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Effects of the addition of fumaric acid on the spontaneous alcoholic fermentation of white grape must

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ABSTRACT

Consumer demand is driving the production of wines without added sulfur dioxide or yeast inoculation. However, this approach can lead to organoleptic defects or health risks due to unwanted microbial activity during alcoholic fermentation (AF). Lactic acid bacteria (LAB), responsible for malolactic fermentation (MLF), may also affect wine quality. Fumaric acid (FUM) inhibits LAB growth and could serve as an alternative to SO_2 in controlling MLF. The objective of this work was to study the effects of adding FUM (0.6 g/L) to natural white grape must without SO_2 on the growth of LAB during spontaneous AF and after it. The results showed that during AF, in the absence of FUM, LAB can grow and achieve both MLF and sugar consumption. The ability to prevent MLF after AF was evaluated without and with ageing on lees for 60 days. Without ageing, FUM up to 0.6 g/L effectively inhibited LAB growth and prevented MLF, whereas the residual FUM after AF (0.1 g/L) was insufficient when *Oenococcus oeni* was inoculated. Ageing on lees promoted MLF, and did not reverse the inhibitory effect of FUM. The residual FUM may not be sufficient for complete MLF inhibition after AF. Therefore, it may be advisable to correct the dose of FUM to 0.6 g/L to ensure its complete inhibition.

1. Introduction

In the last decade, winemakers have shown a growing interest in wines with minimal intervention, as some consumers prefer them for ecological reasons, reduced chemical intervention, and health considerations (González & Parga Dans, 2019; Jorge et al., 2020). These wines, which are made without sulfur dioxide (SO_2) and without starter yeast cultures for spontaneous alcoholic fermentation, may undergo organoleptic alterations (Davis et al., 1985; Wei et al., 2022). Additionally, uncontrolled malolactic fermentation (MLF) in minimal intervention wines can lead to increased volatile acidity, the presence of biogenic amines, and microbiological instability due to the development of indigenous lactic acid bacteria (LAB) strains (Davis et al., 1985; Wei et al., 2022). Currently, due to climate change, grape musts have lower organic acid levels and higher sugar contents, reducing the freshness perception and microbiological stability of wines and making MLF even less desirable (Pardo & Ferrer, 2021).

Therefore, treatments such as chitosan, lysozyme, or SO_2 have been studied for their ability to inhibit MLF in wines (Bauer & Dicks, 2004). The most widely used SO_2 completely inhibits MLF, but its residual concentration in wines is controversial, as it may cause health issues for sensitive consumers (Davis et al., 1985; Tchelistcheff et al., 1971). To mitigate these concerns and comply with increasingly strict legislation regarding SO_2 use, alternative strategies are being explored. One such alternative is fumaric acid (FUM) or trans-butenedioic acid [E297], an additive widely used in the food industry as an acidifier, which can be added to wine and is currently regulated in OIV-OENO 581 A-2021 at a maximum recommended dose of 0.6 g/L. This acid has been studied for its ability to inhibit MLF in wine, representing a good strategy to reduce the SO_2 doses (Cofran & Meyer, 1970; Morata et al., 2020, 2023; Pilone et al., 1973; Wagener et al., 1971). Moreover, other studies have shown that the addition of FUM has no negative effect on the organoleptic characteristics of wines, reported more body and acidity (Gancel et al., 2022; Morata et al., 2020). This organic acid is not very soluble in aqueous media (5.3 g/L at 25 °C) but has a pKa1 of 3.03 and a pKa2 of 4.44 (Gancel et al., 2022). Thus, at the pH of the wines, it is in dissociation equilibrium and therefore can penetrate the cells of microorganisms (Barnes & Karatzas, 2020; Lohbeck et al., 2000; Shah et al.,

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2016). Therefore, one of the effects that FUM could have on MLF could be due to the intracellular acidification of LAB (Barnes & Karatzas, 2020). The addition of FUM has been described as having no effect on yeasts that perform AF of grape must or during secondary fermentation in the bottle to produce sparkling wines (Jamalzadeh et al., 2012; Morata et al., 2020). However, although no effect of FUM has been observed during AF, yeasts can transport, consume or store part of it (Pilone et al., 1973; Xu et al., 2013). In addition, the yeast lees of wines obtained by AF in the presence of FUM may contain greater amounts of this acid (Jamalzadeh et al., 2012). Yeast lees are used both in wine ageing to limit oxidation phenomena and improve organoleptic properties, but their use has also been suggested to improve MLF by releasing nitrogen and lipid metabolites, mannoproteins and other compounds (Balmaseda et al., 2018, 2021; Fornairon-Bonnefond et al., 2002).

The main objective of this work was to study the effect of the addition of 0.6 g/L FUM during the spontaneous AF of Muscat Alexandria grape must without SO_2 at pH 3.5. The secondary objective is to study the effect of the addition of FUM to the must on the achievement of MLF by LAB in wines. For this purpose, two types of conditions were studied. The first was to determine whether the FUM concentration after FUM was sufficient to inhibit MLF or if a correction in FUM up to 0.6 g/L was necessary, and the second was to determine the effectiveness of the addition of yeast lees produced in the presence of FUM on LAB growth and performance.

2. Materials and methods

2.1. Natural grape must

Muscat of the Alexandria variety grape was grown in the experimental cellar *Mas dels Frares* (Faculty of Oenology, *Rovira i Virgili* University, Tarragona, Spain) and was harvested in August 2022. After the grapes were pressed, the must was settled with 200 mg/L Lallzyme-C-Max pectinases (Lallemand Inc., Montreal, Canada) at 8 °C without SO_2. After a day of settling, the must was divided into two parts (Figure SD1): must control without FUM and must with 0.6 g/L of FUM (Ravago Chemicals, Barcelona, Spain). The compositions of the two grape musts after settling and the addition of FUM are presented in Table SD1. The pH of the grape must supplemented with FUM, was not corrected, with a decrease to pH 3.39 (Table SD1).

2.2. Alcoholic fermentation

Spontaneous AF was performed in food-grade plastic bottles of 1.5 L at a controlled temperature of 20 °C, in a refrigerated room, statically and in triplicate without any type of inoculation (Figure SD1). Once the wines completed AF, an aliquot of each replicate was stored at −20 °C until analysis. The wines obtained were centrifuged at 10,000 rpm for 10 min in an HB-4 rotor of a Sorvall RC5C centrifuge (Thermo Scientific, Massachusetts, USA) and stored at 4 °C without SO_2 for one week. The lees obtained after AF under each condition from the centrifuged wines were frozen at -20 °C and subsequently freeze-dried for two days using a LyoQuest (Telstar, Barcelona, Spain).

2.3. Malolactic fermentations of wine obtained from must with the addition of fumaric acid

Different MLFs after AF were performed on wine obtained in the presence of 0.6 g/L of FUM during AF (Figure SD1). All MLFs were performed in triplicate in glass jars of 150 mL to 20 °C, statically in a refrigerated room.

2.3.1. Malolactic fermentation without ageing

In this batch, four different conditions were tested after AF: i) wines with residual FUM + spontaneous MLF, ii) wines with residual FUM + inoculated MLF, iii) wines corrected to 0.6 g/L with FUM + spontaneous MLF, and iv) wines corrected to 0.6 g/L with FUM + inoculated MLF. *Oenococcus oeni* Lalvin VP41 strain (Lallemand Inc.) at 10^6 CFU/mL was used in inoculated MLFs (Figure SD1).

2.3.2. Malolactic fermentation with ageing

The other fraction of the wine obtained from the AF in the presence of FUM was stored under two conditions for 60 days at 4 °C under static conditions (Figure SD1), one fraction without the addition of lees and the other fraction with 5 % (*w/v*) lees obtained of AF. To perform the MLF, wines with the same four different conditions as previous section were prepared.

2.4. Monitoring of fermentation and populations of microorganisms

2.4.1. Monitoring of alcoholic fermentation

AF was monitored daily by measuring the density of the grape must using a densimeter (Densito 30PX Portable Density Mettler-Toledo, Cornellà de Llobregat, Spain). AF was considered complete when the residual sugar (glucose + fructose) content, which was enzymatically determined with the Y15 multianalyser (Biosystems S.A., Barcelona, Spain), was less than 2 g/L.

2.4.2. Monitoring of malolactic fermentation

MLF was monitored by determining L-malic acid levels via an enzymatic kit with a Y15 multianalyser. The MLF was considered finished when the L-malic acid content was <0.1 g/L or when the L-malic acid content was stable for more than 7 days.

2.4.3. Enumeration of total microorganisms.

The total viable and culturable yeast population was monitored by counting decimal dilutions on YPD agar medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 17 g/L agar, Panreac Química SLU, Castellar del Vallès, Spain) after incubation at 28 °C for 48 h. Non-*Saccharomyces* yeasts were counted with lysine medium (Oxoid, Hampshire, England) after incubation at 28 °C for 48 h. Viable and culturable LAB were cultured on modified MRS (Margalef-Català et al., 2016) and incubated at 27 °C for 7 days in the presence of 10 % CO_2 (*v/v*).

2.5. Identification of yeast and lactic acid bacteria during alcoholic fermentation

2.5.1. Identification of yeast

Sixty colonies (20 × 3 repetitions) were randomly collected from both musts (without and with FUM) at the beginning (density of 1080 g/L), middle (1040 g/L) and end of AF (approx. 990 g/L) and cultured individually in YPD medium. To study the evolution of the *Saccharomyces* and non-*Saccharomyces* populations, PCR was performed to determine the amplicon size of the ITS-5.8S rDNA region and restriction patterns according to the method described by Esteve-Zarzoso et al. (1999).

2.5.2. Identification of LAB

To identify LAB, sixty colonies were randomly selected from both musts (without and with FUM) at the beginning, middle and end of AF. These isolates were subsequently grown in liquid MRS media at 28 °C with CO_2 for 7 days. The pellet was subsequently separated, and the DNA was extracted with the High Pure PCR Template Preparation Kit (Roche, Barcelona, Spain). The colonies grown on solid MRS media that had a coccus-shaped morphology, as observed using microscopy, were analysed using a specific PCR for *O. oeni* according to the methods of Zapparoli et al. (1998). For the isolated non-*Oenococcus* LAB colonies, strain identification was performed using the ARDRA method, which involves 16S gene amplification followed by *MseI* digestion. The sequencing of the profiles obtained by digestion was subsequently performed following the procedure described by Rodas et al. (2003).

2.6. Analysis of other main by-products of fermentation

The pH was measured by using a Crison micro pH 2002 pH meter (Hach Lange Spain, l'Hospitalet de Llobregat, Spain). D- and L-lactic acid, citric acid and glucose-fructose were analysed via an enzymatic kit with a Y15 multianalyser. FUM was analysed using an enzymatic kit according to the method of Fernández-Vázquez et al. (2021). Acetic acid, glycerol and succinic acid were analysed using HPLC (Agilent 1100, Agilent Technologies, Waldbronn, Germany) with a multiwavelength detector and an RID detector according to Quirós et al. (2014).

The ethanol content of the wines was determined using GC-FID according to the modified method of Wang et al. (2003). Briefly, 100 μL of sample or standard was diluted with 900 μL of acetonitrile (9.96 g/L, Panreac) in deionised water. After agitation, one μL was injected into a CP-Wax 58 CB column (50 m × 0.25 mm × 0.1 μm; Agilent Technologies) in split mode (25/1). The oven temperature was set initially at 60 °C, increased to 100 °C at a rate of 6 °C/min and then increased at a final temperature of 200 °C at a rate of 80 °C/min. The injector and detector temperatures were 220 °C. The flow rate of helium was set at 1.5 mL/min. Individual ethanol standard solutions (12.26, 20.62, 40.14, 80.62, 120.68, 161.88 and 200 g/L) were prepared in deionised water by weighing the ethanol (Panreac) with a precision balance at 20 °C. The peak area ratios of the ethanol vs. acetonitrile solutions were calculated and plotted against the ethanol content (%, *v*/v) to provide a standard curve (repeated each time) that was used for ethanol quantification in fermentation samples. An example of the mean of five replicates of the standard curve is shown in Figure SD2.

2.7. Metabolomic analysis

The method is inspired by that described by Roessner et al. (2000). Briefly, 10 μL of wine plus 10 μL of ribitol at 1 mg/mL (Sigma–Aldrich) diluted in deionised water was dried in an SC110 speed vacuum system (Savant Instruments, USA) for 2 h. The dried residues were first derivatised with 20 μL of 20 mg/mL methoxyamine hydrochloride (Sigma–Aldrich) in pyridine (Sigma–Aldrich) for 30 min at 70 °C. The second derivatisation was performed with 30 μL of *N*-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFUM, Sigma–Aldrich) for 30 min at 70 °C. Three μL samples were injected in split mode (10:1) into a 6890 N GC system (Agilent Technologies) equipped with a DB-5HT column (30 m × 0.25 mm × 0.1 μm; Agilent Technologies). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The compounds were detected with a mass selective detector (MSD, Model 5975, Agilent Technologies). The MSD temperatures were 300 °C, 180 °C and 280 °C for the transfer, quadrupole and source, respectively. The MSD data were acquired in electronic ionisation scan mode at 70 eV within the 35–650 amu range after a solvent delay of 3 min and then analysed via Agilent MSD ChemStation software (Agilent Technologies). The metabolites were identified using an in-house MS and NIST 2017 libraries. The relative abundance of each identified compound was calculated according to the respective chromatographic peak areas corrected in relation to the IS peak area for ribitol. The results are expressed as arbitrary units (AUs).

2.8. Statistical analysis

All the data were analysed using XLSTAT 2022.5.1 software (Addinsoft, Paris, France). Statistical differences in ANOVA with Tukey's test (HSD, honestly significant difference) were taken with a p value <0.05. A heatmap was constructed with metabolites grouped into lines and samples (AF with or without FUM) in columns. All fermentation and chemical analyses were performed in triplicate for statistical analysis.

3. Results & discussion

3.1. Alcoholic fermentation

The AFs of the musts without or with the addition of 0.6 g/L FUM did not significantly differ in terms of time to completion (16 and 15 days, respectively) or in evolution throughout fermentation (Fig. 1A). However, beginning on the fifth day, in the case of the must without FUM, the concentration of L-malic acid decreased compared with that in the medium and was completely consumed on Day 12, completing the MLF during AF (Fig. 1B). When FUM was added, no consumption of L-malic acid was observed, indicating that the addition of FUM was effective in inhibiting MLF during AF.

According to Fig. 1C, the FUM content in the medium decreased to 0.1 g/L over time when FUM was added at initial dose of 0.6 g/L, this could be due to the absorption or metabolism of this acid by yeasts (Jamalzadeh et al., 2012; Pilone et al., 1973). FUM in the must without its addition was not detected, even though this compound can be naturally produced by yeasts via the tricarboxylic acid (TCA) cycle (Pilone, 1975; Yin et al., 2015); This absence may be attributed to the detection limit of the enzymatic method used, as described by Fernández-Vázquez et al. (2021), which for white wine corresponds to a limit of detection of 0.016 g/L and a limit of quantification of 0.054 g/L.

3.2. Evolution of total populations of microorganisms in alcoholic fermentation and identification of yeast and lactic acid bacteria

Throughout AF, the total yeast population remained relatively constant across both musts, starting with an initial population of 10^6 CFU/mL (Fig. 2). Yeasts are generally not sensitive to FUM (Pilone et al., 1973; Vital-Lopez et al., 2013), and species such as *Candida*, *Pichia*, and *Saccharomyces* showed no variations at concentrations of up to 2000 ppm in a study by Romero-Gil et al. (2016). A more pronounced decline in non-*Saccharomyces* yeasts was observed at more advanced stages of AF, with no major differences between conditions. The effects of *S. cerevisiae* on other yeast species during AF have been widely reported (Bagheri et al., 2019). With respect to the evolution of total LAB populations in which MLF was performed (Fig. 2), the initial concentration was 10^4 CFU/mL, increasing to 10^6 CFU/mL without the addition of FUM. However, with the addition of FUM, the LAB population decreased to 10^2 CFU/mL and remained constant during the development of AF, indicating the effectiveness of FUM against LAB (Morata et al., 2020; 2023).

However, the proportion of yeast species varied as the AF progressed (Fig. 3A). At the onset of AF, six non-*Saccharomyces* species were detected in both musts, with no significant differences in the total number of species present. These species included *Torulaspora delbrueckii*, *Schizosaccharomyces pombe*, *Hanseniaspora uvarum*, *Dekkera anomala*, *Pichia delftensis*, and *Candida vinaria*. These species were typically identified in musts without SO_2 at the beginning of AF (Cuijvers et al., 2020). Notably, a greater proportion of *T. delbrueckii* (40 %) and a lower proportion of *H. uvarum* (20 %) were observed in the must without FUM compared to the must with FUM, where the proportions for both species were 30 % and 30 %, respectively. The remaining species were present at approximately 10 % in both musts.

Towards the middle of AF, several non-*Saccharomyces* species, including *H. uvarum*, *D. anomala*, and *P. delftensis*, disappeared, whereas the proportion of *S. cerevisiae* increased in both musts (Fig. 3A), following an evolution typical of spontaneous AF when *S. cerevisiae* begins to establish (Cuijvers et al., 2020; Hierro et al., 2006). In the must without FUM, the proportions of *T. delbrueckii* and *S. pombe* remained stable at 40 % and 10 %, respectively, with *S. cerevisiae* representing 50 % of the yeast population. The presence of *S. pombe* is associated with increased consumption of L-malic acid during AF but disappears at the end of the AF. *S. pombe* L-malic acid is directly decarboxylated to pyruvic acid and CO_2 by the enzyme malate decarboxylase in the cytosol

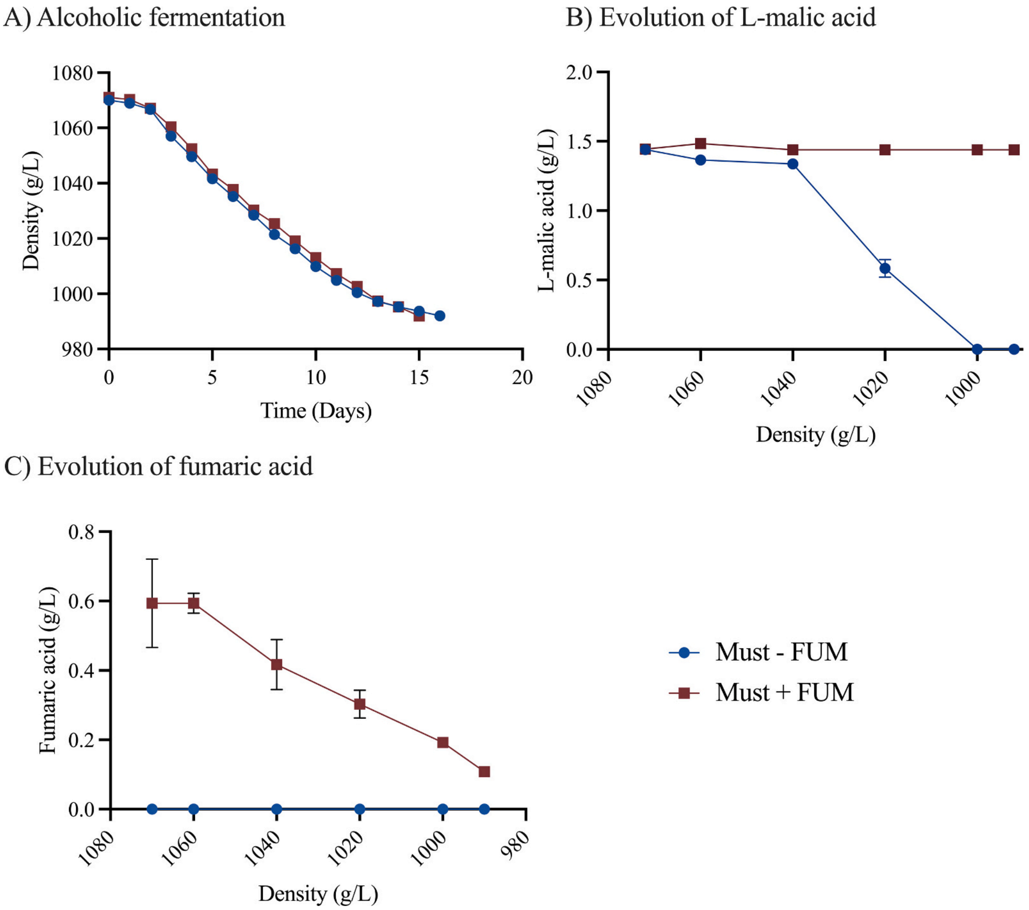


Fig. 1. Evolution of AF expressed in density (A), L-malic acid concentration (g/L) (B) and fumaric acid content (g/L) (C) during the spontaneous AF without sulfur dioxide fermentation of Muscat of Alexandria grape must without fumaric acid (− FUM) and with fumaric acid (+ FUM).

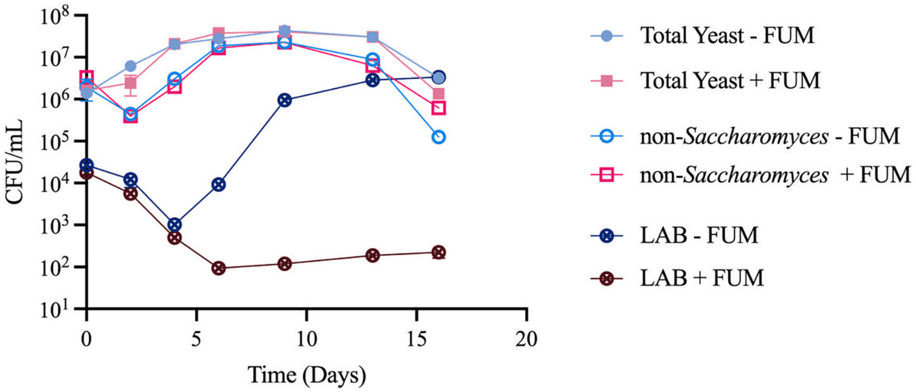
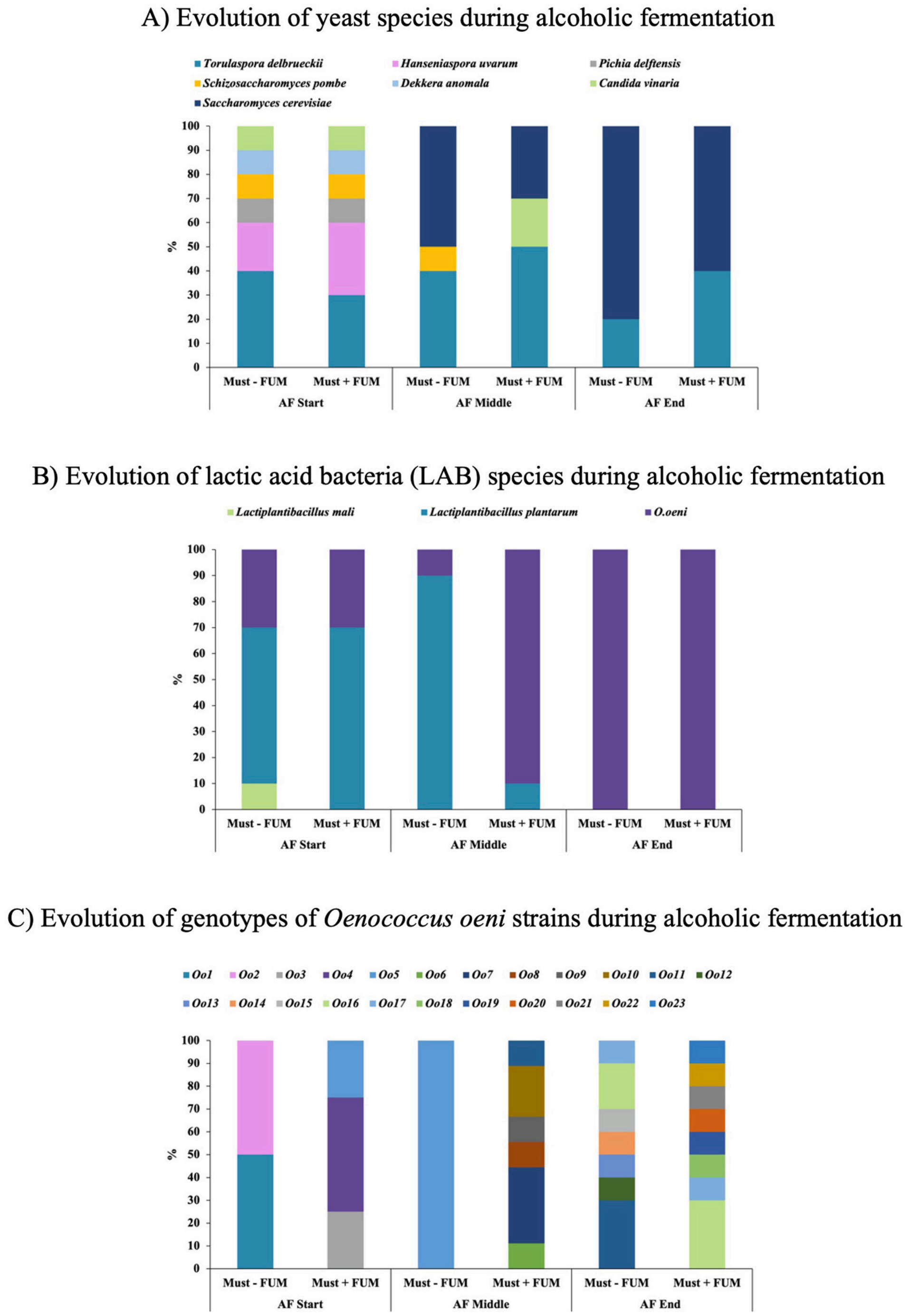


Fig. 2. Evolution of populations of total yeast, non-*Saccharomyces* yeasts and LAB during the spontaneous AF without sulfur dioxide fermentation of Muscat of Alexandria grape must without fumaric acid (− FUM) and with fumaric acid (+ FUM).

(Mylona et al., 2016). However, *S. pombe* was also detected during the initial phase of AF in the treatments with FUM addition (Fig. 3A), yet no L-malic acid consumption was observed under these conditions. Saayman et al. (2000) demonstrated that *S. pombe* possesses a proton, dicarboxylate symporter for L- malic acid, and that FUM acts as a competitive inhibitor of this transporter, potentially limiting malate uptake and its subsequent degradation. On the other hand, in FUM musts, *T. delbrueckii* appeared to be the dominant yeast, representing 50 % of the population, with yeast linked to wines exhibiting organoleptic quality (Loira et al., 2014; Ruiz-de-Villa, Poblet, Bordons, et al., 2023; Ruiz-de-Villa, Poblet, Cordero-Otero, et al., 2023). In addition, *S. cerevisiae* accounted for 30 %, and *C. vinaria* represented 20 % of the population in the must with FUM. At the end of AF, only two yeast species were detected in both musts: *S. cerevisiae* and *T. delbrueckii.* In the must without FUM, *S. cerevisiae* dominated, accounting for 80 % of the total yeast population, and *T. delbrueckii* dominated at 20 %. In contrast, in the must containing FUM, *S. cerevisiae* constituted a lower proportion of the yeast community, accounting for 60 %. *T. delbrueckii* was more prevalent, accounting for 40 % of the total yeast population, one possible explanation is that FUM contributes to a lower pH environment, which may inhibit the growth of more acid sensitive yeast species while allowing acid-tolerant species like *T. delbrueckii* to persist.


A) Evolution of yeast species during alcoholic fermentation

B) Evolution of lactic acid bacteria (LAB) species during alcoholic fermentation

C) Evolution of genotypes of *Oenococcus oeni* strains during alcoholic fermentation

Fig. 3. Evolution of identified non-*Saccharomyces* and *Saccharomyces* yeast species (A), LAB species (B) and genotypes of *O. oeni* strains (C) during spontaneous AF without sulfur dioxide fermentation of Muscat of Alexandria grape must without fumaric acid (− FUM) and with fumaric acid (+ FUM).

Additionally, FUM may exert selective pressure by inhibiting competing microbes, such as *S. cerevisiae* or spoilage bacteria, creating a more favourable niche for *T. delbrueckii*. However, further studies are needed to confirm the specific mechanisms behind this observation.

The evolution of LAB during AF differed distinctly between the two musts throughout the fermentation process. At the start of AF (Fig. 3B), the proportions of non-*Oenococcus* species and *O. oeni* were identical in the two musts, at 70 % and 30 %, respectively, with an initial population of 10^4 CFU/mL. This population decreased during the initial days to 10^3 CFU/mL. The LAB species identified included *Lactobacillus mali, Lactobacillus plantarum*, and *O. oeni,* which are normally identified in musts without SO_2 (Brizuela et al., 2019; Rodas et al., 2003). In the must without FUM, *L. mali* constituted 10 % of the LAB population, whereas *L. plantarum* made up 60 %. In contrast, in the must with FUM, 70 % of the LAB population was identified as L. *plantarum*, with *O. oeni* comprising the remaining 30 %.

However, by mid-fermentation, the LAB population dynamics shifted. In the must without FUM, *L. plantarum* became predominant, accounting for 90 % of the LAB population, which coincided with the onset of L-malic acid consumption and an increase in the LAB population to 10^4 CFU/mL. Conversely, in must with FUM, the population of L. *plantarum* decreased to 10 %, with *O. oeni* dominating at 90 %. At the end of AF, *O. oeni* was the only species detected under both conditions, reflecting its adaptation to wine (Bonomo et al., 2018; González-

Arenzana et al., 2012), with populations of 10^6 and 10^5 CFU/mL in musts without and with FUM, respectively. In total, 23 genotypes of *O. oeni* were identified during AF (Fig. 3C), with the *O. oeni* 16 genotypes being the most prevalent at the end of AF in both musts. This diversity can occur under spontaneous conditions, and one study reported the possibility of finding high variability in *O. oeni* isolates in wine (López et al., 2007).

3.3. Wine composition after alcoholic fermentation

The presence or absence of FUM during AF induced significant differences in the chemical composition of the wines (Table 1). The residual glucose + fructose concentrations, were not significantly different between the two groups, as complete AF was achieved in both groups. A slightly lower ethanol concentration was observed in the wines produced with FUM addition. FUM can induce a moderate stress response, potentially leading to a reduced fermentation efficiency or a metabolic shift towards the production of secondary metabolites such as glycerol or organic acids, rather than ethanol. In our case, glycerol formation was also lower, probably directing yeast metabolism towards the formation of other metabolites. This could be related to the increased presence of *T. delbrueckii* or other non-*Saccharomyces* known to produce less ethanol and glycerol than *S. cerevisiae* under similar fermentation conditions (Mestre et al., 2019; Ruiz-de-Villa, Poblet, Bordons, et al., 2023; Ruiz-de-Villa, Poblet, Cordero-Otero, et al., 2023). With respect to pH, the FUM condition resulted in a lower pH, primarily due to the acidifying effect of FUM itself (Payan et al., 2023). The wine obtained without FUM contained a total consumption of L-malic acid and citric acid, as well as increases in D- and L-lactic acid to values of 0.73 g/L and 0.71 g/L, respectively, indicating that the latter was associated with complete MLF produced in the presence of LAB (Lonvaud-Funel, 1999). This hypothesis is further supported by the observation that, midway through AF, the LAB population began to increase, with a high concentration of *O. oeni*. In the case of wine obtained in the presence of FUM, neither the consumption of L-malic acid nor the formation of L-lactic acid associated with LAB was observed. The concentration of citric acid was preserved, and a slight increase in acetic acid to 0.51 g/L was observed. In addition, the increase in acetic acid to 1.37 g/L observed could be due to the action of yeast (*Saccharomyces* or non-*Saccharomyces*) and LAB (Bartowsky & Pretorius, 2009). However, the succinic acid content significantly differed depending on the presence or absence of FUM in grape musts (Table 1). A significantly greater increase in succinic acid was observed in the case of wines obtained in the presence of FUM, whereas only 0.38 g/L succinic acid was found in the wine without FUM. This phenomenon occurs via the reductive cycle of the TCA cycle when fumarate is reduced to succinate under the action of fumarate reductase by *S. cerevisiae* under oenological conditions (Mendes Ferreira & Mendes-Faia, 2020).

Table 1
Composition of wines obtained by spontaneous AF without sulfur dioxide fermentation of Muscat of Alexandria grape must without fumaric acid (− FUM) and with fumaric acid (+ FUM). Ethanol level expressed in % (*v*/v), Glucose + Fructose, Glycerol, L-malic acid, L-lactic acid, D-lactic acid, Citric acid, Acetic acid, Fumaric acid, Succinic acid are expressed in g/L. Mean and standard deviation (SD) (*n* = 3). Different letters indicate significant differences between conditions for the same parameter.

	Must − FUM	Must + FUM
pH	3.47 ± 0.02^a	3.33 ± 0.04^b
Glucose + Fructose	1.43 ± 0.24^a	0.69 ± 0.11^a
Ethanol	8.83 ± 0.04^a	7.69 ± 0.12^b
Glycerol	6.68 ± 0.02^a	4.06 ± 0.02^b
L-malic acid	0.00 ± 0.00^b	1.44 ± 0.01^a
L-lactic acid	0.71 ± 0.00^a	0.00 ± 0.01^b
D-lactic acid	0.73 ± 0.03^a	0.09 ± 0.01^b
Citric acid	0.00 ± 0.00^b	0.23 ± 0.02^a
Acetic acid	1.37 ± 0.03^a	0.51 ± 0.02^b
Fumaric acid	0.00 ± 0.00^b	0.10 ± 0.00^a
Succinic acid	0.38 ± 0.02^b	0.62 ± 0.02^a

From the results of the chemical parameters analysed after AF in the wine, a heatmap was generated to observe if there were significant differences between the two conditions, with and without FUM addition (Figure SD3). The main by-products of AF were taken, and the data were filtered to improve readability. For this purpose, an interquartile range filter with a threshold of 0.25 was applied, eliminating data with low variability. In addition, the values are represented on a colour scale, where green indicates the highest values and red the lowest values. The results of the heatmap, together with the associated dendrograms, allowed a clear visualisation of the effect of FUM on the final composition of the wines. Indeed, in addition to D- and L-lactate, acetate, citrate, pH and other metabolites mentioned in the previous paragraph, some metabolites detected by GC–MS, such as citramalate, monoethyl succinate, mannitol, tyrosol, and inositol, were more abundant in wines without FUM than in wines with FUM (left side in green).

Among these results, it is interesting to note the relevant contents of mannitol, citramalate and tyrosol, which were present at relatively high concentrations in the wines without FUM. The greater presence of the first metabolite confirms the alterations in LAB (Brizuela et al., 2019), which can metabolize fructose into mannitol (Wisselink et al., 2002). The second metabolite, isopropylmalate, could result from the metabolism of leucine and isoleucine by yeasts and is not metabolised by LAB (Mendes Ferreira & Mendes-Faia, 2020). These results demonstrate the effectiveness of FUM during AF in inhibiting the growth and activity of LAB naturally present in grape must.

3.4. Malolactic fermentations of wine obtained from must with the addition of fumaric acid

To understand the impact on the microbiological stability of FUM after AF and assess its use to prevent MLF development over time, two tests were conducted: one without ageing and one with ageing for 60 days (Figure SD1).

3.4.1. 3.4.1. Malolactic fermentation without ageing

Simple MLF following AF with wine produced in the presence of FUM was monitored over a period of 10 days (Fig. 4). Two different FUM concentration conditions were assessed: one at the residual FUM concentration of 0.1 g/L and another with FUM corrected to 0.6 g/L; this was done to determine whether the residual FUM from AF is sufficient to inhibit LAB growth and, consequently, to prevent MLF, or if it is necessary to increase the FUM dosage to the maximum recommended dose by OIV (OIV-OENO 581A-2021, 2021). Additionally, two inoculation conditions were tested: control spontaneous fermentation and inoculation with 10^6 CFU/mL *O. oeni* strain VP41. This approach aimed to assess whether different FUM concentrations inhibit various LAB populations. Notably, *O. oeni* VP41 is known for its adaptability, strong fermentative capacity, and resistance to stressful wine conditions (Romero et al., 2018; Ruiz-de-Villa et al., 2023).

MLF was fully completed only in the condition without FUM correction, which was inoculated with 10^6 CFU/mL *O. oeni* VP41 (Fig. 4). Under these conditions, the LAB population increased to 5.50 × 10^6 CFU/mL by mid-fermentation (Table 2), leading to the completion of MLF within 10 days. The residual FUM concentration from AF (0.1 g/L) was not sufficient to inhibit LAB growth when the initial population of *O. oeni* VP41 was high. This strain is known for its ability to adapt and complete MLF in less than 2 days under some conditions (Ruiz-de-Villa, Poblet, Cordero-Otero, et al., 2023). In contrast, the spontaneous fermentation condition without FUM correction started with an initial LAB population of 10^2 CFU/mL. Although no L-malic acid was consumed under these conditions, the LAB population still increased to 8.23 × 10^5 CFU/mL after 10 days; this suggests that although the residual FUM concentration of 0.1 g/L exerted some control over the MLF, it did not effectively inhibit LAB growth. As demonstrated by Cofran &

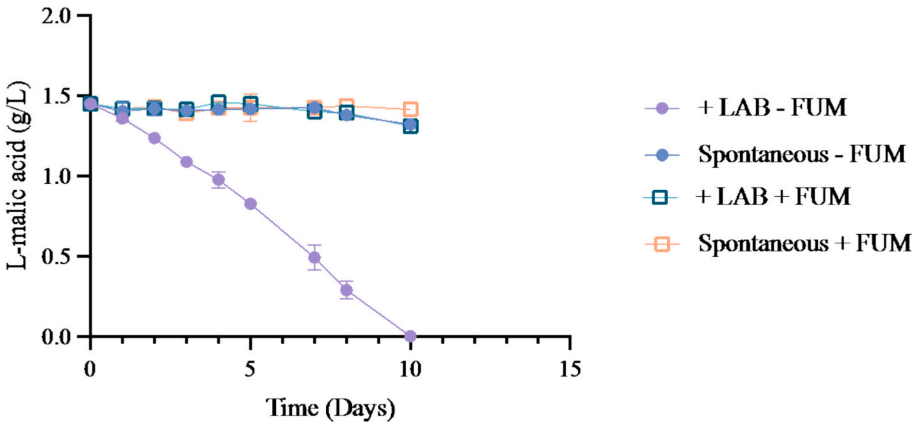


Fig. 4. Evolution of L-malic acid (g/L) during MLF without ageing. +LAB correspond to inoculation with *O. oeni*, while Spontaneous correspond to fermentation without inoculation. - FUM indicates wines without fumaric acid correction, whereas + FUM indicates wines with fumaric acid correction.

Table 2
Populations (CFU/mL) of LAB during MLF without ageing. + LAB correspond to inoculation with *O. oeni*, while Spontaneous correspond to fermentation without inoculation. - FUM indicates wines without fumaric acid correction, + FUM indicates wines with fumaric acid correction up to 0.6 g/L. Mean and standard deviation (SD) (n = 3). nd, indicates no detected.

	- FUM		+ FUM	
	+ LAB	Spontaneous	+ LAB	Spontaneous
Start MLF	$1.05 \cdot 10^6$	$5.57 \cdot 10^2$	$1.06 \cdot 10^6$	$2.23 \cdot 10^2$
Middle MLF	$5.50 \cdot 10^6$	$8.67 \cdot 10^3$	$5.67 \cdot 10^4$	$1.03 \cdot 10^1$
End MLF	$1.45 \cdot 10^6$	$8.23 \cdot 10^5$	$3.77 \cdot 10^4$	nd

Meyer (1970), the inhibitory effect of FUM is contingent on its concentration and can prolong the lag phase but not inhibit MLF.

In the cases where FUM was corrected to 0.6 g/L, a progressive decline in the LAB population was observed under both conditions (Table 2), and L-malic acid was not consumed in either scenario (Fig. 4); this was true for the spontaneous condition, which started with a population of 10^2 CFU/mL, and the inoculated condition with 10^6 CFU/mL *O. oeni* VP41. Therefore, 0.6 g/L FUM was effective in completely inhibiting LAB growth in conditions with a low initial population, although it did not completely inhibit the LAB population in the inoculated condition with 10^6 CFU/mL FUM. However, it did prevent MLF from occurring. Such behaviour has been documented for FUM doses of 0.6 g/L in wine, where it was effective in inhibiting MLF (Morata et al., 2020; 2023). Additionally, the addition of FUM affected the pH of the wine. A dose of 0.6 g/L can lower the pH by up to 0.1 units (Morata et al., 2023), and in this case, the pH decreases to 3.2. Low pH can adversely affect the growth of *O. oeni* (Bauer & Dicks, 2004). Moreover, the first dissociation constant (pKa1) of FUM is 3.03 (Gancel et al., 2022), meaning that at lower pH, the acid is more dissociated and thus more effective (Barnes & Karatzas, 2020; Morata et al., 2023), as it can enter cells more readily (Shah et al., 2016). Consequently, adjusting FUM to a dose of 0.6 g/L after AF is advisable to ensure MLF inhibition.

3.4.2. Malolactic fermentation with ageing

Another experiment was conducted to investigate whether yeast strains produced in the presence of FUM could influence LAB growth and MLF progression. Previous studies have demonstrated the positive impact of yeast lees on the MLF (Balmaseda et al., 2021; Sumby et al., 2019). Moreover, yeast can synthesise succinic acid (Torres-Guardado et al., 2022), FUM (Xu et al., 2013) and other compounds that may affect LAB growth (Bauer & Dicks, 2004) and accumulate in the lees. In this specific case, AF in the presence of FUM was observed to result in an increase in succinic acid (Section 3.3). Consequently, two wines were prepared for MLF: one without lees and another with 5 % lees aged for 60 days. After 60 days, both wines had a pH of 3.23 ± 0.02 and an FUM concentration of 0.086 g/L ± 0.03, indicating a slight decrease in the FUM concentration. Additionally, a reduction in pH was observed, as the pH after AF was 3.33. This pH decrease may have occurred due to tartrate stabilisation or cold stabilisation, as the wine was stored for 60 days at 4 °C (Ribéreau-Gayon, Glories, et al., 2006). To initiate MLF, the two wines were separated into two conditions, including i) without FUM correction and ii) with FUM corrected to 0.6 g/L (recommended dose according to OIV), resulting in a pH decrease to 3.12 after the addition of FUM. Additionally, two inoculation conditions were used: i) spontaneous fermentation and ii) inoculation with 10^5 CFU/mL *O. oeni* strain VP41.

The results (Fig. 5) revealed differences in MLF progression. The wines that started MLF first were those inoculated with *O. oeni* VP41 without FUM correction, indicating that the residual FUM concentration of 0.086 g/L was insufficient to inhibit MLF. Furthermore, in this scenario without correction up to 0.6 g/L of FUM, the wine aged on lees was the first to complete MLF, demonstrating that even when lees were produced in the presence of FUM, MLF was still promoted, as has been observed in various studies (Andújar-Ortiz et al., 2012; Balmaseda et al., 2021; Sumby et al., 2019). The final populations exceeded 10^6 CFU/mL in the conditions with lees, although no significant differences were observed between the final populations of LAB on spontaneous and inoculated MLFs (Table 3). However, in the case of spontaneous MLF without FUM correction, the initial LAB population was 10 CFU/mL in the wine aged without lees and 10^2 CFU/mL in the wine aged with lees. MLF initiation was delayed until Day 12, with the wine aged on lees finishing first. None of the conditions where FUM correction was performed achieved MLF. After 20 days, the LAB populations in these conditions were undetectable.

3.4.3. Composition of wines after malolactic fermentation

Malolactic fermentation without ageing. The wines subjected to MLF were significantly different, as shown in Table 4. Under conditions inoculated with *O. oeni* VP41 without FUM correction, complete L-malic acid consumption was observed, along with an increase in the L-lactic acid concentration (0.83 g/L), indicating successful MLF driven by the LAB *O. oeni* (Davis et al., 1985). Additionally, a reduction in the FUM concentration to 0.03 g/L in the wine was associated with FUM consumption by *O. oeni* (Cofran & Meyer, 1970), and an increase in pH was noted, which is associated with the progression of MLF (Bauer & Dicks, 2004); this suggests that the residual FUM from AF was insufficient to inhibit MLF when the initial LAB population was high. Under other conditions, complete MLF did not occur. Only a minimal partial consumption (0.1 g/L) of L-malic acid was detected in the spontaneous condition without FUM correction and in the inoculated condition with

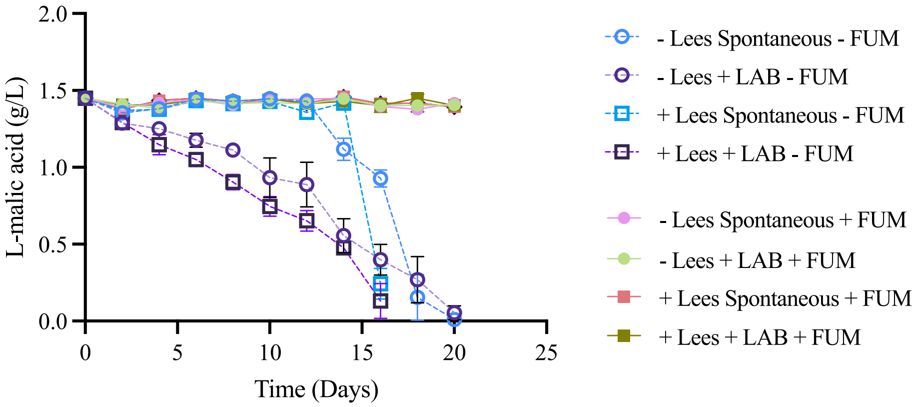


Fig. 5. Evolution of L-malic acid (g/L) of LAB during the MLF with lees ageing, without less (− Lees) or with lees (+ Lees). +LAB correspond to inoculation with *O. oeni*, while Spontaneous correspond to fermentation without inoculation. - FUM indicates wines without fumaric acid correction, whereas + FUM indicates wines with fumaric acid correction.

Table 3
Populations (CFU/mL) of LAB during the malolactic fermentation with lees ageing, without less (− Lees) or with lees (+ Lees). + LAB correspond to inoculation with *O. oeni*, while Spontaneous correspond to fermentation without inoculation. - FUM indicates wines without fumaric acid correction, + FUM indicates wines with fumaric acid correction up to 0.6 g/L. Mean and standard deviation (SD) (n = 3). nd, indicates no detected.

	- Lees				+ Lees			
- FUM			+ FUM		- FUM		+ FUM	
	LAB	Spontaneous	LAB	Spontaneous	LAB	Spontaneous	LAB	Spontaneous
Start MLF	$7.87\cdot10^{5}$	$1.33\cdot10^{1}$	$7.57\cdot10^{5}$	$3.33\cdot10^{1}$	$8.50\cdot10^{5}$	$3.07\cdot10^{2}$	$6.90\cdot10^{5}$	$2.20\cdot10^{2}$
Middle MLF	$3.13\cdot10^{6}$	$2.65\cdot10^{5}$	$3.83\cdot10^{2}$	nd	$8.53\cdot10^{6}$	$4.53\cdot10^{5}$	$3.20\cdot10^{2}$	nd
End MLF	$9.33\cdot10^{5}$	$7.10\cdot10^{5}$	nd	nd	$1.44\cdot10^{6}$	$2.20\cdot10^{6}$	nd	nd

Table 4
Composition of Muscat of Alexandria wines after MLF without and with lees ageing. All compounds except pH are expressed in g/L. –Lees and + Lees correspond with wines without less ageing or with lees respectively. +LAB correspond to inoculation with *O. oeni*, while Spontaneous correspond to fermentation without inoculation. - FUM indicates wines without fumaric acid correction, + FUM indicates wines with fumaric acid correction. Different letters indicate statistically significant differences between conditions within each type of malolactic fermentation (without ageing and with ageing), as determined by separate ANOVA tests for each group. Mean and standard deviation (SD) (n = 3). nd, no detected.

		pH	L-malic acid	L- lactic acid	Citric acid	Fumaric acid	Acetic acid
Malolactic fermentation without ageing	+ LAB – FUM	3.42 ± 0.01^{a}	nd	0.83 ± 0.06^{a}	0.20 ± 0.03^{a}	0.03 ± 0.01^{d}	0.68 ± 0.05^{a}
	Spontaneous – FUM	3.38 ± 0.01^{ab}	1.32 ± 0.01^{b}	0.02 ± 0.01^{c}	0.22 ± 0.04^{a}	0.10 ± 0.00^{c}	0.69 ± 0.01^{a}
	+ LAB + FUM	3.25 ± 0.04^{b}	1.31 ± 0.00^{b}	0.12 ± 0.01^{b}	0.21 ± 0.03^{a}	0.47 ± 0.00^{b}	0.70 ± 0.06^{a}
	Spontaneous + FUM	3.23 ± 0.03^{b}	1.41 ± 0.02^{a}	0.01 ± 0.00^{c}	0.22 ± 0.00^{a}	0.61 ± 0.00^{a}	0.64 ± 0.05^{a}
Malolactic fermentation with ageing	- Lees + LAB – FUM	3.28 ± 0.03^{b}	0.05 ± 0.05^{b}	0.81 ± 0.00^{a}	0.22 ± 0.03^{a}	0.03 ± 0.00^{d}	0.77 ± 0.04^{cd}
	- Lees Spontaneous – FUM	3.34 ± 0.01^{ab}	0.01 ± 0.01^{b}	0.84 ± 0.01^{a}	0.13 ± 0.04^{b}	0.03 ± 0.01^{d}	0.88 ± 0.03^{ab}
	- Lees + LAB + FUM	3.06 ± 0.01^{c}	1.41 ± 0.03^{a}	0.01 ± 0.01^{b}	0.22 ± 0.03^{a}	0.52 ± 0.01^{b}	0.70 ± 0.01^{d}
	- Lees Spontaneous + FUM	3.04 ± 0.01^{c}	1.42 ± 0.03^{a}	0.01 ± 0.00^{b}	0.23 ± 0.00^{a}	0.60 ± 0.01^{a}	0.73 ± 0.01^{d}
	+ Lees + LAB – FUM	3.37 ± 0.02^{a}	nd	0.82 ± 0.05^{a}	0.22 ± 0.04^{a}	0.03 ± 0.00^{d}	0.89 ± 0.08^{ab}
	+ Lees Spontaneous – FUM	3.38 ± 0.05^{a}	nd	0.82 ± 0.04^{a}	0.06 ± 0.05^{c}	nd	0.89 ± 0.02^{a}
	+ Lees + LAB + FUM	3.04 ± 0.01^{c}	0.94 ± 0.08^{a}	nd	0.22 ± 0.04^{a}	0.21 ± 0.01^{c}	0.79 ± 0.03^{bcd}
	+ Lees Spontaneous + FUM	3.05 ± 0.01^{c}	1.39 ± 0.02^{a}	nd	0.22 ± 0.04^{a}	0.52 ± 0.01^{b}	0.85 ± 0.03^{abc}

FUM correction, corresponding to an increase in the LAB population under these conditions (Section 3.4.1). However, a difference in L-lactic acid formation was observed. As shown, under spontaneous conditions without FUM correction, the concentration was lower at 0.02 g/L, which is likely due to fermentation of the initial L-malic acid. However, with FUM correction, the L-lactic acid concentration increased to 0.12 g/L, whereas the FUM concentration decreased to 0.47 g/L. In this case, it is possible that *O. oeni* catalysed the conversion of FUM to L-malic acid via fumarase, followed by MLF of this acid (Pilone et al., 1974). No significant differences were observed in acetic acid formation across the conditions. This may be because, in cases where MLF did not occur, acetic acid formation is typically associated with MLF. In addition, when MLF is complete, the *O. oeni* VP41 strain has a low rate of acetic acid production (Ruiz-de-Villa, Poblet, Cordero-Otero, et al., 2023). The final concentration of citric acid did not significantly differ between the conditions. The consumption of L-citric acid occurs simultaneously with L-malic acid consumption by *O. oeni* but at a much lower rate (Ramos et al., 1995). An increase in the L-lactic acid concentration and a decrease in FUM were observed under the conditions inoculated with *O. oeni* VP41 and corrected with FUM due to the increase in the LAB population, although complete MLF was not achieved. Differences were observed in the final FUM concentration. In the case of MLF, a greater percentage of FUM reduction was noted, depending on the initial LAB population (Cofran & Meyer, 1970). In the conditions inoculated with 10^{6} CFU/mL FUM, both with and without FUM correction, a greater decrease in FUM was observed, possibly due to FUM uptake by the cells (Barnes & Karatzas, 2020), than in the spontaneous conditions with an initial population of 10^{2} CFU/mL LAB.

Malolactic fermentation with ageing. MLF performance, with the total consumption of L-malic acid in the case of wines without FUM correction (Table 4). Significant differences in pH were not detected between spontaneous and inoculated conditions without FUM correction. At the end of fermentation, the residual FUM concentration ranged from 0.00 to 0.03 g/L. Similarly, no significant differences were detected in the formation of L-lactic acid, with levels ranging from 0.81 to 0.84 g/L under both spontaneous and inoculated conditions, corresponding to an MLF performed by *O. oeni* (Bauer & Dicks, 2004). However, partial consumption of citric acid was observed under spontaneous conditions, particularly in wines aged on lees, likely due to the increased LAB population. Under conditions with FUM correction up to 0.6 g/L, MLF did not occur in wines with or without lees. Only in the inoculated condition with lees was partial MLF observed, leading to a reduction in L-malic acid to 0.94 g/L. Consequently, no significant changes in the final pH were observed, which remained between 3.04 and 3.06, as complete MLF did not occur (Bauer & Dicks, 2004; Pardo & Ferrer, 2021). This low pH could negatively impact MLF by LAB (Bauer & Dicks, 2004). Additionally, according to Pilone (1975), a synergistic effect occurs where a lower pH results in a greater inhibitory effect by FUM. Under these conditions without MLF, no significant differences were noted in L-lactic acid formation or L-citric acid consumption. However, significant differences were observed in the final FUM concentration. Wines without lees presented a relatively high concentration of residual FUM, whereas those aged on lees presented a greater decrease in the final FUM, suggesting that lees might act as a FUM scavenger or increase LAB growth (Balmaseda et al., 2021).

4. Conclusions

In conclusion, the addition of FUM to SO_2-free grape must with 10^4 CFU/mL initial LAB populations at pH values near 3.5 may represent an effective strategy to prevent MLF during AF. However, the results also show that FUM supplementation in grape juice at 0.6 g/L is not sufficient to prevent the realization of MLF after AF. This relative ineffectiveness of FUM in protecting wines after AF could be linked to the fact that yeasts largely metabolize this acid and thus, it is necessary to supplement again up to 0.6 g/L (recommended dose according to OIV) with FUM to ensure the complete inhibition of MLF in the event of high populations of LAB by *O. oeni*. On the other hand, the addition of yeast lees positively influences the realization of the MLF, promoting it; however, it does not counteract the inhibitory effect of FUM.

CRediT authorship contribution statement

Violeta García-Viñola: Writing – original draft, Methodology, Investigation. **Montserrat Poblet:** Visualization, Supervision, Data curation. **Albert Bordons:** Visualization, Supervision, Conceptualization. **Cristina Reguant:** Writing – original draft, Visualization, Supervision, Funding acquisition. **Nicolas Rozès:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.117064.

Data availability

Data will be made available on request.

References

Andújar-Ortiz, I., Ángeles Pozo-Bayón, M., Garrido, I., Martin-Álvarez, P. J., Bartolomé, B., & Moreno-Arribas, V. (2012). Effect of using glutathione-enriched inactive dry yeast preparations on the phenolic composition of rosé grenache wines during winemaking. *Journal International Des Sciences de La Vigne et Du Vin, 46*, 241–251. https://doi.org/10.20870/oeno-one.2012.46.3.1516

Bagheri, B., Philipp, C., Horacek, M., Bauer, F. F., & Setati, M. E. (2019). Microbial diversity in grape musts from Austrian and south African grape varieties and regions. *Bio Web of Conferences*. https://doi.org/10.1051/bioconf/20191202028

Balmaseda, A., Bordons, A., Reguant, C., & Bautista-Gallego, J. (2018). Non-*Saccharomyces* in wine: Effect upon *Oenococcus oeni* and malolactic fermentation. *Frontiers in Microbiology, 9*, 534. https://doi.org/10.3389/fmicb.2018.00534

Balmaseda, A., Rozès, N., Bordons, A., & Reguant, C. (2021). Simulated lees of different yeast species modify the performance of malolactic fermentation by *Oenococcus oeni* in wine-like medium. *Food Microbiology, 99*. https://doi.org/10.1016/j.fm.2021.103839

Barnes, R. H., & Karatzas, K. A. G. (2020). Investigation into the antimicrobial activity of fumarate against *Listeria monocytogenes* and its mode of action under acidic conditions. *International Journal of Food Microbiology, 324*. https://doi.org/10.1016/j.ijfoodmicro.2020.108614

Bartowsky, E. J., & Pretorius, I. S. (2009). Microbial formation and modification of flavor and off-flavor compounds in wine. In *Biology of microorganisms on grapes, in must and in wine* (pp. 209–231). Berlin Heidelberg: Springer. https://doi.org/10.1007/978-3-540-85463-0_11.

Bauer, R., & Dicks, L. M. T. (2004). Control of malolactic fermentation in wine. A review. *South African Journal of Enology and Viticulture, 25*(2). https://doi.org/10.21548/25-2-2141

Bonomo, M. G., Di Tomaso, K., Calabrone, L., & Salzano, G. (2018). Ethanol stress in *Oenococcus oeni*: Transcriptional response and complex physiological mechanisms. *Journal of Applied Microbiology, 125*(1), 2–15. https://doi.org/10.1111/jam.13711

Brizuela, N., Tymczyszyn, E. E., Semorile, L. C., Valdes La Hens, D., Delfederico, L., Hollmann, A., & Bravo-Ferrada, B. (2019). *Lactobacillus plantarum* as a malolactic starter culture in winemaking: A new (old) player? *Electronic Journal of Biotechnology, 38*, 10–18. https://doi.org/10.1016/j.ejbt.2018.12.002

Cofran, D. R., & Meyer, B. J. (1970). The effect of fumaric acid on malo-lactic fermentation. *American Journal of Enology and Viticulture, 21*(4), 189–192. https://doi.org/10.5344/ajev.1970.21.4.189

Cuijvers, K., van Den Heuvel, S., Varela, C., Rullo, M., Solomon, M., Schmidt, S., & Borneman, A. (2020). Alterations in yeast species composition of uninoculated wine ferments by the addition of Sulphur dioxide. *Fermentation, 6*(2). https://doi.org/10.3390/FERMENTATION6020062

Davis, C. R., Wibowo, D., Eschenbruch, R., Lee, T. H., & Fleet, G. H. (1985). Practical implications of malolactic fermentation: A review. *American Journal of Enology and Viticulture, 36*(4). https://doi.org/10.5344/ajev.1985.36.4.290

Esteve-Zarzoso, B., Belloch, C., Uruburul, F., & Queroi, A. (1999). Identification of yeasts by RFLP analysis of the 5.85 rRNA gene and the two ribosomal internal transcribed spacers. In. *International Journal of Systematic Bacteriology, 49*.

Fernández-Vázquez, D., Rozès, N., Canals, J. M., Bordons, A., Reguant, C., & Zamora, F. (2021). New enzymatic method for estimating fumaric acid in wines. *OENO one, 55*(3), 273–281. https://doi.org/10.20870/OENO-ONE.2021.55.3.4825

Fornairon-Bonnefond, C., Camarasa, C., Moutounet, M., & Salmon, J.-M. (2002). New trends on yeast autolysis and wine ageing on lees: A bibliographic review. *OENO one, 36*(2), 49–69. https://doi.org/10.20870/oeno-one.2002.36.2.974

Gancel, A. L., Payan, C., Koltunova, T., Jourdes, M., Christmann, M., & Teissedre, P. L. (2022). 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, 56*(3), 137–154. https://doi.org/10.20870/oeno-one.2022.56.3.5455

González, P., & Parga Dans, E. (2019). Natural wine: Do consumers know what it is, and how natural it really is? *Journal of Cleaner Production, 251*, Article 119635. https://doi.org/10.1016/j.jclepro.2019.119635

González-Arenzana, L., López, R., Santamaría, P., Tenorio, C., & López-Alfaro, I. (2012). Dynamics of indigenous lactic acid Bacteria populations in wine fermentations from La Rioja (Spain) during three vintages. *Microbial Ecology, 63*(1), 12–19. https://doi.org/10.1007/s00248-011-9911-y

Hierro, N., González, Á., Mas, A., & Guillamón, J. M. (2006). Diversity and evolution of non-*Saccharomyces* yeast populations during wine fermentation: Effect of grape ripeness and cold maceration. *FEMS Yeast Research, 6*(1), 102–111. https://doi.org/10.1111/j.1567-1364.2005.00014.x

Jamalzadeh, E., Verheijen, P. J. T., Heijnen, J. J., & Van Gulik, W. M. (2012). pH-dependent uptake of fumaric acid in *Saccharomyces cerevisiae* under anaerobic conditions. *Applied and Environmental Microbiology, 78*(3), 705–716. https://doi.org/10.1128/AEM.05591-11

Jorge, E., Lopez-Valeiras, E., & Gonzalez-Sanchez, M. B. (2020). The role of attitudes and tolerance of ambiguity in explaining consumers' willingness to pay for organic wine. *Journal of Cleaner Production, 257*. https://doi.org/10.1016/j.jclepro.2020.120601

Lohbeck, K., Haferkorn, H., Fuhrmann, W., & Fedtke, N. (2000). Maleic and fumaric acids. In W.-V. C. H. GmbH (Ed.), *Ullmann's encyclopedia of industrial chemistry*. Wiley. https://doi.org/10.1002/14356007.a16_053.

Loira, I., Vejarano, R., Bañuelos, M. A., Morata, A., TesFUMye, W., Uthurry, C., … Suárez-Lepe, J. A. (2014). Influence of sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on wine quality. *LWT, 59*(2P1), 915–922. https://doi.org/10.1016/j.lwt.2014.06.019

Lonvaud-Funel, A. (1999). Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology, 76*, 317–331. https://doi.org/10.1023/A:1002088931106

López, I., Tenorio, C., Zarazaga, M., Dizy, M., Torres, C., & Ruiz-Larrea, F. (2007). Evidence of mixed wild populations of Oenococcus oeni strains during wine spontaneous malolactic fermentations. *European Food Research and Technology, 226* (1–2), 215–223. https://doi.org/10.1007/s00217-006-0529-0

Margalef-Català, M., Araque, I., Bordons, A., Reguant, C., & Bautista-Gallego, J. (2016). Transcriptomic and proteomic analysis of *Oenococcus oeni* adaptation to wine stress conditions. *Frontiers in Microbiology, 7*. https://doi.org/10.3389/fmicb.2016.01554

Mendes Ferreira, A., & Mendes-Faia, A. (2020). The role of yeasts and lactic acid bacteria on the metabolism of organic acids during winemaking. *Foods, 9*(9), 1231. https://doi.org/10.3390/foods9091231

Mestre, M. V., Maturano, Y. P., Gallardo, C., Combina, M., Mercado, L., Toro, M. E., … Dellacassa, E. (2019). Impact on sensory and aromatic profile of low ethanol malbec wines fermented by sequential culture of *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* native yeasts. *Fermentation, 5*(3). https://doi.org/10.3390/fermentation5030065

Morata, A., Adell, E., López, C., Palomero, F., Suárez, E., Pedrero, S., … González, C. (2023). Use of fumaric acid to inhibit malolactic fermentation in bottled Rioja wines: Effect in pH and volatile acidity control. *Beverages, 9*(1). https://doi.org/10.3390/beverages9010016

Morata, A., Bañuelos, M. A., López, C., Song, C., Vejarano, R., Loira, I., … Suarez Lepe, J. A. (2020). Use of fumaric acid to control pH and inhibit malolactic fermentation in wines. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 37*(2), 228–238. https://doi.org/10.1080/19440049.2019.1684574

Mylona, A. E., Del Fresno, J. M., Palomero, F., Loira, I., Bañuelos, M. A., Morata, A., … Suárez-Lepe, J. A. (2016). Use of *Schizosaccharomyces* strains for wine fermentation-effect on the wine composition and food safety. *International Journal of Food Microbiology, 232*, 63–72. https://doi.org/10.1016/j.ijfoodmicro.2016.05.023

OIV-OENO 581A-2021. (2021).

Pardo, I., & Ferrer, S. (2021). Malolactic fermentation in white wines. In *White wine technology* (pp. 177–185). Elsevier. https://doi.org/10.1016/B978-0-12-823497-6.00016-8.

Payan, C., Gancel, A. L., Jourdes, M., Christmann, M., & Teissedre, P. L. (2023). Wine acidification methods: A review. *OENO one, 57*(3), 113–126. https://doi.org/10.20870/oeno-one.2023.57.3.7476

Pilone, D. A., Pilone, G. J., & Rankine, B. C. (1973). Influence of yeast strain, pH, and temperature on degradation of fumaric acid in grape juice fermentation. *American Journal of Enology and Viticulture, 5064*. https://doi.org/10.5344/ajev.1973.24.3.97

Pilone, G. J. (1975). Control of malo-lactic fermentation in table wine by addition of fumaric acid, in lactic acid bacteria. In *Lactic acid Bacteria in beverages and food: Proceedings of a symposium*.

Pilone, G. J., Rankine, B. C., & Pilone, D. A. (1974). Inhibiting malo-lactic fermentation in Australian dry red wines by adding fumaric acid. *American Journal of Enology and Viticulture, 25*(2), 99–107. https://doi.org/10.5344/ajev.1974.25.2.99

Quirós, M., Rojas, V., Gonzalez, R., & Morales, P. (2014). Selection of non-*Saccharomyces* yeast strains for reducing alcohol levels in wine by sugar respiration. *International Journal of Food Microbiology, 181*, 85–91. https://doi.org/10.1016/j.ijfoodmicro.2014.04.024

Ramos, A., Lolkema, J. S., Konings, W. N., & Santos, H. (1995). Enzyme basis for pH regulation of citrate and pyruvate metabolism by *Leuconostoc oenos*. *Applied and Environmental Microbiology, 61*(4), 1303–1310. https://journals.asm.org/journal/aem.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Volume 2: The chemistry of wine stabilization and treatments. In *Handbook of Enology*. John Wiley & Sons, Ltd.. https://doi.org/10.1002/0470010398

Rodas, A. M., Ferrer, S., & Pardo, I. (2003). 16S-ARDRA, a tool for identification of lactic acid Bacteria isolated from grape must and wine. *In System. Appl Microbiol, 26*. http://www.urbanfischer.de/journals/sam.

Roessner, U., Wagner, C., Kopka, J., Trethewey, R. N., & Willmitzer, L. (2000). Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. *Plant Journal, 23*(1), 131–142. https://doi.org/10.1046/j.1365-313X.2000.00774.x

Romero, J., Ilabaca, C., Ruiz, M., & Jara, C. (2018). *Oenococcus oeni* in Chilean red wines: Technological and genomic characterization. *Frontiers in Microbiology, 9*(FEB). https://doi.org/10.3389/fmicb.2018.00090

Romero-Gil, V., García-García, P., Garrido-Fernández, A., & Arroyo-López, F. N. (2016). Susceptibility and resistance of lactic acid bacteria and yeasts against preservatives with potential application in table olives. *Food Microbiology, 54*, 72–79. https://doi.org/10.1016/j.fm.2015.10.014

Ruiz-de-Villa, C., Poblet, M., Bordons, A., Reguant, C., & Rozès, N. (2023). Comparative study of inoculation strategies of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on the performance of alcoholic and malolactic fermentations in an optimized synthetic grape must. *International Journal of Food Microbiology, 404*. https://doi.org/10.1016/j.ijfoodmicro.2023.110367

Ruiz-de-Villa, C., Poblet, M., Cordero-Otero, R., Bordons, A., Reguant, C., & Rozès, N. (2023). Screening of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* strains in relation to their effect on malolactic fermentation. *Food Microbiology, 112*. https://doi.org/10.1016/j.fm.2022.104212

Saayman, M., Van Vuuren, H. J. J., Van Zyl, W. H., & Viljoen-Bloom, M. (2000). Differential uptake of fumarate by Candida utilis and *Schizosaccharomyces pombe*. *Applied Microbiology and Biotechnology, 54*, 792–798.

Shah, M. V., van Mastrigt, O., Heijnen, J. J., & van Gulik, W. M. (2016). Transport and metabolism of fumaric acid in *Saccharomyces cerevisiae* in aerobic glucose-limited chemostat culture. *Yeast, 33*(4), 145–161. https://doi.org/10.1002/yea.3148

Sumby, K. M., Bartle, L., Grbin, P. R., & Jiranek, V. (2019). Measures to improve wine malolactic fermentation. *Applied Microbiology and Biotechnology, 103*(5), 2033–2051. https://doi.org/10.1007/s00253-018-09608-8

Tchelistcheff, A., Peterson, R. G., & Van Gelderen, M. (1971). Control of malo-lactic fermentation in wine. *American Journal of Enology and Viticulture, 22*(1), 1–5. https://doi.org/10.5344/ajev.1971.22.1.1

Torres-Guardado, R., Rozès, N., Esteve-Zarzoso, B., Reguant, C., & Bordons, A. (2022). Influence of succinic acid on *Oenococcus oeni* and malolactic fermentation. *OENO one, 56*(3), 195–204. https://doi.org/10.20870/oeno-one.2022.56.3.5403

Vital-Lopez, F. G., Wallqvist, A., & Reifman, J. (2013). Bridging the gap between gene expression and metabolic phenotype via kinetic models. *BMC Systems Biology, 7*. https://doi.org/10.1186/1752-0509-7-63

Wagener, W. W. D., Ough, C. S., Amerine, M. A., Manager, R., Winery, D., & Box, P. O. (1971). The fate of some organic acids added prior to fermentation to grape juice. *American Journal of Enology and Viticulture, 22*(3), 167–171. https://doi.org/10.5344/ajev.1971.22.3.167

Wang, M. L., Choong, Y. M., Su, N. W., & Lee, M. H. (2003). A rapid method for determination of ethanol in alcoholic beverages using capillary gas chromatography. *Journal of Food and Drug Analysis, 11*(2), 133–140. https://doi.org/10.38212/2224-6614.2710

Wei, R., Wang, L., Ding, Y., Zhang, L., Gao, F., Chen, N., … Wang, H. (2022). Natural and sustainable wine: A review. *Critical Reviews in Food Science and Nutrition, 1–12*. https://doi.org/10.1080/10408398.2022.2055528

Wisselink, H. W., Weusthuis, R. A., Eggink, G., Hugenholtz, J., & Grobben, G. J. (2002). Mannitol production by lactic acid bacteria: A review. *In International Dairy Journal, 12*.

Xu, G., Chen, X., Liu, L., & Jiang, L. (2013). Fumaric acid production in *Saccharomyces cerevisiae* by simultaneous use of oxidative and reductive routes. *Bioresource Technology, 148*, 91–96. https://doi.org/10.1016/j.biortech.2013.08.115

Yin, X., Li, J., Shin, H., Dong, D. G., Liu, L., & Chen, J. (2015). Metabolic engineering in the biotechnological production of organic acids in the tricarboxylic acid cycle of microorganisms: Advances and prospects. *Biotechnology Advances, 33*(6), 830–841. https://doi.org/10.1016/j.biotechadv.2015.04.006

Zapparoli, G., Torriani, S., Pesente, P., & Dellaglio, F. (1998). Design and evaluation of malolactic enzyme gene targeted primers for rapid identification and detection of *Oenococcus oeni* in wine. *Letters in Applied Microbiology, 27*(5), 243–246. https://doi.org/10.1046/j.1472-765X.1998.00448.x