± 0.2	17.4a	± 0.1	17.4a	± 0.1	17.0ab	± 0.1	16.9b	± 0.3	4.9a	± 0.0	4.5b	± 0.1	4.9a	± 0.0	5.2a	± 0.2	5.0a	± 0.2	
B1 dimer	2.3a	± 0.1	1.9b	± 0.1	1.9b	± 0.2	2.2ab	± 0.1	2.3a	± 0.2	1.4	± 0.1	1.3	± 0.1	1.3	± 0.1	1.4	± 0.1	1.5	± 0.2	
Cat	46.6	± 0.1	46.2	± 0.2	46.4	± 0.2	46.4	± 0.2	46.7	± 0.3	11.6b	± 0.1	10.0d	± 0.0	114.4c	± 0.0	12.1a	± 0.1	12.1a	± 0.0	
B4 dimer	3.2a	± 0.1	3.0ab	± 0.1	2.9b	± 0.1	3.1ab	± 0.1	3.2a	± 0.1	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	0.1	± 0.0	
B2 dimer	9.7a	± 0.1	9.4b	± 0.1	9.5ab	± 0.0	9.5ab	± 0.0	9.6a	± 0.1	1.6ab	± 0.0	1.4c	± 0.0	1.6b	± 0.1	1.6ab	± 0.0	1.7a	± 0.0	
Epicat	22.4	± 0.0	22.1	± 0.3	22.1	± 0.0	22.4	± 0.1	22.4	± 0.2	4.6a	± 0.0	3.9c	± 0.1	4.4b	± 0.1	4.8a	± 0.1	4.8a	± 0.1	
Total monomers	69.1a	± 0.1	68.3b	± 0.2	68.6ab	± 0.2	68.8ab	± 0.1	69.1a	± 0.4	16.2b	± 0.0	13.8d	± 0.1	15.8c	± 0.0	16.9a	± 0.1	16.8a	± 0.1	
Total dimers	31.9	± 0.0	31.6	± 0.2	31.7	± 0.2	31.9	± 0.2	32.0	± 0.2	8.0b	± 0.0	7.3b	± 0.1	8.0b	± 0.1	8.3a	± 0.1	8.3a	± 0.0	

SB = Sauvignon blanc, CS = Cabernet-Sauvignon, FA = Fumaric acid, TA = Tartaric acid, MA = Malic acid.

[1] Different letters show significant difference between acids with $\alpha = 0.05$.

TABLE 5. Phenolic compounds in SB and CS wines the day of acid addition (T = 0) and 70 days after acid addition (T = 70) (Part 3/3).

CS-Wine																					
Time	T=0										T=70										
Treatment	Control		FA		TA		MA		HCl		Control		FA		TA		MA		HCl		
	Anthocyanins																				
del-3-*O*-glu	7.9	± 0.5	6.8	± 0.3	8.4	± 2.4	5.0	± 1.7	5.9	± 2.7	0.8	± 0.1	1.1	± 0.1	1.6	± 1.7	0.6	± 0.2	0.7	± 0.5	
cya-3-*O*-glu	0.9	± 0.4	0.7	± 0.1	0.9	± 0.5	1.4	± 1.1	0.4	± 0.1	1.2ab	± 0.3	1.4a	± 0.2	0.8b	± 0.1	0.8b	± 0.0	1.0ab	± 0.3	
pet-3-*O*-glu	8.3	± 1.3	8.8	± 0.1	6.7	± 3.5	7.3	± 1.4	8.0	± 0.9	0.8b	± 0.1	1.2a	± 0.1	0.6b	± 0.1	0.7b	± 0.1	0.6b	± 0.1	
peo-3-*O*-glu	4.3	± 0.6	4.3	± 0.2	4.3	± 0.7	4.6	± 0.9	3.4	± 0.8	0.5ab	± 0.1	0.7a	± 0.0	0.4ab	± 0.1	0.3b	± 0.2	0.4ab	± 0.0	
mal-3-*O*-glu	61.5	± 0.3	60.2	± 0.1	59.4	± 1.1	59.7	± 3.5	57.7	± 0.5	5.1b	± 0.2	7.2a	± 0.2	3.6d	± 0.2	4.2c	± 0.1	3.3d	± 0.1	
peo-3-*O*-(6-*O*-acetyl)-glu	2.2a	± 0.2	2.0ab	± 0.2	1.7b	± 0.1	1.7b	± 0.2	1.6b	± 0.2	0.1	± 0.0	0.1	± 0.1	0.1	± 0.0	0.1	± 0.1	0.1	± 0.0	
mal-3-*O*-(6-*O*-acetyl)-glu	19.2	± 0.9	18.0	± 0.3	17.3	± 0.7	17.0	± 0.6	17.6	± 1.3	1.5b	± 0.1	2.2a	± 0.1	1.2bc	± 0.1	1.4b	± 0.1	1.1c	± 0.1	
peo-3-*O*-(6-*O*-p-coum.)-glu	1.0	± 0.2	0.9	± 0.1	1.0	± 0.1	0.8	± 0.1	0.8	± 0.1	0.1	± 0.1	0.2	± 0.1	0.1	± 0.1	0.1	± 0.0	0.1	± 0.1	
mal-3-*O*-(6-*O*-p-coum.)-glu	6.6	± 0.3	6.0	± 0.2	5.1	± 1.6	6.1	± 0.1	6.0	± 0.1	0.3	± 0.1	0.3	± 0.1	0.5	± 0.0	0.4	± 0.0	0.6	± 0.4	
Total glyc. anthocyanins	83.0	± 0.9	80.7	± 0.4	79.8	± 1.3	78.0	± 5.5	75.4	± 2.2	8.3b	± 0.4	11.6a	± 0.3	7.0bc	± 1.8	6.5bc	± 0.1	6.0c	± 0.2	
Total acetyl-glyc. anthocyanins	21.4a	± 1.1	19.9ab	± 0.4	19.0b	± 0.6	18.7b	± 0.8	19.2ab	± 1.1	1.5b	± 0.1	2.4a	± 0.1	1.3cd	± 0.1	1.5bc	± 0.1	1.2d	± 0.1	
Total coum-glyc. anthocyanins	7.6	± 0.4	6.9	± 0.3	6.1	± 1.5	6.9	± 0.2	6.8	± 0.2	0.5	± 0.2	0.5	± 0.0	0.6	± 0.1	0.5	± 0.0	0.7	± 0.4	

SB = Sauvignon blanc, CS = Cabernet-Sauvignon, FA = Fumaric acid, TA = Tartaric acid, MA = Malic acid.

[1] Different letters show significant difference between acids with $\alpha = 0.05$.

In CS-wine, total anthocyanins were not affected by the addition of acid (at equal pH). Regarding the composition of detected anthocyanins at T=0, no important differences were revealed. In contrast, at T = 70, a slight difference was highlighted for total glycosylated and total acetyl-glycosylated anthocyanins. Indeed, the FA acidified wine contained 40 and 60 % more total glycosylated and total acetyl-glycosylated anthocyanins than the control wine, and even more than other acidified wines.

Thus, except for a slight difference observed after 70 days for glycosylated and total acetyl-glycosylated anthocyanins between FA-acidified red wine and others red wines, no differences were generally observed for phenolic compounds, meaning that acidification by fumaric acid, like acidification by others acids, did not affect phenolic compounds (based on similar pH decrease).

3. Taste perception thresholds and organoleptic impact of the addition of fumaric acid to wines

3.1. Detection thresholds

The detection thresholds of fumaric, tartaric, malic, citric and lactic acids were measured in water, hydroalcoholic solution at 12 % ethanol v/v, and in red CS and white SB wines for the first time. To be more exact, when the medium did not contain the acid to be evaluated, the absolute threshold was estimated, whereas when the acid was initially present in the medium (like tartaric acid present in both white and red wines), the difference threshold (also known as the just noticeable difference) was evaluated. The threshold values obtained using two different methods, including BET (Best Estimate Threshold) and sigmoidal regression are shown in Table 6. For two thresholds to be considered as being significantly different, the ratio of the highest one versus the lowest one must be higher than ~2.

Regardless of the media, the detection thresholds found by the BET method were always lower than those found by the sigmoidal regression method.

In water, absolute thresholds of organic acids were found comprise between 47 mg/L (BET = 35 mg/L) for fumaric acid and 91-93 mg/L (BET = 47-93 mg/L) for lactic and citric acids respectively. The fumaric acid threshold was significantly lower than the thresholds for lactic, citric and malic acids, but was close to tartaric acid thresholds (65 mg/L). In water, citric, malic and lactic acids were found to have the highest thresholds, all three being similar. Our BET results were of the same order of those obtained by Fabian and Blum (1943) (unfortunately, fumaric acid was not evaluated in this work) and very close to those of Mao *et al.* (2021), except for malic acid, which was twice as low as in our study here.

Regarding both model media (water and alcoholic solution), the presence of ethanol increased the threshold for the perception of acidity in solution, as previously shown by Berg (1955). The detection thresholds for all acids in water were, on average, two-fold lower compared to the hydroalcoholic solution. The detection threshold of fumaric acid in 12 % ethanol was lower than that of other acids; *i.e.*, its acidity was the most easily perceived. In 12 % ethanol, lactic was the most difficult to detect. Briefly, acid detection thresholds in 12 % hydroalcoholic solution were classified as follows: FA < TA ≈ MA ≈ CA < LA.

The thresholds of the different acids in the white and the red wines (Table 6) were compared, in spite of the fact that there were absolute thresholds or differential thresholds, as here we were interested in the change of perception induced by acid addition to wine (red or white), independently of the initial concentrations of the acids in the wines. In general, the acid detection thresholds in the wines were almost 10-fold higher than the in hydroalcoholic model solution. These differences in thresholds between hydroalcoholic solution and wine may be related to the presence of acids in wines, as well as other compounds, such as tannins and anthocyanins, which could interfere with acid perception. In white wines, acid thresholds ranged between 1346 mg/L for malic acid (BET = 1035 mg/L for tartaric) and 2263 mg/L (BET = 1656 mg/L) for citric acid. The values were comparable to the thresholds from Ough (1963). In red wine, it was the first time that thresholds had been estimated. They ranged between 848 mg/L (BET = 555 mg/L) for malic acid and 1900 mg/L (BET = 1406 mg/L) for lactic acid.

All acids, except lactic acid, had lower thresholds in red wine compared to white wine. This may be related to the higher acidity level (Table 3) of white wine (TitAc = 6.54 ± 0.01 g/L tartaric acid eq.) than red wine (TitAc = 4.76 ± 0.01 g/L tartaric eq.), which may disturb the perception of a specific acid. The order of detection threshold of acids in white

TABLE 6. Taste detection thresholds of organic acids in different media (mg/L).

	Water		12 % Ethanol		SB-Wine		CS-Wine	
Acids	BET	Sigmoidal regression (R^2)	BET	Sigmoidal regression (R^2)	BET	Sigmoidal regression (R^2)	BET	Sigmoidal regression (R^2)
Citric Acid	67	91 (0.94)	114	173 (0.93)	1656	2263 (0.86)	1051	1321 (0.93)
Malic Acid	62	84 (0.99)	117	166 (0.92)	1051	1346 (0.95)	555	848 (0.98)
Lactic Acid	64	93 (0.99)	188	207 (0.95)	1064	1584 (0.84)	1406	1900 (0.81)
Tartaric Acid	42	65(0.94)	114	150 (0.93)	1035	1570 (0.97)	777	1185 (0.97)
Fumaric Acid	35	47 (0.97)	78	100 (0.97)	1275	1897 (0.89)	987	1387 (0.90)

wine and red wine were different: for white wine it was MA ≈ TA ≈ LA < FA < CA, whereas for red wine it was MA < TA < FA ≈ CA < LA. The detection threshold of malic acid was twice as high in white wine as in red wine, probably due to the intrinsic presence of malic acid in white wine, which had not undergone malolactic fermentation, in contrast to the red wine. In the same way, the lactic acid threshold was higher in red wine than in white wine, lactic acid being present only in red wine. In the case of tartaric acid, its threshold in red wine (1185 mg/L) was lower than that in white wine (1570 mg/L). This difference can be explained by the the red and white wines being different matrices, and also by the intrinsic concentration of tartaric acid in both wines: 2.80 ± 0.01 g/L in CS-wine and 3.73 ± 0.01 g/L in SB-wine. Despite the different matrix, the weber ratio (difference threshold of an acid divided by the initial concentration of this acid) of tartaric acid was 0.42 (based on thresholds estimated by sigmoidal regression) for both wines, indicating that the initial concentration of tartaric acid was probably higher than the other compounds of the wines.

To be rigorous, it would be more accurate to compare absolute thresholds and difference thresholds separately. In white wine, the absolute threshold of fumaric acid (1897 mg/L) ranged between thresholds of lactic (1584 mg/L) and citric (2263 mg/L) acids. In the red wine, the fumaric acid threshold (1387 mg/L) was similar to that of citric acid (1321 mg/L), and both were higher than the threshold of malic acid (848 mg/L). Considering Weber ratios for difference thresholds, tartaric acid (0.42) was detectable at lower concentrations than malic acid (0.79) in white wine. This also was the case in red wine, as the Weber ratio for tartaric acid and lactic acid was 0.42 and 1.58 respectively. Applying the hypothesis that the Weber ratios of malic acid and lactic acids are the same in white and red wine, like for tartaric acid, tartaric acid appeared to be the easiest acid to detect, followed by malic acid and lactic acid.

3.2. Organoleptic impact on wines

Several triangular tests were performed on the white and red wines to evaluate significant taste differences between the control wine and the acidified wines on the one hand, and to compare the FA-acidified wine to acidified wines with other acids on the other. To obtain the acidified wines, the pH of the initial wine was first increased by 0.3 units and brought back to the initial pH (pH of the control wine) using the different acids. This precaution was necessary to avoid a pH bias. Table 7 shows the results of these triangular tests.

In the white wine, differences between the control wine and the acidified wines were perceived, except for the wine with added fumaric acid. The largest differences were detected when citric acid and lactic acid were added, which corresponded to the weakest acids. As higher amount of both these acids have to be added to lower the pH by 0.3 units, wines had higher titratable acidity than other acidified wines. Moreover, according to the WineScan analysis (Table 3), lactic acid was weakly present in SB-wine. The combination of both these factors may explain why differences when compared to the control were easily perceived by judges. Although fumaric acid was not present in the wine, no difference was highlighted between the wine acidified with this acid and the control, probably due to the low amount added to decrease the pH by 0.3. When FA-acidified wine was compared to TA- and MA-acidified wines, no differences were noticeable. However, differences were highlighted between FA-acidified wine and wines acidified with the weakest acids; *i.e.*, lactic and citric acids.

In the red wine, differences between the control wine and the added wines were perceived for all the acidified wines.

TABLE 7. Triangle tests between control wine and acidified wines and between wine acidified with fumaric acid and wines acidified with other acids.

Compared wines		Number of correct answers/number of judges	
Wine A	Wine B	SB	CS
Control	FA	21/47	33/48***
	TA	13/24*[1]	14/24*
	MA	14/24*	15/24**
	CA	17/24***	13/24*
	LA	15/24**	14/24*
FA	TA	8/23	9/24
	MA	8/23	13/24*
	CA	13/23*	11/24
	LA	12/23*	13/24*

[1] significant difference: * $\alpha \leq 0.05$, ** $\alpha \leq 0.01$, *** $\alpha \leq 0.001$.

Unlike SB-wine, a very significant difference between the control and the wine acidified with fumaric acid was detected. This may be due to the difference in titratable acidity between both types of wine. Indeed, the titratable acidity of the red wine was lower than that of the white wine, which could imply that acid addition is even easier to detect when absent in the original wine. Other factors must be taken into account regarding the differential perception of each wine, such as the matrix (presence of tannins and anthocyanins in the red wine) and the actual taste or mouthfeel of the acids. Similar to white wine, no difference between the FA-acidified and the TA-acidified red wines was noticeable. However, differences were found between FA-acidified wine and MA- and LA-acidified wines.

Briefly, the addition of organic acid at equivalent pH could cause multifactorial changes in wines. These changes may be induced by the different levels of titratable acidity, by the matrix to which the acid was added (compounds other than the acids present in wines), and by the actual taste of the acid in the corresponding matrix. Ribéreau-Gayon and Peynaud (1961) described tartaric acid as being "hard", malic acid as being "green", citric acid as being "fresh" and lactic acid as being "sourish, but tart". Moreover, sourness is not the only aspect to take into account in terms of mouthfeel when differentiating acids; they can also be perceived as being bitter (Rubico and McDaniel, 1992) and salty (Hartwig and McDaniel, 1995) and can produce somatosensory sensations, like astringency (Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995). The addition of fumaric acid to red or white wines seemed to lead to differential perception compared to the control wine. It would of interest to carry out more in depth studies which compare fumaric acid with other acids in different types of wines to determine any potential benefits in terms of taste.

CONCLUSIONS

Many factors need to be considered when choosing an acid for adjusting pHs which are too high in musts or wines, the most important one being of course the authorisation to use the product. The acidifying power and the cost are also fundamental criteria, and the ability of the acid to be stored and added to the wine must also be taken into account. Moreover, the addition of the acid must neither diminish the general quality of the wine nor negatively modify its organoleptic properties. It must imperatively maintain or even improve the stability of the product during storage. According to our study, fumaric acid fulfils the main criteria, namely its capacity to acidify the musts or the wines, using low quantities, without affecting the intrinsic quality of the products in the case of wine acidification.

Therefore, fumaric acid may be a good candidate for acidifying musts and/or wines. It would be of interest to determine the right moment to add it, depending on the type of wine being produced and the quality objectives, and to more precisely evaluate the sensory modifications it causes in different type of wines. Besides being used to acidify musts and wines, it could also help reduce the amount of SO_2 used during winemaking as a result of the anti-fungal and anti-microbial properties that it is believed to possess. Fumaric acid still raises many questions and further research is needed to understand all the functional characteristics of this acid during winemaking.

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