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Exo-metabolome from novel microbial consortia including lactic acid bacteria, *Starmerella lactis-condensi* and *Candida oleophila* for assessing wine active aroma fraction

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1. Introduction

1.1 Lactic acid bacteria and wine quality

Wine is a fermented beverage produced by numerous biochemical transformations catalysed by different microbial species (Liu et al., 2017). Non-Saccharomyces and Saccharomyces, represent the yeast genera involved during the alcoholic fermentation process; their primary metabolic actions enable the transformation of the sugars present in the grape must into ethanol. Following this phase, in the absence of sugars and in the presence of ethanol, the action of lactic acid bacteria (LAB) enables the degradation of certain substrate-preferring organic acids in a metabolic process known as malolactic fermentation (MLF). Specifically, MLF consists of the transformation of L-malic into L-lactic and CO_2 in the wine (Davis et al 1988); this process occurs thanks to an endogenous enzyme complex called malate-decarboxylase. The action of this enzyme catalyses the elimination of a carboxylic group from malic acid, which is reused within the bacterial cell to produce chemical energy. These transformations lead to profound organoleptic changes that can alter the taste and aroma of wine (Liu et al 2002; Lonvaud-Funel, 1999). Although taste perceptions are mainly changed by a single enzymatic action that can reduce wine acidity (Bartowsky & Borneman, 2011), different endogenous enzyme catalysts are involved in the aroma changes of wines, including glycosidases, β-glucosidases, esterases, phenolic acid decarboxylases and citrate lyases (Grimaldi et al., 2005a, 2005b; Liu, 2002; Matthews et al., 2004).

1.1.1 Glicosidase

Perceptions of wine aromas depend on numerous chemical and physical factors including volatility (bibliography). In some families of volatile organic compounds in wines, volatility is strongly impaired by the presence in the molecule of a glycosidic bond to which a sugar is bound, predominantly glucose in various forms, monosaccharide or disaccharide (Günata et al 1988). Under these conditions, VOCs do not possess any olfactory peculiarities. Odour perceptions are restored by breaking the glycosidic bond by acid or enzymatic hydrolysis. The two processes are distinguished

by their reaction times, the former being closely dependent on the proton activity of the must and much slower than the latter. Therefore, the action of hydrolysis by means of bacterial glycosidases is the only means, during MLF, that can permit the release of the aglycone and consequently the detectability of the aroma to the human nose. Grimaldi et al., 2000, 2005a, 2005b identified many glycosidase activities in *Oenococcus*, *Lactobacillus* and *Pediococcus* strains. Borneman et al., 2010 identified several gene sequences coding for numerous glycosidases, in particular B-glucosidases, in *Oenocuss oeni* (*O. oeni*). The identification of such β-glucosidase activities in *O. oeni* provided important insights into the roles played by this bacterium in wine aroma changes during MLF (Cappello et al., 2017).

1.1.2 Esterase

Esters in wines represent an important aroma family that can influence wine quality (Swiegers et al., 2005). The diversity, number and nature he esters are attributed to the floral and fruity sensory perceptions in wines (Lambrechts and Pretorius, 2000). Esterases are an enzyme class responsible for the hydrolysis or synthesis of esters in wines. Although there is conspicuous information on yeast esterases in the literature (Sumby et al., 2010), there is still little information on this enzyme class in LAB. Scientific evidence has shown an increase in esters during MLF in ethyl acetate, isoamyl acetate, and ethyl lactate (Delaquis et al., 2000; Gambaro et al.,2001; Maicas et al., 1999). Matthews et al. (2007) found that LAB strains have greater activity against short-chain esters (C2-C8) than long-chain esters (C10-C18). Such substrate specificities have the potential to produce important aromatic compounds derived from fatty acids, higher alcohols and organic acids (Matthews et al.,2007). Studies by Antalick et al., 2012; and Costello et al., 2012 report significant increases in fruit perceptions following MLF in red wines.

This evidence also confirms reports by Costello et al., 2013 in which acyltransferase alcohol activities were identified in *O. oeni* and *L. plantarum*. In addition, it has been reported that *O. oeni* has the

ability to metabolise sulphur-containing compounds from activities derived from the metabolism of cysteilderivatives by β/γ-lyase (Knoll et al, 2011; Pripis-Nicolau et al., 2004).

1.1.3 Decarboxylase of phenolic acids

Phenolic acids are natural constituents found in the cell walls of plants (Cappello et al., 2017). These acids, in particular ferulic, p-coumaric and caffeic acid, in grapes are found in the pulp in the form of esters with tartaric acid. However, during fermentation, by cinnamoyl esterase activities (Dugelay et al., 1993), the ester bond is hydrolysed and the phenolic acids are released into the must in free form. Dugelay et al., 1993 reported the presence of substrate-specific decarboxylases in certain lactic acid bacteria capable of bio-converting free phenolic acids into 4-vinyl derivatives, which can also be reduced to 4-ethyl derivatives. This peculiarity mainly attributable to yeasts of the genus *Dekkera* (Ribéreau-Gayon et al., 2006), which can be traced back to the production of off-flavours, was also found in some strains of the genus *Lactoplantibacillus* (Couto et al., 2006; Knockaert et al., 2012). However, depending on the phenolic acid catabolised and the LAB strain, the responses in the aromatic expression of wines may differ (Cappello et al., 2017).

The catabolism of ferulic acid in *Lactoplantibacillus* leads to the formation of small amounts of vanillin and significant productions of 4-vinyl guaiacol; whereas in *O. oeni*, vanillin production is enabled by the bio-conversion of 4-vinyl guaiacol by means of a decarboxylation pathway (Bloem et al., 2007).

1.1.4 Citrate lyase

Citrate lyase is a key enzyme in the citric acid cycle. This enzyme catalyses the acetate and oxaloacetate formation reaction from the citrate ion (Cappello et al., 2017). The action of pyruvate decarboxylase enables the biovoncersion of oxaloacetate into pyruvate. The formation of pyruvate triggers a series of reactions that leads to the formation of VOCs with buttery olfactory hints characterised by different olfactory thresholds. Acetoin and 2,3-butanediol, are compounds with a high olfactory threshold (150 mg/L) that are formed by the reductive degradation of diacetyl. The

non-conversion and consequently the accumulation of diacetyl can also lead, depending on the concentration in solution, to the formation of off-flavours attributable to buttery and/or cheesy scents. These phenomena are mainly due to the low olfactory threshold characterising diacetyl (0.4 mg/L).

2.1 Wines yeasts

Wine is the product obtained from the alcoholic fermentation of sugars present in grape must, a process that is mainly carried out by yeasts. In the late 19th century, Louis de Pasteur showed that the microorganisms responsible for wine fermentations are firstly the yeasts present on the grapes (Barata et al. 2012). As previously stated, the diversity of yeast species present in grape must is directly correlated with the population of yeasts residing on grape skins and, to a lesser extent, those inhabiting the winemaking cellars, particularly on the walls and machinery (Le Jeune et al., 2006). Like other habitats in the phyllosphere, the microbial community on the grape surface is influenced by several factors, including altitude, latitude and longitude (Gao et al., 2019), rainfall, phytosanitary treatments (Čadež, et al., 2010), temperature, ripening stage, and health status (Martins et al., 2012). The environmental conditions exert a selective pressure on the microbiological biodiversity of the grape skin, resulting in the proliferation of specific yeast strains within a given area. In his studies, Louis Pasteur demonstrated that wild yeast strains used in wineries in the same area yield wines with analogous organoleptic characteristics (Suárez-Lepe & Morata, 2012). Furthermore, it is important to consider the role of insects as vectors in the transmission of microbial flora on grape skins. Insects can transport yeasts in their mandibles and on their legs, which is a significant factor influencing the microbial flora on grape skins (Mandl et al., 2015). The ripening stage and the presence of damage to the berries also exert an influence on the abundance and diversity of microbial populations on the peel. In the latter case, the development of undesired microorganisms, such as *Botrytis cinerea*, *Aspergillus* spp. and acetic acid bacteria, is favoured (Romano et al., 2003).

These factors contribute to the determination of the composition of the microbial community in the must, which subsequently gives rise to the initial stages of spontaneous fermentation. Consequently,

the production of wine is the result of complex interactions between fungi, yeasts, and bacteria, which originate at the vineyard level and continue throughout the fermentation process in the winery until the completion of packaging (Fleet, 2003).

Saccharomyces cerevisiae is the most commonly utilized yeast in winemaking. This is due to the indispensable oenological characteristics inherent to these strains.

It is noteworthy that the isolation rate of *S. cerevisiae* strains is relatively low on grapes, especially when healthy berries are considered (Bai et al., 2022). However, the concentration increases in the presence of damaged berries. This variation can be explained by the fact that this organism can occupy the niche of insects, such as wasps (Stefanini et al., 2012).

Saccharomyces spp. are isolated with greater frequency in environments other than vineyards, especially in the wineries (Parapouli et al., 2020). This may be attributed to a previous utilization of *S. cerevisiae* as active dry yeasts (ADY) for managed fermentation, or alternatively, the presence of wild yeasts for spontaneous fermentation (Mandl et al., 2015).

The most frequently occurring non-*Saccharomyces* yeasts in the musts are the species belonging to the genera *Candida, Pichia, Rhodotorula, Debaryomyces, Kluyveromyces, Torulaspora, Zigosaccharomyces, Dekkera, Schizosaccharomyces* and *Metschnikowia* (Marsit & Dequin, 2015). Among these, some species, such as *Starmerella bacillaris* and *Hanseniaspora uvarum* demonstrated a high competitive ability by growing at high densities (10^5-10^7 CFU/mL) and dominating other non-*Saccharomyces* species (Wang et al., 2015). In the early stages of the spontaneous fermentation when the must is still rich in nutrients, yeasts with low fermentative power such as *Pichia, Metschnikowia* and *Hanseniaspora* usually predominate (Torres-Guardado et al., 2022). During this stage, the number of viable yeast populations in the must increases from the initial values of 10^4-10^6 CFU/mL to 10^8 CFU/mL (Fleet, 1993). The growth of non-*Saccharomyces* yeast is usually limited to the first two or three days of must fermentation, after which their population decreases until they completely disappear. During this phase, sufficient sugars and amino acids are consumed to produce the compounds necessary to give the wines a distinctive aromatic quality. However, this process is swiftly

followed by the proliferation of *S. cerevisiae,* which dominates the intermediate and final stages of the process and is often the sole species present in the must fermentation during these periods (Fleet, 2003).

In the context of wine production, two principal methods of fermentation may be employed: managed fermentation and spontaneous fermentation. The first method is based on the inoculation of selected strains of yeasts, including *S. cerevisiae*, alone or with non-*Saccharomyces* yeasts, simultaneously or at different times. This technique permits the process to be repeated, thereby yielding comparable results. In contrast, the product obtained through traditional spontaneous fermentation, in which the yeasts present on the surfaces of the grapes and in the winery carry out the fermentation process, is not reproducible (Mandl et al., 2015). The combined use of non-*Saccharomyces* wine yeasts together with *Saccharomyces* strains in mixed fermentations could be recommended as a means to obtain the advantages of the aromatic complexity of spontaneous fermentation while avoiding the risks of stuck fermentation (Romano et al., 2003, Ciani et al., 2010).

The utilisation of multi-starter fermentation in winemaking is applicable to achieve a multitude of outcomes (Ciani & Comitini, 2015), including the attainment of a more intricate flavor profile, the reduction of alcohol content, the enhancement of wine structure, and the execution of a biocontrol action to curtail the proliferation of undesired microorganisms (Ciani et al., 2017).

Concerning the choice between co-inoculation and sequential inoculation, there is a consensus among authors that sequential inoculation allows a greater expression of the metabolism of non-*Saccharomyces* yeast at the start of fermentation compared to co-inoculation (Loira et al. 2015). Furthermore, additional factors influence the decision between sequential inoculation and co-inoculation. These include the impact of biological interactions between *S. cerevisiae* and non-*Saccharomyces,* and the sensory technological characteristics of the wine to be produced (Renault et al., 2016).

From the point of view of the potential and characteristics of yeasts, new selection criteria have emerged. In addition to the already known technological characteristics, new yeasts must meet other

requirements aimed at improving the technological properties and sensory characteristics of wines. For example, the ability to improve the colour of wine through the metabolic formation of stable pigments and at the same time the absence of β-glucosidase activity to prevent colour degradation; the reduction of off-flavors formation while promoting the production of volatile compounds such as esters and higher alcohols to improve aroma (Suárez et al., 2012).

2.1.1 Non-Saccharomyces yeast

The term "non-*Saccharomyces"* is a common designation among wine microbiologists and in the wine industry, to refer to a group of yeast species that do not belong to the *Saccharomyces* genus (Jolly et al., 2014). Non-*Saccharomyces* yeasts were originally considered the cause of some microbial problems in wine production (Van der Walt & Van Kerken, 1958; Amerine 1980). Consequently, strategies have been devised to mitigate their proliferation, including the utilisation of SO_2 or the sanitisation of winery equipment.

Over the past 25 years, there have been notable advances in understanding of the ecology, biochemistry, physiology and molecular biology of the yeasts that are involved in the production of wine. Furthermore, our comprehension of the impact of these yeasts on wine chemistry, sensory properties, and the overall appeal of the final product has also increased considerably product (Fleet, 2008).

Non-*saccharomyces* yeasts of oenological interest differ from *Saccharomyces* in a series of characteristics, including ethanol tolerance, fermentation power, production of organic acids, enzymes, and secondary metabolites. Recently, research has focused on the potential of using mixed cultures of *Saccharomyces* genus and non-*Saccharomyces* yeasts to improve wine quality (Maicas & Mateo, 2023) and allow the production of wines with distinctive and unique characteristics.

However, thanks to advances in identification techniques and studies conducted to better understand their metabolic activity and consequences in musts, their importance has been revaluated. It has been observed that through the controlled use of selected non-*Saccharomyces* strains, they can contribute

to the improvement of wine quality by influencing the formation of the final taste and flavor (Borren & Tian, 2020). Non-*Saccharomyces* yeasts are widely used in the production of other fermented foods and beverages, including cider, mead and kefir (Maicas & Mateo, 2023).

A number of studies have demonstrated that non-*Saccharomyces* yeast can positively influence various aspects of wine quality, depending on the selected species and strains (Benito et al., 2019). The field of oenology is concerned with the development of strategies to reduce the final ethanol content of wine. While consumers are seeking wines with lower ethanol levels, the phenomenon of climate change (global warming) has resulted in elevated sugar concentrations in grapes, leading to increased alcohol content (Van Leeuwen et al., 2019). One potential solution is the utilisation of non-*Saccharomyces* yeasts in combining with the traditional *S. cerevisiae* to metabolize residual sugar (Garciá et al., 2020). Non-*Saccharomyces* yeasts frequently exhibit a diminished capacity for the conversion of sugars to alcohol in comparison to *S. cerevisiae*. The metabolism of sugars by these yeasts results in the production of a greater quantity of secondary metabolites, including organic acids, glycerol and other aromatic compounds, at the expense of ethanol. Non-*Saccharomyces* species allow us to reduce the initial ethanol content by about 1–2% (v/v), depending on the yeast species and fermentation conditions (Ciani et al., 2016). The effects of climate change have also been observed in the pH of grapes, resulting in a reduction in acidity. Some non-*Saccharomyces* are capable reduce the pH of the must up to 0.5 units (Benito et al., 2019), through the production of organic acids, including lactic acid, malic acid and succinic acid.

2.1.1.1 Lachancea thermotolerans

Lachancea thermotolerans (*L. thermotolerans*) is currently the most reliable microbiological option to increase the acidity of the wines (Vicente et al., 2021), since it is able to produce lactic acid from sugar metabolism during alcoholic fermentation (lactic acid is produced through the activity of lactic acid dehydrogenase LDH from glycolysis-derived pyruvate) (Hranilovic et al., 2021). In contrast to *L. thermotolerans*, *Schizosaccharomyces pombe* has the ability to reduce the acidity of musts. It is

therefore recommended that wines produced in cooler climates undergo de-acidification. *Schizosaccharomyces pombe* is capable of metabolising malic acid into ethanol and CO_2; as a consequence, the total acidity of the wine is reduced (Benito et al., 2018).

The majority of non-*Saccharomyces* species have the effect of increasing the concentration of glycerol in wine. This compound contributes to the wine's overall smoothness and body (Jolly et al., 2014). Of the non-*Saccharomyces* yeasts, *Starmerella bacillaris* is capable of producing the highest concentration of glycerol (up to 14 g/L) during the alcoholic fermentation process (Benito et al., 2019). Non-*Saccharomyces* yeasts positively impact wine aroma through the production of hydrolytic enzymes and esters. Enzymes like β-glucosidase and β-lyase release varietal aromas, such as terpenes and volatile thiols, respectively, while esters such as ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, and isobornyl acetate contribute to floral and fruity characteristics (Tufariello et al., 2021).

The capacity to enhance the bouquet of beverages is a defining trait of numerous non-*Saccharomyces* species. *Pichia kluyveri* is renowned for its ability to produce copious amounts of esters, including 2-phenylethyl acetate and ethyl octanoate, which impart distinctive honey and rose, and peach aromas, respectively. Additionally, it is capable of elevating the concentration of terpenes (Benito et al., 2015; Méndez-Zamora et al., 2020).

2.1.1.2 Torulaspora delbrueckii

Torulaspora delbrueckii (*T. delbrueckii*) has the remarkable ability to release mannoproteins and polysaccharides into wine, which improves the quality of the wine in terms of its mouthfeel properties. When employed in sequential or mixed fermentations with *S. cerevisiae*, it corrects specific defects in wines, such as volatile acidity. In addition, it influences the aromatic characteristics of wines, resulting in the production of fruity esters, thiols, and terpenes (Benito, 2018). A study conducted on Amarone wines with both joint and sequential inoculation with *S. cerevisiae* demonstrated that *T. delbrueckii* imparted heightened aromatic intensity, including the aroma of "ripe red fruit," enhanced sweetness, and astringency (Azzolini et al., 2012).

2.1.1.3 Metschnikowia pulcherrima

Metschnikowia pulcherrima (*M. pulcherrima*) displays a number of advantageous characteristics, including the capacity to elevate glycerol concentration, reduce ethanol content, and impart desirable aromas. Furthermore, it can be employed as a biological control agent due to its capacity to produce natural antimicrobial compounds, notably pulcherrimin. This antimicrobial activity is derived from the precipitation of iron ions, which depletes iron in the environment and creates conditions unsuitable for other microorganisms that require iron for their growth. Pulcherrimin has been demonstrated to effectively inhibit a range of yeast species, including *Candida tropicalis*, *Candida albicans*, *Brettanomyces*, *Hanseniaspora*, and *Pichia*. Moreover, it has been demonstrated to inhibit a number of fungal species, including *Botrytis cinerea*, *Penicillium*, *Alternaria*, and *Monilia* spp. (Morata et al., 2019). In recent years, there have been numerous studies into non-*Saccharomyces* yeasts. Various strains have been isolated from different matrices and selected with a view to improving beverage quality. Currently, a wide range of non-*Saccharomyces* yeast strains is available on the market from leading manufacturers. The development of non-*Saccharomyce*s starter cultures represents an attractive opportunity for companies developing microbial-based biotechnological solutions for the wine industry.

2.2 The role of yeast in wine aroma

The aromatic and flavor profile of wines is the result of a multitude of variables that influence the production process, from the vineyard to the cellar. One of the variables that can be manipulated to define the characteristic profile of a wine is the choice of micro-organisms with which to conduct fermentation (Swiegers et al., 2005). The microbial ecosystem of grapes and wine, consisting of *Saccharomyces*, non-*Saccharomyces* yeasts and bacteria, is considered a decisive factor influencing the aroma of wine and characterizing the product (Belda et al., 2017). The final sensory quality of a wine is the result of the interactions between all chemical components of biotic and abiotic origin present in the wine (Styger et al., 2011). The wine aromas formed during the production process, from

the vineyard to bottling can be classified according to their origin. The grape variety determines the primary aroma, while post-harvest processing of the grapes determines the pre-fermentative aroma (Perestrelo et al., 2020), secondary aromas, which are responsible for the vinous and fruity notes in wines, originate from the metabolic activities of the yeasts during the fermentation process (Romano et al., 2022). These compounds define the bouquet, the set of more complex aromatic compounds. Finally, post-fermentation aromas are generated from the autolytic degradation of dead yeast cells (Capece & Romano, 2019).

The compounds that contribute directly to the secondary aromas of wine are the higher alcohols, acetic and ethyl esters, carbonyl and sulphur compounds. These are often called odour-active compounds (OAC) (Mina & Tsaltas, 2017). Consequently, the highest concentration of OAC is formed during the fermentation process, which subsequently influences the characterise the wine (Alvarez et al., 2012). The metabolism of yeasts, including *Saccharomyces* and non-*Saccharomyces* produce numerous secondary metabolites that are important in the beverage industry, contributing to the overall flavor of beverages (Walker & Stewart, 2016). These secondary metabolites will constitute the distinctive and characteristic aspects of that particular beverage. The formation of different aroma compounds by yeasts during fermentation is a consequence of the production of different types of enzymes (Borren & Tian, 2020) that are capable of interacting with aroma precursors in the wine must making them aromatically active and thus influencing the aromas of fermented beverages. The use of non-*Saccharomyces* yeasts has the potential to impact flavor bouquet, which represents a key quality and aroma factor in the field of food biotechnology (Carrau et al., 2005).

The numerous aromatic metabolites produced by yeasts during fermentation are the result of two distinct processes. The first is the transformation and volatilisation of precursor compounds present in the starting material, which may be fruit juice, cereals, hops, or other substances. The second is the creation of these metabolites *de novo* (Lambrechts & Pretorius, 2000).

Additionally, the final aroma and taste of beers are produced by several hundred volatile compounds, the majority of which are generated during alcoholic fermentation (Pires et al., 2014) by the activity

of unconventional yeasts that can be employed as a tool for aromatic characterisation and the development of beers with a novel flavor profile (Larroque et al., 2021).

Modern gene sequencing techniques have shown that specific non-*Saccharomyces* yeasts are more capable of producing extracellular enzymes compared to *S. cerevisiae* (Lambrechts & Pretorius, 2000).

The most important and characteristic aspect of a wine is surely taste. This encompasses the comprehensive sensorial expression of the aromatic compounds, perceived through the olfactory and gustatory senses. Additionally, it incorporates the most quantifiable attributes, such as acidity, sweetness, alcohol content, astringency, and effervescence.

3. Microbial interaction

Grape must can be traced back to a complex microbial ecosystem in which yeasts and bacteria cooperate with each other (Barata et al., 2012). The direct contact of the grape microbiome with the energy substrates in the must triggers the first microbial interaction phenomena useful for wine production. Therefore, wine constitutes an ideal model for the study of microbial interactions. Alcoholic fermentation (AF) (Ciani et al., 2010), with yeasts, and MLF (Alexandre et al., 2004), with LAB, constitute the most intense technological steps of microbial interaction. However, the introduction of the co-inoculation technique has led to a boost in microbial interaction phenomena between LAB and yeasts for wine production. Ivey et al., 2013 classify the different types of microbial interactions into direct and indirect. Indirect interactions include competitiveness, commensalism, mutualism, amensalism (or antagonism) and neutralism; while indirect interactions include parasitism. Instead, depending on the microbial communities, interactions can be divided into yeast-yeast interaction, bacteria-bacteria interaction and bacteria-yeast interaction (Liu et al., 2017).

3.1 *Yeast-yeast interaction*

These interactions develop during AF; the simultaneous development of yeast during this phase allows numerous interactions of a physiological and metabolic nature to be established (Liu et al., 2017). Sieuwerts et al., 2008 divides yeast-yeast interactions into positive and negative.

3.1.1 Negative interaction

3.1.1.1 Ethanol effect

Ethanol constitutes the main catabolite antagonist of many non-Saccharomyces (Heard & Fleet, 1988). The increasing accumulation of ethanol during alcoholic fermentation leads to a gradual decrease in microbial biodiversity (Beltran et al., 2002; Combina et al., 2005). The increased ethanol productivity, attributable to S. cerevisiae, induces substantial natural selective pressure in musts, inducing one of the largest negative interactions between microbial species (Liu et al., 2017).

3.1.1.2 Killer factor

Killer factors represent an endogenous characteristic of a yeast that can counteract the growth of other individuals belonging to the same genus. Yeasts are able to secrete proteins and/or glycoproteins that can alter the primary metabolisms of sensitive yeasts. This phenomenon called amensalism limits the growth of one strain during coexistence with another strain. These genetic peculiarities have been studied extensively in S. cerevisiae (Musmanno et al., 1999; Gutierrez et al., 2001); however, recent studies have also identified killer factors in non-*Saccharomyces* strains (Ciani and Fatichenti, 2001). Comitini et al., 2004 identified in Pichia anomala and *Kluyveromyce wickerhamiican* ability to secrete toxins capable of counteracting the *Brettanomyces* genus. Similar results were also found by Santoset al. (2009) with *Pichia membranifaciens*.

3.1.1.3 Metabolic catabolites

Negative interactive effects on yeast-yeast interactions can be carried out by certain acidic precursors capable of chelating metal cofactors. Such phenomena have been observed in *M. pulcherrima* (Oro et al., 2014). Studies by Sipiczki, 2006; Turkel and Ener, 2009; Oro et al., 2014 described in strains of *M. pulcherrima* the ability to produce pulcherriminic acid capable of subtracting iron assimilation from must. The difficulty of iron utilization after deactivation of pulcherriminic acid leads to metabolic alterations that compromise the physiology of coexisting yeast cells. Other compounds with antagonistic effects can be short- and medium-chain fatty acids, acetic acid or acetaldehyde (Giannattasio et al., 2005; Ivey et al., 2013).

3.1.1.4 Oxygen level

The amount of oxygen in the must can impair biodiversity levels (Liu et al., 2017). The reductive state created during alcoholic fermentation prevents non-Saccharomyces species holding oxidative and/or weakly fermentative metabolism (Holm Hansens et al., 2001).

3.1.1.5 Nutritive factor

Nutrient factors in musts are essential for the development and growth of microbial biodiversity. The colonisation of the medium by non-*Saccharomyces* strains during the first fermentation stages could adversely affect the subsequent growth of *S. cerevisiae* (Fleet, 2003). Medina et al. (2012) describe this phenomenon as causing significant depletion of must in terms of nutrients such as nitrogen and vitamins. A study by Mortimer (2000) confirms these observations where the subtraction of thiamine (Vit. B1) by *Kloeckera apiculata* is able to impair the growth and dominance of *S. cerevisiae* in the medium.

3.1.1.6 Positive interaction

The positive interactions between yeasts can be traced back to synergistic nutrient actions. The main activity observed between non-*Saccharomyces* and *Saccharomyces* can be traced back to commensalism (Liu et al., 2017). Dizy and Bisson, 2000, report proteolytic activities possessed by some non-*Saccharomyces* species that can assist the release of amino acids into the medium. Such effects would benefit the development of *S. cerevisiae* for reuse within metabolisms to stimulate protein synthesis (Fleet, 2003). Similar effects can be reproduced in sparkling wine bases near draught due to yeast autolysis during the wine maturation phase.

3.2 Bacteria-bacteria interaction

These types of interactions are very often found during spontaneous malolactic fermentation. The bacterial biodiversity of wines, which can be found in alcoholic post-fermentation, consists of the genera *Pediococcus*, *Lactobacillus*, *Leuconostoc* and *Oneococcus* (Liu et al., 2017). Scientific evidence from Osborne & Edwards 2006 reports that possible interactions between bacterial microflora can exert both positive and negative effects on the sensory quality of wines. The trophic peculiarities of bacteria have led to adaptations over time that have enabled them to survive in extreme conditions (Liu et al., 2017). The bacteria's ability to secrete extracellular proteolytic enzymes has

allowed them to generate essential amino acids for their metabolisms from external sources (Remize et al., 2006). However, the same amino acids can also promote the growth of further microorganisms. Fernandez and Nadra, 2006 report a case of mutualism between *O. oeni* and *Pediococcus* via the proteolytic system of *Oenococcus*. Studies by Aredez-Fernandezet al., 2010 report cases of aminebiogen (BA) production by L. hilgardii when it was in mixed culture with *O. oeni* compared to when it was in pure culture. Further effects of LAB interactions during malolactic fermentation are the production of bacteriocins. These compounds play an antagonistic role against other LAB by destabilising the cell membrane (Diez et al., 2012).

3.3 Yeast-bacteria interaction

These types of interactions occur in the case of co-inoculated fermentations between yeasts (*S. cerevisiae*) and LAB. The main effects of the co-inoculation of yeasts and LAB in a fermentation phase are detected on the initiation and completion of MLF. The types of interactions between LAB and *S. cerevisiae* are highly dependent on the strain pair used (Naselli et al 2024). A yeast can inhibit or stimulate a LAB through the production of certain metabolites (Osborne & Edwards, 2006; Guilloux-Benatier et al., 2006). Studies by Naselli et al., 2024 show that these effects have a strong influence on the sensory quality of wine. The main effects on yeast-bacteria interactions were reported by Liu et al., 2017 with: antagonism, amensalism, competition and commensalism.

3.3.1 Amensalism/Antagonism

The inhibitions exerted by yeast depend on metabolites whose inhibitory effects also depend on the interaction between them (Liu et al.,2017):

3.3.1.1 Ethanol

The toxicity of ethanol on bacterial cells increases as the pH of the wine decreases (Chu-Ky et al., 2005). The action on cells of ethanol is to increase membrane fluidity, which consequently leads to a

passive increase in incoming protons together with cell metabolites (from Silveira et al., 2003; Chu-Ky et al., 2005).

3.3.1.2 Sulphur compounds

S. cerevisiae is able to produce sulphite (HSO_3^-) via the sulphate reduction pathway (Ribéreau-Gayon et al., 2006). This peculiarity is a form of yeast cell detoxification (Park & Bakalinsky, 2000). When released into the medium, sulphite, in combination with ethanol, pH and temperature, increases molecular SO_2 in the medium and thus antimicrobial activity (Ribéreau-Gayon et al., 2006). The introduction into the bacterial cell converts molecular SO2 to bisulphite and sulphite, liberates protons and alters the intracellular pH of the bacterium; furthermore, the ability of SO_2 to react with ATPase and NAD^+ leads to the inhibition of bacterial growth and thus also to the arrest of malolactic activity (Henick-Kling, 1993; Lonvaud-Funel, 1999).

3.3.1.3 Medium-chain fatty acids

Medium-chain fatty acids (MCFAs) produced by *S. cerevisiae* are released from the cell to the medium by diffusion (Alexandre et al., 2010). MCFAs play an inhibiting role in bacterial cells due to their ability to deprotonate within the cell. This action leads to the impairment of ATPase and the inhibition of the bacterium's malolactic activity (Alexandre et al., 2004; Tourdot-Marechal et al., 1999). The toxic effect of MCFAs is a function of the concentration in wines but also of the pH and ethanol concentration of the medium (Capucho & San Romao, 1994).

3.3.1.4 *Peptide and protein fractions*

Proteins play important roles within the cells of yeasts and bacteria. However, work by Dick et al. (1992) and Comitini et al. (2005) reported the identification of a protein, secreted by *S. cerevisiae*, with MLF-inhibiting effects. Subsequent work indicated smaller molecular fractions with the same functions. Osborne & Edward (2007) describe SO_2-dependent peptide fractions (< 10 kDa) with antimicrobial action whose mechanism of action involves disruption of the cell membrane.

3.3.1.5 Other metabolites

Many of the metabolites produced by yeasts within the wine matrix perform important sensory functions. However, the same compounds may possess side functions that can impair the functionality of other co-existing cellular individuals. Organic acids such as succinic acid, produced in the Krebs cycle, are known to have savoury organoleptic characteristics (Jackson, 2022). Henick-Kling, 1993 highlighted the ability of yeasts to synthesise succinic acid as a medium for bacteria. The overproduction of succinate in solution would perform an acidifying function such that the medium would be unsuitable for LAB replication (Henick-Kling, 1993). Antagonistic antibacterial actions are also carried out by volatile organic compounds with 2-Phenylethanol (2-PE). This higher alcohol is synthesised by yeasts from L-phenylalanine via the Ehrlich pathway (Ribéreau-Gayon et al., 2006). The antimicrobial action of 2-PE is carried out as an inhibitor of the sugar and amino acid transport system within the cell (Etschmann et al., 2003).

3.3.1.6 Bacteria Antagonising Yeast

Studies by Narendranath et al., 1997 report the ability of *Lactoplantibacillus* to inhibit AF through the production of short-chain carboxylic compounds such as acetic acid. This compound would have an intracellular acidifying action causing metabolic alterations and thus yeast death (Bayrock & Ingledew, 2004). Further recognised abilities of LAB are that of counteracting the growth of certain sensitive yeasts through secondary activities derived from extracellular enzymes diffused in the medium. Guilloux-Benatier et al., 2000 identifies the ability of LAB to secrete enzymes with β-1, 3-glucanase activity capable of degrading the yeast cell wall by inhibiting their growth.

3.3.1.7 Competition for nutrition

LAB have considerable trophic needs due to their inability to synthesise essential amino acids and vitamins for their requirements (Terrade & Mira, 2009). A study by Remize et al., 2006 highlighted the inability of LAB to produce amino acids such as arginine, glutamate and tryptophan; and, essential

vitamins such as biotin and pantothenic acid (LeBlanc et al., 2011). Therefore, a yeast-LAB culture could incur MLF arrest problems if the yeast's nutritional requirements are high (Arnink & Henick-Kling, 2005).

3.3.2 Commensalism

In contrast to antagonisms, the stimulation of LAB metabolisms by yeasts is still lacking in information. However, bacteria benefit from yeast autolysis in the post-AF phase (Liu et al., 2017). Nitrogen fractions, < 1kDa, released in the medium aid the metabolic activities of LAB (Feuillat et al., 1977). Guilloux-Benatier & Chasagne, 2003 report the characterisation of these fractions as rich in arginine, isoleucine, glutamic acid and tryptophan. Larger molecular fractions composed of polysaccharides and proteins may decrease the Lag phase and promote the growth of *O. oeni* (Guilloux-Benatier et al., 1995). In addition, the presence of macromolecules in the lies deposits induce aminopeptidase activities in *O. oeni* (Guilloux-Benatier et al., 1995). This phenomenon would induce the release of mannoproteins in the medium (Guilloux-Benatier et al., 1993) by promoting the adsorption of medium-chain fatty acids and phenols (Vasserot et al., 1997) with LAB-inhibiting action (Reguant et al., 2000). A study by Guilloux-Benatier et al., 2006 also reports that the proteolytic capacity of *O. oeni* promotes nitrogen uptake in the medium.

3.4 Quorum sensing

Quorum sensing is a communicative metabolic process between cells. Its operation is based on gene regulation in response to cell population densities (Dong et al., 2005). Quorum-sensing bacteria produce small signalling molecules that they release into the medium (Choudhary & Schmidt-Dannert, 2010). The perception of these initiate precise information to produce biofilms, secondary metabolites, spores, etc. Several bacterial-derived compounds capable of imparting oridins to cells have been identified with these functionalities, including homoserine N-acyl lactones (AHL), furanosyl borate diester and self-inducing peptides (Cataldi et al., 2013). In contrast, different compounds are used and produced by yeasts to communicate such as acetaldehyde, farnesol,

tryptophol and phenylethanol (Ivey et al., 2013). Nissen et al., 2003 suggests that the synthesis of these molecules could be involved in yeast-yeast interactions for the arrest of non-*Saccharomyces* by *S. cerevisiae*. However, the same author does not exclude that cell-cell contact phenomena are also involved in the antagonistic mechanisms between *Saccharomyces* and non-*Saccharomyces*.

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Chapter 2

- Main R&D activities on wine -

Activity 2.1

Technological affinity index for interaction between lactic acid bacteria and *Saccharomyces cerevisiae* strains to modulate the fruity and floreal aroma of Catarratto wines

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Abstract

Microbial interactions during the fermentation process influence the sensory characteristics of wines. Alongside alcoholic fermentation, malolactic fermentation also plays a crucial role in determining the aromatic traits of wines. The time (t), rate (m) and volatile organic compounds (VOC) of malolactic fermentation are linked to the interaction between yeast and lactic acid bacteria. The study investigated the interactions between *Lactiplantibacillus plantarum* or *Oenococcus oeni* with *Saccharomyces cerevisiae* by using the Technological Affinity Index (TAIndex). The co-inoculation of *L. plantarum*/*S. cerevisiae* resulted in a higher TAIndex than the co-inoculation of *O. oeni*/*S. cerevisiae* conditions. A low TAIndex led to increased aromaticity of the wines. The time and rate of malolactic fermentation have a strong impact on the synthesis of VOCs with a high olfactory impact. Therefore, knowledge of the TAIndex could play a decisive role in improving winemaking planning to produce wines with higher fruit and floral perceptions.

Keywords: Catarratto grape variety; Lactic acid bacteria; Malolactic fermentation; *Saccharomyces cerevisiae*; Technological affinity index; Wine aroma.

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1. Introduction

The aroma of wine is the most important factor that influences consumer acceptance (Morata, 2018). The different aroma components n wine have different origins, including cultivars (Yang et al., 2021), agronomic techniques (Alem, Rigou, Schneider, Ojeda, & Torregrosa, 2019; Coletta et al., 2021), and geographic area.

However, the microbial components of the must plays role in the synthesis of volatile organic compounds, which are responsible for the aroma of wine (Liu et al., 2023).

Lactic acid bacteria (LAB) and yeasts produce a large number of secondary metabolites that create a sensory buffer in wine regulating the antagonisms and synergies of odor perceptions (Ferreira et al., 2016). LAB are microbial entities that most influence the organoleptic and technological framework of wine. For instance, the main technological function of LAB is the conversion of L-malic acid to L-lactic acid, which has deep implications on the gustatory, olfactory and microbial levels (Morata, 2021). The loss of a carboxylic group by L-malic acid leads to a biological deacidification of the wine (Lasik, 2013), which is associated with an improvement in taste. Thus, many of these sensory effects are the direct result of increasing pH and decreasing total acidity. In red wine, this is the case with the decrease in the reactivity of phenolic hydroxyls to salivary proteins. As a result of malolactic fermentation, wines became less tannic and have greater smoothness characters (Costello, Siebert, Solomon, & Bartowsky, 2013).

Malolactic fermentation has been reported to improve aromatic profiles and olfactory complexity of wines (Cappello, Zapparoli, Logrieco, & Bartowsky, 2017). However, the information in the literature is quite discordant. While some authors (Avedovech, Mcdaniel, Watson, & Sandine, 1992; Sauvageot & Vivier, 1997) reported a decrease in the olfactory intensity and fruity characters coming from the cultivar as a result of malolactic fermentation, others like Knoll et al. (2012) and Malherbe, Tredoux, Nieuwoudt, & du Toit (2012) reported an increase in the fruity components of wines due to the production of ethyl esters and acetates. The conflicting information in the bibliography poses uncertainty for technicians who want to adopt malolactic fermentation as a biotechnological means

of maintaining or improving the fruit and floral perceptions of wines while ensuring microbial stability.

The main interfering agent in the fruity and floral perceptions of wines is 2,3-butanedione, which is a by-product of malolactic fermentation. Bartowsky & Henschke (2004) pointed out several factors that lead to the production of 2,3-butanedione, including sulfur dioxide, dissolved oxygen, and temperature of malolactic fermentation performance. Furthermore, Olguín, Bordons, & Reguant (2009) reported that gene expression of the citric acid pathway, as well as 2,3-butanedione production, is stimulated by ethanol in the medium.

Therefore, the different strategies of LAB inoculation, sequential (at the end of alcoholic fermentation, in the presence of ethanol) or co-inoculation (24 h after yeast starter inoculation, in the absence of ethanol), may play a crucial role in the productive suppression of 2,3-butanedione. In fact, many authors (Lasik-Kurdyś, Majcher, & Nowak, 2018; Plavša, Jagatić Korenika, Lukić, Bubola, & Jeromel, 2021; Tristezza et al., 2016) reported that the technique of simultaneous inoculation of LAB at 24 h from the yeast strain has positive effects on fruity perception by shielding them from missed buttery hints resulting from 2,3-butanedione.

The effects of LAB-yeast co-inoculation on the aroma profiles of wines are well documented. During the fermentation phase, the relationships established between LAB and *S. cerevisiae* strains allows the 2,3-butanedione produced by the LAB to be reduced to 2,3-butanediol and acetoin by reductive metabolism of the yeast strains.

Although the interactive metabolic aspect between LAB and *S. cerevisiae* is sufficient to explain the validity of co-inoculation in the technological objective of the intensity of the fruity and floral perceptions of wines, limited information is available in the literature on the metabolic effects of microbial consociation between LAB and *S. cerevisiae* strains in the kinetics of different aromatic classes produced through the co-fermentation of LAB and *S. cerevisiae* during winemaking.

An additional cognitive requirement is represented by the effects of LAB-*S. cerevisiae* consociation on the balance between volatile organic compounds (VOCs) concentration and olfactory threshold, thus determining odor perception in wines.

For the first time, the present study proposes a technological affinity index (TAIndex) for evaluating potential interactions of LAB-*S.cerevisiae* microbial consociation in the aromatic modulation of wines.The applicability of the TAIndex was studied in order to be able to assess its effectiveness, taking into account the instantaneous malic acid degradation rate, the malic acid degradation rate per unit of time and the malolactic fermentation time. Measurements easily available in the cellar and by untrained personnel.

The acquisition of this information would lead to a greater responsiveness of the wine industry to modify wine flavour profiles and accommodate fluctuations in consumer tastes in a very short time.

In particular, in the present research, three commercial LAB strains (two *Oenococcus oeni* and one *Lactiplantibacillus plantarum* strains) were used in different co-inoculation with two *S. cerevisiae* strains. The research activity aimed to evaluate the effects of the LAB–*S. cerevisiae* consociation on: (*i*) kinetics of malolactic fermentation in terms of duration and yield; (*ii*), improvement of high olfactory impact VOC concentration; (iii) sensory characterization of aroma of Catarratto after malolactic fermentation; and (iv) TAIndex calculation.

2. Material and methods

2.1. Experimental design and sampling

The experimentation set, as shown in Fig. 1, consisted of co-inoculation of different LAB and *S. cerevisiae* strain during winemaking of Catarratto white grape. The first experimental set comprised the CO1, CO3 and CO5 trials, which were inoculated with the *S. cerevisiae* NF213 strain. After 24 h, the LAB strains were added: MLB6 (*O. oeni*) in the CO1 trial; MLA4 (*O. oeni*) in the CO3 trial; MLPK45H (*L. plantarum*) in the CO5 trial. The control CONT A1 trial was inoculated only with *S. cerevisiae* NF213 strain.

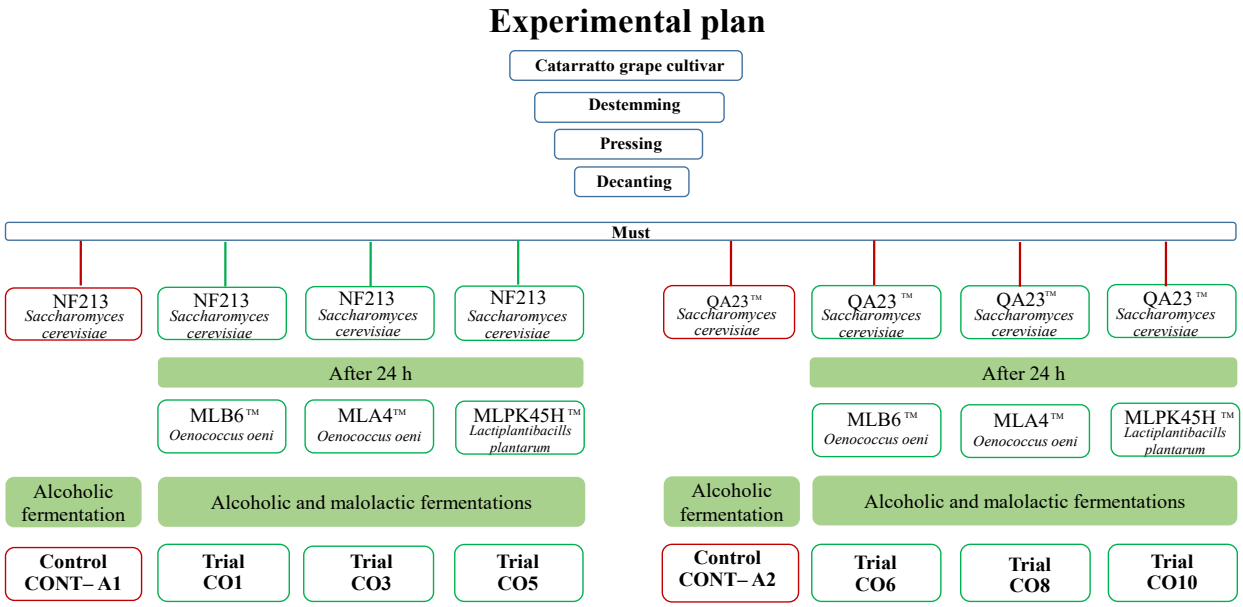


Fig. 1. Experimental plan.

The second experimental set comprised the CO6, CO8 and CO10 trials, which were inoculated with the *S. cerevisiae* QA23™ strain. After 24 h, the LAB strains were added: MLB6 (*O. oeni*) in the CO6 trial; MLA4 (*O. oeni*) in the CO8 trial; MLPK45H (*L. plantarum*) in the CO10 trial. The control CONT A2 was inoculated only with *S. cerevisiae* QA23™ strain.

The QA23™, MLB6™, MLA4™ and MLPK45H™ strains were used as described by the manufacturer (Lallemand Inc. Italia, Castel D'Azzano, Verona, Italy). The strain NF213, which belongs to the strain collection of the Department of Agriculture, University of Palermo, was used at a dose of 20 g/hL. Before yeast inoculation, total nitrogen levels were adjusted as reported by Kemsawasd, Viana, Ardö, & Arneborg (2015) using an organic nutrient. Additionally, in the control trials CONT A1 and CONT A2, 10 g/hL of lysozyme (Esseco s.r.l. San Martino, Novara, Italy) were added before inoculation of *S. cerevisiae* to prevent the development of indigenous LAB.

Samples were taken for analysis from the clarified must before, after *S. cerevisiae* yeast inoculation, after LAB inoculation and at the end of alcoholic fermentation (days 1, 2, 3 and 11).

The samples were collected in triplicate and transported under refrigerated conditions, placed in a climate chamber at 4 °C, and analytically processed within 24 h. Samples for VOCs analysis were collected at the end of sugar or malic acid depletion (days 11, 19, 45, 69).

For brevity of the manuscript and ease of reference of the data by the reader, only data collected at significant sampling points have been reported in the manuscript.

2.2. Winemaking process

The grapes were destemmed and crushed, and 4 g/q of metabisulfite (Laffort, France) was added. Two g/hL of pectolytic enzyme LALLZYME HC™ (Lallemand Inc. Italy, Castel D'Azzano, Verona, Italy) was added to the must during the static settling stage. In addition, a temperature of 10 °C was maintained for 24 h to facilitate the catalytic action of the pectins. Then, the must was aliquoted into 24 (1 hL each) steel tanks to constitute eight different trials, each of which was conducted in triplicates. The trials were inoculated as described in the experimental plan when they reached a temperature of 15 °C. Fermentation took place inside a climatic cell in such a way as to allow the constant and uniform maintenance of 20 °C for the fermentation activities to take place. The addition of potassium metabisulfite (Esseco s.r.l., San Martino, Novara, Italy) was carried out one week after the complete degradation of malic acid. Therefore, microbial inactivity was ensured by 0.8 mg/L molecular SO_2 (Tomasset, 1978).

2.3. Microbial counts and identification of yeasts and LAB strains

During alcoholic fermentation, plate counts were performed to estimate the levels of total yeasts (Pallmann et al., 2001), which were differentiated into *Saccharomyces* and non-*Saccharomyces* colonies as described by Varela (2016). LAB population was monitored according to the procedure described by Tristezza et al. (2016). To make the CFU/mL data in the must during the fermentation and post-fermentation phases as realistic as possible, the mass was homogenized under sterile conditions.

Yeast isolates were purified and phenotypically grouped as reported by Alfonzo et al. (2020). The selection and molecular identification of yeast isolates at species level (Francesca et al., 2024) and genetic strain characterization (Alfonzo et al., 2021) were also performed. The isolation and genetic

characterization of LAB strains were conducted as reported by Solieri et al., 2010. The study of the microbial population, determined by the DNA fingerprinting method, permitted an evaluation of the dominance of all inoculated strains.

2.4. Physico-chemical analysis

The samples were centrifuged at 9000 rpm at 4 °C and then filtered through a polyethersulfone membrane with a pore size of 0.20 μm (VWR®). CO_2 stripping was performed using a vacuum pump to minimize errors during instrumental reading. The pH, total acidity, and ethanol were measured using a FOSS-WineScanTM Flex system (FOSS, Hillerød, Denmark), according to the procedure described in OIV Res. Oeno 390/10 All.2. The values of L-malic, L-lactic, acetic acids, together with reducing sugars, glucose and fructose, and glycerol were measured by means of an iCubio iMagic M9 enzymatic analyser (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China), as reported by Matraxia et al. (2021).

2.5. Analysis of VOCs in wine samples

2.5.1. Standard solutions

Standards for each compound were purchased individually from Sigma-Aldrich (82024 Taufkirchen, Germany). 2,3-butanediol was used as standard for the alcohol fraction, acetoin as standard for the carboxyl-function fraction and ethyl lactate as standard for the ester fraction. In addition, n-alkane standards (C8 to C40) were purchased from Aldrich Chemical Co. (St. Louis, Mo., USA). Standard solutions of each compound were prepared at five different concentrations: 2,3-butanediol, 53.25 mg/L, 112.50 mg/L, 225.00 mg/L, 262.00 mg/L, 450. 00 mg/L; acetoin: 24.70 mg/L, 45.70 mg/L, 64.70 mg/L, 115.60 mg/L, 173.30 mg/L, 289.80mg/L; ethyl lactate, 79.00 mg/L, 134.00 mg/L, 224.00 mg/L, 326.00 mg/L, 477.00 mg/L.

2.5.2. Extraction, identification and quantification of VOCs by GC-MS

To determine the volatile compound composition, wine samples (10 mL) from all trials were mixed with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask. The mixture was stirred at room temperature for 30 min and then centrifuged at 4000 rpm for 10 min using a Low Speed Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS Industrivej 6–8, Vassingerød, DK-3540 Lynge, Denmark). The aqueous phase was removed, and anhydrous sodium sulphate (1 g) was added before centrifugation at 4000 rpm for 5 min. The dichloromethane layer was removed and dried under N_2 gas to 0.3 mL.

Gas chromatographic analyses were performed with Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 °C. Solvent Delay: 3.5 min. Helium was the carrier gas (1 mL/min).

The temperature was initially maintained at 40 °C for 1 min. Then it was gradually increased to 250 °C at a rate of 3 °C/min for 30 min and finally maintained at 250 °C at 10 °C/min. One µL of sample was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra. Quantification was carried out using the three calibration lines. For compounds belonging to other classes than the standards, similarity was used for quantification. A dilution factor was used for the reported data.

To determine which VOCs were actively contributing to the wine's aroma profile, the detected concentrations were transformed into odour activity units (OAV) using the method described by Butkhup et al. (2011). The method reported by Butkhup et al. (2011) was also used to calculate the aroma series per individual wine (fruity, floral, fatty, solvent and sulphurous). The sum of the individual odourant active values (OAV) determined for each volatile organic compound per trial

defined the olfactory intensity of the test wine. The VOCs with OAV > 0.1 were then grouped and reported in tables for ease of reading and processing (Peng, Wen, Tao, & Lan, 2013).

2.6. Sensory analysis

The sensory profiles of the wines were evaluated by submitting the different theses to a trained panel of judges, as described by Jackson (2022). The sensory evaluation was conducted by a 15-member panel of judges, consisting of eight women and seven men, with ages ranging from 27 to 45 years. The panel was preliminarily submitted for organoleptic performance evaluation. The organoleptic profiles of the wines were elaborated in triplicate by three different wine tasting committees for both test batteries. Quantification of the different descriptors detected was performed through a 9-point intensity scale, as described by Alfonzo et al. (2020). Sensory analysis was performed as reported by Naselli et al. (2023).

2.7. Determination of technological affinity index

The technological affinity index (TAIndex) of LAB - *S. cerevisiae* has been proposed by this study and estimated using linear regression with the following formula:

TAIndex= $\{[Vi / (- m)] / t_c\}$, expressed in "t^{-1}";

where:

- "Vi": is the instantaneous speed of the reaction for the conversion of malic acid to lactic acid (malo-lactic fermentation), expressed in "[malic acid] g/L × day $^{-1}{}_{MLF}$";

- "m": is the degradation rate of malic acid in the unit of time, expressed in [malic acid] g/L × day $^{-1}{}_{MLF}$";

- "t_c" is the time of the reaction for the conversion of malic acid to lactic acid (malo-lactic fermentation) corrected by the graphical method modified of Di Stefano & Cravero (1989).

The parameter V_i was calculated using the following formula:

$$Vi = \frac{\Delta[malic\ acid](\mathrm{g/L})}{\Delta[\mathrm{tc}]}$$

$$Vi = \frac{[\text{malic acid}]\text{inoculum LAB}(\mathrm{g/L}) - [\text{malic acid}]\text{end malo\_lactic fermentation}(\mathrm{g/L})}{\text{"tc" end malo\_lactic fermentation LAB inoculum} - \text{"t" inoculum LAB}}$$

The parameter "m" represents the angular coefficient of the straight line obtained from the linear regression equation of the malic acid trend of malolactic fermentation versus time (Fig. 2a, b, c).

The parameter "t_c" is calculated graphically by measuring the distance between the two points on the malic acid concentration trend line as a function of time using the following formula:

$$tc = d\ (A, B) = \sqrt{(X2 - X1)^2 + (Y2 - Y1)^2}$$

where:

- X_1, coordinate point referring to the day malolactic fermentation started (LAB inoculum)
- X_2, coordinate point referring to the day of the end of malolactic fermentation
- Y_1, coordinate point referring to the concentration of malic acid detected at the end of malolactic fermentation
- Y_2, coordinate point referring to the malic acid concentration detected at the start of malolactic fermentation (LAB inoculum)

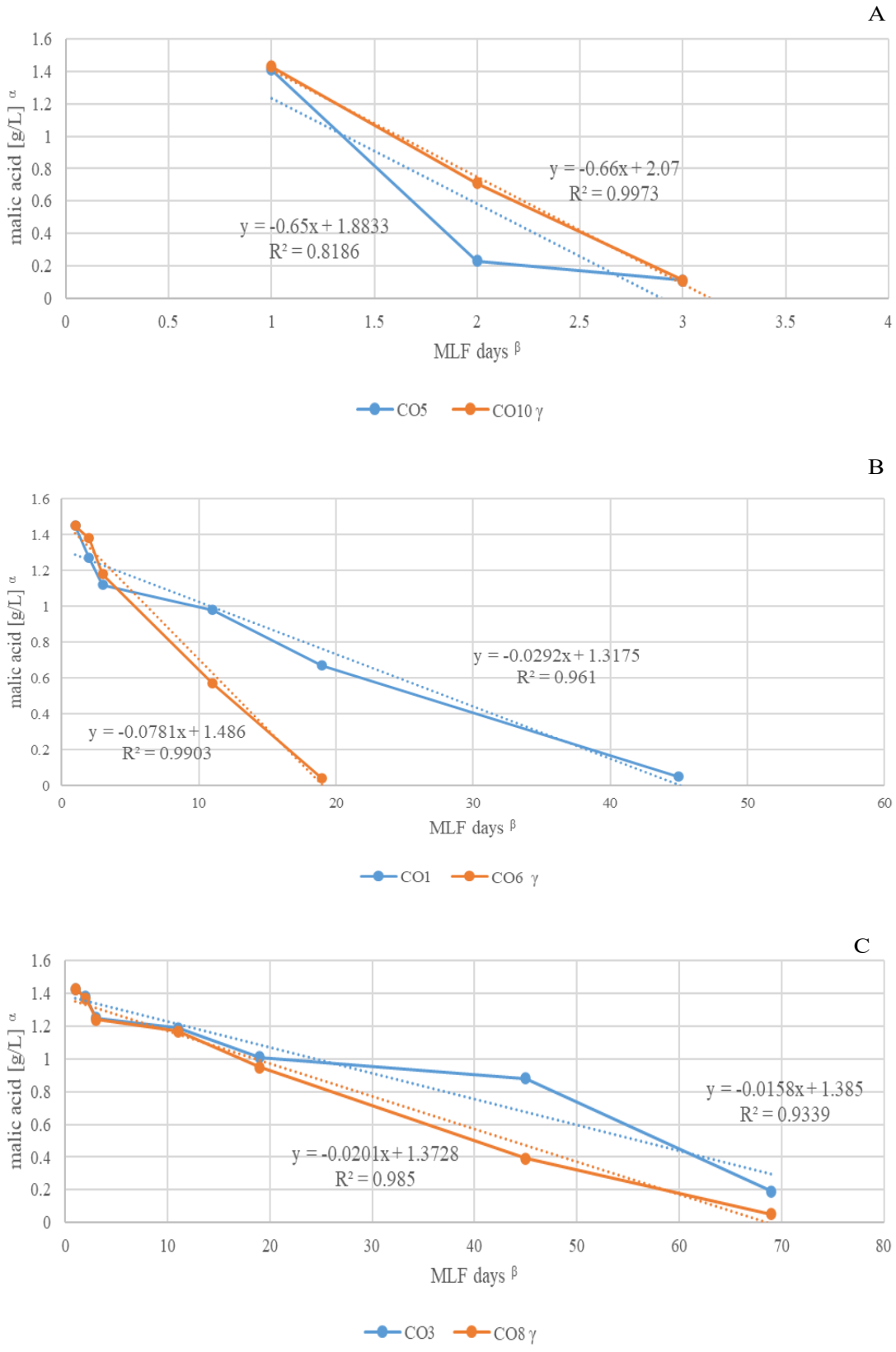


Fig. 2. Trend to degradation of malic acid as a function of time under constant temperature conditions: (A) *Lactiplantibacillus plantarum* MLPK45H with NF213 and QA23 strains *S. cerevisiae*; (B) *Oenococcus oeni* MLB6 with NF213 and QA23 strains *S. cerevisiae*; (C) *O. oeni* MLA4 with NF213 and QA23 strains *S. cerevisiae*. Simbols: $^{\alpha}$ malic acid [g/L]; $^{\beta}$ malolactic fermentation days; $^{\gamma}$ Trials.

2.8. *Statistical analysis*

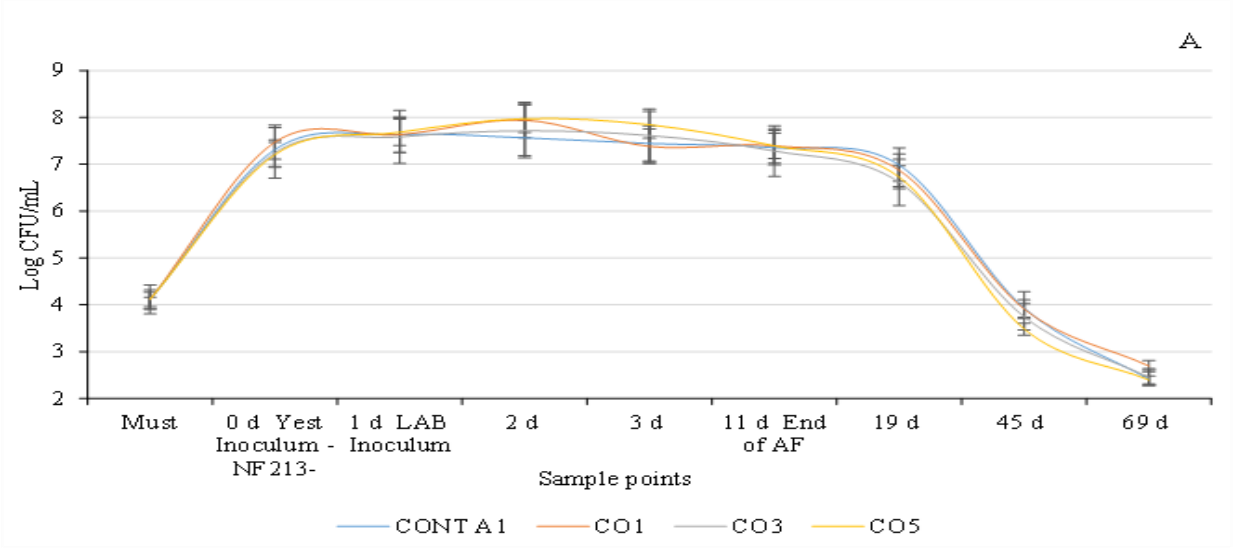
The ANOVA test was applied to determine the significance of the differences between the technological, microbial, VOC, and sensory values of the different tests. In addition, the Tukey's test was used to compare the different data, and values of $P < 0.05$ determined significance. The relationships between VOCs, sensory attributes and biotechnological associations were determined by means of agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) (Naselli et al., 2023).

3. Results and discussion

3.1. Microbial growth dynamics and strain monitoring

The trends of yeast monitoring during the course of fermentation are shown in Fig. S1a, b, c, d. At the beginning of the trial, the detection of *S. cerevisiae* and non-*Saccharomyces* populations in Catarratto must were 4.11 (Fig. S1a, b) and 3.67 Log CFU/mL (Fig. S1c, d), respectively. After yeast inoculation, the cell density of the first experimental set using *S. cerevisiae* NF213 strain ranged from 7.20 and 7.47 Log CFU/mL between trials. The levels of *S. cerevisiae* detected after adding QA23 strain in the second test set were comparable, with a degree of variability between trials of 7.21 and 7.51 Log CFU/mL. The population of non-*Saccharomyces* decreased correspondingly with the increase in *S. cerevisiae*. These decreases were at values of < 2.0 Log CFU/mL on the second day of fermentation. The monitoring of non-*Saccharomyces* was consistent with the findings of Wang, Mas, & Esteve-Zarzoso, (2016).

Before inoculation of the *S. cerevisiae* strains, LAB values of 3.1 Log CFU/mL were detected (Fig. S1e, f). After inoculation of the LAB, which occurred 24 h after the addition of the yeast strain, the bacterial populations in the CO1 and CO3 and CO6 and CO8 trials (inoculated with *O. oeni* strains) were between 5.41 and 5.51 Log CFU/mL. These values were similar to those reported by Celik et al. (2018). In the trials involving the addition of the 24-h MLPK45H strain of *S. cerevisiae*, a cell density of 7.71 Log CFU/mL was found.


A
Log CFU/mL
9
8
7
6
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0 d Yest Inoculum - NF213-
1 d LAB Inoculum
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45 d
69 d
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CONT A1
CO1
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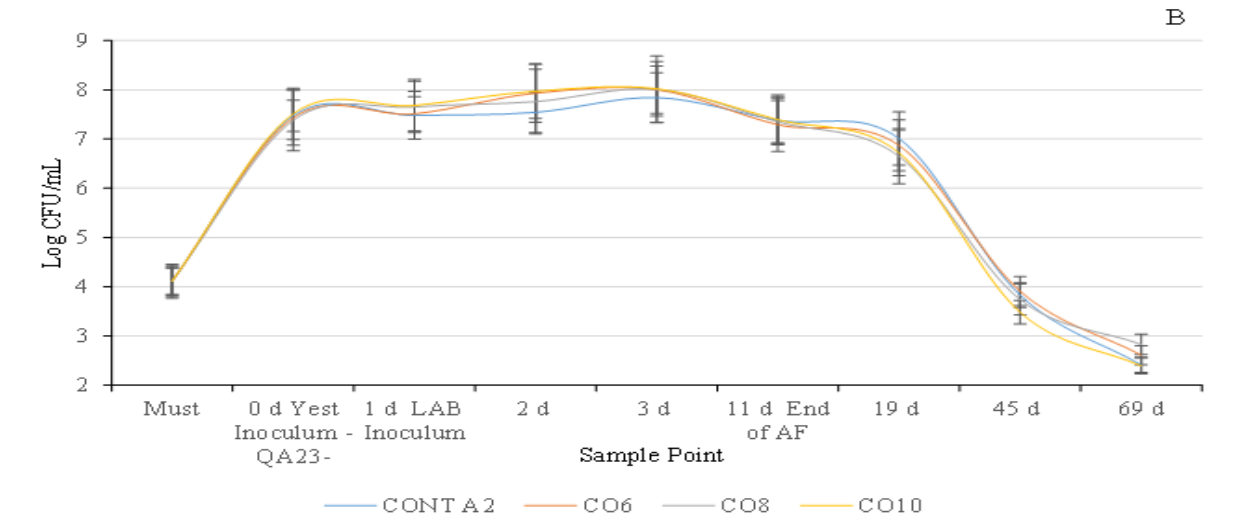
CO5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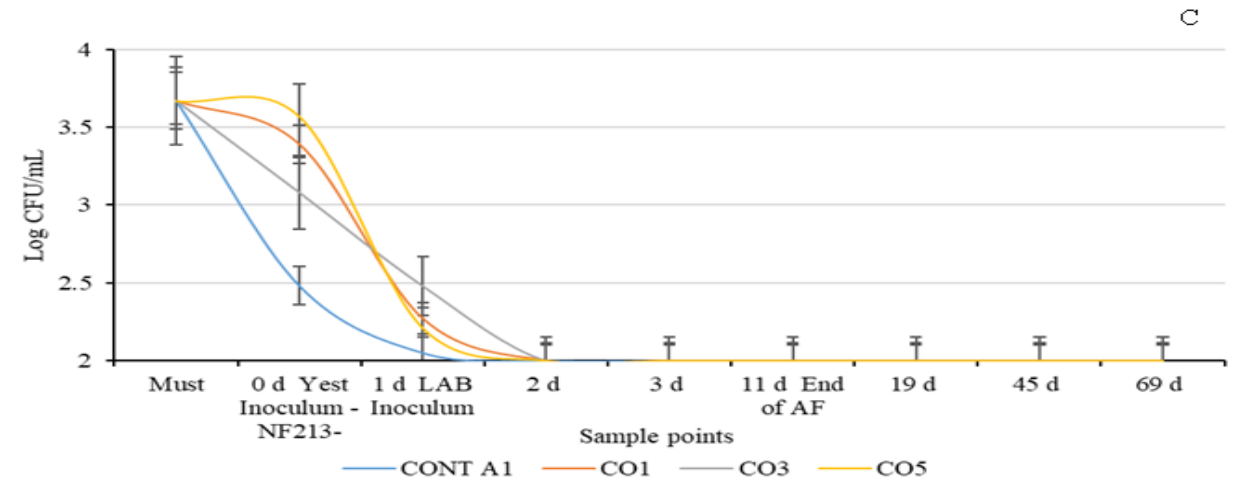
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CO3
CO5

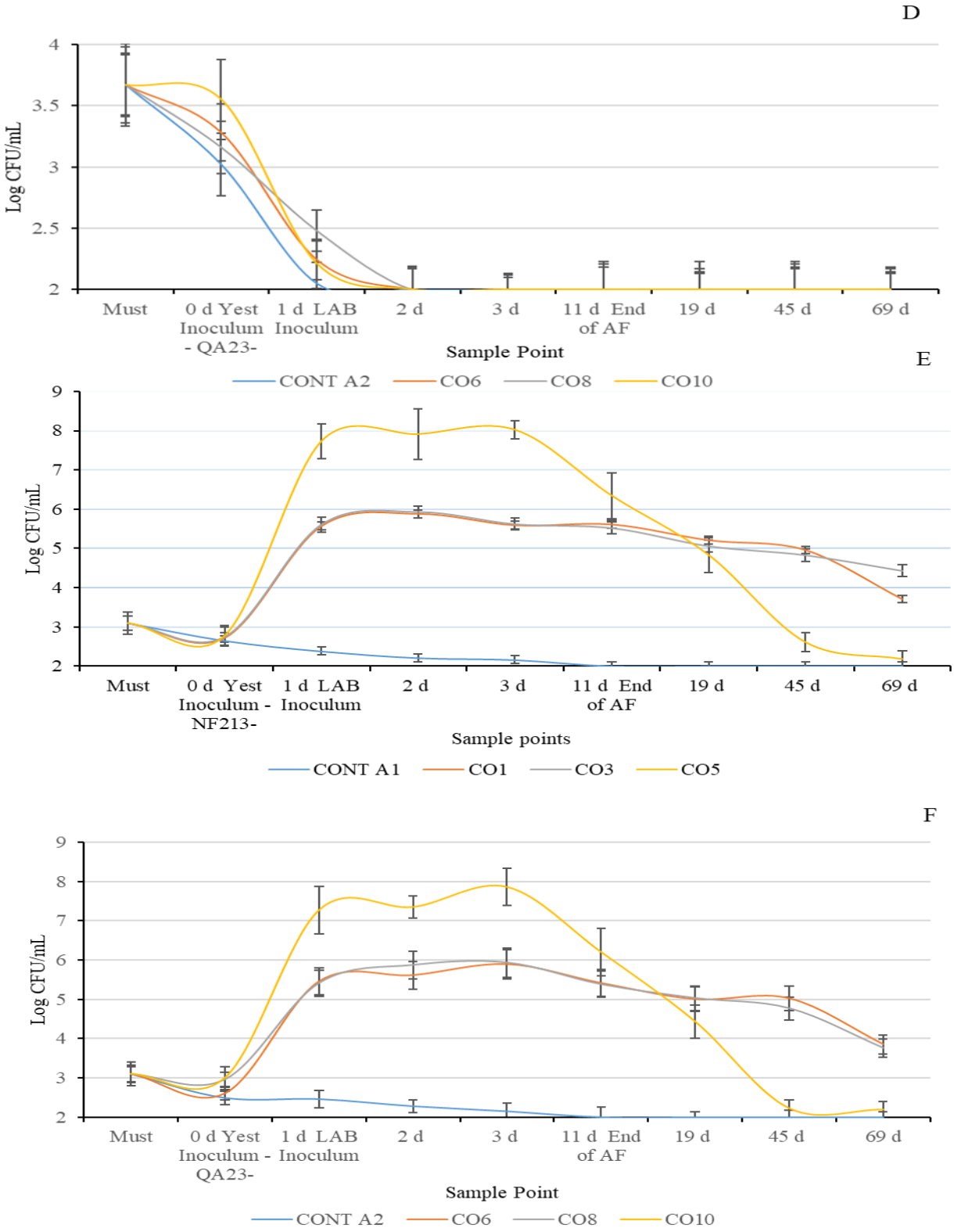

Fig. S1. Microbiological monitoring: (A) *Saccharomcyces cerevisiae* populations Catarratto must in CONT A1, CO1, CO3, CO5 trials; (B) non – *Saccharomyces* in Catarratto must in the CONT A1, CO1, CO3, CO5 trials; (C) *S. cerevisiae* populations in Catarratto must in the CONT A2, CO6, CO8 CO10 trials; (D) non - *Saccharomyces* populations in Catarratto must in the CONT A2, CO6, CO8 CO10 trials; (E) Lactic acid bacteria populations in Catarratto must in the CONT A1, CO1, CO3, CO5 trials; (F) Lactic acid bacteria populations in Cataract must in the CONT A2, CO6, CO8 CO10 trials.

The highest levels of LAB were 5.88 – 5.93 Log CFU/mL for *O. oeni* and 8.0 Log CFU/mL for *L. plantarum*, respectively. At the end of alcoholic fermentation, which occurred for both experimental sets on the 11th fermentation day, the yeasts were at a cell density of 7.39 Log CFU/mL. At the 19th fermentative day, the CO5 and CO10 trials resulted in a lower concentration in LAB than the CO1, CO3 and CO6, and CO8 trials (Fig. S1e, f). This phenomenon is imputable to the depletion of malic acid and to the addition of potassium metabisulphite. At the 19th and 45th fermentation days, corresponding to the end of malolactic fermentation for CO1 and CO6 trials, the levels of LAB were 5.21 and 5.00 Log CFU/mL, respectively. On the 69th day fermentation, the CO3 and CO8 trials completed malic acid degradation and LAB reached densities of 4.43 and 3.76 Log CFU/mL, respectively.

3.2. Kinetics of the main oenological parameters

Table S1 shows the technological parameters of the starting must.

Table S1
Chemical and physical parameters measured at day 0 (yeast inoculum) and day 1 of alcoholic fermentation.

Parameters	*Yeast inoculum*	CONT A1	CO1	CO3	CO5	CONT A2	CO6	CO8	CO10	S.S.
Reducing Sugar $^{\alpha}$	244.71±3.67 ^{a}	228.43± 3.42^{cd}	227.61± 3.18^{d}	226.75 ± 4.08 ^{d}	229.25±2.78 ^{bcd}	238.12±2.78 ^{abc}	239.45±4.27 ^{a}	239.11±4.00 ^{ab}	237.45±3.69 ^{abcd}	***
pH	3.30±0.04 ^{a}	3.31±0.04 ^{a}	3.31±0.04 ^{a}	3.31±0.06 ^{a}	3.31±0.04 ^{a}	3.31±0.06 ^{a}	3.31±0.05 ^{a}	3.31±0.05 ^{a}	3.31±0.04 ^{a}	n.s.
Total Acidity $^{\beta}$	6.01±0.8 ^{a}	5.93±0.09 ^{a}	5.93±0.08 ^{a}	5.96±0.10 ^{a}	5.93±0.07 ^{a}	5.92±0.10 ^{a}	5.97±0.09 ^{a}	5.96±0.09 ^{a}	5.99±0.07 ^{a}	n.s.
L-malic acid $^{\alpha}$	1.46± 0.01^{a}	1.43±0.02 ^{a}	1.45±0.02 ^{a}	1.43±0.02 ^{a}	1.41±0.01 ^{a}	1.42±0.02 ^{a}	1.45±0.02 ^{a}	1.43±0.02 ^{a}	1.43±0.01 ^{a}	n.s.
L-lactic acid $^{\alpha}$	0.00±0.00 ^{d}	0.01±0.00 ^{c}	0.03±0.00 ^{a}	0.02±0.00 ^{b}	0.02±0.00 ^{b}	0.03±0.00 ^{a}	0.01±0.00 ^{c}	0.02±0.00 ^{b}	0.02±0.00 ^{b}	***
Acetic acid $^{\alpha}$	0.01±0.00 ^{h}	0.08±0.00 ^{b}	0.04±0.00 ^{e}	0.07±0.00 ^{c}	0.06±0.00 ^{d}	0.10±0.00 ^{a}	0.02±0.00 ^{g}	0.03±0.00 ^{f}	0.02±0.00 ^{g}	***
Citric acid $^{\alpha}$	0.27±0.01 ^{a}	0.26±0.01 ^{b}	0.25±0.01 ^{c}	0.27±0.01 ^{a}	0.27±0.01 ^{a}	0.27±0.01 ^{a}	0.27±0.01 ^{a}	0.27±0.01 ^{a}	0.27±0.01 ^{a}	***
Ethanol $^{\gamma}$	0.00±0.00 ^{g}	0.91±0.01 ^{bc}	0.94±0.01 ^{b}	1.06±0.02 ^{a}	0.88±0.01 ^{c}	0.35±0.01 ^{e}	0.29± 0.01 ^{f}	0.29±0.01 ^{f}	0.43±0.01 ^{d}	***

Result indicate mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: α, g/L; β, tartaric acid g/L; γ, % v/v. Abbreviations: S.S., statistical significance; P value: ***, P < 0.001; n.s., not significant.

The CO5 and CO10 trials exhibited the most rapid depletion of malic acid, which occurred in two days (Table S2). The consociations with the two different strains of *S. cerevisiae* (QA23 and NF213) did not affect the degradation of malic acid, except for timing; Fig. 2a).

The higher speed of malic acid depletion could be attributed to the TAIndex found when using the LAB strain MLPK45H with the two S. *cerevisiae* strains NF213 and QA23 (Table 1) respectively.

Table 1
Technological affinity index between LAB and *S. cerevisiae* and validity of the method.

Trials [α]	Microbic consociations [β]	TAIndex [γ]	m [δ]	R^2 [ε]	MLF days [ζ]
CO1	MLB6/NF213	0.009	- 0.0292	0.9610	44
CO3	MLA4/NF213	0.013	- 0.0201	0.9903	68
CO5	MLPK45H/NF213	0.351	- 0.6500	0.9339	2
CO6	MLB6/QA23	0.148	- 0.0781	0.9850	18
CO8	MLA4/QA23	0.019	- 0.0158	0.9339	68
CO10	MLPK45H/QA23	0.348	- 0.6600	0.8186	2

[α] Trials;
[β] Microbic consociations: LAB strains – *S. cerevisiae* strains.
[γ] TAIndex. Technological Affinity Index LAB – *S. cerevisiae*.
[δ] m, degradation rate of malic acid [g/L] in the unit of time (day) (angular coefficient, derived from the equation of the straight lines shown in Fig.2.
[ε] R^2, coefficient of determination (derived from the equation of the straight lines shown in Fig. 2).
[ζ] MLF days, days of malolactic fermentation.

Table S2
Chemical and physical parameters measured at day 3 of alcoholic fermentation corresponding to day 2 of malolactic fermentation.

Parameters	CONT A1	CO1	CO3	CO5	CONT A2	CO6	CO8	CO10	S.S.
Reducing Sugar [α]	141.32±2.11 [b]	140.05±1.96 [b]	138.73±2.49 [b]	137.98±1.67 [b]	153.49±2.70 [a]	152.69±2.37 [a]	156.84±2.42 [a]	154.20±1.92 [a]	***
pH	3.34±0.05 [a]	3.36±0.05 [a]	3.34±0.06 [a]	3.38 ±0.04 [a]	3.33±0.06 [a]	3.34±0.06 [a]	3.35±0.05 [a]	3.38±0.04 [a]	n.s.
Total Acidity [β]	5.73±0.09 [a]	5.68±0.08 [a]	5.83±0.010 [a]	4.98±0.06 [b]	5.74±0.10 [a]	5.71±0.10 [a]	5.81±0.09 [a]	4.95±0.06 [b]	***
L-malic acid [α]	1.31±0.02 [a]	1.12±0.02 [d]	1.25±0.02 [b]	0.11±0.01 [e]	1.36±0.02 [a]	1.18 ±0.02[c]	1.24±0.02 [b]	0.11±0.01 [e]	***
L-lactic acid [α]	0.03±0.01 [h]	0.18±0.01 [d]	0.09±0.01 [f]	1.05±0.01 [a]	0.06± 0.01 [g]	0.24±0.01 [c]	0.11±0.01 [e]	1.03±0.01 [b]	***
Acetic acid [α]	0.09±0.01 [f]	0.14±0.01 [c]	0.14±0.01 [c]	0.12±0.01 [d]	0.18±0.01 [a]	0.12±0.01 [d]	0.10±0.01 [e]	0.16±0.01 [b]	***
Citric acid [α]	0.26± 0.01 [bcd]	0.25±0.01 [d]	0.26±0.01 [b]	0.27±0.01 [ab]	0.25±0.01 [cd]	0.27±0.01 [a]	0.26± 0.01 [bc]	0.26±0.01 [b]	***
Ethanol [γ]	5.99±0.08 [d]	6.02±0.08 [c]	6.22±0.10 [c]	6.22±0.07 [b]	5.21±0.05 [e]	6.01±0.11 [a]	5.11±0.08 [d]	5.28±0.06 [d]	***

Result indicate mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: α, g/L; β, tartaric acid g/L; γ, % v/v. Abbreviations: S.S., statistical significance; P value: ***, P < 0.001; n.s., not significant.

Table S3
Chemical and physical parameters measured at day 11 of alcoholic fermentation corresponding to day 10 of malolactic fermentation.

Parameters	CONT A1	CO1	CO3	CO5	CONT A2	CO6	CO8	CO10	S.S.
Reducing Sugar [α]	0.91±0.02 [ab]	0.77±0.01 [c]	0.80±0.01 [bc]	0.80±0.01 [bc]	0.86±0.01 [abc]	0.93±0.01 [a]	0.81±0.01 [abc]	0.90±0.01 [ab]	***
pH	3.35±0.05 [a]	3.37±0.05 [a]	3.36±0.06 [a]	3.38±0.01 [a]	3.36±0.06 [a]	3.36±0.06 [a]	3.38±0.05 [a]	3.38±0.04 [a]	n.s.
Total Acidity [β]	5.51±0.08 [ab]	5.48±0.08 [ab]	5.80±0.10 [a]	4.98±0.06 [b]	5.52±0.10 [ab]	5.12±0.08 [ab]	5.79±0.09 [a]	4.95±0.01 [b]	***
L-malic acid [α]	1.243±0.01 [a]	0.98±0.01 [d]	1.19±0.01 [bc]	0.11±0.01 [f]	1.27±0.01 [ab]	0.57±0.01 [e]	1.17±0.01 [c]	0.11±0.01 [f]	***
L-lactic acid [α]	0.08±0.01 [c]	0.20±0.01 [c]	0.12±0.01 [c]	1.05±0.01 [a]	0.12±0.01 [c]	0.53±0.01 [b]	0.13±0.01 [c]	1.03±0.01 [a]	***
Acetic acid [α]	0.16±0.01 [d]	0.27±0.01 [b]	0.17±0.01 [d]	0.12±0.01 [e]	0.30±0.01 [a]	0.21±0.01 [c]	0.16±0.01 [d]	0.16±0.01 [d]	***
Citric acid [α]	0.247±0.01 [a]	0.23±0.01 [a]	0.25±0.01 [a]	0.27±0.01 [a]	0.24±0.01 [a]	0.26±0.01 [a]	0.25±0.01 [a]	0.26±0.01 [a]	n.s.
Ethanol γ	12.75±0.02 [a]	12.61±0.02 [b]	12.63±0.02 [b]	12.69±0.01 [a]	12.72±0.02 [a]	12.62±0.02 [b]	12.64±0.02 [b]	12.68±0.02 [a]	***

Result indicate mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: α, g/L; β, tartaric acid g/L; γ, % v/v. Abbreviations: S.S., statistical significance; P value: ***, P < 0.001; n.s., not significant.

TAIndex values ranging from 0.348 to 0.351 (Tab. 1) microbiologically stabilized the must before the end of alcoholic fermentation that occurred in 11 days (Table S3).

The kinetics of malic acid degradation were influenced by the association of LAB strain MLB6 with the two different yeasts, QA23 and NF213. In fact, the CO6 trial, (MLB6-QA23) terminated malic acid degradation within day 19 of winemaking (Table S4), compared to the CO1 trial, (MLB6-NF213), that terminated at day 45 (Fig. 2b; Table S5).

Table S4
Chemical and physical parameters measured at day 18 of malolactic fermentation (day 7 from the end of alcoholic fermentation).

Parameters	CONT A1	CO1	CO3	CO5	CONT A2	CO6	CO8	CO10	S.S.
Reducing Sugar α	0.91±0.01 a	0.77±0.01 d	0.80±0.01 cd	0.78±0.01 cd	0.86±0.01 b	0.93±0.01 a	0.81±0.01 c	0.90±0.01 a	***
pH	3.35±0.05 a	3.42±0.05 a	3.41±0.06 a	3.38±0.05 a	3.36±0.05 a	3.41±0.05 a	3.42±0.05 a	3.38±0.05 a	n.s.
Total Acidity β	5.51±0.08 a	5.11±0.07 b	5.63±0.10 a	4.98±0.06 b	5.52±0.10 a	4.59±0.07 c	5.43±0.08 a	4.95±0.06 b	***
L-malic acid α	1.24±0.01 a	0.67±0.01 d	1.01±0.02 b	0.11±0.01 e	1.27±0.02 a	0.046±0.01 f	0.95±0.01 c	0.11±0.01 e	***
L-lactic acid α	0.08±0.01 g	0.42±0.01 c	0.16±0.01 e	1.05±0.01 a	0.12±0.01 f	0.72±0.01 b	0.27±0.01 d	1.03±0.01 a	***
Acetic acid α	0.16±0.01 e	0.30±0.01 a	0.20±0.01 d	0.12±0.01 f	0.30±0.01 a	0.25±0.01 b	0.23±0.01 c	0.16±0.01 e	***
Citric acid α	0.25±0.01 bc	0.23±0.01 d	0.24±0.01 cd	0.27±0.01 a	0.25±0.01 b	0.23±0.01 d	0.23±0.01 d	0.26±0.01 a	***
Ethanol γ	12.75±0.02 a	12.61±0.02 b	12.63±0.02 b	12.69±0.01 a	12.72±0.02 a	12.62±0.02 b	12.64±0.02 b	12.68±0.02 a	***

Result indicate mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: α, g/L; β, tartaric acid g/L; γ, % v/v. Abbreviations: S.S., statistical significance; P value: ***, P < 0.001; n.s., not significant.

Table S5
Chemical and physical parameters measured at day 44 of malolactic fermentation (day 33 from the end of alcoholic fermentation).

Parameters	CONT A1	CO1	CO3	CO5	CONT A2	CO6	CO8	CO10	S.S.
Reducing Sugar α	0.91±0.01 a	0.77±0.01 d	0.80±0.01 cd	0.78±0.01 cd	0.86±0.01 b	0.93±0.01 a	0.81±0.01 c	0.90±0.01 a	***
pH	3.35±0.05 a	3.42±0.05 a	3.41±0.06 a	3.38±0.05 a	3.36±0.05 a	3.41±0.05 a	3.42±0.05 a	3.38±0.05 a	n.s.
Total Acidity β	5.51±0.08 a	4.61±0.06 c	5.45±0.10 a	4.98±0.06 b	5.52±0.10 a	4.59±0.07 c	4.82±0.07 bc	4.95±0.06 b	***
L-malic acid α	1.24±0.01 a	0.05±0.01 e	0.88±0.01 b	0.11±0.01 d	1.27±0.02 a	0.04±0.01 e	0.39±0.01 c	0.11±0.01 d	***
L-lactic acid α	0.08±0.01 f	0.26±0.01 b	0.33±0.01 d	1.05±0.01 a	0.12±0.01 e	0.72±0.01 b	0.62±0.01 c	1.03±0.01 a	***
Acetic acid α	0.16±0.01 d	0.33±0.01 a	0.26±0.01 c	0.12±0.01e	0.30±0.01 b	0.25±0.01 c	0.29±0.01 b	0.16±0.01 d	***
Citric acid α	0.24±0.01 b	0.22±0.01 d	0.16±0.01 f	0.27±0.01 a	0.25±0.01 b	0.23±0.01 c	0.18±0.01 e	0.26±0.01 a	***
Ethanol γ	12.75±0.02 a	12.61±0.02 b	12.63±0.02 b	12.69±0.01 a	12.72±0.02 a	12.62±0.02 b	12.64±0.02 b	12.68±0.02 a	***

Result indicate mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: α, g/L; β, tartaric acid g/L; γ, % v/v. Abbreviations: S.S., statistical significance; P value: ***, P < 0.001; n.s., not significant.

The difference in the timing of malolactic fermentation of the two different consociations, MLB6-QA23 and MLB6-NF213, leads to the hypothesis of a different requirement of the two yeasts to assimilate metal cofactors, such as Mn^{2+} or Mg^{2+} elements, which are essential to trigger the endogenous process of enzymatic decarboxylation in *O. oeni* (Lonvaud-Funel, 2022). On the other hand, *S. cerevisiae* may respond differently to counteract the co-presence of LAB in the medium through the production of volatile catabolites (Alexandre, Costello, Remize, Guzzo, & Guilloux-

Benatier, 2004). In contrast, CO3 and CO8 trials (MLA4-NF213; MLA4-QA23) required 69 days to complete malolactic fermentation. (Fig. 2c; Table S6).

Table S6
Chemical and physical parameters measured at day 68 of malolactic fermentation (day 57 from the end of alcoholic fermentation).

Parameters	CONT A1	CO1	CO3	CO5	CONT A2	CO6	CO8	CO10	S.S.
Reducing Sugar $^{\alpha}$	0.91±0.01 ^{a}	0.77±0.01 ^{d}	0.80±0.01 ^{cd}	0.78±0.01 ^{cd}	0.86±0.01 ^{b}	0.93±0.01 ^{a}	0.81±0.01 ^{c}	0.90±0.01 ^{a}	***
pH	3.35±0.01 ^{a}	3.42±0.01 ^{a}	3.41±0.01 ^{a}	3.38±0.01 ^{a}	3.360.01 ± ^{a}	3.41±0.01 ^{a}	3.42±0.01 ^{a}	3.38±0.01 ^{a}	n.s.
Total Acidity $^{\beta}$	5.51±0.08 ^{a}	4.61±0.06 ^{b}	4.64±0.08 ^{c}	4.98±0.06 ^{c}	5.52±0.09 ^{a}	4.59±0.08 ^{c}	4.58±0.07 ^{c}	4.95±0.0 ^{b}	***
L-malic acid $^{\alpha}$	1.24±.01 ^{a}	0.05±.01 ^{d}	0.19±.01 ^{b}	0.11±.01 ^{c}	1.27±.01 ^{a}	0.04±.01 ^{d}	0.05±.01 ^{d}	0.11±.01 ^{c}	***
L-lactic acid $^{\alpha}$	0.08±0.01 ^{e}	0.70±0.01 ^{bc}	0.68±0.01 ^{c}	1.05±0.01 ^{a}	0.12±0.01 ^{d}	0.72±0.01 ^{b}	0.72±0.01 ^{b}	1.03±0.01 ^{a}	***
Acetic acid $^{\alpha}$	0.16±0.01 ^{e}	0.33±0.01 ^{a}	0.29±0.01 ^{c}	0.12±0.01 ^{f}	0.30±0.01 ^{c}	0.25±0.01 ^{d}	0.31±0.01 ^{b}	0.16±0.01 ^{e}	***
Citric acid $^{\alpha}$	0.24±0.01 ^{b}	0.22±0.01 ^{d}	0.11±0.01 ^{f}	0.27±0.01 ^{a}	0.25±0.01 ^{b}	0.23±0.01 ^{c}	0.13±0.01 ^{e}	0.26± 0.01 ^{a}	***
Ethanol $^{\gamma}$	12.75±0.02 ^{a}	12.61±0.02 ^{b}	12.63±0.02 ^{b}	12.69±0.01 ^{a}	12.72±0.02 ^{a}	12.62±0.02 ^{b}	12.64±0.02 ^{b}	12.68±0.02 ^{a}	***

Result indicate mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test. Symbols: α, g/L; β, tartaric acid g/L; γ, % v/v. Abbreviations: S.S., statistical significance; P value: ***, $P < 0.001$; n.s., not significant.

Table S6 shows the best technological performances were recorded by the trials involving the use of LAB strain MLPK45H, specifically the CO5 and CO10 trials. In spite of the consumption of malic acid, the total acidity values were the highest in each of the trial batteries compared to the trials involving the use of *O. oeni*; 4.98 g/L H_2T versus 4.61 and 4.64 g/L H_2T (for the CO5 trials versus CO1 and CO3, respectively); 4.95 g/L H_2T versus 4.59 and 4.58 g/L H_2T (for the CO10 trials versus CO6 and CO8, respectively). The higher lactic acid yield of *L. plantarum* strains and their low production of acetic acid contributed to these values.

This behaviour is the result of homofermentative metabolism of the MLPK45H strain of *L. plantarum*, as described by Krieger-Weber, Heras, & Suarez (2020).

However, the consumption of citric acid by the heterofermentative *O. oeni* strains, MLB6 and MLA4 (trials CO3, CO5 and CO6, CO8) occurred significantly in comparison to both the control trials, CONT A1 and CONT A2, and the trials involving the inoculation of the *L. plantarum* strain, CO5 and CO10. The acetic acid values, although different between the trials, remained below the technological levels compatible with high quality wines.

3.3. Volatile organic compound composition

3.3.1. Higher alcohols

Alcohols were identified as the most abundant aromatic fraction in the wines object of investigation (Table 2).

Table 2
Volatile organic compounds detected in Catarratto experimental wines (all values in mg/L).

KI [α]	KI [β]	Compounds [γ]	CONT A1 [δ]	CO 1 [δ]	CO 3 [δ]	CO 5 [δ]	S.S. [ε]	→ continued
		∑ Alcohols	**121.74±4.53 [a]**	**111.16±4.14 [b]**	**128.09±4.76 [a]**	**98.2±3.65 [c]**	***	
758	759	3-methyl-1-butanol	55.56±1.30 [a]	44.37±1.04 [c]	50.28±1.18 [b]	42.55±1.00 [c]	***	
765	765	1,2,-propanediol	1.24±0.04 [a]	1.10±0.03 [b]	1.07±0.03 [b]	0.78±0.02 [c]	***	
809	809	2.3-butanediol [ζ]	20.22±0.63 [c]	22.90±0.71 [b]	27.66±0.86 [a]	16.68±0.52 [d]	***	
816	816	2.3-butanediol [η]	5.34±0.08 [b]	5.61±0.08 [b]	7.80±0.11[a]	4.08±0.06[c]	***	
824	824	2.3-butanediol [θ]	3.44±0.09 [c]	9.86±0.25 [b]	10.42±0.26 [b]	11.00±0.28 [a]	***	
848	848	3-ethoxy-1-propanol	0.28±0.01[c]	0.65±0.01[a]	0.29±0.01[b]	0.29±0.01[b]	***	
878	878	1-hexanol	0.52±0.01 [b]	0.49±0.01 [c]	0.56±0.01 [a]	0.39±0.01 [d]	***	
1038	1039	Phenyl methanol	0.20±0.01[a]	0.18±0.01[b]	0.19±0.01 [ab]	0.00±0.00 [c]	***	
1089	1088	1,2,3-propanetriol	1.43±0.06 [a]	0.00±0.00 [c]	0.96±0.04 [b]	1.44±0.06 [a]	***	
1116	1117	Hydroxyethylbenzene	27.04±0.90 [a]	21.79±0.72 [b]	26.87±0.89 [a]	20.59±0.68 [b]	***	
1428	1428	4-(2-hydroxyethyl)-phenol	1.79±0.06 [a]	0.85±0.03 [c]	1.59±0.05 [b]	0.00±0.00 [d]	***	
1502	1503	2,4-di-tert-butylphenol	4.68±0.10 [a]	3.36±0.07 [b]	0.4±0.01 [c]	0.40±0.01 [c]	***	
		∑ Aldehydes	**2.03±0.07 [a]**	**0.93±0.03 [c]**	**0.26±0.01 [d]**	**1.59±0.04 [b]**	***	
1105	1105	Nonanal	1.01±0.03 [a]	0.60±0.02 [b]	0.08±0.01[c]	0.00±0.00 [d]	***	
1211	1211	3.4-dimethylbenzaldehyde	0.00±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]	0.49±0.0 [a]	***	
1271	-	4-propyl benzaldehyde	1.02 ±0.04 [a]	0.33±0.0 [b]	0.18±0.0 [c]	0.00±0.00 [d]	***	
1811	1812	Hexadecanal	0.00±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]	0.33±0.01[a]	***	
-	2020	Octadecanal	0.00±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]	0.77±0.02 [a]	***	
		∑ Carboxylic acids	**8.05±0.27 [bc]**	**7.92±0.24 [c]**	**8.89±0.24 [b]**	**11.08±0.41 [a]**	***	
914	916	4-hydroxybutanoic acid	0.24±0.01[c]	0.38±0.01[b]	0.66±0.02 [a]	0.71±0.03 [a]	***	
928	932	Lactic acid	0.00±0.00 [b]	1.47±0.04 [a]	0.00±0.00 [b]	0.00±0.00 [b]	***	
1013	1016	Hexanoic acid	2.78±0.12 [a]	2.15±0.09 [b]	1.54±0.06 [c]	2.05±0.09 [b]	***	
1189	1188	Octanoic acid	1.93±0.08 [b]	0.86±0.04 [c]	1.82±0.07 [b]	5.83±0.24 [a]	***	
1377	1377	Decanoic acid	3.10±0.06 [b]	3.06±0.06 [b]	4.87±0.09 [a]	2.49±0.05 [c]	***	
		∑ Esters	**14.84±0.46 [a]**	**11.73±0.37 [b]**	**13.87±0.43 [a]**	**9.31±0.57 [c]**	***	
889	884	3-methyl-1-butyl acetate	2.78±0.09 [a]	2.16±0.07 [b]	2.18±0.07 [b]	0.92±0.03 [c]	***	
885	886	2-methyl-1-butyl acetate	0.14±0.01[a]	0.13±0.01[b]	0.08±0.01[c]	0.00±0.00 [d]	***	
941	941	Ethyl 3-hydroxybutanoate	0.12±0.03 [a]	0.18±0.05 [a]	0.14±0.04 [a]	0.13±0.04 [a]	**	
1000	1001	Ethyl hexanoate	1.62±0.04 [a]	0.91±0.02 [d]	1.20±0.03 [c]	1.32±0.03 [b]	***	
1181	1181	Diethyl succinate	0.41±0.01[b]	0.30±0.01[c]	0.48±0.01 [a]	0.50±0.02 [a]	***	
1195	1196	Ethyl octanoate	3.16±0.09 [a]	3.22±0.09 [a]	2.25±0.07 [b]	2.05±0.06 [c]	***	
1206	1205	Monoethyl succinate	4.92±0.19 [a]	2.78±0.11 [b]	5.46±0.21 [a]	2.50±0.10 [b]	***	
1253	1253	Phenylethl acetate	0.62±0.02 [a]	0.53±0.02 [b]	0.60±0.02 [a]	0.54±0.02 [b]	***	
1264	1264	Diethyl malate	0.23±0.01[b]	0.28±0.01[a]	0.14±0.01[c]	0.07±0.01[d]	***	
1390	1392	Ethyl decanoate	0.84±0.04 [b]	1.24±0.05 [a]	0.70±0.03 [c]	0.71±0.03 [c]	***	
1590	1590	Ethyl dodecanoate	0.00±0.00 [c]	0.00±0.00 [c]	0.64±0.03 [a]	0.57±0.23 [b]	***	
		∑ Ketones	**1.08±0.03 [b]**	**1.08±0.03 [b]**	**1.99±0.05 [a]**	**0.76±0.02 [c]**	***	
723	722	3-hydroxy-2-butanone	0.13±0.01 [c]	0.19±0.01 [b]	0.96±0.03 [a]	0.09±0.01 [c]	***	
963	954	4-hydroxy-2-butanone	0.95±0.02 [b]	0.89±0.02 [c]	1.03±0.02 [a]	0.67±0.01 [d]	***	
		∑ Anhydrides	**0.79±0.02 [b]**	**0.63±0.01 [d]**	**1.03±0.02 [a]**	**0.69±0.02 [c]**	***	
993	994	Glutaconic anhydride	0.79±0.02 [b]	0.63±0.01 [d]	1.03±0.02 [a]	0.69±0.02 [c]	***	
		∑ Others	**3.05±0.10 [a]**	**2.91±0.09 [a]**	**0.00±0.00 [b]**	**0.00±0.00 [b]**	***	
1246	1245	1,3-di-tert-butylbenzene	3.05±0.10 [a]	2.91±0.09 [a]	0.00±0.00 [b]	0.00±0.00 [b]	***	

[α] Kovats index obtained through the modulated chromatogram reported for DB-5 MS apolar column;
[β] Kovats index based on literature (https://webbook.nist.gov/);
[γ] compounds are classified in order of Kovats index;
[δ] Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2.3-butanediol;
[ε] statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. . P value: *. P < 0.05; **. P < 0.01; ***. P < 0.001; n.s.. not significant.
[ζ] unidentified stereoisomer
[η] unidentified stereoisomer
[θ] unidentified stereoisomer

→ continued	KI [α]	KI [β]	Compounds [γ]	CONT A2 [δ]	CO 6 [δ]	CO 8 [δ]	CO 10 [δ]	S.S. [ε]
			∑ Alcohols	**147.31±3.45 [a]**	**100.14±2.31 [c]**	**110.79±2.44 [b]**	**86.82±1.95 [d]**	***
	758	759	3-methyl-1-butanol	61.54±1.62 [a]	39.4±1.04 [c]	51.8±1.36 [b]	33.40±0.88 [d]	***
	765	765	1.2.-propanediol	1.72±0.06 [a]	1.19±0.04 [b]	0.00±0.00 [c]	0.00±0.00 [c]	***
	809	809	2.3-butanediol [ζ]	38.01±0.84 [a]	22.44±0.50 [b]	13.94±0.31 [d]	19.74±0.44 [c]	***
	816	816	2.3-butanediol [η]	8.83±0.32 [a]	5.98±0.22 [b]	3.06±0.11 [d]	4.54±0.16 [c]	***
	824	824	2.3-butanediol [θ]	3.37±0.05 [d]	11.24±0.16 [b]	14.01±0.20 [a]	9.17±0.13 [c]	***
	848	848	3-ethoxy-1-propanol	1.15±0.04 [a]	0.41±0.02 [c]	0.64±0.02 [b]	0.35±0.01 [c]	***
	878	878	1-hexanol	0.76±0.02 [a]	0.37±0.01 [c]	0.53±0.01 [b]	0.36±0.01 [c]	***
	983	985	3-methylsulfanyl-1-propanol	1.56±0.04 [a]	0.76±0.02 [c]	0.95±0.03 [b]	0.68±0.02 [c]	***
	1089	1088	Phenyl methanol	0.00±0.00 [d]	0.07±0.01 [c]	0.37±0.01 [a]	0.18±0.01 [b]	***
	1116	1117	Hydroxyethylbenzene	26.42±0.38 [a]	15.98±0.23 [c]	22.54±0.32 [b]	15.58±0.22 [c]	***
	1305	1315	2-methoxy-4-vinyl phenol	1.86±0.05 [a]	0.82±0.02 [c]	1.16±0.03 [b]	1.83±0.05 [a]	***
	1428	1428	4-(2-hydroxyethyl)-phenol	2.09±0.05 [a]	1.15±0.03 [c]	1.27±0.03 [b]	0.63±0.01 [d]	***
	1502	1503	2,4-di-tert- butylphenol	0.00±0.00 [d]	0.33±0.01 [c]	0.52±0.01 [a]	0.36±0.01 [b]	***
			∑ Aldehydes	**1.80±0.06 [b]**	**1.49±0.05 [c]**	**3.11±0.10 [a]**	**1.87±0.06 [b]**	***
	1083	1079	4-methylbenzalddehyde	0.00±0.00 [c]	0.00±0.00 [c]	0.49±0.02 [a]	0.36±0.01 [b]	***
	1211	1211	3,4-dimethylbenzaldehyde	0.00±0.00 [d]	0.45±0.01 [b]	0.53±0.02 [a]	0.40±0.01 [c]	***
	1271	-	4-propyl benzaldehyde	1.80±0.06 [a]	0.00±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]	***
	1811	1812	Hexadecanal	0.00±0.00 [c]	0.34±0.01 [b]	0.66±0.01 [a]	0.35±0.01 [b]	***
	-	2020	Octadecanal	0.00±0.00 [d]	0.70±0.02 [c]	1.43±0.03 [a]	0.76±0.02 [b]	***
			∑ Carboxylic acids	**16.11±0.20 [a]**	**9.49±0.12 [a]**	**11.91±0.15 [b]**	**9.53±0.12 [b]**	***
	914	916	4-hydroxybutanoic acid	0.40±0.01 [b]	0.45±0.01 [a]	0.40±0.01 [b]	0.32±0.01 [c]	***
	928	932	Lactic acid	0.00±0.00 [c]	0.93±0.04 [b]	0.00±0.00 [c]	1.09±0.04 [a]	***
	1013	1016	Hexanoic acid	5.26±0.19 [a]	2.73±0.10 [d]	4.63±0.17 [b]	3.33±0.12 [c]	***
	1189	1188	Octanoic acid	3.09±0.09 [a]	2.59±0.08 [b]	2.97±0.09 [a]	2.29±0.07 [c]	***
	1377	1377	Decanoic acid	7.36±0.31 [a]	2.79±0.12 [c]	3.91±0.17 [b]	2.50±0.11 [c]	***
			∑ Esters	**18. 38±0.48 [a]**	**8.42±0.20 [c]**	**13.91±0.38 [b]**	**7.57±0.20 [c]**	***
	883	884	3-methyl-1-butyl acetate	2.76±0.05 [b]	2.12±0.04 [c]	3.08±0.06 [a]	1.72±0.03 [d]	***
	885	886	2-methyl-1-butyl acetate	0.00±0.00 [d]	0.12±0.01 [a]	0.10±0.01 [b]	0.06±0.01 [c]	***
	941	941	Ethyl 3-hydroxybutanoate	0.14±0.01 [a]	0.15±0.01 [a]	0.15±0.01 [a]	0.15±0.01 [a]	***
	1000	1001	Ethyl hexanoate	1.84±0.03 [a]	1.13±0.02 [c]	1.58±0.03 [b]	1.18±0.02 [c]	***
	1181	1181	Diethyl succinate	0.59±0.02 [a]	0.40±0.01 [b]	0.38±0.01 [b]	0.41±0.01 [b]	***
	1195	1196	Ethyl octanoate	3.69±0.09 [a]	1.91±0.04 [b]	2.01±0.05 [b]	1.69±0.04 [c]	***
	1206	1205	Monoethyl succinate	6.04±0.19 [a]	1.60±0.05 [c]	4.91±0.15 [b]	1.38±0.04 [c]	***
	1253	1253	Phenylethyl acetate	0.58±0.01 [a]	0.44±0.01 [b]	0.59±0.01 [a]	0.41±0.01 [c]	***
	1264	1264	Diethyl malate	0.42±0.02 [a]	0.00±0.00 [c]	0.12±0.01 [b]	0.00±0.00 [c]	***
	1294	-	Ethyl decanoate	1.30±0.03 [a]	0.55±0.01 [c]	0.73±0.01 [b]	0.45±0.01 [d]	***
	1390	1392	Ethyl dodecanoate	0.00±0.00 [c]	0.00±0.00 [c]	0.15±0.02 [a]	0.07±0.01 [b]	***
	1590	1590	Ethyl 5-oxo-2-pyrrolidine-carboxylate	1.02±0.03 [a]	0.00±0.00 [c]	0.15±0.01 [b]	0.05±0.01 [c]	***
			∑ Ketones	**1.62±0.04 [a]**	**0.76±0.03 [d]**	**1.23±0.03 [b]**	**0.91±0.03 [c]**	***
	723	722	3-hydroxy-2-butanone	0.27±0.01 [b]	0.07±0.01 [d]	0.33±0.01 [a]	0.21±0.01 [c]	***
	963	954	4-hydroxy-2-butanone	1.35±0.03 [a]	0.69±0.02b [c]	0.90±0.02 [b]	0.70±0.02 [c]	***
			∑ Others	**2.26±0.07 [a]**	**0.00±0.00 [b]**	**0.00±0.00 [b]**	**0.00±0.00 [b]**	***
	1246	1245	1.3-di-tert-butylbenzene	2.26±0.07 [a]	0.00±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]	***

[α] Kovats index obtained through the modulated chromatogram reported for DB-5 MS apolar column;
[β] Kovats index based on literature (https://webbook.nist.gov/);
[γ] compounds are classified in order of Kovats index;

[δ] Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2.3-butanediol;
[ε] statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: *. $P < 0.05$; **. $P < 0.01$; ***. $P < 0.001$; n.s.. not significant.
[ζ] unidentified stereoisomer
[η] unidentified stereoisomer
[θ] unidentified stereoisomer

The trials that predicted LAB - *S. cerevisiae* microbial consociations showed lower amounts of higher alcohols than the control trials (CONT A1 and CONT A2), with the exception of the CO3 trial (Table 2). These results confirm the findings of Knoll et al. (2012). The CO3 trial stood out by registering a higher total amount of alcohols than the CONT A1 control (Table 2). The production of three different enantiomers of 2,3-butanediol in comparison to the CONT A1 control trial contributed to this result (Table 2). This peculiarity was exclusively recorded in the MLA4 - NF213 consociation (CO3 trial), indicating that this microbial combination has a higher conversion rate of 2,3-butanediol than the corresponding CO8 trial (MLA4 -QA23) and all other microbial interaction trials. Furthermore, the microbial consociation MLB6-NF213 (CO1 trial) exhibited a peculiarity in its favor, recording a significant production of 3-ethoxy-1-propanol in comparison to the CONT A1 trial (Table 2). This peculiarity was attributed to the *S. cerevisiae* yeast strain used in the microbial combination, as it was not produced in the corresponding CO6 trial (MLB6-QA23; Table 2).

The differences in higher alcohols detected between the trials could be due to trophic competitions that occurred between LAB and yeasts during fermentation (Maarman, 2014). The antagonisms between the different microorganisms are presumed to have occurred for the amino acid compounds, in particular, leucine, phenylalanine, tyrosine and methionine (Maarman, 2014). The significant differences between trials in the formation of 3-methyl-1-butanol, hydroxyethylbenzene, 4-(2-hydroxyethyl)-phenol, and 3-methylsulfanyl-1-propanol through the Ehrlich pathway in yeast could support this inference (Ribéreau-Gayon, 2018). Therefore, the varying concentrations of these volatile organic compounds could be a result of a specific physiological nutritional requirement by the LAB strains (Ribéreau-Gayon, 2018).

3.3.2. Esters

3.3.2.1. Acetate esters

Trial showed significant differences in terms of acetate ester amount and composition (Tab. S7, S8). The total acetate ester values were higher in CO8 trials (3.77mg/L) than the CONT A2 control (3.34mg/L). The lowest values were found in the CO6 and CO10 trials (2.68 and 2.19 mg/L, respectively).

Table S7
Acetate and ethyl esters values (mg/L).

	CONT A1	CO 1	CO 3	CO 5	S.S. [α]
3-methyl-1-butyl acetate	2.78±0.09 [a]	2.16±0.07 [b]	2.18±0.07 [b]	0.92±0.03 [c]	***
2-methyl-1-butyl acetate	0.14±0.01 [a]	0.13±0.01 [b]	0.08±0.01 [c]	0.00±0.00 [d]	***
Phenylethyl acetate	0.62±0.02 [a]	0.53±0.02 [b]	0.60±0.02 [a]	0.54±0.02 [b]	***
Total acetate esters	**3.54±0.12 [a]**	**2.82±0.10 [b]**	**2.86±0.10 [b]**	**1.46±0.05 [c]**	***
Total olfactory active acetic esters	**3.40±0.11 [a]**	**2.69±0.09 [b]**	**2.78±0.09 [b]**	**1.46±0.05 [c]**	***
Ethyl hexanoate	1.62±0.04 [a]	0.91±0.02 [d]	1.20±0.03 [c]	1.32±0.03 [b]	***
Ethyl octanoate	3.16±0.09 [a]	3.22±0.09 [a]	2.25±0.07 [b]	2.05±0.06 [c]	***
Ethyl decanoate	0.84±0.04 [b]	1.24±0.05 [a]	0.70±0.03 [c]	0.71±0.03 [c]	***
Ethyl dodecanoate	0.00±0.00 [c]	0.00±0.00 [c]	0.64±0.03 [a]	0.57±0.23 [b]	***
Ethyl 3-hydroxybutanoate	0.12±0.03 [a]	0.18±0.05 [a]	0.14±0.04 [a]	0.13±0.04 [a]	***
Monoethyl succinate	4.92±0.19 [a]	2.78±0.11 [b]	5.46±0.21 [a]	2.50±0.10 [b]	***
Diethyl succinate	0.41±0.01 [b]	0.30±0.01 [c]	0.48±0.01 [a]	0.50±0.02 [a]	***
Diethyl malate	0.41±0.01 [b]	0.30±0.01 [c]	0.48±0.01 [a]	0.50±0.02 [a]	***
Total ethyl esters	**11.3±0.41 [a]**	**8.91±0.34 [b]**	**11.01±0.43 [a]**	**7.85±0.53 [b]**	***
Total olfactory active ethyl esters	**5.62±0.17 [a]**	**5.37±0.16 [a]**	**4.15±0.13 [b]**	**4.08±0.12 [b]**	***

Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2,3-butanediol. Abbreviations: [α] S.S, statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, $P < 0.001$.

Table S8
Acetate and ethyl esters values (mg/L).

	CONT A2	CO 6	CO 8	CO 10	S.S. [α]
3-methyl-1-butyl acetate	2.76±0.05 [b]	2.12±0.04 [c]	3.08±0.06 [a]	1.72±0.03 [d]	***
2-methyl-1-butyl acetate	0.00±0.00 [d]	0.12±0.01 [a]	0.10±0.01 [b]	0.06±0.01 [c]	***
Phenylethl acetate	0.58±0.01 [a]	0.44±0.01 [b]	0.59±0.01 [a]	0.41±0.01 [c]	***
Total acetate esters	**3.34±0.06 [b]**	**2.68±0.06 [c]**	**3.77±0.08 [a]**	**2.19±0.05 [d]**	***
Total olfactory active acetic esters	**3.34±0.06 [b]**	**2.56±0.05 [c]**	**3.67±0.07 [a]**	**2.13±0.04 [d]**	***
Ethyl hexanoate	1.84±0.03 [a]	1.13±0.02 [c]	1.58±0.03 [b]	1.18±0.02 [c]	***
Ethyl octanoate	3.69±0.09 [a]	1.91±0.04 [b]	2.01±0.05 [b]	1.69±0.04 [c]	***
Ethyl decanoate	1.30±0.03 [a]	0.55±0.01 [c]	0.73±0.01 [b]	0.45±0.01 [d]	***
Ethyl dodecanoate	0.00±0.00 [c]	0.00±0.00 [c]	0.15±0.02 [a]	0.07±0.01 [b]	***
Ethyl 3-hydroxybutanoate	0.14±0.01 [a]	0.15±0.01 [a]	0.15±0.01 [a]	0.15±0.01 [a]	***
Monoethyl succinate	6.04±0.19 [a]	1.60±0.05 [c]	4.91±0.15 [b]	1.38±0.04 [c]	***
Diethyl succinate	0.59±0.02 [a]	0.40±0.01 [b]	0.38±0.01 [b]	0.41±0.01 [b]	***
Diethyl malate	0.42±0.02 [a]	0.00±0.00 [c]	0.12±0.01 [b]	0.00±0.00 [c]	***

Ethyl 5-oxo-2-pyrrolidine-carboxylate	1.02±0.03 [a]	0.00±0.00 [c]	0.15±0.01 [b]	0.05±0.01 [c]	***
Total ethyl esters	**15.04±0.42 [a]**	**5.74±0.14 [c]**	**10.14±0.30 [b]**	**5.38±0.15 [c]**	***
Total olfactory active ethyl esters	**6.83±0.15 [a]**	**3.59±0.07 [c]**	**4.32±0.09 [b]**	**3.32±0.07 [c]**	***

Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy 2-butanone, 2,3-butanediol. Abbreviations: [α] S.S, statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, P < 0.001.

3-methyl-1-butyl acetate, phenylethyl acetate, and 2-methyl-1-butyl acetate were the VOCs that contributed the most to the increase in acetate ester content in the CO8 trial compared to the CONT A2 control (Table 2). These increases observed under experimental pH conditions contrast with the findings of Costello, Siebert, Solomon, & Bartowsky, 2013. This phenomenon suggests that wine limiting conditions (Costello, Siebert, Solomon, & Bartowsky, 2013) or those created by the coexistence of LAB and yeasts during fermentation are crucial in activating some biosynthetic processes (Liu et al., 2017). The hypothesis is supported by the varying recorded production of phenylethyl acetate in the CO8 trials and 2-methyl-1-butylacetate in the CO6, CO8 and CO10 trials compared to the control, CONT A2. In such cases, the esterification process due to LAB activity can decrease hydroxybutylbenzene concentration, thereby reducing the potential toxicity against bacterial cells (Table 1, Corre, Lucchini, Mercier, & Cremieux, 1990; Romano, Ciani, & Cocolin, 2022). Indeed, Romano, Ciani, & Cocolin (2022) have reported that hydroxybutylbenzene produced by yeasts is well known to inhibit the transport of sugars and amino acids within the bacterial cell. MLPK45H strain co-inoculated with different strains of *S. cerevisiae* (CO5 and CO10 trials) resulted in the lowest concentration of acetate esters compared to the other consociated trials and the related CONT A1 and CONT A2 controls (Table S7 and S8).

3.3.2.2. Ethyl Esters

Fig. 3a shows that the olfactively active esters depend on the rate of malolactic fermentation "m" as well as the rate of malic acid degradation in the unit of time. Especially ethyl octanoate and ethyl decanoate, seem to be favored by a range of the malic acid degradation rate, "m", between -0.0201 and -0.0292 (Table 1).

It is noteworthy, the timing of malolactic fermentation represents a pivotal factor in enhancing the aromatic profile of a wine.

Although all oenological conditions are standardized, when different strains and/or species of LAB are inoculated into the same grape must, different timing for ending the malolactic fermentation could occur. In order to evaluate the effect of malolactic process on volatile organic compound composition of wines, it is mandatory to sample the wines at the end of malolactic process. It implies that the time factor, meaning the time interval between the end of malolactic process of two experimental trials, should be taken into account to identify the effect of time factor has on the volatile organic compound composition.

Ethyl esters from organic acids are not directly involved in the interactions between LAB-*S. cerevisiae* but they are produced by chemical esterification (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2009) occurring during different time periods.

The principal influence of the biotechnological component can be attributed to the synthesis of specific organic acids, including succinic acid, which resulted in the production of methyl succinate and diethyl succinate. These esters, in conjunction with diethyl malate, contributed to a notable elevation in the total ester content of wine, as a consequence of chemical esterification phenomena (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2009; Shinohara, Shimizu, & Shimazu, 1979). These reactions were catalysed by the availability of ethanol in the medium, the pKa of the acids, the hydrogenionic activity of the wine and, to a greater extent, the temperature (Usseglio-Tomasset, 1978) maintained at 20°C to favour the microbial activities of LAB. In the present study, hwever, the relatively high odor thresholds of the diethyl succinate and diethyl malate esters (200 mg/L and 760 mg/L, respectively) (García-Carpintero, Sánchez-Palomo, Gallego & González-Viñas, 2011) meant that they had only a slight impact on the wine's aroma.

3.3.3. Carboxylic acids

Different trends in fatty acid production were registered depending on the LAB - *S. cerevisiae* consociation (Table 2). In particular, malolactic fermentations conducted at the same time as alcoholic fermentation resulted in a significant increase in decanoic and octanoic fatty acids in the CO3 and CO5 trials (MLA4 -NF213 and MLPK45H-NF213) compared to the CONT A2 control. The concentration of decanoic acid was 4.87 mg/L in CO3 trial, which was higher than the 3.10 mg/L in the CONT A1 trial. Similarly, the concentration of octanoic acid was 5.83 mg/L in CO5 trial, which was higher than the 1.93 mg/L in the CONTA1 trial. These values are consistent with those reported by Knoll et al. (2012) and Sun, Chen, & Jin (2018).

The significant production of decanoic and octanoic acids in the CO3 and CO5 trials involving the combination of LAB MLA4 and MLPK45H with the *S. cerevisiae* strain NF213 could be attributed to an antagonistic response exerted by the *S. cerevisiae* strain NF213 to alter and inhibit the metabolic physiology of the co-fermentative LAB (Rossouw, Du Toit, & Bauer, 2012). Therefore, the lower concentrations of decanoic and octanoic acids found in the CO1, CO6, CO8 and CO10 trials (Table 1) could be explained as an increased response of LAB to matrix detoxification by esterification. However, the different response recorded in the complementary tests of the two different sets (CO3 vs CO8 for decanoic acid; and CO5 vs CO10 for octanoic acid; Table 1) indicates that this is a strongly *S. cerevisiae* strain-dependent effect. The data suggested that only certain consociations of LAB - *S. cerevisiae* strains have a higher technological affinity, as shown in Table 1. Therefore, having noted the inverse correlation between octanoic and decanoic fatty acids with their corresponding ethyl esters (Table S2), the production of fatty acids by the *S. cerevisae* strain as an antagonistic-inhibitory effect (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004; Rossouw, Du Toit, & Bauer, 2012) toward LAB represents a synthesis of aromatic precursors.

3.3.4. Aldehydes

The microbial strains used in this study showed a different response in the synthesis of aldehydes. The LAB microbial consociations with the yeast strain NF213 resulted in significantly reduced aldehyde concentrations compared to the CONT A1 control. These results are consistent with those of Liu (2002). Therefore, the decrease in aldehydes would allow the decrease in herbaceous hints in favour of fruity sensorial intensity. On the contrary, microbial consociations with the yeast strain QA23 resulted in an increase of aldehydes, with the exception of the CO6 trial (Table 2). The concentration of aldehydes in the CO8 trial was 3.11 mg/L, while it was 1.87 mg/L in the CO10 trial (non-significant difference compared to the CONT A2 control, (1.80 mg/L). The concentration of aldehydes in the CO6 trial was 1.49 mg/L, which was lower than the concentration in the CONT A2 trial (1.80 mg/L).

According to ours study, the VOCs that contributed most to the increase in total aldehyde values in the CO8 and CO10 trials were 4-methylbenzaldehyde and 3,4-dimethylbenzaldehyde (Table 2). These compounds result from the methylation of carbons 3 and 4 of the aromatic ring of benzylaldehyde. The formation of the benzylaldehyde during fermentation is due to the degradation of phenylalanine by LAB (Nierop Groot & de Bont, 1998). Thus, the different production of these aldehydes in the various trials could be explained by a trophic competition for phenylalanine between the co-fermenting microorganisms.

3.3.5. The 2,3-butanedione, 2,3-butanediol and 3-hydroxy-2-butanone compounds

2,3-butanedione, also known as diacetyl, is a diketone whose production has always been attended to by technicians because of its olfactory perceptions. In fact, aromas resulting from the production of this compound can also result in off-flavors depending on the concentration produced. In wine, LAB play a key role in the production of diacetyl, which is synthesized due to the degradation of citric acid.

According to Bartowsky & Henschke (2004), using citrate-negative LAB to carry out malolactic fermentation in wines may not always be a valid strategy to prevent diacetyl formation; in fact, its synthesis can also be triggered from pyruvate formed by glycolysis (Ochando, Mouret, Humbert-Goffard, Sablayrolles, & Farines, 2018). The joint creation of a reducing environment by the two *S. cerevisiae* strains and the reductive metabolisms of the different LAB tested favoured the total reduction of 2,3-butanedione to 3-hydroxy-2-butanone and, subsequently, to 2,3-butanediol (Table 2) (Bartowsky & Henschke, 2004) This behaviour was also observed in trials with a significant decrease in citric acid (Table S6) in contrast to that reported by Bartowsky & Henschke (2004).

3.4. Active volatile compound analysis

The aroma profiles of the wines were characterized by VOCs from the metabolic processes of the biotechnology used. The study found that the active volatile component, represented by VOCs above the perception threshold (OAV > 0.1) (Peng, Wen, Tao, & Lan, 2013), was composed of 12 compounds for the trials involving the combination of the LAB MLB6, MLA4, MLPK45H strains with the *S. cerevisiae* NF213 (CONT A1, CO1, CO3 and CO5 trials) (Table 3).

Table 3
Odor activity value of volatile organic compounds detected above the perception threshold in Catarratto experimental wines.

Compounds [α]	Aroma description [β]	Aromatic Series [γ]	Reference Aromatic Series [δ]	Odor threshold [ε]	Reference Odor treshold [ζ]	CONT A1 [η]	CO 1 [η]	CO 3 [η]	CO 5 [η]	S.S. [θ]	CONT A2 [η]	CO 6 [η]	CO 8 [η]	CO 10 [η]	S.S. [ι]
3-methyl-1-butanol	Fusel	4	[1]	40	[2]	1.39±0.03 [a]	1.11±0.03 [c]	1.26±0.03 [b]	1.06±0.03 [c]	***	1.54±0.04 [a]	0.99±0.03 [c]	1.30±0.03 [b]	0.84±0.02 [d]	***
3-ethoxy-1-propanol	Fruity	1	[1]	0.1	[3]	2.80±0.10 [b]	6.50±0.10 [a]	2.90±0.10 [b]	2.90±0.10 [b]	***	11.50±0.40 [a]	4.10±0.20 [c]	6.40±0.20 [b]	3.50±0.10 [c]	***
3-methylsulfanyl-1-propanol	Raw potato, Garlic	5	[4]	0.5	[5,6]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	***	3.12±0.08 [a]	1.52±0.04 [b]	1.90±0.06 [c]	1.36±0.04 [c]	***
Hydroxyethylbenzene	Rose	2	[7,8]	10	[7,8]	2.70±0.09 [a]	2.18±0.07 [b]	2.69±0.09 [a]	2.06±0.07 [b]	***	2.64±0.04 [a]	1.60±0.02 [c]	2.25±0.03 [b]	1.56±0.02 [c]	***
Hexanoic acid	Cheese Fatty	3	[9]	0.4	[3,10]	6.62±0.29 [a]	5.12±0.21 [b]	3.67±0.14 [b]	4.88±0.21 [c]	***	12.52±0.45 [a]	6.50±0.24 [d]	11.02±0.40 [b]	7.92±0.29 [c]	***
Octanoic acid	Rancid, Cheese, Fatty	3	[9]	0.5	[7,8]	3.86±0.16 [c]	1.72±0.08 [b]	3.64±0.14 [b]	11.66±0.48 [a]	***	6.18±0.18 [a]	5.18±0.16 [b]	5.94±0.18 [a]	4.58±0.14 [c]	***
Decanoic acid	Fatty Rancid	3	[9]	1	[11]	3.10±0.06 [b]	3.06±0.06 [b]	4.87±0.09 [a]	2.49±0.05 [c]	***	7.36±0.31 [a]	2.79±0.12 [c]	3.91±0.17 [b]	2.50±0.11 [c]	***
3-methyl-1-butyl acetate	Banana	1	[12]	0.03	[7,8]	92.67±3.00 [a]	72.00±2.33 [b]	72.67±2.33 [b]	30.66±1.00 [c]	***	92.00±1.67 [b]	70.67±1.33 [c]	102.67±2.00 [a]	57.33±1.00 [d]	***
Ethyl hexanoate	Apple, Banana	1	[12]	0.005	[7,8]	324.00±8.00 [a]	182.00±4.00 [d]	240.00±6.00 [c]	264.00±6.00 [b]	***	368.00±6.00 [a]	226.00±4.00 [c]	316.00±6.00 [b]	236.00±4.00 [c]	***
Ethyl octanoate	Pineapple, Pear	1	[12]	0.002	[7,8]	1580.00±45.00 [a]	1610.00±45.00 [a]	1125.00±35.00 [b]	1025.00±30.00 [b]	***	1845.00±45.00 [a]	955.00±20.00 [b]	1005.00±25.00 [b]	845.00±20.00 [c]	***
Phenylethyl acetate	Rosa, Floreal	2	[8]	0.25	[8]	2.48±0.08 [a]	2.12±0.08 [b]	2.40±0.08 [a]	2.16±0.08 [b]	***	2.32±0.04 [a]	1.76±0.04 [b]	2.36±0.04 [a]	1.64±0.04 [c]	***
Ethyl decanoate	Floreal	2	[3]	0.20	[3]	4.20±0.20 [b]	6.20±0.25 [a]	3.50±0.15 [c]	3.55±0.15 [c]	***	6.50±0.15 [a]	2.75±0.05 [c]	3.65±0.05 [b]	2.25±0.05 [d]	***
2,3-butandiol [ζ]	Fruity	1	[13]	150	[12]	0.13±0.00 [c]	0.15±0.00 [b]	0.18±0.00 [a]	0.11±0.00 [d]	***	0.25±0.00 [a]	0.15±0.00 [b]	-	0.13±0.00 [c]	***
Olfactory intensity						2023.95±57.01 [a]	1892.16±52.22 [a]	1462.17 ±44.15 [b]	1350.54±38.17 [b]	***	2358.93±54.36 [a]	1279.00±26.23 [c]	1462.40±34.17 [b]	1164.61±25.81 [d]	***

[α] Compounds with OAV > 0.1
[β] Aroma description
[γ] Aromatic series, 1: fruity; 2: floral; 3: fatty; 4: solvent; 5: sulfurous.
[δ] Reference Aromatic Series: [1] Butkhup et al., 2011; [3] Kelebek et al., 2011; [4] Celik et al., 2019; [7] Selli et al., 2004; [8] Izquierdo-Cañas et al., 2008; [9] Cai et al., 2014; [12] Bayram et al., 2018; [13] García-Carpintero et al., 2011
[ε] Odor threshold (mg/L)

ζ Reference Odor threshold: [2] Herrero et al., 1999; [3] Kelebek et al., 2011; [5] Krieger-Weber et al., 2015; [6] Davis et al., 1985; [7] Selli et al., 2004; [8] Izquierdo-Cañas et al., 2008; [10] Moio et al., 1995;
[11] Delequis et al., 2000; [12] García-Carpintero et al., 2011
η Relative amounts expressed in OAV (odor activity value)
θ Statistical significance among CONT A1, CO1, CO3, CO5 trials; Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, P < 0.001; n.s., not significant.
ι Statistical significance among CONT A2, CO6, CO8, CO10 trials; Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, P < 0.001; n.s., not significant.

For the test set in which the same LAB were combined with *S. cerevisiae* QA23 strain for the initiation of fermentation (trials CONT A2, CO6, CO8 and CO10) the active volatile component was composed of 12 compounds (Table 3). According to Ferreira et al. (2016), the sensorial buffer of wine is composed of a total of three higher alcohols (methyl-1-butanol, 3-ethoxy-1-propanol, hydroxyethylbenzene), three medium chain fatty acids (hexanoic acid, octanoic acid and decanoic acid), two acetate esters and three ethyl esters (3-methyl-1-butyl acetate, phenylethyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate). 3-methylsulfanyl-1-propanol was the VOC that significantly differentiated trials in addition to the amount of OAVs detected (Table 2 and 3).

This study found that the active volatile component is a function of TAIndex; the negative correlation between the two would be explained by 45.96% of the total variance in the PCA analysis (Fig. 3a). Data suggest that a higher degree of esterification by LAB to neutralize the deleterious effect of medium-chain fatty acids in the cell (Costello, Siebert, Solomon, & Bartowsky, 2013) could result in the decrease in TAIndex and increase in olfactory intensity, producing much more aromatic wines. The negative correlation found between octanoic acid and ethyl octanoate (45.96% of the total variance), and decanoic acid with ethyl decanoate (25.93% of the total variance; Fig. 3a) could further confirm this hypothesis. These mechanisms were found to be positively correlated with MLB6-NF213 consociation in the CO1 trial, which had a higher endowment of the fruit and floral component among the trials (Table 4).

Table 4
Aroma profiles of Catarratto experimental wines derived from odor activity values (all values in OAV).

Aroma series [α]	CONT A1 [β]	CO 1 [β]	CO 3 [β]	CO 5 [β]	S.S. [γ]	CONT A2 [β]	CO 6 [β]	CO 8 [β]	CO 10 [β]	S.S. [δ]
Fruity	1999.59 ± 56.10 [a]	1870.65 ± 51.43 [a]	1440.74 ± 43.43 [b]	1322.67 ± 37.10 [b]	***	2316.75 ± 53.07 [a]	1255.92 ± 25.53 [c]	1430.07 ± 33.20 [b]	1141.96 ± 25.10 [d]	***
Floreal	9.38 ± 0.10 [b]	10.49 ± 0.40 [a]	8.58 ± 0.32 [c]	7.77 ± 0.30 [d]	***	11.46 ± 0.23 [a]	6.11 ± 0.11 [c]	8.26 ± 0.12 [b]	5.45 ± 0.11 [d]	***
Fatty	13.58 ±0.51 [b]	9.90 ± 0.35 [c]	12.18 ± 0.37 [b]	19.03 ± 0.74 [a]	***	26.06 ± 0.94 [a]	14.47 ± 0.52 [c]	20.87 ± 0.75 [b]	15.01 ± 0.53 [c]	***
Solvent	1.39 ± 0.03 [a]	1.11 ± 0.03 [c]	1.26 ± 0.03 [b]	1.06 ± 0.03 [c]	***	1.54 ± 0.04 [a]	0.99 ± 0.03 [c]	1.30 ± 0.03 [b]	0.84 ± 0.02 [d]	***
Sulfurous	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]	***	3.12 ± 0.08 [a]	1.52 ± 0.04 [c]	1.90 ± 0.06 [b]	1.36 ± 0.04 [c]	***

[α] Aroma series
[β] Aroma profile calculated by summing and of the odorous activity values (OAV) of the aromatic series from Table 3
[γ] Statistical significance among CONT A1, CO1, CO3, CO5 trials
[δ] Statistical significance among CONT A2, CO6, CO8, CO10 trials

The increase in TAIndex found among the trials associated with the MLPK45H, CO5 and CO10 trials (Table 1) resulted in a significant decrease in active volatile components (Table 3) and, thus, in olfactory intensity. This is probably due to the rapid degradation of malic acid that occurred when the culture medium had low limiting factors, low concentrations of ethanolic substrate (Table S2) and, presumably, low concentrations of medium-chain fatty acids by the metabolism of *S. cerevisiae*.

The limited concentrations of these two compounds in solution during the first three days of vinification would not have led LAB to catalyse the octanoic and decanoic acid esterification reactions. Thus, the failure of LAB to contribute to esterification resulted in a decrease in octane intensity. This hypothesis formulation would also explain the positive correlation of octanoic acid with the CO5 and CO10 trials (Fig. 3a).

The delayed times of potential esterification by LAB and octanoic acid synthesis by the *S. cerevisiae* strain favored the accumulation of octanoic acid in the medium. These hypotheses are confirmed by the negative correlations recorded between: the days of malolactic fermentation and ethyl octanoate and olfactory intensity (25.97% of the total variance); TAIndex and olfactory intensity (45.96% % of the total variance); and TAIndex and days of malolactic fermentation (45.96% % of the total variance) (Fig. 3a).

Furthermore, the dynamics described above made it possible to discriminate three groups of wines according to their TAIndex values (Fig. 3b). Values between 0.009 and 0.013 (CO1 and CO3 trials) (Table 1) marked a single grouping with partially overlapping olfactory profiles (Fig. 3b). This

similarity is explained by 45.96 % of the total variance from the production of ethyl octanoate and ethyl decanoate (Fig. 3a, b) for the MLB6-NF213 and MLA4-NF213 consociations. Single clustering resulted for the CO8 trial. The TAIndex value of 0.019 in this thesis allowed the olfactory active VOCs to be distinguished from the other theses (Fig. 3b). In contrast, TAIndex values above 0.148 outlined partially overlapping olfactory profiles. These peculiarities were found among the CO5, CO6 and CO10 trials, which formed the third group (Fig. 3b).

This group was found to be closely related to octanoic acid. This result suggests that the production of ethyl octanoate, in a microbial consociation, is enabled by a malic acid degradation rate of less than -0.0292 g/L × day^{-1} and a malolactic fermentation of more than 18 days. The technological affinity between LAB and *S. cerevisiae* is calculated by using data from the relationship between malic acid degradation as a function of time. The resulting trend reveals the extent to which the decarboxylative activity of LAB is affected by the activity of the *S. cerevisiae* strain during the co-fermentation phase (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004).

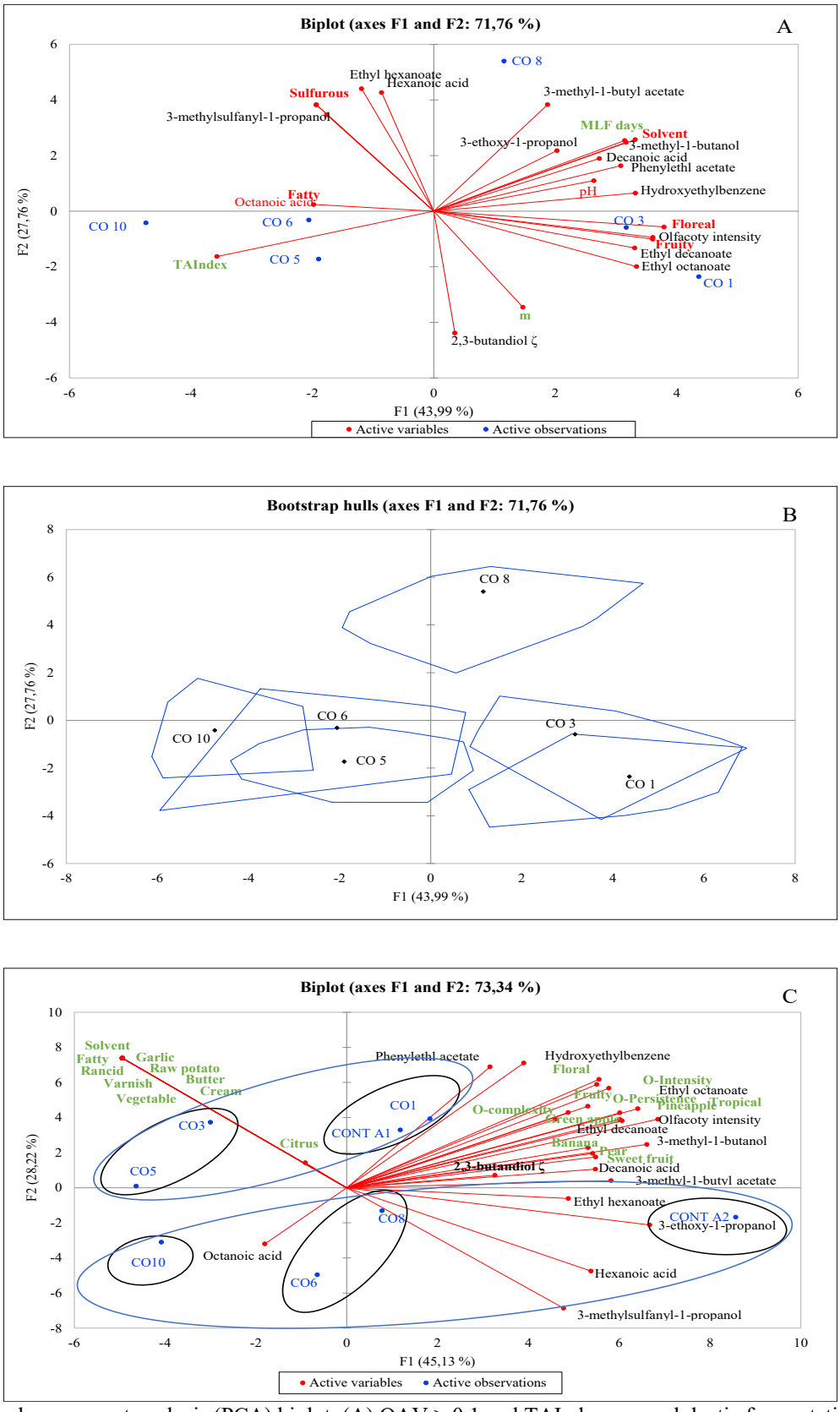

Fig. 3. Principal component analysis (PCA) biplot: (A) OAV > 0.1 and TAIndex; m, malolactic fermentation days and pH; (B) Bootstrap hull's for OAV > 0.1; (C) OAV > 0.1 and aroma ttributes.

This interference would consequently affect the ability of LAB to detoxify the culture medium from *S. cerevisiae* catabolites (medium-chain fatty acids and higher alcohols). Thus, the timing of malolactic fermentation and the ability of LAB to detoxify the culture medium from *S. cerevisiae* catabolites are closely related.

Therefore, by identifying the malic acid trend over time with its graphical representation, it was possible to obtain an equation of the type $y = mx + q$ from the linear regression (Bevilacqua, Speranza, Petruzzi, Sinigaglia, & Corbo, 2023; Caponigro et al., 2010; Hsiao & Siebert, 1999). The corresponding coefficient of determination R^2 indicates the link between the variability of the data and the correctness of the statistical model.

3.4. The sensory analysis

Table 5 presents the data from the sensory measurements.

The microbial consociation trials in the two experimental sets resulted in significantly different sensory profiles. The appearance of the wine was influenced by the variability of colour attributes. The yellow colour showed a variability between the trials ranging from 7.12 and 7.47. This is consistent with the findings of Naselli et al. (2023).

The green reflections in the trials were similar to each other, but had higher values than those described by Scacco et al. (2012). The CO1 trial stood out for having the highest value of green reflections compared to the control and compared to all trials in the two different experimental sets (Table 5). The wines were submitted to a panel, which outlined different olfactory profiles depending on the LAB - *S. cerevisiae* microbial consociation used. Among the microbial consociation trials, the CO1 trial (MLB6 - NF213) stood out for its high values of intensity, persistence and tropical scents with values of 8.69, 7.61 and 8.31, respectively (Table 5). These olfactory attributes appeared not to differ from the CONT A2 control. However, the panel detected higher levels of the fruitiness and pineapple than the CONT A2 control.

The wines were subjected to a panel that outlined different olfactory profiles depending on the LAB – *S. cerevisiae* microbial association used. Among the microbial association tests, the CO1 trial (MLB6 - NF213) stood out for its high intensity, persistence and tropical aroma values of 8.69, 7.61 and 8.31 respectively (Table 5).

Table 5

Sensory score for experimental Catarratto wines.

Attributes [α]		Trials								Statistical [γ] Significance	
		CONT A1 [β]	CO1 [β]	CO3 [β]	CO5 [β]	CONT A2 [β]	CO6 [β]	CO8 [β]	CO10 [β]	Judge	Wine
Apparence	Yellow colour	7.26 ± 0.02 ^{bc}	7.21 ± 0.02 ^{cd}	7.41 ± 0.02 ^{a}	7.37 ± 0.02 ^{a}	7.12 ± 0.02 ^{e}	7.21 ± 0.02 ^{cd}	7.18 ± 0.02 ^{d}	7.28 ± 0.02 ^{b}	***	***
	Green reflexes	6.14 ± 0.11 ^{b}	6.71 ± 0.12 ^{a}	6.31 ± 0.11 ^{b}	6.24 ± 0.11 ^{b}	6.27 ± 0.11 ^{b}	6.23 ± 0.11 ^{b}	6.16 ± 0.11 ^{b}	6.48 ± 0.12 ^{ab}	***	***
Odour	Green apple	7.70 ± 0.22 ^{a}	6.27 ± 0.18 ^{cd}	6.80 ± 0.19 ^{bc}	6.87 ± 0.19 ^{b}	7.83 ± 0.23 ^{a}	6.11 ± 0.17 ^{d}	6.18 ± 0.17 ^{d}	6.03 ± 0.17 ^{d}	***	***
	Banana	6.61 ± 0.14 ^{ab}	6.10 ± 0.13 ^{c}	6.18 ± 0.13 ^{bc}	4.81 ± 0.10 ^{d}	6.62 ± 0.14 ^{a}	6.12 ± 0.13 ^{c}	6.97 ± 0.15 ^{a}	5.10 ± 0.11 ^{d}	***	***
	Citrus	3.27 ± 0.01 ^{b}	3.12 ± 0.01 ^{e}	3.23 ± 0.01 ^{c}	3.71 ± 0.01 ^{a}	3.30 ± 0.01 ^{b}	3.18 ± 0.01 ^{d}	3.21 ± 0.01 ^{c}	3.00 ± 0.01 ^{f}	***	***
	Fatty	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Floral	6.80 ± 0.08 ^{b}	7.12 ± 0.09 ^{a}	6.76 ± 0.08 ^{b}	6.46 ± 0.08 ^{bc}	7.31 ± 0.09 ^{a}	6.01 ± 0.07 ^{d}	6.61 ± 0.08 ^{bc}	5.83 ± 0.07 ^{d}	***	***
	Fruity	8.12 ± 0.15 ^{b}	8.61 ± 0.15 ^{a}	7.30 ± 0.13 ^{c}	6.84 ± 0.12 ^{d}	8.18 ± 0.15 ^{b}	7.18 ± 0.18 ^{cd}	7.13 ± 0.13 ^{cd}	7.21 ± 0.13 ^{cd}	***	***
	Tropical	8.25 ± 0.12 ^{a}	8.31 ± 0.12 ^{a}	7.10 ± 0.10 ^{b}	6.35 ± 0.09 ^{c}	8.36 ± 0.12 ^{a}	6.12 ± 0.09 ^{cd}	6.38 ± 0.09 ^{c}	6.01 ± 0.09 ^{d}	***	***
	O-Intensity	8.34 ± 0.22 ^{a}	8.69 ± 0.22 ^{a}	7.00 ± 0.18 ^{b}	6.68 ± 0.17 ^{bc}	8.42 ± 0.23 ^{a}	6.51 ± 0.17 ^{bc}	7.00 ± 0.18 ^{b}	6.23 ± 0.15 ^{c}	***	***
	Pear	6.58 ± 0.17^{ab}	6.08 ± 0.15 ^{b}	6.12 ± 0.16 ^{b}	5.21 ± 0.13 ^{c}	6.42 ± 0.16 ^{ab}	6.11 ± 0.16 ^{b}	6.74 ± 0.17 ^{a}	5.20 ± 0.13 ^{c}	***	***
	O-Persistence	7.49 ± 0.15 ^{a}	7.61 ± 0.15 ^{a}	6.54 ± 0.13 ^{b}	6.36 ± 0.12 ^{b}	7.64 ± 0.15 ^{a}	6.54 ± 0.12 ^{b}	6.38 ± 0.12 ^{b}	6.37 ± 0.12 ^{b}	***	***
	Pineapple	8.20 ± 0.02 ^{c}	8.40 ± 0.02 ^{b}	6.20 ± 0.02 ^{h}	6.32 ± 0.02 ^{g}	8.60 ± 0.02 ^{a}	6.62 ± 0.02 ^{f}	7.48 ± 0.02 ^{d}	6.68 ± 0.02 ^{e}	***	***
	Sweet fruit	6.59 ± 0.12 ^{ab}	6.09 ± 0.11 ^{b}	6.15 ± 0.11 ^{c}	5.01 ± 0.09 ^{d}	6.52 ± 0.12 ^{b}	6.12 ± 0.11 ^{c}	6.86 ± 0.12 ^{a}	5.15 ± 0.09 ^{d}	***	*
	Solvent	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Garlic	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Raw potato	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Cream	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Butter	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Rancid	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Varnish	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	Vegetable	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	1.00 ± 0.00 ^{a}	***	*
	O-complexity	7.21 ± 0.18 ^{b}	7.66 ± 0.19 ^{a}	6.23 ± 0.16 ^{c}	6.41 ± 0.18 ^{c}	7.18 ± 0.16 ^{b}	6.43 ± 0.16 ^{c}	6.27 ± 0.16 ^{c}	6.31 ± 0.16 ^{c}	***	***
Taste	Sweet	5.36 ± 0.11 ^{d}	6.12 ± 0.12 ^{a}	6.18 ± 0.12 ^{a}	5.63 ± 0.11 ^{cd}	5.42 ± 0.11 ^{d}	6.01 ± 0.12 ^{ab}	6.06 ± 0.12 ^{ab}	5.78 ± 0.11 ^{bc}	***	***
	Sour	6.70± 0.02 ^{b}	4.81 ± 0.01 ^{g}	4.92 ± 0.01 ^{e}	5.21 ± 0.01 ^{c}	6.80 ± 0.02 ^{a}	4.91 ± 0.01 ^{e}	4.86 ± 0.01 ^{f}	5.16 ± 0.01 ^{d}	***	***
	Salty	6.56 ± 0.12 ^{a}	5.60 ± 0.10 ^{bc}	5.78 ± 0.10 ^{b}	5.61 ± 0.10 ^{bc}	6.71 ± 0.12 ^{a}	5.21 ± 0.09 ^{d}	5.46 ± 0.10 ^{cd}	5.57 ± 0.10 ^{bc}	***	***
	Bitter	4.62 ± 0.14 ^{abc}	4.65 ± 0.14 ^{ab}	4.71 ± 0.14 ^{ab}	4.68 ± 0.14 ^{ab}	4.35 ± 0.13 ^{bc}	4.84 ± 0.14 ^{a}	4.24 ± 0.12 ^{c}	4.35 ± 0.13 ^{bc}	***	***
	Body	6.12 ± 0.13 ^{a}	6.21 ± 0.13 ^{a}	6.36 ± 0.14 ^{a}	6.28 ± 0.14 ^{a}	6.25 ± 0.14 ^{a}	6.23 ± 0.13 ^{a}	6.18 ± 0.13 ^{a}	6.23 ± 0.13 ^{a}	***	**
	Balance	7.10 ± 0.02 ^{a}	6.21 ± 0.02 ^{f}	6.27 ± 0.02 ^{e}	6.70 ± 0.02 ^{b}	7.15 ± 0.02 ^{a}	6.36 ± 0.02 ^{d}	6.21 ± 0.02 ^{f}	6.5 ± 0.02 ^{c}	***	***
Flavour	Banana-like	7.69 ± 0.01 ^{b}	7.6 ± 0.01 ^{c}	6.49 ± 0.01 ^{e}	5.83 ± 0.01 ^{h}	7.86 ± 0.01 ^{a}	6.29 ± 0.01 ^{f}	6.94 ± 0.01 ^{d}	5.93 ± 0.01 ^{g}	***	***
	F-Citrus	3.32 ± 0.04 ^{b}	3.12 ± 0.04 ^{de}	3.23 ± 0.04 ^{bcd}	3.48 ± 0.04 ^{a}	3.25 ± 0.04 ^{bc}	3.14 ± 0.04 ^{cde}	3.16 ± 0.04 ^{cde}	3.06 ± 0.04 ^{e}	***	***
	F-Fruity	7.45 ± 0.20 ^{a}	7.04 ± 0.19 ^{ab}	6.55 ± 0.18 ^{bc}	5.97 ± 0.16 ^{cd}	7.54 ± 0.20 ^{a}	6.29 ± 0.17 ^{d}	6.82 ± 0.18 ^{bc}	5.81 ± 0.16 ^{d}	***	***
	F-Intensity	8.61 ± 0.13 ^{a}	8.57 ± 0.13 ^{a}	6.76 ± 0.10 ^{b}	6.47 ± 0.10 ^{bc}	8.64 ± 0.13 ^{a}	6.41 ± 0.09 ^{c}	6.74 ± 0.10 ^{b}	6.23 ± 0.09 ^{c}	***	***
	F-Persistence	7.12 ± 0.18 ^{a}	7.32 ± 0.19 ^{a}	6.21 ± 0.16 ^{b}	6.03 ± 0.15 ^{b}	7.18 ± 0.18 ^{a}	5.92 ± 0.15 ^{b}	5.98 ± 0.15 ^{b}	5.83 ± 0.15 ^{b}	***	***
	F-Complexity	7.31 ± 1.85 ^{a}	7.76 ± 1.97 ^{b}	6.23 ± 1.58 ^{a}	6.36 ± 1.61 ^{b}	7.43 ± 1.88 ^{a}	6.42 ± 1.63 ^{b}	5.58 ± 1.42 ^{c}	6.14 ± 1.56 ^{b}	***	***
	F-Pineapple	7.80 ± 0.15 ^{a}	8.10 ± 0.16 ^{a}	5.90 ± 0.12 ^{c}	6.03 ± 0.12 ^{c}	7.90 ± 0.16 ^{a}	5.70 ± 0.11 ^{c}	7.20± 0.14 ^{b}	5.80 ± 0.11 ^{c}	***	***
	F-Sweet fruit	6.38 ± 0.02 ^{a}	6.16 ± 0.02 ^{c}	6.00 ± 0.02 ^{d}	5.80 ± 0.02 ^{d}	6.31 ± 0.02 ^{b}	5.96 ± 0.02 ^{d}	6.29 ± 0.02 ^{b}	5.30 ± 0.01 ^{f}	***	***
	Overall quality	8.69 ± 0.02 ^{b}	8.91 ±0.02 ^{a}	7.53 ±0.02 ^{h}	6.91 ±0.02 ^{g}	8.59 ±0.02 ^{c}	7.62 ±0.02 ^{e}	7.78 ±0.02 ^{d}	7.46 ±0.02 ^{f}	***	***
	Odour	8.81 ± 0.16 ^{a}	8.76 ± 0.16 ^{a}	6.25 ± 0.11 ^{bc}	6.16 ± 0.11 ^{bc}	8.86 ± 0.16 ^{a}	6.26 ± 0.11 ^{bc}	6.54 ± 0.12 ^{b}	6.12 ± 0.11 ^{c}	***	***
	Taste	7.64 ± 0.22 ^{a}	7.36 ± 0.22 ^{a}	7.21 ± 0.21 ^{a}	7.18 ± 0.21 ^{a}	7.56 ± 0.22 ^{a}	7.18 ± 0.21 ^{a}	7.46 ± 0.22 ^{a}	7.36 ± 0.22 ^{a}	***	***
	Mouth-feel	7.46 ± 0.16 ^{a}	7.26 ± 0.16 ^{a}	7.18 ± 0.16 ^{a}	7.16 ± 0.15 ^{a}	7.36 ± 0.16 ^{a}	7.18 ± 0.16 ^{a}	7.34 ± 0.16 ^{a}	7.12 ± 0.15 ^{a}	***	***
	Flavour	7.48 ± 0.02 ^{ab}	7.43 ± 0.02 ^{b}	6.22 ± 0.02 ^{d}	6.00± 0.02 ^{e}	7.51 ± 0.02 ^{a}	5.94 ± 0.02 ^{f}	6.28 ± 0.02 ^{c}	5.8 ± 0.02 ^{g}	***	***
Finish	After-smell	8.46 ± 0.01 ^{b}	8.56 ± 0.01 ^{a}	7.26 ± 0.01 ^{d}	7.12 ± 0.01 ^{f}	8.48 ± 0.01 ^{b}	7.21 ± 0.01 ^{e}	7.45 ± 0.01 ^{c}	7.11 ± 0.01 ^{f}	***	***
	After-taste	8.23 ± 0.00 ^{a}	8.18 ± 0.10 ^{a}	7.18 ± 0.09 ^{b}	7.18 ± 0.09 ^{b}	8.36 ± 0.10 ^{a}	7.18 ± 0.09 ^{b}	7.35 ± 0.09 ^{b}	7.14 ± 0.09 ^{b}	***	***

Results indicate mean value of three replicate sessions.
[α] Sensorial attribute;
[β] Relative amounts expressed in on a numerical scale of 1 to 9;
[γ] Statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: *, P < 0.05; **, P < 0.01; ***, P < 0.001; n.s., not significant.

These olfactory attributes do not appear to differ from the CONT A1 control. Nevertheless, the panel identified elevated levels of fruitiness and pineapple aromas, which differed from the results of the sensory analysis (Table 5). The OAV values (Table 3) were inconsistent with the sensory analysis

results on pineapple and fruity perceptions. This phenomenon may be attributed to the inhibitory effects of 3-methyl-1-butanol and medium-chain fatty acids on the perception of fruitiness and pineapple hint (Cameleyre, Lytra, Tempere, & Barbe, 2015). The dynamics of sulphur olfactory perceptions differed according to the detection analysis adopted. Detection by means of OAV, a system that omits any interactive influence between VOCs (Gómez-Míguez et al., 2007), enabled the effects that LABs – *S. cerevisiae* QA23 strain associations exert on these scents to be demonstrated. Indeed, the decreases observed in the CO6, CO8 and CO10 trials in comparison to the CONT A2 control were statistically significant (Table 3). These decreases could be attributed to suppression effects exerted by the LAB against the yeast. Indeed, a partial deactivation of the biosynthetic pathway leading to the production of 3-ethoxy-1-propanol within the yeast cell cannot be excluded (Irwin, 1992). In contrast, sensory analysis, a dynamic system that takes into account interactions between VOCs (Gómez-Míguez et al., 2007), did not detect any sulphur perception related to the olfactory attributes of garlic or raw potato (Table 5). These effects were found to be common to the rancid and solvent olfactory attributes derived from fatty acids and 3-methyl-1-butanol. This absence of olfactory manifestations undetected by the human nose suggests that the role played by 3-methyl-1-butanol and medium-chain fatty acids, while above the threshold of perception, is to counterbalance olfactory perceptions with synergy or masking phenomena (Ferreira et al., 2016) within the sensory buffer (Ferreira, Escudero, Campo, & Cacho, 2008).

The use of LAB–*S. cerevisiae* microbial associations plays a pivotal role in enhancing the wine's fruitiness.

In the CO1 trial, MLB6 was employed to create a wine with pronounced pineapple and pear aromas, which were attributed to the synthesis of ethyl octanoate, 1610 olfactory units (Table 3; Fig. S2a). In contrast, the CO8 trial exhibited a more diverse fruit profile, including pineapple, pear, green apple, and banana, which can be attributed to the synthesis of ethyl octanoate, ethyl hexanoate, and 3-methyl-1-butyl acetate, with olfactory units of 1.005, 316 and 102.67, respectively (Table 3; Fig. 4b).

The findings were validated by the sensory analysis, which recorded olfactory descriptors for banana, pear and sweet fruit in the CO8 (MLA4-QA23) trial at 6.97, 6.74 and 6.86 respectively (Table 5).

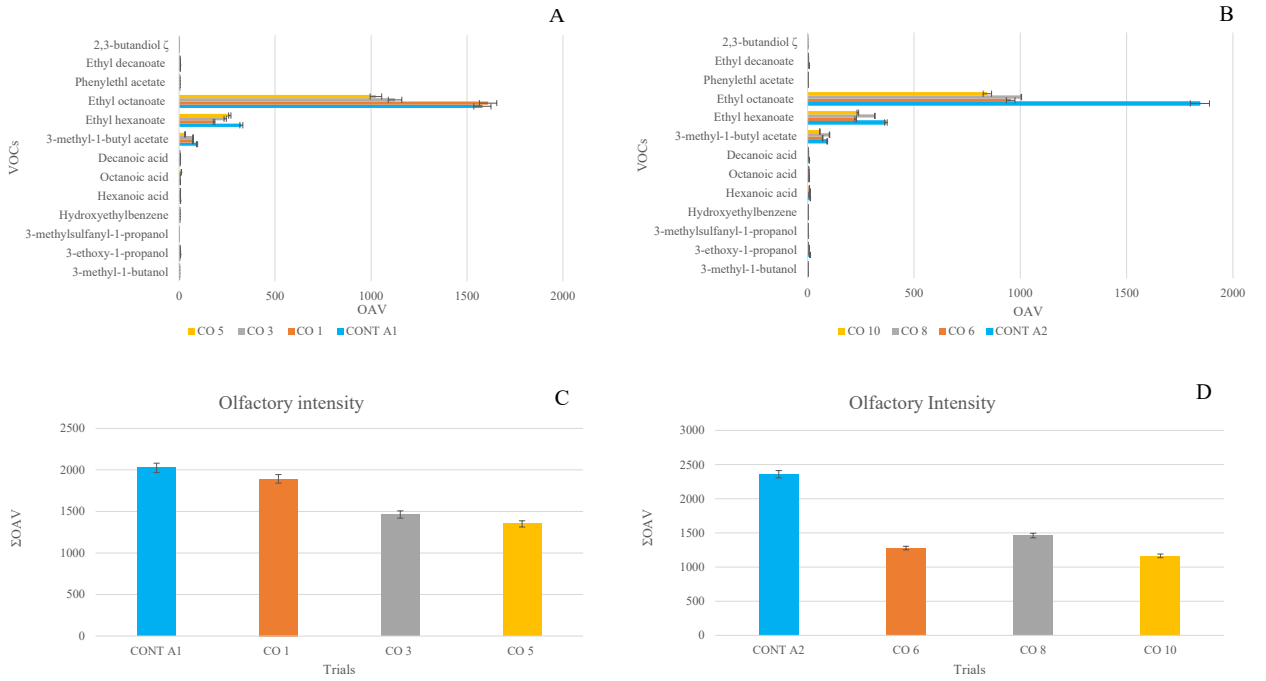

Fig. S2. Olfactory active VOCs: (A) for trials CO1, CO3, CO5 and CONTA1 expressed in OAV per single VOC; (B) for trials CO6, CO8, CO10 and CONTA2; (C) expressed in olfactory intensity (ΣOAV) per single trials (CO1, CO3, CO5 and CONTA1); (D) expressed in olfactory intensity (ΣOAV) per single trials (CO6, CO8, CO10 and CONTA2). The statistical analysis is shown in Table 3.

These values were compared to those of the CONT A2 control and to other trials conducted in the two experimental groups.

The CO1 trial exhibited the highest olfactory complexity value, distinguishing itself from the CONT A1 control and the other microbial association trials (Table 5). This could be attributed to the higher floral perception recorded by the judges (Table 5) and the increase, in comparison to the CONT A1 trial, of ethyl decanoate as evidenced in the OAV findings (Table 3).

Furthermore, the synergistic effect of the VOC mixture, which was below the perception threshold, may have contributed to this result (Atanasova et al., 2005).

In terms of olfactory intensity, the CO1 trial stood out from the other LAB-*S. cerevisiae* consociation tests. Both OAV and sensory analysis attributed the highest scores, 1892 and 7.61, respectively.

The data showed in Tables 3 and 5 indicate a strong correlation between this result and the selected *S. cerevisiae* strain along with its TAIndex value in relation to LAB.

The taste fraction of the different trials was evaluated, and a further distinctive aspect emerged between the trials. Specifically, the CO1 and CO3 trials were characterized by a higher sweetness sensation than the CONT A1 control. Meanwhile, trials CO5 and CO10 were the most acidic of the trials that included microbial association. This sensory effect could result from the increased lactic acid synthesis and lack of citric acid degradation recorded when the *L. plantarum* MLPK45H strain was used (Table S6).

The judges perceived the bitter taste component differently in the different trials of the two experimental groups. The trials including microbial consortia were less bitter than the CONT A1 and CONT A2 controls. Specifically, the CO3 and CO8 trials reported the lowest bitterness value compared to the CONT A1 and CON A2 controls (4.28 and 4.24 *vs* 6.08 and 6.11) and the different microbial consociations CO1, CO5 and CO6, CO10 (4.28 and 4.24 *vs* 4.65 - 4.68, and 4.58 - 4.71, respectively). This phenomenon could be attributed to the protease enzymes synthesized by the LAB, which could promote yeast autolysis from the fermentation phase (de Andrade Bulos et al., 2023).

The panel judges detected clear differences in the flavor of the wines between the trials. Among the trials inoculated with different microbial combinations, the CO1 trial showed the highest values of flavor intensity and persistence (Table 5). However, no difference was felt with the CONT A1 and CONT A2 controls. For taste sensations, the CO1 trial was shown to be different from the CONT A1 and CONT A2 controls in complexity and for the pineapple descriptor (Table 5). The CO1 trial also differed from the other trials in aftertaste (8.56). Although the aftertaste (8.18) was higher among the sister trials, it did not differ from the CONT A1 control. The panel judges concluded that the CO1 trial (MLB6-NF213) had the best overall quality with the distinction of 20 sensory descriptors. This result was higher than the CONT A1 control (15 sensory descriptors) and significantly different from the homologous CO6 trial (MLB6-QA23).

3.5 Sensory profiles associated with volatile organic compounds

To highlight the correlations between aromatic attributes and OAV values, the principal component analysis technique was employed. The F1 and F2 values explained 45.13% and 28.22% of the total variance, respectively (Fig. 3c). This analysis enabled the tests to be separated into two distinct macro-groups according to the *S. cerevisiae* strain used. The use of LAB strains in the microbial association allowed further distinction into subgroups. A variance of 45.13% explained the positive correlation between the two subgroups CONT A1, CO1 and CONT A2 (Fig. 3c) with olfactory intensity and persistence. In turn, these were closely dependent on the odour perceptions of tropical and pineapple. These olfactory attributes detected by the jury showed a correspondence with the OAVs (Table 3). Indeed, the tests and CONT A2, CONT A1 and CO1 were positively correlated with the higher alcohol 3-ethoxy-1-propanol and the ethyl esters hexanoate and ethyl octanoate. These findings are consistent with those reported by Ugliano & Moio (2005) and Vilanova & Martínez (2007). The subgroups CONT A1, CO1 and CONT A2 demonstrated further positive correlations between OAV and olfactory sensory attributes. Floral perception was positively correlated with phenylethylacetate, hydroxyethylbenzene, decanoic acid and 3-methyl-1-butanol.

These results are in agreement with those of previous studies conducted by Cañas, Romero, Alonso, & Herreros (2008); Selli et al., (2004) and Ferreira et al. (2016). However, the attribute of olfactory complexity was positively correlated with the CO1 trial in accordance with the sensory analysis (Table 5). PCA analysis attributed this result to the positive correlation of the floral and fruity perceptions recorded respectively. In particular, the role played by 2,3-butanediolζ in amplifying the fruity notes was significant. Indeed, the olfactory perceptions of sweet fruitiness, banana and pear were positively correlated with it (García-Carpintero, Sánchez-Palomo, Gallego & González-Viñas, 2011and Etievant et al., 1991). These findings align with those previously reported by Liang et al. (2023) and Escudero et al. (2004), which highlighted the significance of VOCs with OAV values between 0.1 and 1. However, no olfactory correspondence was observed between 3-methyl-1-butanol, hexanoic acid, and decanoic acid and the corresponding scents of solvent, rancid, and fatty.

In fact, PCA analysis revealed an inverse correlation between sensory attributes and VOCs. Consequently, the judges were unable to discriminate between the correlated scents, despite the fact that they were detected above the threshold of perception. The further subgroup formed was represented by the CO3 and CO5 tests (MLA4 -NF213 and MLPK45H-NF213) (Fig. 3c). The odour perceptions associated with these tests (rancid, grease, butter, paint, solvent, etc.) had not correspondence with the VOCs associated with them and detected above the perception threshold. It is probable that in a mixture characterised by low olfactory threshold VOCs and ethanol, hexanoic and octanoic medium-chain fatty acids and the higher alcohols 3-methyl-1-butanol and 3-methylsulfanyl-1-propanol, play a role in defining certain synergy and antagonism relationships (Ferreira, 2012; Ferreira et al., 2016) or incorporation (Naselli et al., 2023). These compounds may not be perceptible to the human nose. Although the odour perceptions of cream and butter were positively correlated with the CO3 and CO5 tests, no identifications were observed either by judges or by gas chromatographic analysis for 2,3-butanedione. However, statistical PCA analysis showed a potential productivity of 2,3-butanedione by the MLA4 strain. Celik, Cabaroglu, & Krieger-Weber (2019) have already observed such evidence. The CO6 and CO8-CO10 strains were the two subgroups associated with the use of *S. cerevisiae* QA23 strain in different consociations with LAB strains. These strains were negatively correlated with fruity and floral perceptions. The correlations were explained with 45.13% of the variance for the CO6 and CO10 trials and with 28.22% of the variance for the CO8 trial.

4. Conclusions

The TAIndex was determined and the effects of interactions between LAB and *S. cerevisiae* strains were clarified by its calculation. The application of the TAIndex made it possible to identify the behaviour of LAB strains when associated with different *S. cerevisiae* strains.

The kinetics of malolactic fermentation were the main technological effects associated with the variability of the TAIndex. Marginal deviations of TAIndex in microbial consortia using the same

LAB indicated a lower ability of the *S. cerevisiae* strain to influence the lactic acid bacterium in the degradation of malic acid.

In contrast, a high variability in TAIndex values marked different effects in the timing and speed of malolactic fermentation. The latter was found in the application of the LAB MLB6 strain (*O. oeni*) in different combinations with NF213 and QA23 strains (*S. cerevisiae*). TAIndex values of 0.009 - 0.148 t^{-1} resulted in different degradation times of malic acid in the wine, 44 and 18 days respectively. However, significant effects on the active volatile component of the wines were observed as a result of these differences. TAIndex values tending towards 0 (0.009-0.013) t^{-1} were associated with wines with a higher production of the ethyl esters ethyl octanoate and ethyl decanoate. TAIndex values of close to 0.02 (0.019) t^{-1} indicate wines with olfactory VOCs mainly attributable to acetate esters and higher alcohols. In contrast, TAIndex values above 0.148 showed a negative correlation with the main olfactory VOCs. This aspect suggested that fast malolactic fermentations lead to a decrease in olfactory VOCs in wines.

In conclusion, the wines obtained from the Catarratto cultivar after malolactic fermentation were found to have an olfactory profile mainly characterized by a fruity and floral component that contributed to olfactory complexity. It is noteworthy that the buttery hints attributable to the production of 2,3-butanedione werenotdetected in the final Catarratto wines. This aspect validates the microbial association technique as a biotechnological strategy capable of modulating the aromatic profiles of wines and guaranteeing their microbial stability. At the same time, it contributes to validate the significant role of malolactic process on fruity and floral aroma formation.

Further investigations will be carried out with non-targeted metabolomics approaches to probe the effects on VOCs highlighted by the TAIndex. Finally, it cannot be ruled out that TAIndex can be integrated into artificial intelligence (AI) calculation software to predict and diversify the aroma profiles of wines according to consumer taste.

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Activity 2.2

Application of *Lactiplantibacillus plantarum* in the large-scale winemaking process of Catarratto, Nero d'Avola and Petit Verdot grape varieties

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Abstract

Malolactic fermentation represents a critical step in the winemaking process. The introduction of lactic acid bacteria among the bietechnologies for oenological use represents a major breakthrough in the world of the wine industry. The application of new strains of non-*Oenococcus* lactic acid bacteria opens up interesting production scenarios in the prudutive wine-making process. The study validated the application of *Lactiplantibacillus plantarum* ML Prime^{TM} on an industrial scale and investigated, for the first time, the existence of a cultivar-LAB relationship with effects on the sensory profile of wine.

The use of ML Prime^{TM} achieved the best technological performance with the lowest production rates of acetic acid and rapid microbiological stabilisation. Furthermore, the impacts recorded by the use of ML Prime^{TM} on the volatile fraction of the wine were significant; the use, at 24 hours from the strain of *Saccharomuces cerevisiae*, significantly increased the floral notes of the Catarratto, Nero d'Avola and Petit Verdot wines.

The application of statistical graphical systems on the volatile fractions of the wines confirmed the existence of a cultivar-LAB relationship but further investigations must be carried out using precision research means.

Keywords: malolactic fermentation; lactic acid bacteria; *Lactiplantibacillus plantarum*; wine; aroma.

1. Introduction

During alcoholic fermentation (AF) the presence of lactic acid bacteria (LAB) correlates with their sensitivity to increasing ethanol concentrations and resistance to low pH values (Kunkee, 1984). Once AF is completed, yeast activity diminishes and only LAB resistant to the stressing conditions are able to survive (Wibowo et al., 1985). The survival of these LAB plays a significant role in winemaking, because they drive a secondary biological process known as MLF. This process converts L-malic acid into L-lactic acid and CO_2 and is carried out by one or more LAB species (Lonvaud-Funel, A., 1995; du Toit et al., 2011).

Malic acid degradation leads to the achievement of microbiological stability of wines by removal of fermentable energy substrates (Sumby et al., 2019). The metabolisms implemented by LAB during MLF result in a number of positive changes in the aromatic profiles of wines (Naselli et al., 2024). MLF might occur spontaneously or driven by malolactic starter cultures added during vinification (Brizuela et al., 2019). Oenological LAB present in the must can carry out MLF spontaneously after growing to a critical population required to initiate malic acid degradation (Solieri et al., 2010). Their activities depend on the physicochemical characteristics of the wine (Smit et al., 2013). LAB involved in MLF are mainly represented by the species *Oenococcus, Lactobacillus, Pediococcus, Leuconostoc and Weissella* (Bae et al., 2006; Dicks and Endo, 2009; Lonvaud-Funel, 1999). *Oenococcus oeni* (*O. oeni*) has historically been the predominant species associated with MLF due to its tolerances to high ethanol concentrations, low pH and limited nutrient content (Ribéreau-Gayon et al., 2006).

However, *O. oeni*-like technological peculiarities in *Lactiplantibacillus plantarum* (*L. plantarum*) have paved the way for the introduction of this LAB as a new generation of starter cultures for MLF in wine (Cappello et al., 2017; Krieger-Weber et al., 2020; Lucio et al., 2017).

Moreover, *L. plantarum* strains exhibit a multitude of advantageous biological characteristics when compared to *O. oeni*, including a superior growth rate and the capacity to inhibit the formation of several undesirable compounds (Brizuela et al., 2019; Lucio et al., 2017). Given that MLF typically occurs subsequent to AF, the proliferation of wine LAB and the efficiency of MLF are subject to

influence by the composition of the wine. The production and release of compounds by yeasts, including ethanol, SO_2, fatty acids, peptides, polysaccharides and other macromolecules, impact the growth of wine bacteria and MLF. Furthermore, yeast strains have nutritional requirements that can influence the course of MLF. Guilloux-Benatier et al., 1998 reported the ability of *Saccharomyces cerevisiae* (*S. cerevisiae*) with low nutritional requirements in favouring the course of MLF by LAB. Technologically, the initiation of the MLF process may be controlled through the inoculation of a selected strain of *O. oeni* or *L. plantarum*. However, under favourable conditions, MLF can occur spontaneously (Borneman et al., 2012a; Ribéreau-Gayon et al., 2006). Compared to spontaneous MLF, the use of commercial LAB starters in a controlled fermentation process offers the advantage of ensuring a rapid and reliable fermentation process, as well as providing consistent wine quality (Qiu et al., 2023).

The marketing and approval for the use of wine LAB follows procedures well regulated by the International Oenological Codex.

Sensitive aspects taken care of by the regulatory bodies mainly concern certifying protocols in order to certify full food safety.

However, although of a non-food nature, no obligation is placed on LAB producers to provide useful information on the potential of LAB to modulate wine aroma according to the cultivar used.

The lack of information in the literature led to start this research with the objectives of evaluating: (i) the validity of the use of ML Prime^{TM} strain with respect to a spontaneously conducted MLF; (ii) the impact that a cultivar can play in the interactions with LAB in the aromatic expression of wines.

In order to be able to investigate the cultivar-LAB issue, it was necessary to extend the period of maceration of the skins in the wine up to 150 days in order to be able to monitor the vital dynamics of LAB over time.

2. Materials and methods

2.1. Experimental design and sample collection

The experimental plan of the winemaking process with Cataratto (white grape), Nero d'Avola and Petit Verdot (red grapes) varieties is reported in Fig. 1.

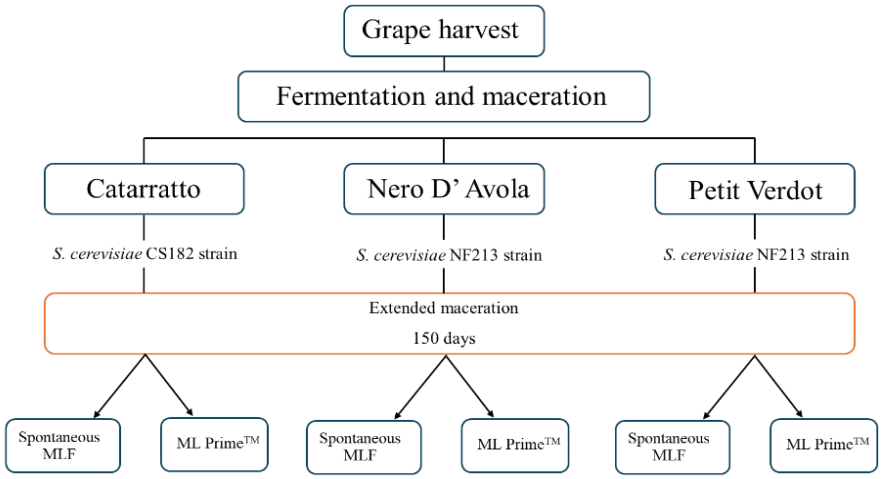


Fig. 1. Experimental design of winemaking process carried out with *L. plantarum* ML Prime ™ strain.

The agent of MLF *L. plantarum* ML Prime™ strain was inoculated into the grape musts before AF took place. *Saccharomyces cerevisiae* yeast NF213 and CS182 strains, belonging to the oenological yeasts collection of the Department of Agricultural, Food and Forest Sciences (SAAF) (University of Palermo. Italy), was added to conduct AF. Both *S. cerevisiae* strains were selected as wine starters at SAAF Department and are being largely used in winemaking by several companies.

Grapes of Catarratto were harvested in a vineyard located in San Giuseppe Jato (Palermo, Sicily, Italy) at Di Bella Vini s.r.l. wine company (Palermo, Italy); Nero d'Avola vineyard was located in Contrada Buonivini (Noto, Siracusa province, Sicily, Italy), while Petit verdot vineyard in Partinico (Palermo, Sicily, Italy) at the Azienda Agricola Calagna Francesco (Palermo, Italy). The experimental vinification took place at the cellar Cantina Sperimentale G. Dalmasso of the Istituto Regionale Vini e Oli di Sicilia (IRVOS) in Marsala (Trapani, Sicily, Italy). All experimental winemaking processes were carried out in duplicate with the same bulk musts (two vats per variety).

Grape samples were collected at harvest, must samples just after the inoculum of starter yeasts and starter LAB, wine samples during the MLF and AF (grape must, yeast inoculum, AF 1d, 2d, 3d, 4d, 5d, 8d, AF-end, end maceration.

2.2. Winemaking process

The grapes were harvested by hand and destemmed. The crushed grapes were aliquoted in steel tanks (50 hL each) and potassium metabisulphite (20 mg/L) was added. Each trial was conducted in triplicate. Catarratto trials were inoculated with *S. cerevisiae* CS182 strain (Settanni et al., 2012), while Nero d'Avola and Petit verdot trials were inoculated with *S. cerevisiae* NF213 strain (Settanni et al., 2012). Both strains were produced in liquid form with a cell density of [about 7.00×10^{10} colony-forming units (CFU)/g] by Bionova srl (Villanova sull'Arda, Piacenza, Italy) and added to must at 20 g/hl.

L. plantarum ML Prime^{TM} strain inoculation occurred 24 h after the addition of the starter yeast culture, in order to promote MLF. The malolactic starter was characterized by a viable cell concentration of about 5.0×10^{12} CFU/g and were added at a final cell density of 7.0 Log CFU/mL and the trials were coded as follows: CT-MLP, ND-MLP and PV-MLP for Catarratto, Nero d'Avola and Petit verdot grape varieties, respectively. For each variety a trial without MLF starter addition was also included in the experimentation and considered as control (spontaneously fermented) trial. Control trials were coded as CT-C, ND-C and PV-C for Catarratto, Nero d'Avola and Petit verdot grape varieties, respectively.

All trials were added with diammonium phosphate and diammonium sulfate (1:1) (0.15 g/L) and organic nitrogen (0.20 g/L) as yeast nutrient during AF.

The AF of the trials CT-MLP and CT-C occurred at 20 °C, while the trials ND-MLP, ND-C, PV-MLP, PV-C were fermented at 20 °C.

During AF, sugar and temperature were daily monitored through densitometric measurement of Babo's mustimeter.

2.3. Microbiological analysis

Samples collected during wine production were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). The surfaces of cellar equipment (stemmer-crusher, hydraulic press and steel tanks) were analyzed following the methodology of the International Organization for Standardization (2004); the surface of grape berries was analyzed according to Settanni et al. (2012).

Serial decimal dilutions were spread-plated (0.1 mL) onto Wallerstein Laboratory (WL) nutrient agar (Oxoid, Basingstoke, UK) and incubated at 28 °C for 72 h to differentiate *Saccharomyces* and non-*Saccharomyces* yeast counts. LAB were detected by pour plating onto de Man, Rogosa, and Sharpe (MRS) agar (Oxoid) and M17 agar (Oxoid), incubated anaerobically at 30 °C for 48 h and onto *Leuconostoc oenos* (MLO) agar medium (Caspritz and Radler, 1983), incubated at 28 °C for 5 d. The latter medium was used for the enumeration of aciduric LAB. Acetic acid bacteria (AAB) were enumerated onto Kneifel agar medium (OIV, 2010), incubated at 25 °C for 10 d. All analyses were carried out in triplicate.

2.4. Yeast isolation and identification

Yeasts were isolated only from WL differential medium. At least five colonies per morphology were randomly collected from the agar plates, purified to homogeneity after several sub-culturing steps onto WL, and at least three isolates (from each sample) sharing the same morphology were subjected to the genetic characterization. DNA extraction was performed using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. In order to perform a first differentiation of the yeasts, all selected isolates were subjected to restriction fragment length polymorphism (RFLP) analysis of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene as reported by Esteve-Zarzoso et al. (1999).

Five isolates representative of each RFLP group were subjected to an additional enzymatic restriction targeting the 26 rRNA gene as reported by Settanni et al. (2012). One isolate per group was further processed by sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary

identification obtained by restriction fragment length polymorphism analysis. The D1/D2 region was amplified with the primers NL1 and NL4 (Kurtzman and Robnett, 1998). Polymerase chain reaction (PCR) products were visualized as described by Settanni et al. (2012). DNA sequencing reactions were performed at AGRIVET (University of Palermo, Italy). The identities of the sequences were determined by BlastN search against the NCBI non-redundant sequence database located at http:// www.ncbi.nlm.nih.gov.

In order to verify the dominance of the starters CS182 and NF213 during AF, all *Saccharomyces* isolates were characterized by Interdelta analysis following the methodology of Legras and Karst (2003). Interdelta patterns were analyzed using the GelCompar II software (v. 6.1, Applied Maths NV, Sint-Martens-Latem, Belgium) and similarities among patterns were assessed. Profiles showing more than 95% of similarity were considered identical.

2.5. LAB isolation and identification

Presumptive LAB were isolated from MRS and MLO media. The isolation and purification were conducted as reported above for yeasts. The three isolates from each sample were then subjected to phenotypic (Gram reaction and catalase test) and genotypic characterization. The DNA from presumptive LAB was extracted according to Ruzauskas et al. (2015). Dominance of *L. plantarum* ML Prime^{TM} strain was ascertained during fermentation by random amplification of polymorphic DNA-PCR (RAPD-PCR) analysis in 25 µL reaction mix using primer M13 (Stenlid et al., 1994). The electrophoresis runs were carried out at 100 V on 2% agarose gel (w/v) in 1× TBE buffer. The bands were visualized by UV transilluminator using GeneRuler 100 bp DNA Ladder Plus (Thermo Scientific) as molecular weight marker. RAPD profiles were analyzed by Gelcompar II software and the DNA sequencing reactions were performed at AGRIVET. The identities of the sequences were determined by BlastN search versus the NCBI non-redundant sequence database located at http://www.ncbi.nlm.nih.gov and those available at EZTaxon located at http://www.ezbiocloud.net/eztaxon. All strains belonging to the *L. plantarum* group were subjected

to the *recA* gene based multiplex PCR described by Torriani et al. (2001) to distinguish unequivocally among *L. plantarum*, *Lactiplantibacillus paraplantarum* and *Lactiplantibacillus pentosus*.

2.6. Physicochemical analysis

Glucose, fructose, ethanol, glycerol, acetic, tartaric, malic and lactic acids were determined by the enzymatic analyser iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co.,Ltd., Shenzhen, China). This system collects and mixes automatically reagents and samples into the cuvette, performs incubation at a controlled temperature, reads absorbance at the specific wavelength, and calculates the concentration of the chemicals with a calibration curve. The parameters used in the automated photometric systems were: temperature, 37 °C; wavelengths, 340 nm and 415 nm (bichromatic); and optical path, 1 cm. All reagents and standards were purchased from R-Biopharm AG (Darmstadt, Germany).

The values of pH were determined by OIV-MA-AS313-15 method (OIV, 2019a), while total acidity was determined by the methodology described by OIV-MA-AS313-01 (OIV, 2019b). Free and total sulphur dioxide were measured in accordance with the methods described by OIV-MA-AS323-04B (OIV, 2019c).

2.7. Volatile organic compound composition by GC-MS

2.7.1. Sample preparation

The volatile components emitted from the final wines of the trials CT-C, CT-MLP, ND-C, ND-MPL, PV-C and PV-MLP were determined applying the protocol of Reddy and Dillon (2005). Ten millilitres of each wine were mixed with MS SupraSolv® dichloromethane (10 mL) in a 100-mL conical flask and stirred at room temperature for 30 min. The samples were centrifuged at 3000 RPM for 10 min. The aqueous phase was removed from the centrifuge tube and anhydrous sodium sulphate (1 g) was added before centrifuging at 3000 RPM for 10 min. The dichloromethane layer was removed, dried under nitrogen gas to 1 mL and injected (1 μL).

2.7.2. Gas chromatography (GC) and mass spectrometry (GC-MS)

Gas chromatography analyses were performed on an Agilent 7000C GC system fitted with a fused silica Agilent DB-5MS capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 ˚C. Solvent Delay: 5 min. Helium was the carrier gas (1 mL min^{-1}). The other GC analyses were performed in a Shimadzu QP 2010 plus equipped with a AOC-20i autoinjector (Shimadzu. Kyoto. Japan) and with a Supelcowax 10 capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness); ionization voltage 70 eV; transfer line temperature, 280 ˚C. Helium was the carrier gas (1 mL min^{-1}). For both columns, the temperature was initially kept at 40 °C for 5 min then gradually increased to 250˚C at 2 °C min^{-1} rate, held for 15 min and finally raised to 270 °C at 10 °C min^{-1}. One microlitre of sample was injected at 250 ˚C automatically in splitless mode; transfer line temperature, 295 °C.

2.7.3. Data analysis and evaluation

The individual peaks were analysed using the GCMSolution package version 2.72. The identification of the compounds was carried out using Adams NIST 11, Wiley 9 and FFNSC 2 mass spectral databases. These identifications were also confirmed by other published mass spectra and linear retention indices (LRI). Linear retention indices were calculated using a series of n-alkanes (C8-C40). In addition, some of the compounds were confirmed by comparison of mass spectra and retention times with standard compounds available at the Department STEBICEF – University of Palermo, Italy.

2.8. Sensory evaluation

The designed sensory evaluation of the experimental wines consisted of two steps: (i) sensory acceptance tests performed by consumers and experts; (ii) quantitative descriptive analyses carried out by panelists to define aroma and sensory profiles.

The sensory assessments were performed in blind tasting conditions at the tasting room of University of Palermo (Palermo, Italy). The ten experimental wines were brought to ambient temperature, and were first checked for faults and/or off-odors and off-flavors, by three experienced wine professional tasters. Wine samples (50 ml) were served monadically at 20 °C in standard ISO type tasting glasses, labelled with three-digit random codes. Water was provided for rinsing between wines. All evaluations were made between 10.00 and 12.00 a.m. in individual booths (ISO 8589, 2007). The final scores were obtained as a mean of three evaluations with the respective statistical analysis.

2.8.1. Acceptance test

Samples of experimental wines were evaluated for their overall acceptability (Villanueva et al., 2005; Villanueva and Da Silva, 2009; Biasoto et al., 2014; Ribeiro et al., 2018; Bednarek et al., 2019). A total of 87 consumers were recruited from the University of Palermo. Following the methodology reported by Biasoto et al. (2014), lecturers, researchers, technicians and graduate students were invited to partecipate by filling in a recruitment form, and from 25 habitual consumers of white wine were selected, 13 women and 11 men, ages ranging from 21 to 42 years old. The same methodology was also followed for red wines. The selection criterion of the subjects was the consumption of at least one glass of white or red wine per week with no experience on wine sensory analysis. As reported by Biasoto et al. (2014), all the consumers evaluated the overall acceptability of the 10 wine samples using a hybrid hedonic scale of 10 cm which included three points: dislike extremely (1), neither like or dislike (5) and like extremely (10). The ten wine samples were evaluated in two distinct tasting sessions, carried out on two consecutive days. The effects of the presentation order and first-order carry-over of the samples were controlled using the crossover design proposed by Wakeling and MacFIE (1995).

2.8.2. Quantitative descriptive analyses

Sixteen judges (9 women and 7 men, ranging from 23 to 41 years old) were recruited from Oenologist Associations, National Organization of Wine Taster (ONAV, Italy), Italian Sommelier Association

(AIS, Italy) and University of Palermo. All judges had experience in winemaking and participated in previous studies as sensory panelists.

The judges were submitted to preliminary tests to determine their sensory performances on basic tastes and the aromas associated with wines. The sensory profiles (ISO 13299, 2016) of the wines obtained from Catarratto grape were constructed using two selected panels (ISO 8586, 2012) each of eleven judges trained over several sessions.

The sensory analysis of wine was conducted following the methodology reported by R.S. Jackson (2017): visual perception (appearance), olfactory sensations [odour (via the nostril, ortho-nasally) and flavour (via the back of the throat, retro-nasally)], oral sensations (taste and mouth-feel), finish and overall quality.

The odour was explained as perception of volatile compounds with the wine outside the mouth [orthonasal (in-glass) odour]; the flavour as perception of volatile compounds with the wine inside the mouth and back of the throat [retro-nasal (mouth-derived) odour]; basic taste as gustatory sensations and mouth-feel sensations as chemical feeling factors with wine inside and after the mouth; finish refers to the lingering of flavour sensations in the mouth (R.S. Jackson, 2017) and overall quality based on global evaluation of odour, taste, mouth-feel and flavour (R.S. Jackson, 2017, Issa-Issa et al., 2020).

The 21 panellists compared the ten experimental wines during different sessions. They consensually generated 38 sensory descriptive attributes regarding appearance, odour, flavour, taste and overall quality in several sessions for Catarratto and Nero D'Avola, while for Petit verdot the attributes were 35. The set of attributes for Catarratto were: appearance (yellow color); odor (intensity, persistency, floral, fruity, peach, apricot, citrus fruits, tropical fruits, pineapple, flinty, spicy, jasmin, mint, cashew); gustatory taste (sweet, sour, salty, astringency and bitter); mouth-feel (acidity, body, balance and velvety); flavour (intensity, persistency, floral, fruity, citrus fruits), overall quality (odor, taste, mouth-feel and flavour) and finish (after-smell and after-taste). The set of attributes for Nero D'Avola were: appearance (red color); odor (intensity, persistency, floral, blackcurrant, blackberry, cherry,

plum, blueberry, carob, eucalyptus, mint, violet); gustatory taste (sweet, sour, salty, astringency and bitter); mouth-feel (acidity, body, balance and velvety); flavour (intensity, persistency, red berries, fruity, floreal, fresh/green, and spices), overall quality (odor, taste, mouth-feel and flavour) and finish (after-smell and after-taste). Finally, the descriptive attributes for Petit Verdot were: appearance (red color); odor (intensity, persistency, floral, blackberry, plum, mint, violet, lilac, and sage); gustatory taste (sweet, sour, salty, astringency and bitter); mouth-feel (acidity, body, balance and velvety); flavour (intensity, persistency, red berries, fruity, floreal, vegetal, and spices), overall quality (odor, taste, mouth-feel and flavour) and finish (after-smell and after-taste).

The panellists were trained (ISO 5496:2006) also for the identification of wine off-odours, off-taste and off-flavours [microbial (moldy, corky, yeastly, buttery, cheese, etc), pungent (vinegary, alcoholic, sulfur, etc), putrid (rancid, rotten egg, rubbery, etc), petroleum (fusel, plastic, solvent), other] (R.S. Jackson, 2017; Issa-Issa et al., 2020). The panellists also generated a consensual descriptive ballot for the wines in which the descriptors were associated with a 9 cm unstructured scale anchored at the left and right extremes with the terms “none/weak” and “strong”, respectively (R.S. Jackson, 2017; Biasoto et al., 2014).

The sensory test was carried out following the procedures reported above at 2.7 paragraph.

The six wine samples were evaluated in distinct tasting sessions carried out on consecutive days. Overall, each judge evaluated each of the six wines with three repetitions. For each repetition, a different wine bottle was opened.

2.9. Statistical analysis

Principal component analisys (PCA) was performed on the data matrix consisted of 2 rows (trials: control and *L. plantarum* ML prime^{TM} strain for each cultivar) × 35 or 38 columns (35 descriptors for sensory analysis of Petit verdot or 38 descriptors for sensory analysis of Catarratto or Nero D’Avola) to explore the correlation between variables and different trials, as well as discrimination among the trials. Agglomerative hierarchical cluster analysis (AHCA) was also performed for the common descriptors of white and red wines according to their dissimilarity, measured by Euclidean distances

and Ward's method. The exploration of the variations and similarities of the wines were in relation to the following descriptors: odours (intensity, persistency, floral, mint and off-odours), taste (sweet, sour, salty, astringency, bitter and off-taste), mouth-feel (acidity, body, balance and velvety), flavour (intensity, persistency, fruity, floreal and off-flavour), overall quality (odor, taste, mouth-feel and floavour) and final (after-smell and after-taste).

Statistical data processing and graphic construction were performed with the XLStat software version 2020.3.1 (Addinsoft, New York, USA) for excel.

The web-based Metabo-Analyst tool (http://www.metaboanalyst.ca/) was used to process the VOCs by heatmap, pairwise t-tests (p-value < 0.0001) and violin plot. For statistical analysis, data were normalised to the median and scaled using the Pareto scale. The distance measure for cluster analysis was Euclidean.

3. Results and discussion

3.1. Dynamics of yeast populations

Plate counts of yeasts during fermentation are shown in Fig. 2.

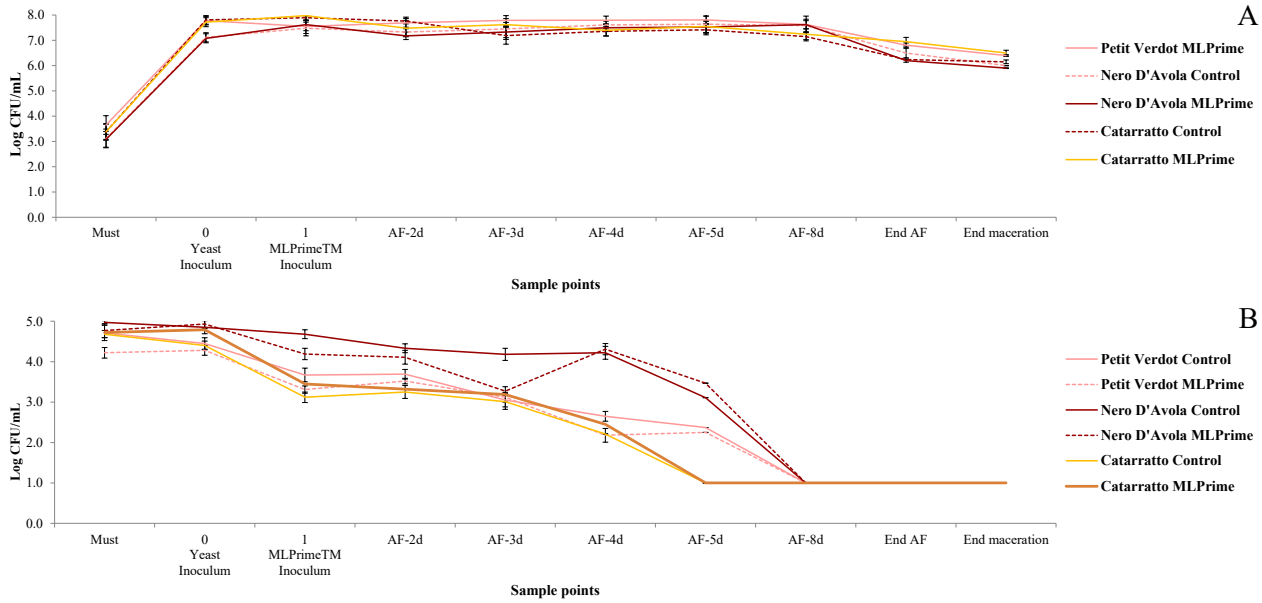


Fig. 2. Yeast concentration (Log CFU/mL) of samples collected during winemaking process: (A) presumptive *Saccharomyces* spp.; (B) non-*Saccharomyces* spp..

Yeast monitoring showed that in Catarratto, Nero d'Avola and Petit Verdot musts presumptive *Saccharomyces* (PS) levels ranged between 3.1 and 3.7 Log CFU/mL (Fig. 2A), while non-*Saccharomyces* (NS) were in the range 4.2 - 5.0 Log CFU/mL (Fig. 2B). *Saccharomyces* strains (CS182 and NF213) were inoculated between 7.1 and 7.8 Log CFU/mL and the initial average PS/NS was around 1.6.

After 3 d of AF an increase of the PS populations was observed for all trials with levels in the range 7.2 - 7.8 CFU/mL, whereas, NS yeasts showed lower cell densities (3.0 - 4.2 Log CFU/mL). At the fifth day of AF, PS numbers increased up to 7 log cycles; this trend was also observed at 8 d of AF. In contrast, NS from the day 8 of AF onwards were found at the same levels of PS and/or below the detection limit. However, at the end of AF, a reduction of PS levels was observed for all trials. Counts were between 6.2 - 7.0 Log CFU/mL.

3.2. Dynamics of LAB population

LAB population growth rates are shown in Fig. 3 A, B, C.

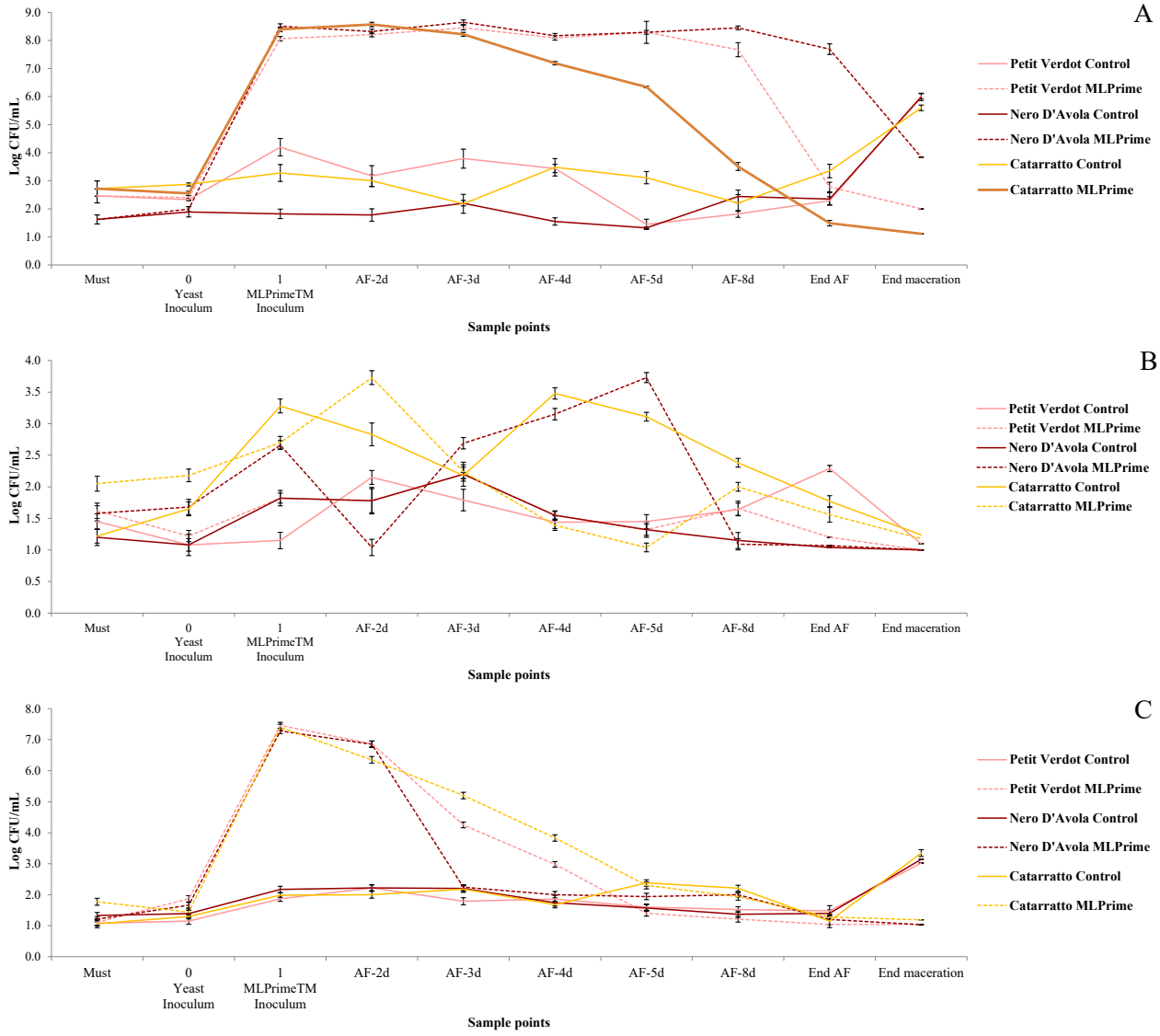


Fig. 3. LAB concentration (Log CFU/mL) of samples collected during winemaking process: (A) MRS; (B) M17; (C) MLO.

LAB populations in must before starter inoculation reached the highest levels on MRS (1.6-2.7 Log CFU/mL, Fig. 3A), while the cell density estimated on M17 (Fig. 3A) and MLO (Fig. 3C) was in the range of 1.1-2.1 Log CFU/mL. LAB population levels after starter yeast inoculation were unchanged. After 24 h, *L. plantarum* ML Prime^{TM} strain was added and the microbiological count values in CT-MLP, ND-MLP and PV-MLP were between 8.1 and 8.5 Log CFU/mL on MRS, almost 1 log cycle

lower on MLO, and barely 1.8 - 2.7 Log CFU/mL on M17. In the control trials (CT-C, ND-C and PV-C) the values were highly variable (1.4-4.2 Log CFU/mL on MRS). LAB levels on M17 and MLO were in the range of 1.2-3.3 Log CFU/mL.

LAB values of the trials ND-MLP and PV-MLP on MRS plates were between 7-8 logarithmic cycles up to 8 d of alcoholic fermentation. A decrease of lactobacilli populations was observed in CT-MLP from day 3 reaching the level of 3.5 Log CFU/mL at day 8. At the end of AF the presence of lactobacilli on MRS was observed only in ND-MLP (7.69 Log CFU/mL) while in CT-MLP and PV-MLP the LAB levels were below 3 log cycles. The count values recorded on M17 and MLO were lower than those registered on MRS. In all control trials, LAB counts were always lower than those of the trials inoculated with *L. plantarum* ML Prime^{TM} strain. Count values from day 2 of alcoholic fermentation until the end were within the range of 1.0-3.5 Log CFU/mL.

3.3. Molecular analysis

3.3.1. Catarratto winemaking

A total of 397 colonies were collected from MRS (294 isolates) and MLO (103 isolates) and subjected to microscopic inspection. The isolates were grouped into 385 rods and 12 cocci. After Gram-stain characterisation and catalase testing, 356 rods were still considered putative LAB cultures (Gram-positive and catalase-negative), while none of the coccus shaped isolates could be further processed. All 356 isolates were subjected to RAPD analysis in order to perform the strain typing. The resulting patterns (n = 356) were used to construct a dendrogram (Fig. 4a).

The analysis revealed 11 main clusters. Cluster G was the most numerous cluster consisting of 319 isolates with the same RAPD profile confirming that the starter strain dominance was 89.61%.

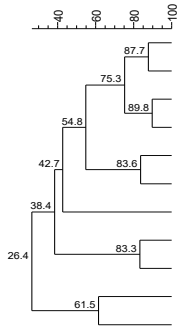
In the control trial CT-C, that did not receive the *L. plantarum* ML Prime^{TM} strain addition, no polymorphic profiles comparable to the starter strain ML Prime^{TM} strain were detected.

3.3.2. Nero D'Avola winemaking

A total of 534 colonies including 368 from MRS and 166 from MLO agar plates were isolated from the experimental thesis with MLF driven by ML Prime^{TM} strain. Five hundred and twenty-four colonies were still considered presumptive LAB. The 501 rod shaped isolates were subjected to RAPD analysis that generated 2 main clusters (A and B) (Fig. 4B). In this case the starter strain dominance was 92.81% and the pattern of the strain ML Prime^{TM} strain was not detected in control trial.

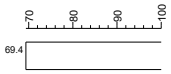
3.3.3. Petit Verdot winemaking

During Petit verdot wine making, 541 colonies were isolated and 498 isolates were considered presumptive LAB. In this case, they were divided into 462 rods and 36 cocci. RAPD analysis revealed 7 strain cluster with cluster C being the most numerous one with 425 isolates and a starter strain dominance of 91.99% was found (Fig. 4c). Also for this trial *L. plantarum* ML Prime^{TM} strain was not found associated to the control production.



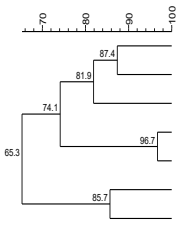
A

Rapd profile	Isolates (number)	Species Blast	% Similarity (Blast)	Accession number (Blast)	Species (EzTaxon)	% Similarity (EzTaxon)	Accession number (EzTaxon)	Species by Multiplex
A	3	*L. plantarum*	99.52	KX261527.1	*L. pentosus*	99.25	DSM 20314	*L. plantarum*
B	7	*L. plantarum*	100.0	MK774602.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*
C	4	*L. pentosus*	99.59	KX057507.1	*L. pentosus*	99.38	DSM 20314	*L. plantarum*
D	5	*L. plantarum*	99.66	KX261527.1	*L. pentosus*	99.25	DSM 20314	*L. plantarum*
E	1	*L. pentosus*	99.46	KX057491.1	*L. pentosus*	99.73	DSM 20314	*L. plantarum*
F	3	*L. plantarum*	99.59	KX261527.1	*L. pentosus*	99.52	DSM 20314	*L. plantarum*
G	319	*L. plantarum*	99.36	KX261527.1	*L. pentosus*	98.63	DSM 20314	*L. plantarum*
H	5	*L. plantarum*	99.80	KX261527.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*
I	2	*L. plantarum*	99.39	KM497500.1	*L. pentosus*	99.25	DSM 20314	*L. plantarum*
J	4	*L. paraplantarum*	98.44	KU315098.1	*L. pentosus*	98.83	DSM 20314	*L. plantarum*
K	3	*L. plantarum*	99.52	KX057688.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*



B

Rapd profile	Isolates (number)	Species Blast	% Similarity (Blast)	Accession number (Blast)	Species (EzTaxon)	% Similarity (EzTaxon)	Accession number (EzTaxon)	Species by Multiplex
A	465	*L. plantarum*	100.00	JN408516.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*
B	36	*L. plantarum*	99.73	KX261527.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*



C

Rapd profile	Isolates (number)	Species Blast	% Similarity (Blast)	Accession number (Blast)	Species EzTaxon	% Similarity (EzTaxon)	Accession number (EzTaxon)	Species by Multiplex
A	11	*L. plantarum*	99.93	KX261527.1	*L. pentosus*	99.79	DSM 20314	*L. plantarum*
B	3	*L. plantarum*	99.46	KX261527.1	*L. pentosus*	99.73	DSM 20314	*L. plantarum*
C	425	*L. plantarum*	99.93	KX261527.1	*L. pentosus*	99.79	DSM 20314	*L. plantarum*
D	5	*L. pentosus*	99.46	KX057507.1	*L. pentosus*	99.73	DSM 20314	*L. plantarum*
E	7	*L. pentosus*	99.80	KX057507.1	*L. pentosus*	99.79	DSM 20314	*L. plantarum*
F	9	*L. paraplantarum*	99.59	KU315098.1	*L. pentosus*	99.59	DSM 20314	*L. plantarum*
G	2	*L. pentosus*	98.91	KX261527.1	*L. pentosus*	98.97	DSM 20314	*L. plantarum*

Fig. 4. Dendrograms obtained from combined RAPD-PCR patterns of LAB strains from samples collected during winemaking process: (A) Catarratto, (B) Nero d'Avola and (C) Petit Verdot grape varieties.

3.4. Chemical composition

The consumption of residual sugars, glucose and fructose during AF graphically reported in Fig.5 A, B, C.

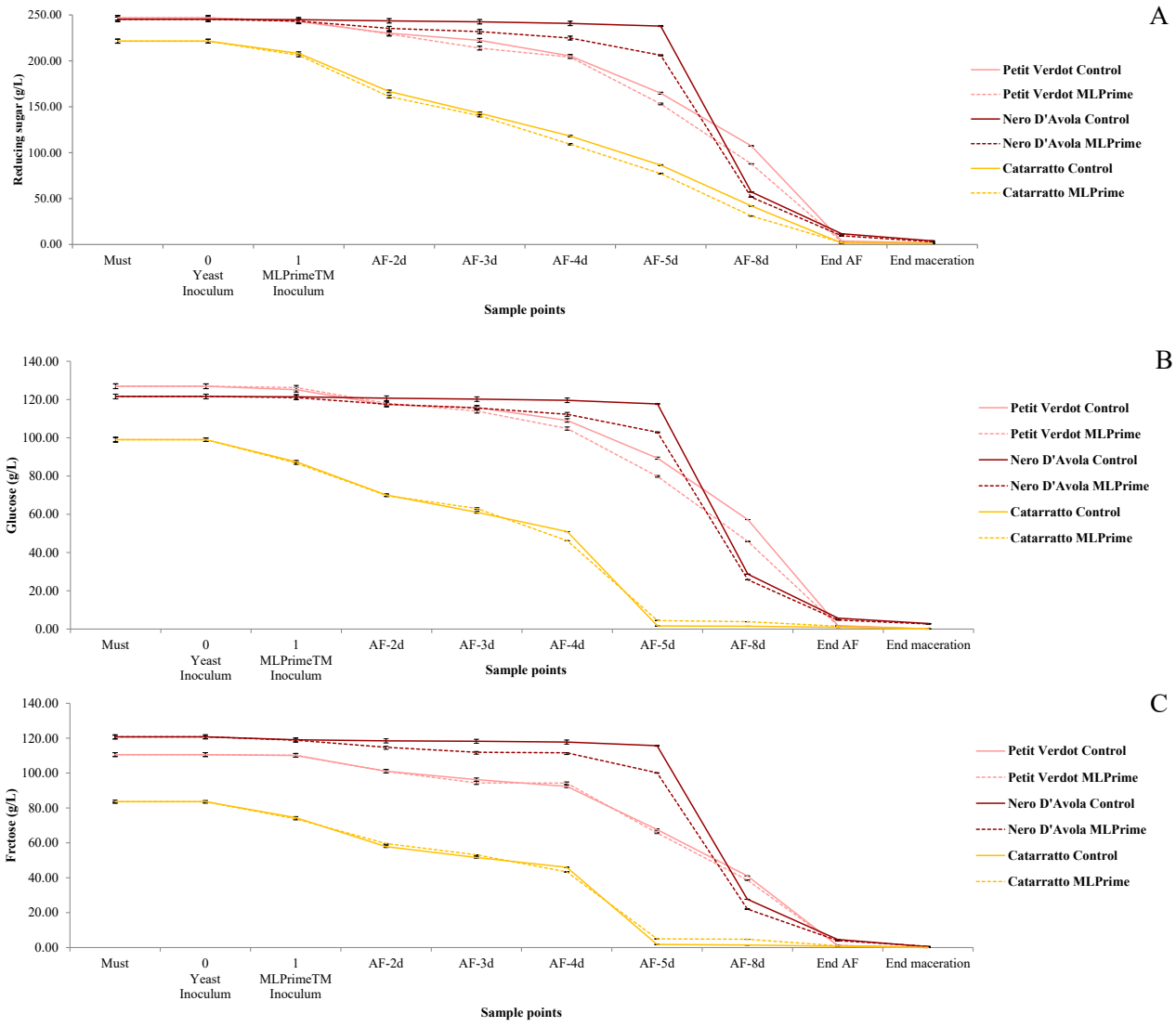


Fig. 5. Concentrations of reducing sugars (A), glucose (B) and fructose (C) of samples collected during winemaking process.

The initial values of residual sugars (Fig. 5A) were similar for the three processes. Glucose values (Fig. 5B) were similar in Nero D'Avola (121.57 g/L) and Petit verdot (126.90 g/L), while lower in Catarratto (99.00 g/L). A similar trend was observed for fructose (Fig. 5C) where the must from red

grapes showed higher values (Nero D'Avola = 120.78 g/L; Petit Verdot = 110.58 g/L) when compared to Catarratto (83.60 g/L).

After yeasts inoculum, a decrease of sugars was registered until the end of AF. All trials concluded the AF in less than 12 d with Catarratto trial in 5 d only.

Ethanol concentrations are reported in Fig. 6A. Ethanol production showed a regular trend during the entire AF. The ethanol values at the end of AF resulted in the range 12.87-15.48 % w/v. From 1 month of the end of AF, no significant variations in ethanol content were observed in Catarratto and Petit Verdot, while this alcohol increased slightly in control Nero D'Avola.

The development of glycerol concentrations during AF was also fairly regular (Fig. 6B).

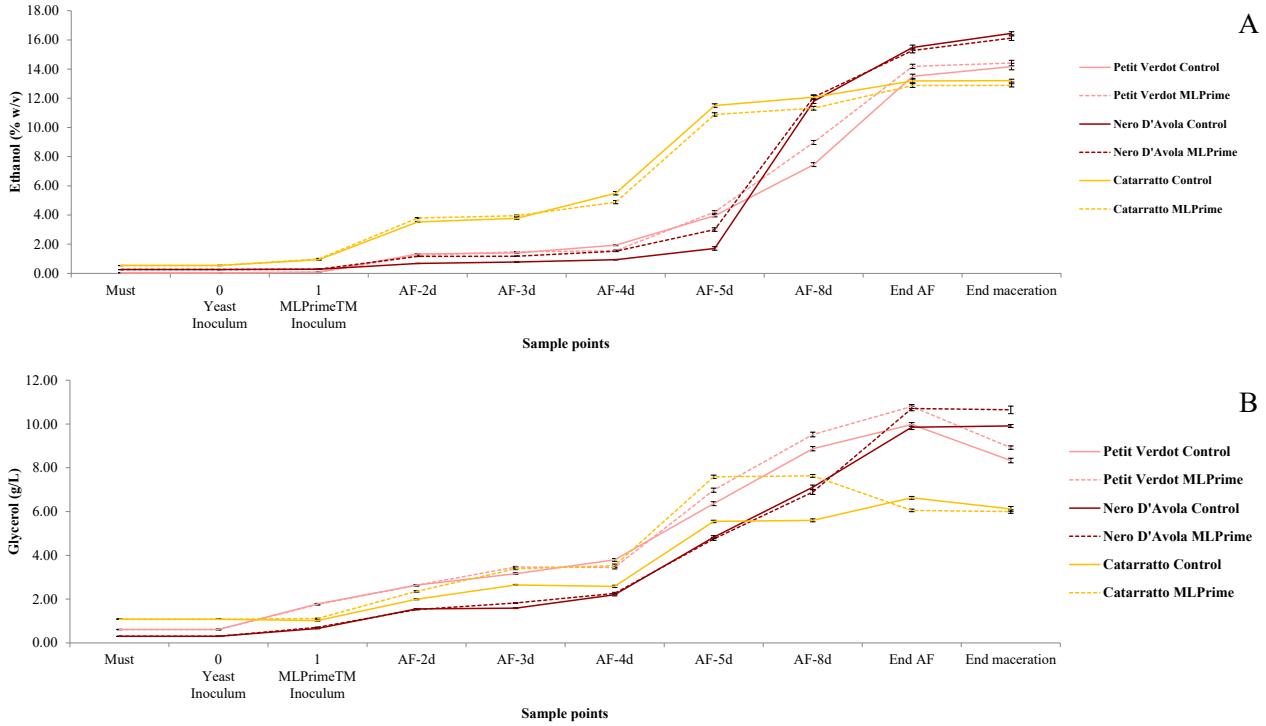


Fig. 6. Concentration of ethanol (A) and glycerol (B) of samples collected during winemaking process.

Values above 5 g/L were observed after 5 d of AF. At the end of AF the highest values were recorded in Nero D'Avola (control = 9.85 g/L; *L. plantarum* ML Prime^{TM} strain = 10.71 g/L) and Petit Verdot (Control = 9.98 g/L; *L. plantarum* ML Prime^{TM} strain = 10.80 g/L). For Catarratto trial glycerol values

were 6 g/L for both control and started process. After 1 month after the end of AF no significant changes in glycerol content were observed for all trials.

The trend in malic acid, lactic and acetic acids concentrations is shown in Fig. 7 A, B, C.

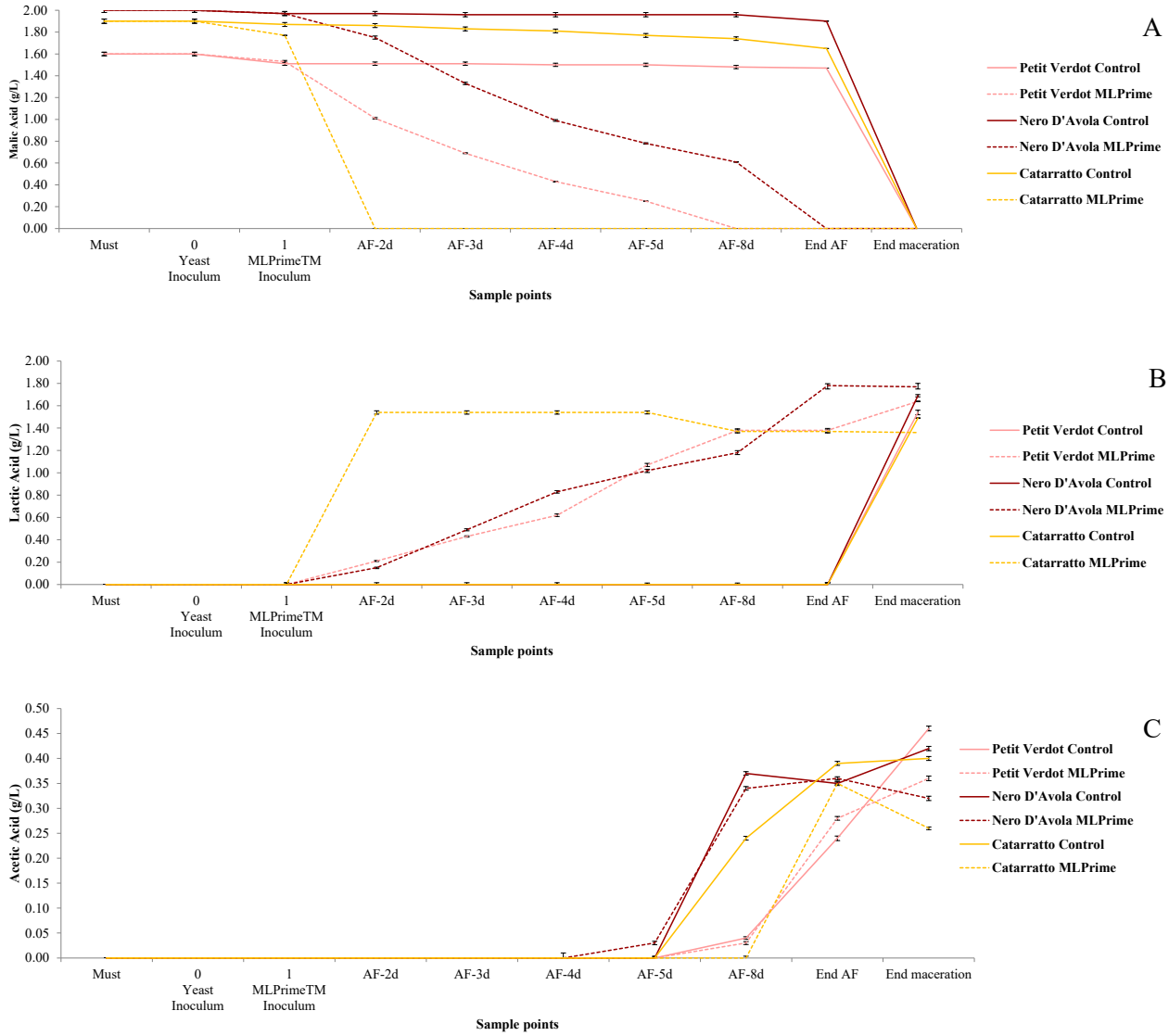


Fig. 7. Concentration of malic (A), lactic (B) and acetic (C) acids of samples collected during winemaking process.

The initial malic acid content in the must varied in relation to the grape variety (Fig. 7A). After 24 h from the addition of ML Prime^{TM} strain, the values of malic acid were completely zeroed in Catarratto, while lactic acid reached values of 1.54 g/L (Fig. 7B). For the red varieties, MLF trend was different. In Petit Verdot, the complete degradation of malic acid occurred after 7 d of MLF,

reaching lactic acid values of 1.38 g/L, while in Nero D'Avola MLF occurred after 10 d. Indeed, the highest values of lactic acid (1.78 g/L) were recorded at the end of AF.

In the control trials, spontaneous MLF was carried out after the end of AF. The lactic acid contents recorded were not statistically different from those obtained from the trials inoculated with ML Prime^{TM} strain.

The volatile acidity was detected at the end of the AF in all trials. The values of this parameter was in the range 0.24-0.39 g/L of acetic acid. After 1 month from the end of the AF, the values of acetic acid were 0.40 g/L (Fig. 7C). All these values were lower than the maximum limits established by the current normative.

The values of pH of the must are shown in Fig. 8.

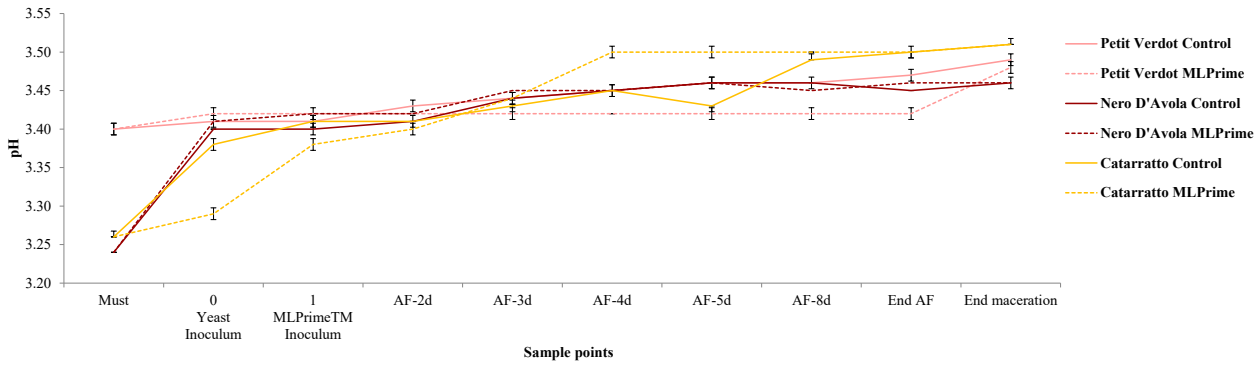


Fig. 8. Values of pH of samples collected during winemaking process.

The values were 3.26 (Catarratto), 3.24 (Nero D'Avola) and 3.40 (Petit Verdot). When ML Prime^{TM} strain was added, pH was ≥3 in all musts except CT-MLP (3.38). During AF the pH values increased slightly, reaching values between 3.42 (PV-MLP) and 3.50 (Catarratto) at the end of AF. At 1 month after the end of AF no significant variations in pH were observed.

3.5. Volatile organic compounds

The composition of the VOCs generated by the wines is shown in Table 1. 41 VOCs were identified in the application of the experimental plan shown in Fig. 1.

The liquid-liquid extraction allowed several aromatic classes to be identified, including alcohols, ethers, aldehydes, carboxylic acids, esters, sulfur compounds, nitriles, lactones, amides and terpenes. The VOCs most represented in the volatile fractions of wines were alcohols and esters. This result is in line with that described by Ribéreau-Gayon et al., (2006).

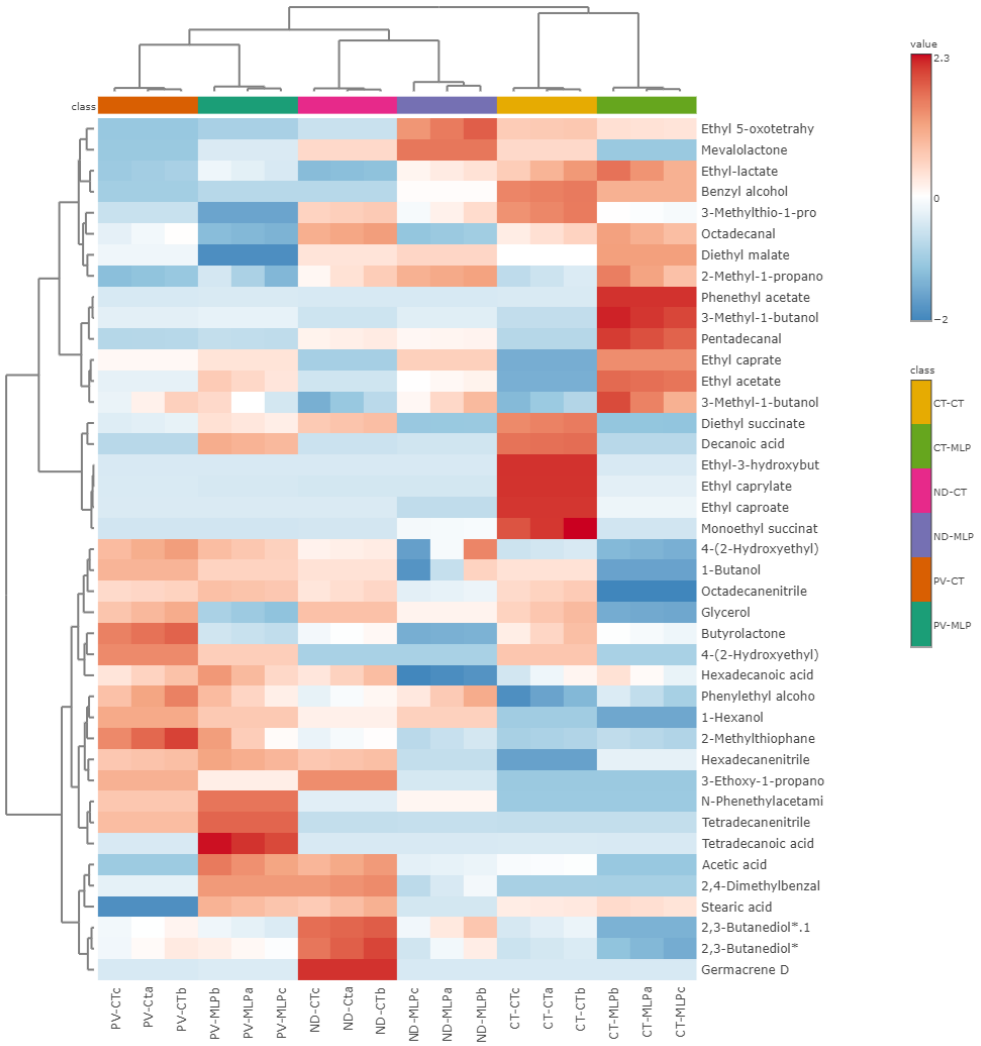


Fig. 9. Heatmap of Volatil organic compounds. The normalized median values of each triplicate were used to color the heatmap. Clustering based on Euclidean distances between samples and variables.

Heatmaps analysis of VOCs made it possible to discriminate the variables to be tested. Two macro-groupings made it possible to distinguish the incidence of days of maceration, the use of two different *S. cerevisiae* strains and the use of different cultivars between white and black; the sub-populations of experimental trials allowed the distinction of the use of ML Prime™ strain *vs* spontaneous MLF

in the wines (Fig. 9). This differentiation made it possible to assess the impact of *L. plantarum* under the different experimental conditions.

Comparable mean values between C *vs* MLPrime™ strain in the three different experimental conditions necessitated the application of the t-test. The use of this statistical approach with threshold values > 4 -log(FDR.p) helped to decrease the false positive rate within the VOCs population for the different LAB contributions. Furthermore, default p-values <0.0001 discriminated the most significant VOCs for the experimental blocks C *vs* ML Prime™ strain. The Catarratto trials conducted with *S. cerevisae* CS182 strain were distinguished by the higher number of characterising VOCs compared to those produced with *S. cerevisae* NF213 strain (N. D'Avola and P. Verdot); 14 vs. 4 and 4 respectively (Fig. 10 A-B, C-D, E-F). Violin plot analysis defined that the use of ML Prime™ strain contributed significantly to the production of ethyl acetate and 3-methyl-1-butanol acetate compared to the control (Fig. 10B). These compounds, limited to a concentration of less than 60 mg/L of ethyl acetate (Jackson, 2022) would contribute to increased olfactory complexity and banana sensory attributes in wines (Jackson, 2022; Butkhup et al., 2011). Wines produced with *S. cerevisiae* NF213 strains were characterised by four compounds for both Nero D'Avola and Petit Verdot cultivars; however, results differed between the two experimental trials (Fig. 10 C-D vs. E-F). This result suggests that the cultivar also plays a primary role in LAB interactions. Amino acid components possessed by the cultivar and released into the musts during skin maceration probably contribute to these phenomena. The effect of cultivar was also highlighted by Bootstrap hulls analysis (Fig. 11): the aroma profiles of Petit Verdot wines, delineated as areas of polygons, were found to be superimposable between the control trial and that conducted with MLPrime™. Tuttavia, le prove inoculate con MLPrime™ sono risultate nettamene distinte rispetto le prove controllo. PCA analysis ha evidenziato le correlazioni negative fra le tesi ND-C e ND-MLP; e, CT-C e CT-MLP (Fig. 12).

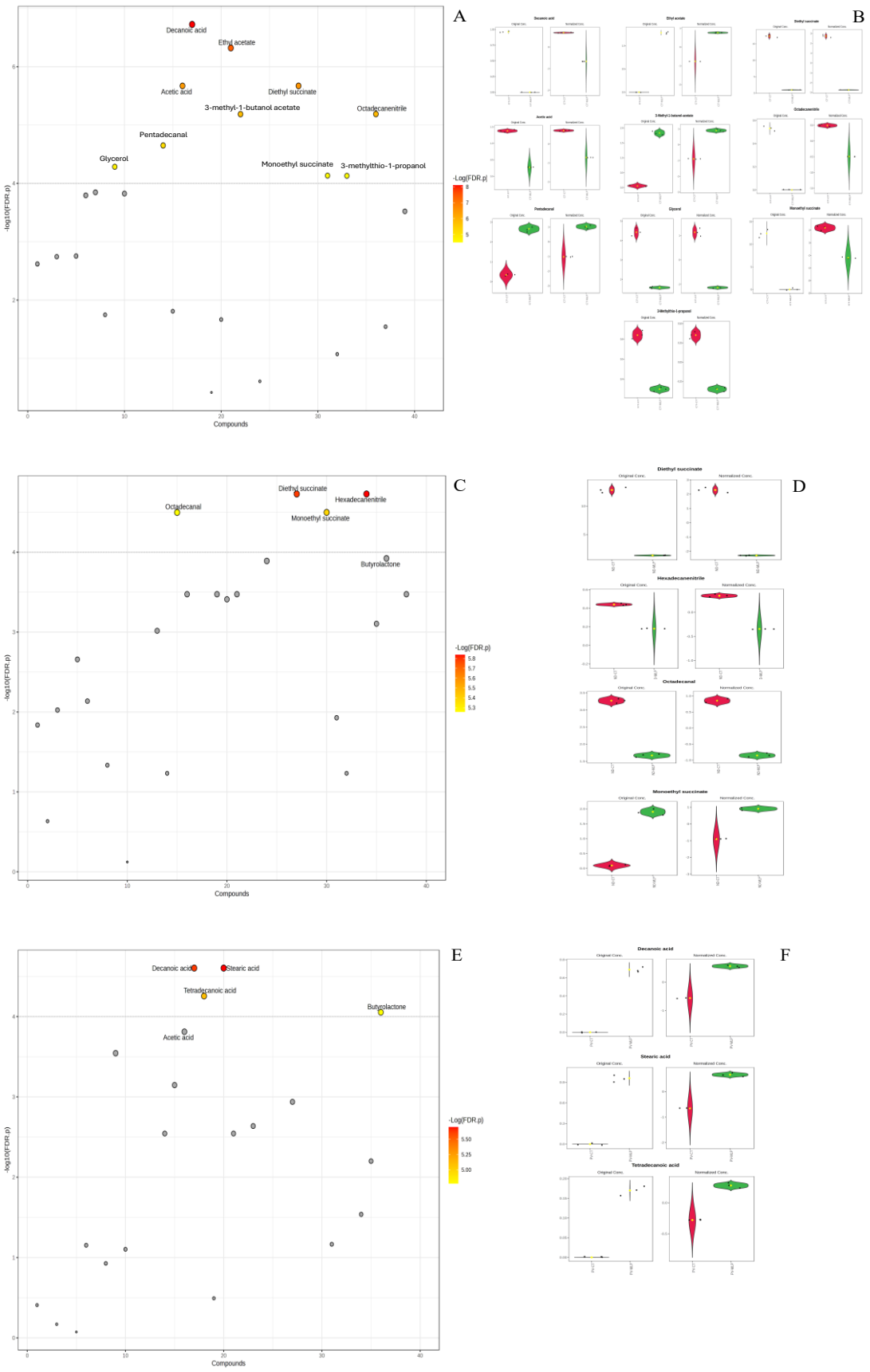


Fig. 10. T-test and violin plot VOCs of Catarratto C *vs* ML Prime^{TM} strain, Nero D' Avola C *vs* ML Prime^{TM} strain, Petit Verdot C *vs* ML Prime^{TM} strain

This phenomenon was explained by 37.86% variance in Nero D'Avola, and 27.26% variance with Catarratto (Fig. 12). Noteworthy are the positive correlations found between the theses that included the use of MLPrime and most of the VOCs with high olfactory relevance (Fig. 12). In particular, phenylethyl alcohol, phenylethyl acetate, ethyl caprate, ethyl acetate, 3-methyl-1-butanol acetate, 3-ethoxy-1-propanol, would significantly contribute to increased rose and floral, fruity and olfactory complexity scents (Butkhup et al., 2011; Jeckson, 2022; Selli et al., 2004; Canas et al., 2008; Bayram & Kayalar, 2018). However, the co-presence among the positively correlated compounds of 3-methyl-1-butanol would contribute to intensifying the floral hints of wines due to the antagonistic effects exerted on the fruity perceptions of the wines (Naselli et al., 2024).

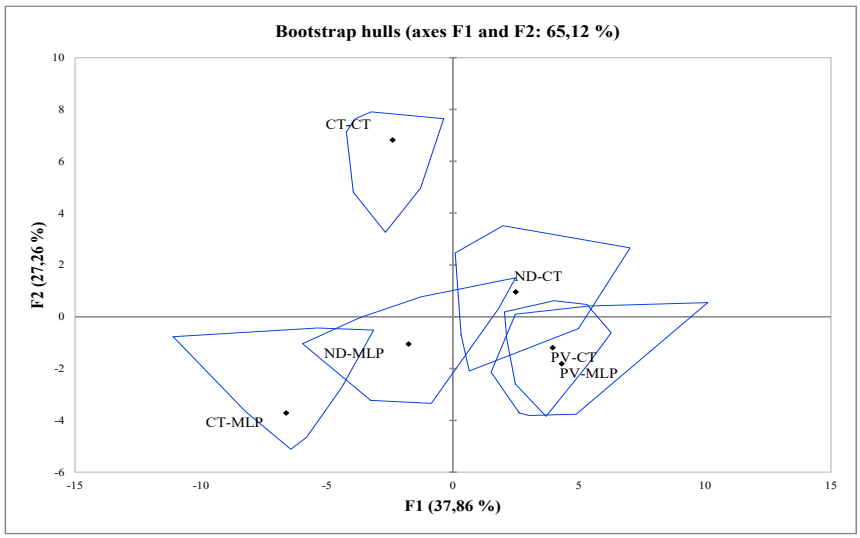


Fig. 11. Bootstrap hulls of VOCs

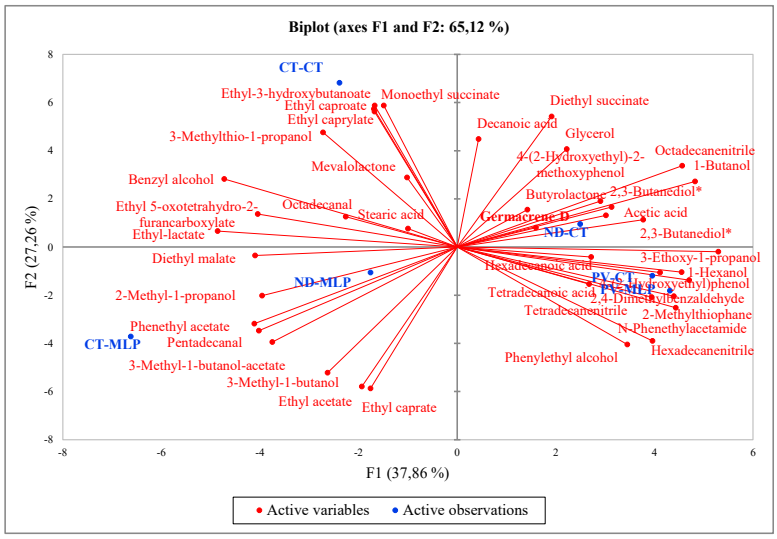


Fig. 12. PCA analysis of VOCs

Table 1. Analysis of volatile organic compounds emitted from experimental wine after 1 month of end alcoholic fermentation.

Compounds[b]		Samples[a]						
		CT-C[d]	CT-MLP[d]	ND-C[d]	ND-MLP[d]	PV-C[d]	PV-MLP[d]	S.S.
Σ Alcohols	**LRI[c]**	**58.83±3.03b**	**72.96±4.42a**	**70.25±3.34ab**	**78.32±5.53a**	**78.19±4.52a**	**72.08±4.90a**	***
2-Methyl-1-propanol	1075	2.94±0.11c	4.16±0.26a	3.60±0.19b	4.11±0.06ab	2.50±0.05c	2.69±0.31c	***
1-Butanol	1150	0.12±0.00 ab	0.02±0.00c	0.12±0.00ab	0.07±0.06 bc	0.15±0.00a	0.13±0.00ab	***
3-Methyl-1-butanol	1189	29.43±1.40c	44.39±2.83a	29.23±2.08c	38.56±2.32ab	36.74±2.42b	35.67±2.83bc	***
1-Hexanol	1322	0.11±0.00e	0.00±0.00f	0.35±0.00d	0.43±0.00c	0.53±0.00a	0.45±0.00b	***
2,3-Butanediol*	1515	5.62±0.11b	4.47±0.21c	8.72±0.28a	6.08±0.50b	6.35±0.29b	6.39±0.16b	***
2,3-Butanediol*	1552	1.77±0.10c	0.84±0.00d	3.56±0.06a	2.30±0.38b	2.05±0.12bc	1.81±0.09c	***
Benzyl alcohol	1852	0.52±0.01a	0.42±0.00b	0.07±0.00d	0.23±0.00c	0.03±0.00e	0.07±0.00d	***
Phenylethyl alcohol	1916	12.59±1.07d	16.19±1.05c	18.46±0.71bc	21.36±1.49ab	23.17±1.36a	20.83±1.28ab	***
Glycerol	2304	4.43±0.20a	1.58±0.04d	4.52±0.00a	3.70±0.00b	4.66±0.20a	2.18±0.17c	***
4-(2-Hydroxyethyl)phenol	2976	1.30±0.03ab	0.89±0.03b	1.62±0.02ab	1.48±0.72ab	2.01±0.08a	1.86±0.06a	***
Σ Ethers		**0.17±0.00d**	**0.00±0.00f**	**0.44±0.00b**	**0.11±0.00e**	**0.61±0.01a**	**0.39±0.00c**	***
3-Ethoxy-1-propanol	1374	0.00±0.00e	0.00±0.00e	0.44±0.00a	0.11±0.00d	0.37±0.01b	0.23±0.00c	***
4-(2-Hydroxyethyl)-2-methoxyphenol	2170	0.17±0.00b	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.24±0.00a	0.16±0.00c	***
Σ Aldehydes		**3.08±0.12c**	**5.85±0.26a**	**4.77±0.12b**	**2.86±0.11c**	**2.78±0.12c**	**2.22±0.07d**	***
2,4-Dimethylbenzaldehyde	1715	0.00±0.00c	0.00±0.00c	0.31±0.01a	0.07±0.04b	0.09±0.00b	0.30±0.00a	***
Pentadecanal	2042	0.34±0.00c	2.66±0.13a	1.19±0.04b	1.12±0.02b	0.34±0.01c	0.44±0.02c	***
Octadecanal	2323	2.74±0.12b	3.19±0.13a	3.27±0.07a	1.67±0.05d	2.35±0.11c	1.48±0.05d	***
Σ Carboxylic acids		**5.15±0.20c**	**2.98±0.17d**	**6.33±0.40b**	**2.92±0.09d**	**2.51±0.11d**	**7.61±0.53a**	***
Acetic acid	1454	1.38±0.03b	0.00±0.00c	2.87±0.21a	1.11±0.05b	0.09±0.00c	3.21±0.27a	***
Decanoic acid	2283	0.96±0.01a	0.00±0.00d	0.08±0.00c	0.10±0.00c	0.00±0.00d	0.69±0.03b	***
Tetradecanoic acid	2694	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.17±0.00a	***
Hexadecanoic acid	2900	2.06±0.15b	2.18±0.15b	2.43±0.13ab	1.22±0.04c	2.42±0.11 ab	2.58±0.19a	***
Stearic acid	3123	0.75±0.01b	0.80±0.02b	0.95±0.06a	0.49±0.00c	0.00±0.00d	0.96±0.04a	***
Σ Esters		**42.00±2.45a**	**16.11±1.16b**	**16.77±0.58b**	**12.37±0.57c**	**11.07±0.51c**	**17.28±1.01b**	***
Ethyl acetate	890	0.00±0.00f	1.28±0.02a	0.38±0.00e	0.63±0.03c	0.48±0.00d	0.80±0.07b	***
3-Methyl-1-butanol-acetate	1125	0.06±0.00d	1.83±0.07a	0.14±0.00c	0.27±0.00b	0.29±0.00b	0.32±0.00b	***
Ethyl caproate	1236	0.44±0.00a	0.11±0.00b	0.08±0.00	0.04±0.00d	0.08±0.00c	0.08±0.00c	***
Ethyl-lactate	1310	10.10±1.03a	11.27±1.03a	2.91±0.13d	7.77±0.39b	3.73±0.20d	5.96±0.40c	***
Ethyl caprylate	1425	0.79±0.00a	0.09±0.00b	0.04±0.00e	0.04±0.00e	0.06±0.00c	0.05±0.00d	***
Ethyl-3-hydroxybutanoate	1519	0.57±0.00a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	***
Ethyl caprate	1642	0.02±0.00f	0.13±0.00a	0.04±0.00e	0.10±0.00b	0.08±0.00d	0.09±0.00c	***
Diethyl succinate	1694	17.38±0.42a	0.94±0.04e	12.82±0.45b	1.34±0.06e	6.15±0.31d	9.98±0.54c	***
Phenethyl acetate	1778	0.00±0.00b	0.10±0.00a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	***
Diethyl malate	2053	0.22±0.00d	0.36±0.00a	0.26±0.00c	0.28±0.00b	0.20±0.00e	0.00±0.00f	***
Monoethyl succinate	2368	12.42±1.00a	0.00±0.00c	0.10±0.00b	1.90±0.09a	0.00±0.00c	0.00±0.00c	***
Σ Sulfur compounds		**2.94±0.06cd**	**2.78±0.08d**	**3.49±0.12bc**	**2.96±0.17cd**	**4.90±0.27a**	**3.75±0.52b**	***
2-Methylthiophane	1116	2.32±0.04d	2.43±0.07cd	3.02±0.11bc	2.57±0.12cd	4.65±0.27a	3.68±0.52b	***
3-Methylthio-1-propanol	1677	0.62±0.02a	0.35±0.01c	0.47±0.01b	0.39±0.05c	0.25±0.00d	0.07±0.00e	***
Σ Nitriles		**0.53±0.02d**	**0.25±0.00e**	**0.94±0.03c**	**0.54±0.01d**	**1.01±0.02b**	**1.14±0.03a**	***
Tetradecanenitrile	2614	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.05±0.00b	0.08±0.00a	***
Hexadecanenitrile	2919	0.00±0.00e	0.25±0.00c	0.44±0.01b	0.18±0.00d	0.44±0.01b	0.49±0.02a	***
Octadecanenitrile	3094	0.53±0.02b	0.00±0.00d	0.50±0.02b	0.36±0.01c	0.52±0.01b	0.57±0.01a	***
Σ Lactones		**1.93±0.12a**	**1.52±0.04b**	**1.28±0.04c**	**1.57±0.10b**	**1.64±0.05b**	**0.91±0.03d**	***
Butyrolactone	1589	1.12±0.11b	0.89±0.03c	0.92±0.04c	0.40±0.01e	1.53±0.05a	0.71±0.03d	***
Mevalolactone	1765	0.07±0.00b	0.00±0.00d	0.07±0.00b	0.12±0.00a	0.00±0.00d	0.03±0.00c	***
Ethyl 5-oxotetrahydro-2-furancarboxylate	2191	0.74±0.01b	0.63±0.01c	0.29±0.00d	1.05±0.09a	0.11±0.00e	0.17±0.00e	***
Amide		**0.00±0.00e**	**0.00±0.00e**	**0.05±0.00d**	**0.08±0.00c**	**0.12±0.00b**	**0.18±0.00a**	***
N-Phenethylacetamide	2575	0.00±0.00e	0.00±0.00e	0.05±0.00d	0.08±0.00c	0.12±0.00b	0.18±0.00a	***
Sesquiterpenoid		**0.00±0.00b**	**0.00±0.00b**	**0.05±0.00a**	**0.00±0.00b**	**0.00±0.00b**	**0.00±0.00b**	***
Germacrene D	1712	0.00±0.00b	0.00±0.00b	0.05±0.00a	0.00±0.00b	0.00±0.00b	0.00±0.00b	***

[a] Results indicate mean value ± standard deviation of two determinations from three replicates; [b] compounds are classified in order of their retention time on Supelcowax polar colum; [c] linear retention indeces obtained through the modulated chromatogram reported for Supelcowax polar column; [d] relative amounts expressed as relative peak areas (peak area of each compound/total area) × l00.

3.6. Sensory analysis

The sensory analysis was conducted in two steps: sensory acceptance test based on the response of wine consumers; and sensory quantitative descriptive analysis of wines based on the response of well-trained panelists. Furthermore, with the aim of better identifying the effect of the driven MLF, data from the sensory analyses were subjected to multivariate analysis by crossing the data obtained from the control trials (spontaneous MLF) and the trials where *L. plantarum* ML Prime^{TM} strain was added as malolactic starter.

3.6.1. Sensory acceptance test

Wine consumers gave the highest scores for Catarratto, Nero D'Avola and Petit Verdot wines produced with the addition of ML Prime^{TM} strain (Fig. 13).

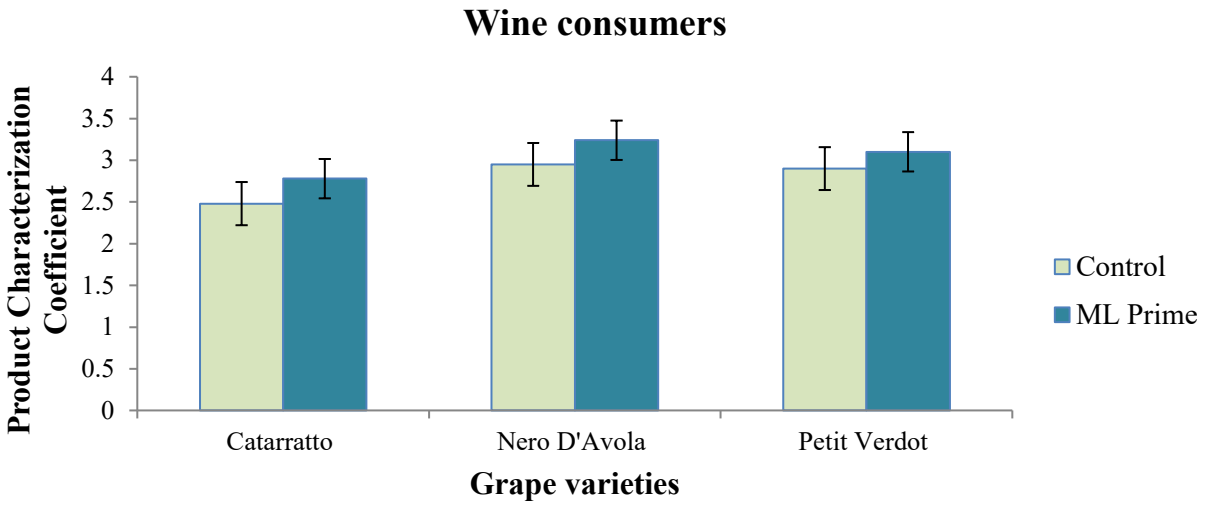


Fig.13. Sensory analysis by wine consumers based on product characterization for overall acceptability of experimental wines.

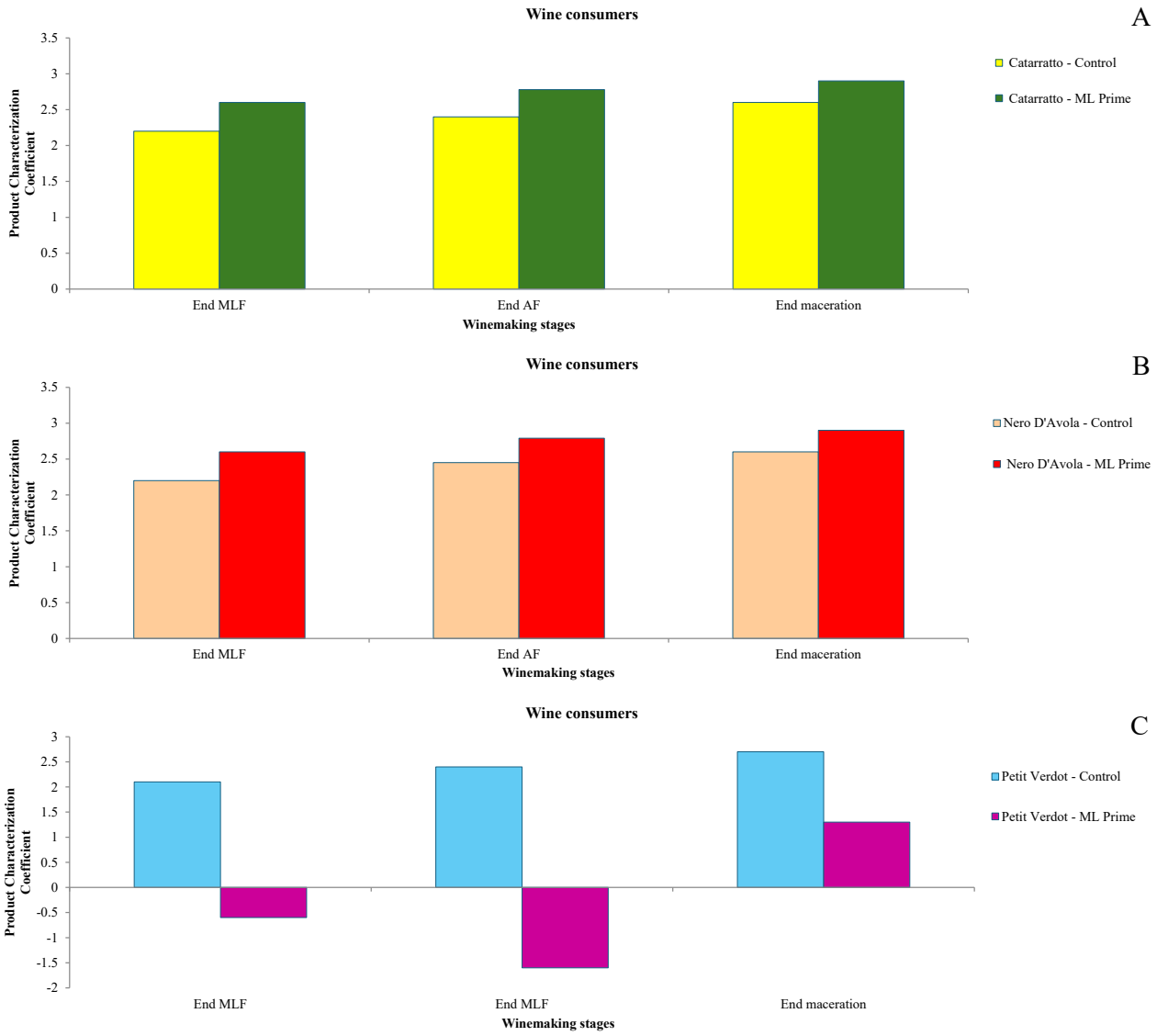


Fig.14. Sensory analysis by wine consumers-based product characterization for overall acceptability of experimental wines, (A) Catarratto, (B) Nero D'Avola and (C) Petit Verdot.

With regard to the Catarratto test, the highest scores were recorded for CT-MLP (Fig. 14A). In particular, they were obtained during stainless steel ageing with values between 3.1 and 3.14. A similar trend was observed for the Nero D'Avola tests, with values between 3.1 and 3.12 in the ND-PLM test (Fig. 14B). In all cases, the ND-PLM trials achieved higher scores than the control trials. A different behaviour was recorded for Petit Verdot when the L. plantarum ML PrimeTM strain was added (Fig. 14C). The attributes olive/vegetal (7.8), muddy (7.1) and rancid (5.1) were perceived at the end of MLF. These defects were strongly perceived after AF. However, no perceptions attributable to olive/vegetable off-flavour, sludge and rancidity were more strongly perceived by the

judges at the end of maceration. This phenomenon could be traced back to adsorption effects carried out by the skin grape or the lies themselves.

3.6.2. Sensory quantitative descriptive analysis

The sensory profiles of all experimental wines are reported in Fig. 15.

	Catarratto			Nero D'Avola			Petit Verdot		
	Control	ML PrimeTM		Control	ML PrimeTM		Control	ML PrimeTM	
Appearance			**Appearance**			**Appearance**			**Appearance**
Yellow color	8.23	8.44	Yellow color	Red color	7.87	7.71	Red color	Red color	7.31	7.25	Red color
Odor			**Odor**	**Odor**			**Odor**	**Odor**			**Odor**
Intensity	3.58	5.75	Intensity	Intensity	7.68	8.10	Intensity	Intensity	6.87	6.91	Intensity
Persistency	3.42	5.62	Persistency	Persistency	7.07	7.61	Persistency	Persistency	6.59	6.71	Persistency
Floral	5.25	5.39	Floral	Floral	3.15	3.18	Floral	Floral	6.12	5.25	Floral
Fruity	3.10	4.17	Fruity	Blackcurrant	2.21	1.54	Blackcurrant	Blackberry	6.55	6.61	Blackberry
Peach	1.48	3.11	Peach	Blackberry	5.17	4.11	Blackberry	Plum	3.87	3.54	Plum
Apricot	3.21	4.33	Apricot	Cherry	6.52	5.74	Cherry	Mint	3.19	5.66	Mint
Citrus fruits	5.26	5.98	Citrus fruits	Plum	2.10	2.01	Plum	Violet	5.48	6.71	Violet
Tropical fruits	1.30	1.40	Tropical fruits	Blueberry	3.12	3.19	Blueberry	Lilac	5.40	6.31	Lilac
Pineapple	2.10	2.00	Pineapple	Carob	4.21	6.71	Carob	Sage	4.56	5.39	Sage
Flinty	2.74	2.63	Flinty	Eucalyptus	4.12	5.22	Eucalyptus	Off-odors	1.00	1.00	Off-odors
Spicy	1.48	1.65	Spicy	Mint	3.12	5.84	Mint	**Taste**			**Taste**
Jasmin	6.67	6.81	Jasmin	Violet	4.10	6.12	Violet	Sweet	2.83	3.54	Sweet
Mint	3.78	5.36	Mint	Off-odors	1.00	1.00	Off-odors	Sour	6.21	5.91	Sour
Cashew	1.89	2.21	Cashew	**Taste**			**Taste**	Salty	4.12	4.87	Salty
Off-odors	1.00	1.00	Off-odors	Sweet	3.12	3.45	Sweet	Astringency	4.38	3.91	Astringency
Taste			**Taste**	Sour	6.77	6.50	Sour	Bitter	3.70	3.21	Bitter
Sweet	1.80	2.58	Sweet	Salty	4.71	5.10	Salty	Off-taste	1.00	1.00	Off-taste
Sour	7.12	6.11	Sour	Astringency	5.10	4.41	Astringency	**Mouth-feel**			**Mouth-feel**
Salty	2.64	2.97	Salty	Bitter	3.74	3.18	Bitter	Acidity	5.71	5.52	Acidity
Astringency	4.14	2.35	Astringency	Off-taste	1.00	1.00	Off-taste	Body	7.12	7.45	Body
Bitter	3.10	2.65	Bitter	**Mouth-feel**			**Mouth-feel**	Balance	6.98	7.32	Balance
Off-taste	1.00	1.00	Off-taste	Acidity	6.10	5.74	Acidity	Velvety	4.11	6.42	Velvety
Mouth-feel			**Mouth-feel**	Body	7.41	8.01	Body	**Flavour**			**Flavour**
Acidity	7.66	6.62	Acidity	Balance	7.10	7.77	Balance	Intensity	6.81	6.77	Intensity
Body	7.25	8.12	Body	Velvety	5.09	6.22	Velvety	Persistency	7.10	7.62	Persistency
Balance	6.87	7.78	Balance	**Flavour**			**Flavour**	Red berriers	6.80	6.61	Red berriers
Velvety	3.41	5.10	Velvety	Intensity	7.55	8.10	Intensity	Fruity	6.80	6.85	Fruity
Flavour			**Flavour**	Persistency	7.77	8.54	Persistency	Floreal	3.65	2.84	Floreal
Intensity	7.45	8.21	Intensity	Red berriers	6.74	6.52	Red berriers	Vegetal	2.21	4.04	Vegetal
Persistency	7.71	8.37	Persistency	Fruity	7.41	7.38	Fruity	Spices	3.45	4.12	Spices
Floral	6.12	6.98	Floral	Floreal	3.71	4.40	Floreal	Off-flavour	1.00	1.00	Off-flavour
Fruity	6.71	7.15	Fruity	Fresh/Green	3.12	5.12	Fresh/Green	**Overall quality**			**Overall quality**
Citrus fruits	4.75	4.68	Citrus fruits	Spices	4.71	5.15	Spices	Odor	6.97	7.21	Odor
Off-flavour	1.00	1.00	Off-flavour	Off-flavour	1.00	1.00	Off-flavour	Taste	7.35	7.40	Taste
Overall quality			**Overall quality**	**Overall quality**			**Overall quality**	Mouth-feel	7.10	7.77	Mouth-feel
Odor	6.27	7.24	Odor	Odor	7.42	8.21	Odor	Flavour	6.77	7.11	Flavour
Taste	7.22	7.77	Taste	Taste	7.70	8.61	Taste	**Finish**			**Finish**
Mouth-feel	7.12	8.20	Mouth-feel	Mouth-feel	7.30	8.10	Mouth-feel	After-smell	7.10	7.64	After-smell
Flavour	7.07	7.87	Flavour	Flavour	7.25	7.82	Flavour	After-taste	7.65	8.21	After-taste
Finish			**Finish**	**Finish**			**Finish**				
After-smell	6.71	6.98	After-smell	After-smell	7.51	7.98	After-smell				
After-taste	7.14	7.84	After-taste	After-taste	7.66	8.21	After-taste				

Fig. 15. Sensory analysis based on quantitative descriptive method by trained panelists.

Regarding Catarratto wines the highest values were recorded for the trial CT-MLP. In particular, scores higher than 8 were recorded for the following descriptors: yellow colour, pesistency and intensity (Flavour), mouth-feel (over quality) and body (mouth-feel). In CT-Control trial, in addition to yellow colour (8.23), the highest values of the descriptors were also registered for acidity (mouth-feel), taste (over quality), and body (mouth-feel).

In case of Nero D'avola trial, the highest values were recorded when *L. plantarum* ML Prime^{TM} strain was added. In particular, scores higher than 8 were found for over quality (taste, odor, mouth-feel), flavour (intensity and persistency), final (after taste) and mouth-feel (body).

Also for Petit Verdot wine when *L. plantarum* ML Prime^{TM} strain was added higher values were registered for the majority of descriptors, including overall quality (flavour, odor, taste and mouth-feel), finish (after-smell and after-taste), appearance (red colour), mouth-feel (balance and body) and flavour (persistence).

3.6.3. Statistical multivariate analysis of sensory scores

The statistical multivariate investigation sensory quantitative data is described in Fig. 16.

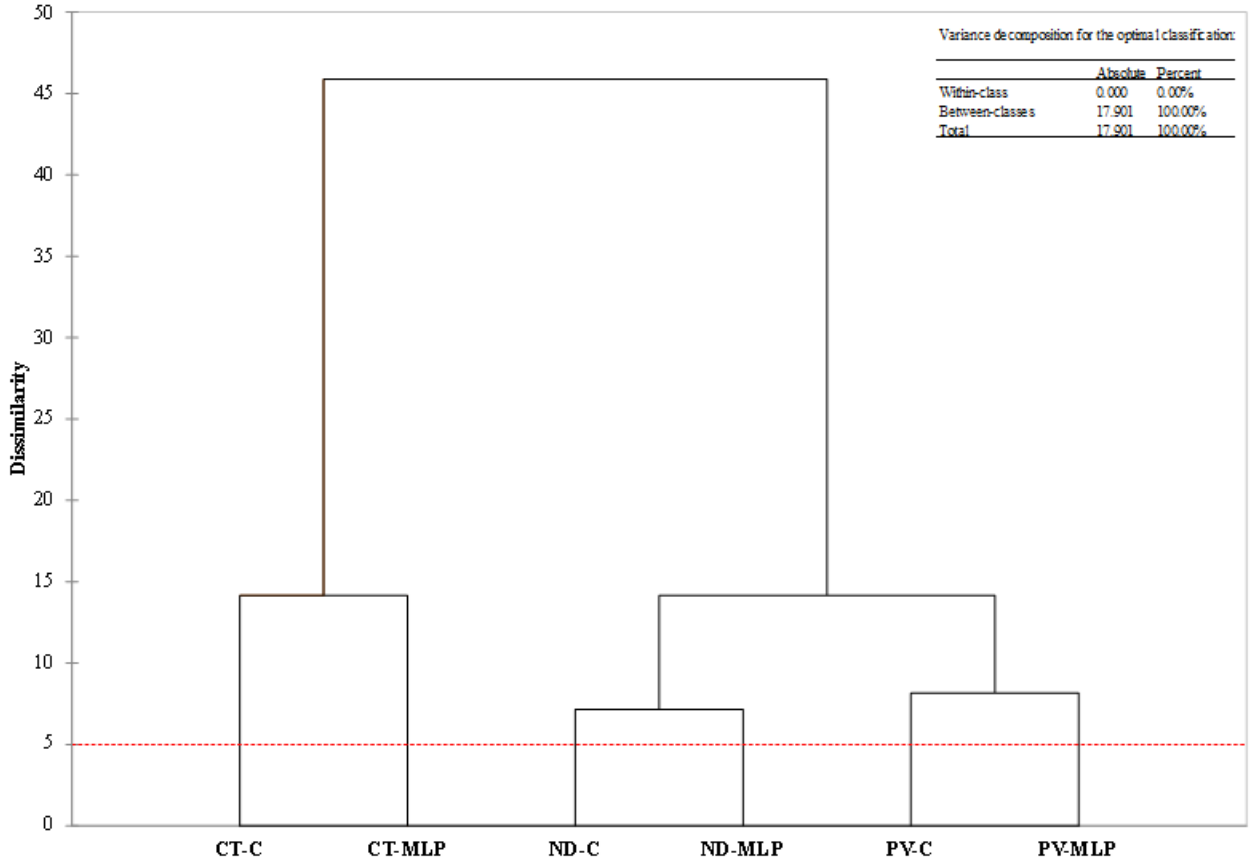


Fig. 16. Dendrogram resulting from HCA based on values of sensory scores from quantitative descriptive method by trained panelists. The dissimilarity among samples was measured by Euclidean distance, whereas cluster aggregation was achieved by Ward's method.

Due to the high numbers of sensory attributes and trials, HCA was applied to identified "who is different form whom" or rather the significant differences among trials. For the elaboration of HCA, the common descriptors of quantitative sensory analysis for all macerated wines (white and red) were considered. HCA analysis allowed the construction of a dendrogram based on 25 variables

distinguishing red and white macerates into two macro-groups. In addition, for each grape variety, a difference was observed between control trial and the trial inoculated with ML Prime^{TM} strain. In Catarratto, the variables that mostly determined a difference between Catarratto-Control and Catarratto-ML Prime^{TM} strain were body (mouth-feel), intensity and persistence (flavour) and mouth-fell (overall quality), while for Nero d'Avola intensity (odour), body (mouth-feel), intensity and persistence (flavour), odor, taste and mouth-feel (overall quality) and for Petit Verdot after-taste (finish), mouth-feel (overall quality) and persistency (flavour).

PCA analysis based on the values of the descriptors is represented in Fig. 17 through biplot graph.

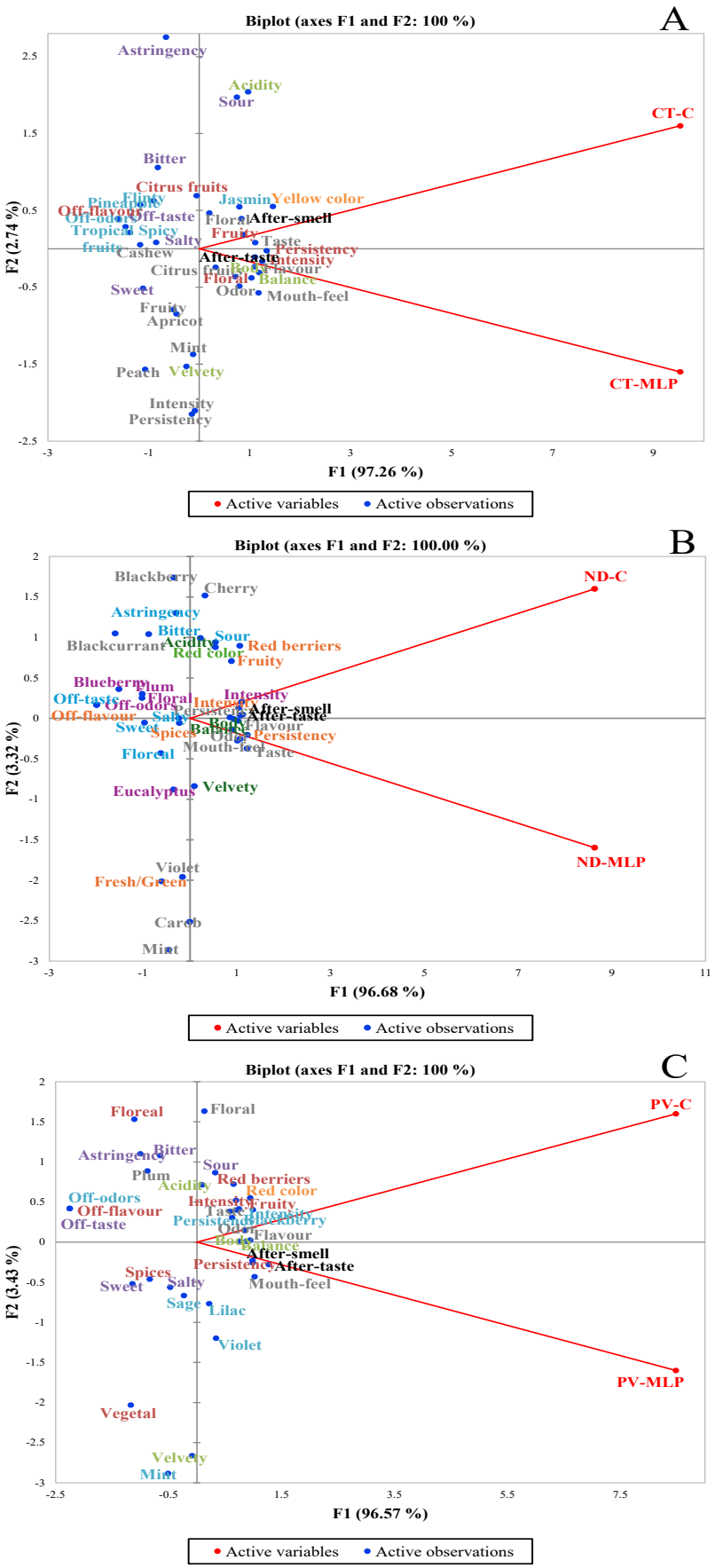


Fig. 17. PCA based on sensory data of experimental wines: (A) Catarratto, (B) Nero D'Avola, (C) Petit Verdot. Biplot graphs show relationships among factors, variables and trials.

For all three grapevarieties the F1 factor explained over 90% of the total variability. A different correlation of variables was observed for Catarratto wine (Fig. 17A) in relation to the addition of ML starter. Catarratto-Control trial was related to yellow colour (appearance), jasmin (odor), sour (taste), acidity (mouth-feel), fruity (flavour), taste and floral (overall quality) and after-smell (finish), while Catarratto-ML Prime™ ™ strain was connected to body and balance (mouth-feel), floral, intensity and persistence (flavour), odor and mouth-feel (overall quality) and after-taste (finish).

A similar trend was observed for Nero D'Avola wine (Fig. 17B). In this case the variables closely related to the control trial were red colour (appearance), intensity (odor), sour (taste), fruity, intensity and red berriers (flavour), cherry and persistency (overall quality), after-smell and after taste (finish). Regarding the trial inoculated with ML Prime™ ™ strain, the most related variables were body, balance and velvety (mouth-feel), persistency (flavour), flavour, mouth-feel, odor and taste (overall quality).

In Petit Verdot wine (Fig. 17C), 15 variables were related to the experimental control wine: red color (appearance); blackberry, intensity and persistency (odor); sour (taste); body and balance (mouth-feel); fruity, intensity and red berries (flavour); floral, odor, flavour and taste (overall quality). For the started trial, after-smell and after-taste (finish), persistency (flavour), mouth-feel (overall quality), lilac and violet (odour) were the main variables related to the final wine.

In conclusion, the wines obtained with the ML starter were extremely different when compared to the respective controls (spontaneous MLF). The differences obtained are related to different descriptors regarding appearance, odor, taste, mouth-feel, flavour, overall quality and finish.

4. Conclusion

The use of *L. plantarum* ML Prime^{TM} strain on Catarratto, Nero D'Avola and Petit Verdot wines was evaluated. Large-scale application made it possible to ascertain the technological reliability of the LAB ML Prime^{TM} strain under industrial conditions. The best performance recorded for low acetic acid values and short malic acid degradation times confirmed the full validity of the use of ML Prime in the different varieties tested.

The impact of the use of ML Prime^{TM} was also significant in the characterisation of the aromatic buffer of the wines obtained.

The use of ML Prime^{TM} in co-inoculation characterised the wines with more pronounced floral perceptions compared to the relative controls conducted with spontaneous fermentation. This peculiarity was achieved through the production of VOCs both associated with floral sensory attributes and suppressing the fruity component.

The investigation also clarified the existence of relationships between cultivar and LAB in the expression of wine aromas.

However, such relationships were only highlighted for the cultivar Petit Verdot. Further investigation through characterisation of the exometabolome will be necessary to clarify the relationships between cultivar and LAB.

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Activity 2.3

Use of *Lactiplantibacillus plantarum* and *Lachancea thermotolerans* strains for modulating Syrah wine acidity and aroma

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Abstract

Wine is composed of many compounds that may influence sensory components. The number and nature of these bio-molecules depend closely on biotechnology and its use. To date, the correct use of biotechnology is the least environmentally impactful way to preserve wine quality from the effects of global warming. This research has identified of *Lachancea thermotolerans* A and B strains, with full 48-hour dominance, as the best trade-offs for acidification and improved wine flavour profiles. Further studies on the characterisation of the exometabolome in wines will clarify the impact of the interactions between *L. thermotolerans* - *Saccharomyces cerevisiae* - *Lactiplantibacillus plantarum* in the aromatic and technological results obtained.

Keywords: Global warming, bio-acidification, eco-sostenibility, wine aroma, *Lachancea thermotolerans*

1. Introduction

Wine contains a multitude of compounds that can influence sensory properties. The quantity and quality of these components are closely dependent on vinification techniques and the biotechnology used in fermentation processes. Recent studies report the influence of the use of non-*Saccharomyces* and their inoculation methods in association with *Saccharomyces* spp. as a means of influencing the perceived quality of wines. Modulation of colour, aromas and taste are therefore the main goals to be achieved for greater consumer acceptance of wines.

Lachancea is a ubiquitous genus associated with a number of ecological niches worldwide. Today, its main use in the oenological sector derives from the main effects that result from its metabolism. For example, the studies that derive from the bio-acidifying impact of *Lachancea thermotolerance* (*L. thermotolerance*) are constantly evolving. However, many of these studies are focusing on the impact that the single non-*Saccharomyces* can bring to the improvement of a single sensory variable (body acidic, or olfactory), whereby the metabolic collaterality influencing the different sensory components is omitted. Therefore, the aim of this research will be to investigate on a broad spectrum the effects of the use of non-*Saccharomyces*, employed through multiple microbial consortia, on the chromatic, volatile and non-volatile components of wine.

2. Material and Methods

2.1 Experimental design and winemaking process

The tests represented in the experimental plan shown in Fig.1, aimed to evaluate the acidifying capacity, through the production of lactic acid, of four different strains of *L. thermotolerans.*

The trials involved setting up nine different tests, including four controls, described and named as follows:

1) LT - 1, inoculation strain *L. thermotolerans*-A 25 g/hl co-inoculated simultaneously at 48 hours with *Saccharomyces cerevisiae* (*S. cerevisiae*) 25 g/hl (Lalvin Persy), Stimula Syrah and ML Prime with the dose of 10 g/hl.

2) LT - 2, inoculation strain *L. thermotolerans*-B 55 g/hl co-inoculated simultaneously with 48 hours of *S. cerevisiae* 25 g/hl (Lalvin Persy), Stimula Syrah and ML Prime with the dose of 10 g/hl.

3) LT - 3, inoculation strain *L. thermotolerans*-C 30 g/hl co-inoculated simultaneously with 48 hours of *S. cerevisiae* 25 g/hl (Lalvin Persy), Stimula Syrah and ML Prime with the dose of 10 g/hl.

4) LT - 4, inoculation *strain L. thermotolerans*-D 20 g/hl co-inoculated simultaneously with 48 hours of *S. cerevisiae* 25 g/hl (Lalvin Persy), Stimula Syrah and ML Prime with the dose of 10 g/hl.

5) LT - 5, inoculation strain *L. thermotolerans*-A 25 g/hl co-inoculated simultaneously at 72 hours of *S. cerevisiae* 25 g/hl (Lalvin Persy), Stimula Syrah and ML Prime with the dose of 10 g/hl.

6) LT - 6, inoculated with *S. cerevisiae* 25 g/hl (Lalvin Persy) and Stimula Syrah, co-inoculated at 24 h by ML Prime with the dose of 10 g/hl

7) LT - 7, acidified with tartaric acid 150 g/hl and inoculated with *S. cerevisiae* 25 g/hl (Lalvin Persy) and Stimula Syrah, co-inoculated at 24 h by ML Prime with the dose of 10 g/hl.

8) Cont-A, simultaneous inoculation of *S. cerevisiae* 25 g/hl (Lalvin Persy), Stimula Syrah and ML Prime with the dose of 10 g/hl.

9) Cont-B, simultaneous inoculation of *S. cerevisiae* 25 g/hl (Lalvin Persy), Stimula Syrah, 2 g/hl Metabisulfite and Lysozyme at a dose of 20 g/hl.

Grapes, Syrah, which generated the fermenting matrix were characterized by a sugar level of 284 g/L with a glucose - fructose ratio in favor of the latter.

The acidity level was characterized by a pH of 3.9, total acidity of 5.5 g/L (expressed as tartaric acid), malic acid 1.5 g/L.

APA stood at 218 mg/L, mainly represented by the alpha amine component at 145.41 mg/L.

Grapes being destemmed saw the addition of 6 g/qle of metabisulphite, attesting the value of total sulphur dioxide at 30 mg/L.

The nutritional plan included the addition of 30 g/hl of Fermeid O shortly after the addition of *L. thermotolerans* strains in trials LT-1, LT-2, LT-3, LT-4, LT-5.

In these same trials at 48 h, for LT-1, LT-2, LT-3, LT-4 and 72 h for LT-5, they received a 40 g/hl dose of Stimula Syrah simultaneously with inoculation with Persy.

While the remaining trials were supplemented with Stimula Syrah (40 g/hl) upon filling the tank simultaneously with Persy.

Afterwards, when all the trials reached about 1/3 of the alcoholic fermentation, 20 g/L were added in concomitance with a pumping over in the air of the fermenting mass.

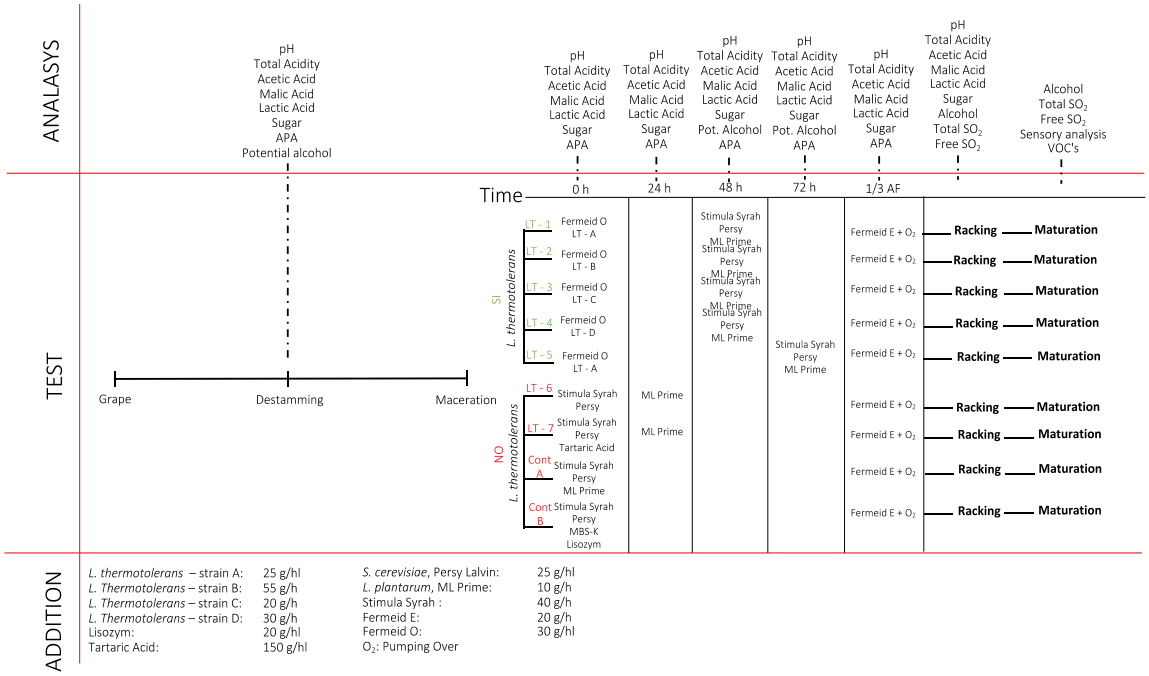


Fig.1 – Experimental plan

2.2. Physico-chemical analysis

The samples were centrifuged at 9000 rpm at 4 °C and then filtered through a polyethersulfone membrane with a pore size of 0.20 μm (VWR®). CO_2 stripping was performed using a vacuum pump to minimize errors during instrumental reading. The pH, total acidity, and ethanol were measured using a FOSS-WineScanTM Flex system (FOSS, Hillerød, Denmark), according to the procedure described in OIV Res. Oeno 390/10 All.2. The values of L-malic, L-lactic, acetic acids, together with reducing sugars, glucose and fructose, and glycerol were measured by means of an iCubio iMagic M9 enzymatic analyser (Shenzhen iCubio Biomedical Technology Co. Ltd. Shenzhen, China), as reported by Matraxia et al. (2021).

2.3. Microbial counts and identification of yeasts and LAB strains

During alcoholic fermentation, plate counts were performed to estimate the levels of total yeasts (Pallmann et al., 2001), which were differentiated into *Saccharomyces* and non-*Saccharomyces* colonies as described by Varela (2016). LAB population was monitored according to the procedure described by Tristezza et al. (2016). To make the CFU/mL data in the must during the fermentation and post-fermentation phases as realistic as possible, the mass was homogenized under sterile conditions.

Yeast isolates were purified and phenotypically grouped as reported by Alfonzo et al. (2020). The selection and molecular identification of yeast isolates at species level (Francesca et al., 2024) and genetic strain characterization (Alfonzo et al., 2021) were also performed. The isolation and genetic characterization of LAB strains were conducted as reported by Solieri et al., 2010. The study of the microbial population, determined by the DNA fingerprinting method, permitted an evaluation of the dominance of all inoculated strains.

2.4. Analysis of VOCs in wine samples

2.4.1. Standard solutions

Standards for each compound were purchased individually from Sigma-Aldrich (82024 Taufkirchen, Germany). 2,3-butanediol was used as standard for the alcohol fraction, acetoin as standard for the carboxyl-function fraction and ethyl lactate as standard for the ester fraction. In addition, n-alkane standards (C8 to C40) were purchased from Aldrich Chemical Co. (St. Louis, Mo., USA). Standard solutions of each compound were prepared at five different concentrations: 2,3-butanediol, 53.25 mg/L, 112.50 mg/L, 225.00 mg/L, 262.00 mg/L, 450. 00 mg/L; acetoin: 24.70 mg/L, 45.70 mg/L, 64.70 mg/L, 115.60 mg/L, 173.30 mg/L, 289.80mg/L; ethyl lactate, 79.00 mg/L, 134.00 mg/L, 224.00 mg/L, 326.00 mg/L, 477.00 mg/L.

2.4.2. Extraction, identification and quantification of VOCs by GC-MS

To determine the volatile compound composition, wine samples (10 mL) from all trials were mixed with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask. The mixture was stirred at room temperature for 30 min and then centrifuged at 4000 rpm for 10 min using a Low Speed Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS Industrivej 6–8, Vassingerød, DK-3540 Lynge, Denmark). The aqueous phase was removed, and anhydrous sodium sulphate (1 g) was added before centrifugation at 4000 rpm for 5 min. The dichloromethane layer was removed and dried under N_2 gas to 0.3 mL.

Gas chromatographic analyses were performed with Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 °C. Solvent Delay: 3.5 min. Helium was the carrier gas (1 mL/min).

The temperature was initially maintained at 40 °C for 1 min. Then it was gradually increased to 250 °C at a rate of 3 °C/min for 30 min and finally maintained at 250 °C at 10 °C/min. One μL of sample was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra. Quantification was carried out using the three calibration lines. For compounds belonging to other classes than the standards, similarity was used for quantification. A dilution factor was used for the reported data.

To determine which VOCs were actively contributing to the wine's aroma profile, the detected concentrations were transformed into odour activity units (OAV) using the method described by Butkhup et al. (2011). The method reported by Butkhup et al. (2011) was also used to calculate the aroma series per individual wine (fruity, floral, fatty, solvent and sulphurous). The sum of the individual odourant active values (OAV) determined for each volatile organic compound per trial defined the olfactory intensity of the test wine. The VOCs with OAV > 0.1 were then grouped and reported in tables for ease of reading and processing (Peng, Wen, Tao, & Lan, 2013).

2.5. *Statistical analysis*

The ANOVA test was applied to determine the significance of the differences between the technological, microbial, VOC, and sensory values of the different tests. In addition, the Tukey's test was used to compare the different data, and values of $P < 0.05$ determined significance. The relationships between VOCs, sensory attributes and biotechnological associations were determined by means of agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) (Naselli et al., 2023).

3. Results and discussion

3.1 Chemical composition

3.1.1 Fermentation trend

Fermentations took place with regularity. The biotechnologies used allowed to carry out all the sugars available to the cultivar.

The endogenous nitrogen framework of the grapes and the nutritional contributions indicated by the Lallemand experimental section, as per protocol, allowed to respect physiologically the metabolic needs of yeasts and bacteria.

Each biotechnology used during the fermentation process was able to express its own metabolism according to its diversity.

Generally speaking, as shown in Fig.2, Fig.3, Fig.4, Fig.5, it could be said that the different strains of *L. thermotolerans* have a slight affinity towards fructose compared to glucose when they alone dominate the first fermentative tract.

The final technological parameters of each fermentation allowed to detect the typical metabolic diversity for each strain.

These diversities were marked in the production of acetic acid and lactic acid.

With reference to acetic acid, as shown in Tab.1, each strain of Lachancea thermotolerans shows a different percentage production of acetic acid depending on the strain and the time of inoculation.

Strains B, C, D, produced more than 50% of total acetic acid; strain A, depending on the different time of inoculation showed a different % impact: 44.8 versus 68.6 respectively going from 48 hours to 72.

The sequencing of inoculation with *S. cerevisiae* Persy was decisive in obtaining limited amounts of acetic acid.

As for the lactic acid produced, the values for most strains of *L. thermotolerans* were around 6 g/L except for strain D, which was able to produce 12.10 g/L of final lactic acid (Fig. 11; Tab. 2).

Each strain showed the highest lactic acid productivity in conjunction with *S. cerevisiae* inoculation, with the exception of strain D where at 48 hours after inoculation it produced only 73.16% of total acetic acid, showing that despite the presence of *S.cerevisiae* it is capable of not stopping metabolically (Tab. 2).

Referring to the values reported in Tab. 2 and the trends depicted in Fig.11b, it is possible to assert that the values of lactic acid produced depend exclusively on the strain and not on the time of inoculation.

In fact, strain A co-inoculated sequentially with *S. cerivisiae* and ML Prime at 48 hours and 72 hours, respectively, showed no significant differences in terms of lactic acid produced.

Contrary to what has just been expressed, however, the time of inoculation appears to be decisive in the inhibition of ML Prime as well as the concentration of lactic acid produced by *L. thermotolerans.*

From the values reported in Tab. 3 it is clear that the dominance of *L. thermotolerans* over *S. cerevisiae* and ML Prime determines a partial inhibitory effect against L. plantarum.

The above is what happened in the LT - 5 trial where S. cerevisiae and ML Prime are inoculated simultaneously at 72 hours (Fig.12a and 12b).

The same inhibitory behavior appears to be carried out by the high concentrations of lactic acid produced by strain D (Fig.13a and 13b).

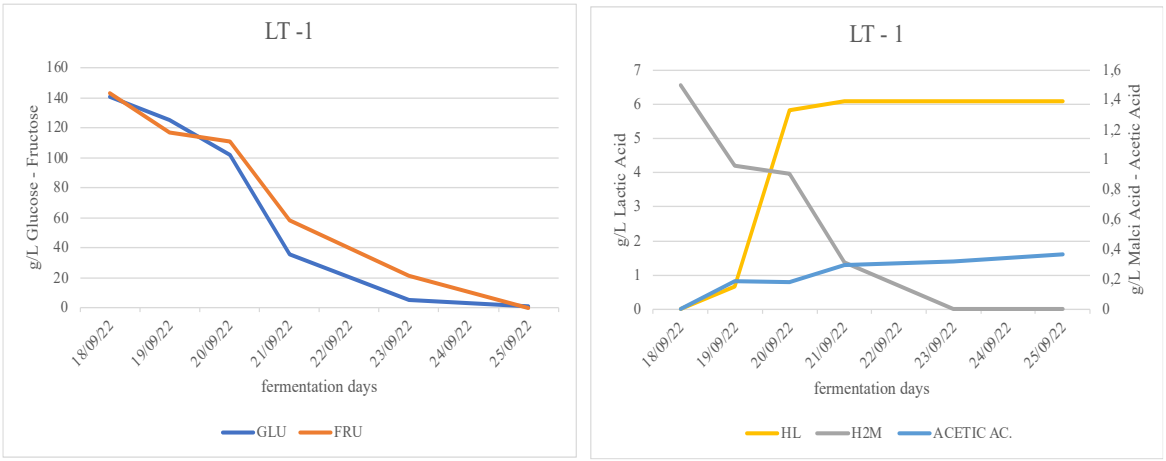


***Fig.2** – Trend of the technological parameters of the LT-1 test*

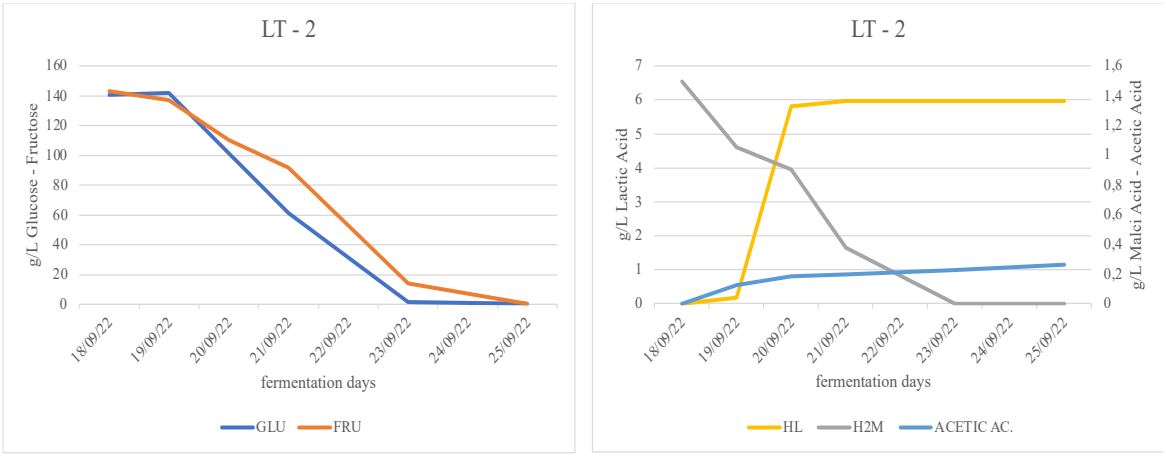


***Fig.3** – Trend of the technological parameters of the LT-2 test*

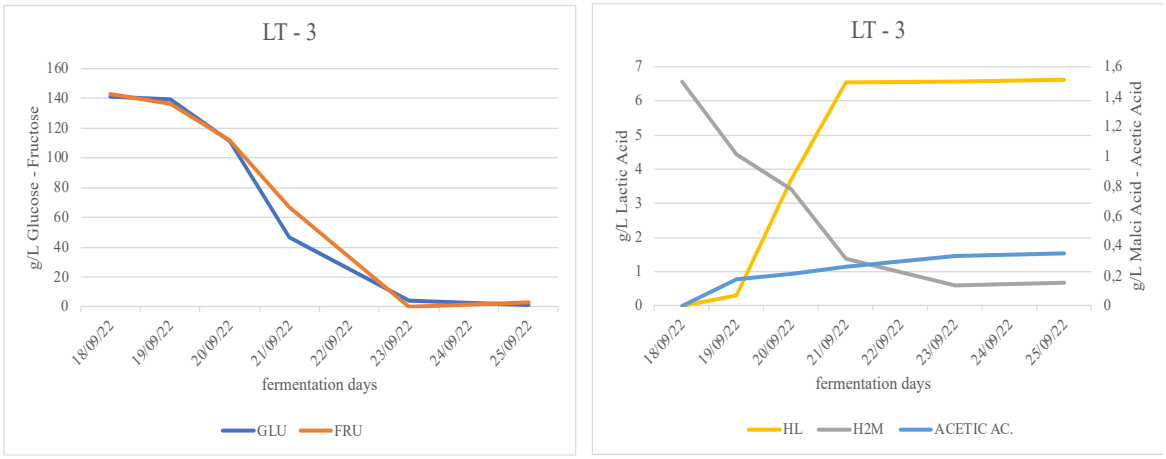


***Fig.4** – Trend of the technological parameters of the LT-3 test*

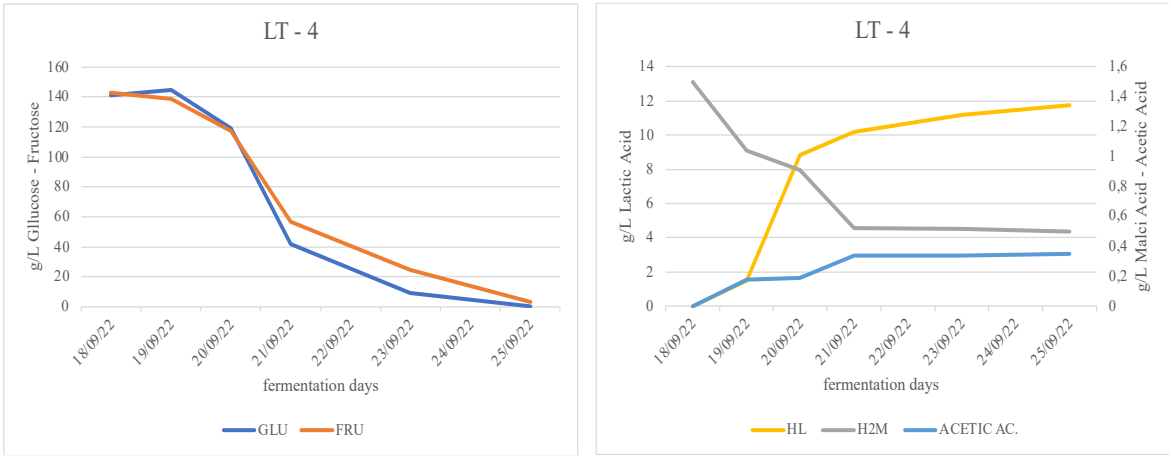


***Fig.5** – Trend of the technological parameters of the LT-4 test*

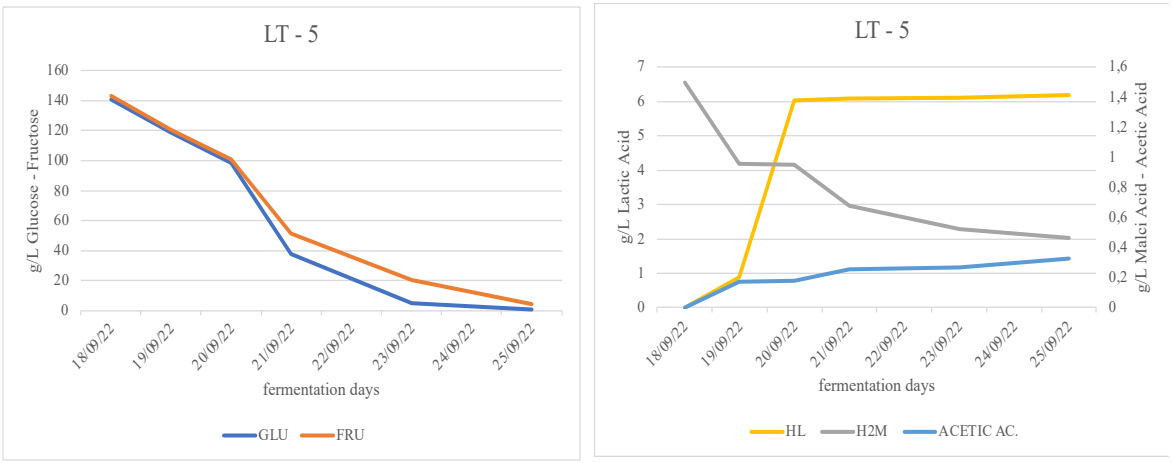


***Fig.6** – Trend of the technological parameters of the LT-5 test*

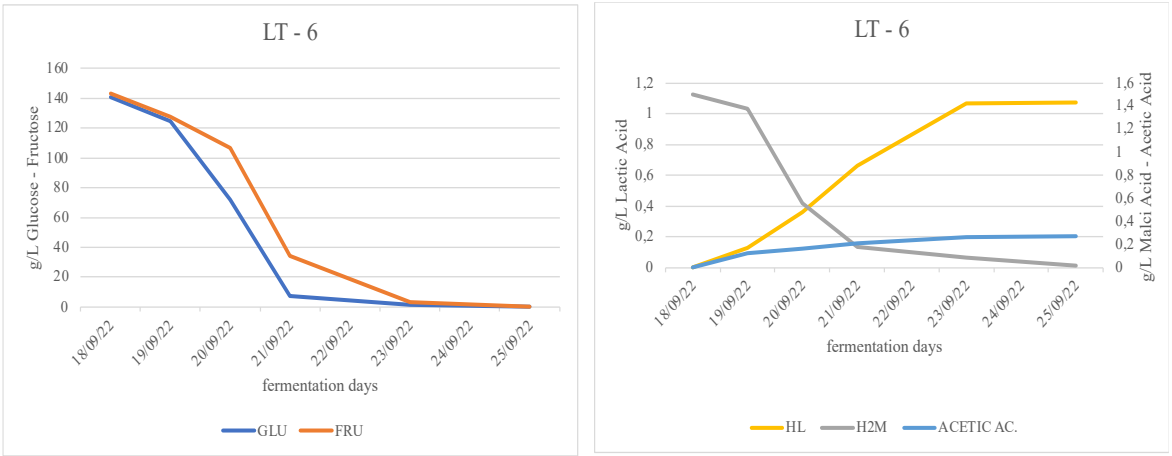


***Fig.7** – Trend of the technological parameters of the LT-6 test*

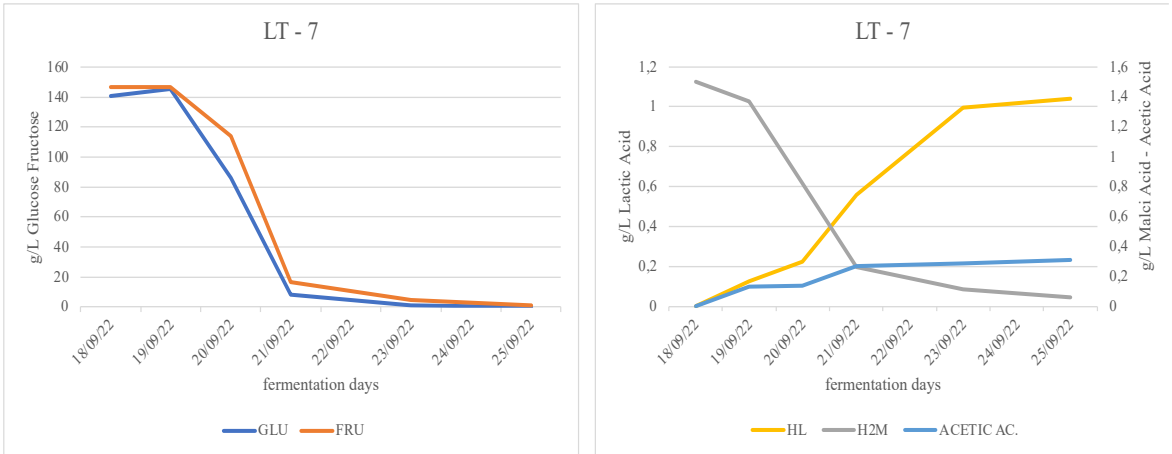
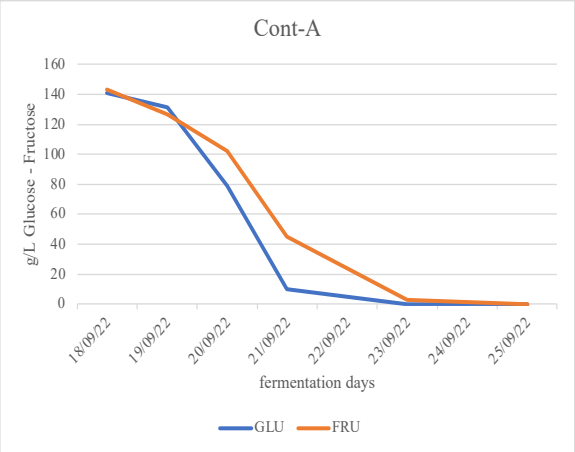


***Fig.8** – Trend of the technological parameters of the LT - 7 test*



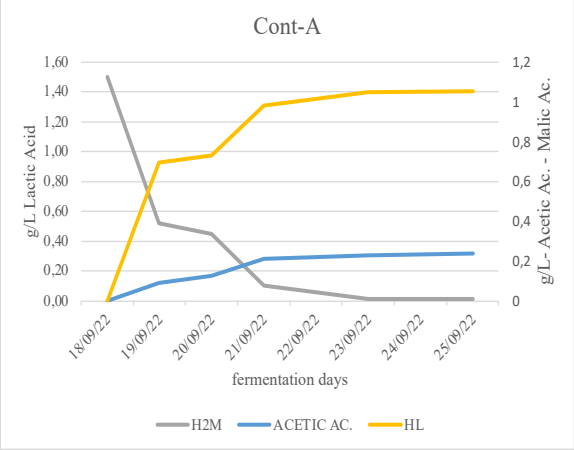
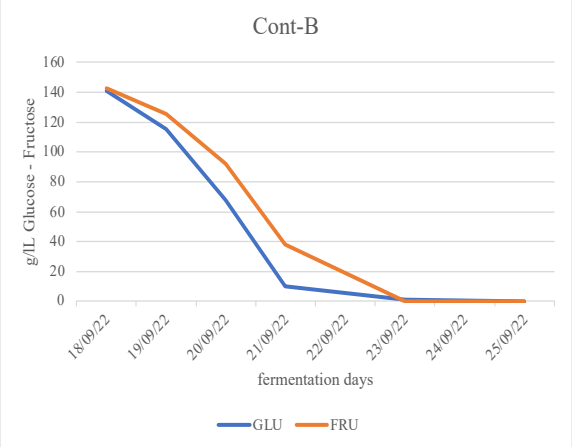


***Fig.9** – Trend of the technological parameters of the Cont-A test*



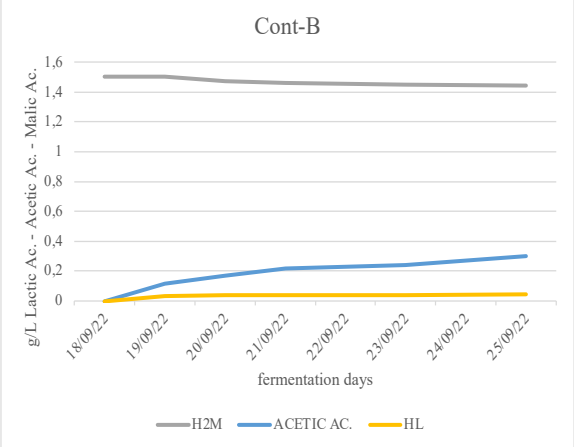


***Fig.10** – Trend of the technological parameters of the Cont - B test*

L. thermotolerans strains		Trials	Acetic Ac. "inoculum *S. cerevisiae*"	Acetic Ac. Final	% Impact
A - 48 h		LT-1	0.18±0.01	0.40±0.01	44.78
B - 48 h		LT-2	0.19±0.01	0.32±0.01	59.37
C - 48 h		LT-3	0.21±0.01	0.36±0.01	58.24
D - 48 h		LT-4	0.19±0.01	0.36±0.01	51.65
A - 72 h		LT-5	0.25±0.01	0.37±0.01	68.55
		LT-6	-	0.30±0.01	
		LT-7	-	0.34±0.01	
		Cont A	-	0.33±0.01	
		Cont B	-	0.33±0.01	

Tab. 1 - Acetic acid values produced by non-*Saccharomyces*

L. thermotolerans strains	Trials	Lactic Ac. "inoculum *S. cerevisiae*"	Lactic Ac. "Final"	% Impact
A - 48 h	LT-1	5.82±0.12	6.10±0.08	95.39
B - 48 h	LT-2	5.82±0.11	5.96±0.14	97.62
C - 48 h	LT-3	3.70±0.13	6.62±0.12	55.94
D - 48 h	LT-4	8.85±0.12	12.10±0.11	73.16
A - 72 h	LT-5	6.04±0.10	6.18±0.10	97.73
	LT-6	-	1.07±0.02	
	LT-7	-	1.04±0.03	
	Cont A	-	1.05±0.02	
	Cont B	-	0.05±0.00	

Tab. 2 - Values of lactic acid produced and impact % at the time of inoculation

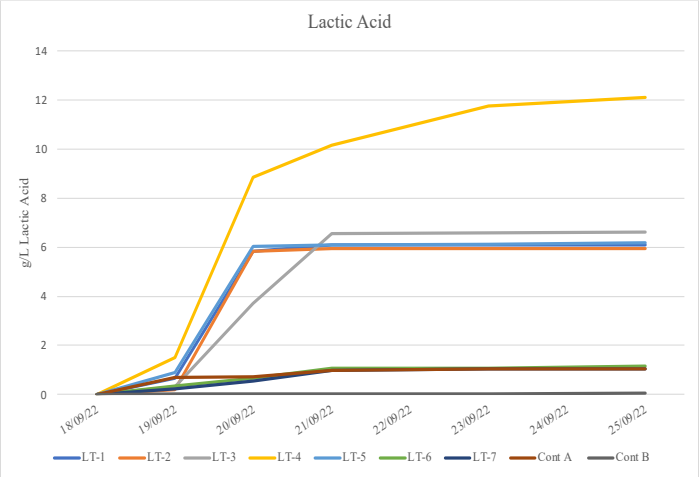


Fig. 11 a – Lactic acid produced in the different trials.

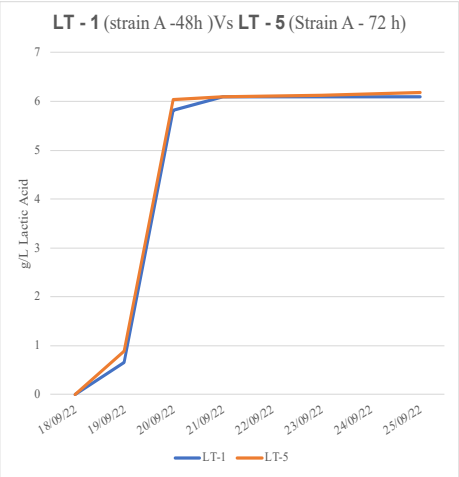


Fig. 11b - Lactic acid produced in the trials LT1 and LT-5

L. thermotolerans Strains	Trials	Lactic Ac. Final	Malic Ac. Final
A - 48 h	LT-1	6.10±0.08	0.08±0.01
B - 48 h	LT-2	5.96±0.14	0.15±0.01
C - 48 h	LT-3	6.62±0.12	0.16±0.01
D - 48 h	LT-4	12.10±0.11	0.50±0.02
A - 72 h	LT-5	6.18±0.10	0.46±0.02
	LT-6	1.07±0.02	0.02±0.00
	LT-7	1.04±0.03	0.06±0.00
	Cont A	1.05±0.02	0.02±0.00
	Cont B	0.05±0.00	1.44±0.03

Tab. 3 – Malic and lactic acid values

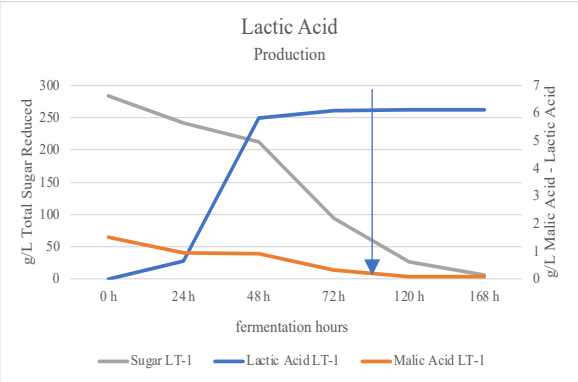


Fig. 12a - Sugar, malic acid and lactic acid values

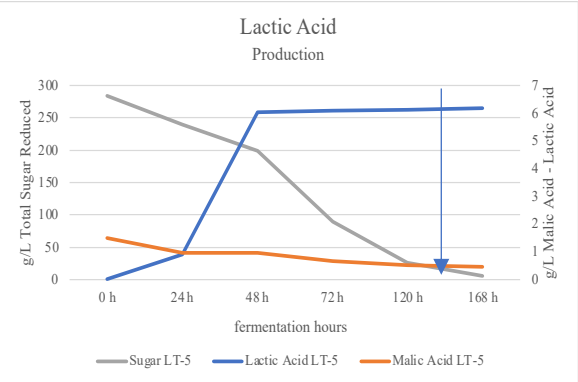


Fig. 12 b – Sugar, malic acid and lactic acid values

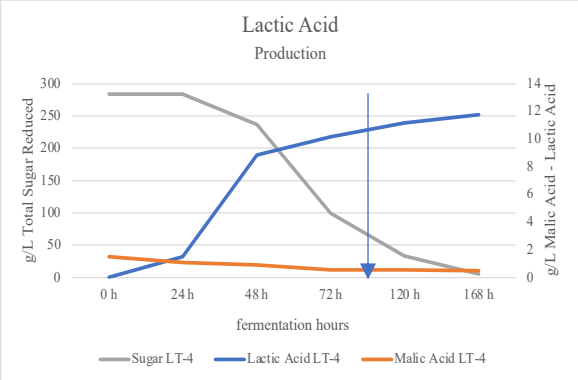


Fig. 13a - Sugar, malic acid and lactic acid values

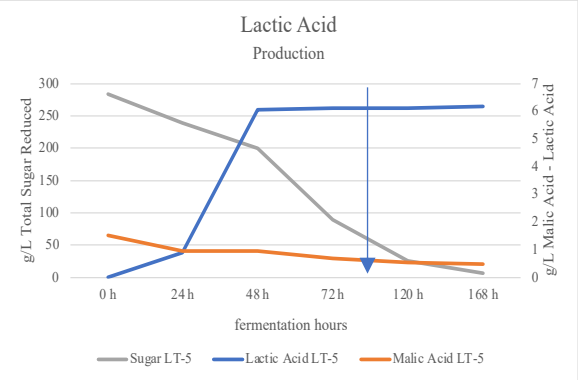


Fig. 13 b – Sugar, malic acid and lactic acid values

3.1.2 ***Acidifying capacity***

The *L. thermotolerans* strains tested showed different acidifying behavior depending on strain and time of inoculation.

The effects on pH and total acidity were also different.

The L.T. strains with the highest acidifying capacity were the C and D strains, sequentially inoculated at 48 hours from S.C., and the A strain sequentially inoculated from S.C. at 72 hours (Tab 4, and Tab.5), compared to the A and B strains, also sequentially inoculated.

With respect to pH, the best acidifying strain was strain D inoculated at 48 hours with an acidifying capacity of 17.4%.

There was no difference between strain C and A, both inoculated at 48 hours; their capacity was 13.8%.

Even though the percentages of acidifying capacity in relation to pH could seem small (Tab. 4) compared to the one verified in relation to total acidity (Tab.5), it should be remembered that wine is a buffer solution.

Therefore, the deviations of pH detected (Tab. 4), result to be strongly significant, as well as strong inorganic acids.

All strains had a higher acidifying capacity than that detected by tartaric acid in the pre-fermentative phase.

The acidifying capacity calculated with respect to total acidity was different.

The latter, since it represents the sum of neutralizable organic acids, results to be strictly related to the production of lactic acid, in our case produced by *L. thermotolerans* (and to a lesser extent by *L. plantarum* which had a deacidifying capacity).

The highest acidifying capacities were recorded for *L. thermotolerans* strains C and D, sequentially inoculated at 48 hour *S. cerevisiae*, and A sequentially inoculated from *S. cerevisiae* at 72 hour.

The most acidifying strain increased total acidity contents by 195.6%.

In general, all *L. thermotolerans* strains had significant increases in total acidity (Tab. 5).

Strain and inoculum time	**Trials**	**18/09/22**	**18/09/22**	**25/09/22**	**% *Acidifying capacity***
A - 48 h	LT-1	---	3.90±0.01	3.43±0,01	13.7
B - 48 h	LT-2	---	3.90±0.01	3.50±0,01	11.4
C - 48 h	LT-3	---	3.90±0.01	3.36±0,01	16.1
D - 48 h	LT-4	---	3.90±0.01	3.22±0,01	21.1
A - 72 h	LT-5	---	3.90±0.01	3.36±0,01	16.1
NO *L. therm*	LT-6	---	3.90±0.01	3.92±0,01	0.0
NO *L. therm* - Tart. Ac.	LT-7	3.90±0.01	3.90±0.01	3.62±0,01	8.3
NO *L. therm*	Cont A	---	3.90±0.01	3.89±0,01	0.3
NO *L. therm*	Cont B	---	3.90±0.01	3.87±0,01	0.8

Tab. 4 - pH variation and acidifying capacity in experimental syrah wines

Strain and inoculum time	**Trials**	**18/09/22**	**25/09/22**	**% *Acidifying capacity***
A - 48 h	LT-1	5.50±0.01	10.20±0,01	85.5
B - 48 h	LT-2	5.50±0.01	10.10±0,01	83.6
C - 48 h	LT-3	5.50±0.01	10.80±0,01	96.4
D - 48 h	LT-4	5.50±0.01	16.26±0,01	195.6
A - 72 h	LT-5	5.50±0.01	10.60±0,01	92.7
NO *L. therm.*	LT-6	5.50±0.01	5.08±0,01	0
NO *L. therm*- AT	LT-7	7.00±0,01	6.59±0,01	0
NO *L. therm.*	Cont A	5.50±0.01	5.06±0,01	0
NO *L. therm.*	Cont B	5.50±0.01	5.48±0,01	0

Tab. 5 - variations in total acidity (g/L tartaric acid) and experimental syrah wines

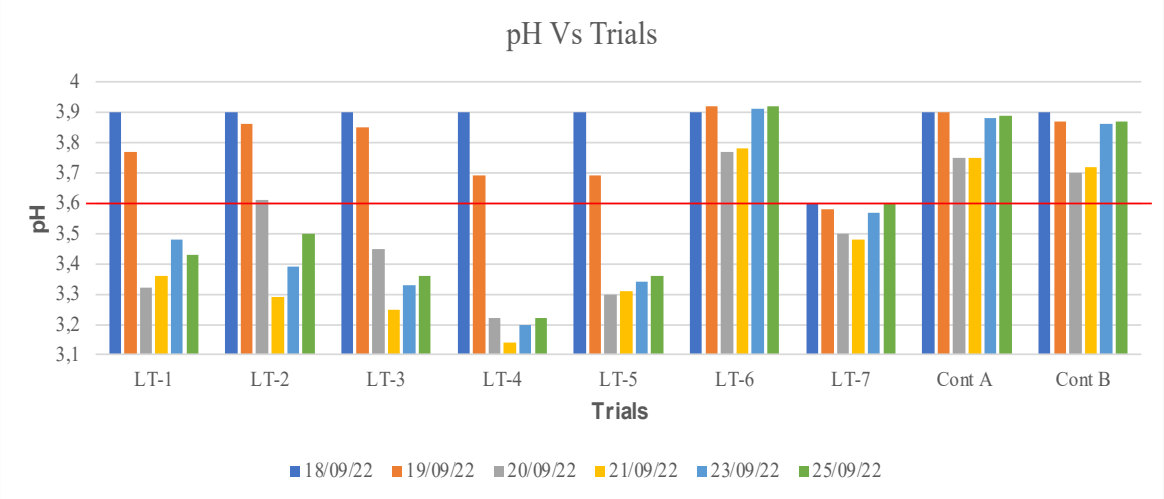


Fig. 14 - Trends of pH values measured during fermentation

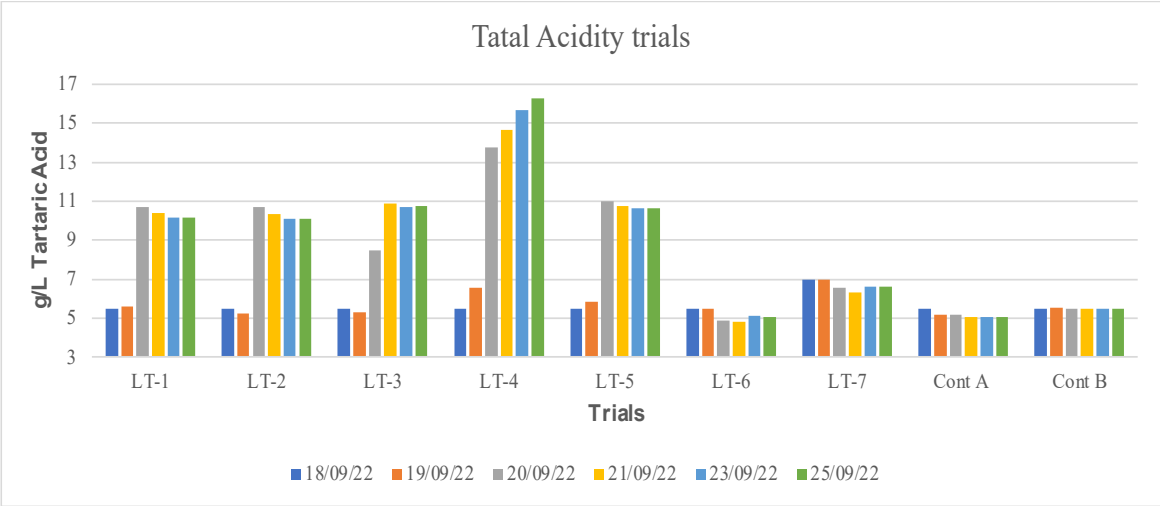


Fig. 15 - Trends of total acidity values measured during fermentation

3.1.3 Statistical analysis

The biplot analysis identifies three groups:

- the group of LT-6, LT-7, Cont-A, and Cont-B controls (untreated with *L. thermorolerans*) negatively correlated to sugars, malic, lactic, and acetic acid

- the group LT-3, LT-4, LT-5, referring to the most acidifying strains (C and D co-inoculated sequentially at 48 hrs from S.C. and ML Prime; and strain A, co-inoculated sequentially at 72 hrs from *S.C.* and ML Prime) that are positively correlated to lactic, malic and acetic acid

- the group LT-1 and LT-2 (strain A and B, co-inoculated sequentially at 48 hours by *S.C.* and ML Prime) that with an intermediate behavior between the two groups are positively correlated to sugars (with a greater propensity to fructose as mentioned above) and to acetic, malic and lactic acid even if to a lesser extent than the second group.

The analysis of clusters highlights the significance of all those theses treated with different strains of *L. thermotolerans*, however, not being able to detect intra-strain diversity.

According to what is expected below the threshold of significance are placed the control samples not inoculated by *L. thermotolerans.*

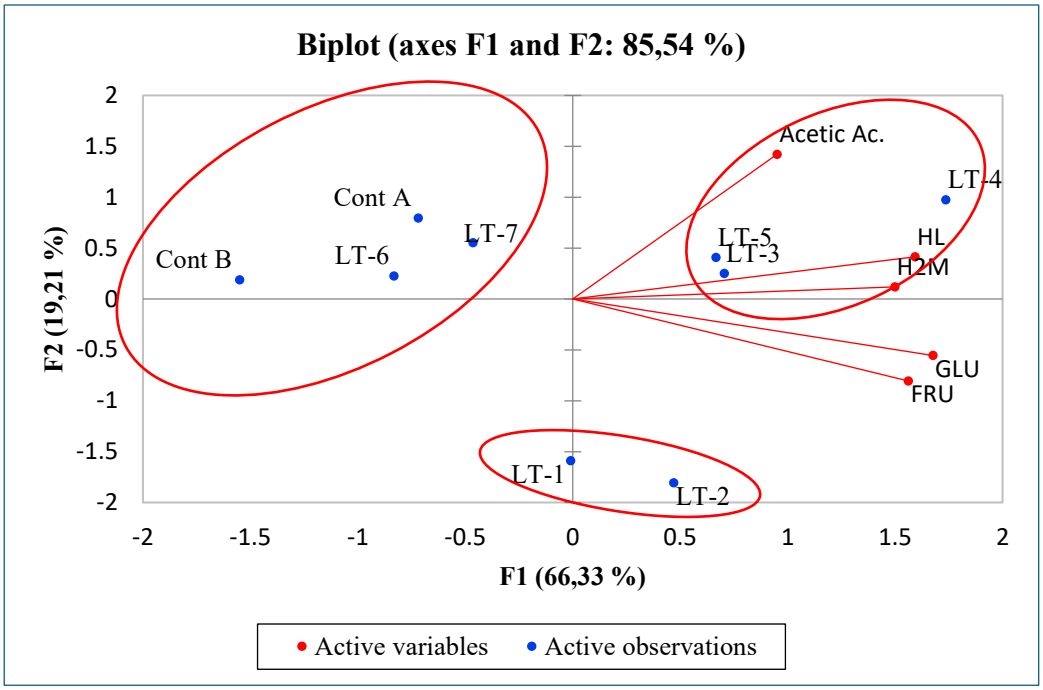


Fig. 16 - Biblot analysis of the main technological parameters

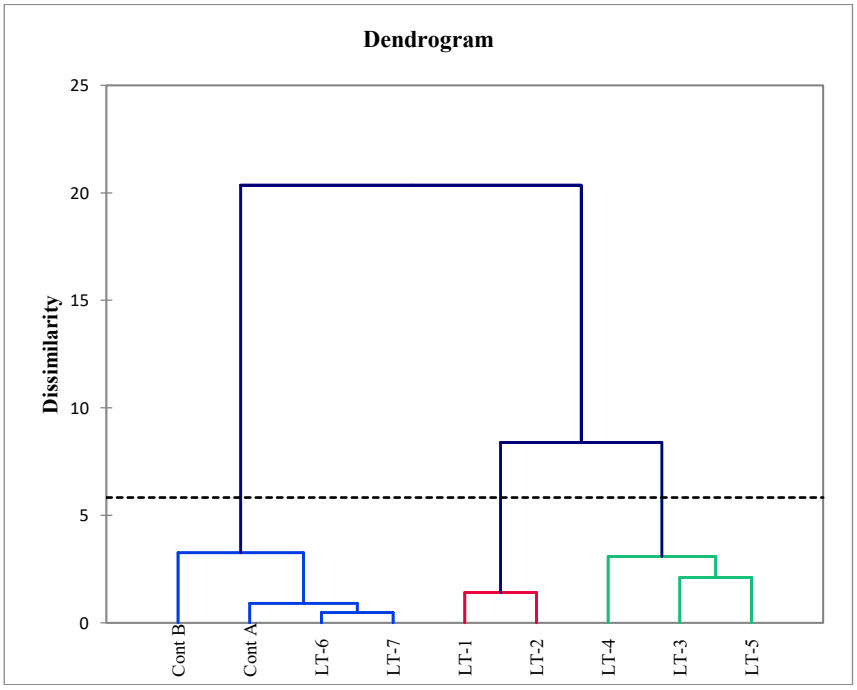


Fig. 17 - Claster analysis chemical-technological parameters

3.1.4 Volatil organic compounds

Table 5 presented the results of VOC detection. Alcohols (79.45% - 97,05%), esters (0,17% – 20.06%), aldehydes (0.06% - 0.74%), ketones (0.15 – 0.18%), carbossilic acid (0.10 – 5.78%) other minor VOCs (2.00% - 3.27%) represented the total volatile fraction of wine (Tab. 6).

The heatmaps subdivide the thesis populations into two macrogroups by not discriminating the presence-absence of the different strains of *L. thermotolerans* used in the microbial consortia (Fig. 18).

In the first macrogroup, it compares the LT-1 and LT-2 trials as similar (Fig. 18); this result suggests that the *L. thermotolerans* A and B strains do not produce significant differences in terms of wine volatile fraction. However, the subgrouping of the LT-1 and LT-2 trials with the CONT_A test is noteworthy (Fig. 18). This behaviour shows that the use of *L. thermotolerans* A and B strains in the first 48 few differences are produced in comparison to the non-use of the same strains in the microbial consortia. Although included in the same macrogroup, the position of the CONT_B control trials is different (Fig.18). Its diversity is marked by the significant presence in the wines of the esters diethyl succinate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl (11E)-11-hexadecanoate, ethyl hexadecanoate, ethyl-9,12-octadecadienoate, (E)-Ethyl 9-octadecenoate, ethyl eicosanoate. The absence of these compounds found in the control thesis CONT_A suggests that the simultaneous inoculation of *S. cerevisiae* persy and the *L. plantarum* ML Prime strain induced LAB has an intense esterase activity. This activity was of lower intensity when simultaneous inoculation between *L. plantarum-S.cerevisiae* occurred at 48 hours after inoculation of *L. thermotolerans* A and B strains. The second macrogroup was divided into two subgroups: LT-6, LT-7; and, LT-3, LT-4, LT-5. The first subgroup shows that there are no significant differences in terms of VOCs when using tartaric acid as an exogenous acidifying additive. However, a noteworthy result is emphasised in the second subgroup and between the two macrogroups: the increase in the residence time of *L. thermotolerans* A strain from 48 to 72 hours produces significant differences in VOCs production (LT-1 vs LT5). The longer residence time of the LT-A strain resulted in similar behaviour in the production of VOCs

when comparing this strain to the LT-C and LT-D strains used in the first 48 hours of fermentation, respectively.

The aromatic class of higher alcohols was the most representative in percentage terms (Table 5). This result is in line with that reported by Ribéreau-Gayon, 2006. The microbial consociations adopted had a significant influence on the VOCs concentrations quantified for each individual test (Fig. 18). 3-methyl-1-butanol and 2-phenethyl alcohol were the main compounds produced. The formation of these compounds is attributed to the Ehrlich pathway of *S. cerevisiae* from the aminoacids leucine and phenylalanine respectively (Hazelwood et al., 2008; Li et al., 2024). The different production of higher alcohols between the tests suggests a different amino acid requirement between the different microbial consociations. However, noteworthy data concern the detection of the higher alcohols Tyrosol and Tryptophol (Tab. 5). These compounds according to Nissen et al., 2003 are involved in yeast-yeast interactions. According to Ivey et al., 2013 the synthesis of tyrosol and tryptophol occur for communication purposes between cells. PCA analysis positively correlated the production of these compounds with CONT_B, CONT_A, LT-6 and LT-7; this correlation was explained by 36.79% variance (Fig. 19). Therefore, it appears evident that these communicative inputs were used by *S. cerevisiae* Persy strain to communicate with other cells of the same strain. In addition, dichloromethane extraction of the volatile fraction of the wines allowed another form of tryptophol to be identified (Tab. 5). The identification of a further peak at a different retention time suggests a different stereoisomeric form of tryptophol. The positive correlation of this form with LT-1, LT-2, LT-3, LT-4 and LT-5 (Fig. 19) suggests a cell-cell communication between *S. cerevisiae* and *L. thermotolerans.* Esters proved to be the aromatic class with the widest variability among VOCs (tab.6). The trials LT1, LT2, LT3, LT4 and LT5 stood out for showing the highest percentages in terms of ethyl 2-hydroxypropanoate productivity (Table 5). The significant lactic acid concentrations recorded (tab. 2) contributed to the esterifications of ethyl 2-hydroxypropanoate. PCA analysis explained these productions with 24.46% of the variance (Fig. 19)

RT	Compounds	CONT_A	CONT_B	LT-1	LT-2	LT-3	LT-4	LT-5	LT-6	LT-7	S.S.
3,905	3-Methyl-1-butanol	53.59±0.97ab	51.66±0.94bc	48.80±0.89d	45.16±0.82e	54.47±0.99a	47.63±0.87de	55.99±1.02a	49.77±0.90cd	50.19±0.91cd	***
5,055	2,3-Butanediol	6.14±0.13b	2.19±0.04e	6.47±0.14	9.96±0.21a	2.80±0.06d	3.52±0.07c	1.63±0.03f	6.21±0.13b	6.25±0.13b	***
5,855	2,3-Butanediol	1.49±0.03a	0.13±0.01h	0.86±0.02e	1.42±0.03b	0.32±0.01f	0.00±0.00i	0.25±0.01g	1.34±0.03c	1.18±0.01d	***
6,055	Ethyl 2-hydroxypropanoate	0.00±0.00g	0.00±0.00g	8.21±0.12d	6.49±0.09e	10.94±0.16b	19.41±0.29a	8.61±0.12c	0.91±0.01f	0.91±0.01f	***
7,004	3-Ethoxy-1-propanol	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.13±0.01a	0.00±0.00b	0.00±0.00b	***
8,504	Octanal	0.31±0.01b	0.00±0.00c	0.55±0.01a	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
8,554	1-Hexanol	0.00±0.00g	0.53±0.01b	0.00±0.00g	0.22±0.01f	0.35±0.01d	0.28±0.01e	0.70±0.01a	0.55±0.01b	0.49±0.01c	***
8,854	3-Methylbutyl acetate	0.17±0.01d	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.09±0.01e	0.29±0.01c	0.09±0.01e	0.38±0.01a	0.34±0.01b	***
8,954	2-Methyl-1-butyl acetate	0.00±0.00c	0.00±0.00c	0.03±0.01b	0.09±0.01a	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
10,553	4-Hydroxy butanoic acid	0.10±0.01f	0.08±0.01g	0.36±0.01b	0.23±0.01d	0.39±0.01a	0.27±0.01c	0.18±0.01e	0.05±0.01h	0.06±0.01h	***
11,853	(3-Methyl-2-oxiranyl)methanol	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.05±0.01b	0.09±0.01a	***
12,203	Ethyl 3-hydroxybutanoate	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.06±0.01b	0.07±0.01a	***
13,200	(S)-2-Hydroxypropanoic acid	0.00±0.00d	0.00±0.00d	4.84±0.07a	4.91±0.08a	0.00±0.00d	2.28±0.04b	0.68±0.01c	0.00±0.00d	0.00±0.00d	***
13,602	4-Hydroxy-2-butanone	0.25±0.01e	0.15±0.01f	0.67±0.01c	0.67±0.01c	0.78±0.01b	1.23±0.02a	0.75±0.02b	0.66±0.01c	0.57±0.01d	***
15,102	3-(Methylsulfanyl)-1-propanol	0.18±0.01a	0.14±0.01c	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.05±0.01d	0.15±0.01b	***
16,652	Ethyl hexanoate	0.00±0.00g	0.18±0.01a	0.06±0.01f	0.11±0.01d	0.09±0.01e	0.14±0.01c	0.09±0.01e	0.15±0.01b	0.09±0.01e	***
17,250	2-Propylmalonic acid	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.13±0.01a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	***
18,951	Benzylalcohol	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.05±0.01d	0.08±0.01b	0.07±0.01c	0.09±0.01a	0.09±0.01a	***
20,850	o-Acetylserine	2.13±0.03cd	2.00±0.03d	2.09±0.04cd	2.22±0.04c	3.04±0.04a	2.13±0.04 cd	2.74±0.05b	3.07±0.05a	2.78±0.05b	***
21,500	Pentyl methoxyacetate	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.13±0.01d	0.16±0.04c	0.22±0.01a	0.21±0.01b	0.00±0.00e	0.00±0.00e	***
22,300	Glycerol	0.23±0.01e	0.00±0.00g	0.34±0.01b	0.14±0.01f	0.00±0.00g	0.00±0.00g	0.26±0.01d	0.36±0.01a	0.32±0.01c	***
24,999	Phenethyl alcohol	35.00±0.51b	37.43±0.55a	22.82±0.33fg	23.91±0.35f	25.25±0.37e	21.90±0.39g	26.84±0.39d	33.42±0.50c	34.03±0.50bc	***
26,649	N-Isopentylacetamide	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.12±0.01b	0.00±0.00c	0.00±0.00c	0.20±0.01a	0.20±0.01a	***
29,848	Diethyl succinate	0.00±0.00d	0.18±0.03a	0.09±0.01c	0.12±0.01b	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	***
30,198	Monoethyl succinate	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.39±0.01a	0.00±0.00e	0.27±0.01d	0.37±0.01b	0.35±0.01c	***
30,948	Ethyl octanoate	0.00±0.00d	0.49±0.01a	0.07±0.01c	0.26±0.01b	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	***
31,697	3,4-Dimethylbenzaldehyde	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.14±0.01a	0.13±0.01b	0.04±0.01c	0.13±0.01b	0.00±0.00d	***
44,444	Ethyl decanoate	0.00±0.00c	0.56±0.01a	0.00±0.00c	0.22±0.01b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
46,453	Tyrosol	0.26±0.01c	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.70±0.01b	0.76±0.01a	***
56,640	Ethyl dodecanoate	0.00±0.00c	0.19±0.01a	0.00±0.00c	0.08±0.01b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
64,788	Tryptophol	0.00±0.00f	0.00±0.00f	0.16±0.01d	0.15±0.01e	0.24±0.01b	0.21±0.01c	0.30±0.01a	0.00±0.00f	0.00±0.00f	***
64,888	Tryptophol (2-(1H-Indol-3-yl)ethanol)	0.16±0.01c	0.12±0.01d	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.62±0.01a	0.57±0.01b	***
68,987	Ethyl (2E)-3-(4-hydroxyphenyl)-2-propenoate	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.57±0.01a	0.23±0.01b	***
76,385	Ethyl (11E)-11-hexadecenoate	0.00±0.00d	0.14±0.01a	0.12±0.01b	0.07±0.01c	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	***
77,585	Ethyl hexadecanoate	0.00±0.00c	1.47±0.02a	1.24±0.02b	1.26±0.02b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
78,734	n-Octadecanal	0.00±0.00f	0.06±0.01e	0.19±0.01c	0.10±0.01d	0.38±0.01a	0.27±0.01b	0.19±0.01c	0.27±0.01b	0.19±0.01c	***
85,082	Ethyl-9,12-octadecadienoate	0.00±0.00c	0.70±0.01a	0.68±0.01b	0.67±0.01b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
85,432	(E)-Ethyl 9-octadecenoate	0.00±0.00c	1.12±0.03a	0.95±0.02b	0.97±0.02b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	***
86,732	Ethyl eicosanoate	0.00±0.00d	0.50±0.01a	0.24±0.01c	0.28±0.01b	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	0.00±0.00d	***

Tab. 5 Volatile organic compounds (VOCs) detected in Syrah experimental wines (all values in % area).

Compounds	CONT_A	CONT_B	LT-1	LT-2	LT-3	LT-4	LT-5	LT-6	LT-7	S.S.
∑ alcohols	97.05 ± 1.68a	92.2 ± 1.57b	79.45 ± 1.40d	80.96 ± 1.44d	83.48 ± 1.46cd	73.62 ± 1.36e	86.17 ± 1.50c	93.16 ± 1.63 ab	94.12 ± 1.62ab	***
∑ aldehyde	0.31 ± 0.01cd	0.06 ± 0.01b	0.74 ± 0.02a	0.10 ± 0.01ef	0.52 ± 0.02b	0.40 ± 0.02bc	0.23 ± 0.16de	0.40 ± 0.02 bc	0.19 ± 0.01def	***
∑ carbossilic acid	0.10 ± 0.01g	0.23 ± 0.01f	5.87 ± 0.08a	5.94 ± 0.1a	1.17 ± 0.01d	3.78 ± 0.05b	1.61 ± 0.02c	0.71 ± 0.01e	0.63 ± 0.01e	***
∑Ketones	0.25 ± 0.01e	0.15 ± 0.01f	0.67 ± 0.01c	0.67 ± 0.01c	0.78 ± 0.01b	1.23 ± 0.02a	0.75 ± 0.02b	0.66 ± 0.01c	0.57 ± 0.01d	***
∑ esters	0.17 ± 0.01g	5.53 ± 0.15e	11.69 ± 0.23b	10.75 ± 0.23c	11.67 ± 0.23b	20.06 ± 0.32a	9.27 ± 0.02d	2.44 ± 0.06f	1.99 ± 0.01f	***
∑other	2.13 ± 0.03de	2,00 ± 0.03e	2.09 ± 0.04 de	2.22 ± 0.04d	3.16 ± 0.05a	2.13±0.04 de	2.74 ± 0.05b	3.27 ± 0.06a	2.98 ± 0.06b	***

Tab. 6 Volatile organic compounds (VOCs) total detected in Syrah experimental wines

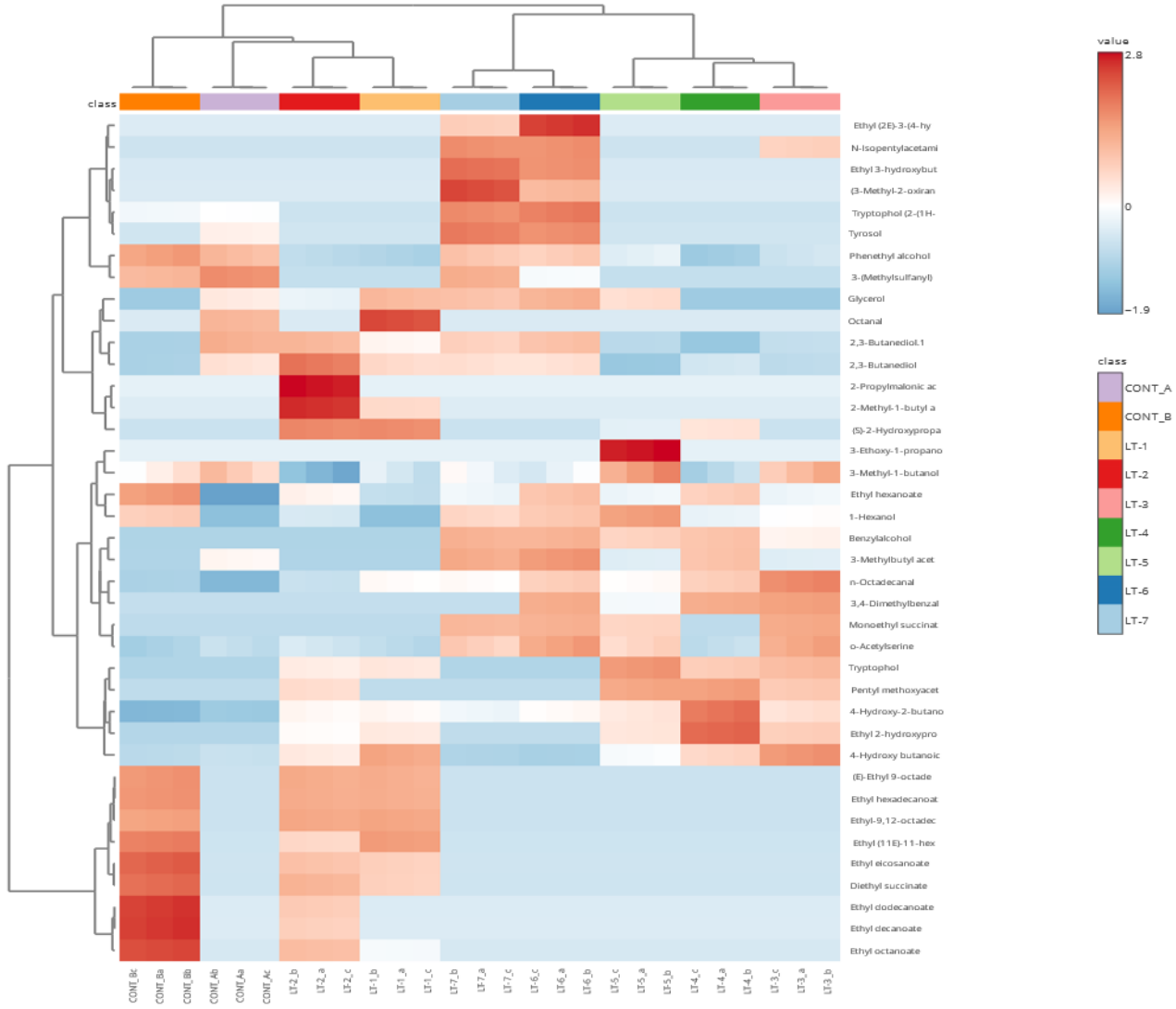


Fig. 18. Heatmaps

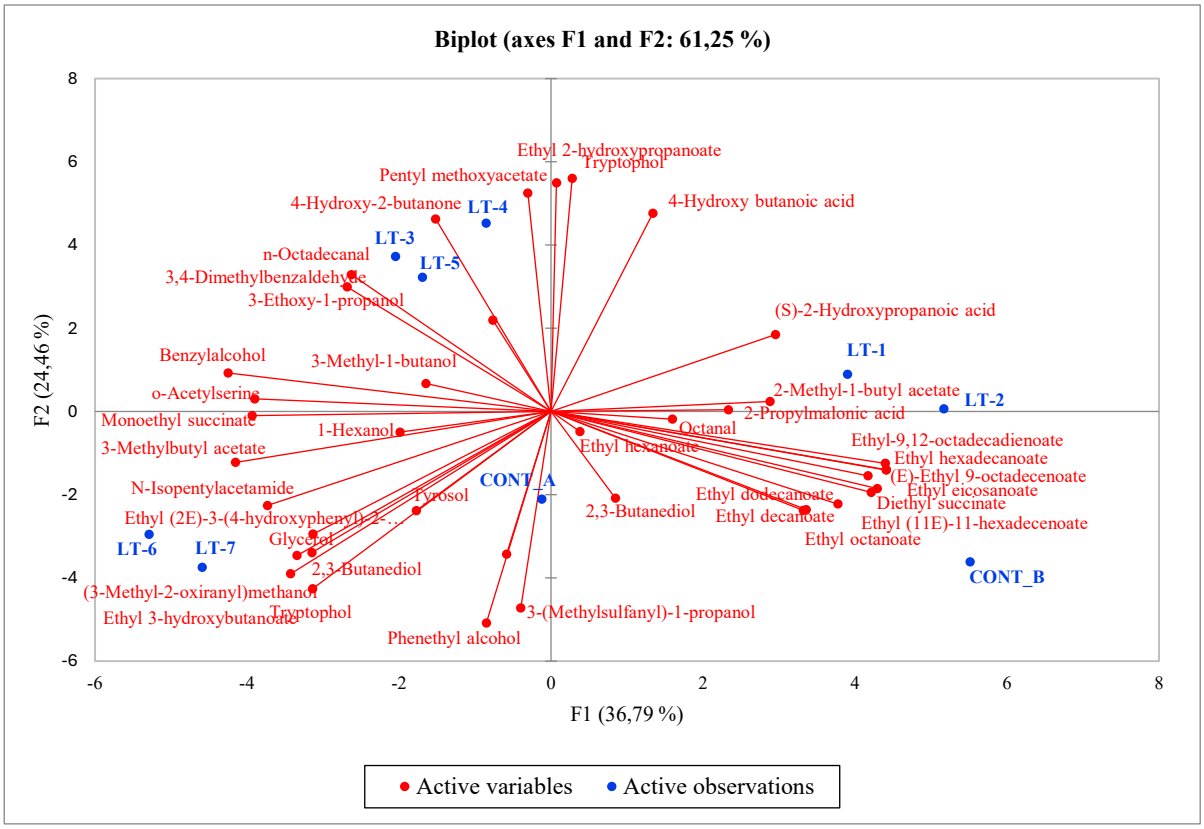


Fig. 19 - Principal component analysis (PCA) biplot VOCs

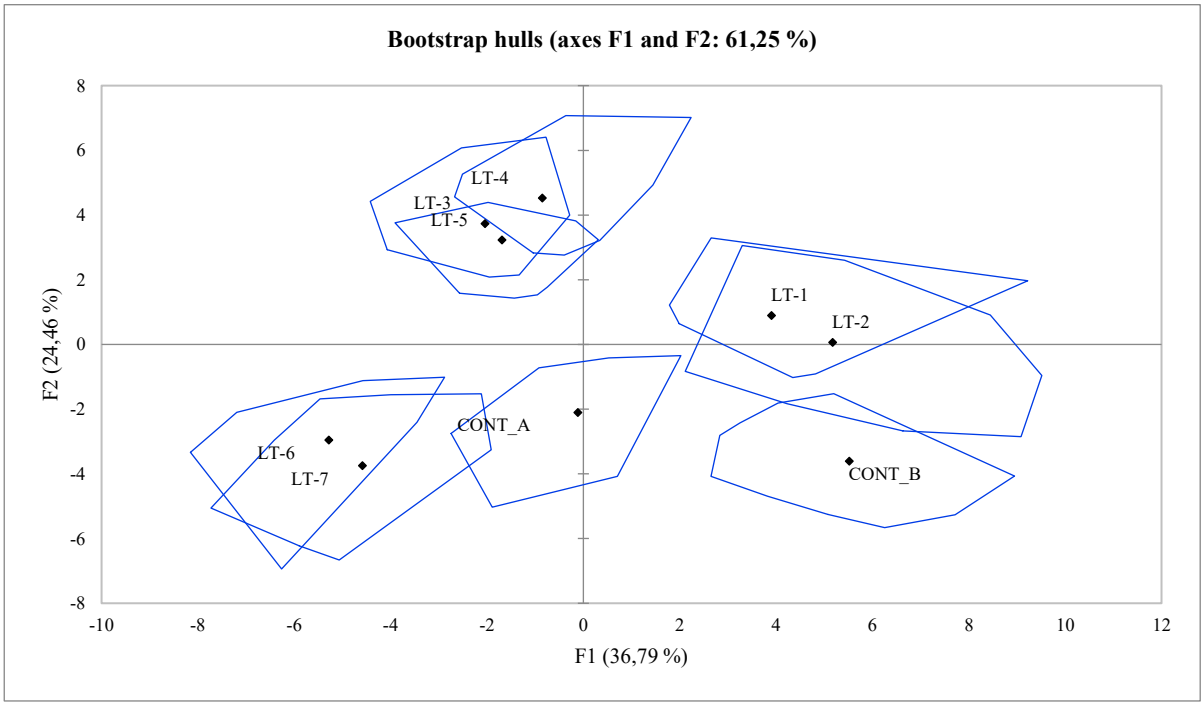


Fig. 20 - Bootstrap hulls of VOCs

4. Conclusion

The strains of *L. thermotolerans* tested showed good acidifying abilities.

The choice of the *L. thermotolerans* strain and the timing of the early fermentation stages are of crucial importance for achieving the objectives.

The strains A, B and C of *L. thermotolerans* inoculated sequentially at 48 hours from *S. cerevisiae* and *L. plantarum* performed best in terms of technological parameters and acidifying capacity.

The use of strains A, B and C of *L. thermotolerans* resulted in microbiologically stable wines with low levels of acetic acid.

However, the best aromatic pictures were recorded from strains A and B of *L. thermotolerans*, showing similar profiles to the control without *L. thermotolerans* and ML Prime. This diversity of results highlights the importance of the timing of inoculation and how this technique can influence changing metabolisms in biotechnology.

Wines produced with strain D, inoculated sequentially at 48 hours from *S. cerevisiae*, and strain A inoculated sequentially at 72 hours from *S. cerevisiae* are those that are highly acidified and microbiologically unstable. The high concentrations of L-lactic acid produced by *L. thermotolerans* contributed to the inhibition of the metabolic activities of *L. plantarum* by excess catabolite in the wines.

In conclusion, this evidence should be regarded as initial insights based on the evaluation of the chemical and VOCs parameters of wines.

Further in-depth studies focusing in particular on the study of the total hexo-metabolome will allow useful information to be outlined in the use of microbial consortia with *L. thermotolerans*. The analysis of whole metabolome has been carried out and the data will be reported in the future scientific papers.

Activity 2.4

A metabolomic study for assessing the active aroma fraction of Catarratto wine produced by novel microbial consortia: *Starmerella lactis-condensi*, *Lactobacillus plantarum*, *Oenoccus oeni* and *Saccharomyces cereivisiae*

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Abstract

The sensory profiles of wines are influenced by the effects of microbial interactions during alcoholic fermentation. In terms of diversity, the active volatile fraction of wine is the component most influenced by the metabolic activities of microbial strains. However, these compositional complexities, which are difficult to represent graphically, do not always correspond to olfactory perceptions. This study analysed the effects of interactions among *Starmerella lactis-condensi*, lactic acid bacteria (*Oenococcus oeni – O. oeni*; *Lactiplantibacillus plantarum – L.plantarum*) and *Saccharomyces cerevisiae* on the active volatile fraction of wine (OAV > 1). Very limited information have been reported on the application of *Starm. lactis-condensi* in wine production and no data have published on its inoculum in combination with lactic acid bacteria strains. Based on the OAVs, new methodological approaches were developed with the aim of clarifying the real contribution of each microbial component. The formulation of indicative relationship values (RI-values) and their graphic representation in 3D-plot surfaces made it possible to explain numerous real-life conditions relating to olfactory perceptions. In addition, interactions between non-volatile wine components were investigated by means of a non-targeted metabolomic study. The microbial associations LABs - *S. cerevisiae* with *Starm. lactis-condensi* showed full compatibility between them and the Catarratto must. The use of *Starm. lactis-condensi* with *S. cerevisiae* resulted in the highest levels of trans-resveratrol and resveratrol-2-O-beta glucopyranoside. The same microbial consortium showed the highest variability in ethyl esters above the level of RI-values. The PCA analysis explained this aromatic condition of the wine with the highest degree of olfactory complexity; the 3D-plot surface revealed possible contributions from synergistic effects between OAV > 1. In contrast, potential additive effects between the OAVs 3-ethoxy-1-propanol, hidroxyethylbenzene and 1-pentanol explained the lavender smell as a contribution of *L. plantarum* MLPK45H™ in microbial consortia

with *Starmerella lactis-condensi* and *S. cerevisiae* QA23™. The RI-values and their representation in 3D-plot surface may be a valuable, user-friendly investigative tool that can help explain many of the sensory attributes from synergistic, additive or antagonistic effects among VOCs in a multiple microbial consortia.

Keywords: Catarratto, Starmerella lactis-condensi, metabolomic, Volatile organic compounds, IR-values

Data currently undergoing submission and peer revision for publication in *Food Chemistry*

1. Introduction

Wine is a fermented beverage whose production is rooted in human traditions. Although time and human socio-cultural differences may have influenced its production dynamics (Pretorius, 2020), fermentation remains an unchanged technological step to this day. Fermentation is an anaerobic metabolic pathway applied by microorganisms to synthesise chemical energy (adenosine triphosphate, ATP) (Zamora et al., 2009). In must, energy substrates from grapes are converted into ethanol, glycerol, acids and other minor compounds (Moreno-Arribas, 2009). These transformations, catalysed by yeasts and bacteria to recover NAD^+ from NADH, play a crucial role in determining the main chemical and physical changes in wine (Fleet, 2008). Recent studies have identified the pivotal role of microorganisms in the fermentation process, highlighting their influence on the sensory qualities of wine (Ferreira, 2010). Winemaking involves numerous yeast species. As in a spontaneous fermentation, the fermentation phase is initiated by the simultaneous growth of various non-*Saccharomyces* species. However, these are distinguished from *Saccharomyces* spp. by their low fermentative power (Fleet, 2008). Consequently, their use in pure culture is not desirable for quality wine production. Only recently are non-*Saccharomyces* strains gaining importance in the application in grape must transformation processes. Ciani et al. (2010) have conducted studies which demonstrate the biotechnological value of non-*Saccharomyces* yeasts when used in association with other yeasts. Consequently, the use of mixed cultures during the fermentation phase substantiates the significance of interactions between different species on wine composition and its volatile fraction (Ciani, Comitini, Mannazzu, & Domizio, 2010; Jolly, Varela, & Pretorius, 2014). The literature contains a multitude of studies describing the consequences of microbial interaction mechanisms. It is generally observed that direct effects on LAB-*S. cerevisiae* interactions occur during the initiation and completion of malolactic fermentation (Liu, Forcisi, Harir, …& Alexandre, 2016; Alexandre, Costello, Remize, & Guilloux-Benatier, 2004). Indeed, numerous authors have documented the ability of yeasts to hinder or decelerate malolactic fermentation through the production of inhibitory compounds (Hennick-Kling, 1993; Arnink & Hennick-Kling, 2005; Rosi et al., 2003; Comitini et al.,

2005; Guilloux-Benatier et al., 2006; Osborne & Edwards, 2006; Osborne & Edwards, 2007). However, it has also been observed that the indirect effects caused by LAB - *S. cerevisiae* interactions (Tristezza, di Feo, Tufariello, ...& Grieco, 2016; Versari, Patrizi, Parpinello, ...& Longhini, 2016) play a key role in constituting the aromatic profile of wine. Studies conducted so far have demonstrated the importance of microbial interactions in the dynamics of creating the aromatic buffer of wine (Ferreira, 2010). However, the most widespread information from the scientific community so far concerns yeast-yeast (non-*Saccharomyces* - *Saccharomyces* or *Saccharomyces* - *Saccharomyces*) microbial interactions (Benito, Hofmann, Laier, . ..& Rauhut, 2015; Sadaoudi et al, 2012) and LAB-*S. cerevisiae* (*Oenococcus oeni* - *S. cerevisiae* or *Lactiplantibacillus plantarum* - *S. cerevisiae*) (Alexandre, Costello, Remize, & Guilloux-Benatier, 2004; Devi, A., & Anu-Appaiah, K. A., 2021); whereas, the effects of non-*Saccharomyces* - LAB - *S. cerevisiae* interactions, although present in the bibliography, limited represent "cause-and-effect" information on wine volatilome.

It is known that wine aroma depends on the metabolisms of yeasts and LAB and that these are activated by various physiological compounds present in the must. In this respect, this study proposes for the first time an investigation into the effects of non-S*accharomyces* - LAB - *S. cerevisiae* interactions caused by their metabolisms on the non-volatile and volatile fraction of Catarratto wine. In order to achieve these objectives, the use of *Starm. lactis-condensi* will be introduced for the first time, whose application in musts will take place in microbial consociation with LAB and *S. cerevisiae,* which are already widely spread on the biotechnology market. Then, as application background information, the biotechnological compatibility between the strains and their suitability to provide alternative aroma products to those obtainable from a pure conventional culture will be assessed. Subsequently, the implications of the different microbial combinations in the wine matrix (non-volatile liquid fraction) and on the wine aromas of the wine itself (gaseous, volatile fraction) will be investigated. The experimental procedure will be characterised by the application of different methodological approaches. A non-targeted metabolomic survey system will be employed with the

aim of clarifying the impact of microbial interactions in the non-volatile fraction of wine. In this regard, the impact of microbial interactions with the must matrix of Catarratto will also be assessed. In addition, different methodological approaches will be used in wine to assess the influence of *Starm. lactis-condensi* and the consequences of interactions with LAB (*Oenococcus oeni* and *Lactiplantibacillus plantarum*) and *S. cerevisiae*. An untargeted metabolomics approach was employed with the aim of clarifying the impact of microbial interactions in the non-volatile fraction of wine. In this respect, the impact of microbial interactions with the must matrix of Catarratto will also be assessed. Furthermore, in order to highlight the contribution of each individual biotenchological component to the active volatile profile of the wine, an attempt will be made to introduce a new evaluation system specifically for multiple microbial consortia (non-Saccharomyces - Lab - *Saccharomyces*). In particular, an attempt will be made to set up a graphical-statistical representation developed on the three dimensions of space "x"', "y", "z", each representing a biotechnological variable. The formulation of this new evaluation criterion will be developed on the basis of the information already available in the literature on olfactory active value (OAV). The application to the OAV data of the Z -Score normalisation will make it possible to create a new input data set, "relationship indicator values" (RI-values) capable of bringing the different active VOCs into synergy. The procedure will simulate the actual process that occurs in the aromatic buffer of wine, thereby clarifying the results of numerous authors (Ferreira, Sáenz-Navajas, Campo, ... & Fernández-Zurbano, 2016; Cameleyre, Lytra, Tempere, ... & Barbe 2015; Ferreira et al, 2012; Atanasova, Thomas-Danguin, Chabanet, ...& Etiévant, 2005;Atanasova, Thomas-Danguin, Langlois, ...& Etievant, 2004).

2. Material and methods

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2.1. Strain preparation, experimental plan and sample collection

The MN412 strain (*Starmerella lactis-condensi*) strain forms part of the oenological yeast collection of the Department of Agricultural, Food and Forestry Sciences (SAAF; University of Palermo, Italy). Non-*Saccharomyces* strain was reactivated from a stock at -80 °C in Yeast Peptone Dextrose (YPD; Condalab, Torrejón de Ardoz, Madrid, Spain) at 28 °C for 48 h and reproduced in a concentrated liquid suspension by Bionova srl (Villanova sull'Arda, Piacenza, Italy). The QA23™, MLB6™, and MLPK45H™ strains were employed in accordance with the specifications outlined by the manufacturer (Lallemand Inc. Italia, Castel D'Azzano, Verona, Italy). Catarratto grapes were cultivated within the agro-ecological zone of the municipality of Monreale (PA) utilising organic farming methods (37° 57' 22.4, N 13° 15' 16.2 coordinates). The experimental plan, as illustrated in Figure 1, was designed to investigate the effects of microbial consortia on the sensory attributes of wine. The experimental design included the following trials: S1, sequential inoculum Stram. lactis-condensi MN412 strain - *S. cerevisiae* QA23™ strain; S2, sequential inoculum *Stram. lactis-condensi* MN412 strain - simultaneous inoculum *L. plantarum* MLPK45H™ strain - *S. cerevisiae* QA23™ strain; S3, sequential inoculum *Stram. lactis-condensi* MN412 strain - simultaneous inoculum O. oeni MLB6™ strain- *S. cerevisiae* QA23™ strain.

As control trials: S7, S. cerevisiae QA23 strain; S8, simultaneous incubation of *L. plantarum* MLPK45H™ strain and *S. cerevisiae* QA23 strain; S9, simultaneous incubation of *O. oeni* MLB6™ strain and *S. cerevisiae* QA23™ strain. The vinification processes were conducted at the SAAF department of the University of Palermo, Italy, on medium-scale volumes (100 litres per trials). Each trial was conducted in triplicate. The sampling plan provided for data to be collected at regular intervals, with samples taken every 1, 2, 3, 6 and 9 days. Volatile organic compounds (VOCs) were analysed in the wines at the conclusion of the fermentation process.

2.2 Winemaking process

The grapes were destemmed and crushed and 4 g/q of metabisulphite (Laffort, France) was added. The static settling process was ensured by the addition of two g/hL of the pectolytic enzyme LALLZYME HC™ (Lallemand Inc. Italy, Castel D'Azzano, Verona, Italy). Maintaining a temperature of 10 °C for 48 hours facilitated the pectin degradation process. Subsequently, the must was aliquoted into 18 steel tanks to constitute six different trials, each of which was conducted in triplicate. Inoculation schedules were performed as illustrated in Fig. 1. The inoculum dosages of strains was conducted to reach a concentration of around 7 log cycles (cell/ml) in to grape must for *Starm. lactis-condensi* (MN412) and *S. cerevisiae* (QA23™). Instead, LAB were inculcated at 10 g/hl and 1 g/hl repeatedly for *L. plantarum* and *O. oeni*. Fermentations were conducted at 20 °C. The addition of potassium metabisulphite at the end of fermentation (Esseco s.r.l., San Martino, Novara, Italy) was carried out one week after complete degradation of the malic acid (according to the LAB manufacturer's instructions (Lallemand Inc. Italy, Castel D'Azzano, Verona, Italy). Microbial inactivity was ensured by reaching 0.8 mg/L molecular SO_2 (Tomasset, 1978).

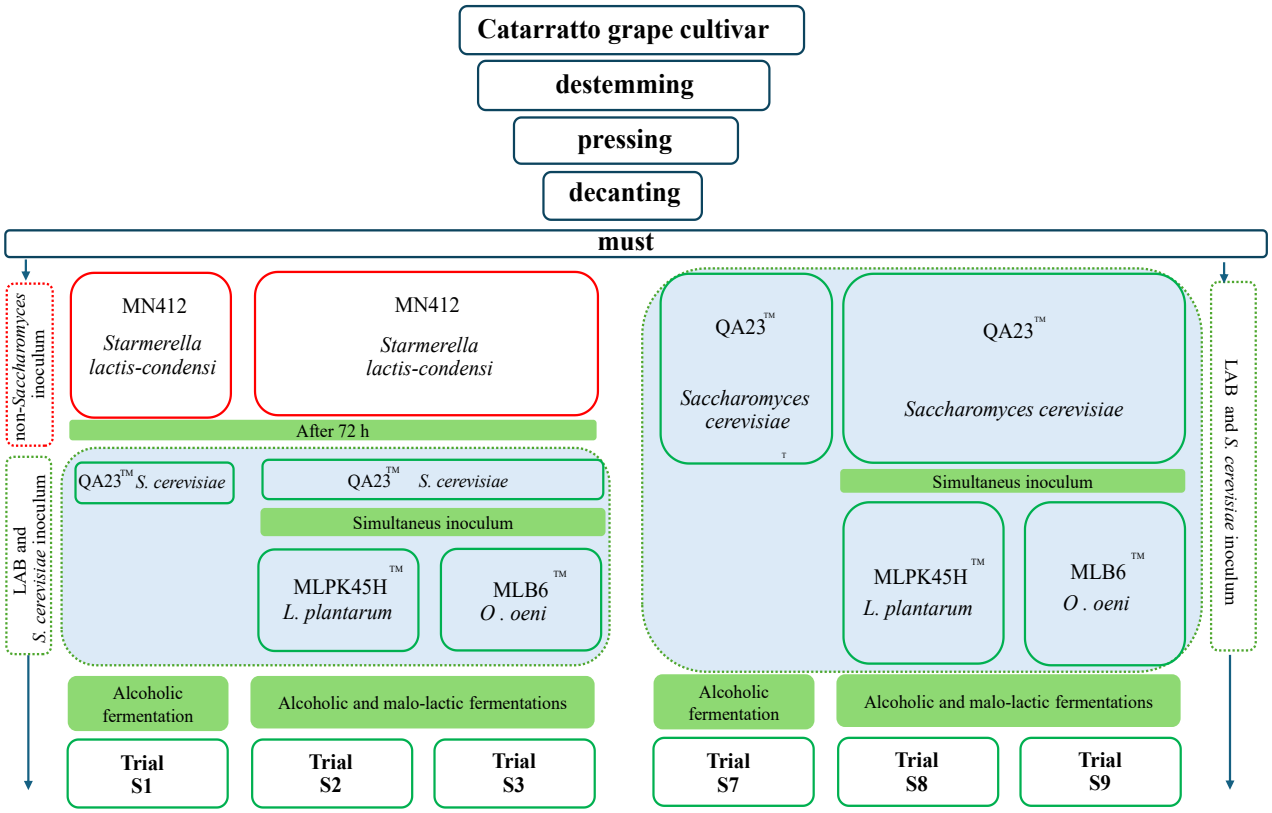


Fig. 1. Experimental plan.

2.4. Physico-chemical analysis

The samples were subjected to centrifugation at 9,000 rpm and 4 °C for 10 minutes. Subsequently, a filtration process utilizing a polyethersulphone membrane (with a pore size of 0.20 μm; VWR®) was employed to eliminate any suspended particles present in the musts. The CO_2 stripping procedure was conducted under a vacuum to minimize potential errors in instrumental readings. The pH, total acidity, ethanol and acetaldehyde levels were determined utilizing an FOSS-WineScanTM Flex system (FOSS, Hillerød, Denmark), in accordance with the methodology outlined in OIV Res. Oeno 390/10 All.2. The values of L-malic, L-lactic and acetic acids, together with the reducing sugars, glucose and fructose, and glycerol, were measured using an iCubio iMagic M9 enzyme analyser (Shenzhen iCubio Biomedical Technology Co. Ltd., Shenzhen, China), as reported by Matraxia et al. (2021). The same analytical instrument was employed to quantify acetaldehyde in musts (Morata et al., 2003).

2.5 Volatilome analysis

Two separate forms of high-performance gas chromatography were used to quantify volatile compounds, as previously documented (Bordet, Romanet, Ferreira, ... & Alexandre, 2024; García-Carpintero, Sánchez-Palomo, Gallego, & González-Viñas, 201 Compounds with high volatility, such as esters, were quantified by headspace solid-phase microextraction (Rodrıguez-Bencomo et al., 2002; Ortega-Heras. et al 2002). In contrast, low-volatile compounds such as alcohols, ketones, carboxylic acids and aldehydes were determined by liquid-liquid extraction, including (Ortega-Heras., et al 2002). Both techniques were applied for determination by gas chromatography with mass spectrometry.

2.5.1. Standard solutions

2.5.1.1 Liquid analysis

Individual standard of each compound: 2,3-butanediol (standard for the alcoholic fraction), acetoin (standard for the carboxylic function fraction) and ethyl lactate (standard for the ester fraction) were purchased from Sigma-Aldrich (82024 Taufkirchen, Germany). n-Alkane standards (C8 to C40) were purchased from Aldrich Chemical Co. (St. Louis, Mo., USA). Standard solutions of each compound were prepared at five different concentrations (56 ppm, 112 ppm, 225 ppm, 262 ppm, 450 ppm for 2,3-butanediol, 79 ppm, 134 ppm, 224 ppm, 326 ppm, 477 ppm for acetoin and 46 ppm, 65 ppm, 116 ppm, 173 ppm, 290 ppm for ethyl lactate).

2.5.1.2. SPME analysis

Ethyl octanoate (standard for the ester fraction) and 2 heptanol (standard for the alcoholic fraction), (Fisher Scientific S.L.C, 28108 – Alcobendas-Madrid) were used as standard for calibration line. Standard solutions were prepared at five different concentrations (38 ppm, 82 ppm, 138 ppm, 170 ppm, 250 ppm for ethyl octanoate and 14 ppm, 62 ppm, 100 ppm, 159 ppm, 222 ppm for 2 heptanol).

2.5.2. Analysis of VOCs in wine samples

2.5.2.1. Liquid-liquid extraction

Volatile compound composition was determined following this protocol. Wine samples (10 mL) from all trials were mixed with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask, stirred at room temperature for 30 min, and centrifuged at 4000 rpm for 10 min by Low Speed Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS Industrivej 6–8, Vassingerød, DK-3540 Lynge, Denmark). The aqueous phase was removed and was added anhydrous sodium sulphate (1 g) before centrifuging at 4000 rpm for 5 min. The dichloromethane layer was removed and dried under N_2 gas to 0,3 mL.

2.5.2.2. SPME analysis

Wine samples (10 mL) were placed in a 20 mL SPME glass vial (Gerstel, 75.5 x 22.5mm) together with 1 g of sodium chloride**.**

2.5.3. Identification and quantification of VOCs by GC-MS

Gas chromatographic analyses were performed with Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 °C. Solvent Delay: 3.5 min. Helium was the carrier gas (1 mL/min).

2.5.3.1 Liquid analysis

The temperature was initially maintained at 40 °C for 1 min. Then it was gradually increased to 250 °C at a rate of 3 °C /min for 30 min and finally maintained at 250 °C at 10 °C /min. One µL of sample was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C. The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra. Quantification was carried out using the three calibration lines. For compounds belonging to other classes than the standards, similarity was used for quantification. A dilution factor was used for the reported data.

2.5.3.2 SPME analysis

The column temperature was initially kept constant at 40 °C for 2 min (during splitless injection), subsequently, increasing the temperature by 4 °C/min was set to 60 °C, at which it was kept constant for 2 min. Increasing the temperature by 2 °C/min., it was raised to 90 °C, from 190 °C to 230 °C, increasing by 5 °C/min and finally left at 230 °C for 15 min. The analytes in the fiber were

automatically injected at 250 °C with the splitless mode. The mass spectrometer was set in MS mode in order to acquire all mass-to-charge ratios from 35 to 450 amu (0.1 amu). Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra. Quantification was carried out using limonene calibration line.

2.6. OAV elaborations

In order to ascertain which VOCs were exerting a significant influence on the wine's aroma profile, the concentrations detected were transformed into odour activity units (OAV) using the methodology described by Butkhup et al. (2011).

2.7. Relationship indication values "RI- values" of OAVs

The relationship indicator values of the active volatile components in wine were determined using the following formula (Henderi, Wahyuningsih, & Rahwanto, 2021):

$$RI\ value = \frac{Xi - \mu}{\sigma}$$

Where:

RIvalue = relationship indicator value single VOC (OAV>1);

Xi = VOC to be normalised within distribution 'x'; (expressed in OAV);

(x = distribution of OAV>1; entire active aroma profile of the wine);

μ = mean value of the VOC distribution with OAV>1 (expressed in OAV);

σ = mean square deviation of the values of the VOC distribution with OAV>1 (expressed in OAV).

2.8 Non-volatile metabolome analysis

Once the AF was complete, the wine samples were analysed using ultra-high-pressure liquid chromatography (Dionex Ultimate 3000, Thermo Fisher Scientific, Waltham, MA USA) coupled to a MaXis plus MQ ESI-Q-ToF mass spectrometer (Bruker, Bremen, Germany). Non-polar compounds

were analyzed in reverse phase using Acquity BEH C18 1.7 %m, 100 × 2.1 mm column (Waters, Guyancourt, France). The mobile phase was ultrapure water from a Milli-Q system (Merck, Darmstadt, Germany) acidified by the addition of 0.1% (v/v) formic acid (MS grade from Acros A. Palud et al. Food Microbiology 117 (2024) 104386 3 Organic, Morris Plains, NJ, USA) for eluent A, 95% (v/v) acetonitrile (MS grade from Biosolve, Dieuze, France) acidified by the addition of 0.1 (v/v) formic acid for eluent B. The elution temperature was 40 ◦C following a gradient: 0–1.10 min 5% (v/v) of eluent B and 95% of eluent B at 6.40 min. The flow rate was 400 µl min^{-1} . The nebulizer pressure was 2 bar and a nitrogen dry gas flow of 10 L/min was maintained. Ionization was performed by electrospray in positive and negative ion mode. The ion transfer parameters and the injection of calibrant at the beginning of each run was performed according to Evers et al. (2023). To verify the stability of the UHPLC-Q-TOF-MS system, quality controls (mixing of all the samples analyzed in the batch analysis) were analyzed at the beginning, the end and every 10 samples during the batch analysis. All the samples were analyzed randomly. Compass DataAnalysis v4.3 software (Bruker, Bremen, Germany) was used for pre-treatment analysis as developed by Evers et al. (2023). Isolated significant features were annotated using the online database of Metlin, KEGG, YMDB, and online tools, MassTrix and Oligonet. Identification confidence was determined according to Schymanski et al., 2014.

2.9 Sensory analysis

The sensory profiles of the wines were evaluated by submitting the different trials to a trained panel of judges, as outlined by Jackson (2022). A 15-member panel of judges, comprising eight women and seven men with an average age of 28 years, was initially subjected to an organoleptic evaluation. The organoleptic profiles of the wines were established by three different wine tasting committees on three separate occasions, in accordance with the methodology described by Alfonzo et al. (2020). The intensity of the different descriptors was quantified on a nine-point scale. The sensory analysis was carried out in accordance with the protocol outlined by Naselli et al. (2023).

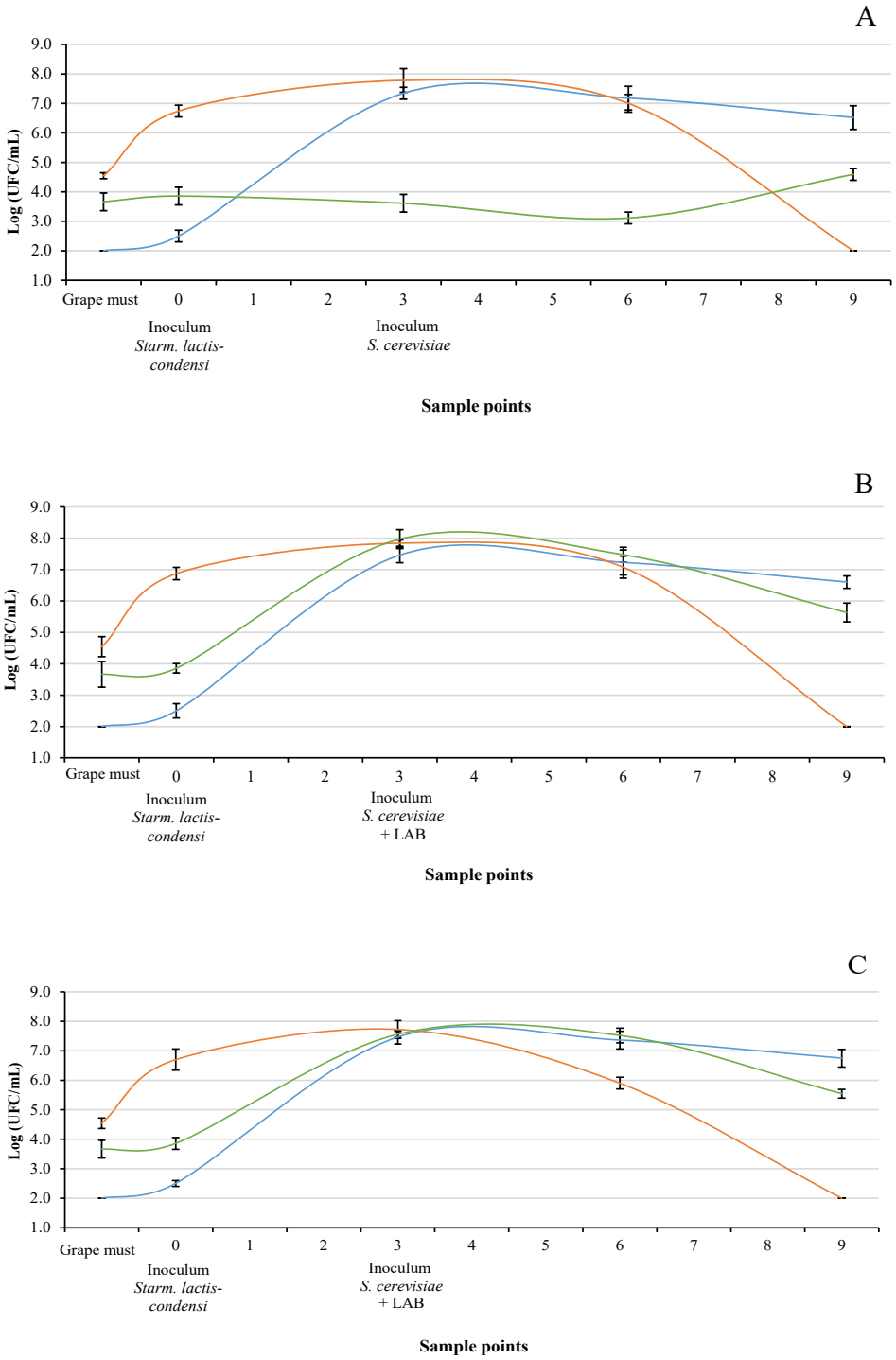
3.0. Statistical analysis

The ANOVA test was employed to ascertain the statistical significance of the discrepancies between the technological, microbial, VOC, and sensory characteristics observed in the various tests. Additionally, the Tukey's test was utilized to compare the disparate data sets, with significance determined by $P < 0.05$. The interrelationships of the VOCs, sensory attributes and biotechnological associations were established through agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) (Naselli et al., 2023). The normalized data set was subjected to PCA and cluster analysis. The web-based Metabo-Analyst tool (http://www.metaboanalyst.ca/) was used for metabolomic data processing (Sun, Lu, Joseph, Ma, ...& Liu, 2024). Interquartile range (Liang, Lin, Chen...& Yang, 2016) and pairwise t-tests (p-value < 0.001) were used to search for biomarkers (Lebleux, Alexandre, Romanet... & Rouiller-Gall, 2023). For statistical analysis, data were normalised to the median and scaled using the Pareto scale. The distance measure for cluster analysis was Euclidean.

3.3 Result and discussion

3.3.1 Microbial growth dynamics

The viable cell counts obtained during the fermentation process are presented in Fig. S1.



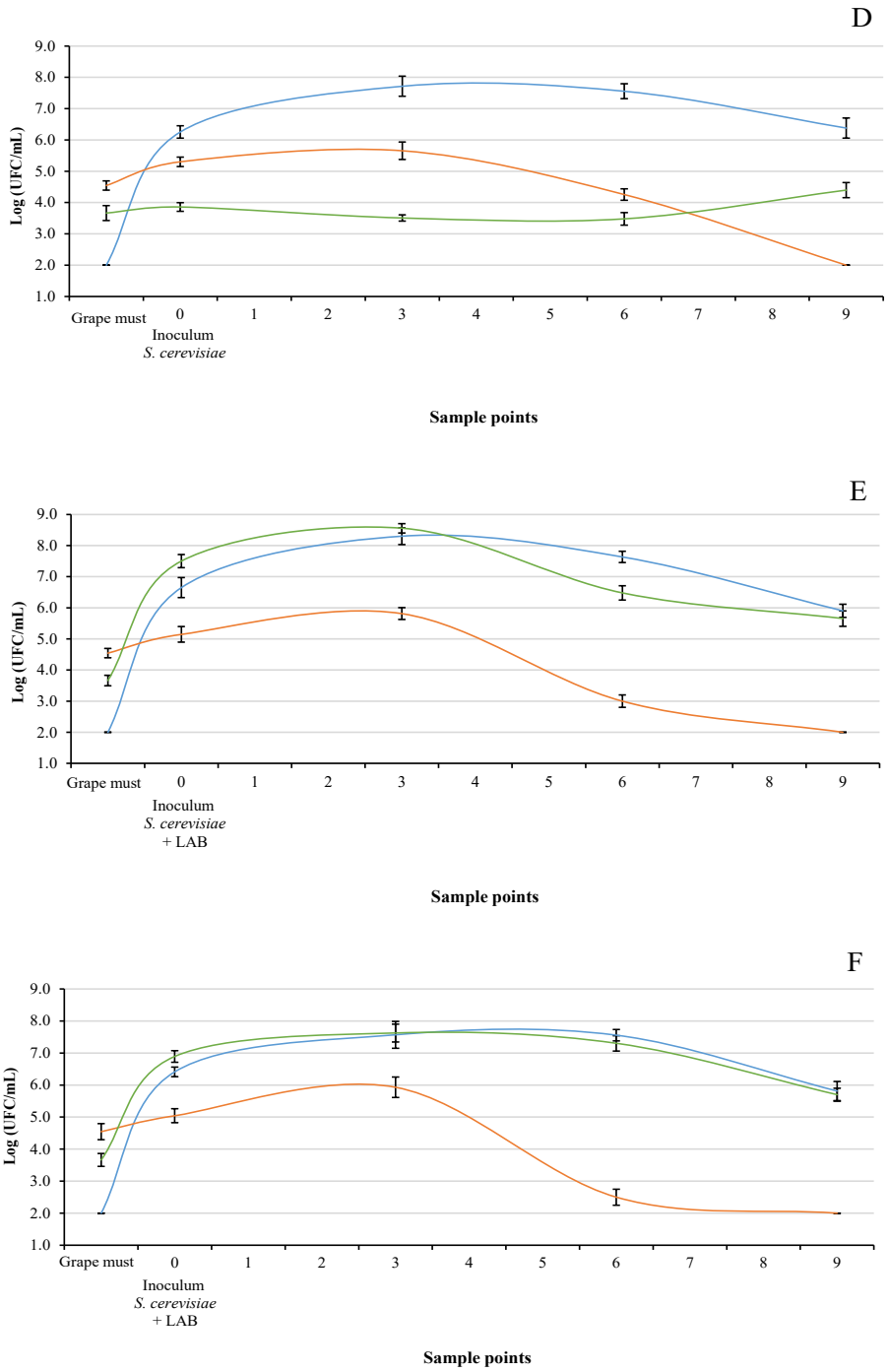


Fig. S1. Evolution of yeast populations of presumptive *Saccharomyces cerevisiae*, non-*Saccharomyces* and Lactic acid bacteria populations during alcoholic and malolactic fermentation: A, sequential inoculum *Starmerella lactis-condensi* MN412 - *Saccharomyces cerevisiae* QA23 strain (S1); B sequential inoculum *Starmerella lactis-condensi* MN412 strain – *Lactiplantibacillus plantarum* MLPK45H strain /*Saccharomyces cerevisiae* QA23 strain (S2); C, sequential inoculum *Starmerella lactis-condensi* MN412 strain – *Oenococcus oeni* MLB6 strain/*Saccharomyces cerevisiae* QA23 strain (S3); D, single inoculum *Saccharomyces cerevisiae* QA23 strain (S7, control); simultaneum inoculum *Lactiplantibacillus plantarum* MLPK45H strain /*Saccharomyces cerevisiae* QA23 strain (S8); *Oenococcus oeni* MLB6 strain /*Saccharomyces cerevisiae* QA23 strain (S9). Legend: (blue)—,presumptive *Saccharomyces*; (orange)—, presumptive non-*Saccharomyces*; (green)—, presumptive lactic acid bacteria.

Prior to inoculation, the musts exhibited microbial loads of 2.00, 4.54 and 3.44 Log colony-forming units per milliliter (CFU/mL) for *Saccharomyces* spp, non-*Saccharomyces* spp and LAB spp, respectively. Following the inoculation of strain MN412, the cell density in the musts exhibited a range of values between 6.74 and 6.88 Log CFU/mL. The number of colonies achieved is sufficient for the sensory characterisation of wines using non-*Saccharomyces*.

The microbial growth of *Starm. lactis-condensi* MN412 strain was characterised by an absence of the Lag phase. (Fig. S1 A, B, C). This behaviour suggests an easy implantation of *Starm. lactis-condensi* MN412 strain in Catarratto grape must; also confirmed by the rapid degradation of sugars (Table S1).

Table S1

Chemical and physical parameters measured at day 0 (non-*Saccharomyces* inoculum) and day 3 of fermentation (LAB-*S. cerevisiae* inoculum).

Parameter	U.M.	Winemaking 0 day must	3 days of fermentation S1	S2	S3	S7	S8	S9	S.S.
pH		3.25 ± 0.01 [a]	3.25 ± 0.01 [a]	3.25 ± 0.01 [a]	3.25 ± 0.01 [a]	3.25 ± 0.01 [a]	3.25 ± 0.01 [a]	3.25 ± 0.01 [a]	n.s.
Total acidity	g/L tartaric acid	6.21 ± 0.03 [a]	6.18 ± 0.04 [ab]	6.13 ± 0.03 [b]	6.16 ± 0.02 [ab]	6.18 ± 0.03 [ab]	5.75 ± 0.03 [c]	5.72 ± 0.05 [c]	***
Total sugar	g/L	261.81 ± 3.41 [a]	163.76 ± 4.76 [cd]	161.26 ± 4.81 [cd]	165.34 ± 7.74 [c]	184.04 ± 8.44 [b]	176.70 ± 4.57 [b]	153.57 ± 7.97 [d]	***
Fructose	g/L	156.15 ± 2.31 [a]	65.77 ± 2.46 [e]	63.46 ± 3.46 [e]	67.22 ± 3.59 [e]	119.33 ± 6.55 [b]	110.53 ± 6.04 [c]	103.01 ± 4.51 [d]	***
Glycerol	g/L	0.00 ± 0.01 [f]	9.18 ± 0.01 [b]	9.23 ± 0.01 [a]	9.16 ± 0.02 [b]	5.58 ± 0.02 [e]	6.41 ± 0.02 [d]	6.91 ± 0.03 [c]	***
Acetic acid	g/L	0.00 ± 0.00 [c]	0.04 ± 0.02 [b]	0.06 ± 0.03 [ab]	0.05 ± 0.02 [ab]	0.06 ± 0.02 [ab]	0.06 ± 0.02 [ab]	0.07 ± 0.01 [a]	***
L-malic acid	g/L	1.73 ± 0.03 [a]	1.69 ± 0.02 [b]	1.56 ± 0.01 [c]	1.68 ± 0.02 [b]	1.67 ± 0.02 [b]	0.24 ± 0.02 [e]	1.16 ± 0.02 [d]	***
L-lactic acid	g/L	0.00 ± 0.00 [d]	0.01 ± 0.00	0.09 ± 0.01 [c]	0.01 ± 0.00 [d]	0.01±0.00 [d]	1.01 ± 0.04 [a]	0.26 ± 0.02 [b]	***
Acetaldehyde	mg/L	0.00 ± 0.00 [c]	2.63 ± 0.04 [b]	2.71 ± 0.06 [b]	2.58 ± 0.07 [b]	12.73 ± 0.52 [a]	12.94 ± 0.63 [a]	13.58 ± 0.78 [a]	***
Ethanol	% v/v	0.00 ± 0.00 [f]	4.55 ± 0.01 [cd]	4.57 ± 0.01 [c]	4.53 ± 0.01 [de]	4.53 ± 0.01 [e]	5.07 ± 0.01 [b]	6.42 ± 0.01 [a]	***

Result indicates mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test.

Abbreviations:

S.S., statistical significance; P value: ***, $P < 0.001$; n.s., not significant.

Table S2

Chemical and physical parameters measured at day 6 of alcoholic fermentation corresponding to day 3 of malolactic fermentation.

Parameter		Winemaking 6 days of fermentation						
	U.M.	S1	S2	S3	S7	S8	S9	S.S.
pH		3.25 ± 0.02 [a]	3.26 ± 0.01 [a]	3.27 ± 0.01 [a]	3.25 ± 0.01 [a]	3.26 ± 0.01 [a]	3.27 ± 0.01 [a]	n.s.
Total acidity	g/L tartaric acid	6.16 ± 0.03 [a]	5.82 ± 0.03 [b]	5.75 ± 0.02 [b]	6.16 ± 0.03 [a]	5.81 ± 0.02 [b]	5.63 ± 0.04 [c]	***
Total sugar	g/L	24.48 ± 0.18 [c]	28.78 ± 0.49 [b]	33.89 ± 1.40 [a]	22.21 ± 1.38 [c]	9.93 ± 0.49 [e]	13.12 ± 0.40 [d]	***
Fructose	g/L	9.44 ± 0.81 [cd]	9.95 ± 1.08 [cd]	20.63 ± 1.52 [a]	17.71 ± 1.08 [b]	8.95 ± 0.48 [d]	12.37 ± 0.52 [c]	***
Glycerol	g/L	12.12 ± 0.16 [a]	12.68 ± 0.18 [a]	11.38 ± 0.04 [a]	9.02 ± 0.16 [b]	9.26 ± 0.06 [b]	9.20±0.04 [b]	***
Acetic acid	g/L	0.19 ± 0.03 [b]	0.24 ± 0.01 [ab]	0.27 ± 0.01 [a]	0.24 ± 0.03 [ab]	0.26 ± 0.01 [a]	0.28 ± 0.01 [a]	**
L-malic acid	g/L	1.67 ± 0.03 [a]	0.12 ± 0.01 [cd]	0.57 ± 0.02 [b]	1.68 ± 0.05 [a]	0.20 ± 0.02 [c]	0.11±0.02 [d]	***
L-lactic acid	g/L	0.01 ± 0.00 [d]	1.09 ± 0.05 [a]	0.68 ± 0.03 [c]	0.01 ± 0.00 [d]	1.06 ± 0.04 [a]	0.95±0.04 [b]	***
Acetaldehyde	mg/L	8.27 ± 0.35 [c]	10.34 ± 0.78 [c]	9.71 ± 0.43 [c]	48.62 ± 1.16 [a]	47.23 ± 1.34 [a]	39.12 ± 1.14 [b]	***
Ethanol	% v/v	11.95 ± 0.01 [d]	11.46 ± 0.01 [f]	11.64 ± 0.01 [e]	12.38 ± 0.01 [c]	12.91 ± 0.01 [a]	12.73 ± 0.01 [b]	***

Result indicates mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test.
Abbreviations:
S.S., statistical significance; P value: ***, $P < 0.001$; **, $P < 0.01$ n.s., not significant.

Table S3

Chemical and physical parameters measured at day 9 of alcoholic fermentation corresponding to day 6 of malolactic fermentation.

Parameter	U.M.	Winemaking End fermentation S1	S2	S3	S7	S8	S9	S.S.
pH		3.27 ± 0.02 [a]	3.29 ± 0.01 [a]	3.31 ± 0.01 [a]	3.25 ± 0.01 [a]	3.30 ± 0.01 [a]	3.31 ± 0.01 [a]	n.s.
Total acidity	g/L tartaric acid	6.13 ± 0.03 [a]	5.84 ± 0.02 [b]	5.64 ± 0.03 [c]	6.14 ± 0.02 [a]	5.82 ± 0.02 [b]	5.62 ± 0.02 [c]	***
Total sugar	g/L	0.76 ± 0.06 [a]	0.79 ± 0.08 [a]	0.81 ± 0.04 [a]	0.82 ± 0.04 [a]	0.83 ± 0.03 [a]	0.83 ± 0.07 [a]	n.s.
Fructose	g/L	0.50 ± 0.02 [b]	0.28 ± 0.04 [c]	0.59 ± 0.02 [b]	0.72 ± 0.05 [a]	0.76 ± 0.04 [a]	0.80 ± 0.01 [a]	***
Glycerol	g/L	12.48 ± 0.07 [b]	12.92 ± 0.02 [a]	11.62 ± 0.03 [c]	9.23 ± 0.03 [d]	9.34 ± 0.02 [d]	9.25 ± 0.13 [d]	***
Acetic acid	g/L	0.21 ± 0.03 [c]	0.28 ± 0.01 [ab]	0.31 ± 0.02 [ab]	0.26 ± 0.02 [bc]	0.31 ± 0.02 [ab]	0.32 ± 0.01 [a]	n.s.
L-malic acid	g/L	1.64 ± 0.02 [a]	0.12 ± 0.00 [b]	0.12 ± 0.02 [b]	1.62 ± 0.04 [a]	0.18 ± 0.02 [b]	0.11 ± 0.02 [b]	***
L-lactic acid	g/L	0.01 ± 0.00 [d]	1.09 ± 0.05 [a]	0.98 ± 0.02 [bc]	0.01 ± 0.00 [d]	1.07 ± 0.04 [ab]	0.95±0.04 [c]	***
Acetaldehyde	mg/L	16.36 ± 0.81 [a]	18.64 ± 1.42 [a]	17.96 ± 0.60 [a]	33.17 ± 1.30 [b]	35.62 ± 1.59 [b]	34.03 ± 1.29 [b]	***
Ethanol	% v/v	13.38 ± 0.01 [c]	13.26 ± 0.01 [d]	13.52 ± 0.02 [b]	13.63 ± 0.01 [a]	13.54 ± 0.01 [b]	13.52 ± 0.01 [b]	***

Result indicates mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test.

Abbreviations:

S.S., statistical significance; P value: ***, $P < 0.001$; n.s., not significant.

The intervals in question occurred at microbial loads between 7.34 and 7.18 CFU/mL between the third and sixth fermentation days (Fig. S1 A, B, C). From the third fermentation day onwards, the population of non-*Saccharomyces* began to decrease in the S1, S2 and S3 samples. This result is consistent with the findings of Binati et al. (2020). However, the growth of the non-*Saccharomyces* population, attributable to strain MN412, in the S3 trial was characterised by an early onset of decline (Fig. XX). The decline observed in the MN412 population is attributable to trophic competition exerted by the *O. oeni* strain, MLB6™, as proposed by Wang et al. (2019). Simultaneous inoculation of LABs (*L. plantarum* MLPK45H™ strain; and *O. oeni* MLB6™ strain) with *S. cerevisiae* (QA23™ strain), carried out 72 h after inoculation of *Starm. lactis-condensi*, resulted in a cell load of 7.97 and 7.46 CFU/mL (test S2) versus 7.56 and 7.46 CFU/mL (test S3) for LAB and *S. cerevisiae*, respectively. The comparability of the evolutionary dynamics of the microbial populations of LAB and *S. cerevisiae* between tests S2, S3 *vs* S8, S9 (control tests) suggests optimal compatibility of LABs and *S. cerevisiae* QA23™ strains with *Starm. lactis-condensi* MN412 strain. This hypothesis is further supported by the temporal correspondence of the malic acid degradation times between the two sets of tests, with and without *Starm. lactis-condensi*. In fact, tests S2, S3 complementary to control tests S8 and S9, were distinguished by a complete malolactic fermentation development in 3 and 6 days respectively from the simultaneous inoculation of LABs and *S. cerevisiae*. It is possible that the affinities, LABs *vs Starm. lactis-condensi*, result from metabolites produced by *Starm. lactis-condensi* itself. Englezos et al. (2018) report that the genus *Starmerella* is able to release amino acids into the culture medium via the glyceraldehyde-tri-phosphate pathway. It is therefore possible that LABs, and in particular *O. oeni*, exploit this distinctive trait of *Starmerella*. Indeed, studies have demonstrated that amino acid compounds are essential for *O. oeni* to overcome cellular stresses derived from the medium (Guerrini, Mangani, Granchi, & Vincenzini, 2002; Lonvaud-Funel, 2001). However, although LABs may benefit from the metabolites of *Starm. lactis-condensi* MN412 strain, the latter did not show a particular affinity towards *O. oeni* MLB6™ strain. In fact, cell growth dynamics in the S3 trials showed a decline of MN412 strain upon inoculation of *O. oeni* MLB6™ in

consociation with QA23™ strain (Fig. S1 C). This would also explain the lower glycerol concentrations derivable from the S3 strain (Tables S1, S2, S3). At the end of the fermentation process, in both experimental sets, the non-Saccharomyces population was found to be below the detection limit, with a count of less than 2 log CFU/mL, as reported by Binati et al. (2020).

3.3.2 Chemical monitoring

The chemical and physical parameters resulting from the fermentations were presented in Tables S1, S2 and S3. The grape must just after pressing was characterized by a pH of 3.25, total acidity of 6.16 g/L tartaric acid, malic acid of 1.73 g/L and a total sugar concentration of 261.76 g/L (Table S1). At 72 hours post-inoculation of *S. cerevisiae*, in the S1, S2 and S3 trials, the *Starm. lactis-condensi* MN412 strain exhibited a preferential degradation of fructose over glucose (Tables S1, S2, S3). Furthermore, trials S1, S2 and S3 demonstrated the highest glycerol concentrations from the third fermentation day onwards, in comparison to trials S7, S8 and S9, which did not include the application of the *Starm. lactis-condensi* strain MN412 (Tables S1, S2, S3). The aptitude of the genus *Starmerella* for preferential fructose degradation and increased glycerol productivity has been previously described by several other authors (Oliva et al., 2012; Lemos Junior et al., 2018; Englezos et al., 2018; Francesca et al., 2024).

The bioavailability of glucose and glycerol in the must, on the third fermentation day (Table S1), as a technological effect of the *Starm. lactis-condensi* MN412 strain, may have contributed to limiting the ethanol stresses present at the time of simultaneous inoculation of lactic acid bacteria (LAB) and *S. cerevisiae* strains (Du et al., 2012; D'Amato et al., 2006). Consequently, these technological conditions may have contributed to the rapid adaptation of S. *cerevisiae* and LABs, as well as to the enhanced performance of the latter. This hypothesis was developed based on the observation that the malic acid degradation times observed for the *L. plantarum* MLPK45H™ strain and the *O. oeni* MLB 6™ strain were similar. Consequently, the ethanol content in the medium did not seem to influence the metabolic activity of the LAB strains. In contrast to the results of Englezos et al. (2018) on

Starmerella bacillaris, the use of the *Starm. lactis-condensi* MN412 strain resulted in no pH deviation (Table S1). This suggests that this peculiarity is species-dependent. Specifically, as Englezos et al. (2018) have previously observed, the pH deviations observed in their study were likely due to the significant pyruvic acid production observed in the tested samples. The absence of pH deviations observed in the S1, S2 and S3 trials indicate that this strain is capable of directing a significant portion of the pyruvic acid produced by the glycolytic pathway towards alternative metabolic pathways (Yang et al., 2024). Consequently, the concentrations of SO_2 combined with the carbonyl groups of pyruvate could be lower when MN412 strain is employed (Ochando et al., 2020; Blasi et al., 2007). The same effects could result from the reduced acetaldehyde concentrations observed in microbial consortia utilizing *Starm. lactis-condensi* (Table S8). The significant differences in acetaldehyde concentrations observed between the two inoculation protocols (with and without *Starm. lactis-condensi*) may be attributed to the preference of *Starm. lactis-condensi* for the reduction of dihydroxyacetone phosphate to glycerol 3-phosphate, which serves to restore the redox potential within the cell (Englezos et al., 2018). This metabolic mechanism would result in the increased production of glycerol at the expense of acetaldehyde, which would then be converted to ethanol (Wang et al., 2001). This hypothesis would also explain the lower ethanol production observed in S1, S2 and S3 trials. In particular, the MN412 - MLPK45H™/QA23™ microbial consortium would have resulted in a reduction in the ethanol concentration in solution by 0.37% (v/v). This is in line with the findings of Francesca et al. (2024).

3.3.3 Non-volatile metabolome analysis

Using UHPLC-q-ToF-MS analysis, a total of 8437 features were detected in samples wine (Fig. 2A), of which 5000 were extracted based on interquantile range (Fig. 2B) (Liang, Lin, Chen…& Yang, 2016).

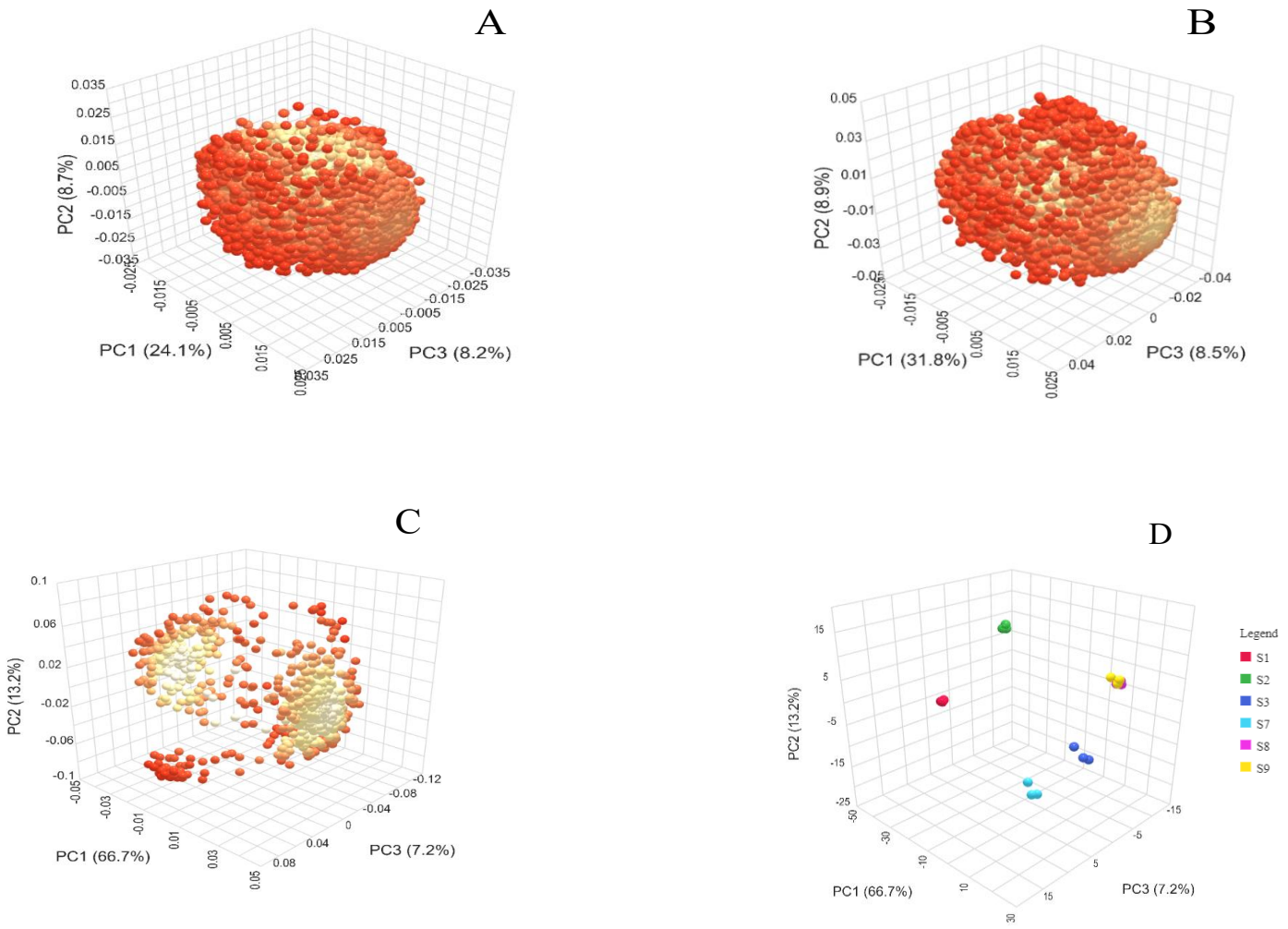


Fig.2. – Total metabolome detected in samples wine (8437 features) (A); total metabolome subtracted from features of equal intensity from the Catarratto grape and biotechnology consociation (5000 features) (B); metabolic biomarkers from biotenologies (879 features) (C); PCA analysis of experimental studies based on the 879 identified metabolic biomarkers (D)

This reduction in features has eliminated many metabolic interferents of equal intensity from the Catarratto grape and biotechnology consociations (Lebleux, Alexandre, Romanet…& Rouiller-Gall, 2023). Subsequently, a statistical analysis was performed (t-test, p-value ≤ 0.001) to reveal the significantly more intense features (Bordet, Roullier-Gall, Ballester, ... & Hlexandre, 2021). Of the 5000 features (Fig. 2B), 4121 were found to be common to the different inocula in the experimental plan. The 879 characteristics identified as statistically highly significant may be considered the biomarkers of each microbial consortium adopted (Fig. 2C). These biomarkers were represented in the PCA (Fig. 2D) and heatmaps (Fig. 3A). The PCA analysis was employed to elucidate the compositional characteristics of the wines based on their metabolomic footprint. The combination of principal components (PC) 1, 2 and 3 (Fig. 2D) accounted for 87.1% of the total variance of the data set. The PC1 axis (Fig. 3A) allows us to explain the different effects attributable to the presence-absence of the *Starm. lactis-condensi* MN412 strain among the different microbial consociations; this was explained by 66.7% variance. The contribution of the PC2 axis (Fig. 3A) explains with 13.2% variance the different contribution of the non-*Saccharomyces* - LAB strains interactions. Whereas, the PC3 axis explains with 7.2 % of the variance the incidence of *S. cerevisiae*-LAB interactions. Based on the different arrangements of the trials (Fig. 2D), it is noteworthy that, in the non-volatile fraction of the wine, the use of *Starm. lactis-condensi* made it possible to distinguish the contribution of the different LAB species (*O. oeni and L. plantarum*) used in the microbial consortia. However, the use of heatmaps as a graphical representation of metabolomic fingerprinting made it possible to explain the effects of the presence and absence of *Starm. lactis-condensi* between the experimental trials (Fig. 3A). The S1 and S2 tests clustered together. This effect suggests that the presence of *L. plantarum* MLPK45H™ strain in the microbial cluster does not metabolically interfere with *Starm. lactis-condensi* MN412strain. However, the effects of the *L. plantarum* MLPK45H™ strain - *S. cerevisiae* QA23™ strain interaction with *Starm. lactis-condensi* were evident within the cluster of featuers one, in which two compounds were identified (Fig. 3B α and β).

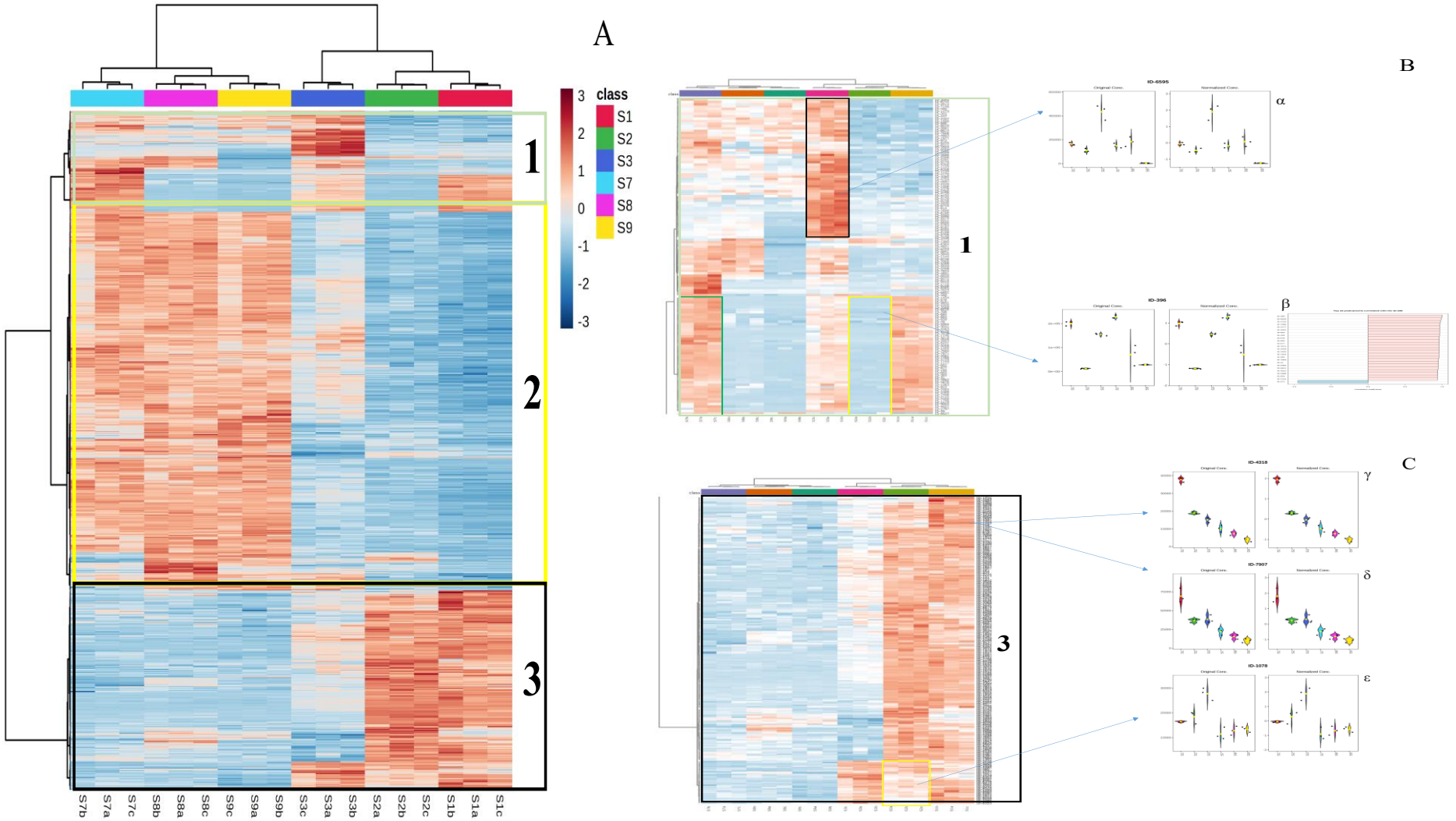


Fig. 3. - Heatmap of the 879 features (biomarkers) detected in negative and positive modes (A). The normalized median values of each triplicate were used to color the heatmap. Clustering based on Euclidean distances between samples and variables. Enlargement cluster one and violin plot feauteres: α, (neamine), β, (5-valerolactone); (B). Enlargement cluster three and violin plot feuteres; γ, (trans-resveratrol); δ, (resveratrol-2-O-beta glucopyranoside); ε, (N-carbamoylputrescine); (C

A metabolomic fingerprint fraction of 48 traits in test S2 was of lower intensity than in test S7 (Fig. 3B yellow box *vs* green box).

In this group of features, 5-valerolactone (ID-396; mass, 100.0524 Da; brute formula $C_5H_8O_2$; rit.t.: 1.43 min) was identified (Fig. 3B β). This compound was negatively correlated with hydroxypyruvic acid (ID-471; mass, 104.011 Da; brute formula $C_3H_4O_4$; rit. t.: 0.68 min), which is involved in the metabolism of glycine, serine and threonine. It is noteworthy that, among the biotechnological and Catarratto grape must interactions, the use of *Starm. lactis-condensi*, in consociation with the *S. cerevisae* QA23™ strain, contributed to the highest levels of intensity of trans-resveratrol characteristics (ID-4318; mass, 228. 0781 Da, brute formula $C_{14}H_{12}O_3$; rit.t.: 3.38) (Fig. 3C γ) and resveratrol-2-O-beta glucopyranoside (ID-797; mass, 390.1240 Da, brute formula $C_{20}H_{22}O_8$; rit. t.: 3.38) (Fig. 3C δ). For the first time, these compounds are reported in Catarratto. Although the hydroxystilbenes can be traced back to the secondary metabolisms of *Vitis* spp. (Marant, Crouzet, Flourat, ...& Courot, 2024), the different intensities of the features identified among the different theses suggest the degradative role played by the biotechnological component towards these non-flavonoids. The use of *Starm. lactis-condensi* allowed for its maximum reserve in its active antioxidant, trans, and picied, bound form. Test S3 was distinguished from tests S1 and S2 in a single cluster. A group of 36 features (Fig. 3C - yellow box) within cluster number three enabled the metabolic fractions affected by the *O. oeni* - *Starm. lactis-condensi* interaction to be distinguished. Among the most intense compounds was identified N-carbamoylputrescine (ID-1078; mass, 131.1056 Da; brute formula $C_5H_{13}N_3O$; t. rit.: 0.70 min) (Fig. 3C ε), a metabolic intermediate of putrescine (Mete, Coşansu, Demirkol, & Ayhan, 2017). This result is in accordance with the assumptions made in section 4.1. The bioavailability of certain amino acid components in the medium, enabled by the co-presence of *Starm. lactis-condensi*, allows the activation of metabolic pathways capable of counteracting cellular stress in *O. oeni*, thereby increasing the compatibility of *O. oeni* with *Starm. lactis-condensi*.

Furthermore, the identification of 35 high-intensity features in cluster number one (Fig. 3B – black box) permitted the differentiation of S3 trials from S2 and S1. This cluster was composed of several nitrogen molecules, including neamine (ID-6595; mass, 322.1865 Da, brute formula $C_{12}H_{26}N_4O_6$; t. rit.: 4.50) (Fig. 3B α). Violin plot analysis attributes the formation of neamine to the *O.oeni-Starm. lactis-condensi* metabolic interaction (Fig. 3B α). It is probable that the antibacterial action carried out by neamine (Zimmermann, Bussière, Ouberai... & Décout, 2013) represents a form of counteracting the colonization of the *Starm. lactis-condensi* medium. However, it is noteworthy that the presence of neamines in the must had no side effect in the primary course of malic acid degradation of *O. oeni* MLB6™ strain. Finally, cluster 2 (Fig. 3A) distinguished between use and non-use of *Starm. lactis-condensi*. Tests S1, S2 and S3 were exhibited lower feature intensities than tests S7, S8 and S9, attributable to peptidides with masses between 117.0789 and 578.1399 Da.

3.3.4 Volatile organic compounds composition

Table 1 presented the results of VOC detection. Alcohols (54.69% - 73.67%), esters (17.02% – 39.55%), carboxilic acid (2.80% – 8.19%), aldehydes (1.13% - 2.38%), ketones (0.94% - 2.47%) and other minor VOCs (0.21% - 1.09%) represented the total volatile fraction of wine (Fig. S2). Alcohols were the most representative aromatic class in VOC profiles (Ribéreau-Gayon, 2006) (Table 1). The microbial consociations adopted had a significant influence on the number and diversity of VOCs per individual trials (Fig. 4). However, those of amino acid origin were the most frequently identified compounds (Table S4). The formation of these compounds is attributed to the Ehrlich pathway of *S. cerevisiae* (Hazelwood et al., 2008; Li et al., 2024). The different production of higher alcohols between the trials (Table S4) suggests that the potential amino acid productivity of *Starm. lactis-condensi* MN412 strain is higher (Englezos et al., 2018b) respect *S. cerevisiae* QA23™ strain (Table S4). This hypothesis would be supported by the negative correlation recorded between acetaldehyde and the compounds 2-methyl-1-propanol, 1-pentanol, 3-ethoxy-1-propanol, hydroxyethylbenzene and 4-hydroxyphenyl ethanol. It would be more beneficial to direct pyruvate toward amino acid synthesis with the *Starm. lactis-condensi* MN412 strain than to continue the reaction and produce acetaldehyde (Englezos et al., 2018).

Table 1

Volatile organic compounds detected in Catarratto experimental wines (all values in mg/L).

K [α]	K [β]	Volatil organic compounds [γ]	S1 [δ]	S2 [δ]	S3 [δ]	S7 [δ]	S8 [δ]	S9 [δ]	S.S [ε]	→ Continued
1234	1234	**∑ alcohols**	**190.08 ± 3.10** [b]	**195.65 ± 3,95** [a]	**139.67 ± 3.62** [c]	**85.45 ± 1.91** [e]	**118.12 ± 2.85** [d]	**143.85 ± 3.25** [c]	***	
607	602	2-methyl-1-propanol	24.32 ± 0.29 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	***	
665	670	1-butanol	0.69 ± 0.01 [a]	0.00 ± 0.00 [d]	0.62 ± 0.01 [b]	0.43 ± 0.01 [c]	0.40 ± 0.01 [c]	0.60 ± 0.01 [b]	***	
764	765	1-pentanol	93.4 ± 0.97 [a]	87.54 ± 0.81 [b]	71.23 ± 0.71 [d]	51.78 ± 0.56 [f]	65.90 ± 0.72 [e]	76.81 ± 0.76 [c]	***	
-	-	3-methyl-1-hexanol	0.55 ± 0.01 [a]	0.43 ± 0.01 [c]	0.55 ± 0.01 [a]	0.21 ± 0.01 [e]	0.30 ± 0.01 [d]	0.48 ± 0.01 [b]	***	
809	806	2,3-butanediol [ζ]	7.16 ± 0.89 [e]	17.45 ± 2.01 [b]	19.63 ± 2.21 [a]	5.76 ± 0.84 [f]	10.89 ± 1.52 [d]	14.37 ± 1.73 [c]	***	
816	815	2,3-butanediol [η]	9.10 ± 0.08 [b]	23.79 ± 0.21 [a]	7.75 ± 0.07 [c]	1.28 ±0.02 [f]	2.77 ±0.03 [e]	3.68 ± 0.04 [d]	***	
824	822	2,3-butanediol [θ]	6.41 ± 0.06 [b]	12.39 ± 0.14 [a]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	***	
848	849	3-ethoxy-1-propanol	0.58 ± 0.01 [a]	0.52 ± 0.01 [b]	0.32 ± 0.01 [d]	0.42 ± 0.01 [c]	0.54 ± 0.01 [b]	0.59 ± 0.01 [a]	***	
856	859	3-methyl-1-pentanol	0.15 ± 0.01 [bc]	0.15 ± 0.01 [bc]	0.17 ±0.01 [b]	0.14 ±0.01 [c]	0.17 ± 0.01 [b]	0.20 ± 0.01 [a]		
878	878	1-hexanol	1.28 ± 0.02 [c]	2.32 ± 0.05 [a]	1.70 ± 0.03 [b]	0.63 ± 0.01 [f]	0.90 ± 0.02 [e]	1.15 ± 0.02 [d]	***	
983	980	3-methyl-thio-1-propanol	0.29 ± 0.01 [e]	0.64 ± 0.01 [c]	0.34 ± 0.01 [d]	0.36 ± 0.01 [d]	0.68 ± 0.01 [b]	0.72 ± 0.01 [a]	***	
1125	1123	Hydroxyethylbenzene	45.15 ± 0.72 [ab]	47.05 ± 0.67 [a]	35.13 ± 0.53 [c]	23.69 ± 0.41 [d]	34.42 ± 0.49 [c]	43.82 ±0.63 [b]	***	
1162	1160	2-methoxy-4-methyl-phenol	0.58 ± 0.01 [d]	0.85 ± 0.01 [a]	0.62 ± 0.01 [c]	0.36 ± 0.01 [f]	0.44 ± 0.01 [e]	0.69 ± 0.01 [b]	***	
1436	1435	4-hydroxyphenyl ethanol	1.14 ± 0.01 [c]	2.52 ± 0.01 [a]	1.61 ± 0.01 [b]	0.39 ± 0.01 [f]	0.71 ± 0.01 [e]	0.74 ± 0.01 [d]	***	
		∑Aldehydes	**4.90 ± 0.06** [b]	**7.15 ± 0.08** [a]	**2.46 ± 0.05** [e]	**2.08 ± 0.03** [f]	**3.12 ± 0.05** [d]	**4.29 ± 0.04** [c]	***	
1001	1001	Octanal	0.00 ± 0.00 [e]	0.00 ± 0.00 [e]	0.18 ± 0.01 [b]	0.06 ± 0.01 [d]	0.10 ± 0.01 [c]	0.20 ± 0.01 [a]	***	
1225	1224	3,4-dimethylbenzaldehyde	1.65 ± 0.02 [a]	1.51 ± 0.02 [b]	0.55 ± 0.01 [f]	0.77 ± 0.01 [e]	0.96 ± 0.01 [d]	1.31 ± 0.01 [c]	***	
2020	2020	Octadecanal	3.25 ± 0.04 [b]	5.64 ± 0.06 [a]	1.73 ± 0.03 [e]	1.25 ± 0.01 [f]	2.06 ± 0.03 [d]	2.78 ± 0.02 [c]	***	
		∑Ketones	**7.29 ± 0.11** [a]	**2.97 ± 0.06** [c]	**3.04 ± 0.05** [bc]	**1.49 ± 0.03** [e]	**2.47 ± 0.04** [d]	**3.22 ± 0.04** [b]	***	
722	724	3-hydroxy-2-butanone	5.05 ± 0.08 [a]	0.00 ± 0.00 [e]	1.28 ± 0.02 [b]	0.47 ± 0.01 [d]	0.72 ± 0.01 [c]	0.72 ± 0.01 [c]	***	
964	965	4-hydroxy-2-butanone	1.45 ± 0.02 [b]	1.83 ± 0.04 [a]	1.06 ± 0.02 [d]	0.54 ± 0.01 [e]	1.01 ± 0.02 [d]	1.28 ± 0.01 [c]	***	
1285	1284	2-hydroxy-2-methyl-1-phenyl-1-propanone	0.79 ± 0.01 [c]	1.14 ± 0.02 [b]	0.70 ± 0.01 [d]	0.48 ± 0.01 [e]	0.74 ± 0.01 [d]	1.22 ± 0.02 [a]	***	

[α] Kovats index obtained through the modulated chromatogram reported for DB-5 MS apolar column;
[β] Kovats index based on literature (https://webbook.nist.gov/);
[γ] compounds are classified in order of Kovats index;
[δ] Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2.3-butanediol;
[ε] statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: *. P < 0.05; **. P < 0.01; ***. P < 0.001; n.s.. not significant.
[ζ] unidentified stereoisomer
[η] unidentified stereoisomer
[θ] unidentified stereoisomer

→ continued									
1234	1234	**∑Carboxylic acids**	**4.60 ± 0.06** [c]	**6.79 ± 0.08** [a]	**6.25 ± 0.09** [b]	**1.96 ± 0.04** [e]	**4.80 ± 0.07** [d]	**3.87 ± 0.04** [e]	***
792	790	2-methyl propanoic acid	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.22 ± 0.01 [a]	0.00 ± 0.00 [b]	***
916	918	4-hydroxy butanoic acid	0.93 ± 0.02 [b]	1.88 ± 0.02 [a]	0.80 ± 0.01 [c]	0.00 ± 0.00 [e]	0.12 ± 0.01 [d]	0.00 ± 0.00 [e]	***
1000	1000	Hexanoic acid	0.80 ± 0.01 [d]	1.37 ± 0.02 [c]	1.89 ± 0.04 [a]	0.49 ± 0.02 [e]	1.45 ± 0.03 [b]	0.82 ± 0.01 [d]	***
1190	1190	Octanoic acid	2.87 ± 0.03 [c]	3.54 ± 0.04 [a]	3.56 ± 0.04 [a]	1.47 ± 0.02 [d]	3.01 ± 0.02 [b]	3.05 ± 0.03 [b]	***
		∑Esters	**73.10 ± 1.25** [a]	**51.20 ± 0.92** [c]	**50.20 ± 0.85** [c]	**62.40 ± 1.15** [b]	**49.20 ± 0.84** [c]	**38.20 ± 0.70** [d]	***
589	592	Ethyl acetate	6.10 ± 0.11 [a]	2.90 ± 0.05 [c]	3.30 ± 0.06 [b]	2.00 ± 0.04 [d]	1.50 ± 0.02 [e]	2.00 ± 0.04 [d]	***
764	765	Isobutyl acetate	0.30 ± 0.01 [a]	0.20 ± 0.01 [b]	0.20 ± 0.01 [a]	0.10 ± 0.01 [c]	0.10 ± 0.01 [c]	0.10 ± 0.01 [c]	***
799	801	Ethyl butanoate	0.40 ± 0.01 [a]	0.20 ± 0.01 [c]	0.30 ± 0.01 [b]	0.20 ± 0.01 [c]	0.20 ± 0.01 [c]	0.20 ± 0.01 [c]	***
803	806	Ethyl lactate	0.40 ± 0.01 [e]	0.60 ± 0.01 [c]	0.70 ± 0.01 [b]	0.10 ± 0.01 [f]	0.90 ± 0.01 [a]	0.50 ± 0.01 [d]	***
-	-	Butyl lactate	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	0.00 ± 0.00 [b]	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	***
856	858	Ethyl-3-methylbutanoate	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	0.10 ± 0.01 [a]	
866	867	Isoamyl acetate	2.50 ± 0.03 [d]	2.10 ± 0.03 [e]	2.80 ± 0.05	2.30 ± 0.03 [e]	3.00 ± 0.04 [b]	3.30 ± 0.05 [a]	***
998	998	Ethyl hexanoate	4.30 ± 0.07 [a]	3.20 ± 0.05 [de]	3.90 ± 0.07 [b]	3.60 ± 0.06 [c]	3.30 ± 0.05 [d]	3.10 ± 0.06 [e]	***
1165	1167	Diethyl succinate	1.10 ± 0.01 [b]	0.90 ± 0.01 [d]	1.20 ± 0.01 [a]	0.90 ± 0.01 [d]	1.00 ± 0.02 [c]	0.90 ± 0.01 [d]	***
1199	1200	Ethyl octanoate	24.10 ± 0.36 [c]	18.70 ± 0.31 [d]	15.90 ±0.25 [e]	31.40 ±0.52 [a]	27.20 ± 0.42 [b]	14.80 ± 0.24 [f]	***
1244	1246	Isoamyl hexanoate	0.50 ± 0.01 [a]	0.40 ± 0.01 [b]	0.40 ± 0.01 [b]	0.40 ± 0.01 [b]	0.40 ± 0.01 [b]	0.30 ± 0.01 [c]	***
1256	1258	Phenylethyl acetate	0.90 ± 0.01 [c]	0.70 ± 0.01 [e]	0.80 ± 0.01 [d]	1.00 ± 0.01 [b]	1.10 ± 0.01 [a]	1.00 ± 0.01 [b]	***
1291	1294	Ethyl-trans-4-decenoate	1.30 ± 0.03 [a]	0.70 ± 0.01 [b]	0.60 ± 0.01 [c]	0.40 ± 0.01 [d]	0.20 ± 0.01 [e]	0.40 ± 0.01 [d]	***
1395	1397	Ethyl decanoate	29.60 ± 0.46 [a]	19.80 ± 0.31 [b]	18.60 ± 0.27 [c]	19.30 ± 0.38 [bc]	9.30 ± 0.17 [e]	10.40 ± 0.18 [d]	***
1450	1451	Isoamyl octanoate	0.40 ± 0.01 [a]	0.20 ± 0.01 [c]	0.30 ± 0.01 [b]	0.30 ± 0.01 [b]	0.30 ± 0.01 [b]	0.30 ± 0.01 [b]	***
1592	1594	Ethyl dodecanoate	0.70 ± 0.01 [b]	0.40 ± 0.01 [d]	1.00 ± 0.01 [a]	0.30 ± 0.01 [e]	0.50 ± 0.01 [c]	0.70 ± 0.01 [b]	***
		∑Others	**2.97 ± 0.04** [b]	**3.28 ± 0.03** [a]	**2.34 ± 0.02** [c]	**0.34 ± 0.01** [f]	**0.66 ± 0.01** [e]	**0.99 ± 0.01** [d]	***
1062	1060	3-(2-hydroxyethyl)-indole	1.10 ± 0.01 [b]	1.67 ± 0.02 [a]	1.06 ± 0.01 [b]	0.34 ± 0.01 [e]	0.66 ± 0.01 [d]	0.99 ± 0.01 [c]	***
1098	1100	Diisoproprylsilyl neopententyl ether	0.00 ± 0.00 [c]	1.61 ± 0.01 [a]	1.28 ± 0.01 [b]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	0.00 ± 0.00 [c]	***
1761	1755	2-methyl thiophene	1.87 ± 0.03 [a]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	***

[α] Kovats index based on literature (https://webbook.nist.gov/);
[β] Kovats index obtained through the modulated chromatogram reported for DB-5 MS apolar column;
[γ] compounds are classified in order of Kovats index;
[δ] Relative amounts expressed as mg/L with respect to calibration curves of ethyl lactate, 3-hydroxy-2-butanone, 2.3-butanediol;
[ε] statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: *. P < 0.05; **. P < 0.01; ***. P < 0.001; n.s.. not significant.
[ζ] unidentified stereoisomer
[η] unidentified stereoisomer
[θ] unidentified stereoisomer

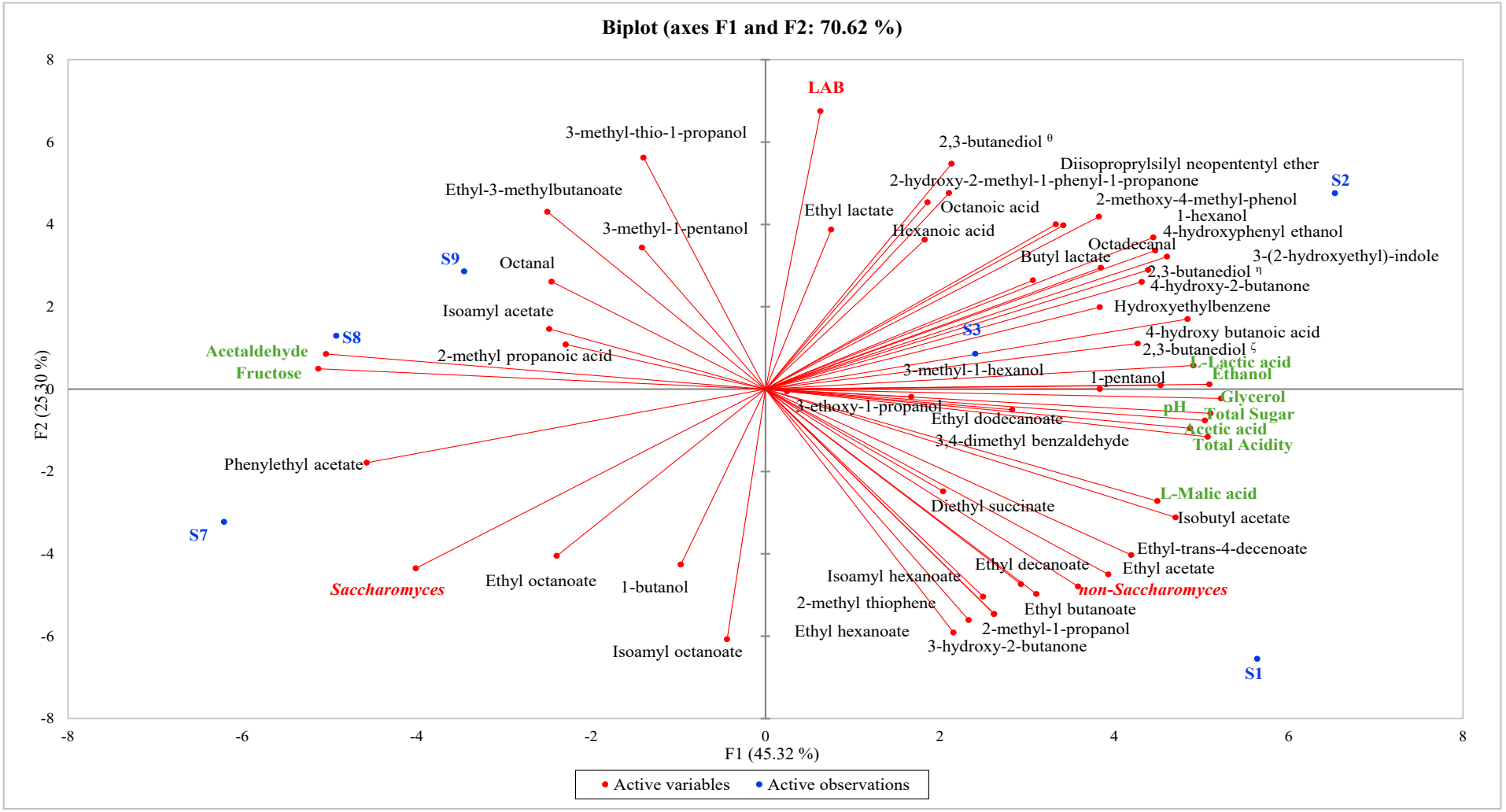


Fig. 4. Principal component analysis (PCA) biplot of VOC, technological parameters, and microbial population

Table S4

Higher alcohols detected at end of fermentation (mg/L)

Higher alcohol	Aminoacid precursos [1] [2]	S1	S2	S3	S7	S8	S9	S.S.
2-methyl-1-propanol	valina	24.32 ± 0.29 ^{a}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	***
1-pentanol	isoleucine	93.4 ± 0.97 ^{a}	87.54 ± 0.81 ^{b}	71.23 ± 0.71 ^{d}	51.78 ± 0.56 ^{f}	65.90 ± 0.72 ^{e}	76.81 ± 0.76 ^{c}	***
3-ethoxy-1-propanol	homoserina	0.58 ± 0.01 ^{a}	0.52 ± 0.01 ^{b}	0.32 ± 0.01 ^{d}	0.42 ± 0.01 ^{c}	0.54 ± 0.01 ^{b}	0.59 ± 0.01 ^{a}	***
3-methyl-thio-1-propanol	metionina	0.29 ± 0.01 ^{e}	0.64 ± 0.01 ^{c}	0.34 ± 0.01 ^{d}	0.36 ± 0.01 ^{d}	0.68 ± 0.01 ^{b}	0.72 ± 0.01 ^{a}	***
Hydroxyethylbenzene	phenylalanina	45.15 ± 0.72 ^{ab}	47.05 ± 0.67 ^{a}	35.13 ± 0.53 ^{c}	23.69 ± 0.41 ^{d}	34.42 ± 0.49 ^{c}	43.82 ±0.63 ^{b}	***
4-hydroxyphenyl ethanol	tirosina	1.14 ± 0.01 ^{c}	2.52 ± 0.01 ^{a}	1.61 ± 0.01 ^{b}	0.39 ± 0.01 ^{f}	0.71 ± 0.01 ^{e}	0.74 ± 0.01 ^{d}	***
∑ Higher alcohol		164.88 ± 2.01 ^{a}	138.27 ± 1.51 ^{b}	108.63 ± 1.27 ^{d}	76.64 ± 1.00 ^{f}	102.25 ± 1.24 ^{e}	122.68 ± 1.42 ^{c}	***

Result indicates mean value ± standard deviation of three determinations. Data within a line followed by the same letter are not significantly different according to Tukey's test.

Symbols:

[1] Irwin, A. J. (1992)

[2] Styger, G., Prior, B., & Bauer, F. F. (2011)

Abbreviations:

S.S., statistical significance; P value: ***, $P < 0.001$; n.s., not significant.

Consequently, this effect represents a potemtial metabolic dynamic implemented by the *Starm. lactis-condensi* MN412 strain to limit the toxic effects of ethanol produced by the reduction of acetaldehyde in the glycerol-pyruvate pathway (Englezos et al., 2018b). This hypothesis is consistent with what has been reported by several authors on the low tolerability of non-*Saccharomyces* genera to ethanol (Lai et al., 2022). This hypothesis may be explained by 45.32% out of 70.62% total variance reported in Figure 4, in which the S1 trial showed a higher concentration of higher alcohols from amino acids than the S7 control trial (164.88 *vs* 76.64 mg/L) (Table S4). This diversity of production would represent a synergistic interaction effect between strains MN412 and QA23™ (Liu, Y. et al. 2016). Indeed, the latter would benefit from the amino acid production of MN412 strain (Englezos et al., 2018b), as would *O. oeni* MLB6™ (described in the previous paragraph). Valine, isoleucine, homoserine, phenylalanine and tyrosine represent the amino acids converted by QA23™ strains (Table S4) (Styger et al., 2011). Conversely, the lower concentrations of higher alcohols (Table XX) observed in the S2 and S3 trials in comparison to the S1 trial suggest that the LAB (MLPK45H™, *L. plantarum*; and MLB6™, *O. oeni*) exhibited greater competitiveness against the *S. cerevisiae* strain, QA23™ (Maarman, 2014). The same trend was not observed between S8 and S9 *vs* S7 trials. This indicates that amino acid assimilability is dependent on the diversity of the microbial consortium. Consequently, the competitiveness between different microbial species plays a pivotal role in determining amino acid uptake in grape must. Furthermore, additional effects of the use of *Starm. lactis-condensi* were identified in the trials. The production of 1-hexanol was found to be statistically significant in the trials that used the non-*Saccharomyces* strain MN412 for 72 hours. The values observed between 1.28 and 2.32 mg/L in the S1, S2 and S3 trials, in comparison to the 0.63 and 1.15 mg/L in the S7, S8 and S9, demonstrate the potential for extracellular enzyme production in *Stram. lactis-condensi*, with the capacity to bioconvert linoleic acid into 1-hexanol. This observation is consistent with the findings reported by Ribéreau-Gayon et al. in 2006. This peculiarity was identified in the genus *Starmerella* by Li et al. (2023) and Englezos et al. (2015). Furthermore, the trials involving the non-*Saccharomyces*-LAB/S*accharomyces* consociation (S2 and S3 trials, 2.32 and 1.70

mg/L, respectively) exhibited a higher biosynthetic activity towards 1-hexanol than the non-*Saccharomyces*-*Saccharomyces* control (S1 trial, 1.28 mg/L). This peculiarity was evident when the MLPK45H™ strain was used in comparison to the MLB6™ strain.

No detectable levels of 3-methyl-1-butanol were observed between the two strains. This phenomenon is consistent with that reported by Francesca et al. (2024). In all experimental cases, 2,3-butanedione was not detected, falling below the limit of the method and instrument. In contrast, 2,3-butanediol and 3-hydroxy-2-butanone, by-products of 2,3-butanedione, were detected (Table 1). The production of esters differed between the trials, as shown in Table 1. The different microbial combinations in the experimental plan significantly influenced the production of esters, as illustrated in Fig. 3A. Test S1 (MN412 - QA23™), characterized by a higher concentration of acetate esters and ethyl esters than the control (S7, QA23™ strain), yielded values of 9.80 and 62.3 mg/L respectively. The same trend was verified in the S1 trial with respect to the further experimental trials (S2, S3, S8, S9) (Fig. XX). These results agree with those reported by Francesca et al. (2024). The use of the *O. oeni* MLB6™ strain, in simultaneous combination with QA23™ strain, with and without MN412 strain (S3 and S9 trials), resulted in the highest acetate ester content among the theses in which LAB was used in microbial intercropping (7.10 and 6.40 mg/L, respectively). The significant difference in terms of total acetate esters (Fig. 3A) observed in favour of the S3 trial, in comparison to the S9 trial, indicates that the utilization of *Starm. lactis-condensi* MN412 strain contributes to an enhancement of this aromatic class in Catarratto wines. In contrast, no statistically significant differences were observed in the total ethyl ester values between the S2 (MN412 - MLK45H/QA23™) and S3 (MN412 - MLB6™/QA23™) tests. However, the total values recorded for this aromatic class in the S2 and S3 tests were found to be lower than in the S1 control (MN412 - QA23™) (Fig. 5A).

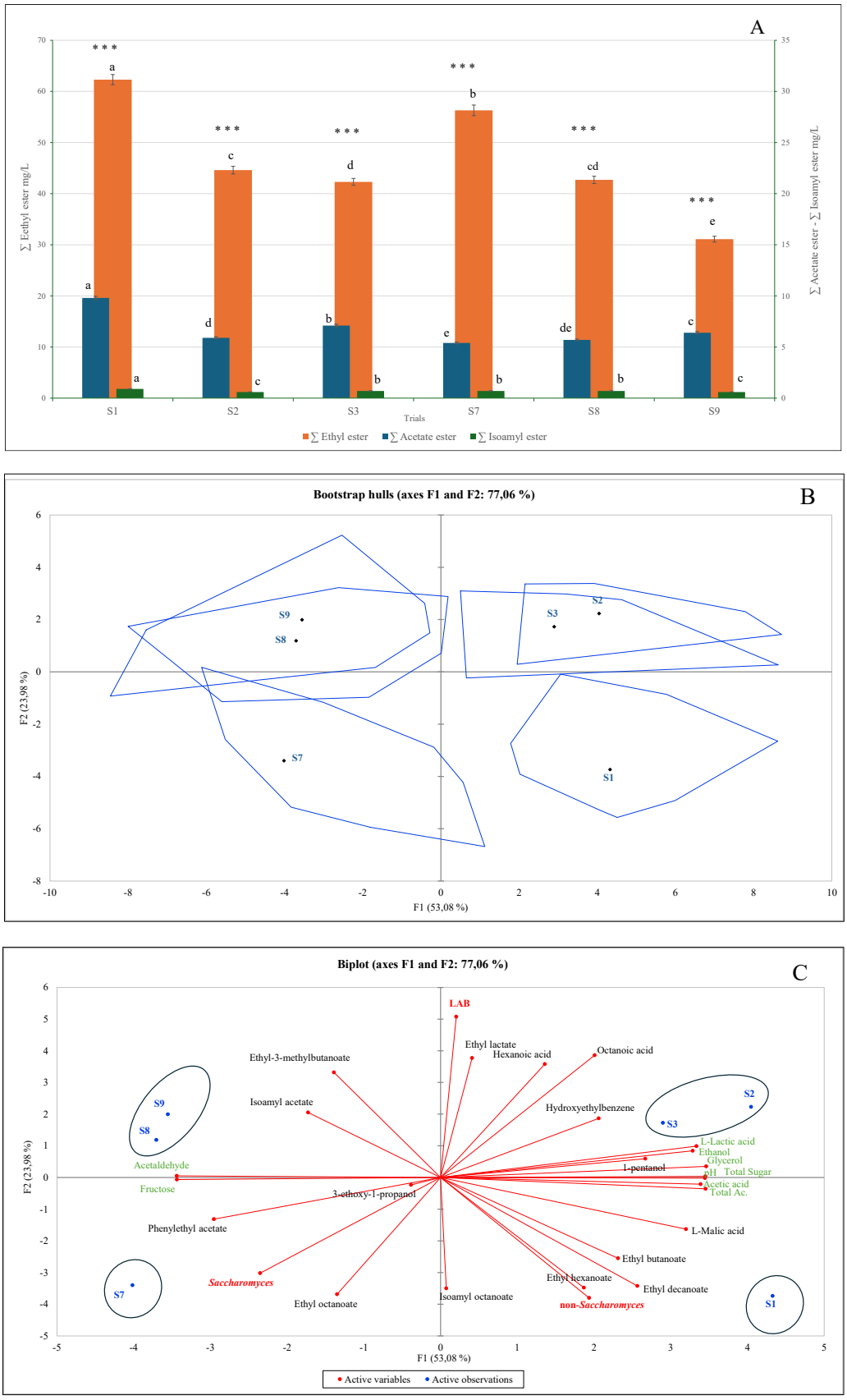


Fig. 5. Graphical representations: (A) Aromatic impact of various concentrations of ethyl esters, acetate esters and isoamyl esters (mg/L); the histograms represent the total concentrations found in wine (mg/L); the error bars indicate the standard deviation; different superscript letters indicate significant differences for $P < 0.001$. (B) Bootstrap hull's for OAV>1; (C) Principal component analysis (PCA) biplot of technological parameters, OAV>1 and microbial populations.

A similar pattern was evident in the S8 (MLPK45H™/QA23™ strains) and S9 (MLB6™/QA23™ strains) tests in comparison to the S7 trial (QA23™ strain) control (Fig. 5A). These observations may be attributed to the hydrolytic reactions of bacterial esterases (Sumby et al., 2009). However, the significant difference in terms of total ethyl esters and acetates between the S3 and S9 tests suggests that the use of the MN412 strain in microbial associations tends to significantly limit the loss in ethyl esters by hydrolysis at the expense of *O. oeni* (Ugliano et al., 2003) (Fig. 5A). The results presented in Table 1 indicate that fatty acid esterification was a prominent metabolic pathway in the production of aromatic profiles in wines. These reactions were found to be positively correlated with the use of the non-*Saccharomyces* strain MN412 (Fig. 4).

In particular, the S1 trial was with the most diverse array of fatty acid esters, in comparison to the S2, S3, S7, S8 and S9 trials, which were conducted with different microbial associations (including the presence of the MN412 strain). In contrast, the use of the pure culture of *S. cerevisiae*, QA23™ strain (S7 trial) was positively correlated with only one ethyl ester (ethyl octanoate) (Puertas et al., 2018). However, the significant production of fatty acid ethyl esters in the S1 trial compared to the S7 trial suggests a marked activity of *Starm. lactis-condensi* to release extracellular enzymes of a potentially lipolytic type into the medium (Charoenchai et al., 1997).

This activity would be beneficial for *Stram. lactis-condensi* in inhibiting yeasts and LAB (Alexandre et al., 2004) in the must by releasing medium-chain fatty acids into the medium. This type of activity has already been reported on *Starmerella* by Charoenchai et al. (1997). Therefore, the bioavailability of medium-chain fatty acids in the medium may stimulate the esterase activity of *S. cerevisiae* (Saerens et al., 2010) and improve the sensory properties of experimental Catarratto wines (Lin et al., 2020). These findings are reinforced by the inverse correlations observed between hexanoic acid and isoamyl hexanoate, as well as ethyl hexanoate (25.30% variance), and octanoic acid and isoamyl octanoate, in addition to ethyl octanoate (45.32% variance). These correlations are depicted in Figure 2, which depicts the VOCs- chemical-physical technological parameters-microbial populationogy correlation. Nevertheless, the potential contribution of *Starm. lactis-condensi* MN412 to the synthesis

of isoamyl esters cannot be ruled out. Indeed, the use *Starmerella* genus for the bioproduction of isoamyl esters in the food industry is already documented in the literature (Liu et al., 2013).

3.3.5 Active volatile compound analysis

Table S5 shows the active odourants (OAV >1) between the trials. Only 29% (14 out of 48 VOCs) of the total compounds detected were above the threshold of perception, which is in line with the findings of Naselli et al. (2023) on Catarratto. The active VOCs are all of fermentation origin. This indicates that the use of appropriate biotechnology for the fermentation of Catarratto musts is fundamental in the constitution of the wine aroma. Bootstrap hulls analysis (Fig. 5B) showed that the use of *Starm. lactis-condensi* MN412 strain in sequential inoculation is capable of diversifying the active aroma profiles (OAV >1) of the wines. This diversity may be explained by a variance of 53.08% out of a total of 77.06% (Fig. 5C). Furthermore, PCA analysis (Fig. 5C) demonstrated that the S2, S3 and S8, S9 trials were separated into two groups. This difference was found to be positively correlated with LAB for the F1 axis, 23.98% variance; and with the *Starm. lactis-condensi* MN412 strain for the F2 axis, 53.08% variance. These results indicate that the diversity of wine aroma profiles is a function of the complexity of the microbial consortium. Consequently, the utilisation of either LAB, namely *O. oeni* or *L. plantarum*, and/or the *Starm. lactis condensi* MN412 strain within a microbial consortium has a significant impact on the aromas observed in Catarratto wines. Although the study of the aromatic profile of wines by means of OAVs has helped to understand the derivation of certain olfactory perceptions in wines, the use of olfactory unit values still remains an indicative tool (Gómez-Míguez et al., 2007).

Table S5

Odor activity value of volatile organic compounds detected above the perception threshold in Catarratto experimental wines.

Compounds [α]	Aroma descriptor [β]	Odor threshold [γ]	S1 [δ]	S2 [δ]	S3 [δ]	S7 [δ]	S8 [δ]	S9 [δ]	S.S. [ε]
1-butanol	Fusel, spirituous [2]	150 [1]	1.46 ± 0.01 [a]	1.37 ± 0.01 [b]	1.11 ± 0.01 [d]	0.81 ± 0.01 [f]	1.03 ± 0.01 [e]	1.20 ± 0.01 [c]	***
3-ethoxy-1-propanol	Fruity [2]	0.10 [2]	5.80 ± 0.10 [a]	5.20 ± 0.10 [b]	3.20 ± 0.10 [d]	4.20 ± 0.10 [c]	5.40 ± 0.10 [b]	5.90 ± 0.10 [a]	***
Hydroxyethylbenzene	Floral, rose [8]	10 [8]	4.51 ± 0.07 [b]	4.70 ± 0.07 [a]	3.51 ± 0.07 [c]	2.37 ± 0.05 [d]	3.44 ± 0.04 [c]	4.38 ± 0.05 [b]	***
Hexanoic acid	Fatty, rancid [6]	0.42 [1]	1.90 ± 0.02 [d]	3.26 ± 0.05 [c]	4.50 ± 0.10 [a]	1.17 ± 0.05 [e]	3.45 ± 0.07 [b]	1.95 ± 0.02 [d]	***
Octanoic acid	Fatty, rancid [6]	0.50 [1]	5.74 ± 0.06 [c]	7.08 ± 0.08 [a]	7.12 ± 0.08 [a]	2.94 ± 0.04 [d]	6.02 ± 0.04 [a]	6.10 ± 0.06 [a]	***
Ethyl butanoate	Fruity, strawberry, sweet, bubble gum [3]	0.125 [3]	3.20 ± 0.08 [a]	1.60 ± 0.08 [c]	2.40 ± 0.08 [b]	1.60 ± 0.08 [c]	1.60 ± 0.08 [c]	1.60 ± 0.08 [c]	***
Ethyl lactate	Fruity, buttery [6]	0.15 [2]	2.66 ± 0.07 [e]	4.00 ± 0.07 [c]	4.66 ± 0.07 [b]	0.66 ± 0.07 [f]	6.00 ± 0.07 [a]	3.33 ± 0.07 [d]	***
Ethyl-3-methylbutanoate	Fruity, strawberry, apple [3]	0.003 [5]	33.33 ± 0.33 [a]	33.33 ± 0.33 [a]	33.33 ± 0.33 [a]	33.33 ± 0.33 [a]	33.33 ± 0.33 [a]	33.33 ± 0.33 [a]	n.s.
Isoamyl acetate	Tropical fruit, banana [1]	0.03 [1]	83.33 ± 1.00 [d]	70.00 ± 1.00 [f]	93.33 ± 1.67 [c]	76.66 ± 1.00 [e]	100 ± 1.33 [b]	110 ± 1.67 [a]	***
Ethyl hexanoate	Fruity, strawberry, pineapple, anise [3]	0.014 [5]	307.14 ± 5 [a]	228.57 ± 3.57 [de]	278.57 ± 5 [b]	257.14 ± 4.29 [c]	235.71 ± 3.57 [d]	221.43 ± 4.29 [e]	***
Ethyl octanoate	Fruity, pineapple [7]	0.005 [5]	**4820.00 ± 72 [c]**	**3740.00 ± 62 [d]**	**3180.00 ± 50 [e]**	**6280.00 ± 104 [a]**	**5440.00 ± 84 [b]**	**2960.00 ± 48 [f]**	***
Phenylethyl acetate	Rose, floreal [2]	0.25 [1]	3.60 ± 0.04 [c]	2.80 ± 0.04 [e]	3.20 ± 0.04 [d]	4.00 ± 0.04 [b]	4.40 ± 0.04 [a]	4.00 ± 0.04 [b]	***
Ethyl decanoate	Floreal [2]	0.20 [1]	148.00 ± 2.30 [a]	99.00 ± 1.55 [b]	93.00 ± 1.35 [c]	96.50 ± 1.90 [bc]	46.50 ± 0.85 [e]	52 ± 0.90 [d]	***
Isoamyl octanoate	Fruity, green, apple,pineapple,waxy [7]	0.028 [4]	14.29 ± 0.36 [a]	7.14 ± 0.36 [c]	10.71 ± 0.36 [b]	10.71 ± 0.36 [b]	10.71 ± 0.36 [b]	10.71 ± 0.36 [b]	***

[α] Compounds with OAV > 1 ,

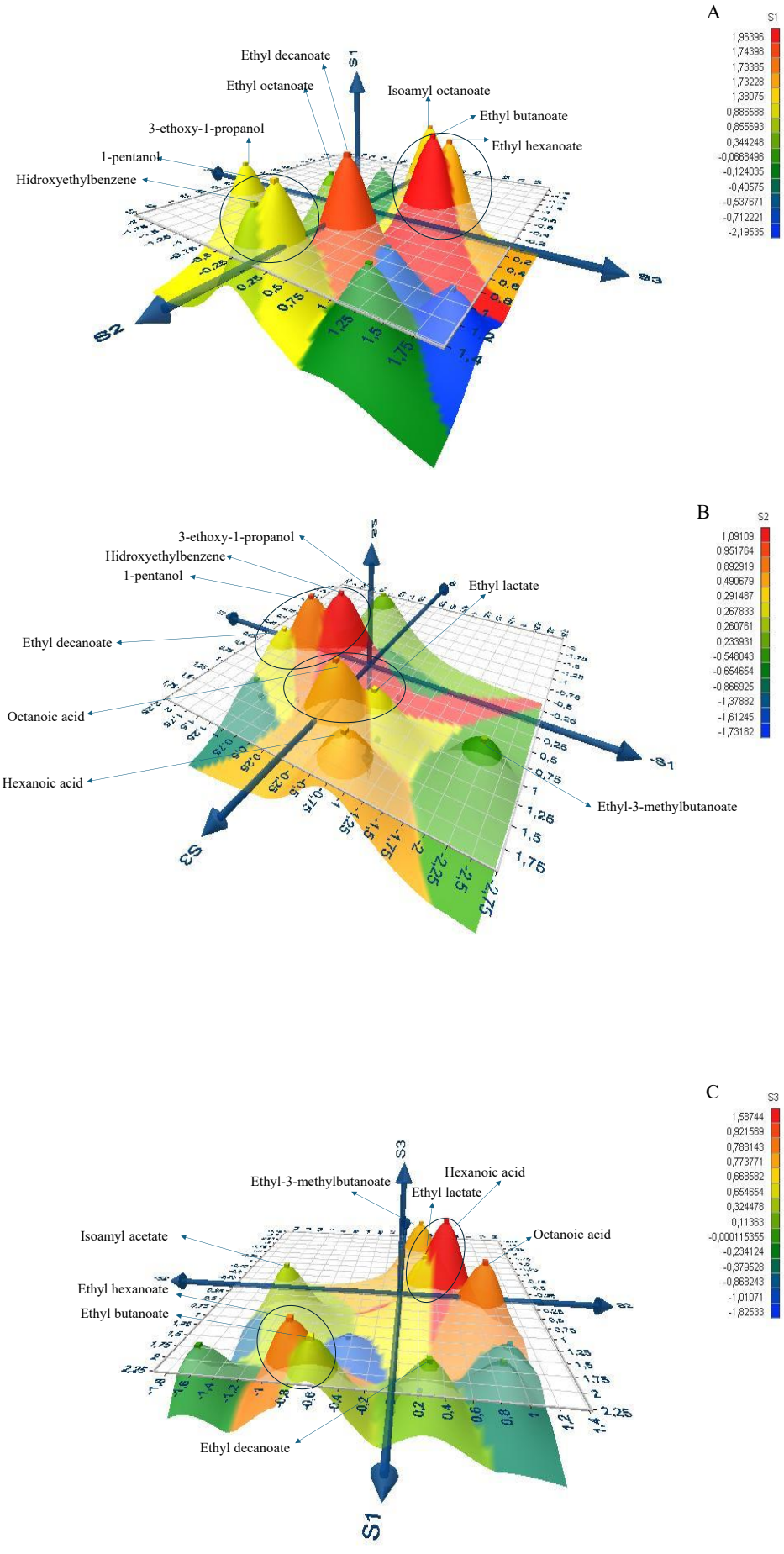
[β] Aroma description and reference: [1] Tronchoni et al., 2018; [2] Celik et al., 2019; [3] De-La-Fuente-Blanco et al., 2020; [6] Peinado et al., 2004; [7] https://www.ventos.com/; [8] Selli et al., 2004;

[γ] Odor threshold (mg/L) and reference: [1] Tronchoni et al., 2018; [2] Celik et al., 2019; [3] De-La-Fuente-Blanco et al., 2020; [4] Philipp et al., 2018; [5] Gómez-Míguez et al., 2017; [8] Selli et al., 2004;

[δ] Relative amounts expressed in OAV (odor activity value)

[ε] Statistical significance among S1, S2, S3, S7, S8, S9 trials; Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: ***, P < 0.001; n.s., not significant.

This results in the exclusion of two key aspects: first, the variability that a given compound may exhibit when its concentration is varied; second, an understanding of the relationships between VOCs. Furthermore, the knowledge of the relationships between VOCs (specifically, how the presence of a single VOC affects the expression of further VOCs) is similarly lacking. This, in turn, has implications for an accurate estimation of the contribution that a microbial consortium may make to wine aroma. Having identified the critical aspects of the method, the OAV profiles were correlated using a three-variable graphic system. This system consisted of an "x" axis, representing the concentration of the compound under investigation, a "y" axis representing the concentration of the microbial consortium, and a "z" axis representing the concentration of the other compounds present in the wine. The profiles were correlated for each experimental protocol, with or without the MN412 strain. Each axis was assigned a single microbial consortium, and the interpretation was carried out in relation to the axis of assignment, which was the microbial consortium under evaluation. The OAV values, subjected to the Z-score normalization method, were entered into a 3D-plot surface diagram and subsequently processed. The application of this procedure made it possible to attribute values that take into account the variability within a finite numerical range. In the context of wine, this interval would represent the totality of the active aromatic profile, with a beginning and an end. This procedure allowed a "relationship indicator value" (RI-value) to be assigned for each VOC in relation to the other VOCs with OAV>1. Consequently, the impact of factors affecting olfactory thresholds (highly dependent on variability of the matrix) was eliminated. This enabled the identification of correlations between VOCs, with an OAV >1, and the specific contribution of individual microbial consortia to the wine aroma (for an OAV >1). Figure 6 A, B, C D, E, F presents the graphical elaborations. The use of the *Stram. lactis-condens*i MN412 strain, which was microbially consociated with the S. *cerevisiae* QA23™ strain (S1 trial), led to an increase in the contribution of the esters ethyl butanoate and ethyl hexanoate compared to the trials fermented in pure culture with the *S. cerevisiae* QA23™ strain (S7 trial) (Fig. 6A *vs* Fig. 6D).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Ethyl butanoate
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S1

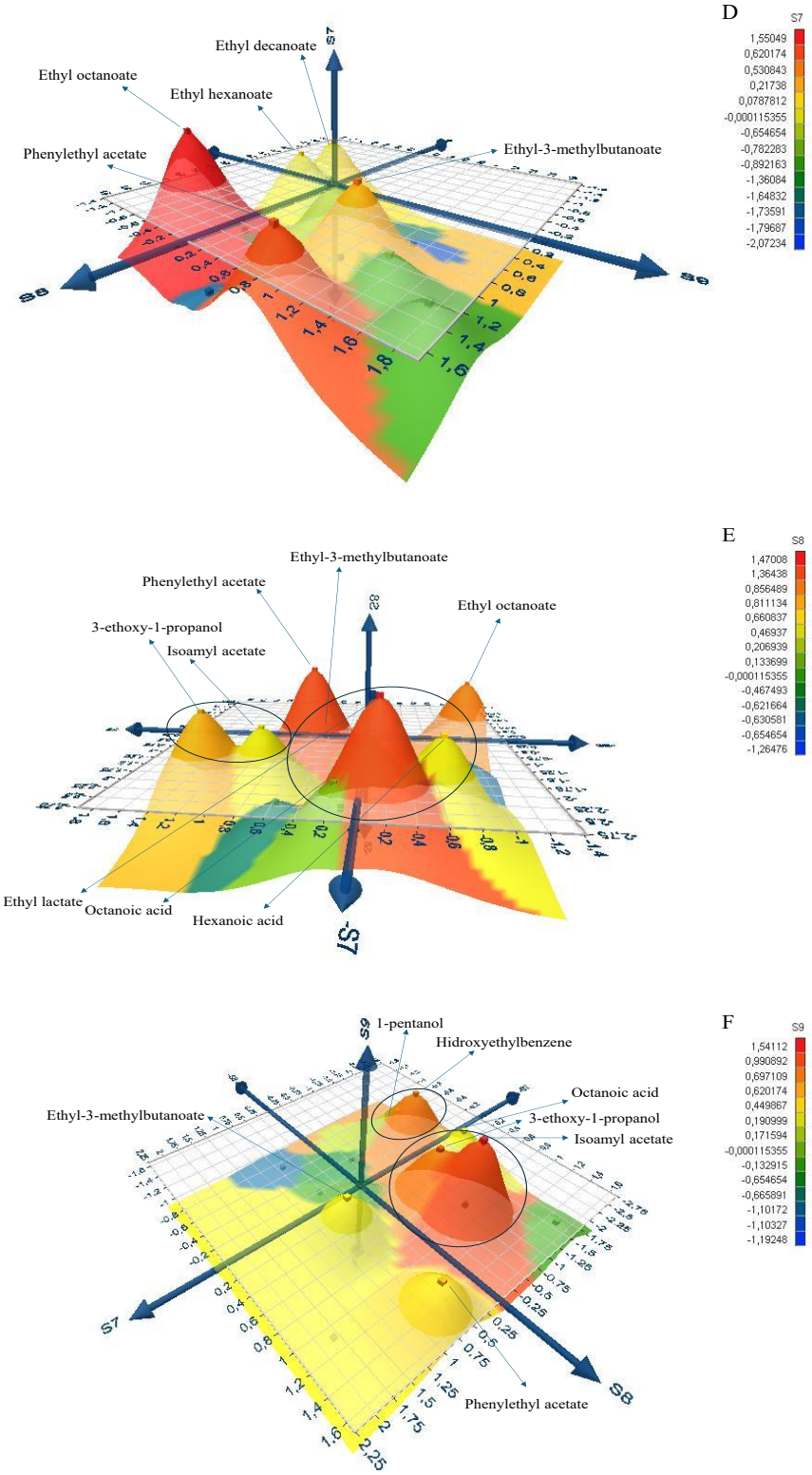


Fig. 6. 3D-plot Surface of relationship value of OAV>1; A, sequential inoculum *Starmerella lactis-condensi* MN412 - *Saccharomyces cerevisiae* QA23 (S1); B sequential inoculum *Starmerella lactis-condensi* MN412 – *Lactiplantibacillus plantarum* MLPK45H/*Saccharomyces cerevisiae* QA23 (S2); C, sequential inoculum *Starmerella lactis-condensi* MN412 – *Oenococcus oeni* MLB6/*Saccharomyces cerevisiae* QA23 (S3); D, single inoculum *Saccharomyces cerevisiae* QA23 (S7, control); simultaneum inoculum *Lactiplantibacillus plantarum* MLPK45H/*Saccharomyces cerevisiae* QA23 (S8); *Oenococcus oeni* MLB6/*Saccharomyces cerevisiae* QA23 (S9).

Furthermore, statistical-graphic analysis revealed that these esters exhibit a common surface area (Fig. 6A). This consideration does not preclude the possibility of an additive effect between their olfactory perceptions, which might generate different sensory attributes from those described in the literature (De-La-Fuente-Blanco et al., 2020). Furthermore, the microbial consociation MN412 strain-QA23™ strain (Fig. 6A) compared to QA23™ strain in pure culture showed a significant representation of ethyl decanoate and isoamyl octanoate. The 3D-plot surface graphical representation of the RI-values made it possible to highlight further VOC groupings; conspicuous numbers were found among the theses where LAB was employed. This suggests that the use of LAB in association with *S. cerevisiae* predisposes the active volatile fraction of the wine to additive, synergistic or masking effects (Fig. 6 B, C, E, F). However, the application of the RI values by means of graphical representation made it possible to explain the diversity of the S2, S3 and S8, S9 trials (resulting as overlapping bootstrap hulls - Fig. 5B), and to clarify the contribution of each LAB species, in consociation or not with *Starm. lactis-condensi*, to the active aroma profile of the wine. The main differences observed between the microbial consortia MN412-MLPK45H™-QA23™ (S2 trial) and MN412-MLB6™-QA23™ (S3 trial) (Fig. 1B and 1C) may be attributed to the effect of the MN412 strain on the active volatile compounds produced by the LAB-*S. cerevisiae* interactions. The effect of MN412 strain was to incorporate, among the active compounds with negative relationship values, isoamyl acetate in the S2 trial (Fig. 6B) and 3-ethoxy-1-propanol in the S3 trial (Fig. 6E). On a sensory level, this effect may correspond to a greater differentiation between the perceptions of tropical fruit and banana (Tronchoni et al., 2018) and fruity aromas (Celik et al., 2019) in the S3 and S2 trials, respectively. In contrast, different dynamics were recorded for hexanoic, octanoic and ethyl lactate acids. The impact of the *Starm. lactis-condensi* strain MN412 in consociation with the *O. oeni* - *S. cerevisiae* strain MLB6™-QA23™ (S3 trial), was an increase in RI values for hexanoic acid and ethyl lactate compared to those observed for the MLB6™-QA23™ microbial consortium (S9 trial). The corresponding figures may be seen in Figure 6C and Figure 6F. This phenomenon resulted in the group of VOCs exceeding the positive threshold of the RI-values (Fig. 6C). This effect could

correspond to an increased distinctiveness of olfactory perception derived from the OAV group (Fig. 6F). By contrast, the use of *Starm. lactis-condensi* MN412 strain with *L. plantarum* - *S. cerevisiae* MLPK45H™-QA23™ strains (S2 trial), allowed the separation of the hexanoic acid from the octanoic acid group and ethyl lactate (Fig. 6B *vs*. 6E). The formation of the OAVs hexanoic acid, octanoic acid and ethyl lactate was facilitated by the use of the microbial consortium MLPK45H™-QA23™ (S8 trial) (Fig. 4E). Further effects of the use of *Starm. lactis-condensi* MN412 strains, *L. plantarum* MLPK45H™ and *O. oeni* MLB6™ strains in consociation with *S. cerevisiae* QA23strain were observed at the higher alcohols. Among the graphical representations of the active olfactory profiles (OAV>1) (Figure 6 A-F), the representative dynamics in terms of hydroxyethylbenzene and 1-pentanol differed between the two cases. The presence or absence of these compounds above the plane of positivity of the RI-values (Table S6 and S7) in the graphical representations is influenced by the trophic competition exerted by LAB against *S. cerevisiae* (QA23™ strain) and the ability of MN412 to stimulate ester synthesis by *S. cerevisiae* (QA23™ strain).

Table S6
Relationship Indicator values (RI-values) of OAVs experimental protocol 1

OAV	Trials		
	S1	S2	S3
1-pentanol	1.380749	0.951764	-0.234123768
3-ethoxy-1-propanol	0.886588	0.260761	-1.825327999
Hydroxyethylbenzene	0.855693	1.091092	-0.379528131
Hexanoic acid	-0.71222	0.490679	1.587439947
Octanoic acid	-0.06685	0.892919	0.921569189
Ethyl butanoate	1.963961	-0.65465	0.654653671
Ethyl lactate	-0.53767	0.267833	0.668581775
Ethyl-3-methylbutanoate	-2.19535	0.233931	0.773770611
Isoamyl acetate	-0.40575	-1.37882	0.32447806
Ethyl hexanoate	1.733849	-0.86692	0.788143301
Ethyl octanoate	0.344248	-0.54804	-1.010712517
Phenylethyl acetate	-0.12403	-1.61245	-0.868243142
Ethyl decanoate	1.743981	0.291487	0.113630483
Isoamyl octanoate	1.732281	-1.73182	-0.000115355

Table S7
Relationship Indicator values (RI-values)of OAVs experimental experimental 2

OAV	Trials		
	S7	S8	S9
1-pentanol	-1.64832	-0.62166	0.171594
3-ethoxy-1-propanol	-0.78228	0.46937	0.990892
Hydroxyethylbenzene	-1.79687	-0.46749	0.697109
Hexanoic acid	-1.36084	0.660837	-0.66589
Octanoic acid	-2.07234	0.133699	0.190999
Ethyl butanoate	-0.65465	-0.65465	-0.65465
Ethyl lactate	-1.73591	1.470078	-0.13291
Ethyl-3-methylbutanoate	0.530843	0.206939	0.449867
Isoamyl acetate	-0.89216	0.811134	1.541119
Ethyl hexanoate	0.078781	-0.63058	-1.10327
Ethyl octanoate	1.550494	0.856489	-1.19248
Phenylethyl acetate	0.620174	1.364382	0.620174
Ethyl decanoate	0.21738	-1.26476	-1.10172
Isoamyl octanoate	-0.00012	-0.00012	-0.00012

Collectively, these two factors would have influenced the relationship between the OAVs intra-aromatic profiles and their olfactory contributions. Nevertheless, the utilisation of *Starm. lactis-condensi* and LAB resulted in the emergence of further effects on the active volatile component. In

particular, co-presence of MN412 and MLPK45H™ strains with QA23™ strain, in comparison to the MN412 and MLB6™ strains consociation with QA23™ strain, resulted in an increased relationship value of ethyl decanoate, which was observed when incorporated together with hydroxyethylbenzene and 1-pentanol (Fig. 6B *vs* Fig. 6C). This effect could be reflected in a diversification of the floral perception of the OAV group as a consequence of an additive effect (Gómez-Míguez et al., 2007).

3.3.6 The sensory analysis

Table S8 shows the data obtained from the sensory analysis. The diverse microbial associations adopted led to a spectrum of sensorial profiles. The variability of the colour attributes influenced the appearance of the wine. Scores ranging between 7.12 and 7.46 defined the yellow colouring of the wine. This range is consistent with that reported by Naselli et al. (2023) on Catarratto wines. Conversely, the values pertaining to green hues exhibited comparable results. The variability of the olfactory attributes recorded by the panel was a function of the microbial association adopted. Test S1 exhibited the highest floral score. This olfactory perception was found to be positively correlated with the OAVs of ethyl decanoate, hydroxyethylbenzene and 1-pentanol (37.08% and 28.00% of the variance out of 65.08% of the total variance, respectively) (Fig. 7A). The explanation for this correlation may lie in the role played by higher alcohols in the interaction phenomena between odour perceptions within a mixture of VOCs. Cameleyre et al. (2015) and De-La-Fuente-Blanco et al. (2016) have reported that a mixture of higher alcohols can suppress the fruity aroma. Consequently, the observed increase in floral perception in the S1 trials may be attributed to the antagonistic effect of the higher alcohols on fruity perception. Indeed, 1-pentanol was found to be negatively correlated with the pineapple perception of ethyl octanoate, which in turn was responsible for the intensity and persistence of the smell in the S7 trial (fermented in pure *S. cerevisiae* culture) (Fig. 7A). Nevertheless, it is possible that the synergistic effect of other higher alcohols present below the perception threshold may also contribute to these observations (Gómez-Míguez et al., 2007) The

olfactory attributes of balsamic, pear and kiwi were perceived by the panel as positive and contributed to a more favourable sensory profile for the S1 trial in comparison to the other trials.

Table S8

Sensory score for experimental Catarratto wines.

Attributes [α]		Trials						Statistical [γ] Significance	
		S1 [β]	S2 [β]	S3 [β]	S7 [β]	S8 [β]	S9 [β]	Judge	Wine
Taste	Yellow colour	7.26 ± 0.16 [a]	7.21 ± 0.18 [a]	7.41 ± 0.25 [a]	7.37 ± 0.24 [a]	7.12 ± 0.21 [a]	7.21 ± 0.16 [a]	***	n.s.
	Green reflexes	6.42 ± 0.11 [a]	6.51 ± 0.16 [a]	6.47 ± 0.14 [a]	6.16 ± 0.13 [a]	6.27 ± 0.18 [a]	6.31 ± 0.22 [a]	***	*
Odour	O-Banana	6.61 ± 0.18 [c]	6.12 ± 0.12 [d]	7.11 ± 0.15 [b]	6.27 ± 0.18 [cd]	7.21 ± 0.22 [b]	7.83 ± 0.19 [a]	***	***
	O-Kiwi	6.87 ± 0.12 [a]	6.12 ± 0.10 [b]	6.43 ± 0.16 [b]	6.28 ± 0.11 [b]	6.26 ± 0.13 [b]	6.25 ± 0.11 [b]	***	***
	O-Fatty	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	n.s.
	O-Floral	7.73 ± 0.21 [a]	6.79 ± 0.16 [b]	6.66 ± 0.14 [b]	6.77 ± 0.12 [b]	6.12 ± 0.15 [c]	6.36 ± 0.16 [bc]	***	***
	O-Lavander	7.01 ± 0.18 [ab]	8.34 ± 0.19 [a]	6.32 ± 0.15 [cd]	5.62 ± 0.12 [e]	6.10 ± 0.12 [d]	6.71 ± 0.15 [bc]	***	***
	O-Fruity	7.87 ± 0.23 [a]	7.15 ± 0.18 [b]	6.81 ± 0.15 [bc]	8.22 ± 0.21 [a]	7.23 ± 0.18 [b]	6.58 ± 0.12 [c]	***	***
	O-Tropical fruity	7.12 ± 0.11 [ab]	6.63 ± 0.18 [c]	6.99 ± 0.12 [bc]	7.56 ± 0.22 [a]	7.11 ± 0.21 [abc]	7.24 ± 0.18 [ab]	***	**
	O-Intensity	7.83 ± 0.21 [b]	7.63 ± 0.16 [b]	7.41 ± 0.15 [bc]	8.62 ± 0.32 [a]	7.87 ± 0.29 [b]	6.97 ± 0.22 [c]	***	***
	O-Pear	7.81 ± 0.27 [a]	6.81 ± 0.19 [bc]	7.12 ± 0.15 [b]	6.51 ± 0.18 [c]	6.67 ± 0.21 [bc]	6.62 ± 0.22 [bc]	***	***
	O-Persistence	7.39 ± 0.23 [ab]	6.93 ± 0.16 [bc]	6.76 ± 0.15 [cd]	7.78 ± 0.23 [a]	7.33 ± 0.18 [ab]	6.36 ± 0.21 [d]	***	***
	O-Pineapple	7.51 ± 0.26 [bc]	7.11 ± 0.12 [cd]	6.93 ± 0.10 [cd]	8.41 ± 0.12 [a]	8.01 ± 0.14 [ab]	6.56 ± 0.11 [d]	***	***
	O-Sweet fruit	7.01 ± 0.11 [bc]	6.52 ± 0.21 [a]	6.98 ± 0.23 [ab]	6.63 ± 0.26 [c]	7.02 ± 0.16 [ab]	6.96 ± 0.19 [ab]	***	**
	O-Balsamic	7.51 ± 0.12 [a]	6.86 ± 0.09 [bc]	7.16 ± 0.16 [b]	7.01 ± 0.15 [bc]	6.81 ± 0.12 [c]	6.83 ± 0.10 [c]	***	***
	O-Butter	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	n.s.
	O-Rancid	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	1.00 ± 0.00 [a]	***	n.s.
	O-complexity	8.33 ± 0.22 [a]	7.52 ± 0.25 [b]	7.02 ± 0.26 [bc]	6.88 ± 0.14 [c]	6.99 ± 0.11 [bc]	6.92 ± 0.15 [c]	***	***
taste	Sweet	7.24 ± 0.21 [b]	7.93 ± 0.23 [a]	6.70 ± 0.13 [c]	6.10 ± 0.22 [d]	6.23 ± 0.15 [cd]	6.18 ± 0.12 [d]	***	***
	Sour	5.83 ± 0.23 [a]	5.91 ± 0.11 [a]	5.98 ± 0.24 [a]	5.97 ± 0.22 [a]	5.76 ± 0.18 [a]	5.83 ± 0.12 [a]	***	n.s.
	Salty	6.56 ± 0.25 [a]	5.60 ± 0.18 [bc]	5.78 ± 0.11 [b]	5.61 ± 0.16 [bc]	6.71 ± 0.16 [a]	5.21 ± 0.25 [c]	***	**
	Bitter	4.71 ± 0.12 [ab]	4.65 ± 0.14 [ab]	4.71 ± 0.19 [ab]	4.68 ± 0.21 [ab]	4.35 ± 0.15 [b]	4.84 ± 0.22 [a]	***	*
	Body	8.2 ± 0.21 [ab]	8.50 ± 0.23 [a]	7.73 ± 0.18 [b]	6.22 ± 0.18 [c]	6.51 ± 0.22 [c]	6.52 ± 0.19 [c]	***	***
	Balance	8.23 ± 0.23 [a]	7.69 ± 0.25 [ab]	7.42 ± 0.18 [b]	7.34 ± 0.16 [b]	7.48 ± 0.11 [b]	7.27 ± 0.23 [b]	***	**
Flavour	F-Banana-like	7.12 ± 0.18 [abc]	6.57 ± 0.14 [c]	7.11 ± 0.23 [abc]	6.83 ± 0.27 [bc]	7.16 ± 0.18 [ab]	7.45 ± 0.21 [a]	***	**

	F-Pear	7.41 ± 0.14 [a]	6.91 ± 0.18 [bc]	7.22 ± 0.21 [ab]	6.62 ± 0.16 [c]	6.87 ± 0.25 [bc]	6.71 ± 0.12 [c]	***	**
	F-Fruity	7.75 ± 0.18 [a]	7.53 ± 0.24 [ab]	6.93 ± 0.16 [c]	7.62 ± 0.22 [ab]	7.12 ± 0.19 [bc]	7.32 ± 0.21 [abc]	***	**
	F-Intensity	8.32 ± 0.26 [a]	7.92 ± 0.21 [ab]	6.76 ± 0.18 [c]	8.28 ± 0.16 [a]	7.72 ± 0.19 [b]	7.68 ± 0.20 [b]	***	***
	F-Persistence	7.72 ± 0.14 [b]	7.92 ± 0.18 [a]	7.71 ± 0.16 [ab]	7.42 ± 0.23 [bc]	7.22 ± 0.16 [bc]	7.12 ± 0.19 [c]	***	***
	F-Complexity	7.91 ± 0.23 [a]	8.42 ± 0.21 [a]	7.30 ± 0.18 [b]	6.36 ± 0.19 [c]	7.26 ± 0.24 [b]	7.28 ± 0.22 [b]	***	***
	F-Pineapple	7.80 ± 0.18 [ab]	7.42 ± 0.14 [bc]	7.21 ± 0.23 [c]	8.12 ± 0.15 [a]	7.90 ± 0.21 [ab]	6.92 ± 0.19 [c]	***	***
	F-Sweet fruit	8.20 ± 0.23 [ab]	8.62 ± 0.25 [a]	7.87 ± 0.18 [b]	7.10 ± 0.21 [c]	7.32 ± 0.16 [c]	7.28 ± 0.15 [c]	***	***
	Overall quality	8.69 ± 0.21 [a]	8.91 ± 0.24 [a]	7.53 ± 0.14 [b]	6.91 ± 0.19 [c]	7.82 ± 0.22 [b]	7.62 ± 0.16 [b]	***	***
	Odour	8.81 ± 0.26 [a]	8.31 ± 0.16 [ab]	8.27 ± 0.18 [b]	8.12 ± 0.15 [b]	7.87 ± 0.19	7.93 ± 0.14 [b]	***	**
	Taste	7.64 ± 0.19 [a]	7.36 ± 0.15 [a]	7.21 ± 0.16 [a]	7.32 ± 0.19 [a]	7.46 ± 0.23 [a]	7.51 ± 0.18 [a]	***	*
	Mouth-feel	8.43 ± 0.18 [a]	8.10 ± 0.16 [ab]	7.69 ± 0.18 [b]	7.67 ± 0.21 [b]	7.84 ± 0.23 [b]	7.64 ± 0.23 [b]	***	**
	Flavour	9.23 ± 0.34 [a]	8.51 ± 0.24 [b]	8.26 ± 0.18 [bc]	7.87 ± 0.16 [c]	8.18 ± 0.18 [bc]	8.23 ± 0.19 [bc]	***	***
Finish	After-smell	8.23 ± 0.15 [a]	7.91 ± 0.18 [ab]	7.82 ± 0.16 [ab]	7.52 ± 0.19 [bc]	7.72 ± 0.21 [bc]	7.28 ± 0.18 [c]	***	**
	After-test	8.21 ± 0.23 [ab]	8.71 ± 0.27 [a]	7.91 ± 0.18 [b]	6.67 ± 0.19 [c]	6.96 ± 0.19 [c]	7.11 ± 0.21 [c]	***	***

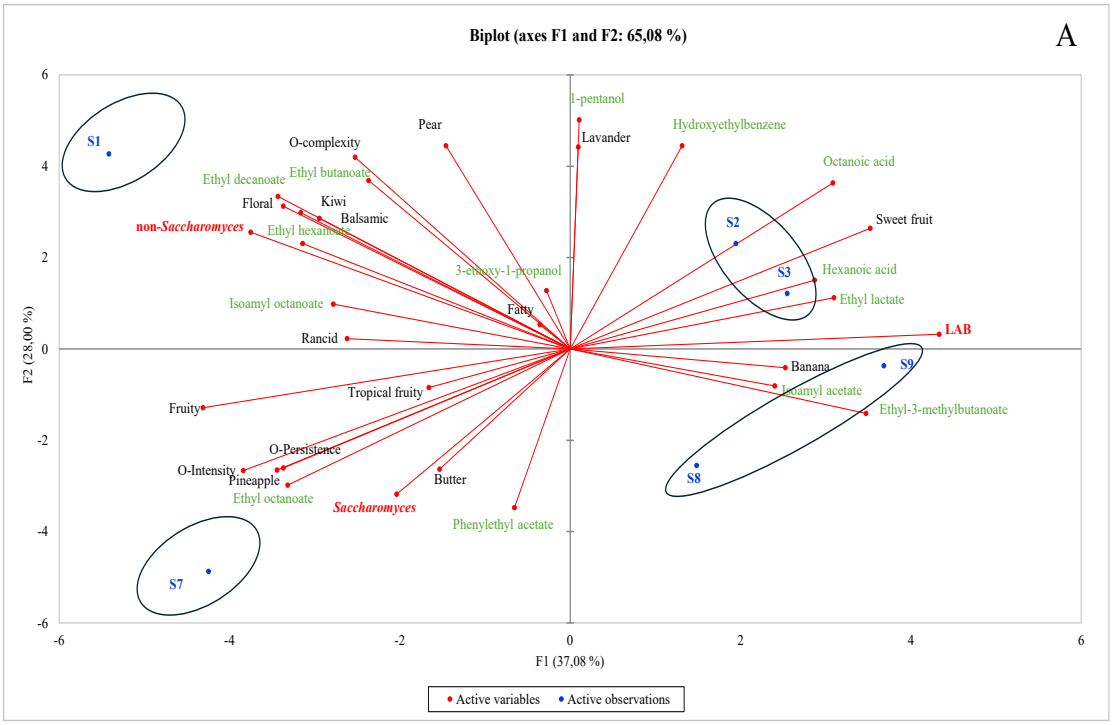
Results indicate mean value of three replicate sessions.

[α] Sensorial attribute;

[β] Relative amounts expressed in on a numerical scale of 1 to 9;

[γ] Statistical significance. Data in the same line followed by the same letter are not significantly different according to Tukey's test. P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.

The results of the PCA analysis indicated that these perceptions and the floral attributes were positively correlated with the olfactory complexity observed in the S1 trial (Figure 7A). Furthermore, additional sensory distinctions were observed between the S1 trial and the S7 trial (*Starm. lactis-condensi* - *S. cerevisiae* versus *S. cerevisiae*, respectively). Fruity odour perceptions of kiwi and pear distinguished the S1 trial from the S7 control trial. In particular, the olfactory attribute of pear detected by the judges was found to be closely correlated with ethyl decanoate, ethyl hexanoate, ethyl octanoate, isoamyl octanoate and ethyl butanoate (Fig. 7A). This relationship was explained by 37.08% of the total variance, which equates to 65.08% of the total variance. It is likely that the pear scent is the result of a synergistic effect of the ethyl esters mentioned above.



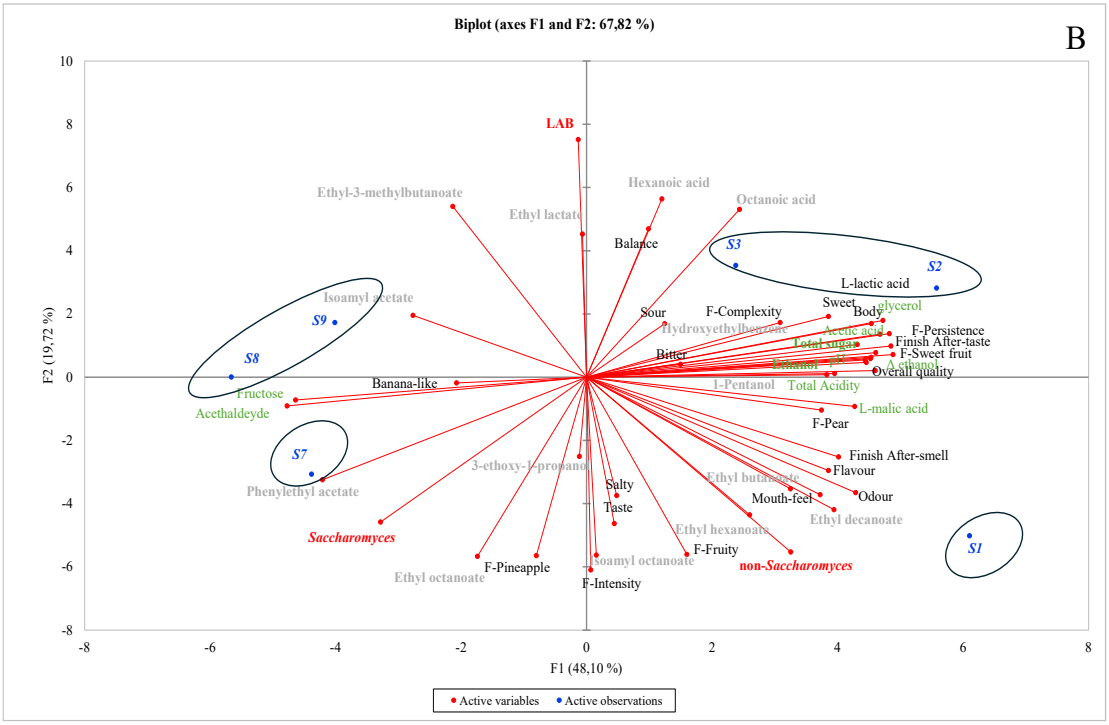


Fig. 7. Principal component analysis (PCA) biplot: (A) OAV>1, aroma attributes (volatil attributes) and microbial populations; (B) OAV>1, flavour, taste and finish attributes (non-volatil attributes) and microbial populations.

This hypothesis is consistent with that proposed by Philipp et al. (2018). It would appear that trials using LABs have shown a sensory difference that is strictly dependent on the use of the MN412 strain (S2, S3 *vs* S8, S9 trials) (Fig. 7A). S2 trials exhibited the highest lavender floral smell score compared to the S1 and S7 trials (Table S8). Hydroxyehtylbenzene and 1-penanol exerted a considerable influence on the perception of this aroma. PCA analysis demonstrated that these two compounds account for 37.08% of the variance in the positive correlation between the two variables (Fig. 7A). Some discrepancies were observed between the OAV values (Table S5) and the olfactory judgments of the sensory panel. No judge was capable of distinguishing between the buttery, rancid and fatty perceptions (Table S8). In fact, there was a negative correlation between these perceptions and ethyl lactate and medium-chain fatty acids, namely hexanoic and decanoic. This result is in line with what has already been found by García-Carpintero, Sánchez-Palomo, Gallego, & González-Viñas, 2011. The S9 test showed the highest score for banana scent (Table S8). This result is consistent with the analysis of the OAVs and their correlation with isoamyl acetate (Fig. 7A). This association is further corroborated by the observation that isoamyl acetate appears to predominate over the other OAVs in

Fig. 6F. Furthermore, the surface sharing detected by the 3D-plot surface (Fig. 6F) between isoamyl acetate and 3-ethoxy-1-propanol leads to the hypothesis that the perception of banana felt by the judges was amplified by a potential synergistic effect between the two compounds. However, the use of LAB had a significant impact in determining differences in the olfactory attributes of wines. In the trials involving the application of LAB in microbial consortia, the biplot analysis associated the olfactory perception of F-sweet fruit (Fig. 5A). However, the association was greater when they were used in consort with MN412 strain. The synergistic effect that medium-chain fatty acids are able to turn with fruity perceptions would be a valid explanation for the LAB-F-sweet fruit association. This is in line with what was reported by San-Juan et al., 2011. This hypothesis was also confirmed by the PCA analysis of the direct correlation between hexanoic acid, octanoic acid and F-sweet fruity (37.08% out of 65.08% total variance) (Fig. 7A). Further effects of MN412 include those attributable to taste sensory fractions. The tactile sensation (Jackson, 2022) of the body was positively correlated with the use of the non-*Saccharomyces* strain in the microbial consortium. This association was explained by 48.10% of the variance out of 67.82% of the total variance. However, the sensory analysis (Table S8) gave the S2 trial the highest score over the S1 and S3 trials (8.50 *vs* 8.20 and 7.83 respectively) (Table S8). This differentiation was subsequently validated by PCA (Fig. 7B), which demonstrated a positive correlation between body perception and glycerol (Jackson, 2022), both in the S2 trial. Furthermore, sweetness was also identified to be closely dependent on glycerol. This taste sensation was more pronounced in the S2 trial than in the S1 and S3 trials (6.42 *vs* 6.30 and 6.11) (Table S8). Moreover, the S2 trial exhibited the highest sweet fruity taste score relative to all the trials (Table S8). The PCA analysis (Fig. 7B) demonstrated a positive correlation between this taste attribute and the decrease in ethanol recorded for this trial (Table S8). Furthermore, principal component analysis demonstrated how the decrease in ethanol contributed to enhanced F-persistence, post-test finish, and overall quality perception in the S2 trial (Fig. 7B; Table S8). One potential explanation for this phenomenon may lie in the lower availability of hydroxyl groups in the wine, derived from ethanol, capable of binding the acidic fraction of esters (Jackson, 2022). This results in

a higher availability of volatile esters than the other trials. The combined effect of human body temperature positively influenced the transition to the vapor state in the oral cavity, thereby increasing the perceptibility of taste to the taster (Jackson, 2022).

3.4 Conclusion

The utilization of microbial consortia was found to be of critical importance for the characterization of wine sensory profiles. The results of chemical-physical analyses and non-targeted metabolomic studies of wines demonstrated the biotechnological compatibility of the *Starm. lactis-condensi* MN412 strain, *O.oeni* MLB6™ strain and *L. plantarum* MLPK45H™ strain with the *S. cerevisiae* QA23™ strain. Furthermore, the development of UHPLC-q-ToF-MS analysis indicated that the microbial consociation of *Starm. lactis-condensi* MN412 strain and *S. cerevisiae* QA23™ strain was fully compatible with Catarratto grape must, maintaining the highest levels of bioactive substances, such as trans-resveratrol. The chemical-physical results and non-targeted metabolomic investigation of the wines demonstrated the biotechnological compatibility between Starm. lactis condensi MN412, *O. oeni* MLB6™ and *L. plantarum* MLPK45H™ and *S. cerevisiae* QA23™. Furthermore, the development of UHPLC-q-ToF-MS analysis indicated that the microbial association *Starm. lactics-condensi* MN412 strain - *S. cerevisiae* QA23™ strain was fully compatible with Catarratto grape must, maintaining the highest contents of bioactive substances such as trans-resveratrol. The taste and smell profiles of the wines exhibited a significant impact from microbial interactions. Consequently, the results obtained indicate the validity of the application of microbial consortia as an alternative biotechnological tool to pure culture. For the confirmation of these effects, the contribution of the calculation of RI-values and the graphical representation by means of 3D-plot surfaces was fundamental.

The application of this novel approach to the active volatile fraction of the wine, which constitutes the aroma buffer, enabled the differentiation of the disparate microbial contributions, which appeared similar when subjected to further statistical representations.

Furthermore, the correlation of the OAVs and sensory attributes with the subsequent comparison of the RI-values in 3D-plot surfaces, conducted via PCA analysis, led to the explanation of many of the sensory effects resulting from the synergistic effects among different VOCs. These effects included the fruity and floral smell of pear and lavender, respectively.

This was corroborated by the hypotheses previously proposed by numerous authors, but with the use of intricate testing and analysis systems in GC-O.

It can therefore be argued that the introduction of RI-values and 3D-plot surface representation represents a computational system capable of bridging the "knowledge gap" between what is perceived by the human sense of smell, through the detection of sensory attributes, and the models currently used in the representation of wine aromas.

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Activity 2.5

Impact of two new non-conventional yeasts (*Starmerella lactis-condensi* and *Candida oleophila*), isolated from sugar-rich substrates, on Frappato wine aroma

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Abstract

The interest of non-*Saccharomyces* yeasts in wine fermentation increased constantly in last years. This study reports for the first time the enological potential of two strains *Starmerella lactis-condensi* MN412 and *Candida oleophila* YS209. In an innovative way, these strains were used in winemaking to improve floral and fruity aroma of Frappato red wine, which has not been explored. The enological performances of the two non-*Saccharomyces* strains were compared to a wine strain of *Starmerella bacillaris*, namely Cz3, previously characterized in winemaking conditions. In these three cases, the non-*Saccharomyces* strain was sequentially inoculated with *S. cerevisiae* wine strain NF213, used as control. The *St. lactis-condensi* MN412 was isolated from Sicilian manna, a sugar-rich matrix, extracted from *Fraxinus angustifolia* trees (Oleaceae). The strain *C. oleophila* YS209 was isolated from honey by-products. Microbiological counts showed the ability of MN412 and YS209 to maintain high counts up to 6 days of alcoholic fermentation. Regarding chemical parameters, Cz3 showed the highest glycerol production. Analysis of VOCs revealed that the trials with non-*Saccharomyces* yeasts were characterized by a higher concentration of esters that contributed positively to the fruity aroma of the wines. The sensory analysis confirmed that the use of MN412 and YS209 impacted positively the final wines in terms of fruity and floral intensity, respectively, while did not generate sensory defects. In conclusion, non-conventional yeasts represent strategy to improve floral-fruity freshness of wine aroma and sugar-rich matrices such as manna ash and honey might represent novel ecological niches as source of potential oenological yeast.

Keywords: *Starmerella lactis-condensi, Candida oleophila*, *Starmerella bacillaris*, Wine fermentation, aroma, Honey Sugar-rich matrix

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1. Introduction

Non-*Saccharomyces* yeasts *constitute the largest microbial* group present on grape berries (Borren and Tian, 2021). Generally, they play an important role in the first days of fermentation when the levels of ethanol are quite low (Benito et al., 2019a). During the alcoholic fermentation, the composition of non-*Saccharomyces* yeast populations changes in relation to the evolution of ethanol concentration with the species sensitive replaced by those moderately resistant to the increasing levels of ethanol (Zhao et al., 2021), with *Saccharomyces* yeasts being predominant in the last stages of alcoholic fermentation (Mateus et al., 2020). Due to the key role played by yeasts on the sensory traits of wines (Romani et al., 2020; Varela, 2016), in the last decade, studies on oenological microbiology have focused also on the impact of non-*Saccharomyces* yeasts (Benito et al., 2019b).

Among the different non-*Saccharomyces* species, *Candida* and *Starmerella* species have recently been successfully used in mixed fermentation with *S. cerevisiae* to reduce ethanol content (Englezos et al., 2016a), increase glycerol concentration (Giaramida et al., 2013) and generating pleasant esters in wine (Englezos et al., 2016b). Currently, most of the *Candida* and *Starmerella* species used in winemaking are derived from oenological sources, mainly grapes and must (Di Maio et al., 2012). Recent studies proved that matrices with a high sugar content (e.g. honey by-products) are rich in microorganism, in particular *Saccharomyces* and non-*Saccharomyces* yeasts (Gaglio et al., 2017; Sinacori et al., 2014). Consequently, some of these strains present in these matrices have shown good aptitude for use in fermentation processes (Francesca et al., 2022). Moreover, Prestianni et al. (2022) applied *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* isolate from honey by-product to improve and stabilize the quality of mead. Alfonzo et al. (2021) also tested the suitability of *S. cerevisiae* strains from non-oenological sources in winemaking and evidenced consistent differences with *S. cerevisiae* of grape origin.

A previous study conducted by Guarcello et al. (2019) analysed the cultivable microbial ecology of Sicilian manna ash, a sugar-rich matrix, and isolated several non-*Saccharomyces* yeasts, including *S. lactis-condensi* strains.

S. lactis-condensi were isolated from different oenological sources such as *Vitis labrusca* grapes (Čadež et al., 2020), botrytized Tokaj Essence wines (Csoma et al., 2021). Battistelli et al. (2021) have found a high presence of *S. lactis-condensi* investigating the cultivable microbiota of "mothers" of *Vino cotto*. Recently, Csoma et al. (2023) clarified the fructosophilic role of the dominant species *S. lactis-condensi* in Essences, a typical sweet wine from the Tokaj wine region in Hungary.

Franco et al. (2021) isolated *C. oleophila* in spontaneous fermentations of grape musts, tested its fermentative capabilities, and conducted sequential fermentation with *S. cerevisiae* in laboratory bioreactors. The same authors found high acetic acid production by *C. oleophila* but did not investigate the impact of this yeast on the composition of volatile organic compounds (VOC) or the sensory profile of wines. Other authors, Lachance et al. (2011) found the ability of *C. oleophila* to metabolize glucose at various levels, and Aplin et al. (2019) described *C. oleophila* under laboratory winemaking conditions finding high acetic acid production. Therefore, to date, *C. oleophila* has been not used as starter or co-starter in any winemaking process.

Sicily is among the main Italian regions active in the production of red and rosé wines. In 2020, about 2 million hL/year of red wines were produced in Sicily (ISTAT, 2020). Among red grapes, Frappato is an autochthonous cultivar mainly cultivated within the provinces of Ragusa and Trapani with a total surface of about 750 ha for the production of Controlled and Guaranteed Denomination of Origin wine "Cerasuolo di Vittoria" (Asciuto and Bacarella, 2008). Frappato wines are characterised by a light ruby red color, brilliant, vinous, fruity and floral notes (Leder, 2020), but very little is known about the evolution of physicochemical, microbiological and aromas parameters of these wines. Frappato wines are commonly produced with commercial strains of *S. cerevisiae*, the species that ensures fermentation reproducibility and wine balancing.

However, many other yeast species with secondary importance during fermentation persist for the entire process. The positive effect of non-*Saccharomyces* yeasts in developing high taste-olfactory complexities has been highlighted (Fazio et al., 2023). This aspect well encounters the current consumer demand for novel wine styles (Comitini et al., 2023).

To our knowledge, however, no previous work has evaluated the effect of *S. lactis-condensi* and *C. oleophila* strains in sequential inoculation with *S. cerevisiae* during wine fermentation and investigated for their capability to improve aroma. Both *S. lactis-condensi* and *C. oleophila* strains have been isolated from novel ecological niches, such as manna ash and honey by-products with high sugar content.

Based on the above considerations, the present study aimed to: (i) to evaluate two non-conventional yeast strains (*S. lactis-condensi* MN412 and *C. oleophila* YS209) isolated from "natural environments" (manna and honey) for their potential in Frappato winemaking; (ii) to deepen our knowledge on VOCs composition of Frappato red wine.

2. Material and methods

2.1 Strain preparation, experimental plan and sample collection

Non-*Saccharomyces* strains *S. lactis-condensi* MN412 isolated from manna (Guarcello et al., 2019), *C. oleophila* YS209 isolated from honey by-product, and *S. cerevisiae* NF213 isolated from grape must (Settanni et al., 2012) belong to the oenological yeast collection of the Department of Agricultural, Food and Forestry Sciences (SAAF; University of Palermo, Italy). All strains were reactivated from -80°C stock in Yeast Peptone Dextrose (YPD) at 28°C for 48 h and were reproduced in a concentrated liquid suspension by Bionova srl (Villanova sull'Arda, Piacenza, Italy). *St. bacillaris* Cz3 is a strain of oenological origin (Di Maio et al., 2012) deposited in the yeast collection of the Sicilian Regional Institute of Wine and Oil (IRVO, Palermo, Italy) and marketed by Bioagro srl (Thiene, Vicenza, Italy). Grape of "Frappato" cultivar were donated by the winery "Caruso & Minini srl" located in Marsala (Italy).

The experimental plan of the present study (Fig. 1) consisted of four treatments: N1, sequential inoculum of *S. lactis-condensi* MN412/*S. cerevisiae* NF213; N2, sequential inoculum of *C. oleophila* YS209/*S. cerevisiae* NF213; N3, sequential inoculum of *St. bacillaris* Cz3/*S. cerevisiae* NF213; N4,

single inoculum of *S. cerevisiae* NF213. In trials N1-N3, *S. cerevisiae* NF213 was inoculated 72 h after the addition of non-*Saccharomyces* strains.

All vinification were conducted at Department SAAF of University of Palermo, Italy and samples were collected at different stages of vinification: after grape pressing, after yeast inoculation, during alcoholic fermentation at day 1, 2, 3, 6, and at the end of fermentation (14 days). All analyses were performed in triplicate.

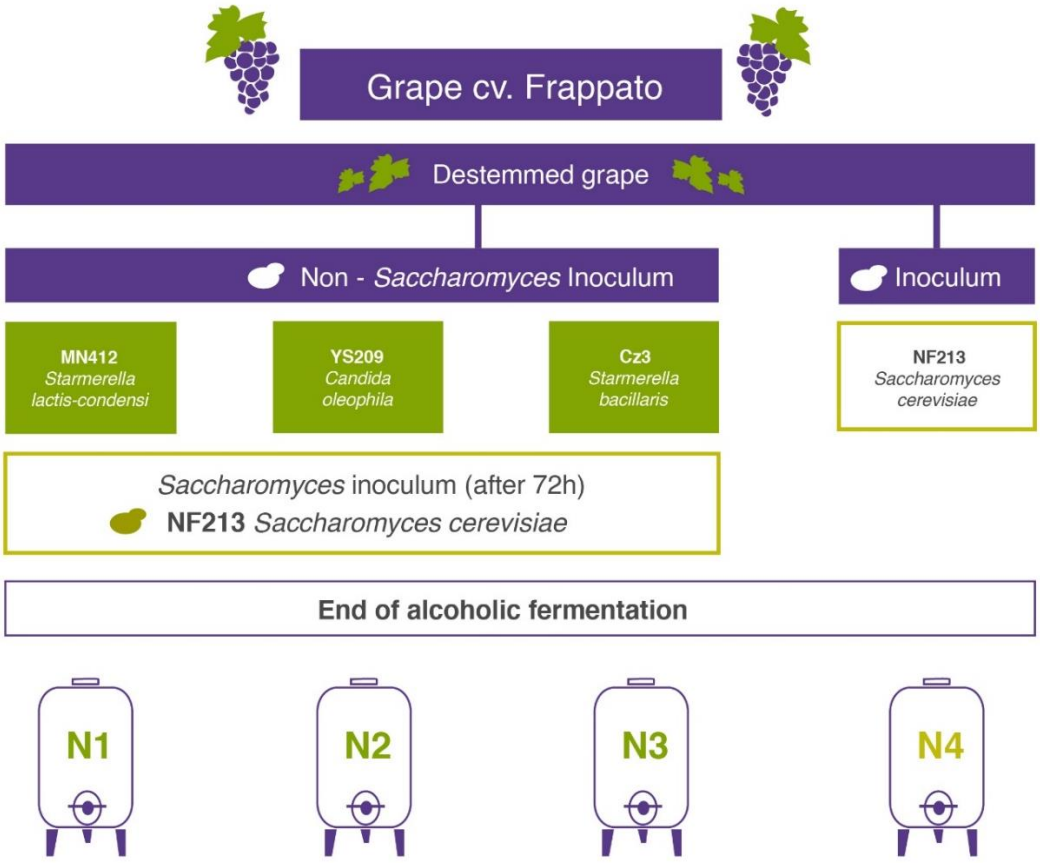

Figure 1. Experimental design of Frappato wines vinified with different non-*Saccharomyces* yeast strain.

2.2 Winemaking

Grapes were stemmer-crushed and supplemented with 2 g/hL of potassium metabisulphite (Chimica Noto s.r.l., Partinico, Italy). Bulk grape must was used to fill three test tanks (250 L each) for a total of 12 vats. Before yeast inoculation, 20 g/hL of diammonium phosphate (Chimica Noto SRL) and 20 g/hL of Fermaid E^{TM} (Lallemand, Castel D'Azzano, Italy) were added to each vat. All strains in

concentrated liquid suspension [approx. 7.00×10^{10} colony-forming units (CFU)/g] were inoculated (20 mL/hL) according to the experimental plan; the alcoholic fermentation was conducted at 22 °C. At the end of alcoholic fermentation, 5 g/hL of potassium metabisulphite was added. The wines were aged in steel tanks 18 °C for two months. At bottling, free sulfur dioxide was adjusted to an approximate concentration of 30 mg/L. Bottled wines were kept at 15 °C. The winemaking process followed an oenological protocol used extensively by several wine companies. The process was performed at experimental wine cellar of University of Palermo based in Palermo city, Sicily (Italy).

2.3 Microbiological analysis

All samples collected during alcoholic fermentation were analysed for yeast colonies forming units, using various selective/differential culture media. Ten milliliters of each must sample were diluted in 90 mL of Ringer's solution (Sigma-Aldrich, Milan, Italy) and plated on Wallerstein Laboratory (WL) nutrient agar (incubated at 28 ºC for 72h) for *Saccharomyces* yeast quantification, and on lysine agar (incubated at 28 ºC for 5 days) for non-*Saccharomyces* (Di Maio et al., 2011). All media and supplements were purchased from Oxoid (Basingstoke, UK).

2.3.1 Yeast isolation, molecular identification and strain typing.

The dominance of the three non-*Saccharomyces* strains selected for this study was verified after three days of alcoholic fermentation, while that of *S. cerevisiae* was investigated at the end of alcoholic fermentation. At least five colonies of each yeast group with different morphology were selected from the respective culture media using the morphological criteria described by Cavazza et al. (1992) and Pallmann et al. (2001). All isolates were purified by successive sub-cultures on YPD agar (Lai et al., 2022) and their purity was verified by light microscopy (Carl Zeiss LTd, Berkochen, Germany). Three isolates with the same morphology from a given sample were then subjected to genetic characterization.

Genomic DNA for PCR assays was extracted by InstaGene Matrix kit (Bio-Rad, Hercules, CA, USA) following the protocol provided by the manufacturer. Yeasts differentiation was performed by RFLP

using the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene (Esteve-Zarzoso et al., 1999). One isolate per group was further analysed by sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary identification obtained by RFLP analysis as indicated by Alfonzo et al. (2020). DNA sequencing reactions were performed at AGRIVET (University of Palermo, Italy). Sequence identity was determined by BlastN search against the NCBI non-redundant sequence database (http://www.ncbi.nlm.nih.gov). Sequences were manually corrected using Chromas 2.6.2. (Technelysium Pty Ltd., Australia).

The dominance of *S. cerevisiae* NF213 was confirmed by comparing the interdelta profile of the isolates from the highest cell dilution of musts with that of the pure strain. Interdelta analysis was conducted as described by Legras and Karst (2003). The persistence of non-*Saccharomyces* was carried out by comparing randomly amplified polymorphic DNA(RAPD)-PCR patterns of the isolates with those of the pure strains. RAPD-PCR was performed with primers M13 (Francesca et al., 2014) and XD5 (Di Maro et al., 2007). PCR products were visualised and compared as reported by Alfonzo et al. (2021).

2.4 Physicochemical analysis of musts and wines

The concentration of glucose, fructose, ethanol, glycerol, ammoniacal nitrogen, alpha-amino nitrogen, malic acid, lactic acid, and acetic acid were evaluated by means of the enzymatic analyser iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co. Ltd., Shenzhen, China) as described by Matraxia et al. (2021). Samples were centrifuged (9000 rpm, 10 min) and analysed following the manufacturer's protocol. All reagents were purchased from R-Biopharm AG (Darmstadt, Germany). The values of pH were determined by OIV-MA-AS313-15 method (OIV, 2020a), total acidity was determined by the methodology described by OIV-MA-AS313-01 (OIV, 2020b), and free and total sulfur dioxide were measured in accordance with the methods described by OIV-MA-AS323-04B (OIV, 2020c). All chemical analyses were carried out in triplicate.

2.5 Analysis of VOCs in wine samples

2.5.1 Liquid-liquid extraction

Volatile compound composition of wine samples right after the end of alcoholic fermentation was determined with the following protocol: wine samples (10 mL) from all trials were mixed with MS SupraSolv® dichloromethane (5 mL) in a 50-mL conical flask, stirred at room temperature for 30 min, and centrifuged at 4000 rpm for 10 min by low Speed Centrifuge (ScanSpeed 416) with Swing Rotor (LaboGene ApS Industrivej 6–8, Vassingerød, DK- 3540 Lynge, Denmark); the aqueous phase was removed, added with anhydrous sodium sulphate (1 g), and centrifuged at 4000 rpm for 5 min; dichloromethane layer was removed, and dried under N_2 gas to 0.2 mL.

2.5.2 Identification and quantification of VOCs by GC-MS

Gas chromatographic analyses were performed with Agilent 7000C GC system, fitted with a fused silica Agilent DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness), coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 295 ◦C. Solvent Delay: 3.5 min. Helium was the carrier gas (1 mL/min).

The temperature was initially maintained at 40 °C for 1 min, gradually increased to 250 °C at a rate of 3 °C/min for 30 min, and finally maintained at 250 °C at 10 °C/min. One microliter of each sample was injected at 250 °C automatically and in the splitless mode: transfer line temperature, 295 °C. The individual peaks were analysed using the GC MS Solution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database (Adams, 2007; Nist, 2008). These identifications were also confirmed by other published mass spectra and linear retention indices (LRI). LRI were calculated using a series of n-alkanes (C8-C40). Quantification was carried out using the three calibration lines. For compounds belonging to other classes than the standards, similarity was used for quantification. A dilution factor was used for the reported data.

2.6 Sensory analysis

Sensory evaluation of experimental wines was performed by quantitative descriptive analysis. Fourteen judges (8 men and 6 women, ranging from 26 to 45 years old) were recruited from University of Palermo. All judges had experience in winemaking and participated in previous studies as members of panels judging wines. Besides, they were subjected to preliminary tests to determine their sensory performances on basic tastes and aromas of wines. Sensory analysis of wine was conducted as described by Jackson (2016). The 14 panellists compared the four experimental wines during different sessions. They consensually generated 16 sensory descriptive attributes regarding appearance (colour), odour (intensity, complexity, floral, fruity, spicy, balsamic, and overall odour quality), flavour, taste (intensity, persistence, sour, salty and smoothness, overall taste quality), and overall quality. The panellists also generated a consensual descriptive ballot (Biasoto et al., 2014; Jackson, 2016) and the descriptors were associated to a 9 cm unstructured scale (1 = extremely low, 5 = moderate intensity, 9 = extremely high). The four wine samples were evaluated in distinct tasting sessions carried out on successive days. Overall, each judge evaluated each of the four wines with two repetitions. A given bottle represented a single technical repeat.

2.7 Statistical analysis

ANOVA test was applied to identify significant differences among physicochemical parameters (pH, total acidity, acetic acid, residual sugars, glucose, fructose, alpha-amino nitrogen, ammoniacal nitrogen, ethanol, glycerol, malic acid, lactic acid, free and total SO_2), levels of *Saccharomyces* and non-*Saccharomyces* and sensory analysis. The post-hoc Tukey's method was applied for pairwise comparison of all data. Statistical significance was attributed to $P < 0.05$ (Mazzei et al., 2010). A heat map clustered analysis (HMCA), based on hierarchical dendrogram with heat map plot, was generated from VOCs and the individual content values of data matrix are graphically represented as colours (Martorana et al., 2017). The relative values of VOCs concentration were depicted by colour intensity from yellow (lowest quantity) to red (highest quantity). Heat map analysis was performed

using the autoscaled data using ascendant hierarchical clustering based on Ward's method and Euclidian distance (Gaglio et al., 2017) to show the similarities between VOCs and wine obtained with different yeast starter strains and nutrition regimes.

Sensory Product Characterization Analysis (SPCA) was applied in order to determine the sensory differences of the wines produced by means of an analytical method based on the attributes describing each trial. For each session, the score was evaluated considering product, judge and session effect. A histogram chart of different colours was created for each wine. Blue is associated with coefficients that show a significant positive value and the red color with coefficients showing a significant negative value. Differences between trials were represented graphically with a sensory profile plot.

Statistical data processing and graphic construction were performed with the XLStat software version 2019.2.2 (Addinsoft, New York, USA) for Excel.

3 **Results and discussion**

3.1 Kinetics of yeast populations during fermentation

The growth of yeasts during the alcoholic fermentation is graphically shown in Fig. 2. The levels of non-*Saccharomyces* and *Saccharomyces* populations of Frappato must, at the beginning of monitoring, were 5.3 Log CFU/mL and < 2.0 Log CFU/mL, respectively. Cell density of non-*Saccharomyces* increased at 6.0-7.3 Log CFU/mL just after inoculation; these densities are considered adequate to influence the sensory characteristics of wines (Du Plessis et al., 2017). After 3 d, the trials N1-N3 were inoculated with *S. cerevisiae* NF213 until 7.3 to 8.3 Log CFU/mL. After further 3 d, all trials showed a decrease of non-*Saccharomyces,* a trend already registered by Binati et al. (2020), who followed a sequential inoculum of *S. bacillaris* and *S. cerevisiae.* Specifically, values lower than 2-3 Log CFU/mL were registered for trial N1, N2 and N3, after 6 d from the beginning of fermentation. The decrease of non-*Saccharomyces* populations in sequential inoculum with *S. cerevisiae* is determined by several events, mainly increased ethanol concentrations, secretion of inhibitory substances, and competition phenomena (Wang et al., 2016). According to Binati et al.

(2020), at the end of alcoholic fermentation (14 d), non-*Saccharomyces* populations were at levels lower than the detection limit. On the contrary, *Saccharomyces* were in the range 7.9-8.6 Log CFU/mL for all trials.

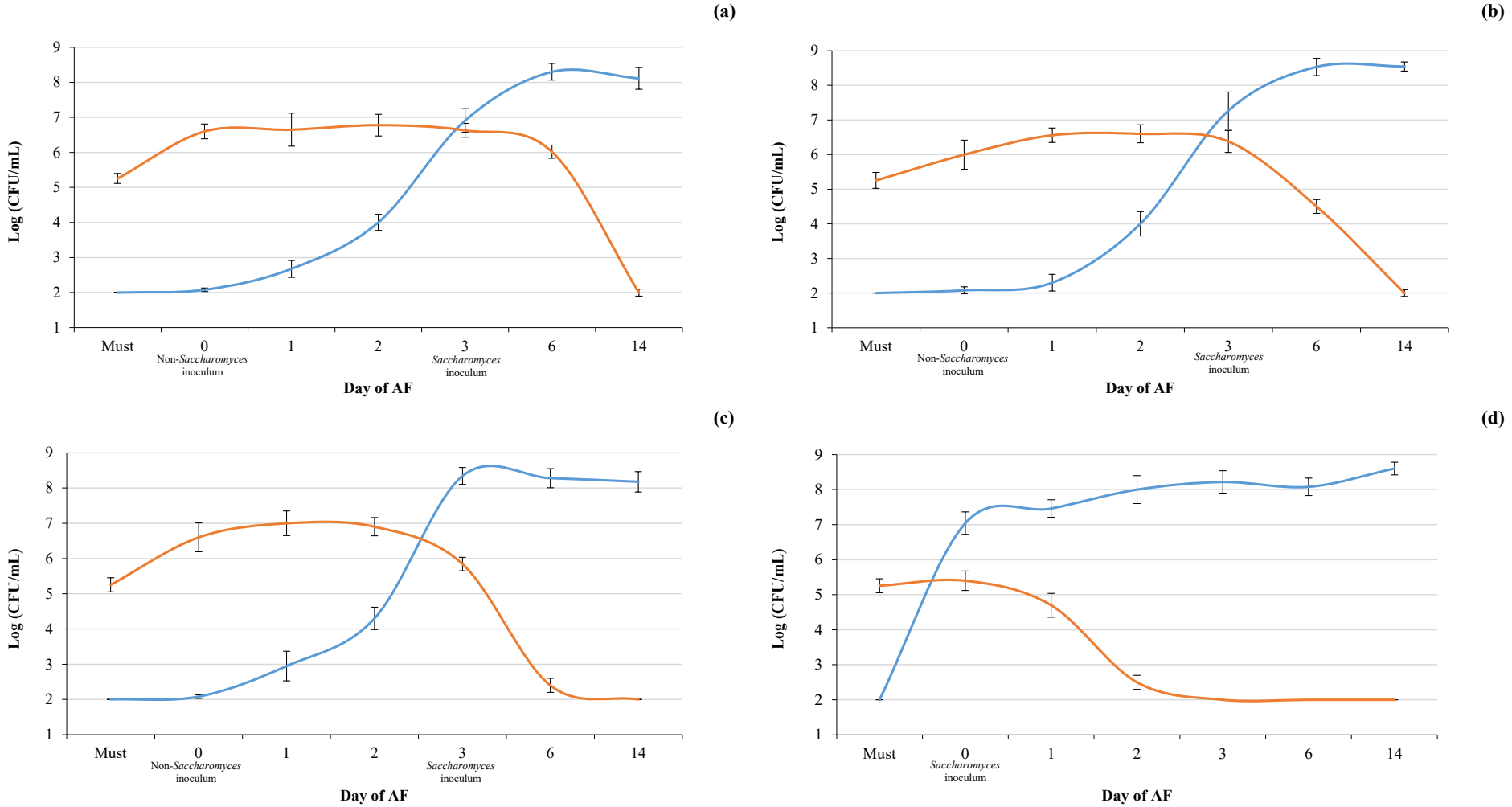

Figure 2. Evolution of yeast populations of presumptive *Saccharomyces cerevisiae* and non-*Saccharomyces* populations during alcoholic fermentation: **(a)** sequential inoculum *Starmerella lactis-condensi* MN412/*Saccharomyces cerevisiae* NF213 (N1); **(b)** sequential inoculum *Candida oleophila* YS209/*Saccharomyces cerevisiae* NF213 (N2); **(c)** sequential inoculum *Starmerella bacillaris* Cz3/*Saccharomyces cerevisiae* NF213 (N3); **(d)** single inoculum *Saccharomyces cerevisiae* NF213 (N4; control). Legend: —, presumptive *Saccharomyces*; —, non-*Saccharomyces*.

3.2 Dominance of inoculated yeasts

A total of 1003 colonies that had grown on WL were isolated, sequentially re-propagated on WL and checked for their colony colour, colony topography and microscopic observations (Cavazza et al., 1992; Pallmann et al. 2001); 748 colonies were classified as *Saccharomyces*. The analysis of 5.8S-ITS amplicons confirmed that all these isolates shared a 5.8S-ITS region of 880 bp typical of *S. cerevisiae* and the profile of the restriction fragments obtained with C*fo*I, *Hae*III and *Hinf*I confirmed that these isolates were *S. cerevisiae*. (Guillamón et al., 1998).

The other unclassified 255 isolates were assigned to the non-*Saccharomyces* yeast group.

Eighty-nine isolates were characterized by an ITS amplicon of 480 bp and were presumptively identified and *S. lactis-condensi*. In fact, the same ITS amplicon sizes were found by Solieri et al. (2006), who worked on *S. lactis-condensi*. Eighty-two isolates showed ITS amplicons of 630 and were considered presumptive *C. oleophila* (n=82) while 67 were allocated to the species *S. bacillaris* (n=67) based on the 430 bp amplicon (Gordún Quiles et al., 2018; Wang et al., 2019). The remaining isolates (n=17) showed an ITS amplicon between 750 (n=11) and 760 (n=6) bp with a colony morphology on WL agar similar to that of yeasts of the genus *Hanseniaspora*, which are very common in sicilian Frappato musts (Romancino et al., 2008). RFLP profiles of non-*Saccharomyces* species confirmed what observed by other authors who identified yeasts (Esteve-Zarzoso et al., 1999; de Llanos Frutos et al., 2004; Solieri et al., 2006; Wang et al., 2019).

Interdelta analysis confirmed the presence of three different strains of *S. cerevisiae*. The different interdelta profiles also indicated the presence of indigenous grape *S. cerevisiae* (Aponte et al., 2020). The direct comparison of the interdelta profiles showed that *S. cerevisiae* NF213 was the strain most frequently isolated (>96%). RAPD pattern comparison indicated that each non-*Saccharomyces* inoculated strains showed a dominance percentage higher than 90%. Yeast genotypic identification was completed by pairwise alignment of D1/D2 sequence of the 30% isolates with those of type strains (*C. oleophila* CBS2219^T, *S. cerevisiae* CBS 1171^T, *S. lactis-condensi* CBS 52^T and *S. bacillaris*

CBS9494^T); D1/D2 sequence from the strains Cz3, MN412, NF213 and YS209, and used in this study showed 100% homology with type strains.

3.3 Chemical monitoring

The results of the chemical analyses are summarized in Table 1. The initial sugar content of Frappato grape must of this study was 231.83 g/L (114.18 g/L glucose and 117.65 g/L fructose), total titratable acidity (TTA) of 8.11 g/L tartaric acid, 2.13 g/L malic acid and pH 3.15.

After 72 h, the majority of chemical parameters showed significant differences among trials, while pH and concentrations of malic acid, lactic acid, free and total SO_2 were quite comparable.

Table 1.

Chemical parameters determined during the winemaking process.

Parameters	Musts	Vinification									
		3 d of alcoholic fermentation					End of alcoholic fermentation				
		N1	N2	N3	N4	S.S.	N1	N2	N3	N4	S.S.
Ammoniacal nitrogen$^{\alpha}$	249.17±0.12	210.98±0.14^{c}	218.45±0.09^{a}	215.75±0.13^{b}	89.22±0.18^{d}	***	48.59±0.14^{d}	88.13±0.09^{a}	73.84±0.17^{b}	51.26±0.18^{c}	***
Alpha-amino nitrogen$^{\alpha}$	192.94±0.15	192.48±0.12^{b}	192.51±0.11^{b}	207.92± 0.19^{a}	59.61±0.04^{c}	***	97.86±0.12^{d}	105.68±0.11^{b}	102.58±0.11^{c}	116.47±0.04^{a}	***
Residual sugars$^{\beta}$	231.83±0.26	174.90±0.12^{a}	181.73±0.20^{b}	172.31±0.15^{b}	90.01±0.26^{c}	***	0.14±0.02^{a}	0.07±0.01^{b}	0.07±0.03^{b}	0.12±0.02^{ab}	*
Glucose$^{\beta}$	114.18±0.10	112.49±0.08^{a}	109.55±0.06^{b}	110.86±0.10^{b}	29.32±0.05^{c}	***	0.03±0.01^{a}	0.02±0.00^{a}	0.07±0.03^{a}	0.03±0.01^{a}	n.s.
Fructose$^{\beta}$	117.65±0.15	62.41±0.21^{a}	72.18±0.12^{a}	61.45±0.08^{b}	60.69±0.14^{c}	***	0.11±0.02^{a}	0.05±0.02^{ab}	0.00±0.00^{b}	0.09±0.04^{a}	**
Acetic acid$^{\beta}$	0.02±0.02	0.06±0.02^{b}	0.04±0.03^{b}	0.21±0.04^{a}	0.09±0.02^{b}	**	0.31±0.02^{a}	0.28±0.04^{a}	0.31±0.06^{a}	0.26±0.01^{a}	n.s.
Malic acid$^{\beta}$	2.13±0.03	2.08±0.02^{a}	2.02±0.03^{a}	2.11±0.06^{a}	2.10±0.02^{a}	n.s.	1.90±0.02^{b}	1.87±0.03^{b}	1.99±0.04^{a}	1.91±0.02^{b}	*
Lactic acid$^{\beta}$	0.04±0.01	0.02±0.01^{a}	0.04±0.01^{a}	0.05±0.02^{a}	0.03±0.01^{a}	n.s.	0.06±0.02^{a}	0.07±0.01^{a}	0.07±0.02^{a}	0.06±0.02^{a}	n.s.
Glycerol$^{\beta}$	0.35±0.01	0.75±0.14^{c}	0.70±0.17^{c}	2.76±0.05^{b}	6.68±0.11^{a}	***	7.90±0.16^{b}	8.26±0.10^{b}	10.31±0.17^{a}	8.29±0.14^{b}	***
Ethanol$^{\gamma}$	0.01±0.01	2.87±0.02^{c}	2.99±0.01^{b}	2.22±0.06^{d}	7.13±0.01^{a}	***	11.70±0.06^{bc}	11.78±0.03^{b}	11.65±0.02^{c}	11.99±0.03^{a}	***
pH	3.15±0.01	3.16±0.02^{a}	3.15±0.01^{a}	3.17±0.00^{a}	3.15±0.01^{a}	n.s.	3.13±0.02^{a}	3.14±0.01^{a}	3.14±0.00^{a}	3.16±0.01^{a}	n.s.
Total titratable acidity$^{\delta}$	8.11±0.09	6.82±0.12^{b}	6.84±0.10^{b}	6.88±0.10^{b}	7.17±0.10^{a}	*	6.35±0.10^{b}	6.30±0.10^{b}	6.35±0.10^{b}	6.70±0.10^{a}	**
Free-SO_2^{α}	8.00±0.00	8.00±0.50^{a}	8.50±0.50^{a}	8.00±0.00^{a}	8.75±0.50^{a}	n.s.	17.00±0.50^{a}	16.50±0.50^{a}	17.50±1.00^{a}	18.00±0.50^{a}	n.s.
Total-SO_2^{α}	9.50±0.50	10.00±1.50^{a}	10.50±1.00^{a}	10.00±0.50^{a}	12.00±1.00^{a}	n.s.	32.00±1.50^{a}	28.00±1.00^{b}	30.00±1.00^{ab}	32.00±1.00^{a}	*

Parameters	Vinification				
	Bottling				
	N1	N2	N3	N4	S.S.
Ammoniacal nitrogen$^{\alpha}$	n.d.	n.d.	n.d.	n.d.	n.d.
Alpha-amino nitrogen$^{\alpha}$	n.d.	n.d.	n.d.	n.d.	n.d.
Residual sugars$^{\beta}$	0.04±0.03^{a}	0.02±0.01^{a}	0.03±0.01^{a}	0.05±0.02^{a}	n.s.
Glucose$^{\beta}$	0.00±0.00^{b}	0.00±0.00^{b}	0.03±0.01^{a}	0.02±0.01^{ab}	**
Fructose$^{\beta}$	0.04±0.03^{a}	0.02±0.01^{a}	0.00±0.00^{a}	0.03±0.01^{a}	n.s.
Acetic acid$^{\beta}$	0.33±0.04^{a}	0.29±0.04^{a}	0.35±0.03^{a}	0.28±0.01^{a}	n.s.
Malic acid$^{\beta}$	1.84±01^{c}	1.83±0.01^{c}	1.91±0.01^{a}	1.88±0.01^{b}	***
Lactic acid$^{\beta}$	0.06±0.02^{a}	0.08±0.01^{a}	0.07±0.02^{a}	0.07±0.03^{a}	n.s.
Glycerol$^{\beta}$	7.67±0.07^{c}	8.02±0.11^{b}	10.13±0.08^{a}	8.00±0.07^{b}	***
Ethanol$^{\gamma}$	11.75±0.05^{b}	11.74±0.03^{b}	11.68±0.03^{b}	11.94±0.02^{a}	***
pH	3.18±0.02^{a}	3.16±0.01^{a}	3.19±0.00^{a}	3.20±0.01^{a}	n.s.
Total titratable acidity$^{\delta}$	6.20±0.10^{a}	6.20±0.10^{a}	6.30±0.10^{a}	6.40±0.10^{a}	n.s.
Free-SO_2^{α}	31.00±1.50^{a}	30.50±1.00^{a}	29.50±1.50^{a}	30.50±1.00^{a}	n.s.
Total-SO_2^{α}	55.00±1.00^{a}	49.00±1.50^{a}	50.00±0.50^{a}	51.00±1.00^{a}	n.s.

Result indicates mean value ± standard deviation of three determinations from three replicates. Data within a line followed by the same letter are not significantly different according to Tukey's test.

Symbols: $^{\alpha}$, mg/L; $^{\beta}$, expressed in g/L; $^{\gamma}$, % v/v; $^{\delta}$, tartaric acid g/L.

Abbreviations: S.S., statistical significance; P value: *, P < 0.05; **, P < 0.01; ***, P < 0.001; n.s., not significant; n.d., not determined.

The trials inoculated with *S. lactis-condensi* MN412 (N1) showed the lowest values of residual sugars (174.90 g/L). The trials N1 – N3 showed a higher consumption of fructose rather than glucose, compared to the trial N2. Fructose preference is a common characteristic of certain *Candida* strains (Englezos et al., 2019; Magyar and Tóth, 2011). The highest concentrations of ethanol and glycerol were registered for trial N4 [7.13 % (v/v) and 6.68 g/L, respectively]. Among the sequential inoculation trials carried out, N2 was the trial showing the highest ethanol concentration [2.99 % (v/v)], while trial N3 showed the highest glycerol concentration (2.76 g/L). No decrease in the production of ethanol was found in the trial inoculated with *S. bacillaris* (N3), as also determined by Giaramida et al. (2013).

All the fermentations were completed in two weeks and the wines obtained were characterised by a residual sugar content of less than 1 g/L. *S. lactis-condensi* (N1) and *C. oleophila* (N2) did not cause any change in the oenological parameters in terms of acetic acid content. Also, no statistically significant differences were observed between trials in terms of glucose concentration, acetic acid, lactic acid and pH values.

Trial N2 inoculated with *C. oleophila*, contrary to what reported by Franco et al. (2021), produced little acetic acid (0.28 g/L). Aplin et al. (2019) tried to select *C. oleophila* as a co-starter, but the strain produced acetic acid higher than 0.8 g/L, for this reason it was discarded and never applied in vinification. To our knowledge, the present work is the first report on application of *C. oleophila* in wine fermentation under real winemaking condition, since the previous authors used strain of *C. oleophila* only in bioreactor and/or *in vitro* investigation (Aplin et al., 2019).

Significant differences were found for TTA values, which were lower for the trials inoculated with non-*Saccharomyces* (6.30-6.35 g/L tartaric acid). At the end of fermentation, ethanol concentrations ranged between 11.65 and 11.99% (v/v). The highest values in ethanol were observed in the control trial N4 and the use of non-*Saccharomyces* in sequential inoculation with *S. cerevisiae* can promote the reduction of ethanol content of wines. In this study, differences in ethanol content ranged from

0.21-0.34 % (v/v). Benavides et al. (2022) who tested different sequential inoculum combinations to lower ethanol content in wines observed similar results.

Malic acid concentration decreased slightly from the beginning of monitoring (2.13 g/L) to the end of fermentation for all trials (1.87-1.99 g/L). The negligible decrease of malic acid concentration observed during the alcoholic fermentation could be due to *Saccharomyces* spp. strains, which can degrade malic acid initially, present in must from 3% to 45% (Saayman and Viljoen-Bloom, 2006). Lactic acid was present in trace amount for all trials. Regarding glycerol, a much higher content than in the other trials was found in trial N3 inoculated with *S. bacillaris* (10.31 g/L), a similar increase was found by Giaramida et al. (2013). This compound influences wine sensory properties, especially in red wines where it positively contributes to smoothness, sweetness, and complexity (Comitini et al., 2011). The increase in glycerol content of wines produced with *C. oleophila* and *S. bacillaris* is a common phenomenon (Englezos et al., 2018; Franco et al., 2021). At bottling, chemical parameters changed insignificantly. For the first time, *C. oleophila* has been used in a grape must obtain bottled wine according to commercial protocols. To our knowledge, *S. lactis-condensi* strains have only recently been selected for oenological applications and have shown greater efficiency in fructose utilisation and tolerance to sugar, alcohol and sulphur content compared to *S. bacillaris* (Csoma et al., 2023).

3.4 Volatile organic compounds of wines

The VOCs of wines are listed in Table 2. Quantitative differences were found among trials. The 29 identified compounds were grouped into alcohols, ethers, aldehydes, carboxylic acids, esters and other compounds. The heat map reports the differences between the aromatic profiles and VOC amounts (Fig. 3).

Alcohols are dominant wine VOCs resulting from yeast fermentation (Kotseridis and Baumes, 2000). The compounds mainly detected in this study were phenylethyl alcohol and 1-penthanol with values varying from 2.08 ppm (N2) to 40.63 ppm (N4) and from 47.94 ppm (N4) to 67.16 ppm (N2), respectively. These higher alcohols are responsible for floral notes (Cordente et al., 2021). Trial N3

inoculated with *S. bacillaris* distinguished from the others for the high concentrations of 3-hexenol and 2-butanol, both related to fusel note (Juan et al., 2012; Furdíková et al., 2014). Among ethers, 3-Ethoxy-1-propanol, a compound that gives a fruity aroma (Velázquez et al., 2015), was the only compound detected and the concentrations varied from 0.30 ppm (N2) to 1.15 ppm (N3).

Due to their rancid and cheesy smells (Călugăr et al., 2020), carboxylic acids are undesirable in wines and the experimental wines obtained in this study were characterized by very low concentrations (< 0.06 ppm).

Esters compounds are released during fermentation and directly influence the aromatic complexity of wines (Tempère et al., 2018). Within this class, ethyl acetate was significantly higher in wines processed with the sequential inoculum (N1-N2-N3) than single culture of *S. cerevisiae* (N4).

Ethyl acetate, which is also associated with the development of fruity flavours (Renault et al., 2015). Englezos et al. (2019) found a similar increase in ethyl acetate content during mixed fermentation of *S. bacillaris*/S. *cerevisiae*.

Among the treatments, N1 and N2 were distinguished from the others by higher levels of ethyl octanoate, 0.90 ppm and 1.15 ppm respectively.

Table 2.
Volatile organic compounds detected in the four Frappato wines (all values in ppm).

LRI	Compounds[1] (Common name)	Aroma description[2]	N1[3]	N2[3]	N3[3]	N4[3]	S.S.[4]
\	**Σ Alcohols**						
625	2-Methyl-2-butanol	Plastic, solvent, fly spray	0.71±0.02^{b}	1.07±0.03^{a}	0.76±0.01^{b}	0.61±0.02^{c}	***
664	2-Butanol	Alcoholic	0.00±0.00^{b}	0.00±0.00^{b}	8.28±0.07^{a}	0.00±0.00^{b}	***
760	1-Pentanol	Fusel	50.84±1.14^{bc}	67.16±1.23^{a}	51.71±1.35^{b}	47.94±1.21^{c}	***
796	2.3-Butanediol[6]	Buttery, creamy	0.17±0.01^{a}	tr	tr	tr	***
796	2.3-Butanediol[6]	Buttery, creamy	tr	0.00±0.00	tr	0.03±0.01	n.s.[5]
829	3-Methyl-1-pentanol	Fusel	0.10±0.01^{a}	tr	0.00±0.00^{b}	0.11±0.01^{a}	***
857	3-Hexenol	Grass, moss	0.06±0.01^{b}	0.00±0.00^{c}	0.15±0.02^{a}	0.05±0.01^{b}	***
872	1-Hexanol	Green	2.33±0.04^{b}	2.25±0.03^{c}	3.93±0.02^{a}	1.30±0.02^{d}	***
1039	Benzyl alcohol	Sweet, flower	0.00±0.00^{b}	0.17±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}	***
1108	Phenylethyl alcohol	Floral, rose	28.33±1.34^{b}	2.08±0.02^{d}	14.18±0.54^{c}	40.63±1.76^{a}	***
1442	p-Thyrosol	Sweet, floral, fruity	0.28±0.02^{b}	0.44±0.03^{a}	0.06±0.01^{c}	tr	***
1502	2.4-Di-tert-butylphenol	Unknown	1.11±0.08^{b}	4.54±0.05^{a}	0.89±0.07^{c}	0.59±0.03^{d}	***
	Σ Ethers						
816	3-Ethoxy-1-propanol	Fruit	0.86±0.02^{b}	0.30±0.01^{d}	1.15±0.04^{a}	0.55±0.03^{c}	***
	Σ Aldehydes						
960	Benzaldehyde	Bitter almond, nutty, smoky	0.00±0.00^{b}	0.00±0.00^{b}	0.06±0.01^{a}	0.00±0.00^{b}	***
	Σ Carboxylic acids						
875	3-Methyl-butanoic acid	Cheese, rancid	0.00±0.00^{b}	0.03±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}	*
976	Hexanoic acid	Mild, fatty	0.00±0.00^{b}	0.03±0.01^{a}	tr	tr	*
---	4-Ethoxy-4-oxobutanoic acid	Unknown	0.06±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	***
	Σ Esters						
613	Ethyl acetate	Ethereal, fruity	10.57±0.23^{b}	13.91±0.21^{a}	13.95±0.18^{a}	4.56±0.10^{c}	***
713	Propyl acetate	Pear	0.03±0.01^{a}	0.04±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}	***
800	Ethyl butanoate	Apple	0.19±0.01^{b}	0.25±0.02^{a}	0.13±0.01^{c}	0.14±0.01^{c}	***
876	Isopentyl acetate	Banana, fruity tropical	0.30±0.02^{a}	0.00±0.00^{c}	0.00±0.00^{c}	0.20±0.01^{b}	***
879	2-Methylbutyl acetate	Fruity	0.04±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	***
937	Ethyl 3-hydroxybutyrate	Fruity, grape, green	0.19±0.02^{a}	0.02±0.01^{c}	0.13±0.01^{b}	0.16±0.02^{ab}	***
999	Ethyl Hexanoate	Sweet fruity, pineapple, green apple	0.77±0.04^{b}	0.74±0.03^{bc}	1.07±0.05^{a}	0.66±0.04^{c}	***
1153	Diethyl butanedioate (Diethyl succinate)	Fruit	0.82±0.06^{a}	0.47±0.05^{c}	0.68±0.03^{b}	0.82±0.01^{a}	***
1188	Ethyl octanoate (Ethyl caprylate)	Fruity, pear	0.90±0.04^{b}	1.15±0.06^{a}	0.69±0.02^{c}	0.57±0.03^{d}	***
1296	Ethyl nonanoate	Fruity, fatty	0.18±0.02^{b}	0.24±0.01^{a}	tr	0.05±0.01^{c}	***
	Σ Others						
1245	1.3-Di-tert-butylbenzene	Unknown	0.90±0.02^{c}	3.80±0.04^{a}	1.57±0.03^{b}	0.91±0.04^{c}	***
-----	Tryptophan	Unknown	0.06±0.01^{c}	1.05±0.07^{a}	0.49±0.02^{b}	0.00±0.00^{c}	***

[1]Compounds are classified in order of retention time; [2]Aroma descriptions are reported in the online database of Good Scents Company Information (http://www.thegoodscentscompany.com/), Flavornet (http://www.flavornet.org/) and LRI & Odour Database (http://www.odour.org.uk/);[3] Relative amounts, expressed as ppb; [4] statistical significances; [5] not significative; [6] stereoisomers not identified.
Abbreviations: tr: trace amount < 0.01 ppm.
Data within a line followed by the same letter are not significantly different according to Tukey's test.
P value: ***, P < 0.001

For the first time the impact on VOCs by *C. oleophila* (N1) was studied. Previously, Franco et al. (2021) used this species as a co-starter *in vitro* winemaking experiment, analysing only the basic chemical-physical parameters. This is the first paper to report a study of VOCs associated with *C. oleophila* for oenological use as a co-starter. Its previous use has been in agriculture as a biocontrol agent (Raspor et al., 2010). The growing interest in non-*Saccharomyces* strains with must bio-protective action (Naselli et al., 2023), offers interesting insights into the selection of new co-starter strains. Further investigations will be necessary to verify the possible bio-protective action of the strain YS209 *C. oleophila.* Matraxia et al. (2021) applying non-*Saccharomyces* strains isolated and

selected from honey by-products, also found an increase in the ester content of beers. To our knowledge, strains of *S. lactis-condensi* have only recently been selected for oenological applications (Csoma et al., 2023).

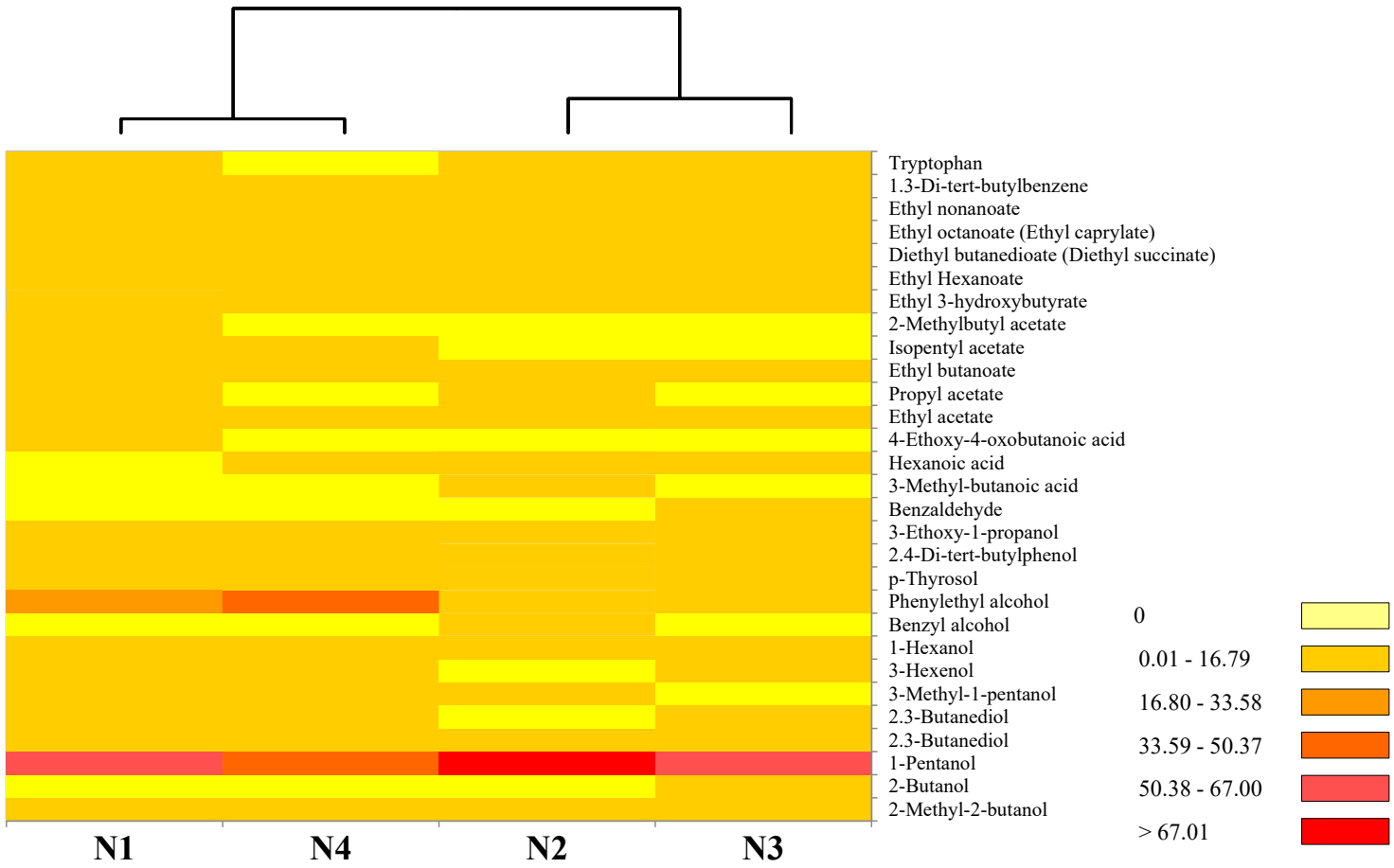

Figure 3. Distribution of volatile organic compounds among wines. The heat map plot depicts the relative concentration of each VOCs. Frappato must fermented by: N1, sequential inoculum *Starmerella lactis-condensi* MN412/ *Saccharomyces cerevisiae* NF213; N2, sequential inoculum *Candida oleophila* YS209/ *Saccharomyces cerevisiae* NF213; N3, sequential inoculum *Starmerella bacillaris* Cz3/ *Saccharomyces cerevisiae* NF213; N4, single inoculum *Saccharomyces cerevisiae* NF213.

3.5 Sensory evaluation

Among the 15 attributes that defined the sensory profile of each wine, SPCA indicated that the highest discriminating power was represented by odour overall quality, flavour overall quality and overall quality, while the lowest discriminating power was shown by colour. The definition of the sensory characteristics of each wine, expressed in model coefficients for each product-descriptor combination is shown in Fig. 4.

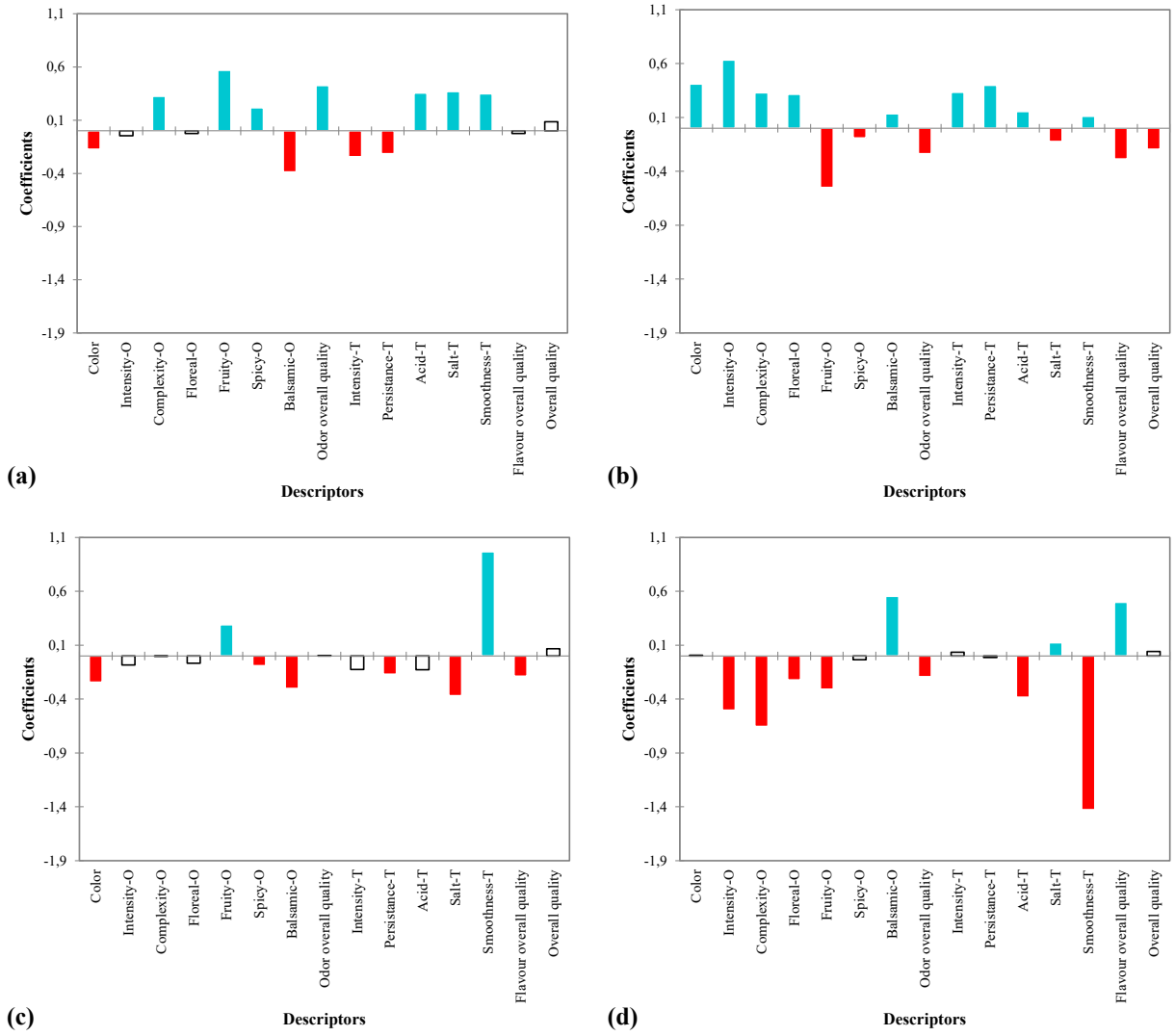

Figure 4. Sensory profiles of Frappato wines obtained with sequential inoculation of: (a) N1, sequential inoculum *Starmerella lactis-condensi* MN412/ *Saccharomyces cerevisiae* NF213; (b) N2, sequential inoculum *Candida oleophila* YS209/ *Saccharomyces cerevisiae* NF213; (c) sequential inoculum *Starmerella bacillaris* Cz3/ *Saccharomyces cerevisiae* NF213; (d) N4, single inoculum *Saccharomyces cerevisiae* NF213. The blue colour is associated to coefficients that have a significant positive value and the red colour is associated to coefficients that have a significant negative value.

Trials N1 and N2 showed a number of attributes with significant positive effect of 7 and 9, respectively. Treatment N3, showed 6 attributes with significant negative effect. The coefficients defining the complexity, fruity and spicy odours of wine from trial N1 produced with sequential inoculation of *S. lactis-condensi* showed the highest coefficients when compared to all other trials.

The wine from trial N3 was characterized by a strong smoothness on the palate (Fig. 4c). The high smoothness values could be related to the amount of glycerol of wine (Ciani and Ferraro, 1998). The activity of *S. bacillaris* before addition of *S. cerevisiae* was sufficient to increase the glycerol until values defining for the smoothness of this wine, a similar behaviour was found by Giaramida et al. (2013). The final wine of the control trial (N4) was characterised by balsamic odours, low intensity and complexity odour, and the taste was neither acidic nor smooth (Fig. 4d). However, none of the wines analysed showed off-odour.

In order to better evaluate the differences among Frappato wines, the data of the sensory analysis performed were illustrated in the sensory profile graph (Fig. 5).

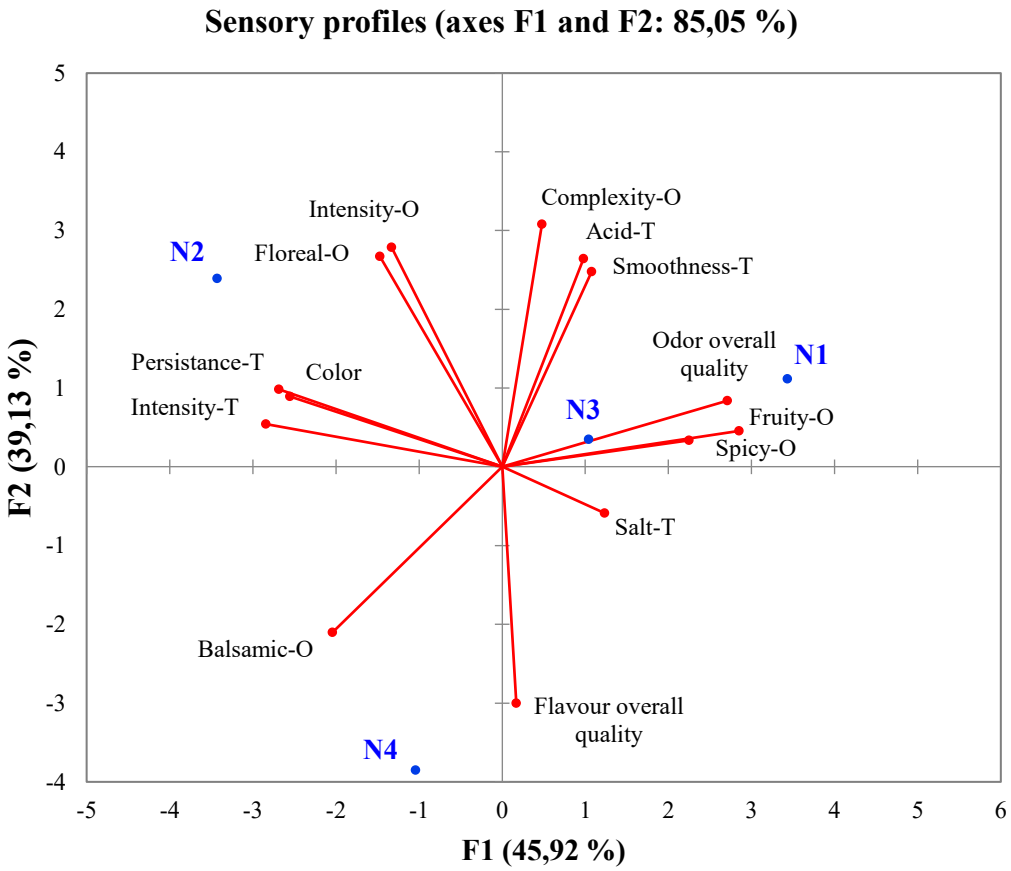

Figure 5. Sensory profiles graph showing the distribution of different Frappato wine in relation to the taste and odour attributes. Codes: N1, *sequential inoculum Starmerella lactis-condensi* MN412/ *Saccharomyces cerevisiae* NF213; N2, sequential inoculum *Candida oleophila* YS209/ *Saccharomyces cerevisiae* NF213; N3, sequential inoculum *Starmerella bacillaris* Cz3/ *Saccharomyces cerevisiae* NF213; N4, single inoculum *Saccharomyces cerevisiae* NF213.

The biplot graph correlates the attributes of wines variables that explained 85.05% of the total variability as function of factor 1 (45.92%) and 2 (39.13%). This graph reveals a clear grouping of the wines into 3 clusters. In the first quadrant, the wines from the trial N1 and N3 were correlated with the attributes of taste (acid and smoothness), odour (complexity, fruity and spicy) and odour overall quality. In the third quadrant, trial N4 wine was associated with balsamic odour attribute. In the last quadrant, trial N2 wine was strongly associated with colour, intensity, and persistence of taste, also for floreal and intensity odour. In all trials, Frappato wines showed different sensory profiles. The wine from trial N1 and N2 wine produced with *S. lactis-condensi* and *C. oleophila* were of considerable interest and showed high scores for most of the descriptors of sensory evaluation.

4. Conclusion

In this research, for the first time, non-*Saccharomyces* yeast isolated from manna and honey by-products were applied to winemaking process. The effect of *S. lactis-condensi* and *C. oleophila* in sequential inoculation with *S. cerevisiae* on the oenological parameters of Frappato wines and the VOCs composition was evaluated in comparison with the non-*Saccharomyces* control strain *S. bacillaris*, previously used in industrial winemaking. Very low production of acetic acid by *C. oleophila* were found in contrast to other author reports.

An increment of glycerol content was registered in wine produced with mixed culture of *S. bacillaris*/*S. cerevisiae* that increased the final smoothness of wines. VOC profiles confirmed wine differences especially for esters that, due to fruity aroma, contributed to the peculiar definition of the resulting wines, mainly by *C. oleophila.* The sensory analysis confirmed that the use of *S. lactis-condensi* and *C. oleophila* influenced positively the final wines in terms of fruity and floral intensity while did not generate sensory defects.

This work reports scientific data on the role of two novel non-conventional yeast species, *S. lactis-condensi* and *C. oleophila*, as potential co-starters to modulate the aromatic and organoleptic profile of wines. Novel information has been also showed on VOCs composition of a Frappato red wine cultivated in southern of Italy.

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Activity 2.6

Improving the aromatic profiles of catarratto wines: impact of *Metschnikowia pulcherrima* and glutathione-rich inactivated yeasts

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Abstract

Catarratto is one of the most widely cultivated grape varieties in Sicily. It is an indigenous non-aromatic white grape variety. Despite its widespread use in winemaking, knowledge of the aroma and chemical and microbiological properties of Catarratto wines is quite limited. The influence of *Metschnikowia pulcherrima* combined with *Saccharomyces cerevisiae* on the aromatic expression of Catarratto wines was investigated with and without the addition of glutathione-rich inactivated yeast. The substance is a natural specific inactivated yeast with a guaranteed glutathione level used to limit oxidative processes. The aromatic profiles of the final wines were determined through analysis of the volatile organic compounds using a solid phase microextraction technique that identified 26 aromatic compounds. The addition of *M. pulcherrima* in combination with the natural antioxidant undoubtedly increased the aromatic complexity of the wines. Dodecanal was exclusively detected in the wines processed with glutathione-rich inactivated yeasts. Furthermore, the presence of this natural antioxidant increased the concentration of six esters above the perception threshold. Sensory analysis was also performed with a panel of trained judges who confirmed the aromatic differences among the wines. These results suggest the suitability of glutathione-rich inactivated yeasts for determining the oxidative stability of Catarratto wines, thus preserving its aromatic compounds and colour.

1. Introduction

Identifying new strategies for the aromatic enhancement of wine produced from non-aromatic grape varieties is one of the major objectives of oenological microbiology research. During alcoholic fermentation (AF), yeast metabolic activity and winemaking techniques determine the biosynthesis of several products that influence wine aroma (Izquierdo Cañas et al., 2014; Alfonzo et al., 2021). The application of non-conventional yeasts isolated during the fermentation of traditional fermented beverages represents an alternative for producing a variety of alcoholic beverages (Varela et al., 2016), including wine.

Raw materials with a high sugar content if subjected to spontaneous fermentation can provide potential starters with interesting traits. Wild *Saccharomyces* and non-*Saccharomyces* spp. may generate flavour profiles with desirable characteristics to be applied at industrial level (Ciani and Comitini, 2011). Several authors have isolated strains of *Saccharomyces cerevisiae* from high-sugar-containing matrices such as manna, honey and honey by-products (Francesca et al., 2022; Guarcello et al., 2019) that were successfully applied in experimental Catarratto cultivar winemaking (Alfonzo et al., 2021).

Recently, the controlled inoculation of selected non-*Saccharomyces* and *S. cerevisiae* strains has permitted the production of higher quality wines. The current trend is to exploit non-*Saccharomyces*/*Saccharomyces* sequential inoculation to achieve a positive impact in terms of aroma (Lappa et al., 2020). The result is closely related to the species of the multi-starter cultures involved in the sequential inoculum (Lappa et al., 2020).

The final aroma of wines can be modulated not only by *Saccharomyces* but also by non-conventional yeasts. The metabolic impact of non-*Saccharomyces* yeasts during the early stages of fermentation is sufficient to trigger significant changes to the wine's volatile profile; they are suitable for inoculation as co-starters with strains of *S. cerevisiae* (Rossouw and Bauer, 2016).

Fermentation processes using mixed strains with the sequential addition of non-*Saccharomyces* and *S. cerevisiae* strains tend to reproduce what happens naturally during spontaneous wine fermentation

concerning population dynamics (Gonzalez and Morales, 2022). Indeed, the levels of non-*Saccharomyces* yeast populations are reduced over time, leaving space for *S. cerevisiae* to dominate and conclude the AF (Fleet, 2003).

The use of non-*Saccharomyces* yeasts has improved the primary aromas of wines, as the production of specific enzymes enables the precursors present in the must to release volatile molecules. Their activity also affects secondary aromas through the production of volatile organic compounds (mainly alcohols and esters) that can influence typical aromatic expressions such as fruity notes (Gonzalez and Morales, 2022).

The impact determined by sequential inoculation can influence various aspects of wine characteristics. In recent oenological studies, strains belonging to the species *L. thermotolerans* and *Starmerella bacillaris* in sequential inoculation with *S. cerevisiae* achieved wines with a significant amount of lactic acid and glycerol, respectively, while strains of *Torulospora delbrueckii* and *Hanseniaspora uvarum* in mixed cultures and in sequential inoculation with *S. cerevisiae*, on the other hand, seemed to influence the composition in terms of higher alcohol and ester contents (Lappa et al., 2020).

Among the non-*Saccharomyces* yeasts, *Metschnikowia pulcherrima* is one of the species most abundant in the initial phase of AF of grape musts. In mixed cultures with *S. cerevisiae*, *M. pulcherrima* rapidly declines due to its low resistance to the ethanol produced by *S. cerevisiae* (Wang et al., 2016). Some strains of *M. pulcherrima* are known to synthesize fruity esters and can increase the concentrations of terpenes or thiols generally masked by higher alcohols (Benito et al., 2015). Non-aromatic grape varieties lack varietal aromatic precursors (terpenes or thiols) and the presence of fruity aromas (pineapple) due to the fact that an increased ethyl octanoate content determines a positive sensorial impact. Some thiol precursors such as 4-methyl-4-sulfanylpentan-2-one, as well as those produced by *S. cerevisiae*, can be synthesized by *M. pulcherrima* at much higher concentrations, thus significantly influencing the characteristics of wine (Ruiz et al., 2018). Recently, *M. pulcherrima* was successfully used in a sequential inoculation with *S. cerevisiae* for a reduction in ethanol content

in Merlot wines (Aplin et al., 2021) and to improve the aromatic complexity in Shiraz and Cabernet Sauvignon wines (Varela et al., 2021).

A solution aimed at limiting the loss of aromaticity in white wines is represented by the addition of a natural antioxidant such as glutathione at the beginning of the vinification process (Kritzinger et al., 2013). The application of glutathione during winemaking has positive effects on the colour and aroma stability of white wines (Badea and Antoce, 2015). The glutathione content naturally present in musts is relatively low, and its quantities are closely related to the reactions that characterise the fermentation process as well as the metabolic activities of the yeasts (Aplin et al., 2021). Its use in oenology provides considerable advantages as its antioxidant activity is capable of limiting browning in white grape must as it inhibits polyphenol polymerisation and severely limits the production of compounds such as sotolone that give wine a fenugreek or curry odour (Binati et al., 2022). Glutathione's degree of protection also extends to the aromatic molecules in wines, especially the esters, volatile thiols and terpenes produced by yeasts during alcoholic fermentation that are present in greater quantities when glutathione is added to the must (Lyu et al., 2021). Some sulphite-free wines are produced by exploiting the antioxidant activity of glutathione in place of potassium metabisulphite, meeting the needs of consumers who are more sensitive to the negative health effects of sulphur dioxide (Ferrer-Gallego et al., 2017).

This study focused on the potential of non-*Saccharomyces* and *Saccharomyces* yeasts for the aromatic improvement of wine produced from non-aromatic grape varieties such as Catarratto. We evaluated the sequential inoculation of a commercial non-*Saccharomyces* yeast strain (*M. pulcherrima*) and *S. cerevisiae* SPF52 isolate from honey by-products to simulate what would occur during spontaneous fermentation. Secondly, we assessed the ability of exogenous glutathione addition during fermentation in the form of inactivated yeast to influence the technological and aromatic properties of wine. The aims of this research were to investigate: (i) the impacts of *M. pulcherrima* associated with *S. cerevisiae*; (ii) the effect of an antioxidant compound on the aroma and sensory profiles of Catarratto wine; and (iii) the volatile organic compound composition of Catarratto white wine.

2. Materlias and methods

2.1 Experimental Drawing and Sampling

The experimental design (Fig. 1) was composed of four treatments: T1, sequential inoculum of FLAVIA® MP346/*S. cerevisiae* SPF52; T2, the use of Glutastar™ to the bulk must and sequential inoculum with FLAVIA® MP346/ *S. cerevisiae* SPF52; C1, single inoculum of *S. cerevisiae* SPF52; C2, the addition of Glutastar™ and fermentation by *S. cerevisiae* SPF52.

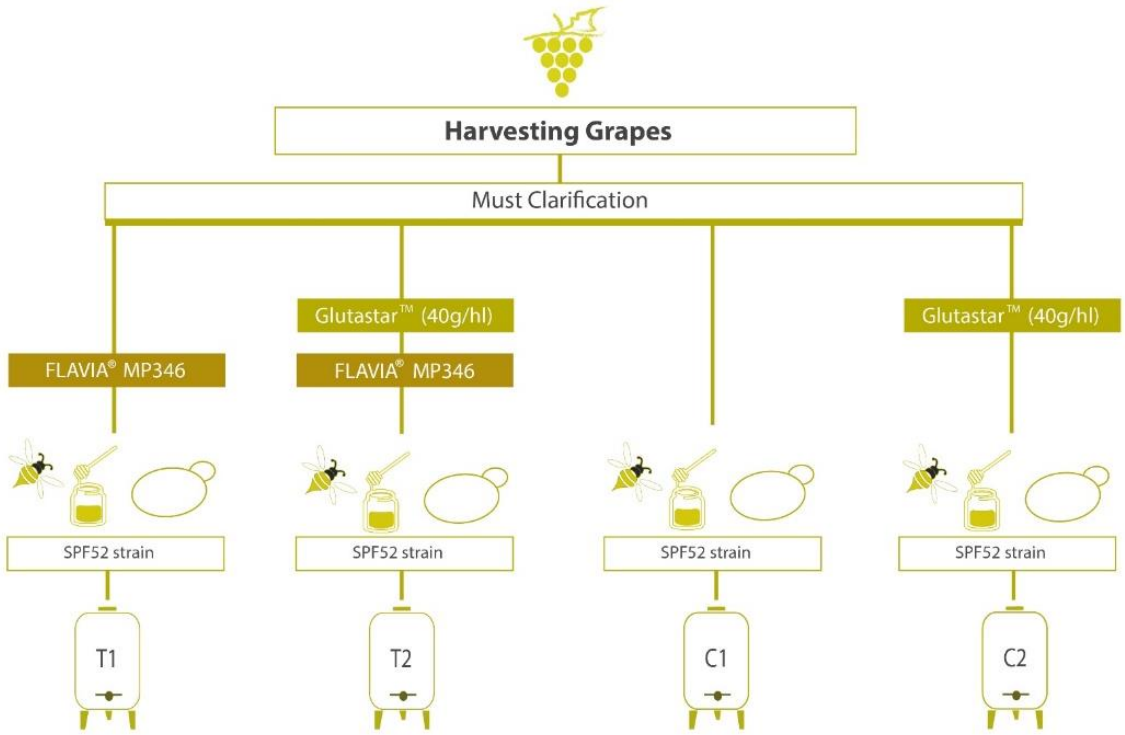

Figure 1. Experimental plan of wines obtained from Catarratto grape must.

FLAVIA® MP346 is a pure culture of *M. pulcherrima* selected by the Universidad de Santiago de Chile (USACH) for its specific capacity to release enzymes with arabinofuranosidase activity (Ganga et al., 2013). Glutathione-rich inactivated yeast (GIY) is an inactivated yeast mass with a guaranteed glutathione level (Bahut et al., 2020). GIY and FLAVIA® MP346 were provided by Lallemand Inc. (Castel D'Azzano, Verona, Italy). The *S. cerevisiae* SPF52 strain used in this study belonged to the yeasts collection of the Department of Agricultural, Food and Forestry Sciences (SAAF; University of Palermo, Italy); it was isolated from fermented honey by-products (Gaglio et al., 2017) and selected for its high performance in fermenting Catarratto grape must (Alfonzo et al., 2021).

Samples were collected from clarified bulk must just after the inoculum of *M. pulcherrima* MP346, after the inoculation of *S. cerevisiae* SPF52, during AF (day 3, 6, 12 and 18), during ageing in a steel tank (1, 3 and 5 months) and at bottling. All samples were transported at 4 °C into a portable fridge and subjected to analysis within 24 h after collection.

2.2 Winemaking

After hand harvesting, grapes were stemmer-crushed and treated with 2 g/hL of potassium metabisulphite (Chimica Noto s.r.l., Partinico, Italy). Clarification of the must was carried out at 4 °C for one day by using pectolytic enzymes [Lallzyme® C-Max (Lallemand Inc. Italia, Castel D'Azzano, Verona, Italy); dosage: 4 g/hL].

T1 and T2 were inoculated with FLAVIA® MP346 at 25 g/hL when the clarified must had reached a temperature of 16 °C. The strain *S. cerevisiae* SPF52 was used in a liquid concentrated form [about 7.00×10^{10} colony-forming units (CFU)/g].

After 24 h, T1 and T2 were inoculated with *S. cerevisiae* SPF52 (20 g/hL), while the controls, C1 and C2, were inoculated immediately with the SPF52 strain at the same dose. Before the inoculum of the starter yeast, GIY (40 g/hL) was added to treatments T2 and C2. The organic nutrient Stimula Chardonnay™ (SC; Lallemand Inc. Italia, Castel D'Azzano, Verona, Italy) was added to all tanks (40 g/hL) prior to *S. cerevisiae* yeast inoculation. The use of Stimula Chardonnay™ with *S. cerevisiae* SPF52 was chosen because of the results obtained by previous vinifications on Catarratto wines (Alfonzo et al., 2021). The fermentation was carried out at 18 °C in 12 steel tanks with a volume of 2.5 hL each. At the end of AF, the wines were cold-settled, their yeast lees were racked off and they were transferred into stainless-steel tanks at 15 °C and topped with nitrogen to avoid oxidation until bottling. During ageing, malolactic fermentation was prevented by keeping the free SO_2 values above 35 mg/L until bottling. Tartaric stability was ensured through the addition of 8 g/hL of metatartaric acid (Chimica Noto s.r.l., Partinico, Italy). Each treatment was performed in triplicate.

2.3 Monitoring Yeast Populations

During the AF, all must samples were microbiologically analysed to determine the total yeast concentration (TY) using the protocol described by Pallmann et al. (2001). *Saccharomyces* and non-*Saccharomyces* yeasts colonies were distinguished as reported by Valera et al. (2016). The analyses were conducted in triplicate.

2.3.1 Yeast Collection and Genotypic Characterization

Yeasts were isolated from WL medium, purified on the same medium and then subjected to morphological analysis, as reported by Pallmann et al. (2001), and genotypic characterisation.

Genomic DNA for PCR assays was prepared from yeast isolates after growth in YPD broth media at 25 °C for 48 h. Cells were harvested, and DNA was extracted using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. According to Sinacori et al. (2014), yeasts were discriminated by RFLP of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene. Species-level identification of each group was confirmed by sequencing the D1/D2 region of the 26S rRNA gene following the procedure described by Guarcello et al. (2019). DNA sequencing reactions were performed at AGRIVET (University of Palermo, Italy). Sequences were manually corrected using Chromas 2.6.2. (Technelysium Pty Ltd., Australia). Nucleotide sequences were compared to GenBank sequences through BLASTn searches.

2.3.2 Dominance of S. cerevisiae *and M. pulcherrima isolates*

The dominance of the inoculated *S. cerevisiae* and *M. pulcherrima* was verified as reported by Legras and Karts (2003), and Barbosa et al. (2012). Fingerprinting profiles were analysed as reported by Alfonzo et al. (2020).

2.3.3 Chemical Properties

Chemical properties such as sugars (glucose and fructose, g/L) and residual sugars (g/L), yeast-assimilable nitrogen (ammoniacal nitrogen and alpha-amino nitrogen, g/L), organic acids (malic acid, lactic acid and acetic acid, g/L), glycerol (g/L) and ethanol (% v/v) were quantified during and at the end of the AF using the methods described by Prestianni et al. (2022).

The pH values were measured using a pH 70 Vio FOOD pH meter (XS Instruments, Carpi, Italy), and total acidity (g/L of tartaric acid) was detected through the procedure proposed by OIV-MA-AS313-01 (2020). Free and total sulphur dioxide was determined in accordance with Alfonzo et al. (2021).

The analysis of the chemical composition of wines analysed included ash alkalinity, buffering power, total extract, total phenols, flavans reactive to 4-(dimethylamino)cinnamaldehyd, oxidation tests, total phenols and extracts were performed as reported by Alfonzo et al. (2021).

2.3.4 Volatile Organic Compounds

All reagents were of analytical grade. Ethyl benzoate was purchased from Sigma-Aldrich (82024 Taufkirchen, Germany). n-Alkane standards (C8 to C40) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA).

An automatic SPME holder (Supelco®, Bellefonte, PA, USA) was used for evaluation of VOC profiles. A fiber 50/30 µm divinylbenzene (DVB)/carbowax (CAR)/polydimethylsiloxane (PDMS) of 1 cm length was used for fractionation of volatile compounds from the headspace (HS) of the conditioned wines. Prior to its use, the fiber was conditioned for 1.5 h at 250 °C in the inlet of the gas chromatograph according to Supelco® Co. Analysis of wine aroma was performed following a slightly modified method proposed by Sagratini et al. (2012). For extraction, each aliquot (10 mL) of the wine samples and 2.2 g of NaCl were placed into a 20 mL vial (75.5 × 22.5 mm) (Supelco, Bellefonte, PA, USA). The samples were equilibrated at 35 °C for 15 min, stirring at 600 rpm. The SPME fiber was exposed to the wine samples for 30 min in the headspace of the sample kept at 35 °C. The flavour compounds were desorbed for 5 min from the fiber to the column through a splitless injector at 250 °C. The SPME fibres were cleaned to prevent cross-contamination by inserting the fibre into the auxiliary injection port at 250 °C for 30 min and were then re-used. All samples were prepared and analysed in triplicates in standard 20 mL volume headspace vials.

Semi-quantification of volatile compounds was performed using an Agilent 7000C GC system fitted with a fused silica apolar DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) (Santa Clara, CA, USA) coupled to an Agilent triple quadrupole Mass Selective Detector MSD 5973. The ionization voltage was 70 eV, the electron multiplier energy was 2000 V and the transfer line temperature was 270 °C. The solvent delay was 0 min. Helium was the carrier gas (1 mL/min). The temperature programme was from 35 °C (0 min) to 270 °C at 8 °C min^{-1}, from 270 °C (2 min) to 300 °C at 15 °C min^{-1} and then 300 °C for 5 min. Volatile compounds were injected at 250 °C automatically in the splitless mode. Linear retention indices were calculated using *n*-alkanes as reference compounds. For the analysis of alkane solutions (C_8-C_{40}) (Sigma-Aldrich, USA), the injector mode was set in the 10:1 split mode. The individual peaks were analysed using the GC-MSolution package, version 2.72. Identification of compounds was carried out using the Adams, NIST 08, Wiley 9 and FFNSC 2 mass spectral databases.

For each volatile organic compound identified, the odour activity value (OAV) as described by Butkhup et al. (2011) was calculated in order to assess which VOCs contributed significantly to the odour series characterising each wine.

2.3.5 Sensory Analysis

A total of 15 judges (7 women and 8 men, ranging from 25 to 46 years old) with previous experience in wine tasting participated in the evaluation of the sensory profile of the wines carried out as described by Jackson (2017). The judges were subjected to preliminary tests to determine their sensory performances in terms of their basic taste and the aromas associated with the wines. The sensory profiles of the wines obtained from Catarratto grapes were constructed using two selected panels each of ten judges trained over several sessions. The fifteen panellists compared the four experimental wines during different sessions. They consensually generated 36 sensory descriptive attributes for appearance, odour, flavour, taste, overall quality and finish in several sessions. The set of attributes were: appearance (green reflexes and yellow colour); odour (banana, citrus, fatty, floral,

fruity, grape, green almond, intensity, pear, persistence, pineapple and sweet fruit); taste (bitter, salty, sour and sweet); mouthfeel (body or balance); flavour (banana-like, cherry pit, citrus, fruity, intensity, mandarin orange, persistence, pineapple, sweet apple and sweet fruit), overall quality (flavour, mouth-feel, odour and taste) and finish (after-smell and after-taste). The different descriptors were quantified using a 9-point intensity scale as reported by Alfonzo et al. (2020).

The sensory test was carried out following the procedures described by Alfonzo et al. (2021).

2.3.6 Statistical Analysis

In order to determine statistically significant differences between the properties monitoring during the AF (chemical and technological data) and in the final wines (sensory analysis and VOCs composition), the ANOVA test was applied. Tukey's test was used for multiple mean comparisons (statistical significance: $p < 0.05$).

Multiple factor analysis (MFA) was carried out in order to distinguish the different treatments from the data acquired during the sensory analysis following the methodology reported by Alfonzo et al. (2021). Agglomerative hierarchical clustering (AHC) was performed to group the trials according to their dissimilarity, as measured by Euclidean distances and Ward's method.

In order to assess the existing correlation between the aromas detected during the sensory analysis and the VOCs with an odour activity value >1, a principal component analysis (PCA) was performed using the XLstat software version 2019.2.2 (Addinsoft, New York, NY, USA) for Excel.

3 Results and discussion

3.1 Microbial Growth Dynamic

The concentrations of yeasts (presumptive *Saccharomyces* (PS), non-*Saccharomyces* (NS) and presumptive *Metschnikowia* (PM)) during the alcoholic fermentation (AF) are shown in Figure 2. The PS and NS levels in the Catarratto must were around four logarithmic cycles (Figure 2a, b), while no isolates attributable to the genus *Metschnikowia* were detected (Figure 2c). Catarratto musts are usually poor for the presence of indigenous *Metschnikowia* spp., although in musts from Sicilian

Catarratto grapes, *M. pulcherrima* has been isolated at percentages ranging from 0.2 to 1.1% (Romancino et al., 2008).

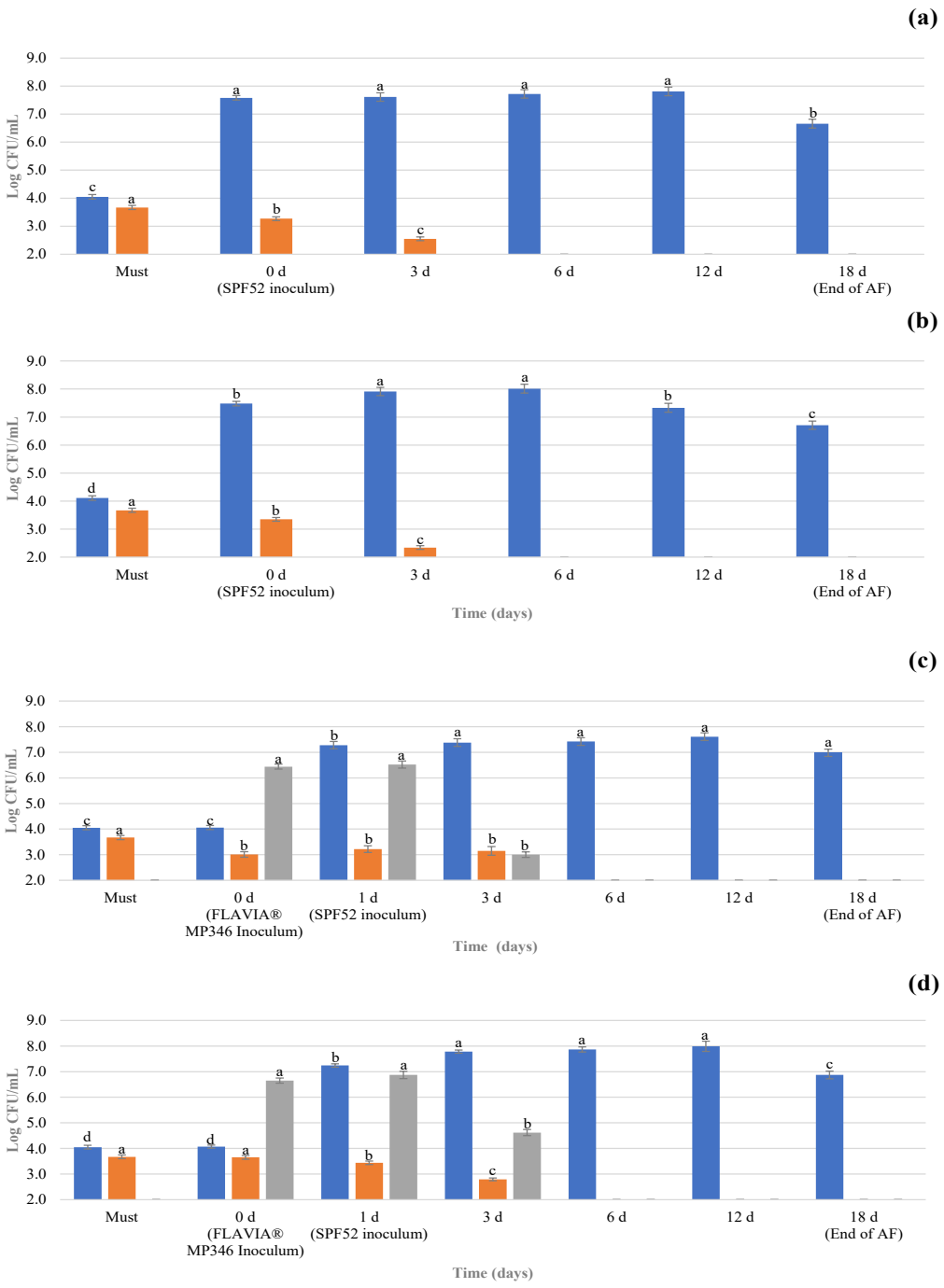

Figure 2. Microbiological concentration (Log CFU/mL) of samples during alcoholic fermentation: (**a**) C1, single inoculum with *S. cerevisiae* SPF52; (**b**) C2, glutathione-rich inactivated yeasts and single inoculum with *S. cerevisiae* SPF52; (**c**) T1, sequential inoculum with *M. pulcherrima* MP346/*S. cerevisiae* SPF52; (**d**) T2, glutathione-rich inactivated yeasts and sequential inoculum with *M. pulcherrima* MP346/*S. cerevisiae* SPF52. For each microbiological group, different letters indicate statistically significant values determined by using Tukey's test ($p \leq 0.05$). Legends: ■, Presumptive *Saccharomyces*; ■, non-*Saccharomyces* (except *Metschnikowia* spp.); ■, Presumptive *Metschnikowia* spp.

The *M. pulcherrima* MP346 inoculum concentration in T1 and T2 was close to 6.5 Log CFU/mL. The concentration of PS after the SPF52 inoculum ranged from 7.3 (T1) to 7.6 (C1) Log CFU/mL in all treatments. On day 3 of AF, PS showed an increase to 7.4–8.0 Log CFU/mL for all trials. The NS populations were lower and in the range of 2.3–3.2 Log CFU/mL. The reduction in the NS yeast populations during AF is a known phenomenon attributable to several causes such as metabolite production by *S. cerevisiae*, nutrient limitation and low resistance to ethanol (Wang et al., 2016). The PM levels were 3.0 Log CFU/mL for T1 and 4.6 Log CFU/mL for T2 after 3 days of AF and were lower than the limit of detection in the C1 and C2 samples. Indeed, the lower microbial load of the PM populations observed at 3 days of AF in T1 and T2 compared with C1 and C2 could be due to the lower ethanol concentration detected in T1 and T2 (Table S1). At day 6 of AF, when the ethanol reached concentrations above 6% v/v, the PS values reached levels in the range of 7.0–8.0 Log CFU/mL, whereas both NS and PM were undetectable in any trials. The absence of *M. pulcherrima* in trials inoculated with the commercial preparation FLAVIA® MP346 (T1 and T2) could be due to the above-mentioned factors. Some authors have recorded a significant decrease in the concentration *of M. pulcherrima* after 9 days of AF when sequential inoculation with *S. cerevisiae* occurred (Ruiz et al 2018).

From the 12th day until the end of AF (18 d), the PS populations decreased slightly from 7.3–8.0 to 6.7–7.0 Log CFU/mL in all treatments. The microbiological count values for *S. cerevisiae* were found by Scacco et al. (2012) on Sicilian Catarratto musts inoculated with selected starter strains of the same species.

3.2 Molecular Analysis

In relation to the macro- and microscopic characteristics, 949 colonies were analysed; from these, 592 isolates showed the typical characteristics of yeasts belonging to the *Saccharomyces* genus. The amplicon size of the 5.8S-ITS region was around 850 bp and confirmed the presumptive species

identity of *S. cerevisiae* for all isolates. The other isolates (n = 357) were assigned to the NS yeast group.

A total of 233 isolates were morphologically identified as *Metschnikowia* spp. and showed an ITS amplicon between 380 and 400 bp. The ITS amplicon sizes were equivalent to those reported in the literature for *M. pulcherrima*. The isolates of the PS group (n = 592) and PM (n = 233) were characterised by RFLP analysis of the 5.8S-ITS region.

The PS RFLP profiles were similar to those indicated by Granchi et al. (1999). Consequently, the PS group represented putative *S. cerevisiae*. The sizes of the RFLP profiles of the PM were equivalent to those described in the literature for the species *M. pulcherrima* (Granchi et al.,1999).

The different profiles may have been caused by the presence of native *S. cerevisiae*, although less representative, being present among the isolates obtained. Indeed, the PS count values detected before SPF52 inoculation (4.1 Log CF/mL) clearly explain the presence of eight additional Interdelta profiles. The Interdelta profile of *S. cerevisiae* SPF52 was the most frequently (>93%) isolated. The strain typing of *M. pulcherrima* was carried out by RAPD-PCR. The results from these analyses showed that all the 233 isolates represented a unique strain.

The genotypic identification of the yeasts was completed by pairwise alignment of the D1/D2 sequence with the type of strain of each species (*S. cerevisiae* CBS 1171^{T} and *M. pulcherrima* CBS 5833^{T}). A comparison of the sequences of the D1/D2 region of the two reference strains showed a 100% similarity to the sequences of the type strains of each species, confirming the identification obtained by the RFLP analysis.

3.3 Kinetics of the Main Oenological Properties

The fermentations carried out in the presence of *S. cerevisiae* SPF52 as the only inoculated strain (C1 and C2) and the corresponding trials with *M. pulcherrima* (T1 and T2) were able to conclude the AF as determined by the complete consumption of sugars.

The trends of the principal oenological data during AF are shown in Table S1. The fermentation was concluded in 18 days on average.

After 3 d of AF, differences in pH, TA and the concentrations of sugars, ethanol, ammonia nitrogen and alpha amine nitrogen were observed among the trials. The highest differences in the sugar, glycerol and ethanol contents were registered at day 6 of AF. Specifically, C2 showed the lowest values in residual sugars (58.79 g/L); glucose was 25.80 g/L and fructose was 32.99 g/L, and consequently, it showed the highest values of ethanol (8.44\% *v*/*v*). The glycerol contents observed in T1, T2 and C2 were similar (5.19–5.28 g/L), whereas the lowest values were found in C1 (5.06 g/L). This trend was observed until the 12th day of AF.

At the end of AF, the glucose concentrations ranged from 1.10 (T1 and T2) to 1.62 g/L (C1), whereas the fructose concentration was slightly higher and within the range of 1.39–2.60 g/L. No differences were observed for TA, whereas VA's values ranged from 0.27 (T1) to 0.31 (C1 and C2) g/L acetic acid. The pH values varied between treatments, where T1 and T2 had slightly lower values (3.41 and 3.43, respectively) when compared to both the control trials C1 (3.47) and C2 (3.51). The ethanol concentrations ranged between 11.35 and 11.43% (*v*/*v*); the comparison between the T1 and T2 and the C1 and C2 treatments showed no significant differences. In contrast, Contreras et al. (2015) reported that some strains of *M. pulcherrima* are able to decrease the amount of ethanol by as much as 1% (*v*/*v*) during fermentation. An analysis of the ethanol production during AF a revealed lower ethanol production in the T1 and T2 trials after 3 d of AF. After AF, differences in the ethanol concentration between the different trials were not statistically significant. This phenomenon could be attributable to the presence of *M. pulcherrima* up to the 3rd day of AF (3.0–4.6 Log CFU/mL).

The malic acid levels decreased in all the treatments from an initial concentration of 1.90 g/L in the must to 1.28–1.50 g/L at the end of AF. Contrary to the reports of Ruiz et al. (2018), no decreases were recorded in T1 and T2 compared to C1 and C2, although these authors showed that in fermentations conducted with *M. pulcherrima*/*S. cerevisiae*, a decrease in the malic acid content of 0.2 g/L occurred in the wines. Lactic acid was absent in all the trials. The highest concentration of

glycerol was found in C2 (6.57 g/L), and lower values (>5 g/L) were detected in the other wines. In this case, the sequential inoculum with *M. pulcherrima*/*S. cerevisiae* did not produce an increase in the glycerol concentration, in contrast to what has been observed in white wines made with the Verdejo variety (Ruiz et al., 2018).

During the five months of ageing in stainless steel tanks, there were no substantial changes in the monitored chemical properties (Table S2). There was a decrease in residual sugars, glucose and fructose, and all the other properties remained constant or showed minimal variations.

3.4 Oenological Data Analysis

The values of the physico-chemical properties of the wines are reported in Table 1.

The free and total SO_2 values were variable in the different wines. In particular, the highest free SO_2 values were observed in T1 and C2 (29 mg/L), while the highest total SO_2 value was observed in T1 (128 g/L).

Table 1. Oenological properties of four Catarratto wines.

Sample	SO_2 Free (mg/L)	SO_2 Total (mg/L)	Total Extract (g/L)	Total Phenols (mg/L Catechins)	p-DACA Flavans (mg/L Catechins)	Absorbance (420 nm)	Oxidation Test (%)	Buffer Power (meq/L)	Ash Alkalinity (meq/L)
T1	29.00 ± 1.00^{a}	128.00 ± 0.00^{a}	18.80 ± 0.09^{b}	92.74 ± 0.84^{a}	19.80 ± 0.08^{a}	0.079 ± 0.000^{c}	5.74 ± 0.09^{a}	31.25 ± 0.08^{b}	12.28 ± 0.05^{c}
T2	22.00 ± 0.00^{b}	115.00 ± 2.00^{b}	19.10 ± 0.07^{a}	93.47 ± 0.39^{a}	10.23 ± 0.11^{b}	0.080 ± 0.003^{c}	1.12 ± 0.03^{b}	31.25 ± 0.17^{b}	12.58 ± 0.07^{b}
C1	16.00 ± 2.00^{c}	109.00 ± 1.00^{c}	18.50 ± 0.08^{c}	84.38 ± 1.13^{c}	1.63 ± 0.08^{d}	0.101 ± 0.001^{a}	0.00 ± 0.00^{c}	31.32 ± 0.12^{b}	12.11 ± 0.07^{d}
C2	29.00 ± 1.00^{a}	105.00 ± 1.00^{d}	19.00 ± 0.11^{ab}	83.36 ± 0.67^{b}	3.12 ± 0.05^{c}	0.093 ± 0.002^{b}	0.00 ± 0.00^{c}	32.34 ± 0.13^{a}	13.43 ± 0.02^{a}
S.S.	***	***	***	***	***	***	***	***	***

Result indicates mean value ± standard deviation of three determinations. Abbreviations: S.S., statistical significance; T1, sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; T2, glutathione-rich inactivated yeasts and sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; C1, single inoculum with *S. cerevisiae* SPF52; C2, glutathione-rich inactivated yeasts and single inoculum with *S. cerevisiae* SPF52. Data in the same column followed by the same letter are not significantly different according to Tukey's test. *p* value: ***, $p < 0.001$.

The total extract was higher than the minimum legal values, which for white wines are fixed at >14 g/L (MIPAAF, 2017). In this study, all the wines exceeded this threshold; the values were in the range of 18.50–19.10 g/L for C1 and T2, respectively, which were comparable to the results described in Scacco et al. (2012) on Sicilian Catarratto wines.

The T1 and T2 trials retained a greater susceptibility to undergo oxidation than the C1 and C2 controls, which was independent of the use or non-use of GIY with oxidation test values of 5.74 and 1.12% (T1 and T2) and 0% (C1 and C2). The presence of *M. pulcherrima* therefore appeared to exert a bio-protective action by predicting oxidations at the pre-inoculation of *S. cerevisiae*. The decrease in polyphenols was not due to the synthesis of polysaccharides by *M. pulcherrima* but to its bioprotective and inhibiting action against grape tyrosinases. In fact, in the pre-fermentative stage in the C1 and C2 controls, the absence of *M. pulcherrima* favoured a significant increase in the optical density at 420 nm. At the same time, in the same controls there would have been a significant decrease in the total polyphenols resulting from the decrease in the phenolic class of the ortho-diphenols detected by means of the p-DACA reagent. The total polyphenol content was independent of the presence/absence of GIY. The null POM test values observed in the controls C1 and C2 may be due to a series of oxidation reactions of polyphenolic compounds that not even the addition of GIY in T2 was able to limit. The colonisation of the must by *M. pulcherrima* in the pre-fermentation phase probably led to a reduction in oxidative activities (Cinquanta et al., 2019).

Regarding buffering power, there were negligible variations, and only the wine C2 reached statistically significant values compared to the other trials. The highest buffering power value was in C2 (32.34 meq/L), which was comparable to those reported in the literature in Sicilian Catarratto wines (Cinquanta et al., 2019). This was similar for ash alkalinity, where C2 had the highest value (13.43 meq/L); the wine values were within the range of 11–17 meq/L, which were similar to those reported in the literature for white wines (Corona, 2010).

3.5 Volatile Organic Compound Composition

The samples showed differences mainly at the quantitative level. Twenty-six compounds were detected, and they were grouped into several classes: alcohols, ethers, aldehydes, ethyl esters of fatty acids (EEFAs), higher alcohol acetates (HAAs), ethyl esters of branched acids (EEBAs), miscellaneous esters (MEs) and other compounds. For clarity, the classification of esters was reported

as described by Alfonzo et al. (2021). The most-concentrated compounds in all the samples were EEFAs (2318.98–1401.74 ppb) followed by MEs (233.83–98.84 ppb) and alcohols (36.48–18.84 ppb).

The must inoculated with *M. pulcherrima* MP346 produced less alcohols than the controls. 3-methyl-1-butanol and phenylethyl alcohol were the compounds detected in the highest quantity in C2. A similar condition was observed in Riesling wines fermented by sequential inoculation with *M. pulcherrima*/*S. cerevisiae* (Benito et al., 2015).

The compound most commonly detected in the aldehyde class was dodecanal. In the wines produced in the absence of GYI, it reached a maximum concentration in C1 (11.06 ppb). Aldehydes, particularly decanal and dodecanal if they are present in high concentrations, can result in the appearance of an unpleasant "green" odour in wines (Liu et al., 2016).

Esters directly and indirectly influence wine aroma by means of highly varied interactions. The fermentation process applied significantly influences the quality and quantity of esters [43,44]. The wine samples inoculated with *M. pulcherrima* MP346 showed a higher content of esters (2318.98 ppb in T1 and 2056.15 ppb in T2) than the controls (1401.74 ppb in C1 and 1848.45 ppb in C2). Among the esters, the most representative was ethyl decanoate, which was produced in amounts over 1000 ppb in the wines inoculated with *M. pulcherrima* MP346. Indeed, in Riesling musts inoculated with the same commercial strain of *M. pulcherrima*, the quantities detected were half of those present in the Catarratto musts (Benito et al., 2015; Mislata et al., 2021).

The ethyl decanoate content reported by Benito et al. (2015) and Mislata et al. (2021) does not appear to have been impacted by the presence of *M. pulcherrima* MP346. However, in the Catarratto wines in this study, the levels of ethyl decanoate were significantly higher in the fermented wines with sequential inoculum.

A different situation was observed for ethyl octanoate, where the second EEFA was detected in greater quantities. Higher levels of ethyl octanoate were found in the experimental wines C1 and T1 without the addition of GIY. The effect of the glutathione-enriched inactivated yeast on ethyl

octanoate was unclear, although these highly volatile hydrophobic esters exhibit significant variations in wines containing yeast-derivative products (Rigou et al., 2021). Among the 2-phenylethyl esters, two opposite situations were found for 2-phenylethyl hexanoate, which was detected only in C1 and C2, while 2-phenylethyl acetate was present exclusively in T1 and T2.

The determination of VOCs in the different wines is reported in Table 2.

However, the 2-phenylethyl acetate concentrations were lower than those determined for Riesling wines produced using *M. pulcherrima* MP346. Most likely, the strain of *S. cerevisiae* used as the starter for AF significantly influenced the levels of this ester (Xu et al., 2022).

Among the twenty-six VOCs, only seven compounds showed an OAV greater than 1 (Table 2), i.e., one aldehyde (dodecanal) and six esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl 9-decenoate, 3-methyl-1-butanol acetate and methyl benzoate). Esters represent a group of compounds of considerable importance that are formed during AF through yeast metabolism and have a strong impact on the aromatic profile of wine (Tempère et al., 2018).

Table 2. Volatile organic compounds detected in the four Catarratto wines (all values in ppb).

t_R (min.s)	LRI [1]	Compounds [2]	Aroma Description [3]	Odour Threshold [3]	C1 [4] (OAV)	C2 [4] (OAV)	T1 [4] (OAV)	T2 [4] (OAV)	S.S. [5]
		Σ Alcohols			33.00±1.32^{b}	36.48±1.46^{a}	18.84±0.75 ^{c}	19.19±0.76^{c}	***
10.55	758	3-methyl-1-butanol	Fusel	40000 [7]	26.65±1.07^{b} (<1)	29.59±1.18^{a} (<1)	12.82±0.51^{d} (<1)	15.59±0.62^{c} (<1)	***
36.49	1110	Phenylethyl alcohol	Floral, rose	125000 [8]	6.35±0.25^{b} (<1)	6.89±0.28^{a} (<1)	6.02±0.24^{b} (<1)	3.60±0.14^{c} (<1)	***
		Σ Ethers			4.75±0.19^{a}	4.16±0.17^{b}	4.24±0.17^{b}	2.53±0.10^{c}	***
32.14	1042	Ethyl benzyl ether	Tropical fruit, pineapple	unknown	4.75±0.19^{a} (n.d.[6])	4.16±0.17^{b} (n.d.[6])	4.24±0.17^{b} (n.d.[6])	2.53±0.10^{c} (n.d.[6])	***
		Σ Aldehydes			17.37±0.69^{a}	4.91±0.20^{c}	11.85±0.47^{b}	2.73±0.11^{d}	***
24.89	958	Benzaldehyde	Bitter almond, cherry	1500 [9]	6.31±0.25^{a} (<1)	4.91±0.20^{b} (<1)	3.60±0.14^{c} (<1)	2.73±0.11^{d} (<1)	***
37.08	1203	Decanal	Floral, orange peel citrus	0.1 [10]	tr (n.d.[6])	tr (n.d.[6])	tr (n.d.[6])	tr (n.d.[6])	n.d.[6]
56.38	1411	Dodecanal	Citrus, floral	2 [11]	11.06±0.44^{a} (5.53)	0.00±0.00^{c} (<1)	8.25±0.33^{b} (4.13)	0.00±0.00^{c} (<1)	***
		Σ EEFAs			1401.74±56.08^{d}	1848.45±73.94^{c}	2318.98±92.76^{a}	2056.15±82.25^{b}	***
27.64	989	Ethyl hexanoate	Sweet fruity, pineapple, green apple	5 [11]	33.79±1.35^{b} (6.76)	48.86±1.95^{a} (9.77)	27.85±1.11^{c} (5.57)	32.14±1.29^{b} (6.42)	***
37.44	1208	Ethyl octanoate	Fruity, pear	2 [11]	901.19±36.05^{a} (450.60)	730.52±29.22^{b} (365.26)	837.67±33.51^{a} (418.84)	596.78±23.87^{c} (298.39)	***
51.00	1379	Ethyl decanoate	Fruity, grape	200 [11]	273.88±10.96^{c} (1.37)	928.14±37.13^{b} (4.64)	1253.71±50.15^{a} (6.27)	1236.22±49.45^{a} (6.18)	***
54.98	1391	Ethyl 9-decenoate	Fruity, fatty	100 [15]	184.44±7.38^{ab} (1.84)	137.73±5.51^{c} (1.38)	199.75±7.99^{a} (2.00)	178.82±7.15^{b} (1.79)	***
67.44	1599	Ethyl dodecanoate	Sweet, waxy, floral	2000 [11]	8.44±0.34^{b} (<1)	3.20±0.13^{c} (<1)	0.00±0.00^{d} (<1)	12.19±0.49^{a} (<1)	***
		Σ HAAs			15.10±0.60^{b}	19.09±0.76^{a}	6.25±0.25^{d}	9.71±0.39^{c}	***
18.59	882	3-methyl-1-butanol acetate	Sweet fruity, banana	0.75 [8]	15.10±0.60^{b} (20.13)	19.09±0.76^{a} (25.45)	6.25±0.25^{d} (8.33)	9.71±0.39^{c} (12.95)	***
		Σ EEBAs			12.94±0.52^{b}	8.02±0.32^{c}	0.00±0.00^{d}	14.56±0.58^{a}	***
58.69	1447	Isopentyl octanoate	Fruity, pineapple, coconut	125 [14]	12.94±0.52^{b} (<1)	8.02±0.32^{c} (<1)	0.00±0.00^{d} (<1)	14.56±0.58^{a} (<1)	***
		Σ MEs			233.83±9.35^{a}	106.27±4.26^{bc}	118.12±4.74^{b}	98.84±3.96^{c}	***
6.80	611	Ethyl acetate	Ethereal, fruity	7500 [11]	65.36±2.61^{a} (<1)	9.10±0.36^{d} (<1)	33.72±1.35^{c} (<1)	38.29±1.53^{b} (<1)	***

34.79	1089	Methyl benzoate	Green almond	10 [12]	36.94±1.48[a] (3.69)	25.00±1.00[b] (2.50)	24.22±0.97[b] (2.42)	14.93±0.60[c] (1.49)	***
46.19	1268	2-phenylethyl hexanoate	Sweet, honey, floral	94 [13]	10.28±0.41[a] (<1)	5.03±0.20[b] (<1)	0.00±0.00[c] (<1)	0.00±0.00[c] (<1)	***
46.24	1542	2-phenylethyl acetate	Rose	250 [11]	0.00±0.00[c] (<1)	0.00±0.00[c] (<1)	3.69±0.15[b] (<1)	5.45±0.22[a] (<1)	***
		Σ Others			121.25±4.85[a]	67.62±2.70[b]	56.49±2.27[c]	40.17±1.61[d]	***
7.50	634	Tetrahydrofuran	Butter, caramel	unknown	40.89±1.64[a] (n.d.[6])	35.68±1.43[b] (n.d.[6])	26.44±1.06[c] (n.d.[6])	23.34±0.93[c] (n.d.[6])	***
18.14	876	1,3-dimethylbenzene	Plastic odour	unknown	12.08±0.48[a] (n.d.[6])	8.03±0.32[b] (n.d.[6])	4.14±0.17[c] (n.d.[6])	2.89±0.12[d] (n.d.[6])	***
29.59	1023	*o*-cymene	Herb	unknown	15.37±0.61[a] (n.d.[6])	9.97±0.40[b] (n.d.[6])	5.41±0.22[c] (n.d.[6])	3.67±0.15[d] (n.d.[6])	***
34.04	1097	1-butenyl benzene	unknown	unknown	2.81±0.11[a] (n.d.[6])	2.05±0.08[b] (n.d.[6])	1.40±0.06[c] (n.d.[6])	0.76±0.03[d] (n.d.[6])	***
44.34	1232	Benzothiazole	Sulfury, rubbery, vegetable	unknown	16.45±0.66[a] (n.d.[6])	0.00±0.00[b] (n.d.[6])	0.00±0.00[b] (n.d.[6])	0.00±0.00[b] (n.d.[6])	***
50.79	1302	6-ethyltetralin (isomer)	unknown	unknown	6.85±0.27 (n.d.[6])	3.10±0.12 (n.d.[6])	3.44±0.14 (n.d.[6])	tr (n.d.[6])	n.d.[6]
51.29	1311	6-ethyltetralin (isomer)	unknown	unknown	7.66±0.31 (n.d.[6])	0.00±0.00 (n.d.[6])	2.97±0.12 (n.d.[6])	tr (n.d.[6])	n.d.[6]
54.53	1368	2-ethenyl-naphtalene	unknown	unknown	11.50±0.46 [a] (n.d. [6])	6.36±0.25[c] (n.d.[6])	10.83±0.43[a] (n.d.[6])	9.51±0.38[b] (n.d.[6])	***
59.64	1485	2,6-di-tert-butylquinone	unknown	unknown	7.64±0.31 (n.d.[6])	2.43±0.10 (n.d.[6])	1.86±0.07 (n.d.[6])	tr (n.d.[6])	n.d.[6]

[1] Linear retention index obtained through the modulated chromatogram reported for DB-5 MS apolar column;
[2] compounds are classified in order of retention time;
[3] Odor thresholds reported in the literature (http://www.thegoodscentscompany.com/; http://www.flavornet.org; http://www.odour.org.uk; Vilanova and Martinez, 2007);
[4] Relative amounts expressed as ppb with respect to calibration curve of ethyl benzoate;
[5] statistical significance;
[6] not determinable. Abbreviations: EEFAs: ethyl esters of fatty acids; HAAs: higher alcohol acetates; EEBAs: ethyl esters of branched acids; MEs: miscellaneous esters; OAV, odour activity value; tr: trace amount < 0.05%; T1, sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; T2, glutathione-rich inactivated yeasts and sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; C1, single inoculum with *S. cerevisiae* SPF52; C2, glutathione-rich inactivated yeasts and single inoculum with *S. cerevisiae* SPF52. Data in the same line followed by the same letter are not significantly different according to Tukey's test. *p* value: ***, $p < 0.001$.
[7] American Industrial Hygiene Association, 1989; [8] Darici et al., 2014; [9] Ferreira et al., 2004; [10] Duan et al., 2015; [11] Pino et al., 2010; [12] Tao and Zhang, 2010; [13] Zhang et al., 2020; [14] Zhang et al., 2013; [15] Xu et al 2022.

3.6 Sensory Analysis

The data from the sensory evaluation are shown in Table 3. The trials revealed some differences correlated with the presence/absence of *M. pulcherrima* MP346 and GIY.

The wines showed variability in terms of the attributes that defined appearance. The yellow colour values were in the range of 7.15–7.29, whereas, the green reflexes ranged between 3.63–4.04. The yellow colour values observed were higher than those shown by Scacco et al. (2012), while the ratings associated with the green reflections attribute were similar.

Table 3. Sensory score for experimental Catarratto wines.

Attributes	Trial				SEM	Statistical Significance	
	C1	C2	T1	T2		Judges	Wine
Appearance							
Yellow colour	7.28 [a]	7.15 [a]	7.21 [a]	7.29 [a]	0.01	n.s.	n.s.
Green reflexes	4.04 [a]	3.63 [b]	3.74 [b]	3.68 [b]	0.02	***	***
Odour							
Banana	3.63 [b]	3.94 [a]	2.79 [d]	3.15 [c]	0.07	***	***
Citrus	2.40 [a]	1.00 [c]	1.74 [b]	1.00 [c]	0.09	***	***
Fatty	1.35 [b]	1.22 [c]	1.62 [a]	1.32 [b]	0.02	***	***
Floral	2.53 [a]	1.00 [c]	1.97 [b]	1.00 [c]	0.10	***	***
Fruity	8.54 [c]	8.02 [d]	8.88 [a]	8.68 [b]	0.05	***	***
Grape	2.97 [c]	2.99 [c]	4.17 [a]	3.43 [b]	0.07	***	***
Green almond	7.67 [a]	6.84 [b]	6.77 [b]	5.71 [c]	0.11	***	***
Intensity	6.68 [c]	7.19 [b]	8.26 [a]	7.40 [b]	0.09	***	***
Pear	5.14 [b]	5.44 [a]	4.76 [d]	4.91 [c]	0.04	***	***
Persistence	7.11 [d]	8.64 [b]	8.12 [c]	8.97 [a]	0.10	***	***
Pineapple	3.62 [a]	3.63 [a]	2.96 [c]	3.44 [b]	0.04	***	***
Sweet fruit	7.25 [b]	7.57 [a]	5.75 [d]	6.59 [c]	0.10	***	***
Taste							
Sweet	3.48 [a]	3.59 [a]	2.78 [b]	2.68 [b]	0.06	***	***
Sour	5.38 [b]	5.37 [b]	8.11 [a]	8.24 [a]	0.21	***	***
Salty	5.70 [c]	5.85 [c]	7.99 [b]	8.39 [a]	0.18	***	***
Bitter	1.10 [c]	1.25 [b]	1.20 [b]	1.42 [a]	0.02	***	***
Mouthfeel							
Body	7.80 [c]	8.42 [b]	8.55 [b]	8.97 [a]	0.06	***	***
Balance	6.50 [d]	7.49 [c]	8.10 [b]	8.65 [a]	0.12	***	***
Flavour							
Banana-like	2.47 [b]	2.75 [a]	1.93 [d]	2.22 [c]	0.07	***	***
Cherry pit	3.67 [a]	3.84 [a]	3.77 [a]	2.70 [b]	0.07	***	***
Citrus	3.92 [a]	1.00 [b]	3.58 [a]	1.00 [b]	0.21	***	***
Fruity	6.15 [c]	6.26 [c]	7.79 [a]	6.80 [b]	0.10	***	***
Intensity	7.80 [c]	7.85 [c]	8.12 [b]	8.56 [a]	0.04	***	***
Mandarin orange	1.74 [a]	1.00 [c]	1.40 [b]	1.00 [c]	0.05	***	***
Persistence	7.70 [c]	8.78 [a]	7.97 [b]	8.94 [a]	0.08	***	***
Pineapple	7.11 [a]	6.89 [b]	6.86 [b]	6.14 [c]	0.05	***	***
Sweet apple	2.51 [c]	2.66 [c]	3.89 [a]	3.54 [b]	0.09	***	***
Sweet fruit	7.12 [b]	7.56 [a]	5.75 [d]	6.58 [c]	0.10	***	***
Overall quality	7.50 [d]	8.57 [b]	8.25 [c]	8.80 [a]	0.07	***	***
Flavour	6.98 [c]	8.81 [a]	8.11 [b]	8.91 [a]	0.11	***	***
Mouthfeel	7.20 [c]	8.32 [a]	7.88 [b]	7.97 [b]	0.06	***	***
Odour	7.20 [c]	8.86 [a]	8.01 [b]	8.74 [a]	0.10	***	***
Taste	7.01 [d]	7.54 [c]	7.82 [b]	8.11 [a]	0.06	***	***
Finish							
After-smell	6.80 [c]	8.15 [b]	8.21 [b]	8.50 [a]	0.10	***	***
After-taste	7.10 [c]	7.96 [b]	8.22 [b]	8.71 [a]	0.09	***	***

Results indicate mean value of three replicate sessions. Abbreviation: SEM, standard error of the mean; T1, sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; T2, glutathione-rich inactivated yeasts and sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; C1, single inoculum with *S. cerevisiae* SPF52; C2, glutathione-rich inactivated yeasts and single inoculum with *S. cerevisiae* SPF52. Data in the same line followed by the same letter are not significantly different according to Tukey's test. *p* value: ***, $p < 0.001$; n.s., not significant.

The T2 sample displayed a high score for 13 descriptors. The *M. pulcherrima* MP346 and GIY wine (T2) had the highest overall quality score (8.80). With regards to the odour attributes, the T1 and T2 wines showed the highest values for intensity and persistence, respectively. In addition, the T1 wine showed high scores for grape, fruity and fatty odours, the C1 wine showed high scores for citrus, floral, green almond and pineapple odours and the C2 wine was characterised by the presence of odours associated with banana, pear, pineapple and sweet fruit. The T2 wine was characterised by

odour attributes with intermediate scores. In wines to which GIY was added (C2 and T2), citrus and floral odours were not perceived. Nevertheless, banana, citrus, floral, fruity and pear aromas were present in the Catarratto wines reported by Scacco et al. (2012) but at lower levels.

The descriptors associated with taste enabled discrimination of the wines. T1 and T2 showed high scores for sour flavours, whereas salty flavours showed high values in T2. In terms of mouthfeel, the T2 wine achieved high values for the body and balance attributes. No unpleasant odours or flavours were revealed for all the wines. The GIY increased the flavour intensity and persistence, confirming. the results described by Alfonzo et al. (2021). Indeed, the treatment with GIY in combination with *M. pulcherrima* MP346 significantly improved the aromatic complexity of the T2 wine.

The T2 wine showed high intensity and persistence scores for flavours. The sensory descriptors with high flavour values were pineapple (C1), sweet fruit (C2) and fruity (T1 and T2). The T2 wine also excelled compared to the other wines for after-smell (8.50) and after-taste (8.71).

Correlations of the sensory analyses were examined by MFA. The number of sensory attributes (thirty-six variables) for the four wines made it possible to define two factors with an Eigen > 1 that represented a total variance of 89.64%. The correlation between the variables and the MFA factor was expressed by the value of the contribution and cos^2. The incidence of the factors F1 (56.41%) and F2 (33.23%) on the total variance discriminated the different wines. Examining the loading plot (Fig. 3), eight variables were located in both quadrants I and IV, ten were located in quadrant II and eleven were located in quadrant III.

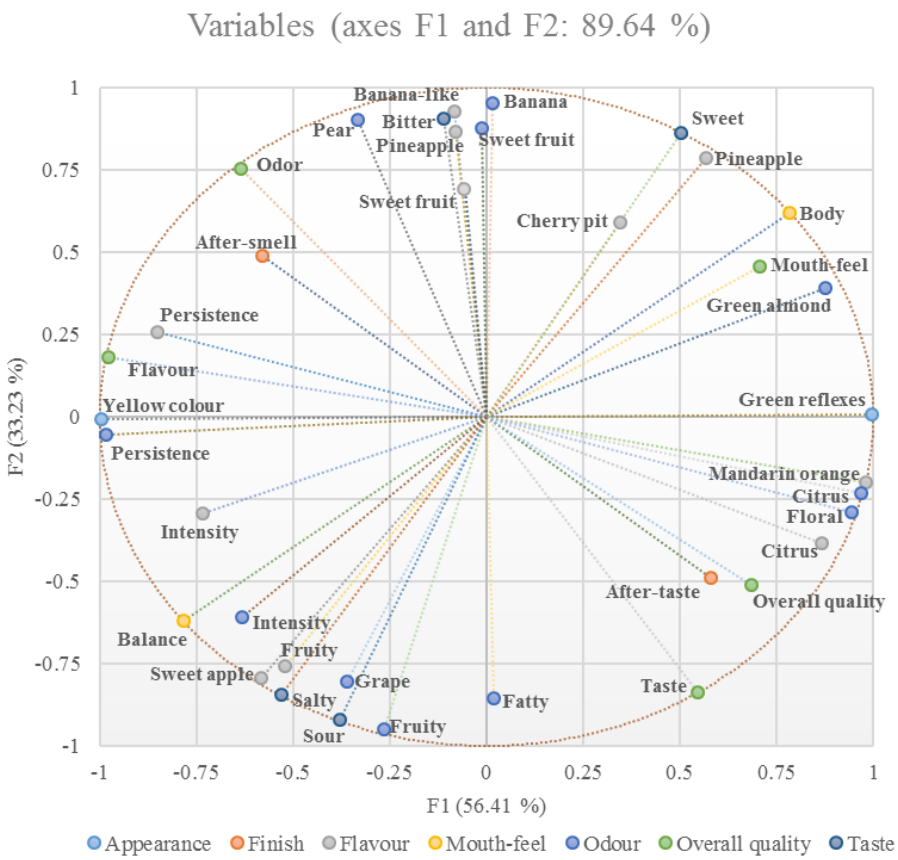

Figure 3. Variable loading plot of MFA.

Figure 4 reveals that the wines were clustered into three groups. In Figure 4a (MFA) and Figure 4b (AHCA), it is possible to observe how T1 and T2 represented a unique cluster. Interestingly, trial C1 did not cluster with trial C2. Indeed, the C1 and C2 trials represented a different cluster.

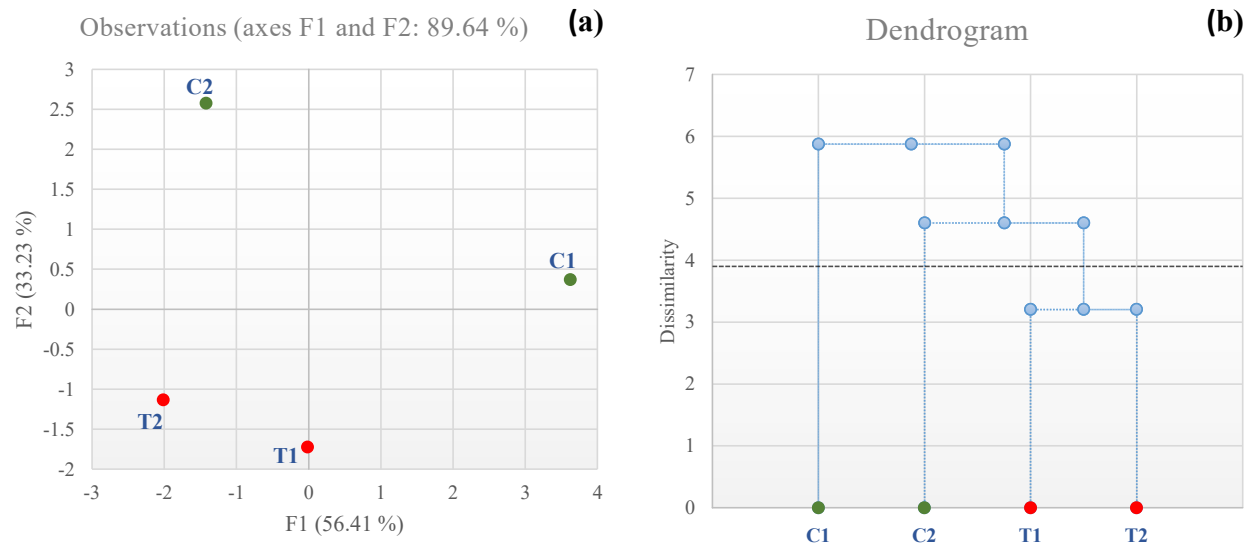

Figure 4. Multiple factor analysis applied to sensory analysis of Catarratto wines: (**a**) sample score; (**b**) agglomerative hierarchical clustering (AHC) dendrogram. Abbreviations: T1, sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; T2, glutathione-rich inactivated yeasts and sequential inoculum with *M. pulcherrima* MP346/*S. cerevisiae* SPF52; C1, single inoculum with *S. cerevisiae* SPF52; C2, glutathione-rich inactivated yeasts and single inoculum with *S. cerevisiae* SPF52.

3.7 Sensory Profiles Associated with Volatile Organic Compounds

A PCA was used to evaluate the correlation between VOCs and aroma attributes. According to Figure 5, the F1 factor contributed 66.11% of the total variance, whereas the F2 factor explained 28.60% of the total variance. Each wine, as can be seen from the biplot graph, was separate from the others.

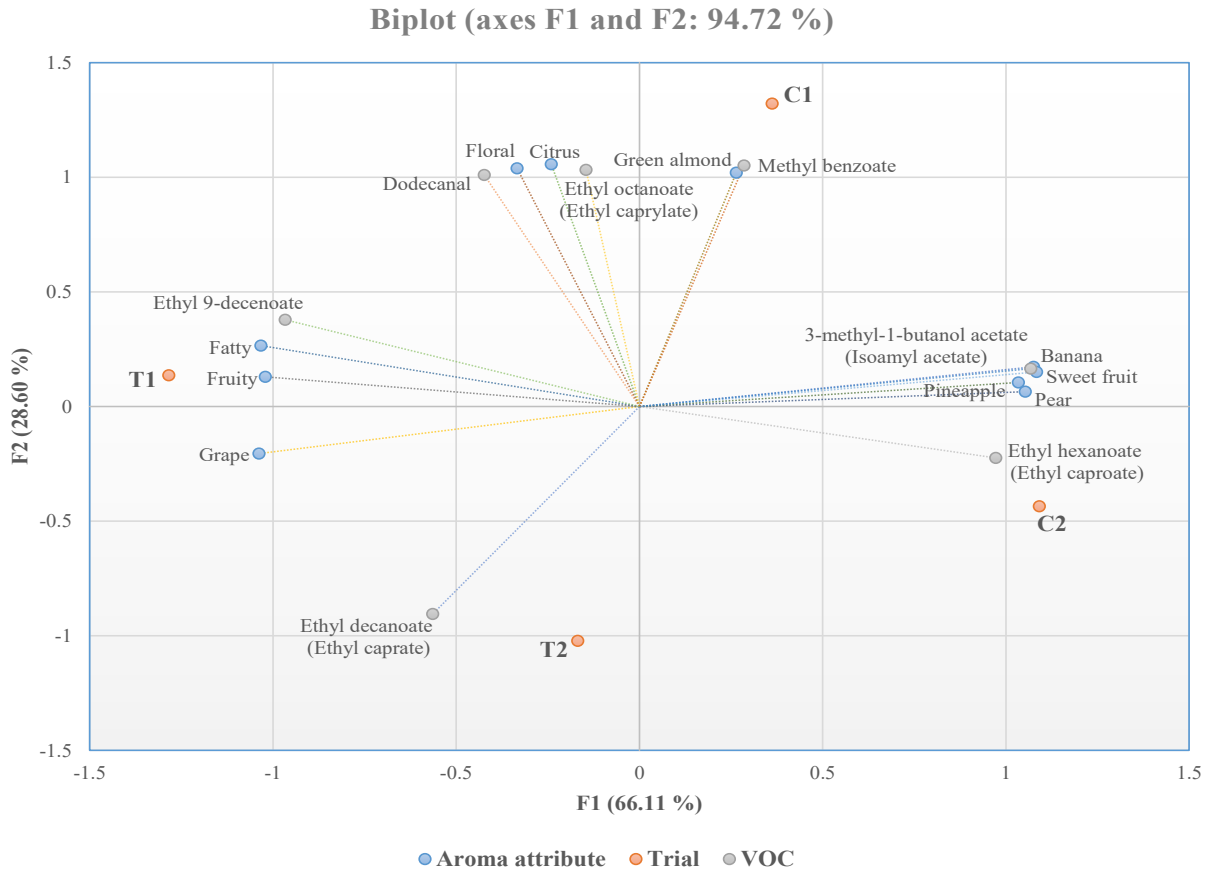

Figure 5. Principal component analysis (PCA) biplot for VOCs and aroma attributes. Abbreviations: T1, sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; T2, glutathione-rich inactivated yeasts and sequential inoculum with *M. pulcherrima* MP346/ *S. cerevisiae* SPF52; C1, single inoculum with *S. cerevisiae* SPF52; C2, glutathione-rich inactivated yeasts and single inoculum with *S. cerevisiae* SPF52.

The C1 wine was associated with methyl benzoate, which produced green almond aromas (Burdock, 2017). A sensory analysis confirmed this attribute, and the highest scores were achieved in this trial. Ethyl 9-decenoate was the compound closely correlated with the T1 wine. This ester produces fruity and fatty odours (Ribéreau-Gayon et al., 2006), which were also detected in the sensory analysis, with the scores of fruity being higher than fatty. The grape aroma emitted by ethyl decanoate (Vázquez-Pateiro et al., 2020) represented the T2 wine. The highest sensory analysis attributes detected in the T2 wine were fruity and sweet fruit, and the grape aroma showed modest values. However, fruity and grape aromas are also associated with the presence of ethyl decanoate (Fang and Qian, 2005). Finally, the C2 wine was closely associated with four odour descriptors (pineapple, sweet fruit, banana and pear). Only 3-methyl-1-butanol acetate and ethyl hexanoate were above the odour threshold and were responsible for the odours detected in the C2 wine by sensory analysis (Burdock, 2017).

The imperfect correlation between the highest OAV values of VOCs and the sensory analysis might be attributable to the synergistic interaction of odour molecules (high OAVs with low OAVs) with each other. As a result, the odours related to specific compounds were absent or very slightly perceived during the sensory analysis.

3. Conclusion

In this research, four treatments were examined in order to investigate the effect of *M. pulcherrima* and an antioxidant on the aroma and sensory profile of Catarratto wines. The use of *S. cerevisiae* SPF52 from a non-winemaking origin confirmed that yeasts from honey and its derivatives can potentially be used as starter strains in oenology. The combined use of *M. pulcherrima* MP346 and GIY had a positive impact on the taste–olfactory complexity of the wines. These differences were also confirmed by a sensory analysis. The VOC profiles generated by the wines obtained in the presence/absence of *M. pulcherrima* MP346 were correlated to the addition of GIY from the point of view of the quantity–intensity effect.

Dodecanal was only detected in the wines without GIY, whereas six esters had an OAV > 1 and actively contributed to the aroma definition of the different wines. Among the esters, ethyl decanoate

was the most abundant in the wines inoculated with *M. pulcherrima* MP346, regardless of the presence/absence of GIY. However, the differences in the VOC profiles enabled the wines produced with the different winemaking protocols to be distinguished.

The modulation of the aromatic profile of each wine was also confirmed by a sensory analysis, which made it possible to differentiate the wines into three groups. The presence of *M. pulcherrima* MP346 and the absence of GIY did not allow the T1 and T2 wines to be discriminated from a sensory profile, while these differences were greater in the C1 and C2 wines, where the only variable was represented by the addition of GIY.

Further studies are needed to evaluate the antioxidant effects of the specific inactive yeast with a guaranteed glutathione content at different times during the pre-fermentation stage (on the crushed-stemmed and drained must during the pressing stage) of Catarratto grapes.

The use of *S. cerevisiae* of a non-oenological origin, *M. pulcherrima* in the pre-fermentation stage and specific inactivated yeast with a high antioxidant power resulted in a better preservation of aromatic the compounds and colour, increasing the positive impact on the oxidative stability of the wines.

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Table S1. Chemical parameters determined during alcoholic fermentation time (days): 3, 6, 12, and 18.

→ continued

Parameters	Must	Vinification									
		3 days of alcoholic fermentation				S.S.	6 days of alcoholic fermentation				S.S.
		T1	T2	C1	C2		T1	T2	C1	C2	
pH	3.30±0.02	3.15±0.01a	3.10±0.01b	3.14±0.01a	3.06±0.02c	***	3.17±0.02bc	3.16±0.01c	3.21±0.01b	3.27±0.02a	**
TA	5.53±0.09	5.48±0.06b	5.56±0.00ab	5.62±0.04a	5.55±0.02ab	*	5.44±0.10a	5.45±0.05a	5.50±0.06a	5.55±0.07a	n.s.
VA	0.00±0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.
Rs	223.99±0.92	172.87±1.20a	171.12±1.93a	146.26±0.24b	117.20±0.35c	***	112.89±0.36a	83.19±0.72c	95.75±0.85b	58.79±0.32d	***
Glu	113.05±0.55	79.97±0.19a	78.32±1.16b	60.38±0.21c	61.93±0.24c	***	64.35±0.24a	41.62±0.20b	44.43±0.15c	25.80±0.11d	***
Fru	110.91±0.36	92.90±1.01a	92.80±0.77a	85.88±0.03b	55.27±0.10c	***	48.54±0.12b	41.57±0.52c	51.32±0.70a	32.99±0.21d	***
Eth	0.01±0.00	2.66±0.01c	2.67±0.01c	3.93±0.09b	5.40±0.08a	***	5.69±0.08d	7.16±0.12b	6.57±0.11c	8.44±0.03a	***
Gly	0.92±0.00	2.00±0.03a	2.00±0.01a	2.02±0.02a	2.01±0.01a	n.s.	5.28±0.01a	5.27±0.04a	5.06±0.04b	5.19±0.07a	**
MA	1.90±0.03	1.65±0.02a	1.66±0.02a	1.69±0.02a	1.66±0.03a	n.s.	1.59±0.02a	1.60±0.02a	1.60±0.02a	1.60±0.03a	n.s.
LA	0.00±0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.
Amm. N	85.42±1.01	161.57±0.31ab	160.86±0.11a	159.51±0.77a	157.27±0.11b	*	146.23±0.28c	147.39±0.31bc	150.96±0.34a	149.91±1.22ab	*
Alpha-AN	33.90±0.39	78.16±0.09c	70.21±0.21d	80.32±0.32b	81.63±0.35a	***	73.44±0.11c	71.39±0.41d	75.97±0.41b	78.66±0.16a	***

Parameters	Vinification									
	12 days of alcoholic fermentation				S.S.	End of alcoholic fermentation (18 days)				S.S.
	T1	T2	C1	C2		T1	T2	C1	C2	
pH	3.24±0.01c	3.21±0.01c	3.31±0.00b	3.35±0.02a	***	3.41±0.02b	3.43±0.01b	3.47±0.00a	3.51±0.01a	***
TA	5.48±0.03a	5.45±0.07a	5.45±0.07a	5.48±0.08a	n.s.	5.41±0.09a	5.40±0.09a	5.42±0.02a	5.50±0.08a	n.s.
VA	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.	0.27±0.00c	0.29±0.00b	0.31±0.00a	0.31±0.00a	***
Rs	49.51±0.34a	28.80±0.12c	47.80±0.20b	27.60±0.30c	***	3.70±0.04a	2.60±0.05d	3.01±0.03c	3.24±0.03b	***
Glu	21.43±0.25a	12.07±0.09b	21.09±0.06a	10.26±0.12c	***	1.10±0.01b	1.10±0.01b	1.62±0.01a	1.11±0.01b	***
Fru	28.08±0.09a	16.73±0.03d	26.71±0.14b	17.34±0.18c	***	2.60±0.03a	1.50±0.04c	1.39±0.02d	2.13±0.02b	***
Eth	9.00±0.11b	10.11±0.06a	9.03±0.03b	10.09±0.08a	***	11.35±0.15a	11.43±0.13a	11.36±0.08a	11.35±0.07a	n.s.
Gly	5.34±0.02b	5.92±0.09a	5.20±0.03b	5.22±0.05b	***	5.58±0.04c	5.31±0.05d	5.85±0.08b	6.57±0.12a	***
MA	1.55±0.01	1.58±0.02	1.47±0.01	1.50±0.01	***	1.49±0.02a	1.50±0.02a	1.36±0.01b	1.28±0.02c	***
LA	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.
Amm. N	119.88±0.43a	112.28±0.10b	110.38±0.44c	109.22±0.37c	***	49.35±0.27a	48.78±0.17a	45.61±0.21b	44.41±0.13c	***
Alpha-AN	67.32±0.08c	68.74±0.18b	63.84±0.13d	69.79±0.15a	***	55.21±0.08b	53.19±0.06c	56.81±0.11a	57.32±0.25a	***

Result indicate mean value ± standard deviation of three determinations. Data in the same line followed by the same letter are not significantly different according to Tukey's test.

Abbreviations: TA, total titratable acidity (tartaric acid g/l); VA, volatile acidity (acetic acid g/L); RS, reducing sugar (g/L); Glu, glucose (g/L); Fru, fructose (g/L); Eth, ethanol (% v/v); Gly, glycerol (g/L); MA, malic acid (g/L); LA, lactic acid (g/L); Amm. N, ammoniacal nitrogen (mg/L); Alpha-AN, alpha-amino nitrogen (mg/ L); n.d., not determined. P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.

Table S2. Chemical parameters determined during steel aging time (month): 1, 3 and 5.

→continued

Parameters	Vinification									
	1 month of steel aging				S.S.	3 month of steel aging				S.S.
	T1	T2	C1	C2		T1	T2	C1	C2	
pH	3.39±0.01b	3.39±0.02b	3.41±0.00b	3.46±0.01a	***	3.40±0.01b	3.39±0.01b	3.43±0.00a	3.44±0.02a	*
TA	5.40±0.01a	5.40±0.08a	5.49±0.03a	5.45±0.10a	n.s.	5.38±0.09a	5.39±0.02a	5.45±0.04a	5.46±0.10a	n.s.
VA	0.30±0.00c	0.30±0.00c	0.35±0.01b	0.43±0.00a	***	0.33±0.00b	0.34±0.01b	0.36±0.00a	0.36±0.00a	*
Rs	3.00±0.02b	2.90±0.01c	2.51±0.00d	3.36±0.02a	***	1.25±0.02b	1.19±0.02c	1.15±0.03c	1.47±0.01a	***
Glu	1.62±0.02a	0.47±0.00c	0.43±0.00d	1.47±0.02b	***	0.31±0.01b	0.30±0.00b	0.35±0.02a	0.21±0.01c	***
Fru	1.38±0.00d	2.43±0.01a	2.08±0.00b	1.89±0.00c	***	0.94±0.01b	0.89±0.02c	0.80±0.01b	1.26±0.00a	***
Eth	11.35±0.08a	11.43±0.13a	11.36±0.08a	11.35±0.07a	n.s.	11.35±0.07a	11.43±0.10a	11.36±0.00a	11.35±0.09a	n.s.
Gly	5.68±0.08bc	5.61±0.07c	5.82±0.03b	6.48±0.08a	***	5.67±0.06bc	5.60±0.09c	5.82±0.00b	6.49±0.08a	***
MA	1.35±0.02a	1.33±0.02a	1.32±0.03ab	1.27±0.01b	**	1.34±0.01a	1.32±0.01a	1.33±0.01a	1.27±0.00b	*
LA	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.
Amm. N	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alpha-AN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Parameters	Vinification				
	5 month of steel aging				S.S.
	T1	T2	C1	C2	
pH	3.38±0.01b	3.38±0.02b	3.41±0.00a	3.43±0.00a	***
TA	5.35±0.06a	5.38±0.05a	5.38±0.10a	5.42±0.05a	n.s.
VA	0.34±0.00c	0.36±0.01b	0.35±0.00b	0.38±0.00a	***
Rs	0.61±0.00a	0.50±0.03b	0.28±0.01d	0.31±0.01c	***
Glu	0.20±0.00a	0.20±0.01a	0.12±0.01b	0.15±0.01b	***
Fru	0.41±0.00a	0.30±0.02b	0.16±0.00c	0.16±0.00c	***
Eth	11.35±0.17a	11.43±0.02a	11.36±0.06a	11.35±0.08a	n.s.
Gly	5.64±0.08bc	5.59±0.09c	5.80±0.01b	6.47±0.06a	***
MA	1.32±0.02a	1.31±0.02ab	1.30±0.00ab	1.27±0.02b	**
LA	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	n.s.
Amm. N	n.d.	n.d.	n.d.	n.d.	n.d.
Alpha-AN	n.d.	n.d.	n.d.	n.d.	n.d.

Result indicate mean value ± standard deviation of three determinations. Data in the same line followed by the same letter are not significantly different according to Tukey's test. Abbreviations: TA, total titratable acidity (tartaric acid g/l); VA, volatile acidity (acetic acid g/L); RS, reducing sugar (g/L); Glu, glucose (g/L); Fru, fructose (g/L); Eth, ethanol (% v/v); Gly, glycerol (g/L); MA, malic acid (g/L); LA, lactic acid (g/L); Amm. N, ammoniacal nitrogen (mg/L); Alpha-AN, alpha-amino nitrogen (mg/ L); n.d., not determined. P value: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.

Chapter 3

- Other R&D training activities on wine in collaboration with other PhDs -

Activity 3.1

Oenological Capabilities of Yeasts Isolated from High-Sugar Matrices (Manna and Honey) as Potential Starters and Co-Starters for Winemaking

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Abstract

Non-*Saccharomyces* yeasts have recently garnered significant interest in oenology. When co-inoculated with *Saccharomyces cerevisiae*, they contribute to the improvement of wine quality from a sensory point of view. In the present study, a group of yeasts previously isolated from manna and honey by-products were subjected to a genotypic identification. The D1/D2 variable domains of the

26-sRNA gene and the ITS region of the 5.8S gene were sequenced. Additionally, a differentiation of strains was carried out by RAPD-PCR. All strains underwent in vitro screening. Subsequently, a micro-vinification experiment was conducted, focusing on strains with favourable technological characteristics: *Lachancea thermotolerans*, *Starmerella lactis-condensi*, and *Candida oleophila*. These strains were sequentially inoculated alongside a control strain of *Saccharomyces cerevisiae*. Technological screening revealed that some strains exhibited limited H_2S production, ethanol tolerance (up to 8% *v*/*v*), resistance to potassium metabisulphite (200 mg/L), osmotic stress tolerance (up to 320 g/L of glucose), and copper resistance (on average 5 mM). The findings from this study can guide the selection of new starters and co-starters for regional wine production.

Activity 3.2

Effects of different yeast strains, nutrients and glutathione-rich inactivated yeast addition on the aroma characteristics of Catarratto wines

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Abstract

Catarratto is one of the most common non-aromatic white grape varieties cultivated in Sicily (Southern Italy). In order to improve the aromatic expression of Catarratto wines a trial was undertaken to investigate the effect of yeast strain, nutrition and reduced glutathione. Variables included two Saccharomyces cerevisiae strains, an oenological strain (GR1) and one isolated from honey by-products (SPF52), three different nutrition regimes (Stimula Sauvignon Blanc™ (SS), Stimula Chardonnay™ (SC) and classic nutrition practice), and a specific inactivated yeast rich in reduced glutathione to prevent oxidative processes [Glutastar™ (GIY)] ensuing in ten treatments (T1-T10). Microbiological and chemical parameters demonstrated the aptitude of strain SPF52 to

successfully conduct alcoholic fermentation. During fermentation, the Saccharomyces yeast populations ranged from 7 to 8 logarithmic CFU/mL. All wines had a final ethanol content ranging between 12.91 and 13.85% (v/v). The dominance of the two starter strains over native yeast populations was higher than 97% as estimated by interdelta analysis. The addition of nutrients SS or SC increased the aromatic complexity of the wines as reflected by volatile organic compounds (VOCs) composition and sensory profiles. In particular, 32 VOCs were identified; alcohols (62.46–81.1%), thiols (0.27–0.87%), ethers (0.09–0.16%), aldehydes (0–1.21%), ketones (0–2.28%), carboxylic acids (4.21–12.32%), esters (0–10.85%), lactones (0.9–1.49%) and other compounds (0.77–6.9%). Sensory analysis demonstrated a significant impact on wine aroma in relation to yeast starter strain used, the type of nutrition (SS, SC or classic nutrition) and the presence/absence of GIY. The wines produced with GR1 yeast strain and SS (T2), SPF52 with SC (T9) both in presence of GIY showed higher overall quality. Trials T2 and T9 showed the highest scores for 13 and 18 attributes, respectively. The different nutrition, addition of GIY and the yeast starter strains diversified and enhanced sensory expression of Catarratto wines.

Activity 3.3

The use of a specific glutathione-rich inactivated yeast to protect organic Catarratto grape must and wine from oxidation in the pre-fermentative phase

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Abstract

This study aimed to investigate the effects of using a specific glutathione-rich inactivated yeast (GIY) during the pre-fermentative stages of winemaking. The antioxidant properties of GIY were evaluated both alone and in combination with a selected strain of Metschnikowia pulcherrima, at various grape pressing stages. The assessment included measuring soluble oxygen levels and color browning in the musts. Additionally, the impact of different oenological protocols on the final aromatic profile of the wines was assessed through volatile organic compounds (VOCs) and sensory analysis. The results showed that inoculating M. pulcherrima during the pre-pressing phase, followed by GIY addition

during the post-pressing stage, effectively reduced O_2 uptake (1.49 mg/L) and minimized browning (0.093 OD at 420 nm) during the pre-fermentative phase. Furthermore, the protocol involving GIY addition during pressing resulted in higher concentrations of specific compounds associated with fruity and floral aromas, such as ethyl octanoate (26.03 mg/L) and ethyl decanoate (26.87 mg/L). Sensory evaluations confirmed that all treatments had no off-odours or off-flavours, but the M. pulcherrima and GIY combination received the highest scores for smoothness and colour attributes. In conclusion, GIY treatments offer a promising alternative to enhance wine colour and mitigate oxidation effects, potentially reducing the reliance SO2 in wine production.

Activity 3.4

Use of *Saccharomyces cerevisiae*, *Metschnikowia pulcherrima* and *Torulaspora delbrueckii* in sequential wine fermentations: effects on yeast kinetics, oenological parameters, amino acids, and volatile organic compounds

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Data currently undergoing peer revision for publication in Food Research International –

Abstract

The aim of this research was to point out the action of non-Saccharomyces and *Saccharomyces cerevisiae* yeasts in sequential fermentations focusing on experiments performed in the same conditions in six different Research Units belonging to the Italian Group of Microbiology of Vine

and 50 Wine (GMVV) to reduce the biological variability. Thus, two non-*Saccharomyces* commercial strains 51 (*Metschnikowia pulcherrima* and *Torulaspora delbrueckii*) were used in sequential fermentations with a commercial S. cerevisiae strain in a red grape juice. The fermentations were monitored by evaluating the following parameters after 2 days (before S. cerevisiae inoculum) and at the end of the fermentative process: cell viable counts, ethanol, glycerol, primary acids (acetic, malic, and tartaric acid), amino acids, phenols, total antioxidant activity, total polysaccharides, and volatile organic compounds (VOCs). *S. cerevisiae* in pure culture attained higher cell levels than in sequential fermentations (7.8 vs 6.5-7.0 log CFU/mL) and showed a marked glucophilic preference in the first two days, as well as a higher glycerol and acetic acid production. All the batches were characterized by a decrease of malic acid and phenolic compounds. Instead, the sequential fermentation *T. delbrueckii/S. cerevisiae* showed a significantly lower amount of acetic acid (12.83 mg/L vs 95-102 mg/L) in comparison with the others. After 2 days of alcoholic fermentation, *S. cerevisiae* pure fermentation showed a significant higher consumption of amino acids than the two sequential fermentations (700 vs 200 mg/L). However, this trend was likely due to a higher cell concentration. In fact, the standardization of the amounts of consumed amino acids per million of cells suggested a lower utilization of amino acids by *S. cerevisiae* than that by M. *pulcherrima* and by *T. delbrueckii* (9 vs 84-116 mg/L). In addition, based on amino acid consumption, yeasts, clustering in four groups, highlighted a different preference by the three yeast species. Finally, VOCs analysis by Principal Component Analysis showed a differential accumulation of compounds, with two-day fermented S. cerevisiae samples exhibiting the highest concentration of all identified compounds. In contrast, higher concentrations of isobutyl alcohol and β-phenylethanol in the samples fermented with *M. pulcherrima* and of 1-propanol in those fermented with *Torulaspora*, were found

Activity 3.5

Diversity of *Saccharomyces cerevisiae* strains associated to racemes of Grillo grape variety

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Abstract

The most important oenological characteristics of high-quality sparkling wines are high content of acidity and low pH. Racemes are late-maturing grapes of Grillo variety characterized by low pH and high content of tartaric and malic acids and, due to their intrinsic characteristics, might represent an interesting technological solution to increase acid quality of base sparkling wine. To this purpose, the use of yeasts able to ferment grape must at very low pH is mandatory for the success of the process. In this work, 261 Saccharomyces cerevisiae isolated from spontaneous vinifications of Grillo grape racemes were subject to intraspecific characterization by interdelta analysis which evidenced a total population consisting of 82 strains which were screened for their basis of technological traits including SO_2 and alcohol tolerance, flocculence, growth at low temperatures and qualitative features such as H_2S production. A total of 11 strains with interesting technological performance in vitro were

inoculated into musts obtained from racemes of Grillo grape variety and microfermentation were monitored. For the first time an ecological investigation of yeast associated to raceme grapes has been carried out and provided an innovative strategy to improve the acidity of a Sicilian sparkling base wine from Grillo grape variety.

Activity 3.6

Use of non-*Saccharomyces* yeasts in the *prise de mousse* of Lambrusco. Microbial evolution through alcoholic fermentation and effect on wine volatile profile

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Abstract

Lambrusco is a sparkling wine largely produced in the north of Italy, especially in the Emilia region. The possible application of two non-*Saccharomyces* yeasts in the secondary bottle fermentation (Champenoise method) was tested in combination with a commercial strain of *S. cerevisiae* to obtain wines having a distinctive volatile profile. Our results demonstrated that the gradual increase of ethanol content in the pied de cuve ensured the adaptation of *Hanseniaspora guilliermondii* and *Torulaspora delbrueckii* at the bottle fermentation, with survival comparable with that of *S. cerevisiae,* in the order of 6 log units. The simultaneous presence of two yeast species reduced the maximum fermentation rate, without any relevant alteration in the main oenological parameters of resultant wines. GC MS-MS analysis of the volatile profile of wines (46 compounds)

highlighted differences in wine made from a pure culture of *S. cerevisiae* from wines obtained by mixed yeasts. Acetates, esters, and fatty acids are the classes of volatile compounds mostly affected by using different yeasts in the bottle fermentation of Lambrusco wines. This work provided for the first time information about the volatile profile of Lambrusco and suggests an innovative application of non-*Saccharomyces* yeast in the production of sparkling wines by champenoise methods.

Chapter 4

- Other R&D training activities on fermented beverages -

Activity 4.1

Technological screening and application of *Saccharomyces cerevisiae* strains isolated from fermented honey by-products for the sensory improvement of Spiritu re fascitrari, a typical Sicilian distilled beverage

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Abstract

"Spiritu re fascitrari" is a Sicilian alcoholic beverage obtained through distillation of a decoction of spontaneously fermented honey by-products (FHP). The production process often leads to sensorial defects due to the unstable alcoholic fermentation. The objective of this work was to select *Saccharomyces cerevisiae* strains from FHP to be used as starter in decoction fermentation. Based on chemical, microbiological and technological data, from a total of 91 strains three S. cerevisae were selected for further testing to produce FHP at laboratory scale level. After FHP distillation, the analysis of volatile organic compounds showed a complex mixture of sensory active molecules,

mainly alcohols and aldehydes. Among the alcohols, 3-methyl-1-butanol, 2-methyl-1-butanol, phenylethyl alcohol, hexadecanol and octadecanol were found at the highest concentrations. Among the carboxylic acids, acetic acid was mainly detected in the spontaneously fermented samples. FHP fermented with the three selected strains were not characterized by the presence of off-odors or off-flavours. The results obtained in this work demonstrate that the selected *S. cerevisiae* strains are promising starters to stabilize the production of distilled alcoholic beverages produced from honey by-products.

Activity 4.2

Use of sequentially inoculation of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* strains isolated from honey by-products to improve and stabilize the quality of mead produced in Sicily

Rosario Prestianni [a], Michele Matraxia [a], **Vincenzo Naselli** [a], Antonino Pirrone [a], Natale Badalamenti [b], Marzia Ingrassia [a], Raimondo Gaglio [a], Luca Settanni a, Pietro Columba [a], Antonella Maggio [b], Maurizio Bruno [b], Nicola Francesca [a],* , Giancarlo Moschetti [a], Antonio Alfonzo [a]

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Data published in *Food Microbiology* – Available 27 May 2022 – https://doi.org/10.1016/j.fm.2022.104064

Abstract

Mead is a beverage produced by alcoholic fermentation of honey-must. The starter yeasts that are commonly used for the alcoholic fermentation of honey-must are oenological *Saccharomyces cerevisiae* strains. The objective of the present work was, for the first time, to apply yeasts of honey by-products origin to evaluate the influences the taste-olfactory attributes of mead. For this purpose, three experimental productions were set up, which included: (i) single inoculation of *S. cerevisiae*; (ii) single inoculation of Hanseniaspora uvarum; (iii) sequential inoculation of *H. uvarum/S. cerevisiae*. Two control trials were performed, using a commercial strain of *S. cerevisiae* of oenological origin and a spontaneous fermentation. The results of the chemical parameters showed

differences between the trials in terms of residual sugars, acetic acid, glycerol, ethanol and volatile organic compounds. Sensorial analysis also showed a high heterogeneity among trials. The attributes of sweetness, honey and floral were found in mead fermented with *H. uvarum*, whereas all meads obtained with *S. cerevisiae* were dry, balanced and without off-odors and off-flavours. The results obtained showed that the controlled application of conventional and non-conventional yeast strains isolated from honey by-products origin could be a promising approach to improve the quality of meads.

Activity 4.3

Influence of indigenous *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* from sugar-rich substrates on the aromatic composition of loquat beer

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Abstract

The demand for unique and exclusive food products and beverages is constantly on the increase. One of the products that mostly evolved to encounter market dynamics in the last decade is craft beer. For a long time, craft breweries have included fruit in beer production to enrich flavour and aroma profile of different beer styles. In this study, for the first time, the use of *Saccharomyces* and non-*Saccharomyces* yeast strains isolated from highsugar matrices (manna and fermented honey by-products) were investigated to diversify fruit craft beer production, in order to improve the fermentation process and highlight the complexity of aroma profiles generated during alcoholic

fermentation. Two yeast strains, *Hanseniaspora uvarum* YGA34 and *Saccharomyces cerevisiae* MN113, were tested as co-starters and starters for their beer production capacity. Commercial yeast strain US-05 was used as control. Loquat juice was added at the end of primary alcoholic fermentation in all trials. Interestingly, *S. cerevisiae* MN113 consumed sugars faster than control strain S. cerevisiae US-05, including maltose, even in the case of sequential inoculation. This strain showed an excellent ability to consume rapidly sugars present. All strains showed their concentrations ranged between 5 and 8 Log cycles during fermentation. The absence of off-odours and the improvement of aromatic perception were observed in experimental trials involving the use of *S. cerevisiae* MN113 as a monoculture and in sequential combination with *H. uvarum* YGA34. Esters and alcohols were the most abundant compounds emitted from the beers. The beers produced with sequential inoculation of *H. uvarum* YGA34 and *S. cerevisiae* MN113 or US-05 are characterised by a higher ester and lower alcohol concentration. These two unconventional yeast strains from high sugar matrices showed great technological properties, representing promising co-starters and starter during craft fruit beer production.

Activity 4.4

A novel microbiological approach to impact the aromatic composition of sour loquat beer

Nicola Francesca [a], Antonino Pirrone [a], Ignazio Gugino [a], Rosario Prestianni [a], **Vincenzo Naselli** [a], Luca Settanni [a], Aldo Todaro [a], Raffaele Guzzon [b], Antonella Maggio [c], Antonella Porrello [c], Maurizio Bruno [c], Vittorio Farina [a], Roberta Passafiume [a], Antonio Alfonzo [a],*, Giancarlo Moschetti [a] , Raimondo Gaglio [a]

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Abstract

The growing interest in novel beer development determined the exploitation of unconventional yeasts isolated from novel ecological niches to generate unexplored sensory profiles. In recent years, there is an increasing interest in generating beers brewed with the addition of fruits. For the first time, *Lachancea thermotolerans* MNF105 and Saccharomyces cerevisiae MN113 isolated from manna, were tested as starter cultures to process loquat beer to improve the sensory profile. Innovatively, the yeast species *L. thermotolerans* was investigated for the production of sour fruit beer. Sour fruit beers produced with *L. thermotolerans* MNF105 were more balanced than the respective control, especially

in terms of perceived acidity during sensory analysis. This could be due to the lower lactic acid production (0.49 g/L) compared to the respective control (1.74 g/L). The overall organoleptic investigation showed a preference for *S. cerevisiae* MN113 (TF1) isolated from manna. Experimental trials conducted with the selected strains demonstrated the absence of off-odour and off-flavour and improved aroma perception. Aldehydes and alcohols were the most abundant compounds emitted from the beers. *S. cerevisiae* MN113 and *L. thermotolerans* MNF105, manna related yeasts, showed great technological properties, representing promising starters for the production of fruit beer and sour fruit beer.

Activity 4.5

Exploring the diversity of native *Lachancea thermotolerans* strains isolated by sugary extracts from manna ash to modulate the flavour of sour beers

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Abstract

The craft beer industry is becoming increasingly interested in the production of innovative beers. A novel approach, designated as "primary souring," employs diverse yeast species, including *Lachancea thermotolerans*, to produce sour beers. Furthermore, there is a growing interest in utilising unconventional yeasts to produce beers with distinctive flavours. For the first time, yeast strains of *L. thermotolerans*, isolated from sugar extracts of manna ash, were evaluated for their ability to produce and improve the sensory properties of sour beers. In particular, five strains exhibited notable

resistance to ethanol, sugar and hops, as well as comparable lactic acid production (ranging from 0.33 to 0.45 g/L). Experimental beers produced using MNF105 (T1) were perceived as the most “fruity". This is the first study to examine the impact of this novel indigenous strain, derived from unconventional matrixes such as manna, on the organoleptic quality of craft sour beers. Consequently, elevated levels of ethyl decanoate, ethyl hexanoate, ethyl octanoate and ethyl nonanoate were found in T1 beer, exceeding the perception threshold. The ability of this strain to perform light bio-acidification is a valuable feature for the development of new brewing techniques, particularly for the creation of sour beers with balanced acidity and innovative flavours. The yeast *L. thermotolerans* MNF105, which is related to manna, has excellent technological properties and is a promising starter for beer production with the ability to light bio-acidify and modulate flavour.

Activity 4.6

Technological and Organoleptic Parameters of Craft Beer Fortified with Powder of the Culinary–Medicinal Mushroom Pleurotus eryngii

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Abstract

Beer is one of the oldest and most popular alcoholic beverages and is currently consumed worldwide. The various components used in the brewing process have a physiological impact on the consumer and current research aims to improve its technological and functional properties through the addition of natural compounds (plants or mushrooms). In this work, the addition of two different amounts (5 and 10 g/L) of Pleurotus eryngii var. eryngii in powder form added at different production stages (PRE and POST alcoholic fermentation) showed the improvement in yeast viability during the alcoholic fermentation, increased the alcoholic content, and improved the sensorial profile. Regarding the organoleptic profile in the experimental samples, cocoa/chocolate and mushroom aromas were found and the samples PRE10 and POST5 received the best ratings with respect to all evaluated parameters.

Chapter 5

- Other R&D training activities on food -

Activity 5.1

Co-inoculation approach combining lactic acid bacteria and yeasts to enhance the production of Nocellara del Belice green split table olives

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Abstract

Table olives are among the most popular fermented foods in the Mediterranean area. In Sicily, split green table olives are produced on a small scale and are traditionally used as an ingredient in various recipes. In order to improve and standardise the production of Nocellara del Belice split table olives, experimental productions inoculated with a commercial LAB strain and two previously selected yeast strains were carried out. The experimental plan included the production of three experimental batches (SO, split olives): SO1, control production inoculated with the commercial strain *Lactiplantibacillus pentosus* OM13; SO2, L. pentosus OM13 was coinoculated with Candida boidinii LC1; SO3, *L. pentosus* OM13 in co-inoculum with C. norvegica OC10. Throughout the 90-day fermentation period, pH, salinity, and microbiological populations were closely monitored. At the conclusion of fermentation, the olives were assessed for colour, pulp hardness, volatile organic compounds, and

sensory characteristics. During the process, the inoculated lactobacilli and yeast strains became the dominant populations, reaching levels above 6 Log CFU/mL and dominance percentages exceeding 80%. In the in the coinoculated trials, brine acidification achieved pH values below 4.5 after 75 days, compared to the control trial, which reached this level after 21 days. The co-inoculation approach effectively limited the growth of undesirable microorganisms, resulting in firmer pulp. This combination of inocula also allowed for aromatic differentiation between the trials. Sensory analysis revealed differences in texture, flavour, and overall appreciation, with no unpleasant odours or tastes detected.

Activity 5.2

Enhancing the quality and safety of Nocellara del Belice green table olives produced using the Castelvetrano method

Antonio Alfonzo [a], Davide Alongi [a], Rosario Prestianni [a], Antonino Pirrone [a], **Vincenzo Naselli** [a], Enrico Viola [a], Claudio De Pasquale [a], Francesco La Croce [b], Raimondo Gaglio [a], Luca Settanni [a], Nicola Francesca [a],*, Giancarlo Moschetti [a]

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[b] *Geolive Belice S.r.l., S.S. 115 Km Dir, Marinella, Castelvetrano, 91022, Italy*

Data published in *Food Microbiology* – Available 11 January 2024 - https://doi.org/10.1016/j.fm.2024.104477

Abstract

The Castelvetrano method is the most widely used among the various table olive processing styles in Sicily. After debittering, the product is stored at low temperatures to prevent the growth of undesirable microorganisms. In an effort to enhance the production process, yeast isolates underwent genotypic characterization and technological screening. The screening process identified two yeast strains *Candida norvegica* OC10 and *Candida boidinii* LC1, which can grow at low temperatures and tolerate high pH values (up to 10) and salinity [10% (w/v)]. During the monitoring period, the inoculated trials showed limited presence of spoilage/pathogenic microorganisms. Additionally, the yeasts limited oxidative phenomena and softening of the drupes. The organic compounds detected were higher in the inoculated trials than in the control, and cold storage induced aromatic decay,

which was less pronounced in the trial inoculated with *C. norvegica*. Sensory analysis revealed that the inoculated trials scored higher in sweetness, hardness and crispness.

Activity 5.3

Application of technological protocols on an industrial scale to improve Seville-style table olive production in Italy and Spain

Antonio Alfonzo [a],*, Valentina Craparo [a], Rosario Prestianni [a], **Vincenzo Naselli** [a], Venera Seminerio [a], Antonino Pirrone [a], Francesco La Croce [b], Luca Settanni [a], Giancarlo Moschetti [a], Nicola Francesca [a]

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Data published in *Applied Food Research* - Available 6 october 2023 - https://doi.org/10.1016/j.afres.2023.100349

Abstract

Improving the fermentation performance of starter strains used in the fermentation of table olives is a biotechnological solution of current interest to improve the quality characteristics of the final product. The aim of this study was to evaluate the use of *Lactiplantibacillus pentosus* OM13 as a starter culture for the fermentation of Seville-type table olives in two different production areas: Italy and Spain. The starter strain *L. pentosus* OM13 was inoculated into two different table olive varieties: Nocellara del Belice in Italy and Manzanilla in Spain. Lactiplantibacillus plantarum Vege-Start 60 was used as a commercial control, while an additional control production was carried out by spontaneous fermentation. The industrial productions consisted of three different protocols, differing in the type of nutrient and the presence/absence of acclimatization of the starter strain. All trials were

subjected to microbiological monitoring, evaluation of acidification dynamics and sensory analysis of the final product. After 90 days, the pH reached values below 5 in the different treatments. The LAB reached microbial loads varying between 6.5 and 8.7 log CFU/mL throughout the monitoring period. The microbial populations of spoilage and/or potential pathogenic microorganisms were variable depending on the microbial group monitored. However, after 12 days of fermentation, Enterobacteriaceae showed values below the detection limit. In contrast, a fluctuating trend was observed for yeasts, Pseudomonadaceae and Staphylococcaceae. Sensory analyses showed variable differences depending on the technological protocol used. Table olives obtained with *L. pentosus* OM13 in the presence of nutrient, activator and acclimatization period achieved higher overall acceptability values compared to the other trials. The use of adjuvants (nutrients and activators) is a strategy used in the production of table olives fermented with *L. pentosus* OM13 to improve the sensory characteristics of table olives.

Activity 5.4

Effect of Glucose and Inactivated Yeast Additions on the Fermentation Performances of *Lactiplantibacillus pentosus* OM13 during the Production of Nocellara del Belice Table Olives

Antonio Alfonzo [1],*, Nicola Francesca [1], **Vincenzo Naselli** [1], Raimondo Gaglio [1], Onofrio Corona [1], Venera Seminerio [1], Luca Settanni [1], Francesco La Croce [2] and Giancarlo Moschetti [1]

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Data published in *Fermentation* – Available 5 July 2023 - https://doi.org/10.3390/fermentation9070634

Abstract

The use of selected strains of lactic acid bacteria is necessary to produce fermented table olives with high hygiene and quality standards at the industrial level. A current tendency is the use of fermentation adjuvants (nutrients and activators) that can satisfy the nutritional needs of starter strains. In this study, five experimental protocols, different for nutrient and activator presence and addition of *Lactiplantibacillus pentosus* OM13 in freeze-dried form and after acclimatization, were tested with the aim of improving the fermentation performances of the commercial starter. The trial inoculated with the starter strain acclimatized in the presence of nutrients and activator showed the most rapid acidification during the first phase of fermentation (third to ninth day), registering a pH loss of 3.40 units. The addition of adjuvants positively influences starter dominance (>89%) and rapid colonization (>7 Log CFU/mL from third d) by indirectly limiting the presence of undesirable

microorganisms. The analysis of volatile organic compounds revealed the presence of 32 chemicals distributed differently in each trial. Sensory evaluation showed that table olives produced with the different treatments were characterized by low bitterness, acidity, and absence of unpleasant odours/flavours. Control production showed slower acidification kinetics and lower sensory pleasantness than the other trials.

Activity 5.5

Use of different nutrients to improve the fermentation performances of *Lactiplantibacillus*

Antonio Alfonzo [1,*], **Vincenzo Naselli** [1], Raimondo Gaglio [1], Luca Settanni [1], Onofrio Corona [1], Francesco La Croce [2], Paola Vagnoli [3], Sibylle Krieger-Weber [4], Nicola Francesca [1] and Giancarlo Moschetti [1]

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Data published in *Microrganism* - Available 23 March 2023 - https://doi.org/10.3390/microorganisms11040825

Abstract

The aim of this study was to evaluate the fermentation performance of the commercial starter *Lactiplantibacillus pentosus* OM13 with four nutrients (A, B, C, and D) that differed in the following ingredients: starch, sugars, maltodextrin, inactivated yeast, inactivated yeast rich in amino acids, inactivated yeast rich in mannoproteins, and salt (NaCl). For this purpose, six different experimental productions of Nocellara del Belice table olives were carried out. During transformation, the fermentation process was monitored by measuring pH and plate counts for lactic

acid bacteria (LAB), yeasts, Enterobacteriaceae, Staphylococcaceae, and Pseudodomondaceae populations. At the end of the production process, each trial was subjected to volatile organic compound analysis and sensory evaluation. The addition of the different nutrients resulted in a significant reduction in pH (around 2.5 points) after 3 days of fermentation. At the same time, a significant increase in the number of LAB populations (> 6.6 log CFU/mL) was observed for all trials. Volatile organic compound (VOC) analysis revealed the presence of 39 compounds. In this study, nutrient C was optimal for improving the fermentation activity of *L. pentosus* OM13. These results provide elements for the implementation of experimental protocols to reduce product losses and improve sensory characteristics.

Activity 5.6

Reuse of almond by-products: Scale-up production of functional almond skin added semolina sourdough breads

Enrico Viola [a], Natale Badalamenti [b,c], Maurizio Bruno [b,c,d], Rosa Tundis [e], Monica Rosa Loizzo [e], Giancarlo Moschetti [a,c], Francesco Sottile [d,f], **Vincenzo Naselli** [a], Nicola Francesca [a], Luca Settanni [a,*], Raimondo Gaglio [a]

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[e] *Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende CS, Italy f Department of Architecture, University of Palermo, Viale delle Scienze, Ed. 14, 90128, Palermo, Italy*

Data published in *Future food* – Available 14 May 2024 - https://doi.org/10.1016/j.fufo.2024.100372

Abstract

The present work reports the application of powdered almond skin (PAS) for industrial bread production. Three trials were conducted involving seven bread shapes, including control production (CTR), and two experimental productions with PAS addition [5–10 % (w/w), 5-PAS and 10-PAS, respectively]. Sourdough inoculum determined the acidification of all doughs and the levels of lactic acid bacteria increased. Spore-forming aerobic bacteria, members of the Enterobacteriaceae family, and total coliforms were not detected until the end of the fermentation process. PAS addition determined a lower weight loss, an increase of firmness, a diminution of specific volume, and a different sensory profile of the breads. Mafalda was the most appreciated bread shape and was subjected to photothermal aging. 10-PAS sample, after nine-day stress, still showed a significant total phenolic compound TPC content (111.0 mg GAE/g extract). The radical scavenging potential increased with PAS with a final IC50 of 103.3 μg/mL in 10-PAS breads. Experimental breads exhibited a notable enhancement in protection against lipid peroxidation. Mold-free shelf life assessment showed a 10-day shelf life for CTR breads, while a 12-day shelf life in presence of PAS. Collectively, the data suggest that PAS holds significant promise as a functional additive for industrial production of bread.

Chapter 6

- Further research support activities on agri-food issue -

Activity 6.1

Boosting Postharvest Quality of "Coscia" Pears: Antioxidant-Enriched Coating and MAP Storage, SI: Eco-friendly technology for postharvest produce quality: New insights on physiology and technology of fresh horticultural crops.

Authors: Ilenia Tinebra, Roberta Passafiume, Alessandra Culmone, Eristanna Palazzolo, Raimondo Gaglio, Giuliana Garofalo, **Vincenzo Naselli**, Antonio Alfonzo, Vittorio Farina

Data currently undergoing peer revision for publication in Journal: AIMS Agriculture and Food , Manuscript ID: Agri-1412

Activity 6.2

Convective hot air-drying for improves the quality of sicilian blood orange. An eco-friendly method for obtaining flavourful dehydrated orange slices from Sicilian cultivars.

Authors: Pasquale Roppolo[1], Roberta Passafiume[1], Alessandra Culmone[1], **Vincenzo Naselli[1]** , Nicola Francesca[1], Vincenzo Guarino[1], Claudio De Pasquale[1], Vittorio Farina[1], Ignazio Gugino[1], Raimondo Gaglio[1].

Data currently undergoing peer revision for publication in *Food Bioscience*

Chapter 7

- Further scientific contributions -

Activity 7.1

Scientific contribution in national wine microbiology book

Titolo: Microrganismi del vigneto e della cantina (CHAPTER 2)

Nome Libro: Microbiologia della vite e del vino

N. di pagine: 11

Data di pubblicazione: 01/09/2022

Nomi autori: Nicola Francesca, **Vincenzo Naselli**, Giancarlo Moschetti

CEA - Casa Editrice Ambrosiana è un marchio editoriale di Zanichelli editore S.p.A.

Codice ISBN/DOI/ISSN: 978-88-08-89978-1

A cura di Patrizia Romano, Maurizio Ciani e Luca Cocolin

Activity 7.2

Scientific papers published in national journal

Lieviti indigeni per le birre alla frutta.

A cura di: Pirrone, A., Prestianni, R., Tinebra, I., Scuderi, D., Farina, V., **Naselli, V**., Cinquemani, G., Alfonzo, A., Moschetti, G., Matraxia, M., & Francesca, N. Birra nostra magazine - settembre-ottobre 2022

Lieviti non-convenzionali: *Hanseniaspora uvarum*.

A cura di Matraxia, M., Pirrone, A., Prestianni, R., **Naselli, V**., Cinquemani, G., Alfonzo, A., Moschetti, G., & Francesca, N. Birra nostra magazine – luglio - agosto 2022

Lieviti per birra: focus su specie non convenzionali.

A cura di Pirrone, A., Prestianni, R., **Naselli, V**., Cinquemani, G., Alfonzo, A., Todaro, A., Cugino, I., Moschetti, G., & Francesca, N. Birra nostra magazine - marzo-aprile 2022

Massimizzare il colore dei vini: il ruolo degli enzimi.

A cura di: Francesca, N.; **Naselli, V**.; Alfonzo, A.; Moschetti, G.; Petegolli,
Rivista L'Enologo Luglio-Agosto 2024

Activity 7.3

Abstract, poster and oral comunication in scientific conferences

Abstract e poster:

Use of *Lachancea thermotolerans* and *Saccharomyces cerevisiae* isolated from manna ash products to improve quality of loquat beer.
Pirrone A., Prestianni R., **Naselli V**., Farina V., Guzzon R., Porello A., Maggio A., Seminerio V., Alfonzo A., Francesca N. 7th International Conference on microbial diversity agrifood microbiota as a tool for a sustainable future. 26-29 Settembre, Parma, Italy

Abstract:

Non-conventional yeasts (*Starmerella lactis-condensi* and *Candida oleophila*) and Lactic Acid Bacteria (*Lactiplantibacillus plantarum* and *Oenococcus oeni*) in sequentially inoculated fermentations: a strategy to improve aroma of Catarratto wine.
Prestianni R., **Naselli V**., Pirrone A., Guzzon R., Vaglica A., Vagnoli P., Krieger S., Notarbartolo G., Alfonzo A., Francesca N. 7th International Conference on microbial diversity agrifood microbiota as a tool for a sustainable future. 26-29 Settembre, Parma, Italy.

Abstract:

Nutraceuticals for food: a biotechnological opportunity for safety and quality foodstufs
Filippo amato, Valeria Guarrasi, Antonella Maggio, **Vincenzo Naselli**, Nicola Francesca, Raimondo Gaglio.

Nutraceuticals and functional foods, Scienze & Industry. 2 February 2023, Palermo.

Poster:

Technological affinity index (TAIndex) for interaction between lactic acid bacteria and *Saccharomyces cerevisiae* strains to modulate the fruity and floreal aroma of Catarratto wines. V Convegno AISSA #UNDER40. 26-27 June 2024. Università degli studi di Firenze – Firenze – Italy.

Abstract:

Saccharomyces cerevisiae – Oenococcus oeni – Lactiplantibacillus plantarum: focus on malolactic fermentation during production of Catarratto and Riesling white wines.
Guzzon, R.; **Naselli, V**.; Francesca, N.; Alfonzo, A;Vagnoli, P.;Krieger, S.; Roman Villegas, T.;Moschetti, G.

Abstract and oral exposition:

Interaction among lactic acid bacteria, *Starmerella lactis-condensi* and *Saccharomyces cerevisiae* strains to modulate the fruity and floral aroma of Catarratto wines in Sicily.
Vincenzo Naselli, Enrico Viola, Antonino Pirrone, Valentina Craparo, Davide Alongi, Venera Seminerio, Micaela Carusi, Antonella Porrello, Antonella Maggio, Luca Settanni, Giancarlo Moschetti, Giuseppe Notarbartolo, Sibylle Krieger-Weber, Paola Vagnoli, Antonio Alfonzo, Stéphanie Weidmann, Nicola Francesca.
FORTHEM Food Science Lab Scientific Workshop; 6 June 2024 – University of Burgundy - Dijon (France).

Activity 7.4

Partecipation in international scientific program

- PRIMA Partnership for Reaserch and Innovative in the Mediterranean Area - Call 2022, Tematic Area 3-Food value chain: Topic 2.3.1-2022 (RIA) Enabling the transition to healthy and sustainable dietary behaviour.
  Titolo Progetto: MEDIET4ALL "Transnational Movement to Support the Sustainable Transition towards a Healthy and Eco-friendly Agri-Food System through the Promotion of MEDIET and its Lifestyle in Modern Society." CUP: B73C23000060001

  Resp.le Scientifico: Prof. Nicola Francesca;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca;

  Partners: Università degli Studi di Palermo e altre sette nazionali dell'area del Mediterraneo (Johannes-Gutenberg-University, of Mainz, Germania – CAPOFILA; University of Bourgogne, Francia; Vitagora, Francia; University of Valencia, Spagna; Università degli Studi di Palermo, Italia; University of Sfax, Tunisia; Microtarians SiS, Lussemburgo; National School of Agriculture of Meknes, Marocco; University Mohammed V of Rabat, Marocco; University of M'hamed Bougara Boumerdès, Algeria.

  Periodo attività: luglio 2023 – luglio 2026

  Totale finanziamento: 2.369.357,00 euro di cui 320.000,00 euro all'Università degli Studi di Palermo

- FORTHEM "Fostering Outreach within European Regions, Transnational Higher Education and Mobility", call of EAC/A03/2018 "European Universities" Erasmus+ Program, Action n.2, under the supervision of Prof. Nicola Francesca and Prof. Paolo Inglese.

Activity 7.5

Partecipation in national scientific program

- LIVING LAB NEBRODI - Partenariato per l'innovazione di cui all' art 65 del D.Lsg. 50/2016 - Progettazione esecutiva e realizzazione e gestione del programma di lavoro correlato allo sviluppo del Living Lab - Area Interna Nebrodi - Azione 1.3.2 del PO FESR SICILIA 2014-2020: Intervento AINEB 50 Strategia interna Nebrodi-Creazione di ambienti di innovazioneaperta: Living e Fab Lab, codice CUP: F47H20003430009;
  Partenariato: -EURIS SRL, (CAPOFILA); ITALIACAMP SRL; UNIVERSITA' DEGLI STUDI DI PALERMO, DIPARTIMENTO di SCIENZE AGRARIE, ALIMENTARI E FORESTALI.
  Responsabile Scientifico: Prof. Nicola Francesca - Importo del finanziamento: 846.300,00
  Durata: 20/01/23 – 31/12/23

- Ministero dello Sviluppo Economico - Fondo per la crescita sostenibile a favore di progetti di ricerca e sviluppo negli ambiti tecnologici identificati dal Programma quadro comunitario "Orizzonte 2020 Progetti di R&S negli ambiti tecnologici di Horizon 2020 – Bando PON I&C 2014-2020". Prog. n. F/050267/01-03/X32 Project title: Approccio integrato per lo sviluppo di prodotti innovativi nei settori vitivinicolo e lattiero-caseario trainanti del comparto agroalimentare siciliano"; CUP Curriculum Vitae Sostituire con Nome (i) Cognome (i) © Unione europea, 2002-2015 | europass.cedefop.europa.eu Pagina 11 / 12 Progetti di rilievo regionale UniPa: B78I17000260008. Total Funding: 3.448.813,32 euros

- Ministero delle Politiche Agricole Alimentari e Forestali - Fondo Dipartimento delle Politiche Europee e Internazionali e dello Sviluppo Rurale - Direzione Generale delle Politiche Internazionali e dell'Unione Europea. Project title: Approccio integrato per il miglioramento qualitativo dei mieli e la valorizzazione dei prodotti dell'apicoltura Siciliana" CUP n. J57G17000030007 Total Funding: 34.508,83 euros

- Ministero delle Politiche Agricole Alimentari e Forestali - Fondo Dipartimento delle Politiche Europee e Internazionali e dello Sviluppo Rurale - Direzione Generale delle Politiche Internazionali e dell'Unione Europea. Project title: "Strategie innovative di miglioramento

tecnologico dei processi fermentativi per la produzione di idromele e la valorizzazione dei prodotti dell'apicoltura Siciliana". CUP n. J51C18000170007 Total Funding: 15.737,70 euros

- Ministero dello Sviluppo Economico - Fondo per la crescita sostenibile a favore di progetti di ricerca e sviluppo negli ambiti tecnologici identificati dal Programma quadro comunitario "Orizzonte 2020 Progetti di R&S negli ambiti tecnologici di Horizon 2020 – Bando PON I&C 2014-2020". Prog. n. Decreto di Concessione Prog n. F/200037/01/X45. Project title: Innovazioni tecnologiche bio based e potenziamento dell'economia circolare nella gestione degli scarti da lavorazione primaria di mandorle biologiche con elevata potenzialità agroindustriale"; CUP UniPa: CUP: B51B19000540008 C Total Funding: 2.012.325,00 euros.

- Progetto FREECO2 – Accordi per l'innovazione MIMIT DM 31/12/21 - Titolo della proposta progettuale "Following, Reducing, Recycling and reuse agrifood chain CO2 footprint".
  Resp.le Scientifico: Prof. Paolo Inglese;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca per le attività R&SS in campo enologico;

  Partners: Università degli Studi di Palermo, TopNetwork s.p.a

  Periodo attività: luglio 2023 – luglio 2026

  Totale finanziamento: 1.899.819,18 euro

- STRAVINA – PSR Sicilia 2014-2020 Misura 16, Sottomisura 16.1, PRJ-055, CUP: G64I20000510009.
  Titolo Progetto: Strategie comparative in ambito ampelografico e botanico per la produzione di vino frappato naturale

  Resp.le Scientifico: Prof.ssa Agata Novara;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca per la parte di trasferimento tecnologico in ambito microbiologia enologia e produzione sperimentale di vino frappato con lieviti non-Saccharomyces

  Partners: Università degli Studi di Palermo, Cantina Caruso&Minini e altre tre aziende agricole conferitrici di uva

  Periodo attività: maggio 2021 – dicembre 2023

  Totale finanziamento: 400.000,00 euro

- Progetto Waste2Value - Programma di ricerca dell'ecosistema dell'innovazione "I-nest – interconnected nord-est innovation ecosystem"- Investimento 1.5. creazione e rafforzamento di "ecosistemi dell'innovazione per la sostenibilità", finanziato dall'unione europea, nextgeneration EU. Titolo della proposta progettuale "Waste2Value (W2V)".
  Resp.le Scientifico: Prof. Filippo Amato – HTS Enologia;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca;

  Partners: HTS Enologia con consulenza di Università degli Studi di Palermo – Dip. SAAF

  Periodo attività: ottobre 2024 – dicembre 2025 (in attesa del decreto da parte della fondazione)

  Totale finanziamento: 250.000,00 euro (sotto revisione)

- Living Lab Sicani - "Partenariato per l'innovazione (art. 65 del D.lgs. 50/2016) per l'individuazione del partner che costituirà uno dei componenti del Living Lab Sicani e che dovrà curare la progettazione esecutiva e la successiva realizzazione e gestione del programma di lavoro correlato allo sviluppo del Living Lab – CUP: J99E20000220006".
  Resp.le Scientifico: Prof. Nicola Francesca;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca;

  Partners: Università degli Studi di Palermo, Italiacamp e Euris srl

  Periodo attività: ottobre 2024 – dicembre 2025 (in attesa del decreto regionale ma gara già aggiudicata ed affidata dal capofila al Dip. SAAF).

  Totale finanziamento: 1.067.744,00 euro

- Contratto di Filiera e di distretto V Bando MIPAAF - Avviso prot. n.182458 del 22/04/2022 recante le caratteristiche, le modalità e le forme per la presentazione delle domande di accesso ai contratti di filiera e le modalità di erogazione delle agevolazioni di cui al DM. n. 673777 del 22/12/2021.
  Titolo Progetto: "White Wine Identity: nuovi orizzonti per uno sviluppo integrato e sostenibile della filiera dei vini bianchi italiani", codice PRJ-1058 - Prot. Ingresso N.0598434 del 22/11/2022;

  Resp.le Scientifico e Project Manager generale: Prof. Nicola Francesca;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca;

  Partners: Università degli Studi di Palermo, dieci imprese viticolo-enologiche, in qualità di soggetti beneficiari diretti, e tre imprese biotecnologie enologico-agroalimentare/enti di ricerca, in qualità di soggetti beneficiari indiretti, ricadenti nel territorio nazionale italiano

Periodo attività: novembre 2023 – novembre 2027 (proposta progettuale in graduatoria utile finanziabile, attività avviate in attesa del decreto ministeriale)

Totale finanziamento: 49.959.324,37 euro

- PSR PROGRAMMA DI SVILUPPO RURALE SICILIA 2014-2022 - SOTTOMISURA 16.1 "Sostegno per la costituzione e la gestione dei gruppi operativi del PEI in materia di produttività e sostenibilità dell'agricoltura" D.D.G. n. 5428 del 29/12/2021.
  Titolo Progetto: GREEN AROMAS "Aromaticità e Longevità del Catarratto: Innovazione in Campo e Cantina basata su Biodiversità, Sottoprodotti e Biotecnologie ad alta Sostenibilità".

  Resp.le Scientifico: Prof. Nicola Francesca;

  Referente Scientifico Dip. SAAF: Prof. Nicola Francesca;

  Partners: Università degli Studi di Palermo, Coreras Ente di Ricerca Regionale, Coribia Ente di Ricerca Regionale, Di Bella Vini srl, HTS enologia e quattro aziende agricole produttrici di uva.

  Periodo attività: agosto 2023 – giugno 2025

  Totale finanziamento: 496.981,30 euro

- Ministero dello Sviluppo Economico - Fondo per la crescita sostenibile a favore di progetti di ricerca e sviluppo negli ambiti tecnologici identificati dal Programma quadro comunitario "Orizzonte 2020 Progetti di R&S negli ambiti tecnologici di Horizon 2020 – Bando PON I&C 2014-2020". Prog. n. Decreto di Concessione Prog n. F/200037/01/X45
  Titolo Progetto: Innovazioni tecnologiche bio based e

  potenziamento dell'economia circolare nella gestione degli scarti da lavorazione primaria di

  mandorle biologiche con elevata potenzialità agroindustriale"; CUP UniPa: CUP: B51B19000540008 C

  Resp. Scientifico: Prof. Francesco Sottile

  Referente. Scientifico Dip. SAAF: Dott. Nicola Francesca.

  Partners: Università degli Studi di Palermo, Bongiovanni Mandorle., Ori di Sicilia.

  Periodo attività: 01 febbraio 2020 – 31 gennaio 2023

Totale finanziamento: 2.012.325,00 euro

- PO FESR Assessorato Att. Prod. Reg. Sicilia è stato prodotto in data 21/08/2019 Prot. N. 52411 con il quale risulta ammesso a finanziamento il progetto
  Titolo e attività ricerca: Approccio integrato per la valorizzazione nella filiera olivicola attraverso la produzione di olive da tavola probiotiche. N. Prog. 07TP1039000074

Resp.li Scientifici: Dott. Francesco La Croce;

Referente Scientifico Dip. SAAF: Dott. Nicola Francesca;

Partners: Geolive sas di Francesco Lombardo; CADA Laboratori di F. Giglio; Rosso Conserve; BMC Santoro, Università di Palermo-Dip. SAAF (Consulenti Scientifici); Università di Catania Dip. D3A (Consulenti Scientifici)

Periodo attività: 2018-2021

Totale finanziamento: 732.201,00 euro.

Activity 7.6

Partecipation in research program funded by international and national enterprises

- Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260 and Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: managing wine flavour novel yeast strains and microbial nutritional strategies to improve wine quality.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: LALLEMAND DANSTAR FERMENT A.G., Università di Palermo-Dip. SAAF con il supporto logistico di Di Bella Vini srl e Cantine Europa sca;

  Periodo attività: ottobre 2019 – ottobre 2020 con proroga attività fino a dicembre 2021

  Totale finanziamento: 20.000,00 euro.

- Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260 and Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: novel Biotechnological Protocol to improve Table Olive Production in Three Mediterranean Countries (Italy, Spain and Greek): focus on debittering process.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: LALLEMAND DANSTAR FERMENT A.G., Università di Palermo-Dip. SAAF con il supporto logistico di Geolive sas di Francesco Lombardo e Aziende Spagnole, Greche e Italiane a gestione Lallemand

  Periodo attività: gennaio 2020 – gennaio 2021 con proroga attività fino a dicembre 2021

  Totale finanziamento: 6.000,00 euro.

- Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260 and

Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
Titolo e attività ricerca: Impact of sequential inoculation of yeasts/lactic acid bacteria on aroma profiles of Catarratto and Nero d'Avola wines. Codice Progetto CON-0302.

Resp.le Scientifico: Dott. Nicola Francesca

Partners: LALLEMAND DANSTAR FERMENT A.G., Università di Palermo-Dip. SAAF con il supporto logistico di Di Bella Vini srl, Cantine Europa Sca, Caruso&Minini Vini già partner del DSAAF.

Periodo attività: ottobre 2020 – ottobre 2021 con proroga attività fino a dicembre 2021

Totale finanziamento: 25.000,00 euro.

- Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260 and Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: Use of enzymes and microbial starters in winemaking of Nero d'Avola. Codice Progetto CON-0303.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: LALLEMAND DANSTAR FERMENT A.G., Università di Palermo-Dip. SAAF con il supporto logistico di Di Bella Vini srl già partner del DSAAF.

  Periodo attività: ottobre 2020 – ottobre 2021 con proroga attività fino a dicembre 2021

  Totale finanziamento: 9.000,00 euro.

- Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260, Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige and Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: Flavorbiota: technical and scientific collaboration agreement on wine

biotechnology. Codice Progetto CON-0364.

Resp.le Scientifico: Dott. Nicola Francesca

Partners: LALLEMAND DANSTAR FERMENT A.G., Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige, Università di Palermo-Dip. SAAF con il supporto logistico di Di Bella Vini srl e Cantine Europa sca già partner del DSAAF.

Periodo attività: aprile 2021 – aprile 2024

Totale finanziamento: 40.000,00 euro.

- Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260, Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige and Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: Enzyme applications to improve quality, chemical stabilization and sustainability of wines. Focus on proteinase, pectinase and glucanase enzymes. Codice Progetto CON-0418.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: LALLEMAND DANSTAR FERMENT A.G. e Università di Palermo-Dip. SAAF con il supporto logistico di Di Bella Vini srl e Cantine Europa sca già partner del DSAAF.

  Periodo attività: novembre 2021 – gennaio 2022

  Totale finanziamento: 11.000,00 euro.

- Convenzione per attività ricerca Azienda Agricola Buonivini, con sede legale in Pachino, Via Francesco Ferruccio, 41 cap 96018, partita IVA 05034020825, rappresentata dal Titolare e Legale Rappresentante Sebastiano Di Bella C.F. DBLSST53L12E017G e Università degli Studi di Palermo Dipartimento Scienze Agrarie, Alimentari e Forestali (SAAF) partita IVA/Codice Fiscale 00605880822.
  Titolo e attività ricerca: Ricerca e Sviluppo per il miglioramento della shelf-life dei vini siciliani. Codice IRIS UNIPA CON-0131

Resp. Scientifico: Dott. Nicola Francesca
Periodo attività: 20 maggio 2019 – 31 maggio 2022
Totale finanziamento: 24.000,00 euro

- Convenzione per attività ricerca tra C.A.D.A. di Giglio Filippo & C snc, Fondazione E. Mach San Michele all'Adige, Di Bella Vini srl e Dipartimento Scienze Agrarie, Alimentari e Forestali Università di Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: Safewine: ricerca e sviluppo nel settore enologico. Codice Progetto CON-0365.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: C.A.D.A. di Giglio Filippo & C snc, Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige, Di Bella vini srl e Università di Palermo-Dip. SAAF

  Periodo attività: aprile 2021 – aprile 2024

  Totale finanziamento: 40.000,00 euro.

- Convenzione per attività ricerca in regime conto terzi tra HTS enologia di Luigi Scavone sede legale Marsala (TP) e Dipartimento Scienze Agrarie, Alimentari e Forestali Università di Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: Accordo per attivita' in ricerca e sviluppo di biotecnologie in ambito viticolo-enologico. Codice Progetto CON-0405.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: HTS enologia di Luigi Scavonee Università di Palermo-Dip. SAAF

  Periodo attività: settembre 2021 – settembre 2024

  Totale finanziamento: 60.000,00 euro.

- Convenzione per attività ricerca tra Azienda agricola G. Milazzo-Terre della Baronia s.r.l. con sede legale a Campobello di Licata (AG) e Dipartimento Scienze Agrarie, Alimentari e Forestali Università di Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: ricerca e sviluppo di biotecnologie in ambito viticolo-enologico - spumantistica. Codice Progetto CON-0405.

  Resp.le Scientifico: Dott. Nicola Francesca

Partners: Azienda agricola G. Milazzo-Terre della Baronia s.r.l e Università di Palermo-Dip. SAAF

Periodo attività: settembre 2021 – settembre 2024

Totale finanziamento: 144.000,00 euro.

- Convenzione per attività ricerca tra Azienda Geolive Belice srl con sede legale a Castelvetrano (TP) e Dipartimento Scienze Agrarie, Alimentari e Forestali Università di Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: ricerca e sviluppo di biotecnologie in ambito olivicolo da mesa e agroalimentare. Codice Progetto CON-0407.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: Azienda Geolive Belice srl e Università di Palermo-Dip. SAAF

  Periodo attività: settembre 2021 – settembre 2024

  Totale finanziamento: 270.000,00 euro.

- Convenzione per attività ricerca tra HTS enologia di Luigi Scavone sede legale Marsala (TP) e Dipartimento Scienze Agrarie, Alimentari e Forestali Università di Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
  Titolo e attività ricerca: Biotecnologie Sostenibili per il Miglioramento della Qualità Tecnologia e Sensoriale dei Vini. Codice Progetto CON-0404.

  Resp.le Scientifico: Dott. Nicola Francesca

  Partners: HTS enologia di Luigi Scavonee Università di Palermo-Dip. SAAF

  Periodo attività: settembre 2021 – settembre 2024

  Totale finanziamento: 450.000,00 euro.

- Convenzione di Ricerca & Sviluppo: "Beer & Bioflavouring: nuove strategie per l'aumento della complessità aromatica della birra artigianale di Sicilia", stipulata dal Dipartimento di Scienze Agrarie, Alimentari e Forestali (SAAF) ed EPICA S.n.c. Società Agricola, prot. N. 4413-20/06/2022.
  Titolo e attività ricerca: Beer & Bioflavouring: nuove strategie per l'aumento della complessità aromatica della birra artigianale di Sicilia
  Resp. Scientifico: Prof. Nicola Francesca

Periodo attività: 20 giugno 2022 – 20 giugno 2025

Totale finanziamento: 00,00 euro

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Titolo e attività ricerca: Safewine: ricerca e sviluppo nel settore enologico. Codice Progetto CON-0365.
Resp.le Scientifico: Prof. Nicola Francesca
Partners: C.A.D.A. di Giglio Filippo & C snc, Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige, Di Bella vini srl e Università di Palermo-Dip. SAAF
Periodo attività: aprile 2021 – aprile 2024
Totale finanziamento: 40.000,00 euro.

Technical and scientific collaboration agreement between LALLEMAND Danstar Ferment A.G., based in Poststrasse 30, 6300 Zug (Switzerland) registered under the code CH-101.456.260, Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige and Dipartimento Scienze Agrarie, Alimentari e Forestali University of Palermo Cod. Fisc. 80023730825, P. IVA 00605880822.
Titolo e attività ricerca: Flavorbiota: technical and scientific collaboration agreement on wine biotechnology. Codice Progetto CON-0364.
Resp.le Scientifico: Prof. Nicola Francesca
Partners: LALLEMAND DANSTAR FERMENT A.G., Fondazione Edmund Mach – Istituto Agrario San Michele all'Adige, Università di Palermo-Dip. SAAF con il supporto logistico di Di Bella Vini srl e Cantine Europa sca già partner del DSAAF.
Periodo attività: aprile 2021 – aprile 2024
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Titolo Progetto: “White Wine Identity: nuovi orizzonti per uno sviluppo integrato e sostenibile della filiera dei vini bianchi italiani”, codice PRJ-1058 - Prot. Ingresso N.0598434 del 22/11/2022;
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