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Article

Combination of Destructive and Non-Destructive Analyses for Microbiological and Qualitative Characterization of Refermented and Yeast-Aged Apple Cider

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Abstract

In Italy, the apple cider market is experiencing significant growth, driven by numerous small-scale artisanal producers who combine local apple varieties with traditional processes to offer complex, and diverse products. However, artisanal production based on spontaneous fermentations often encounters challenges in qualitative reproducibility, particularly related to sensory issues (stability across different vintages and high turbidity of the product). In this context, a methodology has been developed to optimize the technological process of cider production at Contrada Contro in the Monti Sibillini (MC), in Marche region, Italy. The research focused on the isolation and selection of indigenous yeasts from frozen must prepared in the 2023 vintage. Following isolation and preliminary characterization, the indigenous yeasts were used to referment the still cider, followed by 7 months of bottle aging, and a second sampling point was conducted after 14 months of aging on lees. Destructive analyses using HPLC-DAD and GC-MS were conducted to evaluate polyphenols and volatile compounds, while non-destructive analyses with a 12-quartz microbalance electronic nose and NIR spectroscopy allowed for a quicker assessment of production techniques. Chromatographic analysis results showed that the sensory quality of refermented products was strongly influenced by the composition of the yeast strains used. All fermentations inoculated with selected yeasts exhibited lower turbidity compared to spontaneous fermentation. These findings indicate that the selection of indigenous yeasts for cider refermentation enables the production of a high-quality product, enriched with beneficial compounds and characterized by a strong terroir identity, underscoring the importance of microbiological terroir.

Keywords: apple cyder; spontaneous fermentation; microbiological terroir; wet chemistry; non-destructive approaches

1. Introduction

Beverages fermented from plant-based matrices are gaining increasing interest in global consumption [1], both to create new added value from materials that would otherwise have limited value, and for their natural origin. Another advantage of these fermented beverages is their alcohol content, which typically ranges from 2-8%, making them lower in alcohol than traditional alcoholic beverages such as wine and spirits [2]. Among these beverages, we can mention apple cider, fruit ciders, kombucha, or other exotic products now common in consumption. Furthermore, it has been reported that fermentation increases the nutritional value of the final products, which are generally

characterized by lower glucose levels and a high content of antioxidants [3]. This is why a second fermentation in the bottle could further enrich the already fermented matrix, thanks also to the permanence of the yeasts in the product [4]. Bottle aging on lees, also known as “sur lie” in French, is a common practice in various fermented beverages, including cider, wine, and beer. This technique involves leaving the finished product in contact with the yeasts deposited at the bottom of the bottle during the refermentation period. These yeasts, also called “fine lees,” are responsible for the final fermentation and maturation of the product. During bottle aging on lees, several processes occur that contribute to the complexity and richness of the final product. Yeasts consume residual sugars, naturally producing CO₂ and imparting a slight effervescence to the product [5]. Over time, the yeasts deposited at the bottom of the bottle begin to die and undergo a degradative process. During this process, known as autolysis, they release compounds that can positively influence the aroma, structure, and complexity of the cider by adding nuances of toasted bread, hazelnut, or butter to the sensory profile of the cider [6,7]. Another non-negligible aspect is the increased stability; bottle aging on lees can also reduce oxidation and protect the product from potential microbial contamination [8]. Bottle aging on lees is a practice that requires patience and care, as timing and storage conditions can significantly affect the final result in terms of overall quality. However, when executed correctly, this technique can produce ciders of high quality with distinctive depth and complexity. Recent studies have documented situations in which autochthonous biotypes exhibited greater fermentative efficiency than commercial strains, highlighting the limited dominance capacity of some commercial starter cultures during the fermentation process (Aponte et al., 2020). For this reason, in 2025 some researchers evaluated how the production of a fermented pineapple-based beverage can exhibit markedly different physicochemical characteristics depending on the microbial consortium responsible for fermentation.

To further investigate this concept also in secondary fermentation, the focus of the here presented work has been on selecting various yeast blends, isolated from the starting must of the apples, and used to referment the still cider, evaluating the characteristics imparted by each blend after 7 months and after 14 months.

2. Materials and Methods

2.1. Biological Material

For yeast isolation, a total volume of 6 L of frozen must obtained from “pink apples from the Sibillini Mountains” (year 2023), by Contrada Contro, located in the municipality of Gualdo in the province of Macerata, was utilized. The predominant yeast strains isolated were subsequently used as inoculum for the refermentation of the still cider previously produced by the company.

2.2. Cultural Media and Growth Conditions

Two different media were utilized to isolate the predominant yeast strains. Yeast Peptone Dextrose (YPD) agar was used to quantify the total yeast population, whereas the chromogenic differential medium Wallerstein Laboratory Nutrient Agar (WL) was utilized to differentiate yeast isolates based on their acid-producing capacity.

The enumeration of non-*Saccharomyces* yeasts was carried out using the complex synthetic Lysine Medium, which selectively inhibits the growth of *Saccharomyces* species. Microorganisms were cultivated in liquid culture within Erlenmeyer flasks containing YPD broth, maintaining a medium-to-flask volume ratio of approximately 1:5. Culture growth was monitored by measuring the increase in optical density at 600 nm using a spectrophotometer.

2.3. Isolation of Predominant Yeast Strains from the Must

To isolate predominant yeast strains, two 500 mL aliquots of must were thawed and incubated at 18 °C and 28 °C for 48 hours. Subsequently, serial 1:10 dilutions were prepared from 5 mL of the

suspension using sterile peptone water. The resulting plates were then incubated at 28 °C for 48 to 72 hours to facilitate complete colony development.

2.4. Inoculum Preparation and Setup of Different Fermentations

To establish the experimental conditions examined in this study, 500 mL of still cider from the 2023 vintage was utilized for refermentation and aging treatments, designated as CK, A, B, and C. Three of the four treatments (A, B, and C) were inoculated with distinct yeast mixtures isolated from the must, whereas the control treatment (CK) was inoculated with a commercial strain of *S. cerevisiae* (NEW C.E.M. & C. S.R.L; Pomezia, LT). For treatments A, B, and C, the predominant yeast morphotypes were cultured in 30 mL of YPD medium and incubated with agitation (150 rpm) at 30 °C for 48 hours. Following incubation, cells were harvested by centrifugation, and 6 g of wet biomass was resuspended in 10 mL of cider. Subsequently, 0.6 mL of these cell suspensions was added to 500 mL of still cider along with 132 mg/mL of cider. In treatment CK, 2 g of commercial yeast were resuspended in 20 mL of sterile water and incubated at 35 °C for 15 minutes prior to inoculation. For the refermentation and aging process in treatment CK, 0.5 mL of the yeast suspension was added to 500 mL of fresh cider together with 6.6 g of sugar.

2.5. Fermentation Process

Experimental groups CK, A, B, and C were prepared in 0.5 L glass bottles sealed with crown caps. Refermentation and aging were carried out at 15 °C $\pm$ 1 °C, with samples collected at two time points: after 7 months of aging on lees and after 14 months.

2.6. Polyphenols Profile

For the polyphenol profile detected by high-performance liquid chromatography (HPLC) coupled to diode-array detection (DAD), the Watherouse method was used [9]. The HPLC system had four solvent pumps (P680) and PDA 100 as a detector; a C-18 column (Dionex Acclaim 120 C18, 5 μ m, 4.6 $\times$ 250 mm) was used as the stationary phase, maintained at 40 °C with a mobile-phase flow rate of 0.5 mL min⁻¹. The mobile phases used were: Solvent A, 50 mmol L⁻¹ ammonium dihydrogen phosphate adjusted to pH 2.8 with orthophosphoric acid; Solvent B, 20% solvent A with 80% acetonitrile; Solvent C, 0.2 mol L⁻¹ orthophosphoric acid adjusted with NaOH at pH 1.5. All the reagents were purchased from Sigma-Aldrich (Milan, Italy). For qualitative and quantitative analysis of the individual polyphenols, 33 analytical standards were selected and used to build different calibration curves. The measurements were performed in triplicate.

2.7. Aromatic Profile

Aroma profile analysis of wine samples was performed on 5 mL of sample, in triplicate. A PerkinElmer Clarus® SQ 8 GC/MS system with a PerkinElmer Turbomatrix™ HS-40 was used for the analysis. 20 mL falcons were used for headspace sampling (PerkinElmer Inc. Italy) and aluminium crimped caps with PTFE-coated silicone gaskets (PerkinElmer Inc. Italy). The identification of volatile compounds was obtained using the mass spectral database of the National Institute of Standards and Technology (NIST 98, Version 2.0, USA). Only compounds with a match of 80% or more were selected. The selected peaks were then quantified using TurboMass software (TurboMass R, Version 5.4.2 PerkinElmer Inc., USA, 2008) by peak area integration. The area of each peak was then normalised to the sum of the areas and expressed as %. The measurements were performed in triplicate.

2.8. E-nose Analysis

The electronic nose (E-nose) used was designed, developed and assembled at the University of Rome Tor Vergata and is based on an array of 12 quartz microbalances (QMBs). In these sensors, small changes in mass (Δm) on the absorbing layer of the quartz surface lead to a shift in frequency

(Δf) in the electrical output signal of the oscillator circuit. Within a range of minor alterations, the change in frequency (Δf) is directly proportional to the change in mass (Δm). The chosen QMBs are made from AT-cut quartz crystals with a fundamental frequency of 20 MHz, corresponding to a mass resolution of a few nanograms [10].

The same device has been employed in several heterogeneous scientific studies, where it has been used to classify wines originating from different areas within the denomination [11] or to evaluate the quality of hazelnuts subjected to roasting process at different temperatures [12].

Cider measurement was carried out as follows: 10 mL of wine was incubated in 25-mL closed vials (equipped with silicon septum) at 30 °C for 20 min. Then, the equilibrated headspace was extracted for 90 s using a stream of filtered air and delivered into the electronic nose sensor cell. After each measurement, a pure air stream was used to clean the E-nose for an additional 300 s, establishing the reference signal. Sensor signals were calculated as the resonant frequency shift between the two steady conditions corresponding to sensors exposed to pure air and the sample. The ensemble of sensor signals is composed of patterns (fingerprints) encoding the global composition of the headspace.

2.9. Spectral Acquisition

The same ciders used for E-nose assessment were used for NIR spectral analysis with a spectrophotometer with a tunable NIR-acoustic-optical filter (AOTF) (Luminar 5030 miniature hand-held NIR analyser, Brimrose, Baltimore, MD, USA). The same NIR device has also been employed to monitor postharvest grape dehydration [13] or to monitor the controlled development of *Botrytis cinerea* on white grape berries as noble rot [14].

Readings were taken on cider with 5 replicates averaged together in transmittance in the range 1100-2300 nm, in 2 nm wavelength increments. Raw spectra were manipulated for absorbance ($\log 1/T$) transformation using SNAP! 2.03 software (Brimrose). Different spectral filtering and correction methods were tested to evaluate their influence on the performance of the subsequent multivariate analysis. In particular, smoothing and derivative transformations were applied using the Savitzky–Golay algorithm to improve spectral resolution and reduce high-frequency noise. Both first and second derivatives were evaluated to correct baseline shifts and enhance subtle spectral features. Principal Component Analysis (PCA) was subsequently applied to the pre-processed spectral datasets in order to explore the main sources of variability within the samples and to visualize potential clustering patterns. Prior to PCA computation, the spectral variables were mean-centered to ensure that the analysis focused on relative variations among samples.

2.10. Statistical Analysis

The data were analyzed using different statistical software packages. Multivariate statistics computed on autoscaled E-nose data and normalized VOC measurements were performed using Matlab R2013a (MathWorks®, Natick, MA, USA) and the PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA). Specifically, principal component analysis (PCA) was applied to VOCs, polyphenols, and E-nose data, while hierarchical cluster analysis (HCA), performed using Ward's method, was represented as a dendrogram (NIR).

3. Results and Discussion

3.1. Isolation of Autochthonous Yeast Strains

To reactivate and promote the growth of autochthonous yeasts present in the frozen matrix, the must was divided into two aliquots and incubated at two distinct temperatures: 18 °C and 28 °C. The lower temperature (18 °C) approximates the optimal temperature for the fermentation process, whereas the higher temperature (28 °C) corresponds to the optimal growth temperature for *S. cerevisiae*. After 48 hours of incubation, aliquots of the must were plated on YPD and WL agar media to isolate and enumerate predominant yeast morphotypes. As presented in Table 1, the incubation

temperature used to reactivate autochthonous yeasts did not significantly affect the total yeast count. Comparable cell densities, ranging from 0.9 to 1.12×10^{10} CFU/mL, were observed under both conditions (18 °C and 28 °C). These findings suggest that yeast cultivability, which may be influenced by the use of non-optimal temperatures, remains consistent under the conditions tested. Distinct yeast morphotypes were subsequently isolated based on colony morphology and pigmentation on WL chromogenic medium. As presented in Table 2, ten unique yeast morphotypes were identified following the reactivation of the must, with the majority (9 out of 10) originating from the sample reactivated at the suboptimal temperature of 18 °C. These findings underscore the significant impact of temperature on yeast cultivability within the frozen matrix. Reactivation at suboptimal temperatures appears to promote the growth of a more diverse range of yeast strains, thereby aiding in the preservation of the original matrix’s biodiversity. For potential application as starter cultures, it is imperative that the isolated yeasts demonstrate efficient growth at the process temperature of 20–22 °C. Accordingly, in a subsequent experiment (Table 3), the growth capacity of the ten morphotypes was assessed in liquid YPD medium at the refermentation temperature (approximately 22–23 °C). As indicated in Table 3, only six of the ten morphotypes exhibited robust growth, with an absorbance (OD₆₀₀) exceeding 10, at the process temperature. Consequently, further analyses were conducted exclusively on these six morphotypes (A, E, I, L, Lr, and G).

Table 1. Total yeast count (CFU/L) in must incubated at 18 and 28 °C.

Must reactivation temperature (°C)	CFU/mL
18	$0.99 \pm 0.2 \times 10^{10}$
28	$1.12 \pm 0.5 \times 10^{10}$

Table 2. Yeast morphotypes isolated from must samples reactivated at different temperatures.

Morphotype	Must reactivation temperature (°C)	
	18	28
A	+	-
B	+	-
C	+	-
D	+	-
E	+	-
F	+	-
G	-	+
I	+	-
L	+	-
Lr	+	-

Table 3. Cell growth of the 10 isolated morphotypes on YPD medium at 22-23 °C.

Morphotype	Growth on YPD medium
A	+++*
B	+**
C	+
D	+
E	+++
F	+
G	+++
I	+++
L	+++
Lr	+++

*= abundant growth (OD₆₀₀ > 10). **= poor growth (OD₆₀₀ < 5).

3.2. Phenotypic Characterization of the Selected Yeast Strains

In subsequent analyses, the six morphotypes were examined using optical microscopy to assess cell morphology and budding patterns (Figure 1). Concurrently, they were streaked onto lysine medium to differentiate *Saccharomyces* from non-*Saccharomyces*, based on the latter’s ability to grow on media containing lysine as the sole nitrogen source (Table 4). As shown in Table 4, five of the six morphotypes were classified within the *Saccharomyces* group. These findings align with previous studies indicating that, during the fermentation phase, the predominant yeasts are *Saccharomyces* species, such as *S. bayanus* and *S. cerevisiae*, as well as members of the genus *Brettanomyces*, which are known for their high fermentative capacity. The L morphotype, uniquely capable of growth on lysine-containing medium and characterized by colony pigmentation on WL medium and apiculate cell morphology, appears to belong to the genus *Hanseniaspora*. The presence of this apiculate yeast is particularly noteworthy. Commonly referred to as “fruit” yeasts, these organisms are abundant in the must and dominate the early stages of fermentation, substantially contributing to the aromatic profile of the product; however, most do not persist as fermentation progresses. The detection of a *Hanseniaspora* strain in the still cider is therefore significant, as it may serve as a co-inoculum to enhance the aromatic qualities of the fermented product. Subsequently, the six yeast strains were employed as starter cultures in the refermentation of still cider. To evaluate the influence of inoculum composition on the organoleptic and qualitative attributes of the resulting cider, the six yeasts were combined into three mixtures (Table 5), which were used to produce three distinct batches.

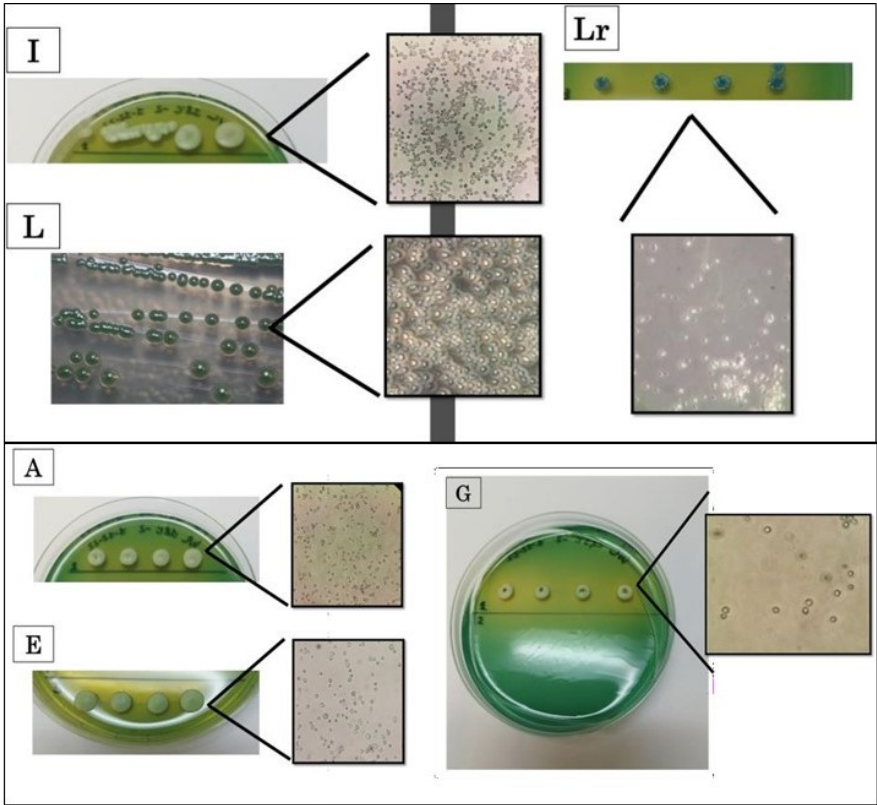


Figure 1. Colony morphology and pigmentation on WL agar medium and cell morphology of the 6 yeast morphotypes selected for refermentation trials.

Table 4. Colony growth of the 6 selected morphotypes on Lysine Agar medium.

Morphotype	Growth on Lysine medium
A	-
E	-

G	-
I	-
L	+
Lr	-

+ = growth. - = no or poor growth.

Table 5. Strain composition of the assembled yeast inocula.

Morphotype	Yeast Mix		
	A	B	C
A	X	-	X
E	X	-	X
G	X	-	X
I	-	X	X
L	-	X	X
Lr	-	X	X

X = presence. - = absence.

3.3. Polyphenol Analysis

Polyphenol monitoring has shown clear differences along fermentation process as well as aging performed with 4 different inocula (Table S1). From still cider to cider after 7 months of refermentation, evident modifications in total procyanidins are already observed. In Ck at T1, a decrease in concentration of about 10% compared to t0 is observed, while in thesis A at T1, the decrease is about 13%, in thesis B about 27%, and finally in thesis C about 37%. The decrease in concentration is also observed in T2: in Ck, from T1 to T2 it was 19%; in thesis A, 29%; and in thesis B and C, 22% and 24%, respectively, compared to T1. Following this decrease in total procyanidins, a lowering in epicatechin content was also detected in all samples, although much more moderate. It dropped from 5.22 mg/L in still cider to 3.85 mg/L in Ck T1, 3.75 mg/L in A T1, 3.51 mg/L in B, and 3.41 mg/L in C at T1. This decrease became more pronounced after 14 months of aging at T2, with Ck at 3.13 mg/L, A reaching the lowest level at 2.90 mg/L, B at 3.51 mg/L, and finally C at 3.38 mg/L. The yeast in the bottle undergoes autolysis, a process in which yeast cells break down and release their intracellular contents, including polysaccharides and mannoproteins [15,16], into the surrounding environment, which can interact with tannins, being a possible cause of modifications in their structure and polymerization process [17]. Aging on lees has been shown to reduce procyanidin concentration in wine. This reduction is due to the adsorption of these compounds by the yeast lees, particularly those with a high degree of polymerization, which can delay polymerization reactions, maintaining low and medium molecular weight tannins in solution [18–20].

Total phenolic acids increased in concentration during the aging phase on the lees. In still cider, a concentration of 6.69 mg/L was measured, which reached 8.14 mg/L in Ck at T1, 7.20 mg/L in A, 7.77 mg/L in B, and 7.71 mg/L in C. At T2, Ck showed a smaller increase, reaching 8.69 mg/L, while the other three theses all reached concentrations of about 10 mg/L. This shows that in Ck, there was a quicker accumulation in the first few months, which then plateaued at T2, while the release of phenolic acids from the other three theses was more pronounced in the last period, after 14 months of aging. Among these acids, the increase in caffeic acid stands out, which in still cider has concentrations of 0.78 mg/L, reaching concentrations of 1.31 mg/L, 1.63 mg/L, and 1.49 mg/L in A, B, and C at T2, respectively. As reported by other authors, this compound tends to increase in concentration during aging on lees in the bottle [21,22], possibly due to the autolysis of yeast and the release of enzymes that were previously cytoplasmic in the yeast cell [23]. Another phenolic acid that showed significant evolution was the 4-hydroxybenzoic acid, which in still cider is at 0.90 mg/L, then increasing by 65% at T2 in Ck and by over 120% in the 3 theses inoculated with selected yeast mixes from the must. This variation is also confirmed by studies carried out on wines aged on lees that reported substantial increases in this benzoic acid concentration [24]. Some studies discuss the

evolution of phenolic acids during aging, showing improved sensory perception over time following aging on lees [25,26].

Resveratrol also underwent a marked change during aging on lees. The starting concentration of this stilbene, highly studied for its health benefits [27–29], in still cider was 0.47 mg/L. The smallest increase at T2 was monitored for ck at 16%, while A and B showed the largest increases at 55% and 51%, respectively, and finally, C increased of 35% compared to T0. Typically, a decrease in this stilbene is observed during wine aging, mainly due to resveratrol adsorption on the mannoproteins released from the yeast, as well as its potential degradation over time [30,31]. In this case, it is plausible that it was released following the second fermentation stage in the bottle due to interactions with the various yeasts employed, as reported by different authors. Figure 2 shows a PCA with all the samples and the influence of phenols to reduce data dimensionality while maintaining the original variability. PC1 explains 68.87% of the total variability, while PC2 explains 20.29%. The cumulative variance for the first two components is 89.16%, and to explain 95% of the total variability, 4 PCs are required. It is possible to observe how the scores for Ck T1 and still cider tend to be placed close to each other in the first quadrant, near phenolic compounds such as epicatechin, ferulic acid, caffeic acid, and procyanidin C1. Other samples at T1 are placed between quadrants 3 and 4, making a single group associated with caffeoyltartaric acid. Ck at T2 is positioned in quadrant 3 associated with gallic acid and 4-hydroxybenzoic acid. Finally, the three theses A, B, and C at T2 are placed in the second quadrant, strongly associated with compounds such as procyanidins of the B class and resveratrol.

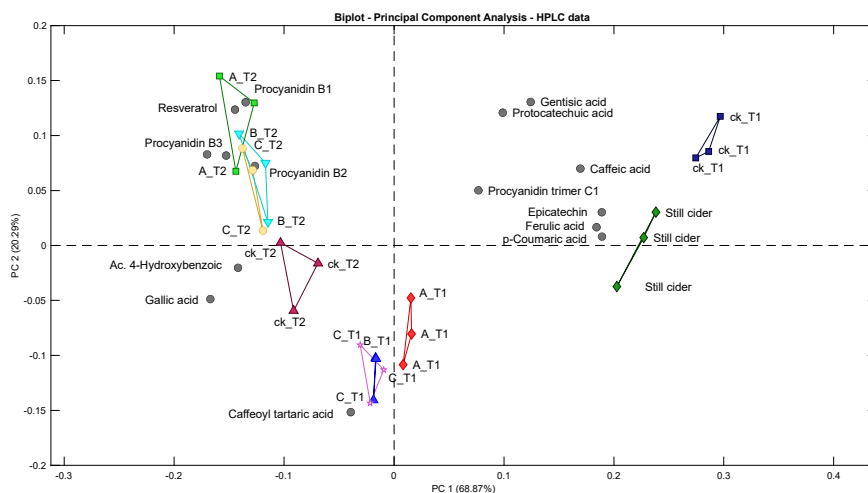


Figure 2. Biplot of PCA (Principal Component Analysis) computed on compound data acquired from High-Performance Liquid Chromatography (HPLC). The data were autoscaled to ensure comparability. The first principal component explains 68.87% of the variance, while the second accounts for 20.29%. In total, four principal components are required to account for more than 95% of the data variance.

3.4. VOCs Analysis

The HS GC-MS detection allowed the identification of 29 compounds belonging to the classes of alcohols, aldehydes, ketones, esters, organic acids, and terpenoids. In Table S2, the relative percentages of the individual compounds detected are shown. The obtained data were also used to compute a PCA model (Figure 3). Using three PCs, the model explains 89.75% of the variability (PC1: 64.75%, PC2: 25%). Four PCs were required to explain 95% of the cumulated variability.

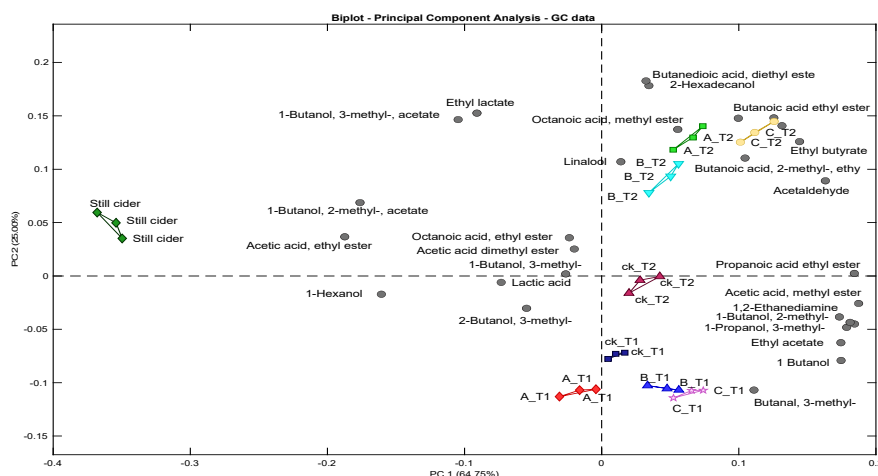


Figure 3. Biplot of PCA (Principal Component Analysis) computed on volatile compound data acquired from Gas Chromatography (GC). The data were autoscaled to ensure comparability. The first principal component explains 64.75% of the variance, while the second accounts for 25%. In total, four principal components are required to account for more than 95% of the data variance.

The still cider is well-separated from the other samples into the 2nd quadrant, demonstrating that the second fermentation strongly influenced the samples' aromatic profile, being significant VOCs related to still cider: 1-butanol-3-methyl acetate, 1-butanol-2-methyl acetate, and ethyl acetate. Treatment A at T1 is well-separated in the 3rd quadrant, associated with 2-butanol-3-methyl, and it looks far from the other samples at T1, which are all grouped in the 4th quadrant. They are correlated with compounds such as butanol-3-methyl, ethyl acetate, 1-propanol-3-methyl, and 1-butanol-2-methyl. The Ck at T2 is the only sample at the second sampling time that falls into the 4th quadrant, associated with compounds like acetic acid methyl ester. On the other hand, the three fermenting samples A, B, and C at T2 are well-separated and distinct in the 1st quadrant, showing that aging for 14 months on yeasts other than the commercial *Saccharomyces cerevisiae* leads to ciders with a unique and characteristic aromatic profile. The compounds most strongly associated with these fermenting processes are octanoic acid, methyl ester, linalool, 2-hexadecanol, ethyl butyrate, butanoic acid 2-methyl-ethyl, and butanedioic acid, diethyl ester. Detected esters and associated with these ciders' samples are likely related to the activity of the enzyme alcohol-O-acetyl/acyltransferase (AATase), which catalyzes the transfer of acyl groups from acyl-CoA to alcohols, forming volatile esters [32]. The activity of this enzyme and the presence of these compounds are related to the overexpression of genes *Atf1* and *Atf2*, which code for these biologically active proteins in the yeasts used for refermentation [33–36], enhancing the presence of fruity aromas in the cider [37,38]. The presence of 2-hexadecanol seems to be linked to the release of fatty acids due to yeast autolysis, which act as precursors, and it appears to increase during bottle aging compared to aging in wood [39].

Another contribution to the aromatic profile comes from non-*Saccharomyces* yeasts. Some studies have reported that co-inoculating a *Saccharomyces* with a non-*Saccharomyces* yeast increases the concentration of linalool by 47% [40]. The presence of this terpene that imparts floral characteristics [41], can also be justified by the overexpression of enzymes in the mevalonate (MVA) pathway, such as HMG-CoA reductase, and the downregulation of competing pathways (e.g., *ERG9*), which can significantly increase linalool yields [42–44].

Some authors report that fermentation by *Hanseniaspora vineae* can increase linalool content, reaching concentrations comparable to those in wines made from aromatic varieties known for their terpenic aromaticity, such as Muscat [45]. Other studies confirm that linalool concentration can initially increase during bottle aging but later decrease, as it is mainly converted into terpin hydrate [46,47], which is consistent with the trend observed in this study during cider aging.

At T1, the wines are characterized by the presence of compounds such as butanol-3-methyl, 1-propanol-3-methyl, and 1-butanol-2-methyl. The detection of these VOCs indicates that, under all tested conditions, the Ehrlich pathway was activated, thereby promoting the catabolism of amino acids such as leucine, isoleucine, and valine to generate higher alcohols. The co-inoculation of non-*Saccharomyces* and *Saccharomyces* yeasts seems to enhance the presence of these compounds [48]. These compounds, after bottle aging at T2, appear to undergo esterification, as confirmed by the proximity of the samples at T2 to various esters, as discussed by various authors. This evolution in the bottle would contribute characteristics recognized as high-quality from an organoleptic perspective, imparting floral and fruity aromas to the ciders [49,50].

3.5. E-nose Computation

The overall aromatic pattern of the ciders was also analyzed using an E-nose based on QCMs. Figure 4 shows the PCA biplot performed on the data derived from the E-nose measurements. The model explains about 96% of the variability on the first two PCs (PC1 66.51% and PC2 31.93%, respectively). The still cider is isolated from the various refermented ciders and is spatially positioned in the 3rd quadrant. The ciders at T1 tend to generate a single cluster between 2nd and 3rd quadrant, with Ck and A samples at T1 look to overlap, showing a suggested similarity in the aromatic fingerprint of the ciders at that time, and approaching QMB 11, functionalized with phosphorus.

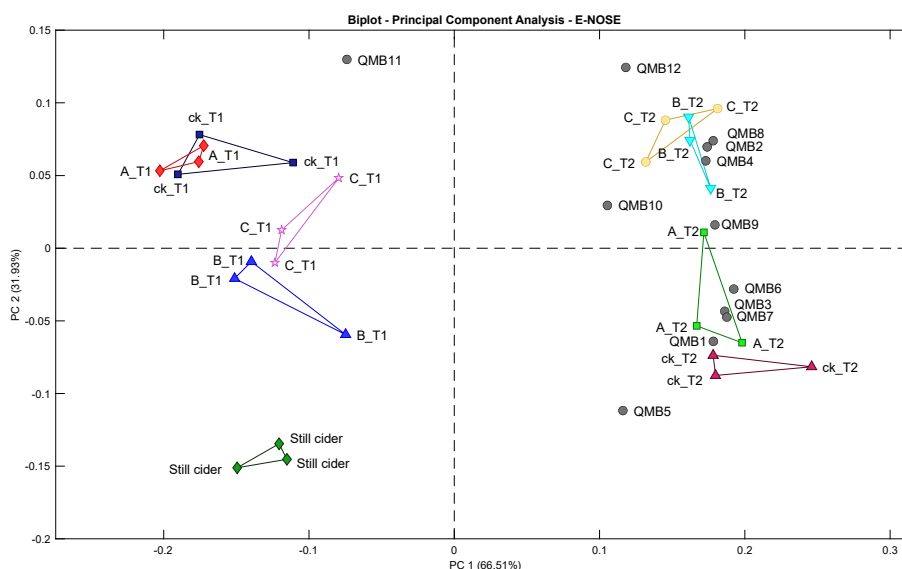


Figure 4. Biplot of PCA (Principal Component Analysis) computed on volatile compound data acquired from the Electronic Nose (E-NOSE). The data were autoscaled to ensure comparability. The first principal component explains 66.51% of the variance, while the second accounts for 31.93%.

At T2, the separation of the different refermented ciders is confirmed, with the Ck and A samples overlapping in the 4th quadrant, along with QMB1, QMB3, QMB5, QMB6, and QMB7. The balance 1 functionalized with Mn mainly interacts with alkanes, while QMB5 and QMB7, functionalized with Co, interact selectively with polar compounds such as ethanol and acetic acid [51,52].

The B and C samples are separated into the 1st quadrant, close to the sensors QMB 2, QMB4, QMB8, QMB9, QMB10, and QMB12. In this case, it looks evident that during the refermentation performed by all 6 selected strains (C treatment), the three strains A, E, and G, also acting in the B treatment, tend to dominate the aromatic pattern, both after 7 months and after 14 months of aging. In this case, QMB 4, functionalized with Zn, mainly binds to aldehydes, within which acetaldehyde

[52], one of the most common in alcoholic fermentations [53], is found near the B and C samples in the GC analysis as well. This molecule can influence the sensory characteristics of the wine, including aroma and taste. Acetaldehyde can impart a fruity aroma, while other aldehydes, such as phenylacetaldehyde, contribute floral notes [54,55]

3.6. NIR Computation

As the final non-destructive evaluation, a dendrogram based on NIR spectra was created (Figure 5) to assess the ability to identify the different samples using only NIR spectroscopy as the detection method. This technology has been used many times to identify wine samples fermented with different inocula [56,57], from different geographic origins [58,59], and with different aging periods [60]. In this case, two large clusters were generated based on spectral responses: one including the still cider, and the other comprising all the refermented ones. The cluster of refermented ciders was further divided into T1 and T2. Only the Ck sample at T1, based on spectral trends, was confused with the T2 samples, implying spectral characteristics similar to those of the C sample at T2.

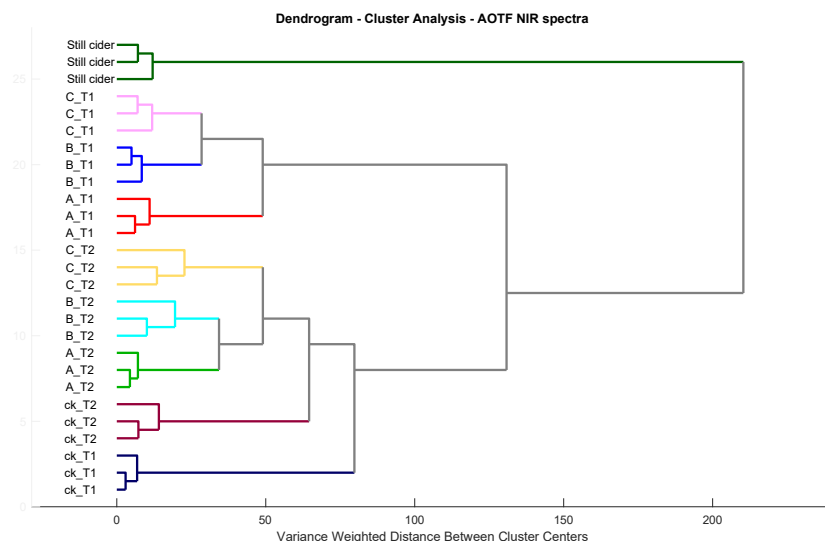


Figure 5. Dendrogram representing the segregation of a cluster analysis performed on spectral data acquired in transmittance using Near-Infrared Acousto-Optic Tunable Filter (NIR-AOTF). The data were subsequently transformed into absorbance ($A=\log(1/T)$) and autoscaled for analysis.

4. Limitation

The most significant limitations fundable in the present work are mainly attributable to the two following issues:

- Microbiological framework included in the experimental trial. In particular, pheno-typic characterization and activity evaluation of yeast strains employed in the different fermenting protocols have been performed based on their morphotyping identification, resulted as *Saccharomyces* and/or non-*Saccharomyces* microorganisms. In terms of response for quality definition of final ciders, this attribution can be considered as satisfactory but a genetic discrimination into the same genus would be desirable for carrying out a deeper investigation in relation to the microbiological aspects.
- In the field of quality definition of elaborated ciders, due to the limited volume available for a valid panel test, a discriminative evaluation of the organoleptic attributes by sensory analysis would be evidently missing. This contribution could be employed both for a direct sensorial

determination as well as for being coupled to non-destructive discrimination performed through E-nose detections.

5. Conclusions

Cider is a beverage strongly influenced by the different inoculation strategies employed for its refermentation. In current study, the microorganisms isolated from the initial must exhibited distinct effects on the inoculated ciders, both in terms of individual polyphenols, volatile organic compounds (VOCs), and the overall aromatic fingerprint monitored using an electronic nose (e-nose). Regarding individual phenols, the ciders inoculated with co-inocula A, B, and C (respectively, 3 *Saccharomyces*, 2 *Saccharomyces* and 1 non-*Saccharomyces*, and finally all six yeasts) showed a significant increase in resveratrol at T2, along with a decrease in procyanidins and epicatechin content, leading to an improvement in the tannic smoothness of the cider. As for phenolic acids, a notable increase in caffeic acid was measured, likely due to the release of enzymes during yeast autolysis at T2. Concerning VOCs, the three ciders refermented with the co-inocula performed in this study were particularly characterized by the synthesis of several esters, such as octanoic acid methyl ester, ethyl butyrate, 2-methyl-ethyl butanoic acid, and diethyl butanedioic acid ester. Additionally, a terpene with a strong aromatic impact, linalool, was also detected. Notably, 2-hexadecanol was found to be associated with the three refermented ciders using the different co-inocula, and it appears to originate from yeast autolysis and the release of fatty acids as precursors for this alcohol. With respect to the aromatic fingerprint monitored by the e-nose, bottle refermentation resulted in a clear separation between samples. The control (Ck) and the cider refermented with co-inoculum A exhibited greater overall similarity, whereas co-inocula B and C clustered together based on their aromatic profiles. Multivariate analysis of NIR spectra from different cider samples allows clear separation from still cider and other fermenting products, all grouped into a single cluster, while no evident segregation was observed across sampling times. In conclusion, the use of microorganisms selected from the original must, preserving a trace of the microbial terroir, appears to be a viable and widely applicable strategy. Future research should focus on a more in-depth characterization of each individual yeast strain isolated from the must and on exploring different co-inoculation strategies to identify the most synergistic combinations to enhance the organoleptic properties of the final product.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: HPLC-DAD analysis aimed at detecting individual phenolic monomers in still cider, and Ck, A, B, and C ciders respectively measured at first (T1) and second (T2) time of fermentation processes. Concentrations of detected phenols are expressed in mg/L $\pm$ standard deviation (sd).; Table S2: Gas-chromatographic analysis, performed through HS GC-MS technique, addressed to monitor volatile organic compounds (VOCs) in still cider, and Ck, A, B, and C ciders respectively measured at first (T1) and second (T2) time of fermentation processes. Data referred to each VOC are expressed as relative % computed on the area of chromatographic peak normalized to the sum of the areas. The measurements were performed in triplicate.

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References

1. Makwana, M., & Hati, S. (2019). Fermented beverages and their health benefits. Fermented beverages, 1-29. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
2. Brugnoli, M., Cantadori, E., Arena, M. P., De Vero, L., Colonello, A., & Gullo, M. (2023). Zero-and Low-Alcohol Fermented Beverages: A Perspective for Non-Conventional Healthy and Sustainable Production from Red Fruits. *Fermentation*, 9(5), 457..
3. Anagnostopoulos, D.A.; Tsaltas, D. Chapter 10–Fermented Foods and Beverages; Woodhead Publishing: Cambridge, UK, 2019.
4. Guiné, R. P., Barroca, M. J., Coldea, T. E., Bartkiene, E., & Anjos, O. (2021). Apple fermented products: An overview of technology, properties and health effects. *Processes*, 9(2), 223.
5. Brum, J. P., Jacobs, B., Ligabue, M. B., Borghi, S. B., Alves, G. B., Kammer, D. D. C., ... & Jacobs, S. A. (2024). Free and encapsulated yeasts for the production of sparkling wines. *OBSERVATÓRIO DE LA ECONOMÍA LATINOAMERICANA*, 22(4), e4410-e4410.
6. Fornairon-Bonnefond, C., Camarasa, C., Moutounet, M., & Salmon, J. M. (2002). New trends on yeast autolysis and wine ageing on lees: A bibliographic review. *OENO one*, 36(2), 49-69.
7. Barcenilla, J. M., Martín-Álvarez, P. J., Vian, A., & Gonzalez, R. (2003). Note: characterization of yeast strains on the basis of autolytic activity and volatile compounds. *Food science and technology international*, 9(2), 95-99.
8. Comuzzo, P., & Zironi, R. (2013). Biotechnological strategies for controlling wine oxidation. *Food Engineering Reviews*, 5, 217-229.
9. Ritchey JG and Waterhouse AL, A standard red wine: monomeric phenolic analysis of commercial cabernet sauvignon wines. *Am J Enol Vitic* 50: 91–100 (1999).
10. Santonico E, Bellincontro A, De Santis D, Di Natale C and Mencarelli F, Electronic nose to study postharvest dehydration of wine grapes. *Food Chem* 121: 789–796 (2010)
11. Alfieri, G., Riggi, R., Modesti, M., Annesi, B., Mencarelli, F., De Santis, D., & Bellincontro, A. (2026). A first characterisation and classification of Montalcino wines in relation to production areas: combination of destructive, non-destructive and chemometrics analyses. *OENO One*, 60(1).
12. Riggi, R., Modesti, M., Alfieri, G., Esposito, G., Cucchiara, P., Ferri, S., ... & Bellincontro, A. (2025). Integration of Destructive and Non-Destructive Analytical Determinations for Evaluating Quality of Fresh and Roasted Hazelnuts Subjected to Different Processing Temperatures. *Food Science & Nutrition*, 13(3), e70095.
13. Bellincontro, A., Cozzolino, D., & Mencarelli, F. (2011). Application of NIR-AOTF spectroscopy to monitor Aleatico grape dehydration for Passito wine production. *American journal of enology and viticulture*, 62(2), 256-260.
14. Modesti, M., Alfieri, G., Chieffo, C., Mencarelli, F., Vannini, A., Catalani, A., ... & Bellincontro, A. (2024). Destructive and non-destructive early detection of postharvest noble rot (*Botrytis cinerea*) in wine grapes aimed at producing high-quality wines. *Journal of the Science of Food and Agriculture*, 104(4), 2314-2325.
15. Alexandre, H., & GUILLOUX-BENATIER, M. I. C. H. È. L. E. (2006). Yeast autolysis in sparkling wine—a review. *Australian journal of grape and wine research*, 12(2), 119-127.
16. Li, X., Shi, X., Zou, M., Luo, Y., Tan, Y., Wu, Y., ... & Li, P. (2015). Characteristics of morphological and physiological changes during the autolysis process of *Saccharomyces cerevisiae* FX-2. *Microbiology and Biotechnology Letters*, 43(3), 249-258.

17. Mazaauric, J. P., & Salmon, J. M. (2005). Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the resulting wines. *Journal of Agricultural and Food Chemistry*, 53(14), 5647-5653.
18. Rodrigues, A., Ricardo-Da-Silva, J. M., Lucas, C., & Laureano, O. (2013). Effect of winery yeast lees on touriga nacional red wine color and tannin evolution. *American journal of enology and viticulture*, 64(1), 98-109.
19. Rodríguez, M., Lezáun, J., Canals, R., Llaudy, M. C., Canals, J. M., & Zamora, F. (2005). Influence of the presence of the lees during oak ageing on colour and phenolic compounds composition of red wine. *Food science and technology international*, 11(4), 289-295.
20. Leskó, A., Kállay, M., Nyúl-Pühra, B., & Nyitrai-Sárdy, D. (2011). The change of polyphenolic composition and tyrosol content of the wine as an effect of sur lie method. *Acta Alimentaria*, 40(Supplement-1), 79-90.
21. Sartor, S., Toaldo, I. M., Panceri, C. P., Caliar, V., Luna, A. S., de Gois, J. S., & Bordignon-Luiz, M. T. (2019). Changes in organic acids, polyphenolic and elemental composition of rosé sparkling wines treated with mannoproteins during over-lees aging. *Food Research International*, 124, 34-42.
22. Sartor, S., Burin, V. M., Panceri, C. P., Dos Passos, R. R., Caliar, V., & Bordignon-Luiz, M. T. (2018). Rosé sparkling wines: influence of winemaking practices on the phytochemical polyphenol during aging on lees and commercial storage. *Journal of food science*, 83(11), 2790-2801.
23. Ibern-Gómez, M., Andrés-Lacueva, C., Lamuela-Raventós, R. M., Buxaderas, S., Singleton, V. L., & De La Torre-Boronat, M. C. (2000). Browning of cava (sparkling wine) during aging in contact with lees due to the phenolic composition. *American journal of enology and viticulture*, 51(1), 29-36.
24. Karathanos, V. T., Syrimbei, C., Chiou, A., Karathanos, A., & Makris, D. P. (2008). Evolution of benzoate derivatives and their hydroxycinnamate analogues during ageing of white wines in oak barrels. *Journal of Food Composition and Analysis*, 21(8), 667-671.
25. Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Williams, P., & Doco, T. (2011). Effect of aging on lees and of three different dry yeast derivative products on Verdejo white wine composition and sensorial characteristics. *Journal of Agricultural and Food Chemistry*, 59(23), 12433-12442.
26. Rinaldi, A., Coppola, M., & Moio, L. (2019). Aging of Aglianico and Sangiovese wine on mannoproteins: Effect on astringency and colour. *Lwt*, 105, 233-241.
27. Ko, J. H., Sethi, G., Um, J. Y., Shanmugam, M. K., Arfuso, F., Kumar, A. P., ... & Ahn, K. S. (2017). The role of resveratrol in cancer therapy. *International journal of molecular sciences*, 18(12), 2589.
28. Penumathsa, S. V., & Maulik, N. (2009). Resveratrol: a promising agent in promoting cardioprotection against coronary heart disease. *Canadian journal of physiology and pharmacology*, 87(4), 275-286.
29. Sawda, C., Moussa, C., & Turner, R. S. (2017). Resveratrol for Alzheimer's disease. *Annals of the New York Academy of Sciences*, 1403(1), 142-149.
30. Jeandet, P., Chaudruc, D., Robillard, B., Peters, F., Tusseau, D., Conreux, A., & Duteurtre, B. (2006). Determination of the trans-resveratrol content of Champagne wines by reversed-phase HPLC. *OENO One*, 40(2), 117-119.
31. Naiker, M., Anderson, S., Johnson, J. B., Mani, J. S., Wakeling, L., & Bowry, V. (2020). Loss of trans-resveratrol during storage and ageing of red wines. *Australian Journal of Grape and Wine Research*, 26(4), 385-387.
32. Zhang, J., Zhang, C., Qi, Y., Dai, L., Ma, H., Guo, X., & Xiao, D. (2014). Acetate ester production by Chinese yellow rice wine yeast overexpressing the alcohol acetyltransferase-encoding gene ATF2. *Genet Mol Res*, 13(4), 9735-9746.
33. Cordente, A. G., Swiegers, J. H., Hegardt, F. G., & Pretorius, I. S. (2007). Modulating aroma compounds during wine fermentation by manipulating carnitine acetyltransferases in *Saccharomyces cerevisiae*. *FEMS microbiology letters*, 267(2), 159-166.
34. Nancolas, B., Bull, I. D., Stenner, R., Dufour, V., & Curnow, P. (2017). *Saccharomyces cerevisiae* Atf1p is an alcohol acetyltransferase and a thioesterase in vitro. *Yeast*, 34(6), 239-251.
35. Steyer, D., Erny, C., Claudel, P., Riveill, G., Karst, F., & Legras, J. L. (2013). Genetic analysis of geraniol metabolism during fermentation. *Food microbiology*, 33(2), 228-234.

36. Tapia, S. M., Pérez-Torrado, R., Adam, A. C., Macías, L. G., Barrio, E., & Querol, A. (2022). Functional divergence in the proteins encoded by ARO80 from *S. uvarum*, *S. kudriavzevii* and *S. cerevisiae* explain differences in the aroma production during wine fermentation. *Microbial Biotechnology*, 15(8), 2281-2291.
37. Ma, Y., Li, T., Xu, X., Ji, Y., Jiang, X., Shi, X., & Wang, B. (2021). Investigation of volatile compounds, microbial succession, and their relation during spontaneous fermentation of Petit Manseng. *Frontiers in Microbiology*, 12, 717387.
38. Lambrechts, M. G., & Pretorius, I. S. (2000). Yeast and its importance to wine aroma.
39. Ancín-Azpilicueta, C., González-Marco, A., & Jiménez-Moreno, N. (2009). Evolution of esters in aged Chardonnay wines obtained with different vinification methods. *Journal of the Science of Food and Agriculture*, 89(14), 2446-2451.
40. Lee, S. B., & Park, H. D. (2020). Isolation and investigation of potential non-Saccharomyces yeasts to improve the volatile terpene compounds in Korean Muscat Bailey A wine. *Microorganisms*, 8(10), 1552.
41. Noble, A. C. (1994). Wine flavour. In *Understanding natural flaVors* (pp. 228-242). Boston, MA: Springer US.
42. Rico, J., Pardo, E., & Orejas, M. (2010). Enhanced production of a plant monoterpene by overexpression of the 3-hydroxy-3-methylglutaryl coenzyme A reductase catalytic domain in *Saccharomyces cerevisiae*. *Applied and environmental microbiology*, 76(19), 6449-6454.
43. Zhang, Y., Wang, J., Cao, X., Liu, W., Yu, H., & Ye, L. (2020). High-level production of linalool by engineered *Saccharomyces cerevisiae* harboring dual mevalonate pathways in mitochondria and cytoplasm. *Enzyme and microbial technology*, 134, 109462.
44. Zhou, P., Du, Y., Xu, N., Yue, C., & Ye, L. (2020). Improved linalool production in *Saccharomyces cerevisiae* by combining directed evolution of linalool synthase and overexpression of the complete mevalonate pathway. *Biochemical Engineering Journal*, 161, 107655.
45. Del Fresno, J. M., Escott, C., Loira, I., Carrau, F., Cuerda, R., Schneider, R., ... & Morata, A. (2021). The impact of *Hanseniaspora vineae* fermentation and ageing on lees on the terpenic aromatic profile of white wines of the Albillo variety. *International Journal of Molecular Sciences*, 22(4), 2195.
46. Cordonnier, R. and Bayonove, C., Mise en evidence dans la baie la raisin var Muscat d'Alexandria de monoterpenes lies revelables pour une ou plusieurs enzymes du fruit. *C.R. Acad. Sci. Ser. D*, 1974, 278, 3387–3390
47. Simpson, R., Aroma and compositional changes in wine with oxidation, storage and aging. *Vitis*, 1978, 17, 274–287.
48. Kim, D. H., Hong, Y. A., & Park, H. D. (2008). Co-fermentation of grape must by *Issatchenkia orientalis* and *Saccharomyces cerevisiae* reduces the malic acid content in wine. *Biotechnology Letters*, 30, 1633-1638.
49. Ancín-Azpilicueta, C., González-Marco, A., & Jiménez-Moreno, N. (2009). Evolution of esters in aged Chardonnay wines obtained with different vinification methods. *Journal of the Science of Food and Agriculture*, 89(14), 2446-2451.
50. Zhang, D., Wei, Z., Han, Y., Duan, Y., Shi, B., & Ma, W. (2023). A review on wine flavour profiles altered by bottle aging. *Molecules*, 28(18), 6522
51. Marques, C., Dinis, L. T., Modesti, M., Bellincontro, A., Correia, E., & Vilela, A. (2024). Exploring the influence of terroir on douro white and red wines characteristics: A study of human perception and electronic analysis. *European Food Research and Technology*, 250(12), 3011-3027.
52. Capuano, R., Paba, E., Mansi, A., Marcelloni, A. M., Chiominto, A., Proietto, A. R., ... & Di Natale, C. (2020). *Aspergillus* species discrimination using a gas sensor array. *Sensors*, 20(14), 4004.
53. Yoncheva, T. R., Spasov, H., & Kostov, G. (2021). THE SYNTHESIS OF CARBONYL COMPOUNDS FROM *SACCHAROMYCES CEREVISIAE* STRAINS IN CABERNET SAUVIGNON WINES. *Journal of microbiology, biotechnology and food*
54. Yao, X. C., Zhang, H. L., Ma, X. R., Xia, N. Y., Duan, C. Q., Yang, W. M., & Pan, Q. H. (2024). Leaching and evolution of anthocyanins and aroma compounds during Cabernet Sauvignon wine fermentation with whole-process skin-seed contact. *Food Chemistry*, 436, 137727.
55. Prusova, B., Humaj, J., Sochor, J., & Baron, M. (2022). Formation, losses, preservation and recovery of aroma compounds in the winemaking process. *Fermentation*, 8(3), 93.

56. Cozzolino, D., McCarthy, J., & Bartowsky, E. (2012). Comparison of near infrared and mid infrared spectroscopy to discriminate between wines produced by different *Oenococcus Oeni* strains after malolactic fermentation: A feasibility study. *Food Control*, 26(1), 81-87.
57. Marsico, A. D., Perniola, R., Cardone, M. F., Velenosi, M., Antonacci, D., Alba, V., & Basile, T. (2018). Study of the influence of different yeast strains on red wine fermentation with NIR spectroscopy and principal component analysis. *J*, 1(1), 133-147.
58. Yu, H., Zhou, Y., Fu, X., Xie, L., & Ying, Y. (2007). Discrimination between Chinese rice wines of different geographical origins by NIRS and AAS. *European Food Research and Technology*, 225, 313-320.
59. Hu, X. Z., Liu, S. Q., Li, X. H., Wang, C. X., Ni, X. L., Liu, X., ... & Xu, C. H. (2019). Geographical origin traceability of Cabernet Sauvignon wines based on Infrared fingerprint technology combined with chemometrics. *Scientific reports*, 9(1), 8256.
60. Pereira, A. C., Reis, M. S., Saraiva, P. M., & Marques, J. C. (2011). Development of a fast and reliable method for long-and short-term wine age prediction. *Talanta*, 86, 293-304.

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