International Journal of Food Microbiology 413 (2024) 110583

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Application of white wine lees for promoting lactic acid bacteria growth and malolactic fermentation in wine

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ARTICLE INFO

Keywords:
Wine lees
Oenococcus oeni
Lactiplantibacillus plantarum
Brettanomyces bruxellensis
Malolactic fermentation
By-product

ABSTRACT

In the context of ecological transition, the use of wine by-products for industrial applications is a major challenge. Wine lees, the second wine by-product in terms of quantity, represent a source of nutrients that can be used for stimulating the growth of microorganisms. Here, white wine lees were used as a stimulating agent for the growth of wine lactic acid bacteria (LAB) and to promote wine malolactic fermentation (MLF) driven out by *Oenococcus oeni*. By adding freeze-dried wine lees to wines under different conditions – including different wine lees at different concentrations and different *O. oeni* strains at various initial populations - it was observed that wine lees can enhance the growth of LAB and reduce the duration of MLF. The chemical composition of wines was also evaluated, proving that wine lees do not compromise the quality of the wines. In addition, wine lees did not seem to promote the growth of spoilage microorganisms like as *Brettanomyces bruxellensis*. Altogether, this work reports the possibility of recovering the lees of white wine to obtain a product favoring the MLF of red wines. More general, we propose a recycling strategy of wine by-products to obtain new products for winemaking.

1. Introduction

In the context of the ecological transition, the valorization of industrial by-products and co-products is a necessity to move towards carbon neutrality. Wine production is a multistep process in which diverse by-products and wastes are generated (Dávila et al., 2017). Wine lees are the second most important in terms of amount of production, after grape pomace, both representing the 10 % and 21 % of the total mass, respectively. Wine lees consist of the solid part that decants to the bottom of wine tanks at the end of the alcoholic fermentation (AF). They are mainly composed of organic acids, carbohydrates, inorganic salts, proteins, phenolic compounds, plant residues from grapes, and the yeast biomass that conducted alcoholic fermentation (AF), depending on environmental conditions, agronomic characteristics, grape variety, and winemaking practices (e. g. racking or time of ageing in wood barrels or tanks). The European regulation (n°1308/2013) authorizes their use to produce alcohol, spirits and "*piquette*" only. However, other valorization strategies are possible.

The complex chemical composition of wine lees is a source of multiple and interesting compounds, which allows to develop new valorization strategies. Wine lees have been investigated with the aim of limiting oxidation of wine, improving its quality, or recovering phenolic compounds or tartaric acid that can be used in diverse applications (Bautista et al., 2007; Del Fresno et al., 2019; Pons-Mercadé et al., 2021; Arboleda-Mejia et al., 2022; Kontogiannopoulos et al., 2017, 2016). Moreover, there is an increasing interest in using wine lees by themself for different applications as additive in animal feeding (Câmara et al., 2020; Sato et al., 2020), as nutrient for vermicomposting (Nogales et al., 2020, 2005), as ingredient in food formulation (Alarcón et al., 2020; De Iseppi et al., 2021; Sharma et al., 2015), as antihypertensive in health (Bravo et al., 2022), or even as raw material in the production of nanomaterials (Varisco et al., 2017).

In the context of wine production, wine lees represent a potentially interesting source of nutrients and protective compounds for the lactic acid bacteria (LAB) which are used to perform the malolactic fermentation (MLF). *Oenococcus oeni* and to a lesser extent *Lactiplantibacillus*

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https://doi.org/10.1016/j.ijfoodmicro.2024.110583
Received 14 November 2023; Received in revised form 9 January 2024; Accepted 14 January 2024
Available online 19 January 2024

plantarum are the main LAB species driving MLF (Lonvaud-Funel, 1999). Most often they develop spontaneously in wine during or after AF and perform MLF when they reach a sufficient population. LAB have complex nutrient requirements and combined with the harsh conditions found in wine (low pH and moderate to high alcohol content), the correct achievement of MLF by indigenous LAB is sometimes compromised (Bech-Terkilsen et al., 2020). The use of selected LAB strains as starter cultures is a common practice to better control MLF (Torriani et al., 2011). Still, their inoculation in wine can reduce their viability and prevent the successful achievement of MLF. Yeast lees produced from diverse strains at laboratory scale could limit these difficulties as it has been shown that their addition in wine stimulate the capacity of *O. oeni* to perform MLF in a synthetic wine (Balmaseda et al., 2021). Their beneficial effect was related with an increase in nitrogenous compounds and mannoproteins released by yeasts. However, the effect of wine lees obtained from winemaking on LAB growth, survival and capacity to perform MLF is still unknown.

Besides a potential positive effect on LAB and MLF, the use of wine lees may have other beneficial or detrimental impacts in wine. For instance, the addition of yeast-assimilable nitrogen in the grape must modulates the sensory perception of wine by increasing the production of substituted esters by LAB during MLF (Lytra et al., 2020). There is a strong correlation between the increase in must nitrogen content, the production of substituted esters during MLF and the increasing perception of fruity aromas of wine (Lytra et al., 2020). However, the use of wine lees could increase the nutrients available for all microorganisms and promote the growth of undesirable yeasts or bacteria responsible of spoilages in wine. Apart from some spoilage LAB that rarely can develop at pH lower than 3.50–4.00 (Ribéreau-Gayon et al., 2006), the most challenging microorganism in wine is the yeast *Brettanomyces bruxellensis*. *B. bruxellensis* usually develops during wine ageing and depreciates considerably the sensory perception of wine by producing volatile phenols (Lleixà et al., 2021). Its ability to grow in wine depends on the strains and the characteristics of the wine, making a thorough understanding of its growing preferences difficult (Cibrario et al., 2020).

The aim of this study was to evaluate the potential of white wine lees to stimulate the growth of LAB and the achievement of MLF. The experiments were carried out mainly with strains of *O. oeni*, which is the main species used for MLF, but also *L. plantarum*. Then, we have evaluated the risk of spoilage linked to the addition of wine lees by analyzing their impact on the growth of *B. bruxellensis* and, we have analyzed their impact on aromatic compounds and the sensory perception of wine.

2. Materials and methods

2.1. Bacterial and yeast strains and growth conditions

Four commercial *O. oeni* strains were used in this study: VF (Vitilactic F, Martin Vialatte, Magenta, France), VP41 (Lalvin VP41, Lallemand Inc., Canada), IOEB-SARCO 277 (SB3, Laffort, Bordeaux, France), and IOEB-SARCO 450 (Lactoenos 450 Preac, Laffort, Bordeaux, France). Also, two L. *plantarum* strains (CRBO 0601 and CRBO 9640) from 'Biological Resources Centre Oenology' of Bordeaux University (CRBO) were used. Three other *B. bruxellensis* strains from CRBO collection were used: CRBO L0611, CRBO L0422, CRBO L0424.

All bacterial strains were grown in liquid grape juice medium composed of (for 1 L) 250 mL of red grape juice, 5 g of yeast extract, 1 mL of Tween 80, adjusted to pH 4.80. The cultures were incubated at 25 °C until late exponential phase. Bacterial enumeration was performed by epifluorescence microscopy for inoculation, and by plating in solid grape juice medium (supplemented with 20 g/L agar and 100 mg/L pimaricin) during the experiments. For inoculation in red wine, commercial freeze-dried *O. oeni* VF was used, hydrated following manufacturer's instructions.

B. bruxellensis strains were grown on YPG plates containing (for 1 L): 10 g of yeast extract, 10 g of peptone, 20 g of glucose and 20 g of agar. Each strain was gradually adapted to wines with successive subcultures.

2.2. Wine lees collection, preparation, and analysis

Three batches of wine lees (named lees 1, 2, and 3) were collected during 2021 vintage, and another one (lees 4) during vintage 2022. Table 1 summarizes the origin and other wine lees related characteristics.

All wine lees were freeze-dried (FreeZone 4.5 plus, Labconco) and stored at 4 °C in a hermetically closed jar until further analysis.

Total nitrogen of wine lees was measured according to the Kjeldahl method (European Commission [EC] regulation No: 152/2009, pp. 15–19) using a Gerhardt Protein and Nitrogen Analysis System, 115 VAC 50/60 Hz. The conversion from total nitrogen to protein depends on the type of protein present in the sample. Usually, the total nitrogen factor used for almost all food is 6.25.

The microbial composition of wine lees was evaluated by resuspending 100 mg of each wine lees in 1 mL of saline solution. Then, 100 μL of each suspension was spread out on grape juice and YPG plates. Isolated colonies were counted and identified at species level by MALDI-TOF mass spectrometry using *MALDI* Biotyper® (MBT) from Bruker and a homemade database (Windholtz et al., 2021).

2.3. Biomass production

The ability of *O. oeni* and *L. plantarum* to develop was tested in a wine by-product medium. First, a combination of different concentrations of commercial red grape juice and wine lees was tested. Grape juice medium containing 25, 50, or 100 mL/L of red grape juice was supplemented with 0, 2.5, 5, or 10 g/L of lees 1. In all cases pH was adjusted to 4.8. Screwed vials of 10 mL (Fisher Scientific, Hampton, New Hampshire, USA ref.: 11981523) were filled with 9 mL of the obtained media and inoculated with *O. oeni* VF for a population of 10^4–10^5 cell/mL. Vials were closed with screw cap-magnetic (Agilent Technologies, hdsp cap 18 mm PTFE/sil 100 pk, Les Ulis, France), perforated by two hypodermic needles: one for allowing CO_2 release (0.8 × 38 mm, Terumo, Tokyo, Japan), and the other (0.6 × 80 mm, B. Braun, Melsungen, Germany) connected to a 2 mL syringe (B. Braun, Melsungen, Germany) for allowing sampling. Fermentations were incubated statically at 25 °C for two weeks. Bacterial population every day. Each condition was tested in triplicate. We observed weak growth in media containing <100 mL/L of grape juice (data not shown), so these conditions were discarded for the following of this study, and the media of 100 mL/L with or without 10 g/L of wine lees at pH 4.80 was evaluated afterwards.

This selected medium was tested with three *O. oeni* strains – VP41, IOEB-SARCO 277, IOEB-SARCO 450 and the two L. *plantarum* strains (CRBO 0601 and CRBO 9640). 9 mL of medium were inoculated with each strain for a population of 10^5 cell/mL into the vial system as previously described. Each condition was incubated statically at 25 °C for two weeks – *O. oeni* – or three days – *L. plantarum* -. Besides, the effect of the temperature and pH was evaluated. For comparison, the same medium was evaluated at 20 °C or adjusting the pH to 3.50 for the three *O. oeni* strains. Each condition was tested in triplicate.

2.4. Malolactic fermentation

2.4.1. Malolactic fermentation performance evaluation

First, a screening of impact of wine lees on LAB growth was performed with wine lees 1 and *O. oeni* VF in wine like medium (WLM). WLM was prepared according to Balmaseda et al. (2023). *O. oeni* VF was inoculated to a population of 10^2, 10^4, or 10^6 cell/mL in presence of 0, 0.25, or 0.5 g/L of lees 1. The inoculated WLM was transferred to a 10 mL syringe (BD, Franklin Lakes, USA) coupled with a hypodermic needle (0.8 × 38 mm, Terumo, Tokyo, Japan) and incubated at 20 °C. Each condition was performed in triplicate. Bacterial population and L-malic acid concentration were monitored periodically. L-Malic Acid

Table 1
Origin and nitrogen amount of wine lees used in this study. The protein content equals N × 6.25, which means that non-protein nitrogen is included. All wine lees came from wines inoculated with *S. cerevisiae* Zymaflore CH9.

	Variety	Winery	Vintage	Collection	Nitrogen (%)*	Proteins (%)*
Lees 1	Viognier	Château Giscours (CDO Medoc, Labarde, France)	2021	After AF completion	3.24 ± 0.01	20.2 ± 0.1 [ab]
Lees 2	Viognier	Château Giscours (CDO Medoc, Labarde, France)	2021	After 3 months of ageing in barrel	4.03 ± 0.03	25.2 ± 0.3 [ab]
Lees 3	Chardonnay	Château Giscours (CDO Medoc, Labarde, France)	2021	After AF completion	2.92 ± 0.01	18.3 ± 0.1 [a]
Lees 4	Sauvignon blanc	Château Couhins (CDO Pessac-Leognan, France)	2022	After AF completion	5.15 ± 0.02	32.2 ± 0.2 [b]

Values shown are the mean of triplicates ± SD. Lower-case letters indicate a significant difference at $P \leq 0.05$ according to a Tukey post-hoc comparison test. n.d.: not determined.

* The percentages shown represent the amount of nitrogen or protein regarding to the amount of freeze-dried wine lees.

Assay Kit (Megazyme, Wicklow, Ireland) was used for L-malic acid quantification.

After the screening, the inoculation of 10^6 cell/mL was chosen for testing the impact of lees 2 and 3 on LAB growth, under the same conditions as lees 1. The MLF performance of two additional *O. oeni* strains – VP41, IOEB-SARCO 450 - was also evaluated in WLM with 0, 0.25, 0.5 g/L of wine lees 1.

The impact of wine lees on MLF was also evaluated in a red wine of Merlot from CDO Saint Emilion (France) containing 12.45 % (vol/vol) of ethanol, 2.07 g/L of L-malic acid, and pH 3.41. This wine had an initial LAB population of around 10^3 CFU/mL. 10 mL of wine were inoculated with 0.01 or 1 g/hL of commercial freeze-dried *O. oeni* VF, corresponding to approximately 10^4 and 10^6 cell/mL, respectively. A control condition without inoculation of *O. oeni* was also assessed. MLF performance was evaluated in the presence of 0, 0.25, and 0.5 g/L of wine lees (1, 2, and 3) at 20 °C. L-malic acid concentration was monitored periodically, and each condition was performed in duplicate.

2.4.2. Impact of wine lees in MLF performance and wine aromas

The impact of wine lees in MLF performance and impact on wine aroma was performed in two red wines at laboratory scale and in a winery. For the study at laboratory scale, a Merlot wine from Château Couhins (14.17 % (vol/vol) of ethanol, 0.99 g/L of L-malic acid, pH 3.42) was used. 0.5 L of wine were inoculated with 1 g/hL of commercial freeze-dried *O. oeni* VF. MLF was evaluated in presence or absence of 0.5 g/L of lees 4 at 20 °C, and bacterial viability and L-malic acid concentration were monitored periodically. Each condition was performed in duplicate.

For the experiment in a winery, the MLF of a red wine of Petit Verdot (Wine A, 14.23 % vol/vol of ethanol, 1.51 g/L of L-malic acid, pH 3.42) was monitored under cellar conditions. The wine was used to fill two barrels of 225 L for MLF: one without addition of wine lees (control) and one supplemented with 0.5 g/L of wine lees 4. MLF was conducted spontaneously at cellar temperature. Bacterial viability and L-malic acid concentration were monitored periodically.

2.4.3. Volatile compound analyses of wines after MLF

The impact of wine lees addition during MLF on esters composition of wine was evaluated. Chromatographic conditions and sample preparation were as optimised by Antalick et al., 2010: 14 esters were determined using solid phase microextraction (SPME) and gas chromatography coupled to mass spectrometry. Gas chromatography analyses were carried out on an HP 6890 GC system coupled to an HP 5972 quadrupole mass spectrometer (Hewlett-Packard), equipped with a Gerstel MPS2 autosampler. The mass spectrometer was operated in electron ionization mode at 70 eV in selected-ion-monitoring (SIM) mode. Esters were characterized by comparing their linear retention indices and mass spectra with those of standards.

Statistical data from chemical evaluations were analyzed using the ANOVA test followed by the Tukey post-hoc test (XLSTAT software), with a statistically significant level of 5 % ($p < 0.05$).

2.5. Brettanomyces bruxellensis growth

The effect of wine lees in *B. bruxellensis* growth was evaluated in two wines with three different strains. 0.5 g/L of wine lees (1 and 4) were added to wine A (Petit Verdot wine from Château Couhins, Section 2.4), and wine B (Cabernet Franc, 14.46 % vol/vol of ethanol, 1.25 g/L of L-malic acid, 0.7 g/L of residual sugars at pH 3.71) from Château Tournefeuille. Each wine without addition of wine lees was used as a control Each wine was inoculated with the three strains of *B. bruxellensis* (CRBO L0611, diploid from wine; CRBO L0422, triploid from beer; CRBO L0424, triploid from wine) to a population of 10^2 cell/mL. Cells were progressively acclimated to each wine from the initial culture medium before the experiment. Similarly, to MLF experiments, wines were aliquoted in syringes and incubated statically at 20 °C. Wine A and wine B had an initial total yeast population of 4.35×10^3 and 3.68×10^3 CFU/mL, respectively. Non-*Saccharomyces* yeasts were not detected by plating (< 10 CFU/mL). *B. bruxellensis* growth was evaluated at day 0, 9, 22, and 35 after inoculation by plating on non-*Saccharomyces* solid medium (20 g/L glucose, 10 g/L yeast extract, 10 g/L peptone, 10 g/L agar, and cycloheximide 500 mg/L). Then, colonies suspected to be *B. bruxellensis* were confirmed by MALDI-TOF. Each condition was evaluated in duplicate.

3. Results and discussion

3.1. Stimulation of lactic acid bacteria (LAB)

Here, we tested the potential use of freeze-dried white wine lees as only nutrient source (apart from the 100 mL/L of grape juice) in a new low-cost medium. Wine lees from different origins were tested. We showed that the growth of both species; *O. oeni* (Fig. 1.A) and *L. plantarum* (Fig. 1.C), was increased in presence of wine lees. Besides, no differences were observed between the growth curves obtained with the two different wine lees. The use of wine lees as nutrient in the development of microbial biomass is one of the proposed recycling alternatives to this byproduct. Indeed, wine lees represent an important source of nutrients (Balmaseda et al., 2021), and mainly nitrogenous compounds that are rather low in grape must. There are different studies that used wine lees in a defined medium to growth different *Lactobacillus* species (Bustos et al., 2004a, 2004b). These studies, using fresh wine lees, reported an increase in biomass production in presence of 20 g/L of wine lees, mainly with white wine lees, and especially after distillation, where fewer polyphenolic compounds are found.

To better characterize the growth of *O. oeni* in wine lees supplemented medium, we altered some parameters that directly impact the microbial growth as pH, and temperature. We observed that lowering the pH from 4.80 to 3.40 (a pH much closer to real grape must), or temperature from 25 °C to 20 °C (an easier operational setting temperature), did not impact on the maximal bacterial population (Fig. 1.B). This adds value to the described new and low-cost medium to be used in low demanding conditions, as it can directly be used by diluting the grape must, adding the wine lees, and incubating at a standard room temperature, around 20 °C.

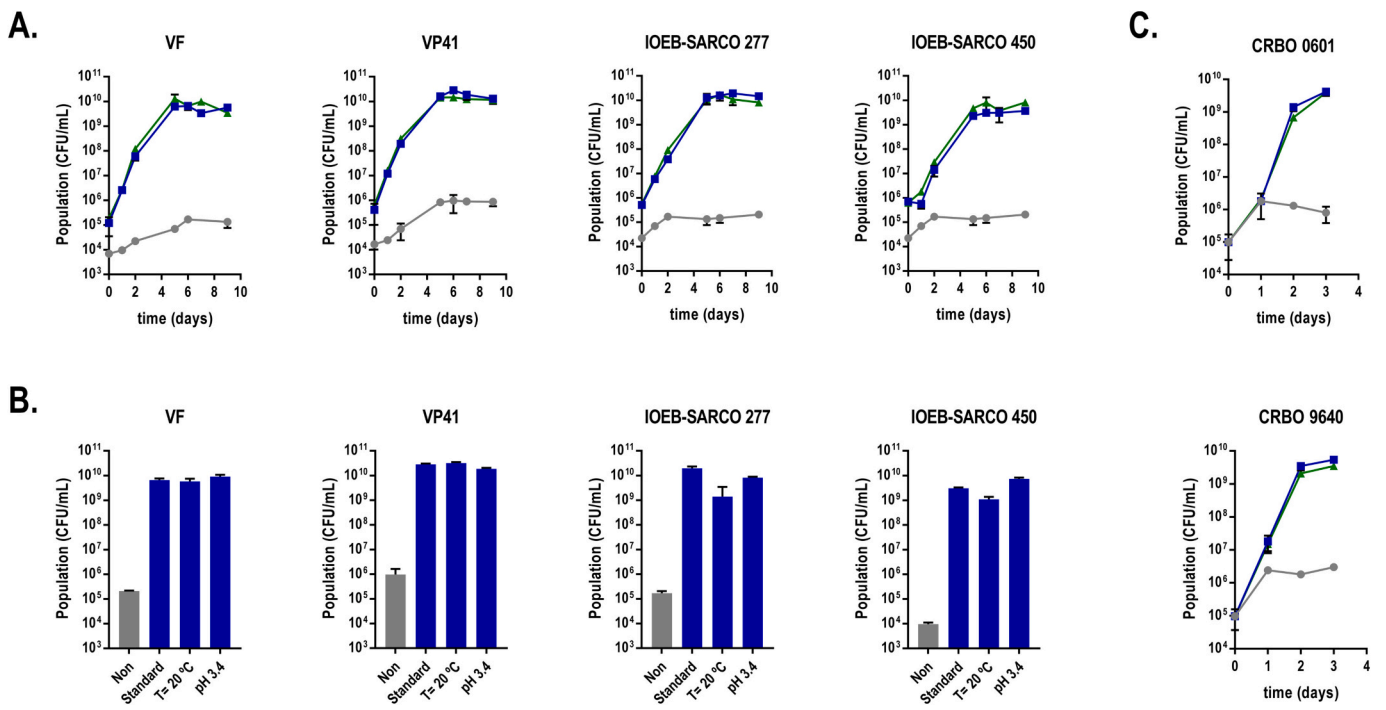

Fig. 1. Use of lees 1 as nutrient source in the designed culture medium containing 100 mL/L of red grape juice. A. Growth curves of *O. oeni* VF, VP41, IOEB-SARCO 277, and IOEB-SARCO 450 strains at 25 °C; (●) without lees, or in presence of 10 g/L of (■) lees 1, or (▲) lees 3. B. Maximal population obtained in the designed culture modifying one parameter at each time; in grey the maximal population obtained without adding lees, and in blue those obtained in presence of lees. C. Growth curves of L. *plantarum* CRBO-0601 and CRBO-9640 strains at 25 °C; (●) without lees, or in presence of 10 g/L of (■) lees 1, or (▲) lees 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The reported results can be related with the increase in nutrient and protective substances of wine lees. Supplementation of wine with wine lees can increase the soluble proteins in wine due to the autolytic process (Martínez-Rodriguez et al., 2001). Table 1 shows the total amount of nitrogen and corresponding protein fraction of the wine lees used for this study. Indeed, the quantity of protein in the wine lees tested was between the 18 and 32 % of the dried matter.

3.2. *Wine lees improves LAB growth and MLF*

In our study we used for the first time, freeze-dried white wine lees with the purpose of enhancing MLF in red wine. Using a regular inoculation regimen of 10^6 cell/mL of *O. oeni* we showed that MLF performance was ameliorated in presence of wine lees 1 with three different commercial bacterial strains (Fig. 2). Even if, all strains finished MLF in presence or absence of wine lees in the tested synthetic wine, in presence of wine lees the LAB growth kinetic is improved (Fig. 2.A). In addition, we observed a linear relationship between the MLF duration, and the wine lees concentration added in the wine (Fig. 2.C). This was true for the three tested strains, thus, reinforcing the hypothesis of that wine lees enhance MLF in wine conditions.

With the aim of reducing the inoculated starter culture concentration in wine with the use of wine lees, lower initial bacterial populations were tested: 10^2 and 10^4 cell/mL. All these conditions were tested with *O. oeni* VF strain, again in synthetic wine with lees 1. Results for the MLF performance, including population and L-malic acid dynamics are shown in Suppl. Fig. 1. Fermentations were followed for >100 days. It is interesting to observe that the tendency in both, 10^2 and 10^4 cell/mL was similar. There was a quick viability loss in those wines without wine less addition, around the day 20. With the addition of 0.25 g/L of wine lees the initial inoculated bacterial population was maintained up to 60 or 120 days for 10^2 and 10^4 cell/mL conditions, respectively. Still, it was not enough to reach the minimal bacterial population to perform the MLF. Finally, the addition of 0.5 g/L of wine lees, enabled a progressive bacterial growth that was enough for performing MLF (Fig. 2.B, Suppl. Fig. 1). Here, we proved that the use of wine lees at a sufficient concentration, can enable the growth of low bacterial population up to 10^8 CFU/mL to perform MLF. These results, also, showed the possibility of reducing the quantity of starter culture needed to inoculate a wine before MLF. This is a promising application, still to be exploited, since the duration of the fermentation can be largely extended, even if it finally concludes.

Apart from testing the effect of wine lees addition in synthetic wine, fermentations in red wine were also evaluated (Fig. 3). Similarly, to the previous tests, this wine was supplemented (or not) with 0.25 g/L and 0.5 g/L of lees 1, 2, and 3. These wines were inoculated with *O. oeni* VF in their commercial freeze-dried form according to manufacturer's instructions to reach a final concentration of around 10^4 cell/mL and 10^6 cell/mL. Also, a wine with no inoculation was performed as a control.

As previously observed in synthetic wine (Fig. 2), the addition of wine lees to red wine enhanced MLF performance (Fig. 3). First, in the wine inoculated with 10^6 cell/mL, all conditions finished MLF, including the control fermentation with no addition of wine lees. Generally, all the three wines supplemented with wine lees at two different concentrations, finished MLF after 12 days. Besides, it was observed that wine lees 2 at 0.25 g/L lasted 15 days, a bit more than the other ones (Fig. 3.B). The wines inoculated with 10^4 cell/mL of *O. oeni* showed a similar tendency. Wines without addition of wine lees did not perform MLF. In these wines, L-malic acid concentration was stuck at 1.5 g/L, which was the initial value (Fig. 2.A). Interestingly, the positive effect on the reduction of MLF time, was observed with all wine lees, in the two inoculation conditions. This supports the idea that the observed effect is dose dependent and relays on the added wine lees quantity, as observed in synthetic wine (Fig. 2.C).

Moreover, considering the origin of lees 1 and 2, which came from the same wine but collected with three months of difference, we can observe interesting results (Fig. 3). When performing MLF with no inoculation or 10^4 cell/mL in presence of 0.5 g/L of wine lees, the

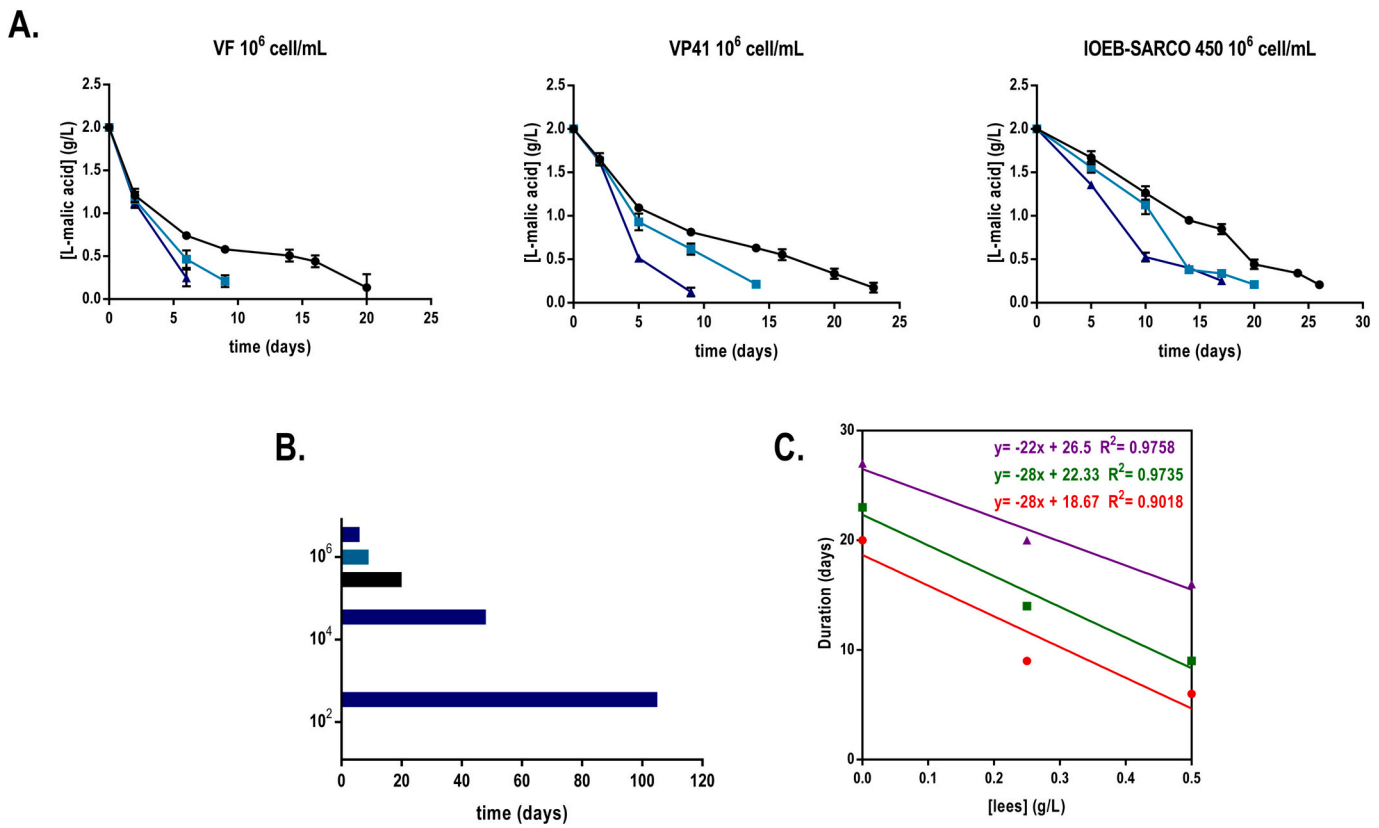

Fig. 2. Malolactic fermentation performance of *O. oeni* in presence of wine lees in WLM. A. MLF of *O. oeni* VF, VP41, and IOEB-SARCO 450 strains inoculated in WLM at an initial population of 10^6 cell/mL: (●) without lees, or in presence of (■) 0.25 g/L, or (▲) 0.5 g/L of lees 1. B. MLF duration of *O. oeni* VF in WLM at an initial population of 10^2, 10^4, and 10^6 cell/mL: (●) without lees, or in presence of (■) 0.25 g/L, or (▲) 0.5 g/L of lees 1. No column means not concluded MLF. C. Correlation of the duration of MLF with the added concentration of lees for the *O. oeni* strains VF (●), VP41 (■), IOEB-SARCO 450 (▲). Each value is the mean of the triplicates.

duration observed is higher in lees 2 (43 days in spontaneous, 40 days for the inoculated one), regarding to lees 1 (33 days in spontaneous, 36 for the inoculated one). In addition, the modality of 0.25 g/L of lees 2 did not enable MLF completion. This points that during ageing, the quality of the recovered wine lees changes and thus their performance on MLF. This fact is not surprising since yeast lees undergo an autolytic process, in which some intracellular and membrane/wall-related compounds are released (Alexandre and Guilloux-Benatier, 2006). We could hypothesize that during autolysis, some compounds related with the positive effect observed in *O. oeni* growth, have been released to the wine, and thus, not found in the collected wine lees. Another hypothesis could be concerning the lysis of nutritive compounds used by the LAB. In this sense, the observed results highlight that autolytic process could impact nutritional properties of wine lees when using them as MLF potentiator.

Overall, the results observed with these wine lees enhanced *O. oeni* growth and MLF performance in synthetic and natural wine. In addition, it enabled the completion of MLF in wines with low bacterial population, which did not finish in the case of no addition of wine lees. We also observed that the drying process did not interfere with the positive effect here observed. Freeze-drying could represent a suitable tool for preserving and storing wine lees and enables an easier dosage comparing to fresh liquid wine lees. The added concentration of wine lees in this study were 0.25 g/L and 0.5 g/L, which corresponds to 25 g/hL and 50 g/hL, respectively. These concentrations are not unfamiliar to other oenological preparations (also commercialized in powder form) used as activators for AF or MLF, which also rage from 20 to 50 g/hL. That is why the use and concentrations proposed in this study are in form for an easy industrial adaptation.

3.3. Volatile compounds

At the end of MLF (for wine samples without or with wine lees treatment) concentrations of several esters increased significantly depending on the matrix (Table 2). More precisely the short- and branched-chain alkyl fatty acid esters levels such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate and ethyl 3-methylbutanoate increased significantly for both red wine matrices, Merlot, and Petit Verdot (Table 2). These results confirm those of other studies that have demonstrated that LAB may increase concentrations of some esters in wine during MLF, thus influencing its aromatic composition and suggesting that lactic acid bacteria possess esterase activity (Gammacurta et al., 2018; Lytra et al., 2020). In addition, for samples analyzed after MLF with wine lees, concentration of some esters is significantly higher from those without wine lees addition, such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoateare for Merlot or 2-methylpropyl acetate concentration for Petit Verdot. These results suggest a positive impact of the white wine lees addition as these short- and branched-chain alkyl fatty acid esters, since they are known to have a great sensory impact on increasing the perception of fruity aromas (Lytra et al., 2017). The increase of some esters' levels may be due to the higher nitrogen levels related to addition of wine lees (Alexandre and Guilloux-Benatier, 2006). Literature provides evidence that after MLF, short- and branched-chain alkyl fatty acid levels increased in wines made from musts with the highest nitrogen content (Lytra et al., 2020). These same authors have also demonstrated that the production of these substituted esters revealed a significant increase in qualitative fruity aromas, opening new perspectives for further experiments to confirm the higher contents in nitrogenous compounds in the samples with wine lees as well as the increase of qualitative fruity notes.

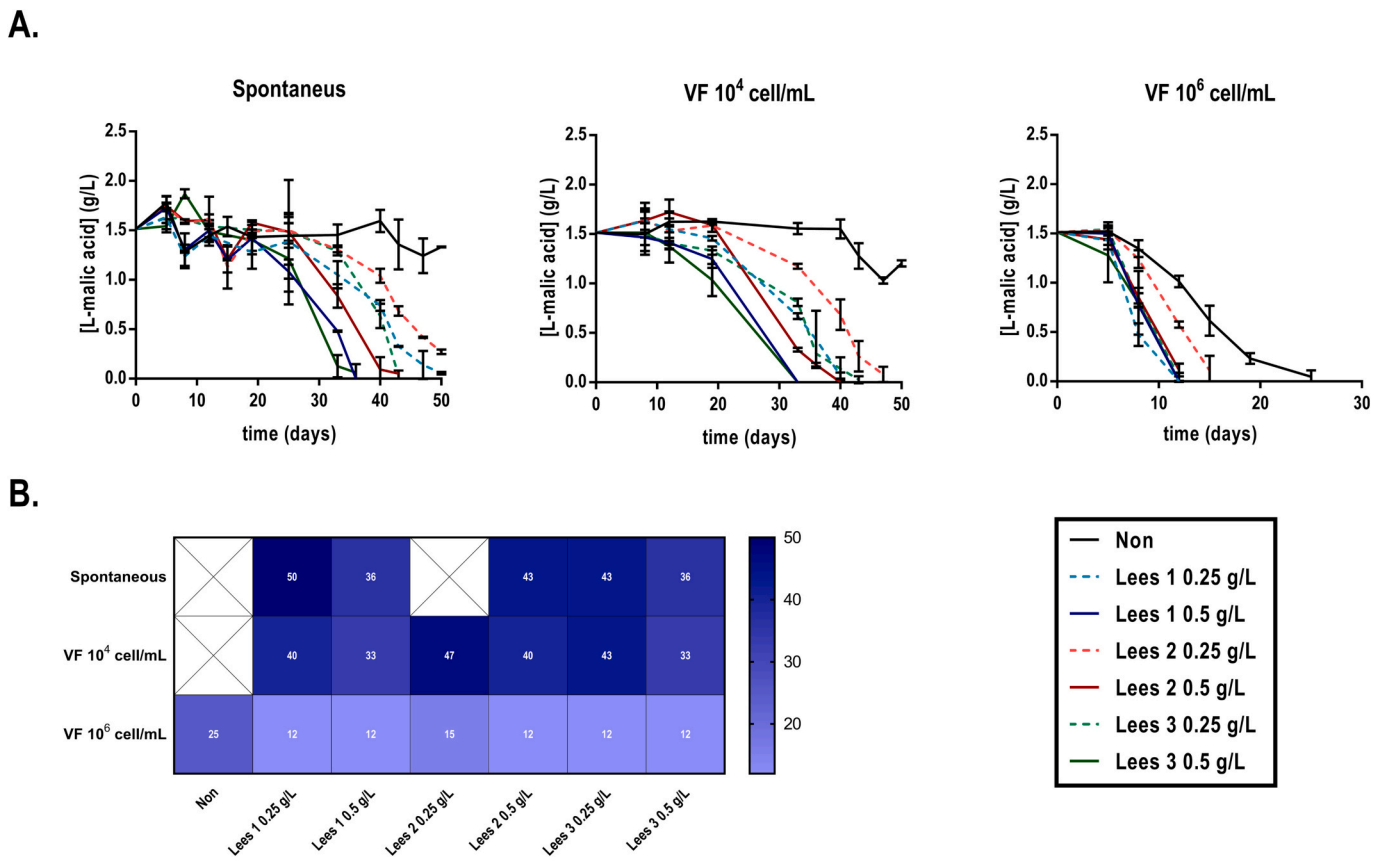

Fig. 3. Malolactic fermentation (MLF) performance of *O. oeni* VF in presence of wine lees in natural red wine. A. L-malic consumption during MLF with no addition of *O. oeni* starter culture, or with 10^4, 10^6 cell/mL of starter culture. Codes for the colour interpretation are present in the figure. Each value is the mean ± SD of the duplicates. B. Heatmap of the duration of MLF in absence/presence of wine lees with no addition of *O. oeni* starter, or with 10^4, 10^6 cell/mL of starter culture. No value corresponds to a non-concluded MLF. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Volatile ester composition (μg/L) of Merlot and Petit Verdot wines before and after malolactic fermentation, without (non) or with lees (lees). t_0, t_f means before and after MLF, respectively.

	Merlot			Petit Verdot		
	t_0	t_f non	t_f lees	t_0	t_f non	t_f lees
Ethyl propanoate	70.4 ± 2.2^a	72.9 ± 2.7^a	84.9 ± 1.1^b	49.9 ± 1.2	51 ± 0.6	53.1 ± 0.2
Ethyl 2-methylpropanoate	31.1 ± 1.4^a	53.4 ± 1.7^b	62.7 ± 0.5^c	38 ± 0.9^a	43.3 ± 0.6^b	44.7 ± 0.1^b
Propyl acetate	103.2 ± 1.2^b	95.4 ± 2.3^a	109.3 ± 1.5^b	56.3 ± 0.7	57.2 ± 0.7	58.6 ± 0.3
2-methylpropyl acetate	45.8 ± 0.5^b	40.6 ± 0.6^a	47.0 ± 0.7^b	64.8 ± 1.2^a	81.8 ± 0.3^b	115.9 ± 0.3^c
Ethyl butanoate	314.8 ± 0.1^a	309.7 ± 9^a	360.4 ± 5^b	171 ± 3.4	174.6 ± 1.5	177.4 ± 1.3
Ethyl 2-methylbutanoate	4.7 ± 0.1^a	7.7 ± 0.1^b	9.0 ± 0.2^c	6.6 ± 0.1^a	7.4 ± 0.1^b	7.4 ± 0.1^b
Ethyl 3-methylbutanoate	8.2 ± 0.2^a	14.8 ± 0.1^b	17.6 ± 0.4^c	7 ± 0.1^a	8.1 ± 0.1^b	8.0 ± 0.1^b
Butyl acetate	n.d.	4.1 ± 0.1	3.6 ± 1.8	1.2 ± 0.3^b	1.1 ± 0.1^b	< LOQ^a
Isoamyl acetate	915.9 ± 110.3	879 ± 2	1000.5 ± 16.9	788.1 ± 20.5	798.6 ± 1.1	760.9 ± 34.9
Ethyl hexanoate	559.3 ± 19^a	557.7 ± 16.1^a	660.2 ± 14.8^b	230.2 ± 2.8^a	238.3 ± 5.2^b	247.6 ± 0.6^b
Hexyl acetate	6.6 ± 0.2^b	5.3 ± 0.1^a	6.1 ± 0.1^b	2.1 ± 0.1^a	2.2 ± 0.1^a	2.7 ± 0.1^b
Ethyl octanoate	596.7 ± 113.2	549.6 ± 69.4	778.3 ± 24.8	350.1 ± 3.8	343.6 ± 12	422.5 ± 31.4
Ethyl phenylacetate	1.6 ± 0.1^a	2.1 ± 0.1^b	2.5 ± 0.1^c	5.6 ± 0.3	5.5 ± 0.1	6.3 ± 0.3
Phenylethyl acetate	69.6 ± 1.4^a	67.4 ± 1.5^a	74.3 ± 0.3^b	112.9 ± 4.5	104.7 ± 2.3	114.8 ± 5.7

Values shown are the mean of duplicates ± SD. Statistics were done comparing wines of each variety independently. Lower-case letters indicate a significant difference at $P \leq 0.05$ according to a Tukey post-hoc comparison test. No lower-case letter indicates no significant differences. n.d.: not detected; < LOQ: under the limit of quantification.

3.4. Microbial composition of wine lees

Wine lees were also analyzed in terms of microbiological composition. Apart from *S. cerevisiae*, no other wine related microbial species were found (data not shown). As an example, the data of wine lees 4 is here reported. Fresh wine lees, before the drying process, were plated on YPD and grape juice plates. The average population on YPD was 5×10^7 CFU/mL, and the MALDI-TOF identification resulted in a 100 % of *S. cerevisiae* yeast population. It is not surprising since wine lees were collected from wines immediately after AF, where *S. cerevisiae* is usually the most abundant yeast species. Bacteria population found in this wine lees was lower. A total population of 1.6×10^2 CFU/mL was detected on

grape juice plates, which corresponded to a 100 % of *Bacillus megaterium*. *B. megaterium*, and generally *Bacillus* genus, is a common environmental bacterium, typically found in the soil (Saxena et al., 2020), not related with wine production. After drying (by freeze-drying), the dominance of *S. cerevisiae* population was maintained but its concentration was reduced to 18 CFU/mg, corresponding to 2.7–3.6 $\times$ 10^3 CFU/mL of fresh wine lees; a reduction of 4 log units from the initial 5 $\times$ 10^7 CFU/mL detected. In the case of the bacterial population found on grape juice agar, the concentration was reduced to 1.1 CFU/mg, increasing its diversity. In this step, *Streptomyces halstedii*, *Bacillus mycoides*, *Bacillus megaterium*, *Paenibacillus pabuli* species were detected. All of them related with the environmental microbiome, probably due to the non-aseptic drying process.

Altogether, wine lees represent a potential microbial load when adding them to a fermentative process. Nevertheless, in the case of wine fermentation, the detected species cannot be considered as a potential risk of contamination since are related to soil, and thus, not participate in the fermentative process. Besides, it is worth noting that the significant *S. cerevisiae* population detected could also participate in AF. Still, the viability of this wine lees could decrease during storage time, and specially from vintage to vintage. Interestingly, non-spoliating microorganisms were found in these wine lees, for instance *B. bruxellensis,* which is also related with the healthy status of the wines from whom they were collected.

3.5. *Brettanomyces bruxellensis*

After studying the positive effect of wine lees in *O. oeni* and *L. plantarum* growth, we evaluated the impact of the addition of wine lees on growth in a very well-known wine spoiling microorganism as *B. bruxellensis*. *B. bruxellensis* growth in wine can compromise the quality of the product due to the production of volatile phenols. In this sense, it was important to address the effect of wine lees addition in *B. bruxellensis* growth. There is not much literature addressing the effects of wine lees on *B. bruxellensis*. However, it is already described that wine lees contains high microbial population during vinification, and usually *B. bruxellensis* is mostly detected (Renouf et al., 2008; Renouf and Lonvaud-Funel, 2004).

In our conditions, we did not observe an impact of wine lees addition – at a concentration of 0.5 g/L – on *B. bruxellensis* growth in tested red wines (Fig. 4). Three *B. bruxellensis* strains were inoculated at an approximately 10^2–10^3 CFU/mL in two different wines. At the same time, wines were supplemented (or not) with lees 1 and 4. Viability of the yeast was monitored for 40 days. These results were confirmed by Kruskal-Wallis test that showed no significant effect of wine lees on *B. bruxellensis* growth (Table 3). Thus, wine lees did not seem to promote the growth of *B. bruxellensis* in wine in this study. However, *B. bruxellensis* is a very diverse yeast and we cannot formally conclude at this step of study. For commercial exploitation of freeze-dried wine lees, it will be essential to explore a large number of *B. bruxellensis* strains on a wider range of wines.

More generally to the findings here explained, we propose a workflow for the application of white wine lees in food industry. The use of wine lees after validation of their bioactivity and health safety has a major interest for the valorization of this by-product. Not only from a circular economy point of view, but also of great economic interest for

Table 3
Results of the Kruskall-Wallis' test for *B. bruxellensis* growth. Test was performed independently for each day, considering all the values of the three yeast strains in the two tested wines.

	9 days	22 days	37 days
No lees	19.79[a]	18.58[a]	16.29[a]
Lees 1	18.50[a]	18.96[a]	20.08[a]
Lees 4	17.21[a]	17.96[a]	19.13[a]

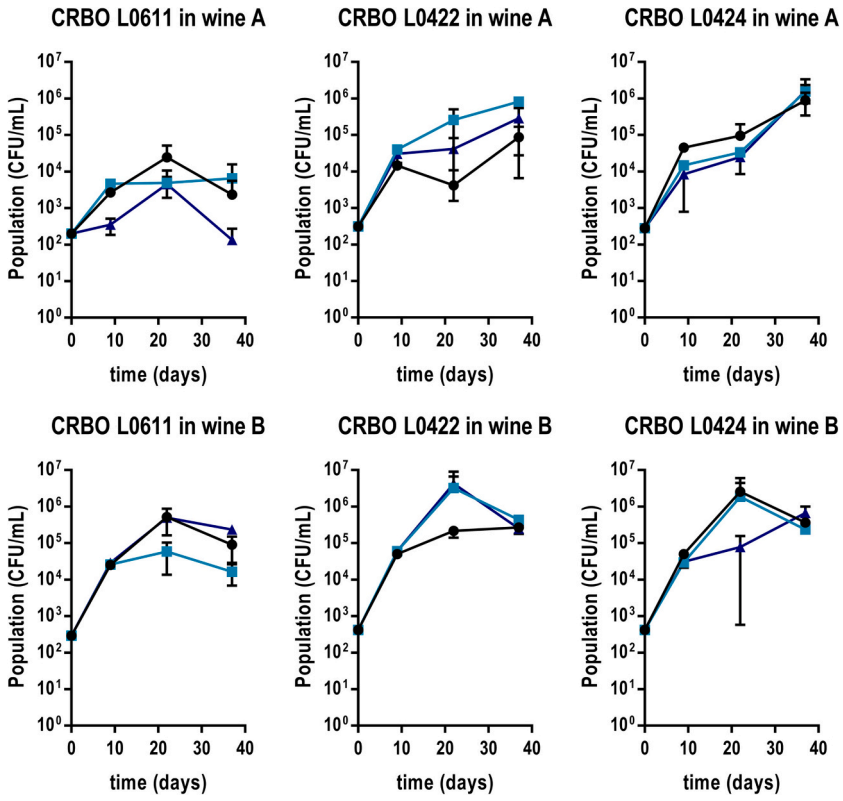

Fig. 4. Growth of *B. bruxellensis* CRBO L0611, CRBO L0422, CRBO L0424 in two different wines (A and B), (●) without lees, or in presence of 0.5 g/L of (■) lees 1, or (▲) lees 4. Each value is the mean ± SD of the duplicates.

the winemakers in comparison with other products on the market that are expensive, and not always efficient. Hence, we first propose their drying by a conventional industrial process used for food additives – spry drying –. Second, an analysis that guarantees the health safety of the lees will have to be conducted in order to promote their use as a new product authorized by the food legislation.

4. Conclusion

The potential exploitation of wine lees in wine industry, particularly with the aim of enhancing wine MLF was studied. First, a new low-cost culture medium based on diluted grape juice and wine lees was tested and proved as suitable for producing wine related LAB, commonly used for performing MLF, as *O. oeni* and *L. plantarum*. A medium containing 100 mL/L of grape juice and 10 g/L of freeze-dried wine lees was sufficient to produce up to 10^{10} CFU/mL. Second, it was demonstrated that the addition of wine lees can reduce MLF duration in high bacterial population wines ($> 10^4$ cell/mL), and at the same time, enable bacterial growth up to perform the MLF, and thus, be able to conclude the fermentation. Third, the addition of wine lees affects positively the quality of wine volatile composition, as the concentrations of some esters, involved in the fruity aromas of red wine, are significantly higher. Fourth, the addition of wine lees in red wine seems to not increase the growth of spoilage microorganisms as *B. bruxellensis*. Altogether, this work represents an integrative study in which wine lees are proposed as LAB growth activator, and it is demonstrated that there is no potential microbial spoilage risk or that it could compromise the aromatic quality of wine.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110583.

CRediT authorship contribution statement

Aitor Balmaseda: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Cécile Miot-Sertier:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. **Georgia Lytra:** Formal analysis, Resources, Writing – review & editing. **Benjamin Poulain:** Investigation, Methodology, Resources. **Cristina Reguant:** Supervision, Writing – review & editing. **Patrick Lucas:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Claudia Nioi:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

Authors are grateful Chateau Giscours for providing wine lees and Château Couhins for allowing the conduction of MLF in presence of wine lees. Also, we want to thank again Château Couhins, and Château Tournefeuille for providing us wine for the experiments.

Funding

The authors thank the ANR project (ANR-21-CE43-0003) for fundings. Aitor Balmaseda is a Margarita Salas postdoc researcher (2021URV-MS-25, Spanish Ministry of Universities financed with European Union- NextGenerationEU).

References

Alarcón, M., López-Viñas, M., Pérez-Coello, M.S., Díaz-Maroto, M.C., Alañón, M.E., Soriano, A., 2020. Effect of wine lees as alternative antioxidants on physicochemical and sensorial composition of deer burgers stored during chilled storage. Antioxidants 9, 1–17. https://doi.org/10.3390/antiox9080687.

Alexandre, H., Guilloux-Benatier, M., 2006. Yeast autolysis in sparkling wine - a review. Aust. J. Grape Wine Res. 12, 119–127. https://doi.org/10.1111/j.1755-0238.2006.tb00051.x.

Antalick, G., Perello, M.C., de Revel, G., 2010. Development, validation and application of a specific method for the quantitative determination of wine esters by headspace-solid-phase microextraction-gas chromatography-mass spectrometry. Food Chem. 121, 1236–1245. https://doi.org/10.1016/j.foodchem.2010.01.011.

Arboleda-Mejia, J.A.A., Ricci, A., Figueiredo, A.S., Versari, A., Cassano, A., de Pinho, M. N., Parpinello, G.P., 2022. Membrane-based operations for the fractionation of polyphenols and polysaccharides from winery sludges. Food Bioprocess Technol. 15, 933–948. https://doi.org/10.1007/s11947-022-02795-3.

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 Microbiol. 99, 103839 https://doi.org/10.1016/j.fm.2021.103839.

Balmaseda, A., Lorentzen, M., Dutilh, L., Bauduin, R., Guichard, H., Ollivier, S., Miot-Sertier, C., Lucas, P.M., 2023. Alcoholic fermentation drives the selection of *Oenococcus oeni* strains in wine but not in cider. Int. J. Food Microbiol. 400, 110276 https://doi.org/10.1016/j.ijfoodmicro.2023.110276.

Bautista, R., Fernández, E., Falqué, E., 2007. Effect of the contact with fermentation-lees or commercial-lees on the volatile composition of white wines. Eur. Food Res. Technol. 224, 405–413. https://doi.org/10.1007/s00217-006-0336-7.

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. Aust. J. Grape Wine Res. 26, 188–206. https://doi.org/10.1111/ajgw.12436.

Bravo, F.I., Mas-Capdevila, A., López-Fernández-Sobrino, R., Torres-Fuentes, C., Mulero, M., Alcaide-Hidalgo, J.M., Muguerza, B., 2022. Identification of novel antihypertensive peptides from wine lees hydrolysate. Food Chem. 366, 130690 https://doi.org/10.1016/j.foodchem.2021.130690.

Bustos, G., Moldes, A.B., Cruz, J.M., Domínguez, J.M., 2004a. Formulation of low-cost fermentative media for lactic acid production with *Lactobacillus rhamnosus* using vinification lees as nutrients. J. Agric. Food Chem. 52, 801–808. https://doi.org/10.1021/jf030429k.

Bustos, G., Moldes, A.B., Cruz, J.M., Domínguez, J.M., 2004b. Evaluation of vinification lees as a general medium for *Lactobacillus* strains. J. Agric. Food Chem. 52, 5233–5239. https://doi.org/10.1021/jf049763m.

Câmara, J.S., Lourenço, S., Silva, C., Lopes, A., Andrade, C., Perestrelo, R., 2020. Exploring the potential of wine industry by-products as source of additives to improve the quality of aquafeed. Microchem. J. 155, 104758 https://doi.org/10.1016/j.microc.2020.104758.

Cibrario, A., Miot-Sertier, C., Paulin, M., Bullier, B., Riquier, L., Perello, M.C., de Revel, G., Albertin, W., Masneuf-Pomarède, I., Ballestra, P., Dols-Lafargue, M., 2020. *Brettanomyces bruxellensis* phenotypic diversity, tolerance to wine stress and wine spoilage ability. Food Microbiol. 87, 103379 https://doi.org/10.1016/j.fm.2019.103379.

Dávila, I., Robles, E., Egüés, I., Labidi, J., Gullón, P., 2017. The Biorefinery Concept for the Industrial Valorization of Grape Processing By-products. Sustainable Solutions. Elsevier Inc., Handbook of Grape Processing By-Products https://doi.org/10.1016/B978-0-12-809870-7.00002-8.

De Iseppi, A., Marangon, M., Vincenzi, S., Lomolino, G., Curioni, A., Divol, B., 2021. A novel approach for the valorization of wine lees as a source of compounds able to modify wine properties. LWT 136, 110274. https://doi.org/10.1016/j.lwt.2020.110274.

Del Fresno, J.M., Morata, A., Ricardo-da-Silva, J.M., Escott, C., Loira, I., Lepe, J.A.S., 2019. Modification of the polyphenolic and aromatic fractions of red wines aged on lees assisted with ultrasound. Int. J. Food Sci. Technol. 54, 2690–2699. https://doi.org/10.1111/ijfs.14179.

European Commission. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009R0152.

European Parliament. Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organization of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32013R1308.

Gammacurta, M., Lytra, G., Marchal, A., Marchand, S., Christophe Barbe, J., Moine, V., de Revel, G., 2018. Influence of lactic acid bacteria strains on ester concentrations in red wines: specific impact on branched hydroxylated compounds. Food Chem. 239, 252–259. https://doi.org/10.1016/j.foodchem.2017.06.123.

Kontogiannopoulos, K.N., Patsios, S.I., Karabelas, A.J., 2016. Tartaric acid recovery from winery lees using cation exchange resin: optimization by Response Surface Methodology. Sep. Purif. Technol. 165, 32–41. https://doi.org/10.1016/j.seppur.2016.03.040.

Kontogiannopoulos, K.N., Patsios, S.I., Mitrouli, S.T., Karabelas, A.J., 2017. Tartaric acid and polyphenols recovery from winery waste lees using membrane separation

processes. J. Chem. Technol. Biotechnol. 92, 2934–2943. https://doi.org/10.1002/jctb.5313.

Lleixà, J., Martínez-Safont, M., Masneuf-Pomarede, I., Magani, M., Albertin, W., Mas, A., Portillo, M.C., 2021. Genetic and phenotypic diversity of *Brettanomyces bruxellensis* isolates from ageing wines. Food Biosci. 40, 100900 https://doi.org/10.1016/j.fbio.2021.100900.

Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Anton. Leeuw. Int. J. Gen. Mol. Microbiol. 76, 317–331. https://doi.org/10.1023/A:1002088931106.

Lytra, G., Franc, C., Cameleyre, M., Barbe, J., 2017. Study of substituted ester formation in red wine by the development of a new method for quantitative determination and enantiomeric separation of their corresponding acids. J. Agric. Food Chem. 65 (24), 5018–5025. https://doi.org/10.1021/acs.jafc.7b00979.

Lytra, G., Miot-Sertier, C., Moine, V., Coulon, J., Barbe, J.C., 2020. Influence of must yeast-assimilable nitrogen content on fruity aroma variation during malolactic fermentation in red wine. Food Res. Int. 135, 109294 https://doi.org/10.1016/j.foodres.2020.109294.

Martínez-Rodriguez, A.J., Carrascosa, A.V., Polo, M.C., 2001. Release of nitrogen compounds to the extracellular medium by three strains of *Saccharomyces cerevisiae* during induced autolysis in a model wine system. Int. J. Food Microbiol. 68, 155–160. https://doi.org/10.1016/S0168-1605(01)00486-X.

Nogales, R., Cifuentes, C., Benítez, E., 2005. Vermicomposting of winery wastes: a laboratory study. J. Environ. Sci. Health B 40, 659–673. https://doi.org/10.1081/PFC-200061595.

Nogales, R., Fernández-Gómez, M.J., Delgado-Moreno, L., Castillo-Díaz, J.M., Romero, E., 2020. Eco-friendly vermitechnological winery waste management: a pilot-scale study. SN Appl. Sci. 2, 1–13. https://doi.org/10.1007/s42452-020-2455-3.

Pons-Mercadé, P., Giménez, P., Gombau, J., Vilomara, G., Conde, M., Cantos, A., Rozès, N., Canals, J.M., Zamora, F., 2021. Oxygen consumption rate of lees during sparkling wine (Cava) aging; influence of the aging time. Food Chem. 342, 128238 https://doi.org/10.1016/j.foodchem.2020.128238.

Renouf, V., Lonvaud-Funel, A., 2004. Racking are key stages for the microbial stabilization of wines. J. Int. Sci. de la Vigne et du Vin 38, 219–224. https://doi.org/10.20870/oeno-one.2004.38.4.914.

Renouf, V., Strehaiano, P., Lonvaud-Funel, A., 2008. Effectiveness of dimethlydicarbonate to prevent *Brettanomyces bruxellensis* growth in wine. Food Control 19, 208–216. https://doi.org/10.1016/j.foodcont.2007.03.012.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A., 2006. Handbook of Enology: Volume 1, The Microbiology of Wine and Vinifications, Handbook of Enology. https://doi.org/10.1002/0470010398.

Sato, Y., Nakanishi, T., Wang, L., Oishi, K., Hirooka, H., Kumagai, H., 2020. In vitro and in vivo evaluations of wine lees as feeds for ruminants: effects on ruminal fermentation characteristics, nutrient digestibility, blood metabolites and antioxidant status. Livest. Sci. 241, 104217 https://doi.org/10.1016/j.livsci.2020.104217.

Saxena, A.K., Kumar, M., Chakdar, H., Anuroopa, N., Bagyaraj, D.J., 2020. *Bacillus* species in soil as a natural resource for plant health and nutrition. J. Appl. Microbiol. 128, 1583–1594. https://doi.org/10.1111/jam.14506.

Sharma, A.K., Kumar, R., Azad, Z.R.A.A., Adsule, P.G., 2015. Use of fine wine lees for value addition in ice cream. J. Food Sci. Technol. 52, 592–596. https://doi.org/10.1007/s13197-013-1007-x.

Torriani, S., Felis, G.E., Fracchetti, F., 2011. Selection criteria and tools for malolactic starters development: an update. Ann. Microbiol. 61, 33–39. https://doi.org/10.1007/s13213-010-0072-x.

Varisco, M., Zufferey, D., Ruggi, A., Zhang, Y., Erni, R., Mamula, O., 2017. Synthesis of hydrophilic and hydrophobic carbon quantum dots from waste of wine fermentation. R. Soc. Open Sci. 4, 170900 https://doi.org/10.1098/rsos.170900.

Windholtz, S., Dutilh, L., Lucas, M., Maupeu, J., Vallet-Courbin, A., Farris, L., Coulon, J., Masneuf-Pomarède, I., 2021. Population dynamics and yeast diversity in early winemaking stages without sulfites revealed by three complementary approaches. Appl. Sci. 11 (6), 2494. https://doi.org/10.3390/app11062494.