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Impact of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts to improve traditional sparkling wines production

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ABSTRACT

In this study the effect of a co-inoculum of *S. cerevisiae* (F6789) with *Torulaspora delbrueckii* (TB1) or *Starmerella bacillaris* (SB48) on the oenological and aroma characteristics of sparkling wines obtained with the *Champenoise* method was investigated. The autolytic outcome and the sensory profile of sparkling wines were also evaluated. The secondary fermentations were completed by all mixed and single starter cultures with the only exception of those guided by *Starm. bacillaris*. Sparkling wines produced with *S. cerevisiae* F6789+*Starm. bacillaris* SB48 showed the highest amounts of glycerol (6.51 g/L). The best autolytic potential was observed in sparkling wines produced with +*Starm. bacillaris* (81.98 mg leucin/L) and *S. cerevisiae*+*T. delbrueckii* (79.03 mg leucin/L). The lowest value was observed for sparkling wines obtained with *S. cerevisiae* F6789 (53.96 mg leucin/L). Sparkling wines showed different aroma and sensory profiles. Esters were mainly present in sparkling wines obtained with *S. cerevisiae* F6789 (88.09 mg/L) followed by those obtained with *S. cerevisiae*+*T. delbrueckii* (87.20 mg/L), *S. cerevisiae* +*Starm. bacillaris* (81.93 mg/L). The content of esters decreased over time, and that might be related to the adsorption on lees and chemical hydrolysis. The highest concentrations of higher alcohols were found in sparkling wines produced with *S. cerevisiae*+*T. delbrueckii* (27.50 mg/L). Sparkling wines obtained with *S. cerevisiae* +*Starm. bacillaris* were well differentiated from the others due to their high score for the descriptor for spicy, bread crust, freshness and floral.

Tailored strains with different autolytic potential might represent an interesting strategy to improve traditional sparkling wine production and favour their differentiation.

1. Introduction

In 2018 the world sparkling wine production reached for the first time 20 mhl, with an overall increase of +57% since 2002 (i.e. +3% per year on average). The global sparkling wines market size was valued at $33.9 billion in 2019, and is anticipated to reach $51.7 billion by 2027 (OIV, 2020 available on line https://www.oiv.int/public/medias/7291/oiv-sparkling-focus-2020.pdf). The *méthode Champenoise* is one of the most famous ways to produce sparkling wine and is generally known as traditional method. It is based on the second fermentation of a base wine with the addition of a *liqueur de tirage* (i.e., wine, sucrose, ethanol-adapted yeast cells, nutrients, and a clarifying agent), in characteristic bottles (Buxaderas and López-Tamames, 2012; Torresi et al., 2011). Inside the bottles yeasts consume sugar producing ethanol and carbon dioxide (about six standard atmospheres) that results in effervescence. Secondary fermentation is generally complete in two to three months after which the aging period in contact with yeast lees starts. This step is considered necessary to develop sensorial complexity (Alexandre and Guilloux-Benatier, 2006; Gnoinski et al., 2021; Todd et al., 2000). The main process occurring during the aging is yeast autolysis which start 2–4 months of secondary fermentation (Lambert-Royo et al., 2022; Perpetuini et al., 2016). This process is favoured by the pressure, alcohol concentration, low pH (3.0–3.5) and low temperature (15 °C) (Gnoinski et al., 2021; Sawyer et al., 2022).

Yeast autolysis positively impacts sparkling wine flavour, composition, and texture through the release of yeast derived compounds

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including amino acids, peptides, mannoproteins, polysaccharides, fatty acids, nucleotides (Charpentier et al., 2005; Martínez-Lapuente et al., 2013). However, yeast autolysis is a very slow process. Yeast proteases favour lysosomal and cytoplasmic membranes hydrolysis increasing cell wall porosity facilitating the release of degraded constituents into the wine. The slow rate of enzymatic activity delays the autolytic process and consequently sparkling wines are left in contact with lees for several months or years to benefit from positive autolytic effects (Alexandre and Guilloux-Benatier, 2006; Gnoinski et al., 2021). Several studies focused on the acceleration of yeast autolysis. The main studies suggested the selection of yeast strains with a good autolytic potential, the combination of killer toxin positive and negative strains, implement tirage solution with spent lees (Perpetuini et al., 2016; Lombardi et al., 2015; Todd et al., 2000; Tofalo et al., 2016; Di Gianvito et al., 2018; La Gatta et al., 2016). Another option could be the combination of yeast strains with different autolytic ability (Raymond Eder and Rosa, 2021). In this sense a possibility could be the exploitation of non-*Saccharomyces* yeasts in combination with *Saccharomyces cerevisiae* strains (Ivit and Kemp, 2018). The interest in non-*Saccharomyces* yeasts for sparkling wine production has increased in recent years in order to improve aroma complexity and differentiate the final products. Actually, very few data are available about the effect of non-*Saccharomyces* on sparkling wines production (Canonico et al., 2018; González-Royo et al., 2015; Ivit et al., 2018; Medina-Trujillo et al., 2017). The majority have been focused on a limited number of non-*Saccharomyces* species including: *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Schizosaccharomyces pombe* and *Saccharomycodes ludwigii* and differences in amino acids, ammonia, volatile aroma compounds, glycerol and proteins, which impact sparkling wine flavor and foaming ability, have been reported (for a review see Ivit and Kemp, 2018). Interesting oenological properties have been described for *T. delbrueckii* and its presence has been reported to increase the presence of some volatile compounds because of its higher β-glucosidase activity and its polysaccharide production capacity (Azzolini et al., 2012; Bely et al., 2008; Benito, 2018). Another non--*Saccharomyces* yeast which is attracting attention from the wine industry is *Starmerella bacillaris* (syn. *Candida zemplinina*). It shows peculiar oenological traits such as high glycerol production, reduced concentration of acetic acid in combination with *S. cerevisiae*, poor ethanol yield from sugars (glucose and fructose), improvement of aroma complexity, ability to metabolize malic acid, toleration of low temperatures, ability to grow at high sugar concentrations and fructophilic character (Raymond Eder and Rosa, 2021b; Russo et al., 2020). Therefore, the purpose of this study was to investigate the effect of a co-inoculum of *S. cerevisiae* with *T. delbrueckii* or *Starm. bacillaris* on the physical-chemical characteristics that might occur during secondary fermentation and over-lees aging of traditional sparkling wines. The evolution of aroma compounds was investigated, as well as the sensory profile of final products. Autolytic outcome was also monitored during aging.

2. Materials and methods

2.1. Yeast strains and culture conditions

The yeast strains used in this study are *S. cerevisiae* F6789, *T. delbrueckii* TB1 and, *Starm. bacillaris* SB48 and belong to the Wine Culture Collection of the Microbial Biotechnology Laboratory – Faculty of BioScience and Technology for Food, Agriculture, and Environment (University of Teramo, Italy). *S. cerevisiae* F6789 has been characterized in previous studies for its flocculent and autolytic capacity, and ability to release aroma compounds (Di Gianvito et al., 2018; Perpetuini et al., 2016, 2021; Perpetuini et al., 2021a,b; Tofalo et al., 2016). Pulverulent non-*Saccharomyces* strains were previously isolated from Montepulciano d'Abruzzo organic must and selected for oenological characteristics, including autolytic ability, resistance to SO_2 and pressure.

The strains were routinely grown on YPD medium (1% w/v yeast extract, 2% w/v peptone, and 2% w/v glucose, all components obtained from Oxoid, Milan, Italy) supplemented or not with 2% agar (w/v) under aerobic conditions at 28 °C for 48 h. Strains were stored at −80 °C in YPD broth supplemented with glycerol (Sigma-Aldrich, Milan, Italy) at final concentration of 20% v/v or on YPD agar at 4 °C for short-term storage.

2.2. Sparkling wine production

Sparkling wines were produced according to the traditional method in 750 mL bottles using a base wine (Trebbiano 85% and Pecorino 15%) provided by VIN.CO cooperative (Ortona, Italy) which comprise 14 wineries producing high quality sparkling wines located in Abruzzo region.

The base wine had the following main analytical composition: 9.15% v/v alcohol; 3.2 pH; 0.15 g/L volatile acidity; 6.48 g/L total acidity; residual sugars <2 g/L. The bottles were filled with the pasteurized base wine, sucrose (final concentration 24 g/L) and the co-adjuvant Oenoactiv tirage (Oenoitalia group s. r.l, Brescia, Italy) at a concentration of 10 g/hL. The starter strains were pre-adapted in the medium described by Penacho et al. (2012) for 10 days and then inoculated at a final concentration of 6 Log CFU/mL. The yeast cell numbers were estimated by plate count. *S. cerevisiae* and non-*Saccharomyces* were used both in pure cultures and mixed cultures (inoculum ratio: 1:1). Secondary fermentation was performed at 15 °C. Five different trials were performed (Table 1) and 3 bottles for each trial were considered and plugged with an aphrometer (Oenoitalia group S. r.l.), to follow the evolution of overpressure during the bottle fermentation phase. The results obtained were normalized by the temperature using Henry's law constant and values were expressed as pressure (bar). All analyses were performed in triplicate at different times (3, 6 and 9 months).

2.3. Oenological parameters of sparkling wines

The main oenological parameters (ethanol, total acidity, titratable acidity, residual sugars) were determined with a FOSS WineScan™ FT120 rapid scanning Fourier Transform Infrared Spectroscopy with FOSS WineScan software version 2.2.1 according to manufacturer's instructions, which was previously calibrated with wine samples analyzed in accordance with official OIV methods. pH was measured using a pH meter (XS Instrument, Modena, Italy). The ninhydrin-cadmium assay was applied to determine the free ammino-nitrogen (AAN) according to Folkertsma and Fox (1992). Results were expressed as g leucine in 100 mL of sparkling wine. Analyses were performed in triplicate.

2.4. Viable counts

Cell viability was checked at different times (3, 6, and 9 months) with methylene blue staining according to Redón et al. (2008). The cell count was carried out using a Bürker counting chamber and an optical microscopy (Olympus BX60, Olympus Optical Co Ltd, Tokyo, Japan).

2.5. Aroma compound analysis

Volatile organic compounds (VOCs) were determined by solid phase microextraction coupled with gas-chromatography (GC-MS-SPME)

Table 1
Secondary fermentation trials performed in this study.

Fermentation trials	Inoculum
SW1	*S. cerevisiae* (F6789)
SW2	*Starm. bacillaris* (SB48)
SW3	*T. delbrueckii* (TB1)
SW4	*Starm. bacillaris* (SB48)+*S. cerevisiae* (F6789)
SW5	*T. delbrueckii* (TB1)+*S. cerevisiae* (F6789)

using a GC-mass spectrometer Clarus SQ8S chromatography/mass (GC-MS) spectrometry (PerkinElmer, Boston, MA) as previously described (Perpetuini et al., 2021a,b). VOCs were identified by comparing the retention time of pure compounds (Sigma-Aldrich) analyzed in the same conditions. 2-Methyl-exanol was used as internal standard (0.1 M). The comparison of MS fragmentation patterns with those present in the National Institute for Standards and Technology database (NIST version 2005) was adopted to achieve a tentative identification and confirmed by retention index (RIndex). All determinations were performed in triplicate.

2.6. Gas sensors array analysis

The samples analysis was carried out using E-nose UTV equipped with 12 Quartz Crystal Microbalance (QCM, 20 MHz) sensor array. 6 QCM was functionalized different pentapeptides (IHRIC, KSDSC, LAWHC, LGFDC, TGKFC and WHVSC), and 6 QCM was functionalized different sequences of hairpin DNA (hpDNA) (CCGATTT, CCCTGTC, AATCAGC, GTCCCTA, GCGAAGG, GTCCGTT). The selection of the pentapeptide and hpDNA sequences were carried out by virtual screening and were reported in previous works (Gaggiotti et al., 2021; Mascini et al., 2018, 2019). Analysis of sparkling wine was carried out using 1 mL of samples in glass lab bottles (10 mL). Measurements were made on sample storage at different time. Sparkling wine were kept for 10 min before starting the measurement to enrich the headspace of volatile compounds. The headspace was then assayed by the E-nose for 2 min. The signal obtained was expressed in terms of Frequency shift (ΔF in Hz) that represented the difference between the frequency value at the beginning of the measurement and the lowest value reached by the sensor during the measurement.

2.7. Sensory analysis

Sparkling wines were riddled and disgorged by the trained staff of VIN.CO cooperative before sensory analysis. Sensory tests were performed at room temperature (20 °C) by a group of 10 trained tasters on the basis of 8 descriptors (fruity, floral, minerality, herbaceous, spicy, bread crust, bitterness, freshness) using a scale from 1 to 10 (ISO, 85864). Each session was conducted in individual booths under white light. Each sparkling wine was opened immediately prior to pouring, and only when panelists were already seated in the evaluation booths. Sparkling wines were presented to the panellists in a randomised order, with each panellist receiving wine poured from the same bottle. In particular, randomized samples of 25–30 mL at 6–8 °C were served in clear glasses marked with random numbers. Unsalted crackers and water were provided as palate cleaners.

2.8. Statistical analysis

Prism 7.0 program (GraphPad Software Inc, La Jolla, CA, USA) was used to analyze data. Results were expressed as mean value ± standard deviation. ANOVA test was performed using XLStat 2014 software (Addinsoft, New York, USA). ANOVA was applied to identify the significant differences among oenological parameters, VOCs, and sensory descriptors and a Bonferroni correction was applied. Hence, the significant results reported were Bonferroni corrected. A level of $p < 0.05$ was considered statistically significant. Principal component analysis (PCA) was performed to investigate the relationship among strains and VOCs released after 9 months of aging. PCA analysis was also used for a multivariate data set from electronic nose sensors array. The analysis was performed using XLStat 2014 software. Dataset of gas sensors array was auto scaled (zero mean and unitary variance) before statistical procedures.

3. Results and discussion

3.1. Fermentation kinetics and oenological parameters

The secondary fermentation kinetics of studied strains are shown in Fig. 1. *S. cerevisiae* pure culture showed the fastest fermentation kinetics and reached the highest values of pressure (5.2 bar). Mixed fermentations were characterized by a slower rate. The secondary fermentation driven by *S. cerevisiae*+*T. delbrueckii* started after 14 days, while that guided by *S. cerevisiae*+*Starm. bacillaris* after 20 days. However, bar pressures of 5.1 bar were reached after different times. The evolution and the final pressure values indicated that *T. delbrueckii* brought forward and completed the secondary fermentation with a final pressure of 4.5 bar. On the contrary, *Starm. bacillaris* pure culture did not complete the second fermentation and only 2 bar were reached inside the bottle. Canonico et al. (2018) observed that selected *T. delbrueckii* strains completed the secondary fermentation reporting a pressure of about 6 bar and observed a slow fermentation kinetics until day 8, and then an increase of the fermentation rate. In this study the lag phase lasted about 20 days, probably because of the strain variability in terms of fermentative capacity. The strain *Starm. bacillaris* was not able to perform the secondary fermentation, probably because it was not able to face the stressing conditions characterizing sparkling wine production.

The oenological parameters of sparkling wines are reported in Table 2. Sparkling wines obtained with *S. cerevisiae* showed the highest ethanol content 10.54% v/v in agreement with the fermentation ability of this strain and the fermentation kinetic (Perpetuini et al., 2021a,b; Di Gianvito et al., 2018). Mixed fermentation allowed to obtain sparkling wines with similar characteristics to those obtained with *S. cerevisiae*. The ethanol content was about 10.5% v/v and a content of residual sugars about 1 g/L was observed in both cases.

As previously observed for still wine, the good metabolic compatibility between *Starm. bacillaris* and *S. cerevisiae* species was confirmed also during the secondary fermentation of sparkling wines. However, it should be underlined that *Starm. bacillaris* and *S. cerevisiae* compatibility could be strain-specific more than species-specific (Raymond Eder and Rosa, 2021).

The positive interaction between *S. cerevisiae* and *T. delbrueckii* is quite interesting, since these 2 species could also establish negative relationships probably due to nutrients competition and a cell-to-cell contact mechanism (Taillandier et al., 2014). Therefore, the choice of tailored *T. delbrueckii* and *S. cerevisiae* strains should be optimized before industrial use. Sparkling wines obtained with *S. cerevisiae*+*T. delbrueckii* showed an ethanol content of 10.51% v/v. Sparkling wines obtained with *T. delbrueckii* had 10.43% v/v and 2.05 g/L of ethanol concentration and residual sugars, respectively.

Canonico et al. (2018) tested 2 strains of *T. delbrueckii* (Td130 and Td313) for the secondary fermentation and the resulting sparkling wines showed 11.65% v/v of ethanol. The little differences observed between our results and those obtained by Canonico et al. (2018) might be a

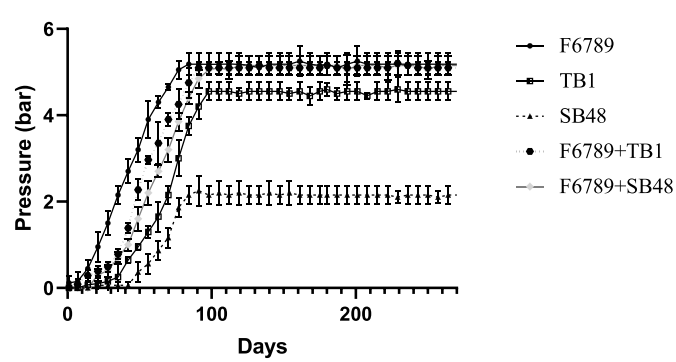

Fig. 1. Secondary fermentation kinetics of tested strains during 270 days.

Table 2
Main oenological parameters of the sparkling wines produced by the pure and mixed fermentations. Different letters in the same column indicate significant differences ($p < 0.05$) according to ANOVA.

Strain	Alcohol (% v/v)	Residual sugars (g/L)	pH	Volatile acidity (g acetic acid/L)	Glycerol (g/L)
F6789	10.54 ± 0.72^{AB}	0.8 ± 0.02^{A}	3.35 ± 0,01^{A}	0.25 ± 0.03 ^{A}	4.76 ± 0.21^{B}
TB1	10.53 ± 0.34^{A}	2.05 ± 0.2^{C}	3.34 ± 0.01 ^{A}	0.26 ± 0.03 ^{A}	4.72 ± 0.22 ^{B}
SB48	9.65 ± 0.23^{B}	15.64 ± 0.10^{D}	3.29 ± 0.03 ^{A}	0.23 ± 0.05 ^{A}	4.35 ± 0.23 ^{A}
F6789 + SB48	10.43 ± 0.45^{AB}	1.09 ± 0.4^{B}	3.34 ± 0.02 ^{A}	0.27 ± 0.04 ^{A}	6.51 ± 0.05^{C}
F6789 + TB1	10.51 ± 0.51^{A}	1.18 ± 0.03^{B}	3.30 ± 0.02 ^{A}	0.28 ± 0.04 ^{A}	4.85 ± 0.17^{B}

strain- specific trait. The fermentation power of *T. delbruekii* has been studied in detail and it ranged from 6% v/v to 12% v/v of ethanol (Comitini et al., 2011; Renault et al., 2009; van Breda et al., 2013). In general, *T. delbrueckii* is considered a relatively powerful fermenter and its ability to complete sparkling base wines secondary fermentation could be due to the relatively low alcohol levels of those beverages compared to still wines (Basso et al., 2016; Canonico et al., 2017; Medina-Trujillo et al., 2017).

In agreement with fermentation kinetics, sparkling wines obtained with *Starm. bacillaris* showed an ethanol content of 9.65% v/v and 15.64 g/L of residual sugars.

Differences were observed for the glycerol content. In fact, sparkling wines produced with *S. cerevisiae*+*Starm. bacillaris* showed the highest amounts of glycerol (6.51 g/L). This data is in agreement with previous observations for still wine production (Perpetuini et al., 2021a,b; Nisiotou et al., 2018; Suzzi et al., 2012; Tofalo et al., 2012). It is a species-specific characteristic. In fact, the over production of glycerol allows *Starm. bacillaris* to maintain the NADH/NAD + redox balance in the cells (Gonçalves et al., 2019). Interestingly, different levels of glycerol production may be associated with alternative alleles of the *GPP*1 gene, encoding glycerol-3-phosphate phosphatase (Lemos Junior et al., 2018). Glycerol accumulation was not observed in sparkling wines produced with *Starm. bacillaris* as pure culture, probably because this strain was not able to complete the secondary fermentation and also its viability was compromised. Volatile acidity (lower than 0.3 g acetic acid/L) and pHs were not significantly different among the sparkling wines produced.

3.2. Autolysis outcome and viable count

The autolysis is the main process characterizing sparkling wines aging. It is a complex process during which yeasts intracellular compounds are released into the wine changing its final composition (Torresi et al., 2011; Perpetuini et al., 2016). Amino acids (AAN) are, generally, considered the major compounds released into the wine during this process which could contribute to the wine's volatile profile and foam properties (Alexandre and Guilloux-Benatier, 2006). Therefore, in this study the autolysis outcome was monitored through the determination of AAN content (Fig. 2).

The highest amount of AAN was found in sparkling wines obtained with *Starm. bacillaris* SB48 strain (188.33 mg leucin/L), followed by those obtained with *T. delbrueckii* TB1 (155.4 mg leucin/L). The good autolytic potential of *T. delbrueckii* has been shown by González-Royo et al. (2015) when this species was used for the fermentation of a base wine. These authors observed that the base wine obtained by sequential inoculation with *T. delbrueckii* and *S. cerevisiae* had a significantly higher protein concentration, than its corresponding control obtained by the

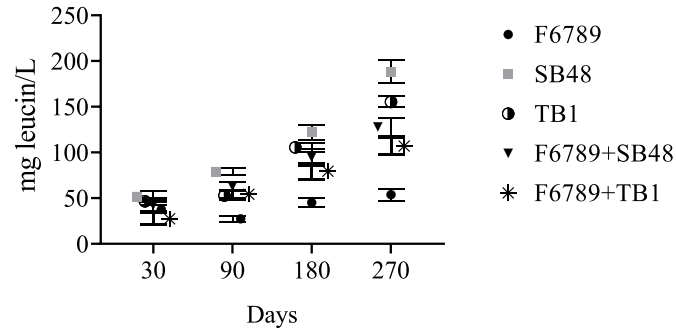

Fig. 2. Cumulative plot showing AAN released by tested strains during secondary fermentation and aging. Data are expressed as mg leucin/L. $p < 0.05$.

conventional inoculation with *S. cerevisiae*. Concerning, *Starm. bacillaris* the highest content of AAN detected could be related to its rapid death and a mis-regulation of the autophagic process. Autophagy takes place during secondary fermentation of sparkling wines, and it is induced by starvation conditions and involves the transport and degradation of cytoplasmic compounds in the vacuole. It is required for yeast survival under stressful conditions (Porras-Agüera et al., 2020). Since autophagy precedes autolysis during aging, and it is essential for cell maintenance and survival under stress conditions, it should be useful to select yeast strains with deregulated autophagy in order to accelerate the autolysis process.

Sparkling wines obtained with the multi-starter cultures showed similar AAN content than the sparkling wines obtained with pure cultures. In particular, AAN values after 270 days were 128 mg leucin/L and 107.03 mg leucin/L in sparkling wines produced with *S. cerevisiae*+*Starm. bacillaris* and *S. cerevisiae*+*T. delbrueckii*, respectively. The low level of AAN found in sparkling wine obtained with *S. cerevisiae* (53.96 mg leucin/L) could be related to the flocculent phenotype of this strain. In fact, flocculation contributes to create specific micro-environmental conditions that favour survival rate under stressful conditions during sparkling wine production. Therefore, it is important to understand the behaviour of flocculent strains alone or in combination with non-*Saccharomyces* yeasts during sparkling wine production. Flocculation mechanism could also explain the amount of AAN content found in sparkling wines obtained with the co-inoculation of *S. cerevisiae* and non-*Saccharomyces* yeasts. In fact, flocs can be formed by a mixed population of flocculent and non-flocculent "cheater" cells that receive the protective benefits. Autolysis outcome results are in agreement with cell count: when the number of viable cells decreased the concentration of AAN increased and *vice versa* (Fig. 3). Viable cells of *Starm. bacillaris* were almost absent already after 30 days in agreement with fermentation kinetics. *T. delbrueckii* viable cells were present until 180 days (1.23 cells/mL). This data agreed with the ability of this yeast to face sparkling wine stressing conditions and complete the fermentation as demonstrated by other authors (Canonico et al., 2018; Medina-Trujillo et al., 2016). Mixed fermentations showed similar cell load to the pure culture of F6789 (about 3 cells/mL). Therefore, the possible formation of multi-species flocs which offer a protective environment for the non-*Saccharomyces* yeasts could be hypothesized.

3.3. Aroma compounds analysis of sparkling wines

Volatile organic aroma compounds (VOCs) characterizing sparkling wines are considered a crucial quality indicator for consumers, with sensory and VOCs analysis being the most widely used methods for its assessment (Martínez-García et al., 2021). In this study the aroma profile of sparkling wines was determined at different times (90, 180, and 270 days) of aging. After 270 days of aging, 42 compounds were identified by GC–MS belonging to the following chemical classes: esters (19), higher alcohols (7), organic acids (6) and 10 other molecules not

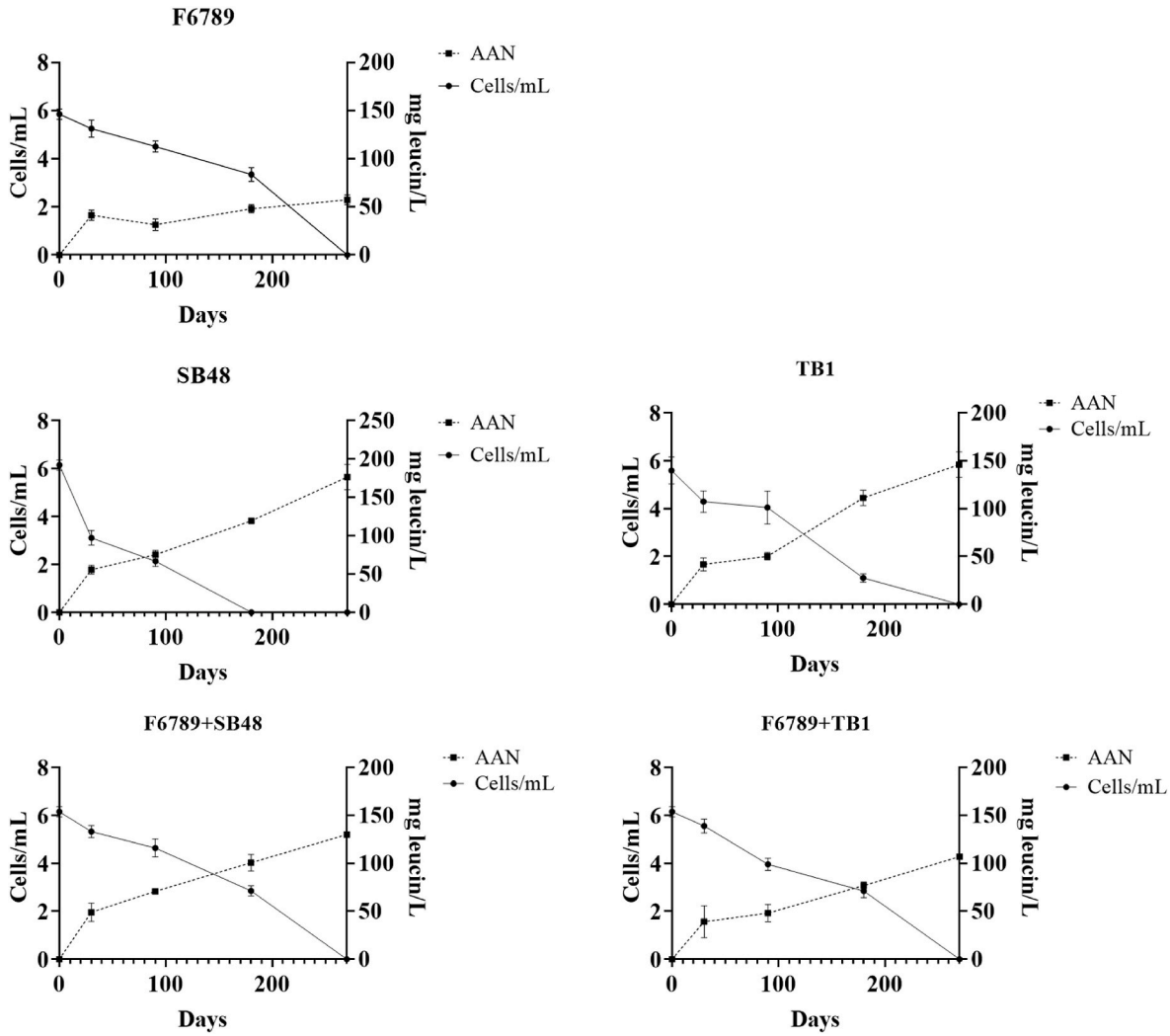

Fig. 3. Correlation between viable cells and autolysis outcome.

classified (Table 3). All siloxane derivatives are not reported since they originate from the fiber and column bleeding. The identified compounds presented different odors whose description was taken from a comprehensive database (www.thegoodscentscompany.com). Fuel, fruity, floral, sweet and waxy were the most frequent odors and were found in the majority of the compounds with an available descriptor.

Differences in volatiles content were detected in relation to the aging time and the strains inoculated as demonstrated in agreement with other authors (Di Gianvito et al., 2018; Ubeda et al., 2019; Martínez-García et al., 2021; 2017). In fact, the volatile profile of sparkling wine is reported to be dependent on the kinetics of volatile compound retention and release by yeast less during the aging process (Torresi et al., 2011).

In particular, the highest concentrations of higher alcohols were found in sparkling wines produced with *S. cerevisiae* F6789+*T. delbrueckii* TB1 (27.50 mg/L). Some authors reported that *T. delbrueckii* produces approximately 70–90 mg/L less higher alcohols than *S. cerevisiae* (Sadoudi et al., 2012; Belda et al., 2017). This difference is mainly due to the different distribution of metabolic flux during alcoholic fermentation and, as a result, parameters such as biomass generation, ethanol production, or by-product synthesis (Milanovic et al., 2012). However, data are controversial. In fact, some studies reported an increase of higher alcohols (Azzolini et al., 2015). A strain-dependent behaviour could be hypothesized, as well as a different regulation of the Ehrlich pathway, which is responsible for the production of these compounds and varies greatly among strains. After 270 days of aging the main higher alcohols detected were: 1-butnol 3-methyl, 1-pentanol, and phenylethyl alcohol. In particular, the concentration of 1-butanol 3-methyl and 1-pentanol increased during aging, while phenylethyl alcohol decreased after 9 months of contact with lees (Tables 3A and 3B and Table 4). Coelho et al. (2009) described an increase of 1-pentanol after 24 months of aging suggesting that this molecule could be used as a marker of aging of sparkling wines. Concerning phenylethyl alcohol Ubeda et al. (2019) reported that its concentration is stable during aging, while other studies reported its increase. In this study its decrease was observed, suggesting that the differences reported in the different papers might be due to the balance between intracellular synthesis and the extracellular lees adsorption-desorption process. In general, the total content of higher alcohols is similar in sparkling wines after 6 and 9 months of aging probably because they are not strongly retained by yeast lees. Similar results were obtained by Ubeda et al. (2019) and Gallardo-Chacón et al. (2010).

Esters were mainly present in sparkling wines obtained with *S. cerevisiae* F6789 (88.09 mg/L) followed by those obtained with *S. cerevisiae*+*T. delbrueckii* (87.20 mg/L), *S. cerevisiae*+*Starm. bacillaris* (81.93 mg/L), *T. delbrueckii* (44.92 mg/L) and *Starm. bacillaris* (22.04 mg/L). Esters play a key role in the determination of sparkling wine aroma compounds because of their direct sensory contribution and synergistic interactions affecting aroma perception (Escudero et al., 2007). Their production is an intracellular process requiring energy

Table 3
Main volatile compounds detected by SPME-GS/MS in sparkling wines after 90 (A) and 180 (B) days of aging. Different letters in the same raw indicate significantly differences ($p < 0.05$) according to ANOVA A.

Volatile compounds	Fermentation trials				
Higher alcohols	**F6789**	**SB48**	**TB1**	**F6789 + SB48**	**F6789 + TB1**
A					
1-butanol, 2-methyl	1.22 ± 0.10^{C}	0.18 ± 0.08^{B}	0.15 ± 0.07^{B}	2.33 ± 0.12^{A}	2.12 ± 0.02^{A}
1-butanol, 3-methyl-	4.30 ± 0.24^{B}	2.40 ± 0.92^{A}	3.20 ± 0.53^{AB}	4.8 ± 0.33^{A}	5.02 ± 1.22^{AB}
1-hexanol	0.16 ± 0.08^{AB}	0.18 ± 0.11^{B}	0.12 ± 0.08^{A}	0.16 ± 0.05^{B}	0.13 ± 0.09^{A}
1-pentanol	4.40 ± 0.22^{AB}	2.60 ± 0.23^{A}	2.40 ± 0.12^{B}	3.77 ± 0.25^{A}	3.15 ± 0.03^{A}
2,3-butanediol	0.12 ± 0.11^{C}	n.d.	0.09 ± 0.01 ^{A}	0.15 ± 0.08 ^{B}	0.11 ± 0.08^{A}
Phenylethyl alcohol	5.55 ± 1.12^{AB}	1.88 ± 0.21^{A}	3.45 ± 0.22^{A}	5.55 ± 0.33^{A}	5.33 ± 0.21^{A}
Total	**15.75**	**7.24**	**9.41**	**16.76**	**15.86**
Organic acids					
2-methylheptanoic acid	3.10 ± 0.23^{A}	n.d.	1.20 ± 0.14^{AB}	n.d.	0.09 ± 0.03^{B}
Glycollic acid	0.10 ± 0.07^{A}	n.d.	n.d.	0.08 ± 0.01^{A}	0.05 ± 0.03^{A}
Hexanoic acid	n.d.	0.44 ± 0.12^{A}	0.12 ± 0.03^{B}	1.12 ± 0.09^{B}	0.33 ± 0.12^{A}
Decanoic acid	0.22 ± 0.04^{C}	0.60 ± 0.53^{A}	0.80 ± 0.23^{AB}	0.44 ± 0.32^{A}	0.99 ± 0.11^{AB}
Octanoic acid	1.20 ± 0.44^{A}	2.64 ± 0.23^{B}	3.22 ± 0.15^{A}	3.20 ± 0.22^{A}	4.22 ± 0.14^{AB}
Total	**4.62**	**3.68**	**5.34**	**4.84**	**5.68**
Esters					
2-phenylethyl acetate	0.24 ± 0.02^{A}	0.12 ± 0.08^{B}	0.22 ± 0.06^{A}	0.30 ± 0.09^{A}	0.12 ± 0.08^{B}
Diethyl succinate	0.22 ± 0.08^{A}	0.33 ± 0.12^{B}	n.d.	0.33 ± 0.23^{A}	0.12 ± 0.23^{B}
Ethyl 4-methylpentanoate	n.d.	n.d.	n.d	0.20 ± 0.13^{A}	0.08 ± 0.02^{A}
Ethyl 9-decenoate	0.06 ± 0.02^{B}	n.d.	n.d.	0.27 ± 0.34^{A}	0.03 ± 0.06^{B}
Ethyl decanoate	1.20 ± 0.14^{B}	0.53 ± 0.23^{AB}	1.40 ± 0.43^{A}	1.30 ± 0.23^{C}	0.30 ± 0.09^{D}
Ethyl hexadecanoate	0.03 ± 0.01^{C}	0.03 ± 0.01^{A}	0.10 ± 0.08^{B}	0.12 ± 0.09^{B}	0.03 ± 0.01^{A}
Ethyl hexoate	2.70 ± 0.32^{A}	2.10 ± 0.38^{A}	2.90 ± 0.12^{A}	2.10 ± 0.88^{A}	2.01 ± 0.45^{A}
Ethyl octanoate	2.20 ± 0.33^{B}	2.10 ± 0.66^{A}	3.20 ± 0.33^{A}	4.30 ± 1.34^{A}	4.90 ± 0.88^{A}
Methyl 2,2-dimethylbutanoate	1.70 ± 0.42^{A}	0.20 ± 0.12^{B}	1.20 ± 0.12^{A}	n.d.	1.80 ± 0.22^{A}
Methyl 2-methylhexanoate	0.20 ± 0.22^{AB}	0.31 ± 0.14^{AB}	0.12 ± 0.05^{B}	0.11 ± 0.09^{A}	0.22 ± 0.12^{A}
Hexyl ethanoate	0.24 ± 0.12^{B}	0.10 ± 0.08^{B}	0.19 ± 0.08^{A}	0.32 ± 0.12^{A}	0.08 ± 0.03^{B}
Total	**8.79**	**5.82**	**9.33**	**9.35**	**9.69**
Others					
2.6-dimethyl heptadecane	n.d.	n.d.	0.62 ± 0.7^{A}	n.d.	n.d.
2.5-ditert-butylphenol	n.d.	0.08 ± 0.04^{A}	0.01 ± 0.01^{C}	0.06 ± 0.01^{A}	0.05 ± 0.01^{B}
3.6-dimethyloxan-2-one	n.d.	n.d.	n.d.	0.04 ± 0.01^{A}	n.d.
2.4-dimethylbenzo [h] quinoline	n.d.	0.02 ± 0.01^{B}	n.d.	0.09 ± 0.04^{A}	0.12 ± 0.08^{A}
α-Ionone	0.04 ± 0.02^{A}	0.08 ± 0.02^{A}	0.14 ± 0.13^{A}	n.d.	0.03 ± 0.01^{B}
Total	**0.04**	**0.18**	**0.77**	**0.19**	**0.20**
B					
1-Butanol, 2-methyl	0.79 ± 0.22^{B}	0.34 ± 0.09^{A}	0.55 ± 0.08^{B}	0.47 ± 0.06^{A}	0.12 ± 0.15^{C}
1-Butanol, 3-methyl-	24.76 ± 1.3^{AB}	8.31 ± 1.45^{B}	7.74 ± 1.18^{B}	13.02 ± 1.07^{A}	9.74 ± 0.72^{AB}
1-Hexanol	0.11 ± 0.04^{C}	0.28 ± 0.08^{A}	0.19 ± 0.05^{B}	0.22 ± 0.1^{A}	0.16 ± 0.05^{B}

Table 3 (*continued*)

Volatile compounds	Fermentation trials				
Higher alcohols	**F6789**	**SB48**	**TB1**	**F6789 + SB48**	**F6789 + TB1**
1-Pentanol	4.83 ± 0.84^{A}	3.94 ± 0.75^{C}	6.09 ± 0.55^{C}	4.60 ± 0.62^{AB}	1.94 ± 0.33^{AB}
2.3-Butanediol	0.57 ± 0.23^{A}	n.d.	0.10 ± 0.08^{B}	0.32 ± 0.13^{A}	0.39 ± 0.08^{A}
3.3-Dimethyl-4-methylamino-butan-2-one	n.d.	0.04 ± 0.01^{A}	0.01 ± 0.01^{B}	0.01 ± 0.01^{B}	n.d.
Phenylethyl Alcohol	4.83 ± 0.77^{B}	3.98 ± 0.84^{C}	4.67 ± 0.85^{A}	15.04 ± 0.64^{BC}	14.02 ± 1.45^{AB}
Total	**35.89**	**16.89**	**19.35**	**33.68**	**26.37**
Organic acids					
2-Methylheptanoic acid	9.69 ± 0.35^{A}	n.d.	6.55 ± 0.99^{A}	n.d.	0.15 ± 0.07^{B}
3-Nitropropanoic acid	0.01 ± 0.01^{B}	n.d.	n.d.	n.d.	0.24 ± 0.05^{A}
Glycollic acid	0.18 ± 0.02^{A}	n.d.	n.d.	0.18 ± 0.12^{B}	0.10 ± 0.09^{A}
Hexanoic acid	n.d.	0.85 ± 0.14^{B}	0.90 ± 0.12^{AB}	0.41 ± 0.09^{A}	0.51 ± 0.43^{A}
Decanoic acid	1.28 ± 0.22^{AB}	1.08 ± 0.65^{A}	2.53 ± 0.85^{A}	0.59 ± 0.33^{A}	1.27 ± 0.94^{A}
Octanoic acid	8.96 ± 0.33^{B}	1.83 ± 0.34^{A}	6.77 ± 0.43^{A}	7.69 ± 0.8^{B}	6.26 ± 1.22^{B}
Total	**20.12**	**3.76**	**16.75**	**8.87**	**8.53**
Esters					
Isoamyl ethanoate	n.d.	0.20 ± 0.09^{A}	n.d.	n.d.	n.d.
2-Methylbutyl pentanoate	0.02 ± 0.01^{B}	0.07 ± 0.03^{A}	0.06 ± 0.03^{A}	0.03 ± 0.01^{B}	n.d.
2-Phenylethyl acetate	0.30 ± 0.13 ^{B}	0.20 ± 0.14 ^{B}	2.27 ± 0.14 ^{A}	0.36 ± 0.17 ^{B}	2.16 ± 0.05 ^{A}
3-Methyl-1-butyl acetate	n.d.	n.d.	n.d.	n.d.	1.10 ± 0.09^{A}
Diethyl succinate	0.28 ± 0.23^{B}	0.64 ± 0.43^{AB}	n.d.	0.57 ± 0.22^{B}	1.27 ± 0.15^{A}
Ethyl 10-undecenoate	0.05 ± 0.01^{A}	n.d.	n.d.	n.d.	0.07 ± 0.02^{A}
Ethyl 4-methylpentanoate	n.d.	n.d.	n.d.	0.20 ± 0.09^{A}	0.08 ± 0.01^{A}
Ethyl 9-decenoate	0.06 ± 0.02^{B}	n.d.	n.d.	0.27 ± 0.08^{A}	1.03 ± 0.02^{A}
Ethyl decanoate	2.95 ± 0.37^{A}	0.54 ± 0.12^{B}	1.85 ± 0.44^{B}	2.45 ± 0.61^{AB}	0.51 ± 0.12^{AB}
Ethyl E−11-hexadecenoate	0.17 ± 0.03^{B}	0.09 ± 0.08^{C}	0.18 ± 0.16^{A}	0.14 ± 0.05^{A}	0.05 ± 0.01^{C}
Ethyl hexanoate	13.96 ± 0.73^{B}	1.28 ± 0.42^{B}	13.50 ± 0.55^{A}	15.98 ± 1.33 ^{A}	16.27 ± 0.8^{AB}
Ethyl octanoate	52.71 ± 1.64^{A}	2.08 ± 0.83^{B}	37.61 ± 1.5^{A}	54.26 ± 0.95^{A}	45.62 ± 0.44^{B}
Methyl 2.2-dimethylbutanoate	2.12 ± 0.94^{A}	0.56 ± 0.13^{B}	2.57 ± 0.74^{A}	n.d.	2.39 ± 0.73^{A}
Methyl 2-methylhexanoate	21.23 ± 0.04^{A}	0.01 ± 0.01^{C}	2.22 ± 0.14^{C}	14.58 ± 1.43^{A}	9.31 ± 0.15^{B}
Hexyl ethanoate	0.42 ± 0.22^{A}	0.23 ± 0.13^{AB}	0.27 ± 0.13^{AB}	0.53 ± 0.69^{A}	0.1 ± 0.09^{C}
3-Methylbutyl acetate	1.31 ± 0.93^{B}	0.49 ± 0.34^{C}	1.63 ± 0.42^{A}	1.78 ± 0.68^{A}	0.32 ± 0.16^{C}
Total	**95.58**	**6.37**	**62.16**	**91.15**	**80.28**
Others					
2.6-dimethyl heptadecane	n.d.	n.d.	0.62 ± 0.73^{A}	n.d.	n.d.
2.5-ditert-butylphenol	n.d.	0.08 ± 0.04^{A}	0.01 ± 0.01^{A}	0.06 ± 0.01^{A}	0.05 ± 0.01^{A}
3.6-dimethyloxan-2-one	n.d.	n.d.	n.d.	0.04 ± 0.01^{A}	n.d.
Benzo [h]quinoline. 2.4-dimethyl	n.d.	0.02 ± 0.01^{B}	n.d.	0.09 ± 0.04^{B}	0.12 ± 0.08^{A}
α-Ionone	0.07 ± 0.03^{B}	0.10 ± 0.23^{A}	0.15 ± 0.85^{A}	n.d.	0.09 ± 0.01^{B}
Total	**0.07**	**0.20**	**0.78**	**0.19**	**0.26**

*Data were expressed as mg/L.
**n.d. as not detected.

Table 4
Main volatile compounds detected by SPME-GS/MS in sparkling wines after 270 days of aging. Different letters in the same raw indicate significantly differences ($p < 0.05$) according to ANOVA.

Volatile compounds		Fermentation trials					
Higher alcols	**Odor description**	**Base wine**	**F6789**	**SB48**	**TB1**	**F6789 + SB48**	**F6789 + TB1**
1-Butanol, 2-methyl-	Fuel, fatty, winey, cocoa	0.66 ± 0.34^{F}	1.01 ± 0.83^{C}	1.31 ± 0.4^{A}	0.93 ± 0.23^{D}	0.87 ± 0.32^{E}	1.04 ± 0.41^{B}
1-Butanol, 3-methyl-	Fuel, oil, whiskey, fruity, banana	2.17 ± 1.52^{F}	9.12 ± 1.43^{B}	5.88 ± 1.3^{E}	8.48 ± 1.92^{D}	8.80 ± 0.42^{C}	10.69 ± 1.43^{A}
1-Hexanol	Sweet, herbaceous, woody	0.25 ± 0.09^{B}	0.32 ± 0.12^{C}	0.29 ± 0.13^{B}	0.21 ± 0.09^{A}	0.24 ± 0.63^{B}	0.29 ± 0.43^{A}
1-Pentanol	Fusel, oil, sweet, balsam	2.24 ± 1.92^{E}	6.62 ± 1.32^{D}	n.d.	8.48 ± 1.22^{B}	7.90 ± 0.45^{C}	9.81 ± 1.52^{A}
2.3-Butanediol	Fruity, creamy, buttery	0.10 ± 0.04^{B}	n.d.	0.01 ± 0.01^{C}	0.29 ± 0.14^{A}	0.05 ± 0.01^{C}	n.d.
6-amino-2-methyl-heptan-2-ol	n.f.	n.d.	0.34 ± 1.45^{A}	n.d.	n.d.	n.d.	n.d.
Phenylethyl alcohol	Sweet, floral, fresh, bready, rose	1.11 ± 1.13^{C}	5.51 ± 1.4^{A}	3.80 ± 1.2^{B}	5.29 ± 1.63^{B}	5.37 ± 0.84^{D}	5.67 ± 0.41^{B}
Total		**6.53**	**22.92**	**11.29**	**23.68**	**23.23**	**27.50**
Organic acids							
Glycollic acid	Very mild buttery	0.17 ± 0.04^{A}	n.d.	n.d.	n.d.	n.d.	n.d.
Guanidineacetic acid	n.f.	n.d.	n.d.	n.d.	0.02 ± 0.01^{A}	0.03 ± 0.01^{A}	0.03 ± 0.01^{A}
Hexanoic acid	Sour, fatty, sweat, cheese	1.19 ± 0.32^{A}	n.d.	0.18 ± 0.51^{C}	1.09 ± 0.22^{AB}	0.99 ± 0.32^{C}	n.d.
Decanoic acid	Rancid, sour, fatty, citrus	1.50 ± 0.55^{B}	1.03 ± 0.2^{A}	1.14 ± 0.33^{A}	0.38 ± 0.13^{B}	0.89 ± 0.23^{C}	1.15 ± 0.44^{C}
Octanoic acid	Fatty, rancid, oily, cheesy	0.72 ± 0.33^{C}	3.61 ± 0.86^{B}	n.d.	n.d.	1.57 ± 0.52^{A}	1.95 ± 0.33^{A}
Propyl-propanedioic acid	n.f.	n.d.	n.d.	1.43 ± 0.82^{A}	n.d.	0.36 ± 0.14^{A}	1.54 ± 0.52^{A}
Total		**3.58**	**4.64**	**2.75**	**1.49**	**3.84**	**4.67**
Esters							
2-Methylbutyl pentanoate	n.f.	n.d.	n.d.	n.d.	2.17 ± 0.7^{A}	n.d.	0.05 ± 0.01^{B}
2-Phenethyl acetate	Floral, sweet, honey, fruity, tropical	0.13 ± 0.12^{D}	0.42 ± 0.23^{C}	0.21 ± 0.15^{B}	0.15 ± 0.1^{B}	0.17 ± 0.13^{A}	0.27 ± 0.14^{A}
3-Methylbutyl 2-ethylhexanoate	n.f.	n.d.	n.d.	n.d.	n.d.	0.30 ± 0.12^{A}	n.d.
Diethyl succinate	Mild, fruity, cooked, apple, ylang	1.07 ± 0.32^{E}	8.92 ± 1.24^{D}	0.74 ± 0.44^{C}	8.16 ± 1.62^{A}	8.30 ± 1.73^{AB}	1.24 ± 0.44^{B}
Ethyl 14-methyl-hexadecanoate	n.f.	n.d.	n.d.	n.d.	n.d.	0.07 ± 0.01^{A}	n.d.
Ethyl 9-decenoate	Fruity, fatty	n.d.	0.26 ± 0.09^{C}	n.d.	0.12 ± 0.07^{A}	0.42 ± 0.12^{B}	0.58 ± 0.34^{A}
Ethyl 9-tetradecenoate	n.f.	n.d.	0.21 ± 0.14^{C}	0.14 ± 0.03^{AB}	0.25 ± 0.08^{B}	0.26 ± 0.23^{A}	0.27 ± 0.22^{A}
Ethyl butanoate	Fruity, pineapple	n.d.	0.08 ± 0.03^{D}	0.12 ± 0.05^{B}	0.05 ± 0.01^{D}	0.08 ± 0.05^{C}	0.34 ± 0.15^{A}
Ethyl dodecanoate	Sweet, waxy, floral, soapy, clean	n.d.	n.d.	n.d.	n.d.	0.47 ± 0.26^{A}	n.d.
Ethyl hexadecanoate	Waxy, fruity, creamy, milky	n.d.	0.09 ± 0.05^{C}	n.d.	0.24 ± 0.18^{A}	n.d.	0.40 ± 0.3^{B}
Ethyl hexanoate	Sweet fruity, pineapple, waxy, banana	1.23 ± 1.23^{E}	12.72 ± 1.6^{C}	0.19 ± 0.05^{D}	5.19 ± 1.17^{A}	10.56 ± 1.85^{B}	12.11 ± 0.9^{AB}
Ethyl decanoate	Sweet, waxy, fruity, apple, grape, oily	n.d.	7.80 ± 1.8^{B}	3.53 ± 1.5^{C}	6.19 ± 1.2^{B}	9.23 ± 0.94^{A}	14.66 ± 1.73^{A}
Ethyl octanoate	Fruity, sweet, apricot, banana, pear	2.76 ± 2.46^{C}	48.10 ± 1.45^{B}	15.56 ± 1.84^{A}	20.02 ± 1.78^{AB}	44.45 ± 2.56^{AB}	48.55 ± 2.88^{A}
Hexyl ethanoate	Fruity, green, apple, banana, sweet	n.d.	0.43 ± 0.23^{B}	0.24 ± 0.12^{B}	0.47 ± 0.27^{C}	0.52 ± 0.69^{AB}	0.56 ± 0.23^{A}
Isoamyl acetate	Sweet, fruity, banana, solvent	n.d.	1.22 ± 0.91^{B}	0.04 ± 0.01^{C}	0.35 ± 0.12^{B}	1.16 ± 0.35^{A}	1.27 ± 0.77^{A}
Isoamyl ethanoate	Sweet, fruity, banana, solvent	n.d.	0.40 ± 0.23^{AB}	1.09 ± 0.4^{B}	0.86 ± 0.2^{AB}	0.36 ± 0.15^{A}	0.49 ± 0.2^{B}
Methyl 2.2-dimethylbutanoate	n.f.	0.27 ± 1.14^{C}	6.68 ± 1.34^{A}	0.13 ± 0.08^{B}	n.d.	5.05 ± 1.37^{A}	5.64 ± 1.77^{A}
Methyl 2-methylhexanoate	n.f.	0.27 ± 0.23^{C}	0.76 ± 0.55^{B}	0.05 ± 0.01^{B}	0.45 ± 0.25^{AB}	0.53 ± 0.54^{A}	0.77 ± 0.24^{A}
Methyl oxoethanoate	n.f.	n.d.	n.d.	n.d.	0.25 ± 0.1^{A}	n.d.	n.d.
Total		**5.73**	**88.09**	**22.04**	**44.92**	**81.93**	**87.20**
Others							
Z.Z.Z-4.6.9-Nonadecatriene	n.f.	n.d.	n.d.	0.05 ± 0.01^{A}	n.d.	n.d.	n.d.
1.2.3-Propatriol. 1-indol-4-yl (ether)	n.f.	n.d.	n.d.	n.d.	0.04 ± 0.01^{A}	n.d.	n.d.
1.3-Dioxan-5-ol	n.f.	n.d.	n.d.	n.d.	0.36 ± 0.16^{A}	n.d.	n.d.
2-Benzylsulfanyl-1H-benzoimidazole	n.f.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.01^{A}
3.6-dimethyloxan-2-one	n.f.	0.06 ± 0.04^{B}	n.d.	0.03 ± 0.01^{B}	n.d.	0.11 ± 0.14^{A}	n.d.
2.4-Dimethylbenzo [h]quinoline	n.f.	0.03 ± 0.01^{C}	0.07 ± 0.01^{C}	0.09 ± 0.02^{AB}	0.23 ± 0.12^{A}	0.09 ± 0.02^{A}	0.17 ± 0.1^{B}
O-(2-methylpropyl)-Hydroxylamine	n.f.	n.d.	n.d.	n.d.	0.07 ± 0.02^{AB}	n.d.	n.d.
2-hydroxy-Propanamide	n.f.	n.d.	0.11 ± 0.04^{AB}	n.d.	0.06 ± 0.01^{A}	0.02 ± 0.01^{A}	0.05 ± 0.03^{AB}
3-ethyl-Quinoline	n.f.	n.d.	n.d.	0.06 ± 0.04^{C}	0.07 ± 0.03^{B}	0.23 ± 0.1^{A}	0.07 ± 0.04^{A}

(*continued on next page*)

Table 4 (*continued*)

Volatile compounds		Fermentation trials					
Higher alcols	**Odor description**	**Base wine**	**F6789**	**SB48**	**TB1**	**F6789 + SB48**	**F6789 + TB1**
α-Ionone	Sweet, woody, floral, violet, tropical	0.14 ± 0.09^{C}	0.09 ± 0.02^{B}	0.12 ± 0.08^{A}	0.17 ± 0.04^{A}	0.02 ± 0.01^{B}	0.11 ± 0.08^{AB}
Total		**0.23**	**0.27**	**0.35**	**1.00**	**0.47**	**0.48**

(Saerens et al., 2010). The role played by these molecules in yeast metabolism is not completely understood. Some authors suggested that esters production is a way to remove fatty acids with a potential toxic effect for the cell (C8–C14 unsaturated fatty acids are considered as toxic metabolites for the yeast cell). Another possibility is that some esters could act as unsaturated fatty acids analogues in the cell membrane, to ensure optimal membrane fluidity during fermentation. However, as most esters (and certainly the shorter ones) are volatile compounds, this hypothesis can only be valid for the longer esters like the ethyl esters (Saerens et al., 2010).

In general, the content of esters increased after 6 months and a slight decrease was observed after 9 months (Tables 3A and 3B and Table 4). Previous studies showed a decrease of ester concentrations during sparkling wine production (Torrens et al., 2010). Lees adsorption could explain this phenomenon (Ubeda et al., 2019). However, other mechanisms have been proposed. Ruiz-Moreno et al. (2017) suggested that the reduction of esters could also be related to their hydrolysis because of their thermodynamical instability. The main esters detected were diethyl-succinate, ethyl-hexanoate, ethyl-decanoate, and ethyl-octanoate with a positive impact on sparkling wine flavour.

Sparkling wines obtained with *T. delbrueckii* showed a content of organic acids of1.49 mg/L, while the others sparkling wines presented values of about 4 mg/L. As expected, sparkling wines obtained with *Starm. bacillaris* had the lowest values (Table 3). A fluctuation of their content can be observed during aging. In fact, a peak is reached after 6 months with values ranging from 8.53 mg/L (SW5) to 20.12 mg/L (SW1); very low values were observed for SW2 (3.76 mg/L). This decrease has been observed also by Martínez-García et al. (2021) showing that aged sparkling wines had a decrease of 83% of their total content in acids. This phenomenon might be the result of lees adsorption since they are characterized by a high hydrophobicity and thermodynamic instability (Gallardo-Chacón et al., 2010). Sparkling wines obtained with *Starm. bacillaris* showed the lowest values for all aroma compounds and could be related to the low number of viable cells detected. PCA analysis was performed to differentiate sparkling wines on the basis of their aroma compounds composition. Principal component analysis allowed 73.27% of the total variance to be explained by the first two PCs (Fig. 4). Sparkling wines fermented with non--*Saccharomyces* were in the left part of graph. Sparkling wines fermented with *T. delbrueckii* were differentiated from the others for 3 esters, 2,3 butanediol, and hexanoic acid. Sparkling wines obtained with *S. cerevisiae* and *S. cerevisiae* + *T. delbrueckii* clustered together and were well differentiated for 9 esters, 2 organic acids and 1-hexanol. Sparkling wines obtained with *S. cerevisiae*+*Starm. bacillaris* clustered alone and were characterized by 7 esters, 3 higher alcohols and an organic acid.

Obtained data suggested that the co-inoculum *S. cerevisiae*+*Starm. bacillaris* exerts a stronger effect in the definition of sparkling wine volatilome than *S. cerevisiae*+*T. delbrueckii*. Probably, the high autolytic potential of *Starm. bacillaris* could provide several aroma precursors to *S. cerevisiae* favouring the production of volatile compounds able to impart a specific aroma footprint to sparkling wines.

3.4. Gas sensor array analysis of sparkling wine samples

The sensorial profile of a wine is the resulting of volatile and not volatile molecule interaction, therefore the gas sensor array analysis was performed. The data from electronic nose were obtained by using different sensors able to detect different classes of compounds, as reported in Materials and Methods, and give a wine sensory evaluation. In this work, sparkling wine analysis was carried out both gas sensors

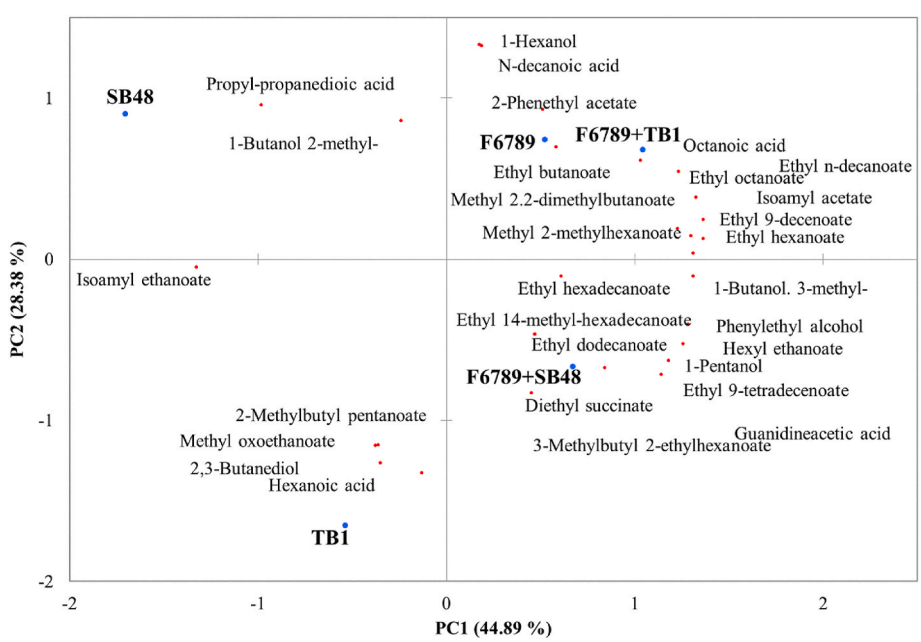

Fig. 4. Principal component analysis (PCA) encompassing aroma compounds released in sparkling wines by the tested strains. The biplot (score and loading) of the first two principal components showed 73.27% of the cumulative variance.

array, because a mixed set of hpDNA and pentapeptides can provide a synergistic response in the detection of VOCs in real samples. No drift of the signal was observed, and reproducibility of the measurement was satisfactory (RSD 15–20%). Humidity values, given by the sensor included in the e-nose, were constant (19–20%) during measurement of the samples.

The raw data obtained were analyzed by PCA. Fig. 5 reports the first and second principal component of sparkling wine analyzed after 90 days of secondary fermentation with 71.81% of total variance (A); after 180 days of secondary fermentation with 74.03% of total variance (B); and after 90 days of secondary fermentation with 94.9% of total variance (C). After 3 months the sparkling wines were not well differentiated. After 6 months a separation can be observed, since pentapeptides were in the II quadrant while the hpDNA was in the III quadrant. This trend is in agreement with GC/MS data, since sparkling wines obtained with *S. cerevisiae*, *S. cerevisiae*+*T. delbrueckii*, and *S. cerevisiae*+*Starm. bacillaris* showed a higher ester content than those fermented with *Starm. bacillaris* and *T. delbrueckii*. Finally, in Fig. 5C a well-defined profile can be observed with a clear separation between pentapeptides and hpDNA. These results might be due to the highest content of esters in sparkling wines obtained with *S. cerevisiae* + *T. delbrueckii*, in fact, pentapeptides sequences have more affinity for this chemical class than hpDNA (Mascini et al., 2018). *S. cerevisaie* and *S. cerevisiae*+*Starm. bacillaris* have a different VOCs profile, characterized by esters, organic acids, and alcohols. In this case, hpDNA have more affinity because VOCs present in the profile pattern were more complex (Gaggiotti et al., 2020; 2021; Mascini et al., 2019). The results showed that the volatile flavor compounds of the sparkling wines obtained had obvious differences. This is consistent with the results detected by GC-MS.

3.5. Sensory analysis

Sensory analysis continues to be an efficient tool for assessing the sensory properties of sparkling wines (Ivit and Kemp, 2018). Mixed fermentations showed the highest scores for the aromatic descriptors (fruity, floral and bread crust) in comparison with the sparkling wines inoculated with the pure cultures. Sparkling wines fermented with *S. cerevisiae* were characterized by minerality, and intermediate scores for the other descriptors (Fig. 6). Sparkling wines obtained with *Starm. bacillaris* showed the lowest scores for all the descriptors considered. *T. delbrueckii* allowed to obtain sparkling wines with intermediate scores for some descriptors e.g. freshness, bread crust, fruity and minerality. The obtained results showed that the mixed fermentation produced balanced sparkling wines with negative and positive attributes arranged in good proportions, showing good aroma descriptors. In particular, the *S. cerevisiae*+*Starm. bacillaris* sparkling wine was significantly different from the others, due to its high score for the descriptor for spicy, bread crust, freshness and floral (Fig. 6). *S. cerevisiae*+*T. delbrueckii* sparkling wines showed the highest cores for fruity. The sensory analysis is in agreement with the differences observed in terms of aroma compounds, since sparkling wines obtained with *S. cerevisiae*+*Starm. bacillaris* were well differentiated from the others.

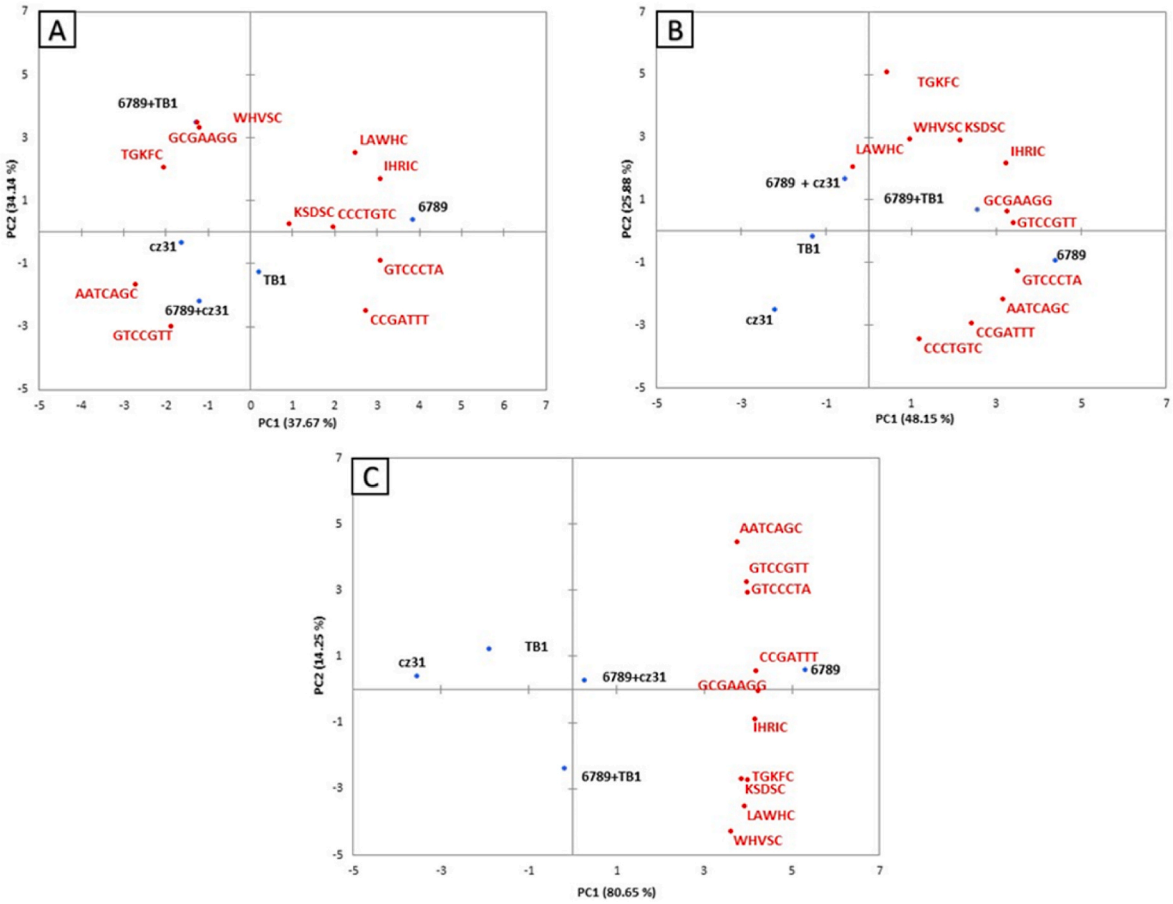

Fig. 5. PCA biplot (Score and Loading) obtained with E-nose responses of the sparkling wine samples. Loading plot was represented by red pentapeptides and hpDNA sequences; Score plot was represented by black samples. (A) Samples after 90 days of secondary of fermentation; (B) Samples after 180 days of secondary of fermentation; (C) Samples after 270 days of secondary of fermentation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

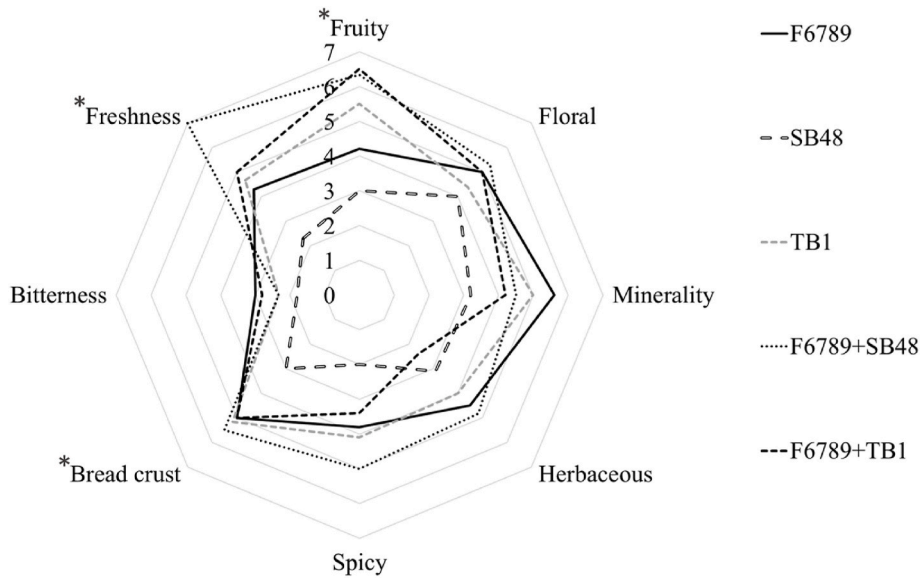

Fig. 6. Sensory analysis of the sparkling wines produced with the pure and mixed starter cultures. Sensorial parameters indicated with an asterisk (*) were significant different ($p < 0.05$).

4. Conclusion

The traditional method of sparkling wine production could be implemented using tailored mixed cultures of non-*Saccaromyces* yeasts and *S. cerevisiae* as starter cultures.

The results obtained in this study highlighted that the use of non-*Saccharomyces* yeasts allowed to obtain sparkling wines with distinctive traits. Promising results were showed by *Starm. bacillaris* strain. Even if, *Starm. bacillaris* was unable to complete the secondary fermentation when was inoculated as pure culture, once it was co-inoculated with *S. cerevisiae* its autolytic potential was a decisive aspect to define the sensorial profile of sparkling wines. These data suggested once again that the selection of strains with different autolytic potential represents an interesting strategy to improve yeast autolysis during sparkling wine aging and modulate sparkling wines' sensory profile. This approach might be useful to valorize autochthonous grape cultivar to be used for sparkling wines production. Further studies are necessary to study the autophagic process in non-*Saccharomyces* yeasts and identify the genes which could promote autolysis in order to reduce sparkling wines production costs and time. Moreover, it should be interesting to test non-*Saccharomyces* strains also for the production of sparkling wines using the *Charmat* method.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alexandre, H., Guilloux-Benatier, M., 2006. Yeast autolysis in sparkling wine - a review. Aust. J. Grape Wine Res. 12 (2), 119–127. https://doi.org/10.1111/j.1755-0238.2006.tb00051.x.

Azzolini, M., Fedrizzi, B., Tosi, E., Finato, F., Vagnoli, P., Scrinzi, C., Zapparoli, G., 2012. Effects of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* mixed cultures on fermentation and aroma of Amarone wine. Eur. Food Res. Technol. 2 (235), 303–313. https://doi.org/10.1007/s00217-012-1762-3.

Azzolini, M., Tosi, E., Lorenzini, M., Finato, F., Zapparoli, G., 2015. Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*. World J. Microbiol. Biotechnol. 31 (2), 277–293. https://doi.org/10.1007/s11274-014-1774-1.

Basso, R.F., Alcarde, A.R., Portugal, C.B., 2016. Could non-*Saccharomyces* yeasts contribute on innovative brewing fermentations? Food Res. Int. 86, 112–120. https://doi.org/10.1016/j.foodres.2016.06.002.

Belda, I., Ruiz, J., Beisert, B., Navascués, E., Marquina, D., Calderón, F., Rauhut, D., Benito, S., Santos, A., 2017. Influence of *Torulaspora delbrueckii* in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. Int. J. Food Microbiol. 257, 183–191. https://doi.org/10.1016/j.ijfoodmicro.2017.06.028.

Bely, M., Stoeckle, P., Masneuf-Pomarède, I., Dubourdieu, D., 2008. Impact of mixed *Torulaspora delbrueckii-Saccharomyces cerevisiae* culture on high-sugar fermentation. Int. J. Food Microbiol. 122 (3), 312–320. https://doi.org/10.1016/j.ijfoodmicro.2007.12.023.

Benito, S., 2018. The impact of *Torulaspora delbrueckii* yeast in winemaking. Appl. Microbiol. Biotechnol. 102 (7), 3081–3094. https://doi.org/10.1007/s00253-018-8849-0.

Buxaderas, S., López-Tamames, E., 2012. Sparkling wines: features and trends from tradition. Adv. Food Nutr. Res. 66, 1–45. https://doi.org/10.1016/B978-0-12-394597-6.00001-X.

Canonico, L., Comitini, F., Ciani, M., 2017. *Torulaspora delbrueckii* contribution in mixed brewing fermentations with different *Saccharomyces cerevisiae* strains. Int. J. Food Microbiol. 259, 7–13. https://doi.org/10.1016/j.ijfoodmicro.2017.07.017.

Coelho, E., Coimbra, M.A., Nogueira, J.M.F., Rocha, S.M., 2009. Quantification approach for assessment of sparkling wine volatiles from different soils, ripening stages, and varieties by stir bar sorptive extraction with liquid desorption. Anal. Chim. Acta 635 (2), 214–221. https://doi.org/10.1016/j.aca.2009.01.013.

Canonico, L., Comitini, F., Ciani, M., 2018. *Torulaspora delbrueckii* for secondary fermentation in sparkling wine production. Food Microbiol. 74, 100–106. https://doi.org/10.1016/j.fm.2018.03.009.

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M., 2011. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. Food Microbiol. 28 (5), 873–882. https://doi.org/10.1016/j.fm.2010.12.001.

Charpentier, C., Aussenac, J., Charpentier, M., Prome, J.C., Duteurtre, B., Feuillat, M., 2005. Release of nucleotides and nucleosides during yeast autolysis: kinetics and potential impact on flavor. J. Agric. Food Chem. 53, 3000–3007. https://doi.org/10.1021/jf040334y.

Di Gianvito, P., Perpetuini, G., Tittarelli, F., Schirone, M., Arfelli, G., Piva, A., Patrignani, F., Lanciotti, R., Olivastri, L., Suzzi, G., Tofalo, R., 2018. Impact of *Saccharomyces cerevisiae* strains on traditional sparkling wines production. Food Res. Int. 109, 552–560. https://doi.org/10.1016/j.foodres.2018.04.070.

Escudero, A., Campo, E., Farina, L., Cacho, J., Ferreira, V., 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J. Agric. Food Chem. 55 (11), 4501–4510. https://doi.org/10.1021/jf0636418.

Folkertsma, B., Fox, P., 1992. Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. J. Dairy Res. 59 (2), 217–224. https://doi:10.1017/S0022029900030466.

Gallardo-Chacón, J.J., Vichi, S., López-Tamames, E., Buxaderas, S., 2010. Changes in the sorption of diverse volatiles by *Saccharomyces cerevisiae* lees during sparkling wine aging. J. Agric. Food Chem. 58 (23), 12426–12430. https://doi.org/10.1021/jf103086e.

Gaggiotti, S., Mascini, M., Cichelli, A., Del Carlo, M., Compagnone, D., 2021. A hairpin DNA-based piezoelectric E-nose: exploring the performances of heptamer loops for the detection of volatile organic compounds. Chemosensors 9 (5), 115. https://doi.org/10.3390/chemosensors9050115.

Gaggiotti, S., Palmieri, S., Della Pelle, F., Sergi, M., Cichelli, A., Mascini, M., Compagnone, D., 2020. Piezoelectric peptide-hpDNA based electronic nose for the detection of terpenes; Evaluation of the aroma profile in different Cannabis sativa L. (hemp) samples. Sens. Actuators B Chem. 308, 127697 https://doi.org/10.1016/j.snb.2020.127697.

Gnoinski, G.B., Schmidt, S.A., Close, D.C., Goemann, K., Pinfold, T.L., Kerslake, F.L., 2021. Novel methods to manipulate autolysis in sparkling wine: effects on yeast. Molecules 26 (2), 387. https://doi.org/10.3390/molecules26020387.

Gonçalves, C., Ferreira, C., Gonçalves, L.G., Turner, D.L., Leandro, M.J., Salema-Oom, M., Santos, H., Gonçalves, P.A., 2019. New pathway for mannitol metabolism in yeasts suggests a link to the evolution of alcoholic fermentation. Front. Microbiol. 10, 2510. https://doi.org/10.3389/fmicb.2019.02510, 2019.

González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., Esteve-Zarzoso, B., Mas, A., Canals, J.M., Zamora, F., 2015. Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. Eur. Food Res. Technol. 240, 999–1012. https://doi.org/10.1007/s00217-014-2404-8.

ISO 85864, 2014. Sensory Analysis - General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors. ISO 8586:2012, Corrected version 2014-06–15).

Ivit, N.N., Kemp, B., 2018. The impact of non-*Saccharomyces* yeast on traditional method sparkling wine. Fermentation 4 (3), 73. https://doi.org/10.3390/fermentation4030073.

Ivit, N.N., Loira, I., Morata, A., Benito, S., Palomero, F., Suarez-Lepe, J.A., 2018. Making natural sparkling wines with non-*Saccharomyces* yeasts. Eur. Food Res. Technol. 244, 925–935. https://doi.org/10.1007/s00217-017-3015-y.

La Gatta, B., Picariello, G., Rutigliano, M., Lopriore, G., Petrella, G., Rusco, G., Tremonte, P., Di Luccia, A., 2016. Addition of lees from base wine in the production of Bombino sparkling wine. Eur. Food Res. Technol. 242 (8), 1307–1317. https://doi.org/10.1007/s00217-016-2634-z.

Lambert-Royo, M.I., Ubeda, C., Del Barrio-Galána, R., Sieczkowski, N., Canals, J.M., Peña-Neira, A., Cortiella, M.G., 2022. The diversity of effects of yeast derivatives during sparkling wine aging. Food Chem. 190, 133174 https://doi.org/10.1016/j.foodchem.2022.133174.

Lemos Junior, W.J., da Silva Duarte, V., Treu, L., Campanaro, S., Nadai, C., Giacomini, A., Corich, V., 2018. Whole genome comparison of two *Starmerella bacillaris* strains with other wine yeasts uncovers genes involved in modulating important winemaking traits. FEMS Yeast Res. 18, foy069. https://doi.org/10.1093/femsyr/foy069.

Lombardi, S.J., De Leonardis, A., Lustrato, G., Testa, B., Iorizzo, M., 2015. Yeast autolysis in sparkling wine aging: use of killer and sensitive *Saccharomyces cerevisiae* strains in co-culture. Recent Pat. Biotechnol. 9 (3), 223–230. https://doi.org/10.2174/1872208310666160414102015.

Martínez-García, R., García-Martínez, T., Puig-Pujol, A., Mauricio, J.C., Moreno, J., 2017. Changes in sparkling wine aroma during the second fermentation under CO_2 pressure in sealed bottle. Food Chem. 237, 1030–1040. https://doi.org/10.1016/j.foodchem.2017.06.066.

Martínez-García, R., Moreno, J., Bellincontro, A., Centioni, L., Puig-Pujol, A., Peinado, R. A., Mauricio, J.C., García-Martínez, T., 2021. Using an electronic nose and volatilome analysis to differentiate sparkling wines obtained under different conditions of temperature, ageing time and yeast formats. Food Chem. 334, 127574 https://doi.org/10.1016/j.foodchem.2020.127574.

Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., Ortega-Heras, M., Pérez-Magariño, S., 2013. Sparkling wines produced from alternative varieties: sensory attributes and evolution of phenolics during winemaking and aging. Am. J. Enol. Vitic. 64 (1), 39–49. https://doi.org/10.5344/ajev.2012.12013.

Mascini, M., Gaggiotti, S., Della Pelle, F., Wang, J., Pingarrón, J.M., Compagnone, D., 2019. Hairpin DNA-AuNPs as molecular binding elements for the detection of volatile organic compounds. Biosens. Bioelectron. 123, 124–130. https://doi.org/10.1016/j.bios.2018.07.028.

Mascini, M., Gaggiotti, S., Della Pelle, F., Di Natale, C., Qakala, S., Iwuoha, E., Pittia, P., Compagnone, D., 2018. Peptide modified ZnO nanoparticles as gas sensors array for volatile organic compounds (VOCs). Front. Chem. 6, 105. https://doi.org/10.3389/fchem.2018.00105.

Medina-Trujillo, L., González-Royo, E., Sieczkowski, N., Heras, J., Canals, J.M., Zamora, F., 2017. Effect of sequential inoculation (*Torulaspora delbrueckii*/*Saccharomyces cerevisiae*) in the first fermentation on the foaming properties of sparkling wine. Eur. Food Res. Technol. 243 (4), 681–688. https://doi.org/10.1007/s00217-016-2781-2.

Milanovic, V., Ciani, M., Oro, L., Comitini, F., 2012. *Starmerella bombicola* influences the metabolism of *Saccharomyces cerevisiae* at pyruvate decarboxylase and alcohol dehydrogenase level during mixed wine fermentation. Microb. Cell Factories 11 (1), 18. https://doi.org/10.1186/1475-2859-11-18.

Nisiotou, A., Sgouros, G., Mallouchos, A., Nisiotis, C.S., Michaelidis, C., Tassou, C., Banilas, G., 2018. The use of indigenous *Saccharomyces cerevisiae* and *Starmerella bacillaris* strains as a tool to create chemical complexity in local wines. Food Res. Int. 111, 498–508. https://doi.org/10.1016/j.foodres.2018.05.035.

OIV Focus, 2020. The Global Sparkling Wine Market. Available online: https://www.oiv.int/public/medias/7291/oiv-sparklingfocus-2020.pdf.

Penacho, V., Valero, E., Gonzalez, R., 2012. Transcription profiling of sparkling wine second fermentation. Int. J. Food Microbiol. 153 (1), 176–182. https://doi.org/10.1016/j.ijfoodmicro.2011.11.005.

Perpetuini, G., Battistelli, N., Tittarelli, F., Suzzi, G., Tofalo, R., 2021a. Influence of *FLO1* and *FLO5* genes on aroma profile of sparkling wines. LWT-Food Sci. Technol. 146, 111407 https://doi.org/10.1016/j.lwt.2021.111407.

Perpetuini, G., Di Gianvito, P., Arfelli, G., Schirone, M., Corsetti, A., Tofalo, R., Suzzi, G., 2016. Biodiversity of autolytic ability in flocculent *Saccharomyces cerevisiae* strains suitable for traditional sparkling wine fermentation. Yeast 33 (7), 303–312. https://doi.org/10.1002/yea.3151.

Perpetuini, G., Rossetti, A.P., Tittarelli, F., Battistelli, N., Arfelli, G., Suzzi, G., Tofalo, R., 2021b. Promoting *Candida zemplinina* adhesion on oak chips: a strategy to enhance esters and glycerol content of Montepulciano d'Abruzzo organic wines. Food Res. Int. 150, 110772 https://doi.org/10.1016/j.foodres.2021.110772.

Porras-Agüera, J.A., Román-Camacho, J.J., Moreno-García, J., Mauricio, J.C., Moreno, J., García-Martínez, T., 2020. Effect of endogenous CO2 overpressure on the yeast "stressome" during the "prise de mousse" of sparkling wine. Food Microbiol. 89, 103431 https://doi.org/10.1016/j.fm.2020.103431.

Raymond Eder, M.L., Rosa, A.L., 2021. Non-conventional grape varieties and yeast starters for first and second fermentation in sparkling wine production using the traditional method. Fermentation 7 (4), 321. https://doi.org/10.3390/fermentation7040321.

Raymond Eder, M.L., Rosa, A.L., 2012. Genetic, physiological, and industrial aspects of the fructophilic non-*Saccharomyces* yeast species, *Starmerella bacillaris*. Fermentation 7, 87. https://doi.org/10.3390/fermentation7020087.

Redón, M., Guillamón, J.M., Mas, A., Rozès, N., 2008. Effect of active dry wine yeast storage upon viability and lipid composition. World J. Microbiol. Biotechnol. 24 (11), 2555–2563. https://doi.org/10.1007/s11274-008-9779-2.

Renault, P., Miot-Sertier, C., Marullo, P., Hernández-Orte, P., Lagarrigue, L., Lonvaud-Funel, A., Bely, M., 2009. Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: potential applications in the wine industry. Int. J. Food Microbiol. 134 (3), 201–210. https://doi.org/10.1016/j.ijfoodmicro.2009.06.008.

Ruiz-Moreno, M.J., Muñoz-Redondo, J.M., Cuevas, F.J., Marrufo-Curtido, A., León, J.M., Ramírez, P., Moreno-Rojas, J.M., 2017. The influence of pre-fermentative maceration and ageing factors on ester profile and marker determination of Pedro Ximenez sparkling wines. Food Chem. 230, 697–704. https://doi.org/10.1016/j.foodchem.2017.03.048.

Russo, P., Tufariello, M., Renna, R., Tristezza, M., Taurino, M., Palombi, L., Capozzi, V., Rizzello, C.G., Grieco, F., 2020. New insights into the oenological significance of *Candida zemplinina*: impact of selected autochthonous strains on the volatile profile of apulian wines. Microorganisms 8 (5), 628. https://doi.org/10.3390/microorganisms8050628.

Sadoudi, M., Tourdot-Maréchal, R., Rousseaux, S., Steyer, D., Gallardo-Chacón, J.J., Ballester, J., Vichi, S., Guérin-Schneider, R., Caixach, J., Alexandre, H., 2012. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. Food Microbiol. 32 (2), 243–253. https://doi.org/10.1016/j.fm.2012.06.006.

Saerens, S.M., Delvaux, F.R., Verstrepen, K.J., Thevelein, J.M., 2010. Production and biological function of volatile esters in *Saccharomyces cerevisiae*. Microb. Biotechnol. 3 (2), 165–177. https://doi.org/10.1111/j.1751-7915.2009.00106.x.

Sawyer, S., Longo, R., Solomon, M., Nicolotti, L., Westmore, H., Merry, A., Gnoinski, G., Ylia, A., Dambergs, R., Kerslake, F., 2022. Autolysis and the duration of ageing on lees independently influence the aroma composition of traditional method sparkling wine. Aust. J. Grape Wine Res. 28, 146–159. https://doi.org/10.1111/ajgw.12527.

Suzzi, G., Schirone, M., Sergi, M., Marianella, R.M., Fasoli, G., Aguzzi, I., Tofalo, R., 2012. Multistarter from organic viticulture for red wine Montepulciano d'Abruzzo production. Front. Microbiol. 3, 135. https://www.frontiersin.org/article/10.3389/fmicb.2012.00135.

Taillandier, P., Lai, Q.P., Julien-Ortiz, A., Brandam, C., 2014. Interactions between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in wine fermentation: influence of inoculation and nitrogen content. World J. Microbiol. Biotechnol. 30 (7), 1959–1967. https://doi.org/10.1007/s11274-014-1618-z.

Todd, B.E.N., Fleet, G.H., Henschke, P.A., 2000. Promotion of autolysis through the interaction of killer and sensitive yeasts: potential application in sparkling wine production. Am. J. Enol. Vitic. 51 (1), 65–72.

Tofalo, R., Perpetuini, G., Di Gianvito, P., Arfelli, G., Schirone, M., Corsetti, A., Suzzi, G., 2016. Characterization of specialized flocculent yeasts to improve sparkling wine fermentation. J. Appl. Microbiol. 120 (6), 1574–1584. https://doi.org/10.1111/jam.13113.

Tofalo, R., Schirone, M., Torriani, S., Rantsiou, K., Cocolin, L., Perpetuini, G., Suzzi, G., 2012. Diversity of *Candida zemplinina* strains from grapes and Italian wines. Food Microbiol. 29 (1), 18–26. https://doi.org/10.1016/j.fm.2011.08.014.

Torrens, J., Riu-Aumatell, M., Vichi, S., López-Tamames, E., Buxaderas, S., 2010. Assessment of volatile and sensory profiles between base and sparkling wines. J. Agric. Food Chem. 58 (4), 2455–2461. https://doi.org/10.1021/jf9035518.

Torresi, S., Frangipane, M.T., Anelli, G., 2011. Biotechnologies in sparkling wine production. Interesting approaches for quality improvement: a review. Food Chem. 129 (3), 1232–1241. https://doi.org/10.1016/j.foodchem.2011.05.006.

Ubeda, C., Kania-Zelada, I., del Barrio-Galán, R., Medel-Marabolí, M., Gil, M., Peña-Neira, Á., 2019. Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method. Food Res. Int. 119, 554–563. https://doi.org/10.1016/j.foodres.2018.10.032.

van Breda, V., Jolly, N., van Wyk, J., 2013. Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains. Int. J. Food Microbiol. 163 (2), 80–88. https://doi.org/10.1016/j.ijfoodmicro.2013.02.011.