



## From bioprotective effects to diversification of wine aroma: Expanding the knowledge on *Metschnikowia pulcherrima* oenological potential

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### ABSTRACT

Microbial diseases are of major concern in vitiviculture as they cause grape losses and wine alterations, but the prevention with chemical substances represents a risk to human health and agricultural ecosystem. A promising alternative is the biocontrol and bioprotection activity of non-*Saccharomyces* yeasts, such as *Metschnikowia pulcherrima*, which also presents positive oenological traits when used in multistarter fermentations. The aim of this study was to assess the impact of a selected *M. pulcherrima* strain in the post-harvest withering and vinification of Garganega grapes to produce the sweet ‘passito’ wine Recioto di Gambellara DOCG (Italy). *M. pulcherrima* was firstly inoculated on grape at the beginning of the withering process, and afterwards in must for multistarter sequential microfermentation trials with *Saccharomyces cerevisiae*. Microbiological, chemical, and sensory analyses were carried out to monitor the vinification of treated and control grapes. Grape bunches during withering were a suitable environment for the colonization by *M. pulcherrima*, which effectively prevented growth of molds. Differences in grape must composition were observed, and the diverse inoculation strategies caused noticeable variations of fermentation kinetics, main oenological parameters, wine aroma profile, and sensory perception. *M. pulcherrima* proved effective to protect grapes against fungal infections during withering and contribute to alcoholic fermentation generating wine with distinguished aromatic characteristics.

### 1. Introduction

*Vitis vinifera* L. cv. Garganega is among the oldest and most important Italian grapes. Its characteristic late ripening, loose bunches, and hard skins make this grape variety suitable for the process of grape withering, used in the area of Gambellara to obtain the distinctive “passito” sweet wines Gambellara Classico Vin Santo DOC and Recioto di Gambellara DOCG (Consorzio Tutela Vini Gambellara, 2014; D’Onofrio et al., 2021; Tomasi & Gaiotti, 2008).

Grape withering (“appassimento”) consists in air-drying the harvested grape bunches in large well-ventilated rooms (“fruttaio”), usually lasting between 60 and 90 days. The slow water evaporation causes the concentration of sugars and acids in berries and also triggers some significant changes in grape metabolism, activating or inhibiting the biosynthesis of phenolic and aromatic compounds, as well as modifying the qualitative and quantitative composition of grape microbiota (Salveti et al., 2016; Slaghenaufi et al., 2020; Torriani et al., 2011).

Grape berry surface is colonized by a complex microbial population, including molds, bacteria, and yeasts, whose abundance and diversity depend on the phenological maturity, climate, viticultural practices, and grape variety (Lorenzini & Zapparoli, 2019). The low water activity and exosmosis on berry surface during withering make it a unique habitat for epiphytic populations (Lorenzini et al., 2013; 2016).

The health of grapes for withering process greatly affects the resident microbiota. The formation of noble rot by *Botrytis cinerea* can positively modify the wine organoleptic profile, but uncontrolled environmental conditions in a fruttaio, above all a high relative humidity, favor the development of grey rot and other mold pathogens, which may seriously impair the grape yield and quality (De Filippis et al., 2019; Lorenzini et al., 2018; Simonato et al., 2019). Skin laceration causes the release of sugary juices and nutrients, supporting the multiplication of yeasts and acetic acid bacteria and attracting potential vectors of microbial pathogens (Barata et al., 2012; Lleixà et al., 2018; Lorenzini et al., 2015).

As the saprophytic growth of filamentous fungi is the main concern

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for winegrowers, when adverse climatic conditions occur, then synthetic chemicals are traditionally employed to reduce the incidence of infections. However, their indiscriminate use has caused increased pollution of the agricultural ecosystem with consequent risks also for human health, in addition to the emergence of resistance by pathogens. Moreover, the persistence of those toxic compounds in grape must may negatively affect yeast activity during alcoholic fermentation (Gava et al., 2021; Lemos Junior et al., 2020; Lorenzini et al., 2016).

Thus, in recent years, natural antagonists or phytosanitary products have arisen as alternative solutions to inhibit mycelial growth and conidia germination. The antagonistic activity of biocontrol agents (BCAs) involves competition for nutrients and space, production of antimicrobial molecules and lytic enzymes, parasitism, and the induction of resistance mechanisms in the host plant (Dukare et al., 2019; Freimoser et al., 2019). In particular, antagonistic yeasts naturally colonize fruits and vegetables, are highly tolerant to possible stress conditions both pre- and post-harvest, have simple nutritional requirements, and easily grow large biomass volumes on inexpensive substrates, generating great interest as BCAs (Agarbatl et al., 2022; Maluleke et al., 2022; Ribes et al., 2018).

When grapes are ready for pressing, the control of possible contaminants is generally achieved with sulphur dioxide (SO<sub>2</sub>), which has, besides a strong antimicrobial effect, important antioxidant and antioxidant activities. This preservative became thus almost indispensable in winemaking process, although more recent health concerns, especially for sensitive people, triggered a rush towards the reduction in the amount of SO<sub>2</sub> in foods and beverages (Lisanti et al., 2019; Nardi, 2020). Likewise pre- and post-harvest biocontrol, the concept of bioprotection is associated with the use of non-*Saccharomyces* yeast species to counteract the development of any contaminants in the pre-fermentation phase and at the beginning of fermentation (Gianvito et al., 2022).

Among the yeast species with antimicrobial activity towards grape and wine spoilage organisms, *Metschnikowia pulcherrima* is one of the best studied (Gore-Lloyd et al., 2019; Pawlikowska et al., 2019; Sipiczki, 2020). This species is a common component of the grape microbiota, but, given its low fermentative power and low tolerance to ethanol (up to 4–5% v/v), it is associated with the first days of alcoholic fermentation. Nevertheless, in multistarter fermentation with *Saccharomyces cerevisiae*, some interesting characters of *M. pulcherrima* in the early fermentation phase can significantly impact the wine quality and aromatic complexity, such as release of hydrolytic enzymes, increased production of glycerol and glutathione, and reduced yield of ethanol, acetaldehyde, and acetic acid (Binati et al., 2022; Morata et al., 2019; Vicente et al., 2020). In the alcoholic fermentation of passito wines, with a high sugar and acid content being slowly fermented at low temperatures (De Filippis et al., 2019; Torriani et al., 2011), the impact of *M. pulcherrima* inoculation on the final characteristics of these wines has yet to be tested.

Therefore, the use of biocontrol and bioprotection technologies in fruttuato and in winery assumes great importance, making it possible to reduce chemical inputs and modulate the wine organoleptic profile, responding to the growing consumer demand for distinguished, healthier, and more sustainable wines. This study aimed at verifying whether the inoculation of a selected strain of *M. pulcherrima* on grapes in post-harvest withering and, subsequently, in must, can lead to significant benefits on the winemaking of sweet 'passito' wine Recioto di Gambellara DOCG.

## 2. Material and methods

### 2.1. Yeast and mold strains

The active dry yeast *M. pulcherrima* Level<sup>2</sup> Initia (Lallemand, Castel D'Azzano, Italy), hereinafter named LI, was tested for antagonistic activity *in vitro* and *in vivo* during grape withering toward molds, and in microvinification trials, as co-starter with *S. cerevisiae* Uvaferm 228

(Lallemand). This cryophilic *S. cerevisiae* strain is routinely used by the winery Tenuta Maule to produce Vin Santo and Recioto di Gambellara DOCG.

For the *in vitro* assay, the type strain of *M. pulcherrima* (*M. pulcherrima* NRRL Y7111<sup>T</sup>) and seven type strains of former species previously assigned to the *pulcherrima* clade, whose identification was updated to *M. pulcherrima* ("*M. andauensis*" NRRL Y48695, "*M. fruticicola*" NRRL Y27328, "*M. leonuri*" NRRL Y6546, "*M. rubicola*" NRRL Y6064, "*M. shanxiensis*" NRRL Y48710, "*M. sinensis*" CBS 10357, and "*M. zizyphicola*" NRRL Y48712), according to recent taxonomic revision (Sipiczki, 2022), were used. All yeasts were provided by the USDA-ARS Culture Collection (NRRL, Illinois, USA).

Colonies were isolated from the rehydrated dried preparations and the yeasts were maintained under cryo-preservation at –80 °C in the Verona University Culture Collection – Department of Biotechnology (VUCC-DBT).

The *Botrytis cinerea* strain B05.10 (ref. D-071295, VTT Culture Collection, Finland) was the mold chosen for the antagonistic assays and was also deposited at VUCC-DBT.

### 2.2. In vitro antagonistic assay against *B. cinerea*

Antimicrobial activity of the *M. pulcherrima* strains against *B. cinerea* B05.10 was evaluated through the method of contemporary growth on agar plates, as described by Lemos Junior et al. (2020). Briefly, yeast cells were grown overnight in YPD broth (10 g/L Yeast extract, 20 g/L bacteriological Peptone, 20 g/L Dextrose) at 27 °C, washed twice, and re-suspended in saline solution (0.9% w/v NaCl). Cells were microscopically counted (Fast Read 102®, Biosigma, Cona, Italy) and diluted to reach a concentration of approx.  $1 \times 10^7$  cells/mL. *B. cinerea* B05.10 was grown on PDA medium (4 g/L Potato extract, 4 g/L Dextrose, 20 g/L Agar) at 25 °C for 5 days. All reagents were purchased from Sigma-Aldrich (Milan, Italy).

A 10-μL droplet from the yeast cell suspension was streaked orthogonally in the diameter of a PDA agar plate. Then, two small discs excised from the edges of the mycelial growth of *B. cinerea* were placed on opposite sides of the streaked yeast culture. Each of the nine *M. pulcherrima* strains was tested in quadruplicate and control plates were streaked with sterile saline solution. After incubation at 25 °C for 5 days, the average radial growth of *B. cinerea* in control plates (C) and the average radial growth of *B. cinerea* in the plates streaked with each yeast (T) were measured. Radial mycelial growth inhibition (I%) was calculated by using the following formula:  $I\% = [(C - T)/C] \times 100$ .

### 2.3. In vivo antagonistic assay against molds

Grape bunches of *Vitis vinifera* L. cv. Garganega were harvested from the vineyards of Tenuta Maule in Capitello located in Selva di Montebello, North-East of Italy (45°47'N, 11°35'E) on the 30th September 2021 and transferred to a fruttuato with natural ventilation. Around 85 kg of grape bunches were split in two batches and each batch was divided into 17 crates, with an average of 2.5 kg of grapes in each.

*M. pulcherrima* LI was used following the manufacturer's instructions, with some modifications for the application on grapes to be withered. The active dried yeast was firstly rehydrated in 10 volumes of water at 25 °C, considering a dosage of 5 g yeast per 10 kg grape. The cell suspension was gently mixed and, after 20 min of resting, was sprayed over the grape bunches of the 17 crates of one batch (labelled TG). Grape of the control batch (CG) was sprayed with the same amount of water. Grapes remained in the fruttuato for around four months, until the 10<sup>th</sup> March 2022, when they were transferred to the cellar for pressing. Dehydration rate during withering was estimated by measuring the weight loss of grapes every two weeks, while room temperature and humidity were monitored with a digital thermo-hygrometer H5075 (Govee, Hong Kong).

## 2.4. Multistarter microvinification trials

The protocol and fermentation aids normally used by Tenuta Maule for the vinification of Recioto di Gambellara were adapted to the laboratory scale. Withered grapes from the TG and CG batches were separately loaded into a basket press at the winery on the 11<sup>th</sup> March 2022. Pectolytic enzyme Zima Blanc Plus (Francy Oenology, San Bonifacio, Italy) was added at a concentration of 20 mg/L to the pressed grapes and clarification was carried out by cold static decantation for 24 h at 14 °C. The musts did not receive any SO<sub>2</sub> addition. Approximately 10 L of the clarified must from each batch were transferred refrigerated to the laboratory and divided into nine sterile 1-L glass bottles filled to the top. Aliquots of both grape musts were frozen at -20 °C until further analysis.

Three different inoculation regimes were prepared for each of the two must batches, in triplicate, identified with the codes CC, CS, CM, TC, TS, and TM. The first letter refers to must obtained from treated (T) or control (C) grapes. The second letter indicates the inoculation strategy: inoculation of *S. cerevisiae* at the beginning of fermentation (CC and TC; control trials); inoculation of *S. cerevisiae* 48 h after the beginning of fermentation (CS and TS); and inoculation of *M. pulcherrima* at the beginning of fermentation and of *S. cerevisiae* 48 h later (CM and TM). The active dry yeasts *M. pulcherrima* LI and *S. cerevisiae* Uvaferm 228 were rehydrated in 10 volumes of water at 25 °C and 38 °C, respectively, following the manufacturer's instructions and inoculated at a dosage of 200 mg/L. Then, musts were supplemented with the amino acid-based fermentation activator Enervin Aromatique (Francy Oenology). All bottles were closed with loose caps to allow CO<sub>2</sub> release, incubated under static conditions at 18 ± 1 °C, and weighed daily during the fermentation.

Four days after the beginning of fermentation, 300 mg/L of the nitrogenous nutrient based on inactivated yeasts OPTI-MUM WHITE™ (Lallemand) and 10 mg/L of the pectolytic enzyme Aromapiù (Francy Oenology) were added to all bottles. On the ninth day, 150 mg/L of amino acid-based nitrogenous nutrient Enervin Flory (Francy Oenology) were added and on the eighteenth day 200 mg/L of inorganic nitrogen-based nutrient Enervin Middle (Francy Oenology).

When the alcohol by volume achieved around 12.5% v/v on each bottle (reaching the alcohol level and the residual sugar set up for Recioto di Gambellara DOCG), as estimated by the weight loss due to CO<sub>2</sub> release, fermentation was stopped by adding 100 mg/L potassium metabisulphite (K<sub>2</sub>S<sub>2</sub>O<sub>5</sub>; Sigma-Aldrich) and the racked wines were kept at 4 °C. One month later, the stabilized wines were clarified by centrifugation and bottled in hermetically sealed glass bottles, maintained at 4 °C until further analyses.

## 2.5. Monitoring of microbial population

The evolution of microbial population was followed throughout grape withering and alcoholic fermentation of musts. During withering, a representative sample of grape bunches (about 100 g) was collected every month from TG and CG batches. Grapes were pressed and juice was serially diluted in saline solution and plated on Wallerstein Laboratory (WL) nutrient agar plates (Sigma-Aldrich), supplemented with 100 mg/L chloramphenicol (Merck, Darmstadt, Germany) to inhibit bacterial growth. Serial dilutions of fermenting musts were plated on the same medium but sampled on a weekly basis. After 48-h incubation at 27 °C, differential microbial plate counting was carried out based on the diverse colony morphologies observed.

## 2.6. Chemical analysis of musts and wines

Must and wine samples were centrifuged (5,000 × g, 10 min) and the clarified supernatants were loaded into the automated Analyzer Y15 (BioSystems S.A., Barcelona, Spain). Specific enzymatic kits were used to measure the following parameters in grape must: residual sugars

(glucose and fructose), primary amino nitrogen (PAN), ammonium, and total acidity. Yeast assimilable nitrogen (YAN) was calculated from the sum of PAN and 0.8225 × ammonium. Besides these parameters, acetic acid, acetaldehyde, glycerol, free sulphites, and total sulphites were measured in wine samples. The pH at 20 °C of must and wine samples was determined using a Crison Basic 20 pH meter (Hach Lange, Barcelona, Spain).

## 2.7. Quantification of volatile compounds

Volatile organic compounds (VOCs) were quantified by gas chromatography coupled to mass spectrometry (GC-MS). Extraction of VOCs from wine samples was carried out with different methodologies for the diverse families of chemical compounds.

Solid Phase Extraction (SPE) through a BOND ELUT-ENV cartridge (Agilent Technologies, Santa Clara, CA, USA) was used for the quantification of alcohols, esters, fatty acids, benzenoids (except methyl salicylate) and p-menthane-1,8-diol, following the procedure described by Slaghenaufi et al. (2019). Headspace Solid Phase Microextraction (HS-SPME) with a 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) was used for the quantification of free terpenes, norisoprenoids and methyl salicylate, using the protocol of Slaghenaufi and Ugliano (2018). For the quantification of volatile sulphur-containing compounds, SPME was applied using a polydimethylsiloxane-divinylbenzene fiber (PDMS/DVB) (Supelco) exposed to sample headspace for 30 min, according to Slaghenaufi et al. (2021).

The GC-MS analyses were performed on an HP 7890A gas chromatograph (Agilent Technologies) coupled to a 5977B quadrupole mass spectrometer, equipped with a MPS3 autosampler (Gerstel, Müllheim an der Ruhr, Germany). Separation was performed using a DB-WAX UI capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent Technologies) and helium (6.0 grade) as carrier gas. Different programs of the GC oven were set up for the SPE and SPME vials, according to the references cited above. Mass spectrometer was operated in electron ionization (EI) and the spectra were acquired in Selected Ion Monitoring (SIM) mode.

Samples were analyzed in random order. For the calibration, each analyte was prepared in seven concentration points and three replicates per point in model wine solution (12% v/v ethanol, 3.5 g/L tartaric acid, pH 3.5), extracted and analyzed as described for the samples in SPE-GC-MS and SPME-GC-MS methods. Linear term for calibration curves were obtained using Chemstation software (Agilent Technologies) by linear regression, plotting the response ratio (analyte peak area/internal standard peak area) against concentration ratio (analyte added concentration/internal standard concentration).

## 2.8. Sensory analysis

A sorting task was performed four months after the end of fermentations to identify the sensory differences among wines, following the procedure described by Alegre et al. (2017), with minor modifications. A group of 13 judges, all considered wine experts according to Parr et al. (2002), participated in the sensory evaluation. Wine samples (20 mL) were poured into ISO 3591:1977 glasses (<https://www.iso.org/standard/9002.html>, accessed on 10<sup>th</sup> March 2023), labelled with random three-digit codes, and covered with plastic Petri dishes. All wines were served at room temperature (22 ± 1 °C) and presented at the same time in random order for each judge. To evaluate panel reproducibility, one sample was served twice. Panelists were asked to sort the wine samples into groups based on aroma similarities, exclusively by orthonasal evaluation. No limits were set for the number of groups to be formed.

## 2.9. Statistical analysis

Statistical analysis was performed using the XL-STAT 2017 software (Addinsoft SARL, Paris, France). Comparisons of grape treatment, inoculation strategy, and their interaction were performed based on one-way and two-way analysis of variance (ANOVA) followed by post-hoc Tukey's HSD (Honestly Significant Difference) test, with a statistical significance threshold set at 95% ( $p$ -value < 0.05). Principal Component Analysis (PCA) was calculated using the results of quantification of volatile compounds. For the sorting task, data was organized into individual similarity binary matrices ( $7 \times 7$  samples; 0 = different and 1 = similar) for each judge. A co-occurrence matrix was calculated by the sum of all panelists and used for obtaining a dendrogram by hierarchical cluster analysis with the Ward criterion.

Furthermore, data from chemical analysis was normalized, hierarchically clustered by Ward's minimum variance method and Euclidean distance metric and plotted in the form of a heatmap using MetaboAnalyst 5.0 (Xia & Wishart, 2011).

## 3. Results and discussion

### 3.1. *In vitro* inhibition of *B. cinerea*

A preliminary evaluation of the antagonistic activity of *M. pulcherrima* against *B. cinerea*, one of the most important fungal pathogens threatening grapes, was conducted *in vitro*. Results of mycelial inhibition by the tested nine strains of *M. pulcherrima* are shown in Fig. 1.

The inhibition of the mycelial growth ranged from 12.4% (strain NRRL Y48695) to 33.9% (CBS 10357), with the commercial strain LI resulting among the most efficient strains. Our results agree with those of Esteves et al. (2023) and Pawlikowska et al. (2019) who reported a remarkable variability among *M. pulcherrima* strains, since the efficacy of yeast against grape and wine contaminants is species- and strain-dependent (Gianvito et al., 2022). Besides *B. cinerea*, recent investigations also showed biocontrol activity of *M. pulcherrima* against other fungal pathogens, such as *Acrostalagmus luteoalbus* (synonym *Verticillium cinnabarinum*), *Alternaria alternata*, *Aspergillus niger*, *Botrytis caroliniana*, *Fusarium fujikuroi*, *Fusarium oxysporum*, *Mucor* spp., and *Penicillium expansum* (Esteves et al., 2023; Gore-Lloyd et al., 2019; Pawlikowska et al., 2019).

Given these results and considering that *M. pulcherrima* LI is a commercial strain carefully characterized in wine fermentation, it was selected for the subsequent trials.

### 3.2. Biocontrol activity during grape withering

Microbial population dynamics during grape withering was monthly monitored (Fig. 2). In particular, the peculiarities of colony morphology allowed to differentially count *M. pulcherrima*, other yeast species, and filamentous fungi (Binati et al., 2019; Polizzotto et al., 2016).

Remarkable differences between the *M. pulcherrima* treated (TG) and control (CG) grapes were found. Since the beginning of the trial, *M. pulcherrima* population was higher on the TG batch than on the CG ones. Interestingly, viable counts of this yeast in both batches remained stable during time, proving that grapes during withering are a suitable environment for its colonization and persistence. Thus, a single inoculation treatment was sufficient to support a high population of *M. pulcherrima* LI during the five months of withering, dispensing the need for further treatments.

The biocontrol activity of *M. pulcherrima* against filamentous fungi was confirmed in this study, as extensively noticed on a laboratory scale, and in multiple vineyard trials (Agarbati et al., 2022; Freimoser et al., 2019). Indeed, an evident difference regarded the counts of molds, which were lower in grapes treated with LI during the entire withering process. In the CG batch, this population showed a constant increase over time, while on TG, after an initial growth, a decrease was observed, reaching the highest gap between the two batches in the fifth month (around  $10^6$  CFU/mL in CG and less than  $10^3$  CFU/mL in TG). These levels were found by Lorenzini et al. (2013), who reported a mold population between  $10^4$  and  $10^6$  CFU/mL in naturally withered grapes. Samples collected on the 160<sup>th</sup> day of withering were from grapes already transferred to the cellar for pressing. The observed increase of mold population in both batches could be related to contaminations from cellar environment, although TG showed 30 times lower mold counts than CG.

Interestingly, *M. pulcherrima* was the consistently dominant species on TG, while other yeast species were more abundant than *M. pulcherrima* on CG. Nevertheless, the quantitative level of non-*Saccharomyces* yeasts was similar in the two grape batches, although, qualitatively, a greater variety of colonies were visually observed on the plates of the CG batch (Supplementary Fig. 1). It is well-known that berries infected by molds have a wider population diversity than healthy grapes (Barata et al., 2012; Lleixà et al., 2018; Lorenzini et al., 2018), but, in the present study, it could be argued that the overpowering presence of *M. pulcherrima* on TG hindered the detection of minor species, which were more evident on CG.

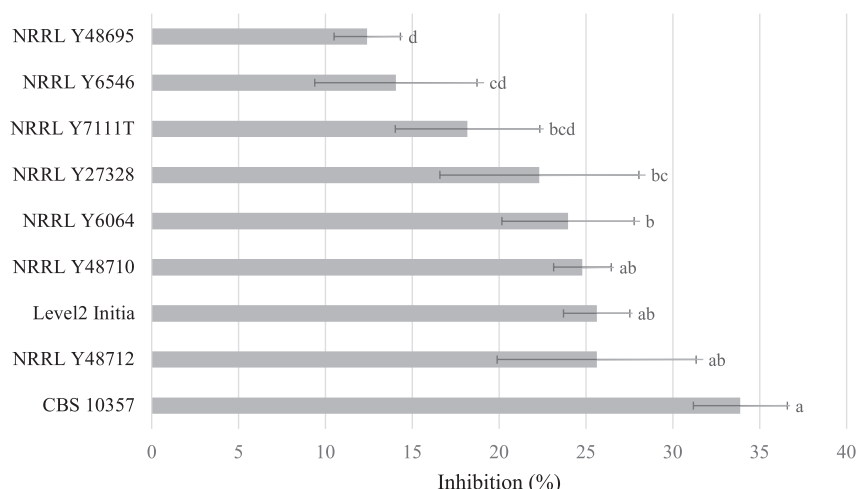


Fig. 1. Mycelial inhibition (%) of *B. cinerea* B05.10 by nine strains of *M. pulcherrima*. Different letters indicate statistically significant differences ( $p < 0.05$ ).

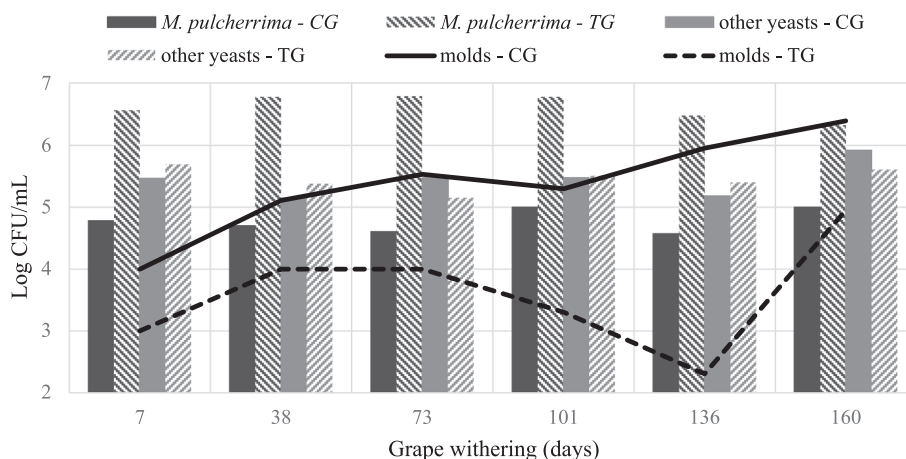


Fig. 2. Microbial population (Log CFU/mL) dynamics during the withering of Garganega grapes, either control (CG) or treated (TG) with *M. pulcherrima*.

### 3.3. Dehydration kinetics during grape withering and must composition

The progress of grape withering was monitored measuring the percentage weight loss of Garganega grape bunches respect to the initial weight (Fig. 3).

Dehydration was faster during the first month, corresponding with the warmest period. The average temperature was above 20 °C at the onset of grape withering in early October, then it slowly decreased during the first 50 days. It stayed below 10 °C from the end of November until mid-February, reaching close to 0 °C by the end of December. The final days of withering, late February/early March, saw a slight acceleration of weight loss, when temperature increased again to about 10 °C and relative humidity was at its lowest, below 50%. During the first four months of withering, relative humidity ranged between 50 and 90% (Supplementary Fig. 2).

Despite a similar dehydration kinetics, at the end of withering the grapes treated with *M. pulcherrima* LI lost about 52% of their weight, while the control grapes reached almost 48%. This difference between the two batches was built already in the first month and maintained throughout the withering until the pressing of grapes. A higher dehydration rate, leading to a faster withering process, could be advantageous, as the vinification process would start earlier and grapes would be less exposed to risks of contamination in the fruttato (Lorenzini et al., 2013), and VOCs emission and retention would be enhanced (Sanmartin et al., 2021).

Since viticultural (skin thickness, size of the berries, compactness of the bunches) and environmental (ventilation, temperature, relative

humidity, light) conditions were the same for the CG and TG batches, the variable that presumably affected withering was the berry surface microbial population (Lorenzini et al., 2013; Sanmartin et al., 2021; Shmulevitz et al., 2023; Zenoni et al., 2016). The variation observed in the dehydration rate was reflected in the chemical composition of grape musts. After pressing and clarification, and before yeast inoculation, the key oenological parameters of the musts were analyzed and are reported in Table 1.

In agreement with the greater weight loss of TG batch, a higher content of sugars and acids was found in the must obtained from these grapes. On the other hand, the levels of organic and inorganic nitrogen were higher in the must from CG, although the quantity was rather low in both musts. To equalize the possible negative effects caused by

Table 1

Concentration of major chemical compounds of withered Garganega grape musts either control (CG) or treated (TG) with *M. pulcherrima* LI, after pressing and decanting. Mean  $\pm$  standard deviation of two replicates.

Must	Sugar (g/L)	PAN (mg/L)	Ammonium (mg/L)	YAN (mg/L)	Total acidity (g/L)
CG	347.25 $\pm$ 0.33	50.50 $\pm$ 0.14	27.60 $\pm$ 8.49	73.20 $\pm$ 6.84	4.60 $\pm$ 0.42
TG	384.34 $\pm$ 0.65	41.50 $\pm$ 0.42	19.10 $\pm$ 2.97	57.21 $\pm$ 2.02	5.25 $\pm$ 0.07

PAN: Primary Amino Nitrogen; YAN: Yeast Assimilable Nitrogen.

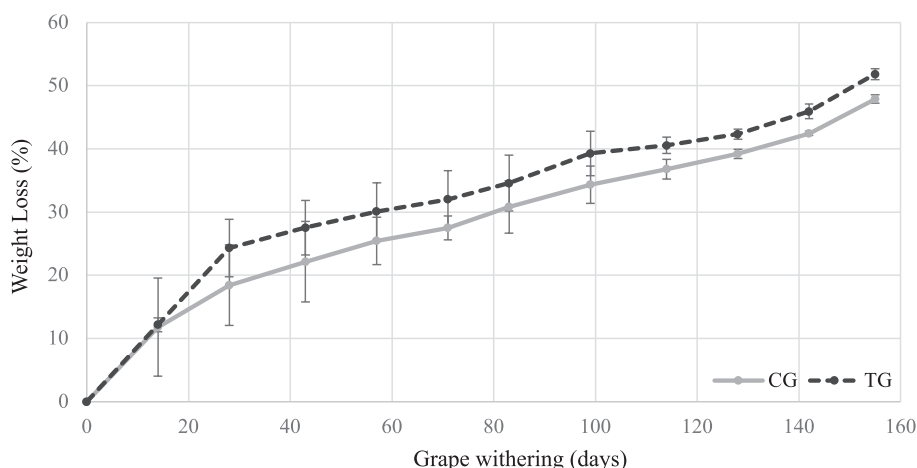


Fig. 3. Weight loss (%) during the withering of Garganega grapes, either control (CG) or treated (TG) with *M. pulcherrima*.

starvation stress, the same regime of nutrient supplementation was followed in the subsequent microvinification trials, aiming to achieve sufficient YAN levels for yeast fermentation.

### 3.4. Fermentation kinetics and yeast population

The musts obtained from the CG and TG batches were further divided into three aliquots, each inoculated with a different scheme, as detailed in Section 2.4. The progress of alcoholic fermentation was monitored in the six fermentations and presented as weight of carbon dioxide produced per 100 g of fermenting must (Fig. 4).

The most evident difference was the fastest fermentation of must from control grapes (CC, CS, and CM), while fermentation of treated grapes (TC, TS, and TM) took around two weeks longer (about 40 days vs 55 days). In all pairwise comparisons, the fermentations of TG musts slowed down compared to their CG must counterparts. This behavior could be related to the stress of higher sugar concentrations and total acidity.

At the beginning of fermentation, a shorter lag phase and more rapid start was observed for the musts inoculated initially with *S. cerevisiae* (CC and TC). In the trials without initial inoculation (CS and TS) and those inoculated with *M. pulcherrima* (CM and TM), the CO<sub>2</sub> production was detected only from the fourth day on, following the sequential inoculation of *S. cerevisiae* on day 2. These data are in line with those of De Filippis et al. (2019) who studied the fermentation of withered Falanghina grapes and observed a quicker start in the musts inoculated with selected strains of *S. cerevisiae*, while in non-inoculated must the lag phase was longer. Previous investigations also evidenced that multi-starter inoculation with *M. pulcherrima* and *S. cerevisiae* showed slower fermentation kinetics compared to single *S. cerevisiae* (Binati et al., 2020; Hranilovic et al., 2020; Varela et al., 2021). Nevertheless, all fermentations, with different timing, reached the expected production of 12.5% v/v ethanol, confirming that *M. pulcherrima* LI, either already present or inoculated in must at a concentration of 6 log CFU/mL, did not hamper the fermentation process.

Interaction between yeast populations during fermentations was analyzed by means of differential plate counting, that allowed the visual distinction of the diverse colony type of *M. pulcherrima*, *S. cerevisiae* and other non-*Saccharomyces* species (Fig. 5) (Binati et al., 2019; Polizzotto et al., 2016).

In all fermentations, *S. cerevisiae* reached high cell counts (more than 10<sup>7</sup> CFU/mL) and dominated the mycobiota. Growth was higher in the first 10 days of fermentation and high cell levels were maintained until the end. The maximum cell population of *S. cerevisiae* (CC, 7.73 Log CFU/mL) was detected in the musts not inoculated with *M. pulcherrima*

(CC and TC, Fig. 5a; CS and TS, Fig. 5b), and in particular in the must from the CG batch. As expected, high cell counts of *M. pulcherrima* were found in CM and TM (6.93 Log CFU/mL, Fig. 5c), but also in the TC (6.22 Log CFU/mL) and TS (6.70 Log CFU/mL) fermentations from the TG must. These results suggest a slight inhibitory effect of *M. pulcherrima* against *S. cerevisiae* when present in high concentrations. This competition could also be considered to explain the slowing down of fermentations of the TG must compared to CG (Fig. 4).

The initial abundance of *M. pulcherrima* in the fermentations of TG batch reflects the population that has colonized the treated grapes, except in CM and TM where an increment of 6 log CFU/mL was given by the starter inoculation. In all fermentations, the population increased in the first days, more intensely in the trials CS, TS, CM, and TM where *S. cerevisiae* was not inoculated at the beginning. It started to decrease right after, and this decline was stronger in CC and TC, probably for the high ethanol production by *S. cerevisiae*. Indeed, the low ethanol tolerance of *M. pulcherrima* can explain its falloff in all fermentations after the 16<sup>th</sup> day, when the alcohol level was above 6% v/v (Binati et al., 2020; Checchia et al., 2021).

As regard other non-*Saccharomyces* yeasts, their behavior was similar between fermentations of TG and CG musts, although the initial population was higher in the must from CG batch. These non-*Saccharomyces* yeast populations remained viable until the 23<sup>rd</sup> day, whereas on the last day of fermentations they were lower than 10<sup>3</sup> CFU/mL, showing a higher tolerance to ethanol compared to *M. pulcherrima*. In a study of the bioprotective activity of *M. pulcherrima* during pre-fermentative cold clarification, it was observed a reduction of 1 log in the population of wild non-*Saccharomyces* yeasts, without affecting *S. cerevisiae* (Canonico et al., 2023).

### 3.5. Chemical composition of wines

Table 2 reports the main oenological parameters analyzed in the sweet wines.

Residual sugars were higher in the wines from TC, TS, and TM fermentations, consistently with the higher grape weight loss and sugar concentration in the must of the TG batch. Residual YAN was statistically equivalent between the different inocula and grape treatments, indicating a similar trend for the consumption of nutrients regardless of microbial interactions.

Higher total acidity and lower pH in wines from the TG batch reflect the differences already observed in the grape musts, with an overall increase in total acidity compared to the grape musts. Acidity is an essential trait in sweet wines to balance the high level of sugars, and in all wines, it was above the minimum required by the product

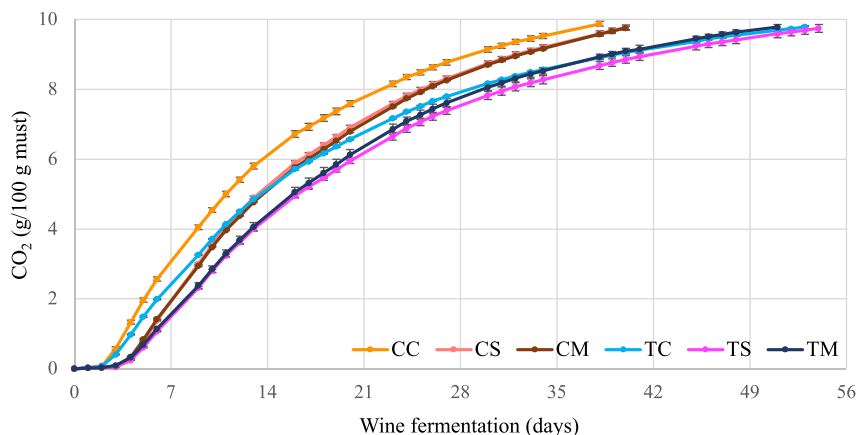
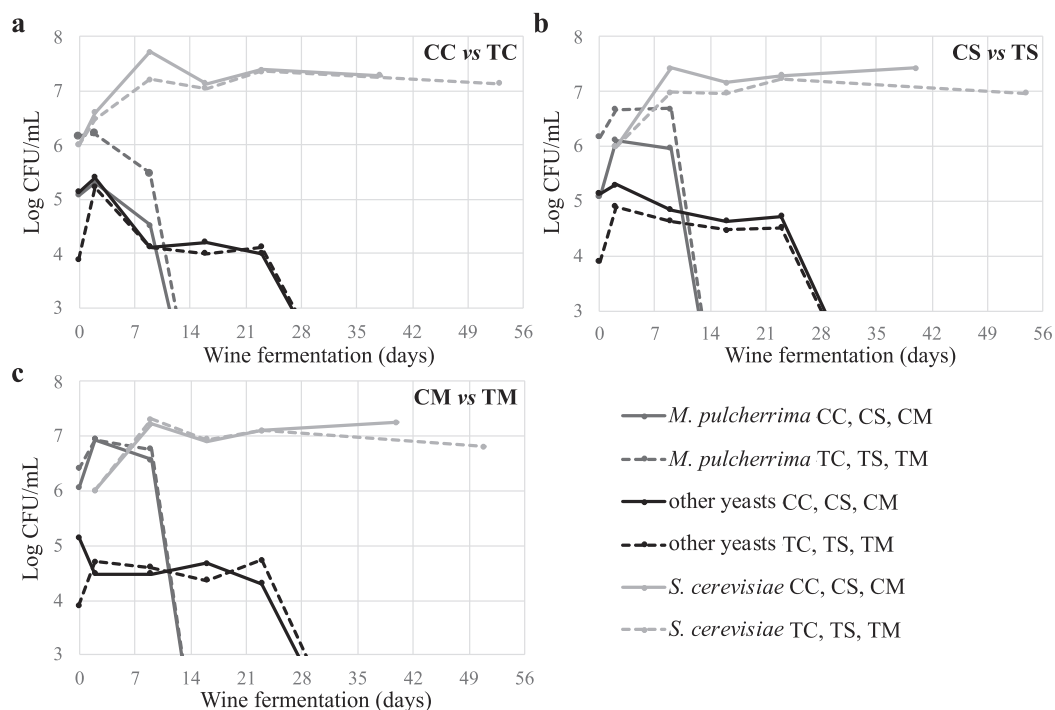


Fig. 4. Fermentation kinetics of the microvinification trials with musts from withered Garganega grapes, either control or treated with *M. pulcherrima*, inoculated with *S. cerevisiae* at the beginning of fermentation (CC and TC), *S. cerevisiae* after 48 h (CS and TS), or *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM and TM).



**Fig. 5.** Yeast population (Log CFU/mL) during the fermentation of musts from withered Garganega grapes, either control or treated with *M. pulcherrima*: a. inoculated with *S. cerevisiae* at the beginning of fermentation (CC vs TC); b. inoculated with *S. cerevisiae* after 48 h (CS vs TS); and c. inoculated with *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM vs TM).

**Table 2**

Chemical analysis of the main oenological parameters at the end of fermentation of musts from withered Garganega grapes, either control or treated with *M. pulcherrima*, inoculated with *S. cerevisiae* at the beginning of fermentation (CC and TC), *S. cerevisiae* after 48 h (CS and TS), or *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM and TM). Mean  $\pm$  standard deviation of three replicates.

	Must inoculation strategy					
	CC	CS	CM	TC	TS	TM
Residual sugars (g/L)	128.11 $\pm$ 1.81 <sup>c</sup>	136.29 $\pm$ 3.14 <sup>b</sup>	134.73 $\pm$ 1.26 <sup>bc</sup>	152.30 $\pm$ 1.93 <sup>a</sup>	156.18 $\pm$ 0.33 <sup>a</sup>	150.83 $\pm$ 5.67 <sup>a</sup>
YAN (mg/L)	22.77 $\pm$ 0.15	22.15 $\pm$ 0.81	23.50 $\pm$ 3.15	23.03 $\pm$ 0.67	21.40 $\pm$ 2.17	20.15 $\pm$ 0.94
Total acidity (g/L)	6.27 $\pm$ 0.06 <sup>b</sup>	6.27 $\pm$ 0.06 <sup>b</sup>	6.13 $\pm$ 0.21 <sup>b</sup>	6.73 $\pm$ 0.12 <sup>a</sup>	6.73 $\pm$ 0.06 <sup>a</sup>	6.80 $\pm$ 0.10 <sup>a</sup>
pH	3.32 $\pm$ 0.03 <sup>a</sup>	3.32 $\pm$ 0.01 <sup>a</sup>	3.31 $\pm$ 0.01 <sup>a</sup>	3.26 $\pm$ 0.01 <sup>b</sup>	3.26 $\pm$ 0.02 <sup>b</sup>	3.26 $\pm$ 0.01 <sup>b</sup>
Acetic acid (g/L)	1.27 $\pm$ 0.04 <sup>b</sup>	1.19 $\pm$ 0.04 <sup>bc</sup>	1.12 $\pm$ 0.02 <sup>c</sup>	1.52 $\pm$ 0.04 <sup>a</sup>	1.46 $\pm$ 0.06 <sup>a</sup>	1.54 $\pm$ 0.08 <sup>a</sup>
Free SO <sub>2</sub> (mg/L)	1.77 $\pm$ 0.21 <sup>c</sup>	2.07 $\pm$ 0.35 <sup>bc</sup>	2.30 $\pm$ 0.52 <sup>abc</sup>	2.63 $\pm$ 0.06 <sup>ab</sup>	2.97 $\pm$ 0.23 <sup>a</sup>	2.43 $\pm$ 0.32 <sup>abc</sup>
Total SO <sub>2</sub> (mg/L)	35.67 $\pm$ 3.79 <sup>b</sup>	38.33 $\pm$ 1.53 <sup>ab</sup>	37.67 $\pm$ 4.16 <sup>ab</sup>	44.33 $\pm$ 4.04 <sup>a</sup>	41.33 $\pm$ 2.52 <sup>ab</sup>	40.67 $\pm$ 1.15 <sup>ab</sup>
Acetaldehyde (mg/L)	21.37 $\pm$ 0.61 <sup>ab</sup>	19.80 $\pm$ 0.44 <sup>b</sup>	19.50 $\pm$ 0.20 <sup>b</sup>	23.07 $\pm$ 1.26 <sup>a</sup>	21.43 $\pm$ 1.19 <sup>ab</sup>	23.73 $\pm$ 1.85 <sup>a</sup>
Glycerol (g/L)	13.71 $\pm$ 0.36 <sup>cd</sup>	13.54 $\pm$ 0.26 <sup>d</sup>	13.92 $\pm$ 0.14 <sup>bcd</sup>	14.21 $\pm$ 0.23 <sup>bc</sup>	14.37 $\pm$ 0.16 <sup>b</sup>	15.13 $\pm$ 0.19 <sup>a</sup>

YAN: Yeast Assimilable Nitrogen.

Different letters in the same row indicate statistically significant differences ( $p < 0.05$ ).

regulations, 4.5 g/L in Recioto di Gambellara DOCG (Mipaaf, 2008).

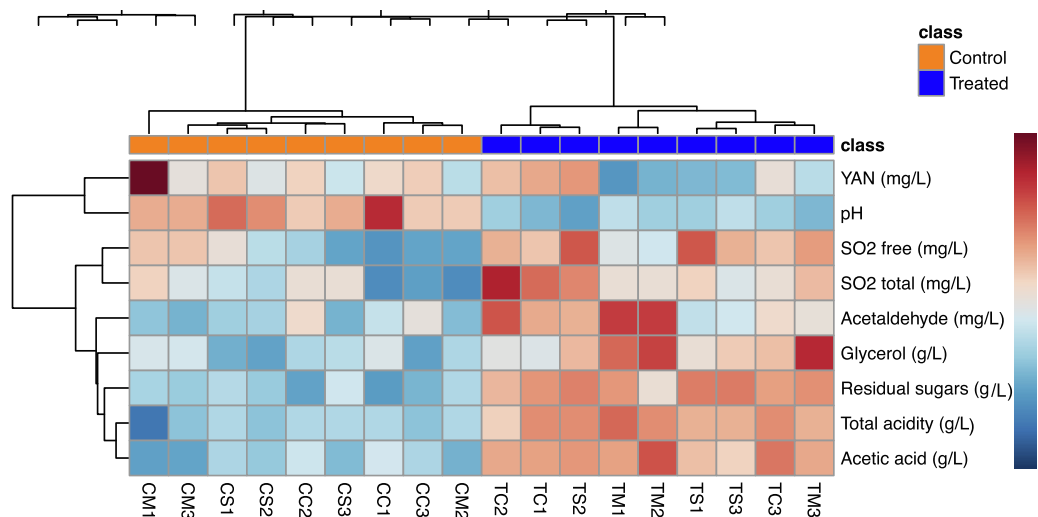
A statistically higher production of acetic acid was found in the three wines from the TG batch (around 1.5 g/L) than those of CG (around 1.2 g/L), which could be related with a higher stress experienced by yeasts in the former, as indicated by the slower fermentation kinetics. Nevertheless, these values of volatile acidity are within the maximum limit of 1.5 g/L allowed for Recioto di Gambellara DOCG (Mipaaf, 2017). Interestingly, in the wines from the CG batch, the multistarter fermentation with *M. pulcherrima* had a concentration of acetic acid lower than the single *S. cerevisiae*, a characteristic previously observed (Canonico et al., 2023; Checchia et al., 2021; Hranilovic et al., 2020).

For the free SO<sub>2</sub>, total SO<sub>2</sub>, acetaldehyde, and glycerol, the values were generally higher in wines produced with TG, but not all differences were statistically significant. High glycerol content, as well as acetic acid, are normally found in passito wines since the water loss stress and increased osmotic potential in cells of dehydrated grapes lead to increased synthesis of glycerol and acetic acid (De Filippis et al., 2019).

Indeed, must from TG with higher dehydration resulted in higher concentrations of both metabolites. Moreover, *M. pulcherrima* in multi-starter fermentations increased the glycerol production, usually associated with sensation of more body and sweetness in wine (Binati et al., 2020; Hranilovic et al., 2020).

These results were used to build a Hierarchical Cluster Analysis together with a heat-map (Fig. 6), which showed a clear separation of wines based on the type of grape must fermented. The cluster of wines from the TG batch achieved a generally higher level of all chemical compounds evaluated, with a lower pH reflecting the higher total acidity.

The yeast metabolic activity during fermentation had a less marked impact on the chemical parameters, as evidenced by the random distribution of replicates of the three different strategies of starter inoculation within the two separate clusters of the CG and TG batches.



**Fig. 6.** Hierarchical cluster analysis and heat-map associated with the main oenological parameters at the end of the microvinification trials with musts from withered Garganega grapes, either control or treated with *M. pulcherrima*, inoculated with *S. cerevisiae* at the beginning of fermentation (CC and TC), *S. cerevisiae* after 48 h (CS and TS), or *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM and TM). Replicates are indicated by the numbers 1 to 3 following the code of each fermentation strategy.

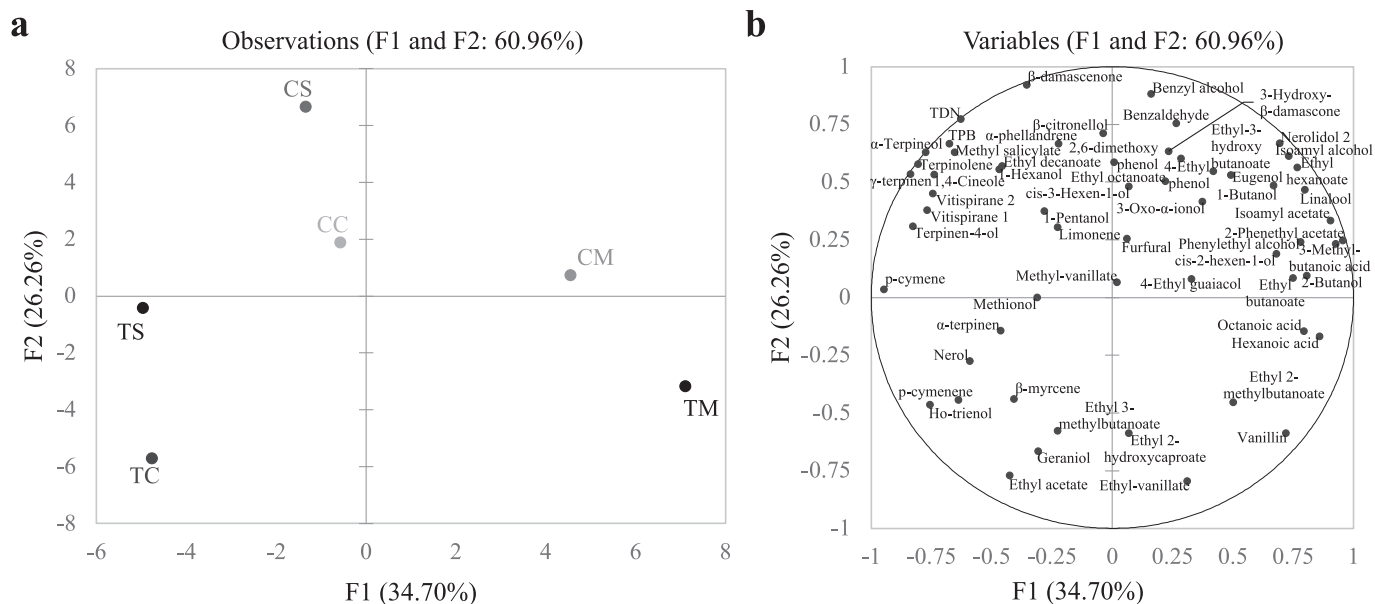
### 3.6. Volatile profile

Analysis of the volatile organic compounds (VOCs) allowed the quantification of 58 molecules, including alcohols, fatty acids, esters, benzenoids, terpenes, norisoprenoids, and sulphur-containing compounds (Supplementary Table 1). All 58 VOCs were subjected to a Principal Component Analysis, using the averages of replicates (Fig. 7).

The first and second components of the PCA accounted for about 61% of the total variation (F1 = 34.70% and F2 = 26.26%). The plots show the differences among the six fermentations (Fig. 7a) and the volatile compounds responsible for the differentiation (Fig. 7b). In agreement with the chemical analysis of the wines (Fig. 6), the differences between the type of grape must were clearly acknowledged in the PCA. Fermentations of musts from the control grapes (CC, CS, and CM) were characterized by positive values on F2, while those from the

treated grapes (TC, TS, and TM) by negative values on F2.

Most VOCs were present in higher concentrations in the wines from CG than those from TG batch. The biotic and abiotic stresses associated with water loss during withering may strongly impact the biosynthesis and release of VOC precursors in the grape berries, with different behaviors for the various molecules (Sanmartin et al., 2021; Zenoni et al., 2016). Some terpenes and norisoprenoids, important aroma compounds contributing “fruity/floral” descriptors, are considered markers of passito wines, since the withering process increase their production and accumulation in the berries, revealed later in wine through yeast fermentation (Negri et al., 2017; Sanmartin et al., 2021; Tosi et al., 2013). Interestingly, volatile compounds with low perception thresholds, such as terpinen-4-ol,  $\beta$ -citronellol, geraniol, and  $\beta$ -damascenone, reached high concentrations in all wines, regardless of grape treatment and must inoculation, showing the preponderance of withering in



**Fig. 7.** (a) Observations and (b) variables of the Principal Component Analysis with the volatile organic compounds quantified at the end of the microvinification trials with musts from withered Garganega grapes, either control or treated with *M. pulcherrima*, inoculated with *S. cerevisiae* at the beginning of fermentation (CC and TC), *S. cerevisiae* after 48 h (CS and TS), or *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM and TM).

determine key characteristics of the wine style.

Besides the higher dehydration of the treated grapes, the variation of some VOCs concentrations might also have been influenced by the mold and yeast metabolism. As a matter of fact, the control grapes had a significantly higher incidence of fungal infection, which could be either noble rot or other fungal pathogens, and the yeast population was also different among the trials.

Fungal infection of grapes is related to the modulation of some key aromatic compounds, as previously shown in Garganega grapes in the Soave-Gambellara appellations (Negri et al., 2017; Simonato et al., 2019). According to previous studies, some grape pathogens could strongly impact yeast metabolism, resulting in important changes in wine aroma (Barata et al., 2011; Tosi et al., 2013). Additionally, the stresses caused by fungal infections in bunch rot affected grapes can lead to changes in the VOCs profile as they might be involved with the plant defense system or as infection potentiators (Lorenzini et al., 2015; Santos et al., 2022; Steel et al., 2013).

Furthermore, the inoculation strategy caused also marked differences in the content of aromatic compounds. The CM and TM wines were characterized by positive values on F1, while CC, CS, TC, and TS by negative values. As for some general trends, sequential fermentations with *M. pulcherrima* (CM and TM) produced higher concentrations of alcohols, fatty acids, and esters, while fermentations with single inoculation of *S. cerevisiae* (CC, CS, TC, and TS) presented increased norisoprenoids, terpenes, and benzenoids. In Garganega wines, the overall quality is generally influenced by the ethyl and acetate ester content with a positive effect on “floral” and “fruity” notes (Tomasi & Gaiotti, 2008; Tosi et al., 2013). In other sweet wines, higher alcohols have been associated with a “chemical” odor, while terpenes give “floral”

descriptors (De Filippis et al., 2019).

For a more in-depth investigation of the separate effects of grape treatment and must inoculation on the wine volatile profile, a two-way ANOVA was carried out, and the compounds showing statistically significant differences are shown in Table 3. Firstly, the three fermentations performed on must from the CG batch (CC, CS, and CM) were compared with the fermentations of must from TG (TC, TS, and TM). Afterwards, fermentations were separated and compared according to the type of inoculation (CC with TC, CS with TS, and CM with TM).

Type of must inoculation had a significant impact on 27 VOCs, while the treatment of grapes was associated with a differential production of 18 VOCs in the wines. As observed in the PCA with all compounds (Fig. 7), most VOCs increased in the fermentations of control must, with the only exception of vitispirane 1. This norisoprenoid, with floral, fruity, woody, or reminiscent of eucalyptus notes, was higher in the wines from the TG batch, probably due to  $\beta$ -glucosidase activity of *M. pulcherrima* (Binati et al., 2019). In previous investigations, benzaldehyde, found three times more concentrated in the CG wines than TG, was strongly correlated with fungal activity on infected grapes (Negri et al., 2017; Simonato et al., 2019; Tosi et al., 2013). On the other hand, a decrease has been reported for 1-hexanol (Simonato et al., 2019), phenylethyl alcohol, and methionol (Tosi et al., 2013) in botrytized wines, while in the present study these molecules were higher in the CG wines, more affected by fungal growth. As regards the norisoprenoid  $\beta$ -damascenone, while Negri et al. (2017) and Simonato et al. (2019) reported a higher concentration in healthy grapes, Tosi et al. (2013) agreed with our study and found it more concentrated in must from fungal affected grape.

As regards the VOCs influenced by must inoculation, 12 were found

**Table 3**

Comparison of the significantly different Volatile Organic Compounds (VOCs;  $\mu\text{g/L}$ ) at the end of fermentation of musts from withered Garganega grapes, either control or treated with *M. pulcherrima*, inoculated with *S. cerevisiae* at the beginning of fermentation (CC and TC), *S. cerevisiae* after 48 h (CS and TS), or *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM and TM). Mean of wine replicates separated either by grape treatment or type of inoculation, higher values in **bold**.

VOCs	Grape treatment			Must inoculation strategy			
	CC CS CM	TC TS TM	S.	CC TC	CS TS	CM TM	S.
$\alpha$ -Phellandrene				0.148 <sup>b</sup>	<b>0.213<sup>a</sup></b>	0.160 <sup>ab</sup>	*
1,4-Cineole				0.795 <sup>b</sup>	<b>0.990<sup>a</sup></b>	0.670 <sup>b</sup>	***
$\gamma$ -Terpinen	<b>15.662<sup>a</sup></b>	14.031 <sup>b</sup>	**	15.637 <sup>b</sup>	<b>17.633<sup>a</sup></b>	11.270 <sup>c</sup>	***
p-Cymene				<b>1.452<sup>a</sup></b>	<b>1.513<sup>a</sup></b>	0.805 <sup>b</sup>	*
Terpinolene	<b>0.256<sup>a</sup></b>	0.227 <sup>b</sup>	*	0.248 <sup>b</sup>	<b>0.289<sup>a</sup></b>	0.187 <sup>c</sup>	***
$\alpha$ -Terpineol				0.652 <sup>ab</sup>	<b>0.781<sup>a</sup></b>	0.483 <sup>b</sup>	**
$\beta$ -Citronellol	<b>5.921<sup>a</sup></b>	4.970 <sup>b</sup>	*				
Nerolidol 2	<b>0.408<sup>a</sup></b>	0.326 <sup>b</sup>	*	0.315 <sup>b</sup>	0.375 <sup>ab</sup>	<b>0.410<sup>a</sup></b>	*
Vitispirane 1	1.473 <sup>b</sup>	<b>1.657<sup>a</sup></b>	*	1.385 <sup>b</sup>	<b>2.498<sup>a</sup></b>	0.813 <sup>c</sup>	***
Vitispirane 2				1.720 <sup>b</sup>	<b>2.795<sup>a</sup></b>	0.858 <sup>c</sup>	***
TPB	<b>0.227<sup>a</sup></b>	0.192 <sup>b</sup>	*	0.190 <sup>b</sup>	<b>0.298<sup>a</sup></b>	0.140 <sup>c</sup>	***
TDN	<b>3.481<sup>a</sup></b>	2.458 <sup>b</sup>	**	3.046 <sup>b</sup>	<b>3.976<sup>a</sup></b>	1.886 <sup>c</sup>	***
$\beta$ -Damascenone	<b>10.439<sup>a</sup></b>	7.177 <sup>b</sup>	***	8.622 <sup>b</sup>	<b>10.711<sup>a</sup></b>	7.090 <sup>b</sup>	***
2-Butanol				8309.017 <sup>b</sup>	9116.181 <sup>ab</sup>	<b>11110.603<sup>a</sup></b>	**
1-Butanol	<b>233.858<sup>a</sup></b>	203.093 <sup>b</sup>	***	210.067 <sup>b</sup>	210.564 <sup>b</sup>	<b>234.797<sup>a</sup></b>	*
Isoamyl alcohol	<b>175057.225<sup>a</sup></b>	152609.846 <sup>b</sup>	**				
Methionol	<b>346.165<sup>a</sup></b>	271.571 <sup>b</sup>	**	<b>416.397<sup>a</sup></b>	252.742 <sup>b</sup>	257.465 <sup>b</sup>	***
Ethyl butanoate				121.320 <sup>c</sup>	140.026 <sup>b</sup>	<b>158.953<sup>a</sup></b>	***
Ethyl hexanoate				197.703 <sup>b</sup>	218.831 <sup>ab</sup>	<b>231.932<sup>a</sup></b>	*
Ethyl octanoate				133.245 <sup>b</sup>	<b>174.297<sup>a</sup></b>	164.978 <sup>ab</sup>	*
Ethyl decanoate				68.020 <sup>b</sup>	<b>100.737<sup>a</sup></b>	72.312 <sup>b</sup>	**
Ethyl-3-hydroxybutanoate	<b>30.974<sup>a</sup></b>	22.809 <sup>b</sup>	*				
Isoamyl acetate	<b>542.373<sup>a</sup></b>	505.894 <sup>b</sup>	*	497.015 <sup>b</sup>	509.853 <sup>b</sup>	<b>565.533<sup>a</sup></b>	*
2-Phenethyl acetate				35.143 <sup>b</sup>	35.911 <sup>b</sup>	<b>38.580<sup>a</sup></b>	**
3-Methylbutanoic acid				278.575 <sup>b</sup>	274.169 <sup>b</sup>	<b>319.813<sup>a</sup></b>	*
Hexanoic acid				1549.745 <sup>b</sup>	1674.016 <sup>b</sup>	<b>1978.678<sup>a</sup></b>	***
Octanoic acid				4028.217 <sup>c</sup>	4294.953 <sup>b</sup>	<b>4724.455<sup>a</sup></b>	***
1-Hexanol	<b>741.154<sup>a</sup></b>	546.191 <sup>b</sup>	***	<b>764.683<sup>a</sup></b>	673.674 <sup>b</sup>	492.660 <sup>c</sup>	***
Methyl salicylate	<b>0.976<sup>a</sup></b>	0.774 <sup>b</sup>	*	<b>0.947<sup>a</sup></b>	<b>0.975<sup>a</sup></b>	0.703 <sup>a</sup>	*
Benzaldehyde	<b>135.012<sup>a</sup></b>	43.714 <sup>b</sup>	***	85.003 <sup>b</sup>	86.804 <sup>b</sup>	<b>96.282<sup>a</sup></b>	*
Phenylethyl alcohol	<b>11976.062<sup>a</sup></b>	9381.169 <sup>b</sup>	*				
2,6-Dimethoxy phenol	<b>5.092<sup>a</sup></b>	4.444 <sup>b</sup>	*				

Different letters in the same row, in the separate columns Grape treatment and Must inoculation, indicate statistically significant difference in the two-way ANOVA ( $p < 0.05$ ). S. (significance): \*\*\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \* =  $p < 0.05$ .

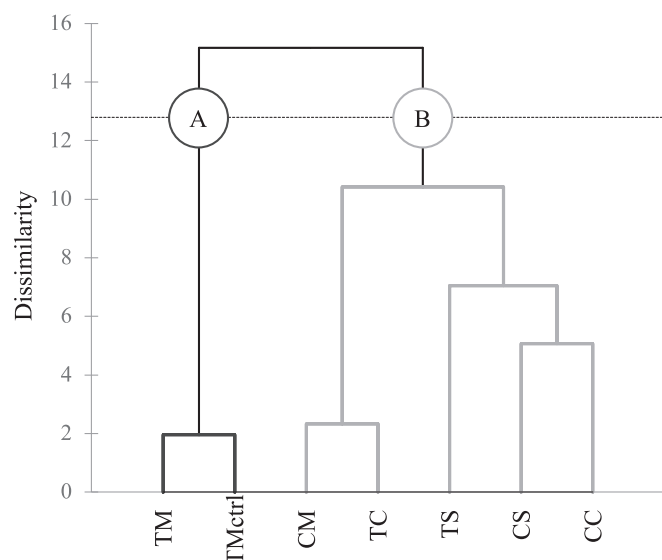
in higher concentrations in the single inoculation of *S. cerevisiae* after 48 h (CS and TS), mostly comprising terpenes and norisoprenoids. The single *S. cerevisiae* inoculated at the beginning (CC and TC) significantly increased only two compounds, namely methionol, a sulphur-containing compound linked with a “potato” note, and 1-hexanol, linked to the enzymatic oxidation of fatty acids. The higher concentration of terpenes and norisoprenoids in the fermentations in which the inoculation of *S. cerevisiae* was delayed, giving more time for the native non-*Saccharomyces* yeasts to develop, could be related to their high  $\beta$ -glucosidases activity that hydrolyze odorless aroma precursors (De Filippis et al., 2019). For p-cymene and methyl salicylate, both CS and TS, and CC and TC had the highest values, without significant difference.

Eleven VOCs achieved the highest levels in CM and TM wines, mainly higher alcohols, esters, fatty acids, and benzaldehyde. These classes of compounds are normally considered as fermentative, associated with a diverse yeast metabolism in the fermentation process, and their increase has already been linked with the presence of *M. pulcherrima* in multi-starter fermentations (Binati et al., 2020; Canonico et al., 2023; Hranić et al., 2020).

### 3.7. Sensory assessment of the wines

Sorting task method followed by agglomerative hierarchical clustering led to the formation of two distinct clusters based on the aromatic similarity of wines (Fig. 8). In general, the panel was reproducible since the TM sample and its analytical replicate (TMctrl) were projected in the same group. Grouping of TM and TMctrl in cluster A showed a significant difference of TM respect to all the other fermentations. The other cluster, B, was composed by the wines CM, TC, TS, CS, and CC.

The remarkable differentiation of TM in the sensory analysis reinforces the significant impact of the combined use of *M. pulcherrima* in post-harvest withering and in multi-starter fermentations on the wine characteristics. Previous fermentation studies with *M. pulcherrima* have also showed a clear sensory impact respect to the control singly inoculated with *S. cerevisiae* (Binati et al., 2020; Varela et al., 2021). Interestingly, the CM and TC wine samples, which were placed in opposite positions in the PCA of VOCs (Fig. 7a), were the most similar ones within



**Fig. 8.** Agglomerative hierarchical clustering of the sorting task scores, at the end of the microvinification trials with musts from withered Garganega grapes, either control or treated with *M. pulcherrima*, inoculated with *S. cerevisiae* at the beginning of fermentation (CC and TC), *S. cerevisiae* after 48 h (CS and TS), or *M. pulcherrima* at the beginning and *S. cerevisiae* after 48 h (CM and TM). Sample of TM was served in duplicate. The dashed line indicates the significance threshold.

the cluster B, while CC and CS, sharing the same quadrant on the PCA, were less similar than CM and TC in the sorting task (Fig. 8). This demonstrates how the aromatic complexity of a wine does not depend only on the quantity of individual VOCs, but also on the presence of some key odorants, their relative concentration with respect to the odor thresholds, the synergistic effect, and the interactions between VOCs and the wine matrix (Cardoso Schwindt et al., 2023; Sarrazin et al., 2007).

## 4. Conclusions

In this study, for the first time, the combined effects of inoculating a selected strain of *M. pulcherrima* on grapes at the beginning of the withering process and in must before alcoholic fermentation were analyzed. Grapes during withering proved to be a suitable environment for the development and persistence of a high population of this yeast. Colonization of berries by the inoculated *M. pulcherrima* effectively counteracted the development of molds, confirming the role of this yeast as a microbial antagonist and the possibility of use as a BCA, reducing the need of SO<sub>2</sub> addition.

Fermentation of musts obtained from grapes treated or not with *M. pulcherrima*, and inoculated with *M. pulcherrima* and/or *S. cerevisiae* showed marked differences. Fermentation kinetics and the non-volatile oenological parameters were mainly influenced by the grape treatment, while the analysis of the aromatic compounds showed a great effect of the inoculation strategy. Moreover, sensory analysis highlighted a remarkable differentiation of wines due to the modulation of aromatic compounds by *M. pulcherrima*, both in the post-harvest withering process and in the multi-starter fermentation.

Future trials could elucidate the mechanisms behind those effects and evaluate the wine quality through detailed sensory analysis. With the role of non-*Saccharomyces* yeasts comprehensively investigated, they will become a valuable asset to achieve more sustainable winemaking processes, especially regarding the reduction of sulphites.

## Ethical statement

This study contains sensory analyses carried out by a trained panel, for which the principles indicated in the 1964 Helsinki Declaration and its later amendments were followed. Informed consent was obtained from all participants. Participants were provided with an information sheet approved by the University Human Ethics' Committee, describing what was involved in the participation. Participants were informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal.

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## CRediT authorship contribution statement

**Renato L. Binati:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Marzia Maule:** Conceptualization, Formal analysis, Investigation. **Giovanni Luzzini:** Formal analysis, Investigation, Writing – original draft. **Francesco Martelli:** Formal analysis, Investigation, Writing – original draft. **Giovanna E. Felis:** Resources, Writing – review & editing. **Maurizio Ugliano:** Resources, Writing – review & editing. **Sandra Torriani:** Conceptualization, Resources, Supervision, 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.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2023.113550>.

## References

- Agarbari, A., Canonico, L., Pecci, T., Romanazzi, G., Ciani, M., & Comitini, F. (2022). Biocontrol of non-*Saccharomyces* yeasts in vineyard against the gray mold disease agent *Botrytis cinerea*. *Microorganisms*, 10, 200. <https://doi.org/10.3390/microorganisms10020200>
- Alegre, Y., Sáenz-Navajas, M. P., Ferreira, V., García, D., Razquin, I., & Hernández-Orte, P. (2017). Rapid strategies for the determination of sensory and chemical differences between a wealth of similar wines. *European Food Research and Technology*, 243, 1295–1309. <https://doi.org/10.1007/s00217-017-2857-7>
- Barata, A., Campo, E., Malfeito-Ferreira, M., Loureiro, V., Cacho, J., & Ferreira, V. (2011). Analytical and sensorial characterization of the aroma of wines produced with sour rotten grapes using GC-O and GC-MS: Identification of key aroma compounds. *Journal of Agricultural and Food Chemistry*, 59, 2543–2553. <https://doi.org/10.1021/jf104141f>
- Barata, A., Malfeito-Ferreira, M., & Loureiro, V. (2012). The microbial ecology of wine grape berries. *International Journal of Food Microbiology*, 153, 243–259. <https://doi.org/10.1016/j.ijfoodmicro.2011.11.025>
- Binati, R. L., Innocente, G., Gatto, V., Celebrin, A., Polo, M., Felis, G. E., & Torriani, S. (2019). Exploring the diversity of a collection of native non-*Saccharomyces* yeasts to develop co-starter cultures for winemaking. *Food Research International*, 122, 432–442. <https://doi.org/10.1016/j.foodres.2019.04.043>
- Binati, R. L., Larini, I., Salvetti, E., & Torriani, S. (2022). Glutathione production by non-*Saccharomyces* yeasts and its impact on winemaking: A review. *Food Research International*, 156, Article 111333. <https://doi.org/10.1016/j.foodres.2022.111333>
- Binati, R. L., Lemos Junior, W. J. F., Luzzini, G., Slaghenaufi, D., Ugliano, M., & Torriani, S. (2020). Contribution of non-*Saccharomyces* yeasts to wine volatile and sensory diversity: A study on *Lachancea thermotolerans*, *Metschnikowia* spp. and *Starmerella bacillaris* strains isolated in Italy. *International Journal of Food Microbiology*, 318, Article 108470. <https://doi.org/10.1016/j.ijfoodmicro.2019.108470>
- Canonico, L., Agarbari, A., Galli, E., Comitini, F., & Ciani, M. (2023). *Metschnikowia pulcherrima* as biocontrol agent and wine aroma enhancer in combination with a native *Saccharomyces cerevisiae*. *LWT*, 181, Article 114758. <https://doi.org/10.1016/j.lwt.2023.114758>
- Cardoso Schwindt, V., Coletto, M. M., Díaz, M. F., & Ponzoni, I. (2023). Could QSOR modelling and machine learning techniques be useful to predict wine aroma? *Food and Bioprocess Technology*, 16, 24–42. <https://doi.org/10.1007/s11947-022-02836-x>
- Checchia, I., Binati, R. L., Troiano, E., Ugliano, M., Felis, G. E., & Torriani, S. (2021). Unravelling the impact of grape washing, SO<sub>2</sub>, and multi-starter inoculation in lab-scale vinification trials of withered black grapes. *Fermentation*, 7, 43. <https://doi.org/10.3390/fermentation7010043>
- Consorzio Tutela Vini Gambellara. (2014). Gambellara, terre e colli da vino. Ed. Centro di Cultura e Civiltà Contadina Biblioteca Internazionale “La Vigna”, Vicenza, Italy, 46 pp.
- D’Onofrio, C., Tumino, G., Gardiman, M., Crespan, M., Bignami, C., de Palma, L., Barbagallo, M. G., Muganu, M., Morcia, C., Novello, V., Schneider, A., & Terzi, V. (2021). Parentage atlas of Italian grapevine varieties as inferred from SNP genotyping. *Frontiers in Plant Science*, 11, Article 605934. <https://doi.org/10.3389/fpls.2020.605934>
- De Filippis, F., Aponte, M., Piombino, P., Lisanti, M. T., Moio, L., Ercolini, D., & Blaiotta, G. (2019). Influence of microbial communities on the chemical and sensory features of Palanghina sweet passito wines. *Food Research International*, 120, 740–747. <https://doi.org/10.1016/j.foodres.2018.11.033>
- Dukare, A. S., Paul, S., Nambi, V. E., Gupta, R. K., Singh, R., Sharma, K., & Vishwakarma, R. K. (2019). Exploitation of microbial antagonists for the control of postharvest diseases of fruits: A review. *Critical Reviews in Food Science and Nutrition*, 59, 1498–1513. <https://doi.org/10.1080/10408398.2017.1417235>
- Esteves, M., Lage, P., Sousa, J., Centeno, F., Teixeira, M. F., Tenreiro, R., & Mendes-Ferreira, A. (2023). Biocontrol potential of wine yeasts against four grape phytopathogenic fungi disclosed by time-course monitoring of inhibitory activities. *Frontiers in Microbiology*, 14, 1146065. <https://doi.org/10.3389/fmicb.2023.1146065>
- Freimoser, F. M., Rueda-Mejia, M. P., Tilocca, B., & Migheli, Q. (2019). Biocontrol yeasts: Mechanisms and applications. *World Journal of Microbiology & Biotechnology*, 35, 154. <https://doi.org/10.1007/s11274-019-2728-4>
- Gava, A., Emer, C. D., Ficagna, E., Fernandes de Andrade, S., & Fuentefria, A. M. (2021). Occurrence and impact of fungicides residues on fermentation during wine production - A review. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 38, 943–961. <https://doi.org/10.1080/19440049.2021.1894357>
- Gianvito, P. D., Englezos, V., Rantsiou, K., & Cocolin, L. (2022). Bioprotection strategies in winemaking. *International Journal of Food Microbiology*, 364, Article 109532. <https://doi.org/10.1016/j.ijfoodmicro.2022.109532>
- Gore-Lloyd, D., Sumann, I., Brachmann, A. O., Schneeberger, K., Ortiz-Merino, R. A., Moreno-Beltrán, M., Schläfli, M., Kirner, P., Santos Kron, A., Rueda-Mejia, M. P., Somerville, V., Wolfe, K. H., Piel, J., Ahrens, C. H., Henk, D., & Freimoser, F. M. (2019). Snf2 controls pulcherriminic acid biosynthesis and antifungal activity of the biocontrol yeast *Metschnikowia pulcherrima*. *Molecular Microbiology*, 112, 317–332. <https://doi.org/10.1111/mmi.14272>
- Hranilovic, A., Gambetta, J. M., Jeffery, D. W., Grbin, P. R., & Jiranek, V. (2020). Lower-alcohol wines produced by *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* co-fermentations: The effect of sequential inoculation timing. *International Journal of Food Microbiology*, 329, Article 108651. <https://doi.org/10.1016/j.ijfoodmicro.2020.108651>
- Lemos Junior, W. J. F., Binati, R. L., Felis, G. E., Slaghenaufi, D., Ugliano, M., & Torriani, S. (2020). Volatile organic compounds from *Starmerella bacillaris* to control gray mold on apples and modulate cider aroma profile. *Food Microbiology*, 89, Article 103446. <https://doi.org/10.1016/j.fm.2020.103446>
- Lisanti, M. T., Blaiotta, G., Nioi, C., & Moio, L. (2019). Alternative methods to SO<sub>2</sub> for microbiological stabilization of wine. *Comprehensive Reviews in Food Science and Food Safety*, 18, 455–479. <https://doi.org/10.1111/1541-4337.12422>
- Lleixà, J., Kioroglou, D., Mas, A., & Portillo, M. D. C. (2018). Microbiome dynamics during spontaneous fermentations of sound grapes in comparison with sour rot and *Botrytis* infected grapes. *International Journal of Food Microbiology*, 281, 36–46. <https://doi.org/10.1016/j.ijfoodmicro.2018.05.016>
- Lorenzini, M., & Zapparoli, G. (2019). Yeast-like fungi and yeasts in withered grape carposphere: Characterization of *Aureobasidium pullulans* population and species diversity. *International Journal of Food Microbiology*, 289, 223–230. <https://doi.org/10.1016/j.ijfoodmicro.2018.10.023>
- Lorenzini, M., Azzolini, M., Tosi, E., & Zapparoli, G. (2013). Postharvest grape infection of *Botrytis cinerea* and its interactions with other moulds under withering conditions to produce noble-rotten grapes. *Journal of Applied Microbiology*, 114(3), 762–770. <https://doi.org/10.1111/jam.12075>
- Lorenzini, M., Cappello, M. S., & Zapparoli, G. (2015). Isolation of *Neofusicoccum parvum* from withered grapes: Strain characterization, pathogenicity and its detrimental effects on passito wine aroma. *Journal of Applied Microbiology*, 119, 1335–1344. <https://doi.org/10.1111/jam.12931>
- Lorenzini, M., Cappello, M. S., Logrieco, A., & Zapparoli, G. (2016). Polymorphism and phylogenetic species delimitation in filamentous fungi from predominant mycobiota in withered grapes. *International Journal of Food Microbiology*, 238, 56–62. <https://doi.org/10.1016/j.ijfoodmicro.2016.08.039>
- Lorenzini, M., Simonato, B., Favati, F., Bernardi, P., Sbarbati, A., & Zapparoli, G. (2018). Filamentous fungi associated with natural infection of noble rot on withered grapes. *International Journal of Food Microbiology*, 272, 83–86. <https://doi.org/10.1016/j.ijfoodmicro.2018.03.004>
- Maluleke, E., Jolly, N. P., Patterson, H. G., & Setati, M. E. (2022). Antifungal activity of non-conventional yeasts against *Botrytis cinerea* and non-*Botrytis* grape bunch rot fungi. *Frontiers in Microbiology*, 13, Article 986229. <https://doi.org/10.3389/fmicb.2022.986229>
- Mipaaf (Ministero delle politiche agricole, alimentari e forestali). (2008). Disciplinare di produzione denominazione di origine controllata e garantita “Recioto di Gambellara”. DM 01.08.2008 G.U. 196 – 22.08.2008. Available online: [http://cata.logoviti.politicheagricole.it/scheda\\_denom.php?t=dsc&q=1055](http://cata.logoviti.politicheagricole.it/scheda_denom.php?t=dsc&q=1055) (accessed on 19 April 2023).
- Mipaaf (Ministero delle politiche agricole, alimentari e forestali). (2017). Elenco aggiornato dei vini DOP e IGP italiani per i quali è consentita la deroga per il limite massimo di acidità volatile, ai sensi dell’Allegato I C 3 del regolamento (CE) n. 606/2009. Available online: <https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/TT/IDPagina/10455> (accessed on 29 March 2023).
- Morata, A., Loira, I., Escott, C., del Fresno, J. M., Banuelos, M. A., & Suárez-Lepe, J. A. (2019). Applications of *Metschnikowia pulcherrima* in wine biotechnology. *Fermentation*, 5, 63. <https://doi.org/10.3390/fermentation5030063>
- Nardi, T. (2020). Microbial resources as a tool for enhancing sustainability in winemaking. *Microorganisms*, 8, 507. <https://doi.org/10.3390/microorganisms8040507>
- Negri, S., Lovato, A., Boscaini, F., Salvetti, E., Torriani, S., Comisso, M., Danzi, R., Ugliano, M., Polverari, A., Tornielli, G. B., & Guzzo, F. (2017). The induction of noble rot (*Botrytis cinerea*) infection during postharvest withering changes the metabolome of grapevine berries (*Vitis vinifera* L., cv. Garganega). *Frontiers. Plant Science*, 8, 1002. <https://doi.org/10.3389/fpls.2017.01002>
- Parr, W. V., Heatherbell, D., & White, K. G. (2002). Demystifying wine expertise: Olfactory threshold, perceptual skill and semantic memory in expert and novice wine judges. *Chemical Senses*, 27, 747–755. <https://doi.org/10.1093/chemse/27.8.747>
- Pawlikowska, E., James, S. A., Breierova, E., Antolak, H., & Kregiel, D. (2019). Biocontrol capability of local *Metschnikowia* sp. isolates. *Antonie Van Leeuwenhoek*, 112, 1425–1445. <https://doi.org/10.1007/s10482-019-01272-w>
- Polizzotto, G., Barone, E., Ponticello, G., Fasciana, T., Barbera, D., Corona, O., Amore, G., Giammanco, A., & Oliva, D. (2016). Isolation, identification and oenological characterization of non-*Saccharomyces* yeasts in a Mediterranean island. *Letters in Applied Microbiology*, 63, 131–138. <https://doi.org/10.1111/lam.12599>

- Ribes, S., Fuentes, A., Talens, P., & Barat, J. M. (2018). Prevention of fungal spoilage in food products using natural compounds: A review. *Critical Reviews in Food Science and Nutrition*, 58, 2002–2016. <https://doi.org/10.1080/10408398.2017.1295017>
- Salvetti, E., Campanaro, S., Campedelli, I., Fracchetti, F., Gobbi, A., Tornielli, G. B., Torriani, S., & Felis, G. E. (2016). Whole-metagenome-sequencing-based community profiles of *Vitis vinifera* L. cv. Corvina berries withered in two post-harvest conditions. *Frontiers in Microbiology*, 7, 937. <https://doi.org/10.3389/fmicb.2016.00937>
- Sanmartin, C., Modesti, M., Venturi, F., Brizzolara, S., Mencarelli, F., & Bellincontro, A. (2021). Postharvest water loss of wine grape: When, what and why. *Metabolites*, 11, 318. <https://doi.org/10.3390/metabo11050318>
- Santos, H., Augusto, C., Reis, P., Rego, C., Figueiredo, A. C., & Fortes, A. M. (2022). Volatile metabolism of wine grape Trincadeira: Impact of infection with *Botrytis cinerea*. *Plants*, 11, 141. <https://doi.org/10.3390/plants11010141>
- Sarrazin, E., Dubourdieu, D., & Darriet, P. (2007). Characterization of key aroma compounds of botrytized wines, influence of grape botrytization. *Food Chemistry*, 103, 536–545. <https://doi.org/10.1016/j.foodchem.2006.08.026>
- Shmulevitz, R., Amato, A., Commisso, M., D'Inca, E., Luzzini, G., Ugliano, M., Fasoli, M., Zenoni, S., & Tornielli, G. B. (2023). Temperature affects organic acid, terpene and stilbene metabolisms in wine grapes during postharvest dehydration. *Frontiers in Plant Science*, 14, 1107954. <https://doi.org/10.3389/fpls.2023.1107954>
- Simonato, B., Lorenzini, M., Cipriani, M., Finato, F., & Zapparoli, G. (2019). Correlating noble rot infection of Garganega withered grapes with key molecules and odorants of botrytized passito wine. *Foods*, 8, 642. <https://doi.org/10.3390/foods8120642>
- Sipiczki, M. (2020). *Metschnikowia pulcherrima* and related pulcherrimin-producing yeasts: Fuzzy species boundaries and complex antimicrobial antagonism. *Microorganisms*, 8, 1029. <https://doi.org/10.3390/microorganisms8071029>
- Sipiczki, M. (2022). Taxonomic revision of the pulcherrima clade of *Metschnikowia* (Fungi): Merger of species. *Taxonomy*, 2, 107–123. <https://doi.org/10.3390/taxonomy2010009>
- Slaghenaufi, D., & Ugliano, M. (2018). Norisoprenoids, sesquiterpenes and terpenoids content of Valpolicella wines during aging: Investigating aroma potential in relationship to evolution of tobacco and balsamic aroma in aged wine. *Frontiers in Chemistry*, 6, 66. <https://doi.org/10.3389/fchem.2018.00066>
- Slaghenaufi, D., Boscaini, A., Prandi, A., Dal Cin, A., Zandonà, V., Luzzini, G., & Ugliano, M. (2020). Influence of different modalities of grape withering on volatile compounds of young and aged Corvina wines. *Molecules*, 25, 2141. <https://doi.org/10.3390/molecules25092141>
- Slaghenaufi, D., Guardini, S., Tedeschi, R., & Ugliano, M. (2019). Volatile terpenoids, norisoprenoids and benzenoids as markers of fine scale vineyard segmentation for Corvina grapes and wines. *Food Research International*, 125, Article 108507. <https://doi.org/10.1016/j.foodres.2019.108507>
- Slaghenaufi, D., Luzzini, G., Samaniego Solis, J., Forte, F., & Ugliano, M. (2021). Two sides to one story—aroma chemical and sensory signature of Lugana and Verdicchio wines. *Molecules*, 26, 2127. <https://doi.org/10.3390/molecules26082127>
- Steel, C. C., Blackman, J. W., & Schmidtke, L. M. (2013). Grapevine bunch rots: Impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry*, 61, 5189–5206. <https://doi.org/10.1021/jf400641r>
- Tomasi, D., & Gaiotti, F. (2008). Gambellara, terre e colli da vino. Ed. Camera di Commercio Industria, Artigianato e Agricoltura, Arcugnano, Italy, 308 pp.
- Torriani, S., Lorenzini, M., Salvetti, E., & Felis, G. E. (2011). *Zygosaccharomyces gambellarensis* sp. nov., an ascosporegenous yeast isolated from an Italian 'passito' style wine. *International Journal of Systematic and Evolutionary Microbiology*, 61, 3084–3088. <https://doi.org/10.1099/ijs.0.031146-0>
- Tosi, E., Azzolini, M., Lorenzini, M., Torriani, S., Fedrizzi, B., Finato, F., Cipriani, M., & Zapparoli, G. (2013). Induction of grape botrytization during withering affects volatile composition of Recioto di Soave, a "passito"-style wine. *European Food Research and Technology*, 236, 853–862. <https://doi.org/10.1007/s00217-013-1943-8>
- Varela, C., Bartel, C., Espinase Nandorfy, D., Bilogrevic, E., Tran, T., Heinrich, A., Balzan, T., Bindon, K., & Borneman, A. (2021). Volatile aroma composition and sensory profile of Shiraz and Cabernet Sauvignon wines produced with novel *Metschnikowia pulcherrima* yeast starter cultures. *Australian Journal of Grape and Wine Research*, 27, 406–418. <https://doi.org/10.1111/ajgw.12484>
- Vicente, J., Ruiz, J., Belda, I., Benito-Vázquez, I., Marquina, D., Calderón, F., Santos, A., & Benito, S. (2020). The genus *Metschnikowia* in enology. *Microorganisms*, 8, 1038. <https://doi.org/10.3390/microorganisms8071038>
- Xia, J., & Wishart, D. S. (2011). Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nature Protocols*, 6, 743–760. <https://doi.org/10.1038/nprot.2011.319>
- Zenoni, S., Fasoli, M., Guzzo, F., Dal Santo, S., Amato, A., Anesi, A., Commisso, M., Herderich, M., Ceoldo, S., Avesani, L., Pezzotti, M., & Tornielli, G. B. (2016). Disclosing the molecular basis of the postharvest life of berry in different grapevine genotypes. *Plant Physiology*, 172, 1821–1843. <https://doi.org/10.1104/pp.16.00865>