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Use of Fumaric Acid to Inhibit Malolactic Fermentation in Bottled Rioja Wines: Effect in pH and Volatile Acidity Control

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Abstract: Fumaric acid (FH2) is an additive allowed by the Codex Alimentarius and the International Organization of Vine and Wine (OIV) that can be used for wine acidification but also to inhibit malolactic fermentation (MLF). FH2 has a positive effect in the reduction in SO_2 doses by controlling LAB and other bacteria and by preserving molecular SO_2 due to pH effect. This article reports the use of FH2 at 600 mg/L in wines produced with 3 varieties of *Vitis vinifera* L. grapes (Tempranillo, Garnacha and Viura) made in vintages 2018, 2020 and 2021. Wines treated with 600 mg/L of FH2 were more stable in the long term and showed lower pH by the preservation of malic acid due to both the absence of MLF (which reduced the pH in 0.1–0.2 units compared with controls) and the effect of FH2 acidification (what produced and additional reduction of 0.05–0.1 pH units). The wines treated with FH2 also remained with very low volatile acidity contents close to 0.2 mg/L or lower. These results corroborate that FH2 can be used to successfully control malolactic fermentation in all still wine types (red, white, and rose) from either of the studied varieties.

Keywords: wine; malolactic fermentation; fumaric acid; pH; volatile acidity

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1. Introduction

Fumaric acid (FH2) has been described as a powerful tool to control malolactic fermentation (MLF) [1–4] because of the capacity to eliminate lactic acid bacteria (LAB) at concentrations of 0.4–1.5 g/L with a pH synergic effect [5]. The main impacts of FH2 in enology are detailed in Figure 1. In addition to the effects on LAB, FH2 has also been described as a strong antimicrobial agent against *E. coli*, *L. monocytogenes* and *Salmonella* sp. [6]. It seems the inactivation is produced by the common inhibition mechanism of weak organic acids, in which at low extracellular pH the undissociated form can diffuse across the membrane and produces a reduction in the pH inside the cell that affects the homeostasis. Additionally, it seems that fumarate is able to inhibit the GAD system which transforms glutamate to γ-amino butyric acid (GABA) removing a proton and working as an acid resistance system [6]. Yeasts such as *Saccharomyces cerevisiae* are not sensible to this mechanism because they have several transporting proteins (Pma1 and Pdr12) able to extrude protons and carboxylate anions to reduce the toxicity [7]. Therefore, FH2 is an interesting tool to inhibit MLF without affecting alcoholic fermentation, so it can be useful to apply in bottle fermentation of natural sparkling wines.

Fumaric acid can be analyzed by LC-DAD after C18 separation and with high sensitivity UV detection due to the intense absorbance of its double bond [8]. Recently, an enzymatic method to analyze FH2 with a high sensibility and selectivity has also been published [9].

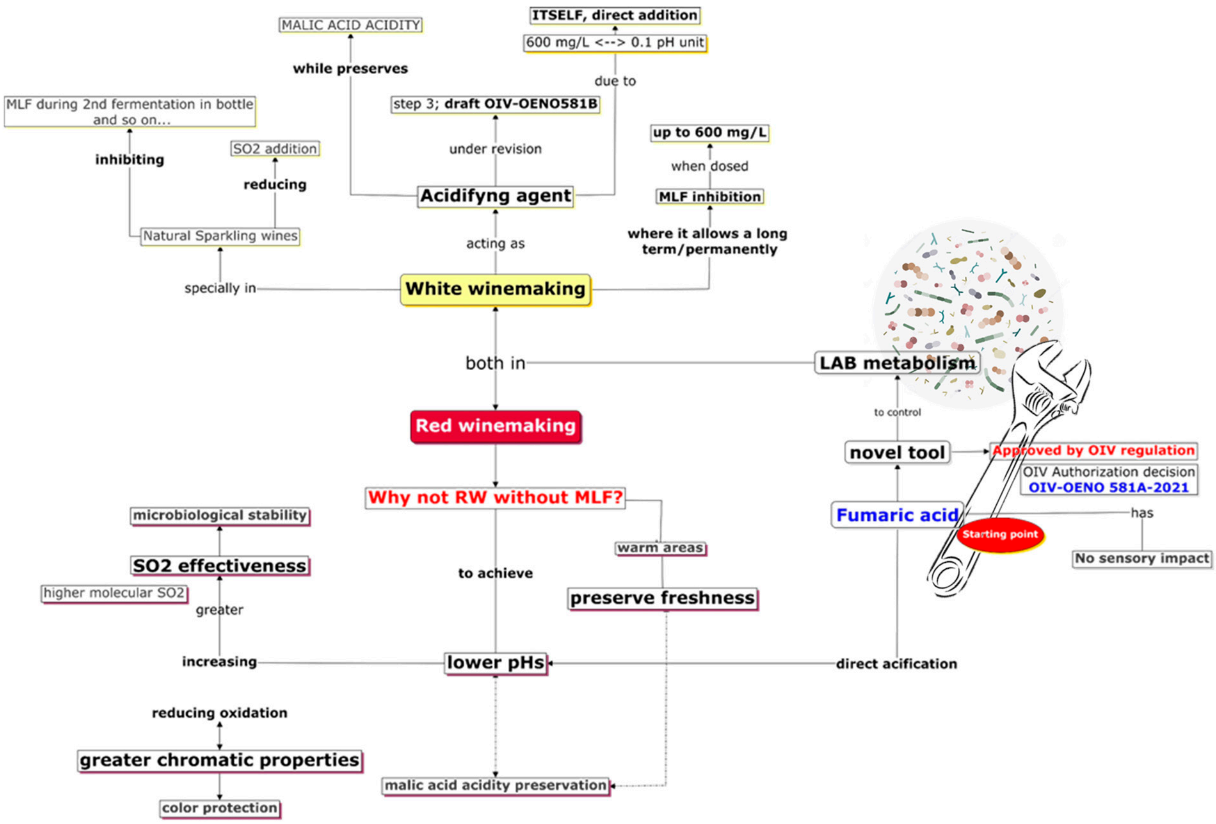

Figure 1. Main impacts in enology of the use of Fumaric acid as inhibitor of malolactic fermentation.

The effectivity of FH2 has been verified against *Oenoccus oeni* strain alpha (Lallemand) at several doses observing that the range 300–600 mg/L is strongly inhibitory while 150 mg/L is able to delay the beginning of MLF for several days [4]. The application of FH2 in wines when the MLF is running with 20–60% of malic acid (MH2) degradation, also inhibits the process keeping the MH2 concentration stable from the moment of addition [4]. The inhibition is also effective in highly inoculated MLFs when we dose >8-log CFU/mL. Lactic acid can also inhibit MLF, however a higher amount (≈4 g/L) is necessary to control LAB [4]. Doses of 2 g/L delay the MLF but are unable to stop it. Therefore, the inhibitory activity of FM2 is ≈10× higher than LH.

Another interesting product to inhibit LAB is chitosan, that is a polysaccharide obtained from fungal sources allowed to control MLF [10]. This product can also be used in organic winemaking and the effectivity is quite good. The only problem is that chitosan is insoluble, so it must be used as a clarification agent and later removed by filtration, hence there is not permanent protection after bottling as with FH2.

FH2 is included as an acidifying agent for some food products in the Codex Alimentarius [11], and recently its use to inhibit MLF in wines has been approved by the OIV in the resolution: OIV-OENO 581A-2021 (https://www.oiv.int/public/medias/8084/en-oiv-oeno-581a-2021.pdf (accessed on 27 September 2022)). This resolution includes as objectives the control of lactic acid bacteria, the inhibition of MLF and the reduction in SO_2 doses. Currently FH2 is under evaluation at OIV (step 3) for its use as an acidification agent at higher doses (draft of resolution OIV-OENO 581B). FH2 is a powerful tool to reduce pH with an effect similar to that of tartaric acid but with better stability in wine [12].

FH2 helps to decrease pH by direct acidification and by preserving MH2 acidity. The direct acidification with 600 mg/L of FH2 can produce a pH reduction of ≈0.1 units depending on the wine buffer capacity [13]. FH2 is also an intermediate of the Krebs cycle, and during the alcoholic fermentation by yeasts it works partially with both a reductive and an oxidative branch [14]. During alcoholic fermentation, FH2 can be partially metabolized by MH2, so it can be a useful strategy to biologically increase malic acidity, and also, if it is well balanced, the residual FH2 can inhibit the MLF protecting wine acidity. The decrease of pH also promotes higher levels of molecular SO_2 increasing its antioxidant and antimicrobial effect. This synergy allows the reduction in SO_2 doses that is a clear trend in the wine sector.

In order to evaluate the impact of FH2 at sensory level, a triangular test has been performed with the addition of doses of 300 and 600 mg/L. The sensory panel was unable to distinguish both treatments from the controls [13]. Therefore, at such doses FH2 do not have sensory impact. When wines with 600 mg/L of FH2 were tasted by trained persons knowing the addition, some of them perceived a slight increase of acidity and body [13].

The objective of this research has been to study the effectivity of FH2 in the control of inoculated and spontaneous MLFs in real white, rose and red wines from La Rioja wine region in Spain, to see the effect in volatile acidity (VA) and pH. The goal is to keep the acidity, thus preserving wine stability and freshness in warm areas.

2. Materials and Methods

2.1. Wines

Ten red, rose and white commercial wines from Bodegas Campoviejo (Rioja, Spain), belonging to the 2018, 2020 and 2021 Vintages, were used for the inhibition trials (Table 1). The wines were made with 3 grape varieties, Tempranillo and Garnacha were used for red wines, Tempranillo also was used to produce rose wines and Viura for white wines. For that, 2, 3 or 12 (*n* in Table 2) 75 cL bottles were analyzed for each trial. Their main initial analytical parameters are shown in Tables 1 and 2.

Table 1. Main enological parameters of the wines used for the inhibition trial with fumaric acid.

Wine	Variety	Year	Ethanol Content	Color Intensity	A280 nm	Residual Sugars
			% vol.	Absorbance units	Absorbance units	g/L
Red	Tempranillo	2018	13.0	19.2	64	2.0
Red	Tempranillo	2020	14.0	21.0	77	2.0
Red	Tempranillo	2020	13.1	13.5	57	1.9
Rose	Tempranillo	2020	14.0	1.0	10	2.3
White	Viura	2020	12.5	-		2.1
Red	Tempranillo	2021	14.2	12.8	60	3.5
Red	Tempranillo **MLF started**	2021	14.4	17.8	74	2.2
Red	Garnacha	2021	14.3	11.6	58	2.4
Rose	Tempranillo	2021	14.2	0.7	10	2.1
White	Viura	2021	13.3	-		1.8

Table 2. Effect of fumaric acid (600 mg/L) in the inhibition of malolactic fermentation (MLF) in red, rose, and white wines: impact in malic acid (g/L), pH and volatile acidity (g/L). MLF started by inoculation with *Oenococcus oeni* strain alpha or spontaneously with wild lactic acid bacteria. When $n = 3$, values are means ± standard deviations. Significant differences are indicated by different letter ($p < 0.05$). One way ANOVA and mean tests have been performed per vintage.

Wine	Variety	Year	MLF	*n*	MH2		pH		Volatile Acidity
					Initial	Final	Initial	Final	
Red	Tempranillo	2018	Spontaneous	12	3.02	<0.05	3.49	3.65	0.22
			Inhibited FH2	12	3.02	3.00	3.49	3.48	0.17
Red	Tempranillo	2020	Spontaneous	2	2.30	<0.05 a	3.62	3.77	0.45 ± 0.01 ef
			Inoculated Oo	2	2.27	<0.05 a	3.62	3.77	0.43 ± 0.00 f
			Inhibited FH2	2	2.26	2.26 ± 0.02 d	3.55	3.52	0.20 ± 0.00 ab
			Inoculated Oo FH2	2	2.27	2.29 ± 0.01 de	3.52	3.51	0.19 ± 0.01 ab

Table 2. *Cont.*

Wine	Variety	Year	MLF	*n*	MH2		pH		Volatile Acidity
					Initial	Final	Initial	Final	
Red	Tempranillo	2020	Spontaneous	2	1.58	<0.05 a	3.67	3.77	0.51 ± 0.06 de
			Inoculated Oo	2	1.55	<0.05 a	3.67	3.78	0.73 ± 0.08 de
			Inhibited FH2	2	1.60	1.52 ± 0.28 c	3.58	3.62	0.21 ± 0.03 ab
			Inoculated Oo FH2	2	1.60	1.17 ± 0.14 b	3.58	3.63	0.21 ± 0.01 ab
Rose	Tempranillo	2020	Spontaneous	2	2.24	<0.05 a	3.63	3.80	0.20 ± 0.02 ab
			Inoculated Oo	2	2.27	<0.05 a	3.63	3.77	1.77 ± 0.11 g
			Inhibited FH2	2	2.36	2.41 ± 0.04 f	3.50	3.51	0.05 ± 0.01 a
			Inoculated Oo FH2	2	2.34	2.18 ± 0.04 d	3.50	3.56	0.15 ± 0.01 ab
White	Viura	2020	Spontaneous	2	2.29	<0.05 a	3.33	3.49	0.28 ± 0.01 bc
			Inoculated Oo	2	2.24	<0.05 a	3.33	3.48	0.55 ± 0.25 cd
			Inhibited FH2	2	2.24	2.21 ± 0.02 d	3.21	3.26	0.14 ± 0.01 ab
			Inoculated Oo FH2	2	2.30	2.18 ± 0.04 d	3.25	3.26	0.15 ± 0.01 ab
Red	Tempranillo	2021	Spontaneous	3	2.30 ± 0.00 c	<0.05 a	3.66	3.85	0.45 ± 0.01 g
			Inoculated Oo	3		<0.05 a		3.80	0.30 ± 0.01 e
			Inhibited FH2	3		2.16 ± 0.01 f		3.59	0.22 ± 0.01 bc
			Inoculated Oo FH2	3		2.16 ± 0.01 f		3.59	0.22 ± 0.01 b
Red	Tempranillo **MLF started**	2021	Spontaneous	3	1.52 ± 0.00 a	<0.05 a	3.61	3.80	0.45 ± 0.01 g
			Inoculated Oo	3		<0.05 a		3.80	0.45 ± 0.01 g
			Inhibited FH2	3		<0.05 a		3.74	0.43 ± 0.01 g
			Inoculated Oo FH2	3		<0.05 a		3.74	0.43 ± 0.01 g
Red	Garnacha	2021	Spontaneous	3	2.10 ± 0.00 b	1.88 ± 0.01 c	3.47	3.40	0.18 ± 0.01 a
			Inoculated Oo	3		<0.05 a		3.49	0.22 ± 0.01 bc
			Inhibited FH2	3		1.82 ± 0.01 b		3.32	0.18 ± 0.01 a
			Inoculated Oo FH2	3		1.84 ± 0.03 b		3.32	0.17 ± 0.01 a
Rose	Tempranillo	2021	Spontaneous	3	2.29 ± 0.00 c	<0.05 a	3.55	3.81	0.43 ± 0.02 g
			Inoculated Oo	3		<0.05 a		3.79	0.25 ± 0.01 d
			Inhibited FH2	3		2.07 ± 0.04 e		3.52	0.17 ± 0.01 a
			Inoculated Oo FH2	3		2.02 ± 0.05 d		3.52	0.17 ± 0.02 a
White	Viura	2021	Spontaneous	3	3.45 ± 0.00 d	<0.05 a	3.32	3.42	0.33 ± 0.07 f
			Inoculated Oo	3		<0.05 a		3.49	0.25 ± 0.01 cd
			Inhibited FH2	3		2.84 ± 0.01 g		3.26	0.17 ± 0.01 a
			Inoculated Oo FH2	3		2.85 ± 0.05 g		3.25	0.17 ± 0.00 a

After fermentation in tank and partial settling, but before the MLF, the wines were bottled and FH2 was added or inoculated with lactic acid bacteria depending on the trial. Controls for spontaneous MLF were bottled and leaved to produce the MLF until full degradation of Malic acid.

2.2. Fumaric Acid

Doses of 600 mg/L were applied to inhibit MLF. The Fumaric acid used was pure, pharma grade (Panreac Applichem®, Barcelona, Spain). It was added to wine and homogenized by shaking until full dissolution.

2.3. Inoculations

The inoculated MLF were carried out with strain Alpha® of the specie *Oenoccocus oeni* (Lallemand, Blagnac, France). The dose was 1 g/hL previously hydrated in water, as recommended by provider. FML proceeded at 20 °C.

2.4. Analysis

Ethanol content, pH, color intensity, total polyphenols measured as A280, and residual sugars were analyzed according the OIV methods. Ethanol was analyzed by distillation and densitometry, pH by potentiometry, and residual sugars by reduction and titration [15]. Color intensity and A280 were analyzed by UV-Visible spectrophotometry using a V-1100 JPSELECTA (Barcelona, Spain) spectrophotometer.

Malic acid, Lactic acid, and volatile acidity measured as acetic acid were analyzed by enzymatic analysis, using a Y15 enzymatic photometric autoanalyzer (Biosystems, Barcelona, Spain) [15]. Briefly, the analysis consisted of using enzymatic tests in which the substance to measure is processed in a sequence of enzymatic reactions in which the variation of absorbance of cofactor NAD+/NADH is proportional to the concentration of the molecule to measure and can be calculated by the Lambert-Beer law. Dosing of reagents and sample, absorbance readings, calibration and calculation of results are performed automatically in the enzymatic autoanalyzer. Malic acid: detection limit: 0.03 g/L; linearity limit: 4.00 g/L; precision: 0.28 g/L mean concentration with 3.4% repeatability (CV). Lactic acid: detection limit: 0.02 g/L; linearity limit: 3.00 g/L; precision: mean concentration 0.25 g/L with repeatability 2.3% (CV). Acetic acid: detection limit: 0.03 g/L; linearity limit: 1.30 g/L; precision: mean concentration 0.30 g/L with repeatability 3.5% (CV). Specific details about the enzymatic tests can be found at https://int.foodquality.bio/en/sectors/productlist/Enology/Organic_Acids/any (accessed on 27 September 2022).

Malic acid concentration, pH and volatile acidity were analyzed by triplicate.

2.5. Statistical Treatments

Means, standard deviations, error bars, graphics, one way ANOVA, LSD tests 95% and other statistical analyses were performed with Excel and PC Statgraphics v.XI software (Graphics Software Systems, Rockville, MD, USA).

3. Results

Main enological parameters of the wines used for the inhibition trials are described in Table 1. The concentration of the different compounds was quite variable because the tests were performed in white, rose and red wines. Therefore, grape type (red or white), variety, degree of maturity, and maceration time strongly affected the ethanol content, color intensity and total polyphenols (A280). The ethanol content of wines ranged from 12.5 to 14.4, with the lower values for white ones and one red from 2020 (Table 1). Color intensities for red wines ranged 11.6–21.0, with the lowest value for the Garnacha variety as expected. Roses ranged 0.7–1.0 as usual in this pale low maceration wines. All wines were dry with concentrations of residual sugars close to 2 g/L or lower, except for one of the Tempranillo 2021 with 3.5 g/L (Table 1).

The MLFs performed for 3 years (2018, 2020 and 2021) are described in Table 2. Tempranillo and Garnacha varieties were used for red wines, also Tempranillo for roses and Viura for white wines. In each case, four experiments were performed: (i) Control 1: spontaneous MLF process; (ii) Control 2: MLF process initiated by inoculating the commercial strain Alpha of *Oenoccocus oeni*; (iii) Inhibition with FH2 during the spontaneous MLF process; (iv) Inhibition with FH2 during a MLF performed by inoculating *Oenocccocus oeni*. In year 2018 just a spontaneous fermentation control was performed. However, in 2020 and 2021 spontaneous and inoculated MLFs as controls or inhibited with FH2 were made. The initial content of malic acid was variable depending on the variety and type of wine ranging from 1.52 to 3.02 in red and rose wines and from 2.24 to 3.45 in white wines. Initial pHs ranged 3.47–3.67 in red wines, corresponding the lower value to Garnacha variety, and 3.21–3.33 in white wines. The addition of FH2 (600 mg/L) in most of the cases reduces the pH $\approx$ 0.1 units. The addition of FH2 inhibited the MLF preserving the malic acidity except for the Tempranillo red wine from 2021 in which the MLF was started. In absence of FH2 all the MLF were finished with residual malic acid contents below 0.05 g/L, except for Garnacha, where spontaneous fermentation did not start. When the MLF was inhibited by FH2, volatile acidity (VA) remained very low in the range 0.05–0.22 g/L. In absence of FH2, MLF proceeded with final VA values in the range of 0.20–1.77 g/L, but with average values close to 0.5 g/L.

The metabolization of malic acid and production of volatile acidity during MLF in spontaneous or inoculated fermentations with or without addition of FH2 shows a strong inhibitory effect of this compound at 600 mg/L (Figure 2). In all wines, reds (Tempranillo and Garnacha, Figure 2A,B), rose (Tempranillo, Figure 2C), and white (Viura, Figure 2D), malic acid remained stable and close to the initial value when MLF was inhibited by FH2. As expected, in all of them the inoculated fermentations with *O. oeni* strain alpha in absence of FH2 started and finished faster than the spontaneous fermentations with a typical delay of several days (Figure 2A,C,D). The only spontaneous fermentation that failed was the one corresponding to Garnacha wine, in which only the trials inoculated with *O. oeni* fermented, even monitoring the fermentations for a longer time (1.5 months approximately, Figure 2B) compared with the others that took less than 1 month (Figure 2A,C,D). The absence of spontaneous fermentation and the delay in the inoculated ones is probably due to the lower pH and some nutritional scarcity. In all trials, the inhibition of MLF using FH2 was observed to produce wines with lower volatile acidity. In addition, a lower pH is observed due to both the acidification by FH2 and the preservation of malic acidity.

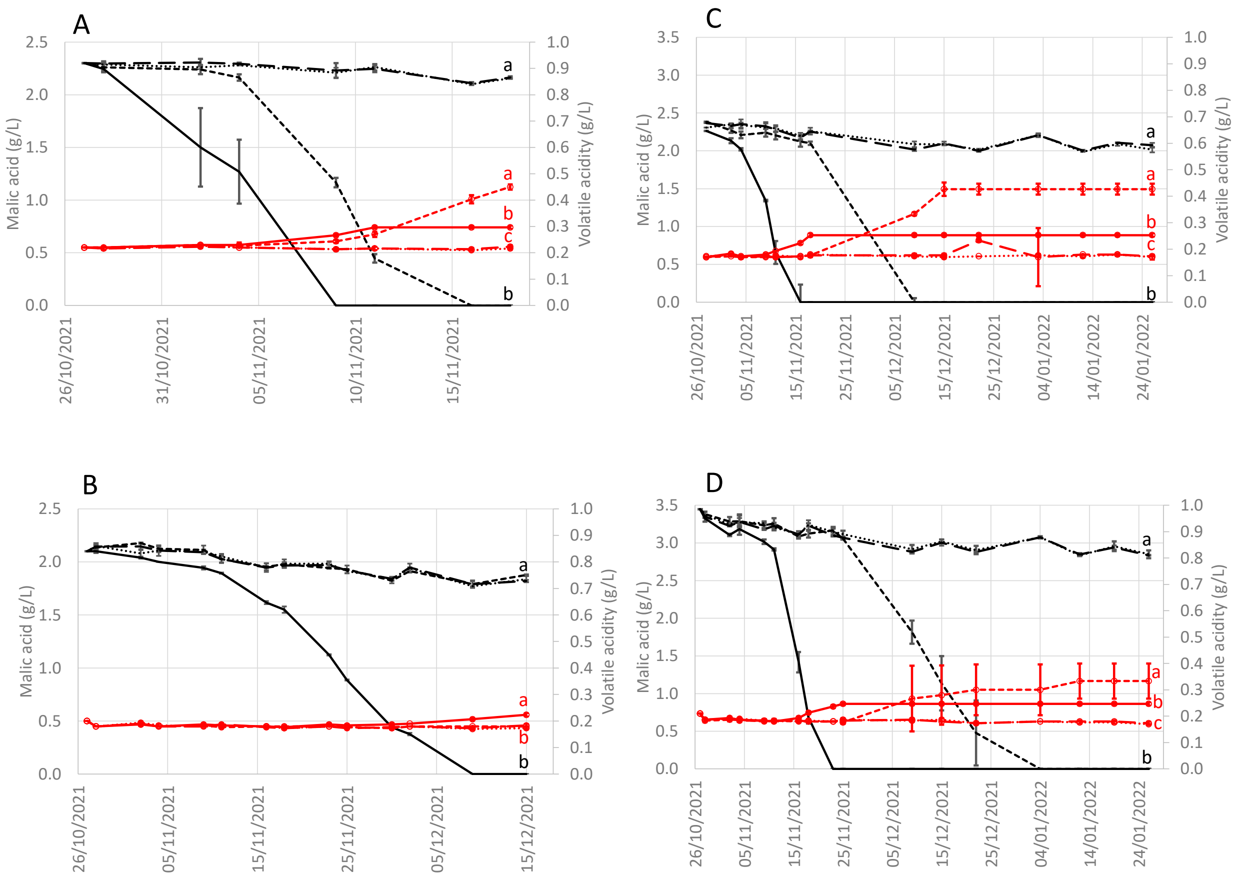

Figure 2. Evolution of malic acid (black lines) and volatile acidity (red lines with circles) (g/L) in spontaneous or inoculated (alpha, *O. oeni*) MLFs with or without FH2 inhibition (600 mg/L) for trials performed in triplicate during 2021. Malic acid in control (----), control with FA (— —), control with LAB (——), and control with FA and LAB (·········). Volatile acidity in control (--ө--), control with FA (—ο—), control with LAB (—ө—), and control with FA and LAB (···ο···). (**A**,**B**) red wines from Tempranillo and Garnacha, respectively, (**C**) rose, (**D**) white wine. Values are the means and bars are standard deviations of three independent fermentations. Letters of last day represent significant differences.

In order to test the effect of FH2 added once the MLF has started, a specific trial with Tempranillo variety was performed in 2021 (Figure 3). In this case the inhibitory effect of FH2 was not enough to stop the MLF both in spontaneous and inoculated fermentations, but it produced a delay in the evolution of the fermentation.

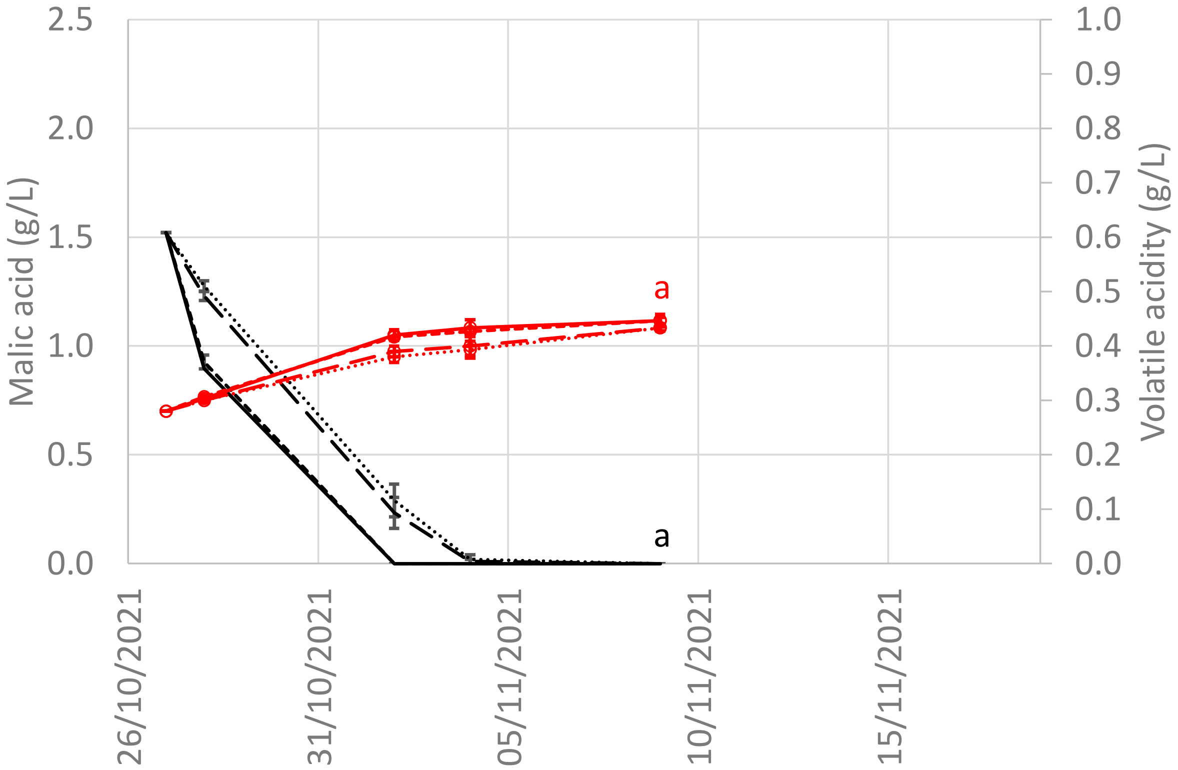

Figure 3. Evolution of malic acid (g/L) and volatile acidity (g/L) during spontaneous or inoculated (alpha, *O. oeni*) MLFs with or without inhibition with FH2 (600 mg/L). Malic acid in control (----), control with FA (— —), control with LAB (——), and control with FA and LAB (·········). Volatile acidity in control (--o--), control with FA (—o—), control with LAB (—o—), and control with FA and LAB (···o···). Values are the means and bars are standard deviations of triplicate independent fermentations. Letters of last day represent significant differences.

When the results of all trials were represented as function of pH and VA, all the fermentations inhibited by FH2 were observed to remain at values close or lower than 0.2 g/L in volatile acidity and most of them below 3.6 in pH (Figure 4). Conversely the controls without FH2 remained close or higher than 0.2 g/L of volatile acidity, many of them showing values above 0.4. Concerning pH most of them were close or higher than 3.5, and many of them showing values above 3.7.

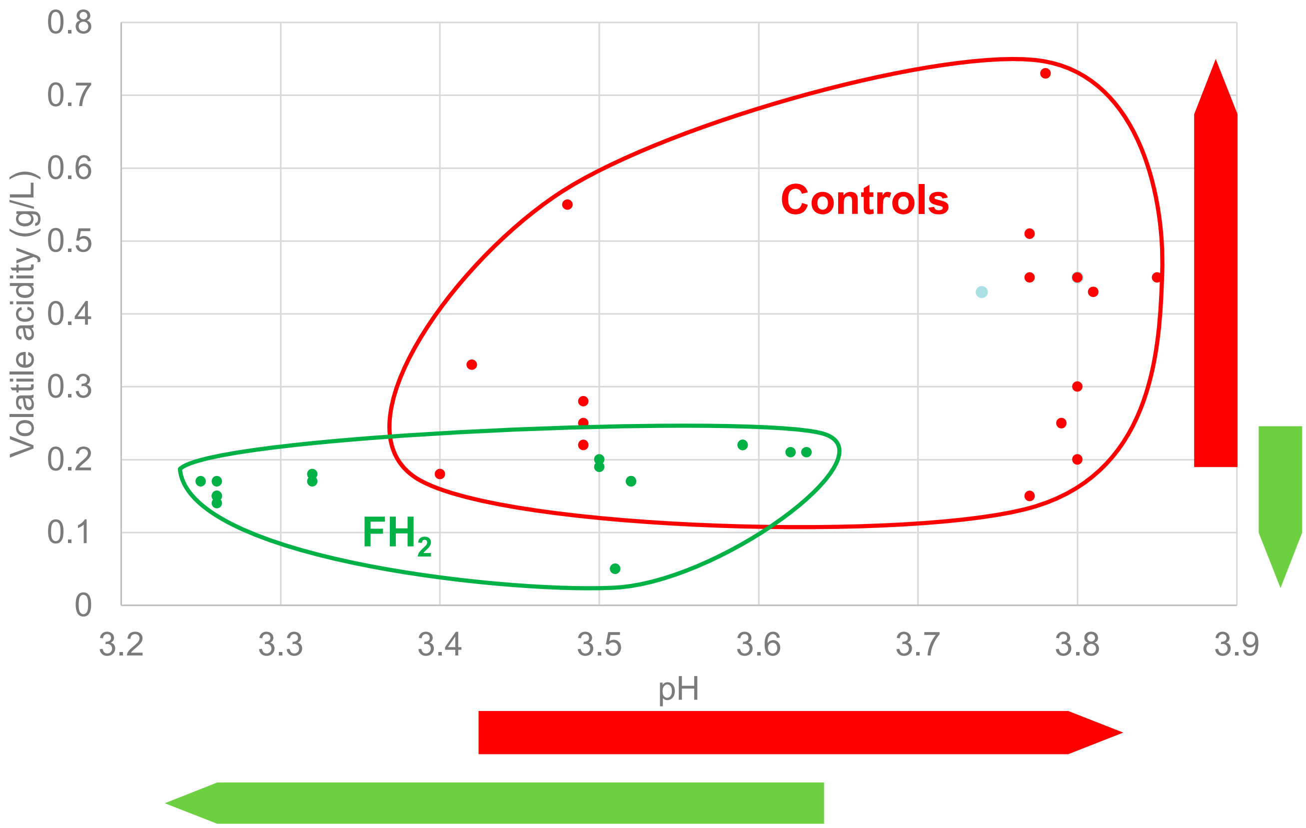

Figure 4. Values of volatile acidity and pH in all trials (years 2018, 2020, and 2021) of controls inoculated or with spontaneous fermentation and treatments with MLF inhibited by FH2.

4. Discussion

This 3-year bottle-scale study involving 3 grape varieties (Tempranillo, Garnacha, and Viura; Table 1) and 3 wine types (red, rose, and white; Table 1) provides a lot of information and practical results that complement previous findings [1–4] concerning the inhibitory capacity of FH2 on MLF. The current results show that FH2 is a powerful tool to control MLF with an effective inhibition of LAB at very low doses (Table 2). In current trials we have used and verified the effectivity of 600 mg/L that previously showed a good inactivation [4] without sensory impact [13]. Usually, MLF produces an elevation of VA which is a negative parameter in wine quality at values higher than 0.5 g/L [16], and has a defective sensory impact in most wines at values above 0.7 g/L [17]. Our results show that most of the wines which were supplemented with FH2 remained with VA concentrations around or below 0.2 g/L (Table 2, Figure 4), which is very low in wines, being a normal range of 0.2–0.6 g/L [17]. These low values are due to the full inhibition of the LAB and the non-existence of MLF.

The acidity and freshness are well connected parameters [18] and strongly affected by MLF. MLF produces a typical deacidification in wines that easily increases pH in 0.1–0.2 units [19]. Even though the deacidification by MLF has been traditionally used to soften mouth perception of acidity in red wines, in the current scenario of global warming, many wines after MLF are excessively warm and flat with pH values close to 4. Therefore, the inhibition of MLF by FH2 improves the sensory profile of these wines. FH2 has a double role in acidity and pH: this compound mainly preserves malic acid, what has an effect of 0.1–0.2 pH units depending on its content (Figure 2A–D), but additionally, at concentration of 600 mg/L, decreases the pH by 0.05–0.1 units depending on the buffer effect of wine (Table 2, initial pHs). FH2 is a strong acid with an effect similar to that of citric or tartaric acid [4,12], but more stable from a microbiological and chemical perspective. The inhibition of MLF is stable over a long time, as malic acid was observed to remain constant for

1.5–3 months (Figure 2A–D), which has also been observed in previous works [4]. Therefore, the use of FH2 is interesting to make fresher wines in warm areas. Furthermore, acidity and pH also affect the chemical stability and color, so it is possible to improve these parameters protecting composition and color.

This research developed under variable enological conditions, —wine composition (12.5 < %Ethanol < 14.4, Table 1; 3.33 < pH < 3.67, Table 2), grape variety (Tempranillo, Garnacha and Viura) and wine type (Red, Rose and white)—, shows that MLF can be controlled precisely and effectively by FH2. The absence of MLF avoids the formation of biogenic amines (BAs) by LABs that are allergenic molecules at high concentration (1–100 mg/L) [20], and are produced mainly during MLF by LAB. The formation of BAs is influenced by nutritional conditions but also is strain dependent [21]. BAs are produced by the bacterial decarboxylase enzymes from amino acids [20]. Low pH also promotes higher levels of molecular SO_2 with more intense protection in wine against microbial spoilage and oxidative degradation. FH2 has a double role in the control of LAB, directly by intracellular diffusion affecting cell homeostasis and indirectly by acidification, thus increasing the molecular SO_2. In this research the addition of FH2 at 600 mg/L decreases the initial pH up to 0.1 units (Figure 2A–D) in average. This reduction in pH implies a 10–15% increase of molecular SO_2 content, being lethal for most LABs at 0.3 mg/L [22]. Contents of 15 mg/L of free SO_2 during 3 h can be deleterious on most of the cell population of *O. oeni* [23], but the effectivity is strongly pH dependent, and deeply affected by the formation of bounded SO_2, being necessary 100 mg/L of bound SO_2 to inhibit the MLF [5]. FH2 has a high effectivity as can be observed in this research from many wines during 3 years, but the effect is not variable in time as happens with SO_2, producing a more permanent protection in bottle, compared with SO_2.

Chitosan, a deacetylated polysaccharide obtained from chitin, mainly of fungal origin in enology (*Aspergillus niger*), is another powerful tool to control MLF without sensory impact [24]. The use of 200 mg/L of chitosan is highly effective against *O. oeni* [25]. The only inconvenience is that chitosan is an insoluble molecule that can be used before bottling but without protective effect for long-term storage in bottle. The simultaneous effect of chitosan and FH2 has shown a synergistic behavior [10], with positive impact in SO_2 reduction, and the antimicrobial activity of FH2 remains during bottle storage. The initial use of chitosan and FH2 can be an interesting protocol in wine making to avoid the MLF, to control LAB, to remove them, and later to keep suitable contents of FH2 to protect the wine during either barrel ageing or bottle storage.

5. Conclusions

FH2 is a powerful tool to control MLF in any type of wine and grape variety. In warm areas the undesired development of MLF in white, rose and sparkling wines reduces the freshness producing flat and inexpressive wines. The traditional improvement in red wine quality after MLF by softening acidity and increasing aroma complexity, is not clear in the current context of global warming in Mediterranean areas. The decreased acidity produces flat and winey wines with low freshness and reduced quality. In these conditions, maybe we should wonder: why not red wines without MLF in warm areas? Especially if malic acidity can be long-term stable in the bottle by the antimicrobial activity of FH2. This new strategy must be specifically useful with some varieties and in young red wines. The use of FH2 can also decrease the use of SO_2 by microbial control and pH improvement, which is another trend in current enology.

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References

1. Cofran, D.R.; Meyer, B.J. The effect of fumaric acid on malo-lactic fermentation. *Am. J. Enol. Vitic.* **1970**, *21*, 189–192.
2. Tchelistcheff, A.; Peterson, R.G.; Van Gelderen, M. Control of malolactic fermentation in wine. *Am. J. Enol. Vitic.* **1971**, *22*, 1–5.
3. Pilone, G.J.; Rankine, B.C.; Pilone, D.A. Inhibiting malo-lactic fermentation in Australian dry red wines by adding fumaric acid. *Am. J. Enol. Vitic.* **1974**, *25*, 99–107. [CrossRef]
4. Morata, A.; Bañuelos, M.A.; López, C.; Song, C.; Vejarano, R.; Loira, I.; Palomero, F.; Suarez Lepe, J.A. Use of fumaric acid to control pH and inhibit malolactic fermentation in wines. *Food Addit. Contam. Part A* **2020**, *37*, 228–238. [CrossRef] [PubMed]
5. Bauer, R.; Dicks, L.M.T. Control of Malolactic Fermentation in Wine. A Review. *S. Afr. J. Enol. Vitic.* **2004**, *25*, 74–88. [CrossRef]
6. Barnes, R.H.; Karatzas, K.A.G. Investigation into the antimicrobial activity of fumarate against *Listeria monocytogenes* and its mode of action under acidic conditions. *Int. J. Food Microbiol.* **2020**, *324*, 108614. [CrossRef] [PubMed]
7. Vital-Lopez, F.G.; Wallqvist, A.; Reifman, J. Bridging the gap between gene expression and metabolic phenotype via kinetic models. *BMC Syst. Biol.* **2013**, *7*, 63. [CrossRef]
8. OIV (International Organization of Vine and Wine). *Method OIV-MA-AS313-04, Organic Acids Detection by HPLC. Compendium of International Methods of Analysis of Wines and Musts 2(OIV-MA-AS313-04)*; OIV (International Organization of Vine and Wine): Paris, French, 2009; pp. 1–3.
9. Fernández-Vázquez, D.; Rozès, N.; Canals, J.M.; Bordons, A.; Reguant, C.; Zamora, F. New enzymatic method for estimating fumaric acid in wines. *OENO One* **2021**, *55*, 273–281. [CrossRef]
10. Morata, A.; Bañuelos, M.A.; Loira, I.; Villegas, A.; González, C.; Suarez Lepe, J.A. Empleo de ácido fumárico y quitosano para inhibir la fermentación maloláctica. *ACE Rev. Enol.* **2020**. Available online: https://www.acenologia.com/fml_fumarico_quitosano_cienc176_0620/ (accessed on 20 September 2022).
11. FAO-WHO. Codex Alimentarius. 2015. Available online: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FShared%2BDocuments%252FArchive%252FMeetings%252FCCFA%252Fccfa47%252Ffa47_10s.pdf (accessed on 20 September 2022).
12. Gancel, A.-L.; Payan, C.; Koltunova, T.; Jourdes, M.; Christmann, M.; Teissedre, P.-L. 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. *OENO One* **2022**, *56*, 137–154. [CrossRef]
13. Morata, A.; Bañuelos, M.A.; López, C.; Adell, E. Use of fumaric acid to control pH and inhibit malolactic fermentation in wines. In Proceedings of the Ives Science Meeting, Margaux-Cantenac, France, 16–17 June 2022.
14. Camarasa, C.; Grivet, J.-P.; Dequin, S. Investigation by 13C-NMR and tricarboxylic acid (TCA) deletion mutant analysis of pathways for succinate formation in *Saccharomyces cerevisiae* during anaerobic fermentation. *Microbiology* **2003**, *149*, 2669–2678. [CrossRef]
15. OIV (International Organization of Vine and Wine). *International Organisation of Vine and Wine. Compendium of International Methods of Wine and Must Analysis*; OIV (International Organization of Vine and Wine): Paris, French, 2022; Volume 1.
16. Bartowsky, E.; Costello, P.; Chambers, P. Emerging trends in MLF. *Aust. J. Grape Wine Res.* **2015**, *21*, 663–669. [CrossRef]
17. Vilela-Moura, A.; Schuller, D.; Mendes-Faia, R.D.; Silva, A.; Chaves, S.R.; Sousa, M.J.; Côrte-Real, M. The impact of acetate metabolism on yeast fermentative performance and wine quality: Reduction of volatile acidity of grape musts and wines. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 271–280. [CrossRef]
18. Morata, A.; Escott, C.; Bañuelos, M.A.; Loira, I.; del Fresno, J.M.; González, C.; Suárez-Lepe, J.A. Contribution of Non-*Saccharomyces* Yeasts to Wine Freshness. A Review. *Biomolecules* **2020**, *10*, 34. [CrossRef] [PubMed]
19. Ugliano, M.; Moio, L. Changes in the concentration of yeast-derived volatile compounds of red wine during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *J. Agric. Food Chem.* **2005**, *53*, 10134–10139. [CrossRef] [PubMed]
20. Sumby, K.M.; Grbin, P.R.; Jiranek, V. Implications of new research and technologies for malolactic fermentation in wine. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 8111–8132. [CrossRef]
21. Capozzi, V.; Tufariello, M.; De Simone, N.; Fragasso, M.; Grieco, F. Biodiversity of oenological lactic acid bacteria: Species- and strain-dependent plus/minus effects on wine quality and safety. *Fermentation* **2021**, *7*, 24. [CrossRef]
22. Krieger-Weber, S.; Heras, J.M.; Suarez, C. *Lactobacillus plantarum*, a new biological tool to control malolactic fermentation: A review and an outlook. *Beverages* **2020**, *6*, 23. [CrossRef]
23. Guzzo, J.; Jobin, M.P.; Delmas, F.; Fortier, L.C.; Garmyn, D.; Tourdot-Maréchal, R.; Lee, B.; Diviès, C. Regulation of stress response in *Oenococcus oeni* as a function of environmental changes and growth phase. *Int. J. Food Microbiol.* **2000**, *55*, 27–31. [CrossRef]

24. Guzzon, R.; Nardin, T.; Larcher, R. The controversial relationship between chitosan and the microorganisms involved in the production of fermented beverages. *Eur. Food Res. Technol.* **2022**, *248*, 751–765. [CrossRef]
25. Bağder Elmacı, S.; Gülgör, G.; Tokatlı, M.; Erten, H.; İşci, A.; Özçelik, F. Effectiveness of chitosan against wine-related microorganisms. *Antonie van Leeuwenhoek* **2015**, *107*, 675–686. [CrossRef] [PubMed]

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