Tiziana Nardin[1], Raffaele Guzzon[1*], Lorenzo Camellini[1], Flavio Bonetti[2], Mario Malacarne[1], Roberto Larcher[1]

Impact of addition of fumaric acid in the first stages of wine production

Effect on the oenological yeasts and wine composition

Affiliations
[1]Centro di Trasferimento Tecnologico. Fondazione Edmund mach. Via Mach 1 38010 San Michele all'Adige (TN), Italy.
[2]Agriculture, Food and Environment Department (C3A). University of Trento. Via Mach 1 38010 San Michele all'Adige (TN), Italy.

Correspondence
Tiziana Nardin: tiziana.nardin@fmach.it; *Raffaele Guzzon: raffaele.guzzon@fmach.it; Lorenzo Camellini: lorenzo.camellini1@gmail.com; Flavio Bonetti: bonetti.1202@gmail.com; Mario Malacarne: mario.malacarne@fmach.it; Roberto Larcher: roberto.larcher@fmach.it.

Summary

Fumaric acid is a promising oenological tool known for its ability to block unwanted malolactic fermentations due to its antibacterial properties. While its use as an acidifier in musts and wines is currently being debated, its interaction with yeast has long been recognized, yet in-depth studies are lacking. In this work, we investigated the impact of fumaric acid on the viability and fermentation performance of a pool of *S. cerevisiae* strains and other yeast species of oenological interest in experimental laboratory fermentations. In addition to collecting kinetic and microbiological data, we thoroughly characterized the resulting wines by analysing all intermediates of the Krebs cycle for possible traces of fumaric acid, which was added to the grape must. Our results demonstrate that fumaric acid does not significantly alter the activity of *S. cerevisiae*, though we were able to identify some markers of its presence. Furthermore, a higher concentration of this molecule was found in the finished wines compared to the controls, suggesting a new approach for tracing fumaric acid in wine. Although preliminary, our tests on non-*Saccharomyces* yeasts revealed divergent behaviours among the species, which paves the way for further research.

Keywords

Wine, fumaric acid, traceability, *S. cerevisiae*, alcoholic fermentation, lactic acid bacteria, antimicrobial agent.

Introduction

Acidity, understood not merely as pH but as the concentration and balance of different organic acids, is the backbone of wine's taste and a key factor in its longevity. Tartaric, malic, and citric acids naturally accumulate in the grape bunch, and from there they are transferred to the wine in different quantities and proportions depending on a multitude of parameters that are not always easily predictable beforehand. The most influential factors include vine cultivar, climatic conditions, and agronomic practices (Gutiérrez-Gamboa *et al.*, 2021). The techniques applied during winemaking also influence wine's acidic profile, such as the duration of grape maceration or the temperature of wine storage (Shanshiashvili *et al.*, 2024; Van Leeuwen *et al.*, 2024; Payan *et al.*, 2023).

A fundamental role in determining the wine's acidic profile is also played by different microbial groups. *Saccharomyces cerevisiae*, the main yeast involved in alcoholic fermentation, slightly alters the wine's acid profile, degrading up to 30% of the malic acid present in grapes, and accumulating modest doses of acetic acid (Vion *et al.*, 2024; Guzzon *et al.*, 2014). Other oenological yeasts can have a much greater impact, altering the concentration of different organic acids in wine. The *Lachancea* genus can increase the L-lactic acid content up to 12 $g \cdot L^{-1}$ through the bioconversion of part of hexose sugars present in grape must (Sizzano *et al.*, 2024). *Schizosaccharomyces* spp. can consume malic acid almost completely without accumulating other organic acids through maloalcoholic fermentation (Loira *et al.*, 2018). These characteristics have suggested the commercial application of these yeasts as active dry yeasts complementary, not alternative to selected strains of *Saccharomyces cerevisiae* to regulate the acidic profile of wines (Vilela, 2019). Some other non-*Saccharomyces* yeasts accumulate significant amounts of acetic acid from glucose or, during wine aging, from the oxidation of ethanol. Except for a few rare cases (Vin de voile, Sherry, Vernaccia di Oristano), this activity is generally considered wine spoilage and is controlled by hygiene and an adequate level of SO_2 (Borren & Tian, 2021).

A more relevant role in defining the wine's acidic profile is played by lactic acid bacteria. Not many genera of bacteria can survive in the harsh conditions of wine, a selective environment due to the high acidity, toxic compounds like alcohol and SO_2, and a lack of nutrients (Bech-Terkilsen *et al.*, 2020). However, these microorganisms can derive energy not only from sugars, which are scarce or absent in wine after alcoholic fermentation, but from the single-enzyme catalyzed decarboxylation of L-malic acid, which is converted into L-lactic acid during the biological process known as malolactic fermentation (Fu *et al.*, 2022). This bioprocess converts L-malic acid into L-lactic acid, resulting in a slight reduction in the

Submitted/accepted for publication: November 4, 2025/February 10, 2026

wine's total acidity, but it also shapes its organoleptic profile. This is due to the different tastes of malic and lactic acid, and at the conversion of citric acid (the third most concentrated acid in grapes) into diacetyl (Eicher *et al.*, 2024; Bartowsky and Henschke, 2004). For this reason, and because of some spoilage activities connected to the uncontrolled presence of lactic bacteria (Bartowsky, 2009), managing these microorganisms and, more generally, maintaining adequate wine acidity are fundamental issues in modern oenology. This is especially true in a global warming scenario that reduces the natural concentration of organic acids in grapes and, with them, the wine's ability to counteract microbial infections.

Until now, the acidification of grape must or wine had to be performed, according to the rules of the International Organization of Vine and Wine (OIV, 1999), solely by adding tartaric, lactic, malic, citric acid, or a mixture of them. This limitation is not based on chemical reasons; in some cases, the addition of strong inorganic acid could be preferable from a strictly technological point of view. Rather, it is related to the tradition of avoiding the introduction of exogenous components into wine, beyond grape derivatives, to preserve its perceived value and authenticity for consumers. Therefore, the evaluation by scientific and technical communities of fumaric acid as a wine acidifier (Payan *et al.*, 2023) represents a notable innovation that requires careful evaluation. Fumaric acid, also known as trans-2-butenedioic acid ($C_4H_4O_4$), is a four-carbon dicarboxylic organic acid. It has numerous uses, but its primary application is in the food sector (Guo *et al.*, 2020; Coban, 2020). As a relatively strong acid, it can significantly reduce the pH of food even at low doses, performing an acidifying and preservative action. In the oenological field, the use of fumaric acid is subject to specific regulation by the OIV, which, until 2021, allowed its use to control lactic bacteria and inhibit malolactic fermentation in wine, with recommended doses between 300 and 600 $mg \cdot L^{-1}$ (OIV, 2023; OIV 2021; Morata *et al.*, 2019) formalizing its antibacterial action has been known since the 1970s (Ough and Kunkee, 1975).

The possible use of fumaric acid as wine acidifier provoked a debate among professionals of wine sectors. The main concerns about the application of fumaric acid in winemaking are due to its non-grape-derived nature. Although it can be produced from hydrocarbons, there are also other possible alternatives, suitable for the food and oenological industries. Fungi such as *Rhizopus oryzae* and *Rhizopus arrhizus* can produce a large amount of fumaric acid via cytosolic reductive TCA pathways under aerobic conditions (Deng and Aita, 2018; Kuenz *et al.*, 2023). Furthermore, the use of *Saccharomyces* cerevisiae was proposed to produce this carboxylic acid (Xu *et al.*, 2013), as well as enzymatic *process* based on the thermoresistant maleate isomerase, derived from *Bacillus stearothermophilus* or *Bacillus brevis* (Tanpong *et al.*, 2024). Another concern regarding fumaric acid as an acidifier in oenology is its interaction with yeasts, during alcoholic fermentation. Already from the first works about the use of fumaric acid in wines (Rankine, 1977; Ough and Kunkee, 1975) it was clear that the permanence of a high amount of this carboxylic acid in the wine depended on the absence of viable yeasts, since *Saccharomyces cerevisiae* can efficiently intake fumaric acid, integrating it into their metabolic cycles and leading to its degradation, even complete, with accumulation of malic acid and/or succinic acid (Yéramian *et al.*, 2007). Therefore, it should not be underestimated that the addition of fumaric acid alters the activity of yeasts during winemaking, given that at present there is no detailed knowledge about this matter. Furthermore, it should be considered the difficulties to trace the addition of fumaric acid in wine because this molecule, not accumulated in grape must by *Vitis vinifera*, is converted by the most common oenological yeast into malic and succinic acid, already naturally present in wines.

Starting with these considerations, we proposed a detailed investigation about the impact of fumaric acid addition on the activity, in terms of viability, fermentative performance and bioproducts, of a pool of commercial strains of *S. cerevisiae*. The survey was also extended to the main genus of non-*Saccharomyces* yeasts of oenological interest, utilizing type strains for each species to ensure generalizability of the results obtained. Microbiological assessments via flow cytometry, integrated with chemical profiling of key metabolic intermediates using HPLC-HQOMS and IC-PAD, provide valuable insights into the impact of this new oenological tool on the experimental wines.

Material and Methods

Microorganisms and microbiological analyses

The following type strains of yeast were employed in this work, according to the experimental plan: *S. cerevisiae* ATCC 13007, *B. bruxellensis* ATCC 36234, *M. pulcherrima* ATCC 20032, *P. membranifaciens* ATCC 16046, *H. uvarum* ATCC 32369, *S. pombe* ATCC 24843, and T. *delbrueckii* DSM 70504. Were also utilized the following Active Dry Yeast (ADY): iYeast® Toro Nero (LAFOOD, I), Mycoferm CRU 811 (EVER, I), LALVIN CH14® (Lallemand, CA), FR-2 (Ferrari, I), UVAFERM HPS (Lallemand, CA), SOEC® 1971 (Station Oenotechnique de Champagne, F), IOC 18-2007 (Perdomini, I), EXOTICS MOSAIC (Anchor, ZA), Andante ADT-36 (Renaissance Yeast, CH), UVAFERM DV10 (Lallemand, CA). Type strains were purchased from international collections, stored in ultra fridge at –80 °C until use, then reactivated and cultured in YM broth Media (Oxoid, UK) at 30 °C for 3-7 days depending to the growth rate of different species. Final cellular concentration in liquid culture was estimated by flow cytometry. ADY were rehydrated according to the OIV method (OIV, 2009) prior the application in the fermentation tests.

Fermentation and growth tests.

Fermentation tests were performed using, according to the experimental plan, up to 7 grape musts made from different grape cultivars (Table S1). All grape musts were pasteurized (90 °C for 5 min.) to avoid microbial contamination and uncontrolled malolactic fermentation. Fermentations were carried out in 1 L glass bottles equipped with a non-return valve to allow CO_2 evolution without contamination of the grape musts. Yeast strains were inoculated at a nominal concentration of 5×10^6 $Cells \cdot mL^{-1}$, fermentation was performed at 25 °C under daily manual stirring (30 sec., two times for day).

As required by the experimental plan, fumaric acid was added 24 hours after yeast inoculation. To allow dissolution of acid, it was previously dissolved in a 1:5 ethanol/water solution, to obtain a 20 $g \cdot L^{-1}$ solution of fumaric acid. This solution was added to the grape must with a dilution rate sufficient to obtain a final concentration of 1 $g \cdot L^{-1}$ of fumaric acid in grape must. During tests pH was checked after complete fumaric acid dissolution using a XS pH 2700 pHmeter (Eutech Instruments, Chelmsford, MA). The evolution of fermentation was followed by measuring weight loss with a CP2202S balance (Sartorius, Göttingen, D). Cellular growth test conducted on non-*Saccharomyces* yeasts was performed in Yeast Medium Broth (Oxoid Ltd, Basingstoke, UK) in a volume of 100 mL. Yeasts were inoculated at a nominal concentration of 10^3 $CFU \cdot mL^{-1}$, then samples were incubated at 25 °C under continuous agitation.

Flow cytometric analysis (FCM).

Samples were diluted with Phosphate Buffered Saline (PBS, pH 7.20) to reach a concentration of 10^3 $cells \cdot mL^{-1}$. One mL of the sample, obtained by appropriate dilution in the PBS buffer, was filtered through a 30 µm filter (CellTrics®, Sysmex, Norderstedt, D) and incubated for 10 minutes at 30 °C in the presence of 10 µL of fluorescein diacetate solution (Sysmex, D). After incubation, samples were mixed and 10 µL of propidium iodide solution (Sysmex, D) was added. The double-stained samples were homogenized and subjected to FCM analysis within 10 minutes. FCM analysis was performed using a CUBE 8 Cytometer (Sysmex, D) equipped with a solid blue laser (488 nm). Thanks to four band-pass filters, we considered the following signals: a combined forward-angle light scatter (FSC), a side-angle light scatter (SSC), and two fluorescence signals. The first fluorescence signal used a 530 nm band-pass filter to collect green fluorescence (FL1 channel), and the second used a 630 nm long-pass filter to collect red fluorescence (FL2 channel). FCM analysis was performed using logarithmic gains and specific detector settings, adjusted using a sample of un-stained *S. cerevisiae* ATCC 13007 to eliminate background and cellular autofluorescence. Data was collected and analyzed using FCS Express 4 software (De Novo Software Inc., CA). The yeast cell population was identified and gated in the FSC/SSC dot plot. Live and dead cell differentiation was performed using an FL1/FL2 dot plot, adjusted for appropriate compensation between the two signals, considering the subpopulation of yeast gated in the FSC/SSC dot plot. Major details are available in Guzzon and Larcher (2015).

Chemical analysis of grape must and wine.

Sample preparation.

The must/wine samples collected daily were pre-centrifuged at 3000 rpm using a ROTINA 380 R (Beckman Coulter, Brea, CA) centrifuge to remove any suspensions. The samples were then diluted 60-fold for analysis: 17 µL of the centrifuged sample were collected in a new 2 mL clear glass vial with a screw cap and a 9 mm PTFE membrane, to which 978 µL of Milli-Q water and 5 µL of 25 $g \cdot L^{-1}$ sodium azide were added to prevent the development of contaminating microflora.

Sugar analysis.

For the quantification of simple sugars (sucrose, glucose, fructose, mannitol, arabinose, rhamnose, galactose, xylose, mannose, lactose, and maltose) an ion chromatography (ICS-5000; Thermo Fisher Scientific) equipped with an autosampler, a quaternary gradient pump, a thermostat-controlled column compartment, and a pulsed amperometric detector (PAD) with a gold working electrode and a palladium reference electrode was used. Monosaccharides and di-saccharides were separated by injecting 5 µL of sample onto an analytical CarboPac PA200 3x250 mm column, preceded by a Car-boPac PA200 3 x 50 mm pre-column (Thermo Fisher Scientific, Waltham, MA). Both the column and the pre-column were maintained at 30 °C. Chromatographic elution was performed at a flow rate of 0.4 $mL \cdot min^{-1}$ using an eluent generator that allowed the automatic production of potassium hydroxide (KOH) from 0.1 to 100 mM starting from deionized water. The sugar standard solution for quantification was prepared in Milli-Q water at 500 $mg \cdot L^{-1}$ and then diluted to 5 $mg \cdot L^{-1}$ for injection into the instrument.

Organic acid analysis.

For the quantification of malic, citric, isocitric, α-ketoglutaric, oxaloacetic, fumaric, and succinic acids, a Thermo UltiMate™ 3000 high performance liquid chromatography (HPLC; Thermo Fisher Scientific) equipped with a pump, autosampler, and column oven, coupled via a HESI II source to a high-resolution mass spectrometer Q-Exactive™ (HRMS, Thermo Fisher Scientific) was used. Chromatographic separation was achieved using a Raptor Biphenyl column, 3×150 mm (2.7 µm particle size; Restek Corporation, Bellefonte, PA), with a mobile phase composed of 2% formic acid, acetonitrile, and H_2O MilliQ in a gradient elution at a flow rate of 0.3 $mL \cdot min^{-1}$ and a temperature of 30 °C. Mass spectra were acquired in Full-MS/dd-MS2 mode, with resolutions of 70,000 FWHM (m/z 200, 1.5 Hz; scan range m/z 100-700) for Full-MS and 17,500 FWHM (12 Hz) for dd-MS2 in negative ion mode. The method was developed *de novo*, and its validation was assessed in terms of Limit of Detection (LOD) and Limit of Quantification LOQ, precision (Relative Standard Deviation, RSD%), and accuracy (recovery). For the quantification of acetic acid and lactic acid a high-performance liquid chromatography (HPLC 1260 Infinity, Agilent Technologies) equipped with a double ternary gradient pump, autosampler, column oven and refractive index detector (IR) (Agilent Technologies, CA) has been used. The chromatographic separation was carried out with an Aminex HPX-87H3 7.8 X 300 mm (9 µm particle size; Bio-Rad Laboratories, CA) with 0.05M sulfuric acid as the mobile phase in isocratic mode, at a flow rate of 0.6 $L \cdot min^{-1}$ and a temperature of 65 °C.

Statistical and Untargeted analysis.

XLSTAT 2023.1.1 software (Addinsoft, F) was used for statistical analysis. The results of the instrumental analysis were processed with a statistical test (One-way ANOVA, t-test for two paired samples/Two-tailed test) to highlight the presence of statistically significant differences (p value < 0.05) between wines treated with fumaric acid and related controlled samples. Compound Discoverer 3.3.3.200 software (Thermo Fisher Scientific) was used for untargeted analysis of metabolites (negative ionization).

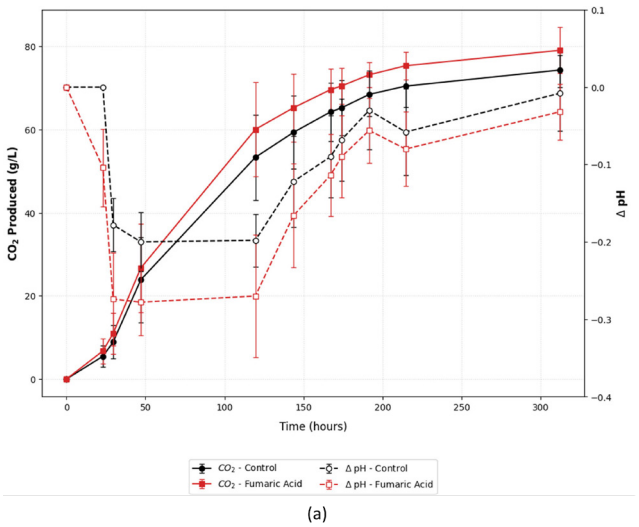
Results

Effect of Fumaric acid on the Saccharomyces cerevisiae viability and fermentation performances.

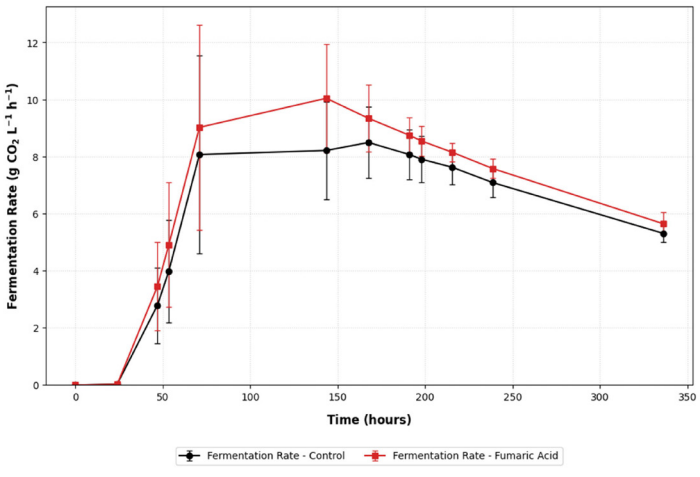
The first series of experiments was performed considering 5 different grape musts (TEST 1, Table S1) and it is aimed to evaluate the impact of adding fumaric acid to musts on the fermentative activity and viability of *S. cerevisiae*, the most widely used yeast in oenology. Figure 1a shows the progress of alcoholic fermentation, expressed as $CO_2 \cdot L^{-1}$ produced. The data represent the average of five tests conducted on different grape musts, whose composition is detailed in Table S1. Fermentations were carried out using the *S. cerevisiae* type strain ATCC 13007. The addition of fumaric acid did not cause significant variations in fermentative activity compared to the control. This was true for both CO_2 production (Figure 1a) and the fermentation rate (Figure 1b) across all 11 observation points conducted over 336 hours (one-way ANOVA, $p < 0.05$). More in detail, the maximum fermentation rate (V_{max}) for tests carried out with fumaric acid supplementation in grape musts was 10.04 ± 1.90 $gL^{-1}h^{-1}$, compared to 8.92 ± 1.72 g $L^{-1}h^{-1}$ for the control test performed without fumaric acid addition. In both cases, V_{max} was achieved 143 hours after yeast inoculation. The differences between these two values of V_{max}, although notable, are not statistically significant (one-way ANOVA, $p < 0.05$) due to the large variability observed in the measurements. Another two parameters were monitored during the experiments: the evolution of wine pH and the number of live/dead yeast cells. Data regarding the evolution of wine pH are also reported in Figure 1a. In both experiments, we observed a lowering of pH during the first 96 hours of fermentation. The addition of fumaric acid (1 $g \cdot L^{-1}$) at 24 hours caused a pH reduction of 0.10±0.05 pH units. The pH decreased until 53 hours of fermentation, after which a progressive increase in pH was observed, reaching a value comparable to the initial one (3.39 ± 0.34). The trend observed in the experiments without fumaric acid addition was similar, but during the first 53 hours of fermentation, the difference between the mean pH of the tests treated with fumaric acid and the control without addition was statistically significant (one-way ANOVA, $p < 0.05$). After this point, the high variability observed made the two curves not significantly different. The trend of pH variation is consistent with the accumulation and salification of organic acids during fermentation, which we will discuss further in the next chapter of the paper. Table 1 lists the concentrations of live (LVC) and dead (DCC) yeast cells measured at three different points of alcoholic fermentation: 24 hours (end of the lag phase), 72 hours (beginning of the exponential growth phase), and 366 hours (stationary phase). Statistical analysis of the data did not reveal differences due to the presence of fumaric acids in the first two observation points. However, at the third observation point, fermentations carried out in the presence of fumaric acids showed an increase in dead cells and a concentration of live cells of an order of magnitude lower than the control test.

Table 1. Concentration (× 107 cells·mL^{-1}) of live and dead cells measured by flow cytometric analysis in different points of fermentation. Mean data ± DS (n = 5). Different letters in caption means that values measured at the same moment are statistically significantly different (one-way ANOVA, $p < 0.05$).

	24 h		72 h		336 h	
	Live	**Dead**	**Live**	**Dead**	**Live**	**Dead**
Test without fumaric acid	2.7 ± 2.5[a]	0.6 ± 0.3[a]	3.2 ± 1.1[a]	0.4 ± 0.2[a]	2.0 ± 1.1[a]	1.5 ± 1.3[a]
Test with fumaric acid (1 $g \cdot L^{-1}$)	2.3 ± 2.6[a]	0.4 ± 0.2[a]	3.7 ± 1.3[a]	0.4 ± 0.3[a]	0.3 ± 0.2[b]	4.1 ± 1.8[b]



(a)



(b)

Figure 1. Effect of fumaric acid addition (1 $g \cdot L^{-1}$) on the S. cerevisiae fermentation performances. (a) Evolution of alcoholic fermentation and pH during fermentation performed by S. cerevisiae ATCC 13007 in 5 different grape musts with addition of fumaric acid, mean data ± SD. (b) Evolution of fermentation rate in the same experiment, mean data ± SD.

A second series of tests was performed utilizing different commercial strains of *S. cerevisiae* in the form of Active Dry Yeast (ADY) for the inoculation of alcoholic fermentation in grape must (number 6 of Table S1). The aim was to confirm the results obtained with the type strains of *S. cerevisiae*. Fig. 2 illustrates the evolution of alcoholic fermentation by 10 different ADY strains. Data are expressed as mean ± standard deviation. Despite the variability observed in this test being lower than that of previous experiments, likely due to the use of the same grape must, the addition of fumaric acid did not significantly alter either the evolution of alcoholic fermentation or the dynamics of the fermentation rate. We observed the start of alcoholic fermentations 24 hours after ADY inoculation, with V_{max} reached in approximately 142 hours and conclusion in about 300 hours in both experiments. V_{max} of test with fumaric acid was 9.86 ± 1.20 $gL^{-1}h^{-1}$, compared to 9.18 ± 0.76 $gL^{-1}h^{-1}$ for the control test performed without fumaric acid addition, according to previous observation the addiction of fumaric non caused significant changes in the yeast activity (one-way ANOVA, $p < 0.05$). Figure 3 showed the concentration of live (Figure 3a) and dead (Figure 3b) cells in 3 different moments of fermentation, both for test with fumaric acid (F+), than for the control test without fumaric acid (F-). According to the data relative at the kinetics of fermentation of Fig. 2 we did not find statistically significant differences between value of live or dead cell of the two theses, in all point of observation although a tendency towards a higher concentration of live cells in F+ samples can be observed.

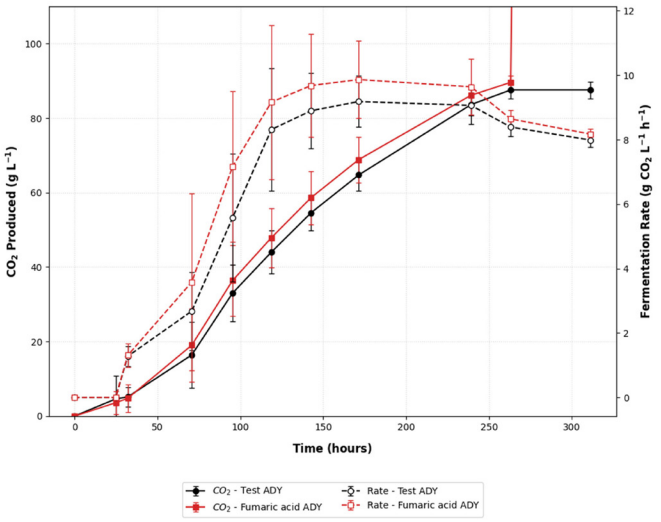


Figure 2. Effect of fumaric acid addition (1 $g \cdot L^{-1}$) on the alcoholic fermentation kinetics and fermentation rates of 10 S. cerevisiae strains, used in Active Dry Yeast (ADY) form and commonly employed in oenology (Mean ± SD, n=10).

Impact of fumaric acid addition on the non-Saccharomyces yeast growth.

The third experiment (TEST 3, Table S1) investigated the potential impact of fumaric acid addition on the viability of various non-*Saccharomyces* yeast species commonly found in the oenological environment. Fermentation assays were not conducted, as several species lack sufficient fermentative vigor and exhibit highly variable ethanol tolerance. To avoid these confounding factors, we instead evaluated growth capacity, expressed through viable cell concentration and the presence of non-viable cells, as a proxy for the potential inhibitory or toxic effects of fumaric acid. Table 2 presents the concentrations of live and dead cells monitored after 2, 4, and 6 days of growth for six non-*Saccharomyces* species. As hypothesized, the yeasts exhibited divergent behavioural responses to fumaric acid supplementation. Notably, these differences persisted despite buffering the pH of both the control (F-) and treatment (F+) solutions to eliminate the confounding influence of increased acidity. *Schizosaccharomyces pombe, Torulaspora delbrueckii*, and *Hanseniaspora uvarum* demonstrated significantly enhanced growth in the presence of fumaric acid across all observation points (one-way ANOVA, $p < 0.05$). Conversely, the remaining species displayed an inverse trend, with viable cell concentrations one to two orders of magnitude lower in the fumaric acid-enriched trials (F+, Table 2). In all instances, the concentration of dead cells did not differ significantly between the F+ and F- experiments but rather increased naturally over the growth cycle; this suggests that fumaric acid does not exert a direct cytotoxic action on the yeasts considered.

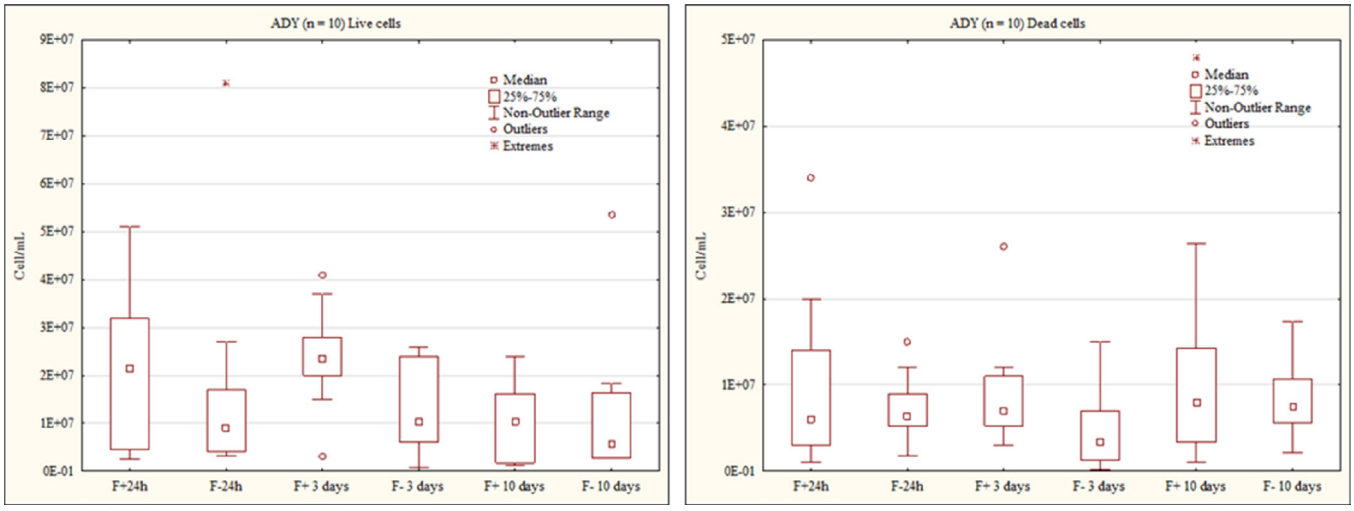


Figure 3. Box plot of concentration of live (Left) and dead (Right) cells measured by flow cytometer during fermentation tests performed by 10 strains of *S. cerevisiae* in form of ADY. F+: test with fumaric acid; F-: test without fumaric acid.

Table 2. Concentration of live and dead cells of some non-*Saccharomyces* yeasts of oenological interest, measured by flow cytometric analysis at 3 different times. Experiments were performed in YM medium broth in presence (F+) or absence (F-) of fumaric acid. Mean data ± DS (n = 3). Different letters in captions mean that value measured at the same moment and for the same yeast are statistically significantly different (one-way ANOVA, $p < 0.05$).

Day		Cells	*B. bruxellensis* ATCC 36234	*M. pulcherrima* ATCC 20032	*P. membranifaciens* ATCC 16046	*H. uvarum* ATCC 32369	*S. pombe* ATCC 24843	*T. delbrueckii* DSM 70504
					× 10^5 cells·mL^{-1}			
2	F+	Live	0.1 ± 0.0^a	2.0 ± 0.3 ^{a}	30 ± 9.6 ^{a}	110 ± 17 ^{a}	900 ± 17 ^{a}	420 ± 27 ^{a}
		Dead	1.1 ± 0.6^a	0.1 ± 0.0 ^{a}	2.0 ± 1.1^a	14 ± 1.6 ^{a}	12 ± 0.9 ^{a}	30 ± 3.7 ^{a}
	F-	Live	110 ± 12^b	24 ± 3.0 ^{b}	100 ± 12 ^{b}	200 ± 13 ^{b}	270 ± 21 ^{b}	80 ± 17 ^{b}
		Dead	1.0 ± 0.5^a	2.0 ± 0.8 ^{b}	20 ± 1.7 ^{b}	20 ± 2.7 ^{b}	10 ±1.6 ^{a}	10 ± 1.1 ^{b}
					× 10^6 cells·mL^{-1}			
4	F+	Live	6.1 ± 0.2 ^{a}	4.8 ± 0.9 ^{a}	3.0 ± 0.7 ^{a}	13 ± 2.7 ^{a}	62 ± 11 ^{a}	100 ± 9.4 ^{a}
		Dead	1.1 ± 0.1^a	0.5 ± 0.2 ^{a}	2.0 ± 0.5 ^{a}	8.0 ± 3.4 ^{a}	2.0 ± 0.9 ^{a}	3.0 ± 1.1 ^{a}
	F-	Live	15 ± 0.2 ^{b}	48 ± 3.7 ^{b}	35 ± 1.1 ^{b}	12 ± 1.8 ^{a}	83 ± 17 ^{b}	120 ± 11 ^{b}
		Dead	1.7 ± 0.4 ^{b}	1.0 ± 0.6 ^{a}	4.0 ± 1.8 ^{b}	1.9 ± 1.1 ^{b}	3.0 ± 1.3^a	4.0 ± 2.7 ^{a}
7	F+	Live	21 ± 2.0 ^{a}	50 ± 6.4 ^{a}	15 ± 2.1 ^{a}	9.0 ± 2.1 ^{a}	6.0 ± 3.2 ^{a}	22 ± 1.8 ^{a}
		Dead	2.7 ± 0.4 ^{a}	6.0 ± 1.2 ^{a}	5.4 ± 0.7 ^{a}	2.5 ± 0.7 ^{a}	23 ± 1.8 ^{a}	11 ± 1.3 ^{b}
	F-	Live	25 ± 1.1^b	51 ± 3.6 ^{a}	31 ± 2.6 ^{b}	10 ± 3.2 ^{a}	46 ± 11 ^{b}	9.8 ± 3.1 ^{b}
		Dead	2.0 ± 0.3^b	3.0 ± 2.2 ^{a}	2.5 ± 2.3 ^{a}	3.4 ± 1.6 ^{a}	20 ± 2.2 ^{a}	18 ± 2.4 ^{b}

Influence of fumaric acid on the sugar consumption by S. cerevisiae.

In the first experiment, aimed at evaluating the impact of addition of fumaric acid to musts fermented with the *S. cerevisiae* ATCC13007 (Figure 1), the fermentative performance was assessed also by measuring the residual sugar concentration in wine at the end of fermentation. For sugar quantification the calibration curves were prepared in water, constructed by plotting the analyte concentrations (mg·L^{-1}) on the x-axis and the peak areas of the different analytes (nC*min) on the y-axis. The resulting functions are quadratic, with determination coefficients (R^2) always exceeding 0.99. Table 3 reports the concentration of sugars found in wine after fermentation with *S. cerevisiae* and the possible addition of fumaric acid. A two-tailed paired t-test revealed a significant difference in fructose consumption between the F- and F+ samples ($p = 0.049$), with a higher fructose consumption observed in the treated samples. In contrast, no significant differences were detected in glucose consumption, despite in the F+ samples the concentration being generally lower than F-samples. Furthermore, sucrose and other minor sugars, including maltitol, arabinose, rhamnose, galactose, xylose, mannose, lactose, and maltose, were found to be below the detection limit (0.01 g·L^{-1}).

Table 3. Concentration of sugar (g·L^{-1}) in wine fermented by S. cerevisiae ATCC 13007 with (F+) and without (F-) fumaric acid addition. Different letters in apex for the same sugar mean a statistically significant difference (Two-tailed paired t-test $p < 0.05$).

Wine	F-		F+	
	Glucose	Fructose	Glucose	Fructose
	g·L^{-1}			
1	2.5	5.6	0.7	3.9
2	0.2	0.5	0.2	0.3
3	1.9	5.7	0.3	2.4
4	0.3	1.0	0.3	0.4
5	0.6	3.7	0.3	1.0
Mean ± SD	1.1 ± 0.4^a	3.3 ± 2.5^a	0.4 ± 0.9^a	1.6 ± 1.5b

Impact of fumaric acid on organic acid concentration of wine at the end of alcoholic fermentation.

Preliminary validation of the HPLC-HQOMS method.

To qualify and quantify the organic acids, the protonated molecular ions $[M-H]^-$ were monitored based on their respective m/z values with a mass accuracy of less than 5 ppm. Identification was confirmed by correlating retention times (RT) and fragmentation patterns (dd-MS/MS) against those of the corresponding analytical standards. The analytical performance of the instrument was evaluated through a linearity study by injecting various concentrations of the analytical standards within a range of 0.005 to 5 $mg \cdot L^{-1}$. The lowest concentrations were injected in triplicate to determine the LOD and the LOQ. Calibration curves in the solvent were constructed by plotting the analytes' areas (counts*min) against their respective concentrations ($mg \cdot L^{-1}$). All regression curves showed a good degree of linearity, with an $R^2 > 0.99$. Table 4 reports main validation data, the method's precision and accuracy parameters, otained by injecting the same sample in quadruplicate to calculate the Relative Standard Deviation (RSD) and by spiking approximately 0.5 $mg \cdot L^{-1}$ of each analyte into another sample to calculate the recovery percentage in the matrix. All analytes showed excellent performance, with RSD consistently below 10 % and recovery values between 80 % and 120 %, except for citric acid, which had a recovery of 144 %.

Organic acid content of wines after alcoholic fermentation in presence of fumaric acid.

Fumaric acid is involved in numerous metabolic pathways, but its most important role is in the Krebs cycle. In fact, the first experiment investigated the behaviour of the acids involved in this pathway (citric acid, isocitric acid, α-ketoglutaric acid, succinic acid, fumaric acid, malic acid, and oxaloacetic acid). Samples were collected at different stages of fermentation to monitor changes in acid concentrations (Table S2). The first point to highlight is that there is a natural content of fumaric acid in the must, always below 10 $mg \cdot L^{-1}$. Where fumaric acid had been added, the starting value in the must was 1 $g \cdot L^{-1}$. The consumption of fumaric acid during fermentation is evident, especially in the first six days, and is reported as an average in the graph (Figure 4) reaching final values ranging between 41 and 189 $mg \cdot L^{-1}$ in F+ wines. Despite all degradation curves showing a consistent and continuous consumption of fumaric acid, its final concentration in F+ test remains significantly higher than that of untreated wines. For the F– samples, starting from much lower values, final concentrations below 4 $mg \cdot L^{-1}$ were reached (Table 5). Concerning the other organic acids (Table 5), malic acid decreased progressively from initial values of around 1.0 – 1.8 $g \cdot L^{-1}$ to final levels of approximately 0.5 – 1.3 $g \cdot L^{-1}$. This decrease is not attributable to malolactic fermentation, since grape musts were pasteurized, lactic acid content in the wine never exceeded 0.2 $g \cdot L^{-1}$, and no significant differences were found between F+ and F- samples. Citric acid showed minimal changes, remaining below 0.5 $g \cdot L^{-1}$ in all cases. Succinic acid concentrations decreased gradually in both experiments (F+ and F-), stabilizing at 0.3–0.5 $g \cdot L^{-1}$ by the end of fermentation. Similarly, α-ketoglutaric acid and oxaloacetic acid increased in concentration from a few $mg \cdot L^{-1}$ to several hundred $mg \cdot L^{-1}$ for α-ketoglutaric acid and from 200 to 600 $mg \cdot L^{-1}$ for oxaloacetic acid, also in this case independently from the presence of fumaric acid. No variations were observed in the concentration of isocitric acid during fermentation, with values consistently ranging between 30 to 90 $mg \cdot L^{-1}$ in both studies. Acetic acid remained below 0.6 $g \cdot L^{-1}$. The mixed model analysis revealed that malic acid ($p = 0.044$) and α-ketoglutaric acid ($p = 0.046$) were significantly affected by the fumaric acid treatment. Fig. 5 shows the increases or decreases in the concentrations of these acids in response to fumaric acid treatment, including succinic acid, which is relevant to fumaric acid metabolism. Unfortunately, none of the acids examined showed significant differences (p-values ranging from 0.059 to 0.2 in paired t-tests) in the increase or decrease of concentration.

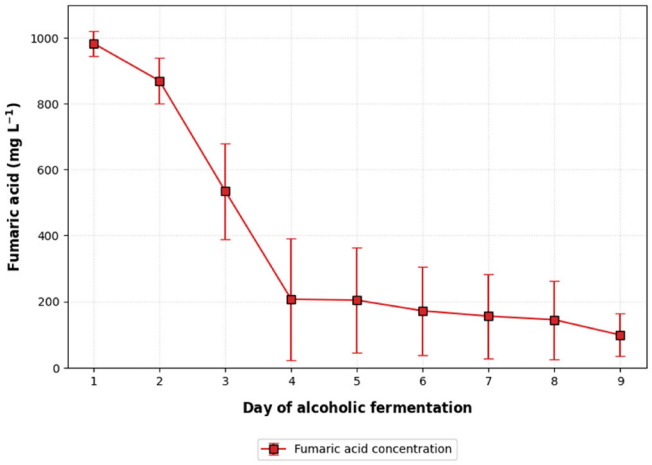


Figure 4. Evolution of fumaric acid during alcoholic fermentation. Concentration of fumaric acid during alcoholic fermentation performed by *S. cerevisiae* ATCC 1007 in 5 grape musts (Mean data ± SD).

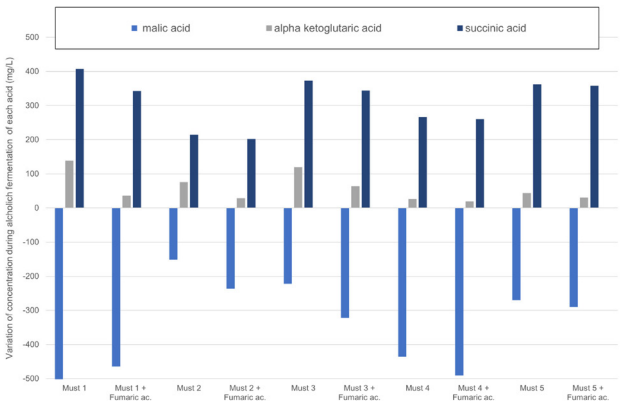


Figure 5. Variation of content of main organic acid (malic, α-ketoglutaric, and oxaloacetic ac.) during alcoholic fermentation performed by *S. cerevisiae* ATCC 1007. The experiment was repeated in 5 grape musts (Table S1, TEST 1). (F+: with fumaric acid; F-, without fumaric acid).

In the second test, 10 strains of *S. cerevisiae* in the form of Active Dry Yeast (ADY) were used to inoculate grape must, with and without fumaric acid addition (1 $g \cdot L^{-1}$). Since the focus was on the most relevant acids, only fumaric, malic, and succinic acids were quantified in the final wine (Table S3). Following a mixed model analysis, the random effect of the must, as well as of the yeast, is present but not significant ($p = 0.169$), although it may still influence the results. For the final malic acid content, the treatment (F- and F+) is not significant, but $p = 0.099$ indicates a trend, the addition of fumaric acid increases malic acid, although the statistical evidence is limited. As regards succinic acid, the treatment is even less impactful ($p = 0.173$). Fumaric acid was always less than 4 $mg \cdot L^{-1}$ in F- and less than 135 $mg \cdot L^{-1}$ in F+. The third experiment considers the possible impact of fumaric acid addition on the viability of different non-*Saccharomyces* yeast. Even in this case malic, succinic and fumaric acid were evaluated (Table 6). The mixed model analysis in this case revealed a random effect attributable to the yeasts ($p = 0.049$). For the final malic acid and succinic acid contents, the treatment (F– vs. F+) was not significant ($p = 0.85$ and 0.12, respectively). In this study, the consumption of fumaric acid in F+ appeared to be less evident for *Pichia membranifaciens* and *Schizosaccharomyces pombe*, with final values of 336 and 476 $mg \cdot L^{-1}$, respectively. Moreover, there even seems to be a formation of fumaric acid in the F– samples, which normally show values < 10 $mg \cdot L^{-1}$, but in this case reached 82, 48 and 92 $mg \cdot L^{-1}$ for *Metschnikowia pulcherrima, Pichia membranifaciens* and *Schizosaccharomyces pombe*, respectively.

Discussion

The purpose of this work is to verify the effects of the addition of fumaric acid into the grape musts on the activity of wine yeasts, primarily *S. cerevisiae*. This objective proved to be anything but simple from the outset, because, although it has long been known that fumaric acid is metabolized by yeasts (Ough and Kunkee, 1975), this molecule is a common intermediate of the Krebs cycle and is therefore not easily monitored along the yeast's metabolic pathways. Furthermore, the dose of fumaric acid that could reasonably be added to grape musts as acidifier agent is modest, in our case 1 $g \cdot L^{-1}$, therefore largely overshadowed by the amount produced by the metabolism of carbon compounds, which in grape musts easily exceed 180-200 $g \cdot L^{-1}$. In the case of lactic acid bacteria, the effectiveness of fumaric acid was easily monitored by evaluating bacterial viability, or indirectly, by measuring the residual malic acid (García-Viñola *et al.*, 2025), but this path is also not feasible for yeasts, as no significant effects on the reduction or increase of the fungal population have been already reported in the literature. It is therefore necessary to rely on a broad-spectrum investigation that takes into consideration the greatest possible number of indicators, microbiological, chemical, and kinetic, to highlight possible deviations from the "native" behaviour of the yeasts, due to the presence of fumaric acid.

In the present work, this approach was carried out through 3 different experiments. The first experiment considered the behaviour of a standard *S. cerevisiae* strain in five different grape musts, with the addition of a standard amount of fumaric acid (1 $g \cdot L^{-1}$). As expected, the addition of fumaric acid did not significantly alter the yeast's fermentative performance, neither in terms of CO_2 accumulation, and therefore alcohol, or in terms of fermentation rate (Fig. 1). The acidification induced by the addition of fumaric acid is temporary (Fig. 4), which is also a known fact (Gancel *et al.*, 2022), but this does not reduce its potential effectiveness as an oenological coadjutant aimed at preventing microbial alterations, especially in the early stages of fermentation, when *S. cerevisiae* does not yet dominate the oenological microbiota, by effectively countering harmful microorganisms by an increase in the must's acidity (Moreira *et al.*, 2024, Bartowsky, 2009). However, a more accurate observation reveals some signs of possible metabolic effects from an over-presence of this organic acid.

Table 4. Validation parameters of HPLC-HRMS analytical method. RT: retention time; LOD: limits of detection; LOQ: limits of quantification; RSD: relative standard deviation.

Compound	RT (min)	*m/z* $[M-H]^-$	Fragments (*m/z*)	LOD ($mg \cdot L^{-1}$)	LOQ ($mg \cdot L^{-1}$)	Precision (RSD %)	Accuracy (%)
Malic acid	2.44	133.0143	115.004-71.014	0.1	0.3	1.92	84
Isocitric acid	2.46	191.0197	111.009-85.029	0.1	0.3	3.22	128
α-ketoglutaric acid	2.72	145.0143	101.025	0.1	0.3	5.22	95
Oxaloacetic acid	2.75	130.9986	-	1.0	3.0	3.72	118
Citric acid	2.80	191.0197	111.009-85.030	0.1	0.3	1.93	144
Fumaric acid	3.18	115.0037	114.933-71.014	0.1	0.3	3.56	98
Succinic acid	3.55	117.0193	73.030	0.1	0.3	3.08	116

Table 5. Concentration of organic acid (mg·L^{-1}) in wine after fermentation with the strain of S. cerevisiae ATCC 13007 with (F+) and without (F-) fumaric acid addition. Different letters in apex for the same organic acid mean a statistically significant difference between mean of F+ and F- samples (Two-tailed paired t-test $p < 0.05$).

	Wine	Malic ac.	Isoctric ac.	*a*-ketoglutaric ac.	Oxaloacetic ac.	Citric ac.	Fumaric ac.	Succinic ac.
F+	1	565.8	41.8	154.2	370.9	386.9	99.6	549.8
	2	838.7	41.8	71.9	326.4	313.6	51.8	216.0
	3	784.9	28.1	126.3	230.4	297.7	144.9	445.6
	4	1346.3	73.7	28.1	554.1	499.8	36.0	278.2
	5	788.6	30.6	35.3	296.7	256.0	182.4	301.3
	Mean	864.9[a]	43.2[a]	83.2[a]	355.7[a]	350.8[a]	102.9a	358.2a
	SD	289.0	18.2	55.6	122.1	95.8	61.6	136.2
F-	1	518.1	40.6	54.3	329.3	382.8	3.7	501.0
	2	792.7	38.4	31.5	345.4	304.1	1.4	215.6
	3	695.7	25.7	71.4	157.6	272.8	3.9	410.7
	4	1378.5	80.0	23.3	656.1	538.9	2.4	287.8
	5	719.7	33.8	31.8	273.2	275.9	1.3	374.8
	Mean	820.9[a]	43.7[a]	42.5[a]	352.3[a]	354.9[a]	2.5b	358.0a
	SD	327.6	21.0	19.9	185.1	112.0	1.2	110.3

Table 6. Concentrations of malic acid (g·L^{-1}), succinic acid (mg·L^{-1}) and fumaric (mg·L^{-1}) acid after a 6-day growth period of non-Saccharomyces yeasts in grape must at 25 °C.

Yeast	Treatment	Malic acid	Fumaric Acid	Succinic acid
Brettanomyces bruxellensis	F-	26.7	13.7	72.6
	F+	22.9	4.4	56.9
Kloeckera apiculata	F-	17.8	1.4	188
	F+	19.4	40.2	211
Metschnikowia pulcherrima	F-	20.3	82.2	318
	F+	18.9	170.0	349
Pichia membranifaciens	F-	21.5	48.2	52.1
	F+	21.9	336.0	49.5
Schizosaccharomyces pombe	F-	0.02	91.5	320.0
	F+	0.04	476.0	353.0
Torulaspora delbrueckii	F-	19.5	6.3	273.0
	F+	23.4	105.0	352.0

In our experiments, yeasts generally fermented more quickly in the presence of fumaric acid, although the statistics do not reveal this effect, likely due to the considerable variability induced by the composition of the five musts considered. This tendency towards greater activity is somewhat confirmed by the measurement of cellular viability, which is higher in the fumaric acid-added variant (F+, Table 1), as if under these conditions the yeasts exhausted the available nutrients more quickly, entering a phase of cellular decline. Furthermore, the measurement of residual sugars at the end of fermentation seems to support this idea (Table 3). The addition of fumaric acid does not alter the well-known glucophilic character of *S. cerevisiae* (Díaz-Hellín *et al.*, 2016), but both glucose and fructose consumption are faster and more complete in the presence of fumaric acid, with even statistically significant differences. According to the metabolic reactions of the Krebs cycle, it is reasonable to suppose that fumaric acid can be converted into malic or succinic acid. However, current data do not confirm this hypothesis. Due to the nature of the Krebs cycle, intermediates are not only continuously transformed but also strategically exchanged with other pathways. Specifically, they can be withdrawn for biosynthetic processes or replenished through anaplerotic reactions, which maintain the pool of metabolites necessary for the cycle's stability. The mechanism of the Krebs cycle is well understood, yet numerous regulatory processes and alternative pathways contribute to the metabolism of its main intermediates. Indeed, these intermediates can be transported out of the mitochondria into the cytoplasm, where they serve as precursors for other biosynthetic reactions. For example, oxaloacetate and malate are involved in gluconeogenesis, α-ketoglutarate and oxaloacetate serve as precursors for amino acid synthesis, and citrate can be used for the synthesis of fatty acids and isoprenoids (Chemler *et al.*, 2006).

In the second experiment, the intraspecific variability of *S. cerevisiae* was explored, considering ten commercial strains commonly used in oenology. The results obtained are generally in agreement with the previous experiment. As shown in Fig. 2, the addition of fumaric acid did not cause significant variations in yeast activity; the variability in fermentative performance between different strains was much more significant than the exogenous presence of fumaric acid, as evidenced by the high variability associated with measurements performed under the same conditions and within the same time interval. However, even in this case, the trials conducted in the presence of fumaric acid showed a tendency towards more significant performance. Furthermore, the measurement of organic acids at the end of fermentation, while not revealing significant differences due to the addition of fumaric acid, shows a greater accumulation of malic acid in the treated samples. This evidence is in good agreement with the metabolism of fumaric and malic acids in the Krebs cycle (Pines *et al.*, 1996). This phenomenon could have interesting technological implications, as malic acid is a fundamental compound for the acid profile of wines, and its concentration has been reduced in recent years because of climate change (Hewitt *et al.*, 2023; Picariello *et al.*, 2019). However, once again, the ability to accumulate malic acid appears to be highly strain-dependent and suggests the selection of specific *S. cerevisiae* strains. What is certain is that the presence of *S. cerevisiae* causes the rapid degradation of fumaric acid, which anyway was found in the range of a hundred milligrams per liter in wines derived from treated grape must. This value is consistently higher than naturally found in wines after alcoholic fermentation, as reported in Table 5, suggesting a possible approach at the traceability of this additive under the oenological productive chain.

Finally, we sought to determine the effect of fumaric acid on other yeast species commonly found in the oenological environment. We recognize that this topic would require extensive and specific work, given the large number of genera and species involved. However, we believe the results we obtained, which are original and being presented to the scientific community for the first time, offer some interesting insights. Among the many yeasts of oenological interest, we selected several for our study: *I)* beneficial yeasts already used in co-fermentation with *Saccharomyces cerevisiae*, such as *Hanseniaspora uvarum, Schizosaccharomyces pombe,* and *Torulaspora delbrueckii* (Cheng *et al.*, 2025; Benito, 2019; Hu *et al.*, 2019). *II)* yeasts typical of the early stages of winemaking, including *Metschnikowia pulcherrima* and *Pichia membranifaciens* (Puyo *et al.*, 2023; Vincente *et al.*, 2021). *III)* Spoilage yeast capable of causing serious defects in wines, such as *Brettanomyces bruxellensis* (Le Montagner *et al.*, 2024). Our evaluation of the effect of fumaric acid focused on the growth capacity of these different species. Yeasts known as vigorous fermenters (*H. uvarum, S. pombe,* and *T. delbrueckii*) showed increased cell growth in the presence of fumaric acid, a behaviour consistent with our findings for *S. cerevisiae*. Conversely, other yeasts, including *B. bruxellensis, M. pulcherrima,* and *P. membranifaciens*, were inhibited by the acid, at least during the initial observation period, exhibiting a significantly lower cell concentration compared to the control. The percentage of dead cells in both cases remained within the normal physiological range for yeast cultures under these conditions, confirming that fumaric acid does not have a direct toxic effect on the yeasts.

Consistent with previous tests, the concentration of the organic acid related to the Krebs cycle was quantified at the end of the experiment. The mixed model analysis revealed a random effect attributable to the different yeasts, which is a reasonable result considering the significant differences already among the six species studied. *P. membranifaciens, M. pulcherrima,* and *S. pombe* preserved the amount of fumaric acid better than the other species. Furthermore, a slight accumulation in the F+ trials with these three species indicates a partial biosynthesis that could be exploited in the future. The reduction in the growth of *B. bruxellensis* is also of great interest, given its increasing prevalence in the early stages of winemaking. These observations, while preliminary, open an interesting field of study regarding the use of this acid during winemaking, but they must be validated with specific future experiments.

Supplementary Material

Supplementary Material can be found online:
https://doi.org/10.5073/vitis.2026.65.05.

Acknowledgements

The authors would like to thank the colleagues of the "Dipartimento Trasformazione Alimentare" of the Edmund Mach Foundation for their valuable assistance and support throughout this research.

Author contribution

Conceptualization (Guzzon R., Larcher R.) Data curation (Nardin T., Guzzon R.) Formal analysis (Nardin T., Guzzon R., Camellini L., Bonetti F., Malacarne M.) Investigation (Camellini L., Bonetti F.) Methodology (Guzzon R., Nardin T., Larcher R.) Resources (Larcher R.) Writing – original draft (Guzzon R., Nardin T., Larcher R.) Writing – review & editing (Guzzon R.).

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

References

Bartowsky, E. J., 2009: Bacterial spoilage of wine and approaches to minimize it. Letters in Applied Microbiology 48(2), 149–156. DOI: 10.1111/j.1472-765X.2008.02505.x.

Bartowsky, E. J., Henschke, P. A., 2004: The 'buttery' attribute of wine, diacetyl desirability, spoilage and beyond. International Journal of Food Microbiology 96(3), 235-252. DOI: 10.1016/j.ijfoodmicro.2004.05.013.

Bech-Terkilsen, S., Westman, J. O., Swiegers, J. H., Siegumfeldt, H., 2020: *Oenococcus oeni*, a species born and moulded in wine: a critical review of the stress impacts of wine and the physiological responses. Australian Journal of Grape and Wine Research 26(3), 188-206. DOI: 10.1111/ajgw.12436.

Benito, S., 2019: The impacts of *Schizosaccharomyces* on winemaking. Applied Microbiology and Biotechnology 103, 4291-4312. DOI: 10.1007/s00253-019-09827-7.

Borren, E., Tian, B., 2021: The important contribution of non-saccharomyces yeasts to the aroma complexity of wine: a review. Foods 10, 13. DOI: 10.3390/foods10010013.

Chemler, J. A., Yan, Y., Koffas, M. A., 2006: Biosynthesis of isoprenoids, polyunsaturated fatty acids and flavonoids in *Saccharomyces cerevisiae*. Microbial Cell Factories 5, 20. DOI: 10.1186/1475-2859-5-20.

Cheng, Y., Geng, S., Zhang, J., Zhao, X., Jiang, J., Liang, Y., Mu, H., Li, W., Qin, Y., Liu, Y., Song, Y., 2025: A comprehensive study on fermentation and aroma contributions of *Torulaspora delbrueckii* in diverse wine varieties: Insights from pure and co-fermentation studies. Food Research International 199, 115340. DOI: 10.1016/j.foodres.2024.115340.

Coban, H. B., 2020: Organic acids as antimicrobial food agents: applications and microbial productions. Bioprocess Biosystem Engineering 43, 569–591. DOI: 10.1007/s00449-019-02256-w.

Deng, F., Aita, G. M., 2018: Fumaric acid production by *Rhizopus oryzae* ATCC® 20344™ from lignocellulosic syrup. Bio-Energy Research 11, 330–340. DOI: 10.1007/s12155-018-9899-y.

Díaz-Hellín, P., Naranjo, V., Úbeda, J., Briones, A., 2016: *Saccharomyces cerevisiae* and metabolic activators: HXT3 gene expression and fructose/glucose discrepancy in sluggish fermentation conditions. World Journal of Microbiology and Biotechnology 32, 196. DOI: 10.1007/s11274-016-2154-9.

Eicher, C., Coulon, J., Favier, M., Alexandre, H., Reguant, C., Grandvalet, C., 2024: Citrate metabolism in lactic acid bacteria: is there a beneficial effect for *Oenococcus oeni* in wine? Frontiers in Microbiology 14, 1283220. DOI: 10.3389/fmicb.2023.1283220.

Fu, J., Wang, L., Sun, J., Ju, N., Jin, G., 2022: Malolactic fermentation: new approaches to old problems. Microorganisms 10, 2363. DOI: 10.3390/microorganisms10122363.

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. DOI: 10.20870/oeno-one.2022.56.3.5455.

García-Viñola, V., Poblet, M., Bordons, A., Reguant, C., Rozès, N., 2025: Effects of the addition of fumaric acid on the spontaneous alcoholic fermentation of white grape must. Food Research International 220, 117064. DOI: 10.1016/j.foodres.2025.117064.

Guo F., Wu M., Dai Z., Zhang S., Zhang W., Dong W., Zhou J., Jiang M., Xin F., 2020: Current advances on biological production of fumaric acid. Biochemical Engineering Journal 153, 107397. DOI: 10.1016/j.bej.2019.107397.

Gutiérrez-Gamboa, G., Zheng, W., Martínez de Toda, F., 2021: Current viticultural techniques to mitigate the effects of global warming on grape and wine quality: A comprehensive review. Food Research International 139, 109946. DOI: 10.1016/j.foodres.2020.109946.

Guzzon, R., Larcher, R., 2015: The application of flow cytometry in microbiological monitoring during winemaking: two case studies. Annals of Microbiology 65, 1865–1878. DOI: 10.1007/s13213-014-1025-6.

Guzzon, R., Nicolini, G., Nardin, T., Malacarne, M., Larcher, R., 2014: Survey about the microbiological features, the oenological performance and the influence on the character of wine of active dry yeast employed as starters of wine fermentation. International Journal of Food Science and Technology 49(9), 2142–2148. DOI: 10.1111/ijfs.12610.

Hewitt, S., Hernández-Montes, E., Dhingra, A., Keller, M., 2023: Impact of heat stress, water stress, and their combined effects on the metabolism and transcriptome of grape berries. Scientific Reports 13, 9907. DOI: 10.1038/s41598-023-36160-x.

Hu, K., Jin, G., Xu, Y., Xue, S., Qiao, S., Teng, Y., Tao, Y., 2019: Enhancing wine ester biosynthesis in mixed *Hanseniaspora uvarum/Saccharomyces cerevisiae* fermentation by nitrogen nutrient addition. Food Research International 123, 559-566. DOI: 10.1016/j.foodres.2019.05.030.

Kuenz, A., Eidt, L., Prüße, U., 2023: Biotechnological Production of Fumaric Acid by *Rhizopus arrhizus*—Reaching Industrially Relevant Final Titers. Fermentation 9, 588. DOI: 10.3390/fermentation9070588.

Le Montagner, P., Etourneau, L., Ballestra, P., Dols-Lafargue, M., Albertin, W., Maupeu, J., Moine, V., Renouf, V., Masneuf-Pomarède, I., 2024: Critical areas for *Brettanomyces bruxellensis* contamination and biofilm formation in the cellar: on the origin of wine spoilage. OENO One 58(3). DOI: 10.20870/oeno-one.2024.58.3.8015.

Loira, I., Morata, A., Palomero, F., González, C., Suárez-Lepe, J. A., 2018: *Schizosaccharomyces pombe*: a promising biotechnology for modulating wine composition. Fermentation 4, 70. DOI: 10.3390/fermentation4030070.

Morata, A., Bañuelos, M. A., López, C., Song, C., Vejarano, R., Loira, I., Suarez Lepe, J. A., 2019: Use of fumaric acid to control pH and inhibit malolactic fermentation in wines. Food Additives & Contaminants: Part A 37(2), 228–238. DOI: 10.1080/19440049.2019.1684574.

Moreira, L., Milheiro, J., Filipe-Ribeiro, L., Cosme, F., Nunes, F. M., 2024: Exploring factors influencing the levels of biogenic amines in wine and microbiological strategies for controlling their occurrence in winemaking. Food Research International 190, 114558. DOI: 10.1016/j.foodres.2024.114558.

OIV, 1999: International Code of Oenological Practices. Section.3.1.1.1 Chemical Acidification (OENO 4/99, OENO 14/01).

OIV, 2009: International Code of Oenological Practices. COEI-2-CONBAC: methods of microbiological analysis bacteriological control analysis common to all monographies.

OIV, 2021: Resolution OIV-OENO 581A. Treatment with fumaric acid in wine to inhibit malolactic fermentation.

OIV, 2023: Resolution OIV-OENO 690. Monograph on fumaric acid.

Ough, C. S., Kunkee, R. E., 1975: Effect of fumaric acid on malolactic fermentation in wines from warm areas. American Journal of Enology and Viticulture 25(4), 188-190.

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

Picariello, L., Rinaldi, A., Martino, F., Petracca, F., Moio, L., Gambuti, A., 2019: Modification of the organic acid profile of grapes due to climate changes alters the stability of red wine phenolics during controlled oxidation. Vitis 58, 127–133. DOI: 10.5073/vitis.2019.58.special-issue.127-133.

Pines, O., Even-Ram, S., Elnathan, N., Battat, E., Aharonov, O., Gibson, D., Goldberg, I., 1996: The cytosolic pathway of L-(-) malic acid synthesis in *Saccharomyces cerevisiae*: the role of fumarase. Applied Microbiology and Biotechnology **46**, 393–399. DOI: 10.1007/BF00166235.

Puyo, M., Simonin, S., Bach, B., Klein, G., Alexandre, H., Tourdot-Maréchal, R., 2023: Bio-protection in oenology by *Metschnikowia pulcherrima*: from field results to scientific inquiry. Frontiers Microbiology 14, 1252973. DOI: 10.3389/fmicb.2023.1252973.

Rankine, B. C., 1977: Developments in malolactic fermentation of Australian red table wines. American Journal of Enology and Viticolture 28, 27-33. DOI: 10.5344/ajev.1977.28.1.27.

Shanshiashvili, G., Baviera, M., Fracassetti, D., 2024: Exploring grape pressing for sparkling wine production: A comprehensive literature review on physicochemical parameters and technological applications. Applied Food Research 4(2), 100454. DOI: 10.1016/j.afres.2024.100454.

Sizzano, F., Bianconi, V., Blackford, M., Bieri, S., Vuichard, F., Monnard, C., Amiet, L., Spring, J. L., Dorsaz, E., Pfenninger-Bridy, N., Simonin, S., Bach, B., Bourdin, G., 2024: Use of *Lachancea thermotolerans* for the bioacidification of white grape musts: assays from the bench to the cellar scale. Fermentation 10, 458. DOI: 10.3390/fermentation10090458.

Tanpong, S., Khochamit, N., Pootthachaya, P., Siripornadulsil, W., Unnawong, N., Cherdthong, A., Tengjaroenkul, B., Wongtangtintharn, S., 2024: Citric acid by-product fermentation by *Bacillus subtilis* I9: a promising path to sustainable animal feed. *Veterinary Science* 11, 484. DOI: 10.3390/vetsci11100484.

Van Leeuwen C., Cornelis J. T., Edwards E. J., Fort G. W., Gowdy M., Scanlon T. M., Stolpman P., Templer A., van den Heuvel H. and Wang Y. (2024): Climate change impact on wine production. Nature Reviews Earth & Environment 5, 303-321. DOI: 10.1038/s43017-024-00521-5.

Vilela, A., 2019: Use of nonconventional yeasts for modulating wine acidity. Fermentation 5, 27. DOI: 10.3390/fermentation 5010027.

Vicente, J., Calderón, F., Santos, A., Marquina, D., Benito, S., 2021: High Potential of *Pichia kluyveri* and Other *Pichia* Species in Wine Technology. International Journal of Molecular Sciences **22**(3), 1196. DOI: 10.3390/ijms22031196.

Vion, C., Yeramian, N., Hranilovic, A., Masneuf-Pomarède, I., Marullo, P., 2024: Influence of yeasts on wine acidity: new insights into *Saccharomyces cerevisiae*. OENO One 58(4). DOI: 10.20870/oeno-one.2024.58.4.7877.

Yéramian, N., Chaya, C., Suárez Lepe, J. A., 2007: L-(−) malic acid production by *Saccharomyces* spp. during the alcoholic fermentation of wine. Journal of Agricultural and Food Chemistry 55(3), 912-917. DOI: 10.1021/jf061990w.