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Research article

Sniffing the wine differences: The role of *Starmerella bacillaris* biofilm-detached cells

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ABSTRACT

This study investigated the impact of 10 strains of *Starmerella bacillaris*, co-inoculated as planktonic or biofilm-detached cells with *Saccharomyces cerevisiae*, on the volatilome of a red wine. The wines produced with *St. bacillaris* biofilm-detached cells exhibited a greater concentration of glycerol and a lower quantity of ethanol than the other wines. Furthermore, these wines exhibited elevated levels of higher alcohols, organic acids, esters, terpenes, and norisoprenoids. Based on the odor activity value and relative odor contribution, isoamyl acetate, ethyl octanoate, ethyl isobutanoate, and methyl decanoate were the main aroma components of wines made with planktonic cells. The main compounds characterizing the wines obtained with biofilm-detached cells were: phenethyl alcohol, β-damascenone, citronellol, β-ionone, and nerol. The sensory analysis revealed that the wines produced with biofilm-detached cells had higher scores for mouth-feel, spicy, floral, and raspberry notes than the others. The present study provides evidence that *St. bacillaris* biofilm-detached cells released specific volatile compounds in red wines.

1. Introduction

Several volatile compounds have been detected in wine and their concentrations range from hundreds of mg/L to the μg/L or ng/L level [1]. The aroma and flavor of a wine play a significant role in assessing its quality and distinguishing between various wine styles across different regions, thereby impacting customer preferences [1]. However, it is important to note that not all volatile molecules impact wine aroma profile. The olfactory impact of a compound is contingent upon its presence at concentrations that exceed this perception threshold. The odor activity values (OAV) were introduced to select odorants with significant impact. The perception of odorants is limited to those with OAVs>1 [2].

Wine aromas can be distinguished into primary, secondary, and tertiary aroma components, generated during distinct winemaking and aging phases [3]. The primary aromas originate from the grape variety. The secondary aromas are released during the fermentation, while the tertiary ones are linked to the prolonged age of the wine [3]. These molecules exist both as free volatiles and as non-volatile sugar-bound glycosidic conjugates. Free aroma compounds are released through the physical crushing of grapes and the chemical or enzymatic breakdown of the volatile compounds by yeast, and/or industrial enzymes (such as glycosidases or peptidases). They encompass esters, aldehydes, terpenes, and various other volatile organic compounds. Bound aromas are typically bound to other molecules in the wine and can be converted into volatile compounds by hydrolysis [4].

Several factors influence the aroma of wine, including yeast strains, grape variety, environmental factors like climate, soil

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composition, and light exposure, and the winemaking process, which includes fermentation and aging [5]. Concerning yeasts, it is important to underline the existence of many yeast genera and species other than *Saccharomyces cerevisiae*, which are referred to as non-*Saccharomyces* yeast. However, it is important to note that only yeasts with a beneficial impact on wine production are included in this category. The description typically excludes well-known rotting yeasts, such as *Dekkera/Brettanomyces* [6]. Previously, these yeasts were seen as spoilage yeasts as they were mainly isolated from spoiled wines, showed uncontrolled fermentation kinetics, and produced high levels of volatile acidity and off-aromas. Recently, researchers have re-evaluated their role in wine production. Selected strains can have a positive influence on the fermentation process. However, due to their limited fermentation ability, it is necessary to create mixed starters that include specific non-*Saccharomyces* yeasts with optimum biotechnological traits, together with *S. cerevisiae*, in order to complete the fermentation [6]. Non-*Saccharomyces* strains exhibit greater intraspecific physiological diversity compared to strains of *S. cerevisiae*, and can influence wine characteristics positively or negatively. This is due to their ability to produce metabolites such as glycerol (which improves the mouth-feel of wines), mannoproteins (which improve the mouth-feel, and roundness of wines and decrease the astringency, protein, and tartrate instability), organic acids (which influence wine acidity), and volatile compounds with a positive impact on wine quality [6]. The use of mixed starter cultures made up of selected non-*Saccharomyces* strains and *S. cerevisiae* is considered a promising strategy to improve wine characteristics. In fact, non-*Saccharomyces* yeasts have metabolic features that might impact the aroma profile [6]. In particular, they can hydrolyze the glycosidic bonds of the odorless and non-volatile glycoside leading to the production of aroma compounds such as terpenols and norisoprenoids. As a result, it becomes imperative to employ these non-*Saccharomyces* strains to exploit their unique attributes during wine fermentation [7].

Starmerella bacillaris (syn. *Candida zemplinina*) has remarkable characteristics in winemaking. These include high production of glycerol, reduction of acetic acid and ethanol levels, increased aroma complexity, ability to develop in the presence of high sugar concentrations, and a preference for fructose [8]. Perpetuini et al. [9,10] showed that the use of *St. bacillaris* cells adhered to oak chips improved the characteristics of Montepulciano d'Abruzzo and Trebbiano d'Abruzzo wines by enhancing the content of glycerol, higher alcohols, esters, and terpenes.

Biofilms can be present throughout the food fermentation process, exerting notable impacts on the overall quality and flavor of fermented foods; in fact, the cells embedded in a biofilm (sessile cells) differ from their free-living counterparts (planktonic cells) [11].

However, the majority of the studies have investigated the metabolic features of sessile cells, while the characteristics of biofilm-detached cells have received little attention. Biofilm-detached cells show growth kinetics and cell surface features similar to those of attached biofilm cells [12]. Moreover, these cells exhibit greater stress resistance since they are more similar to sessile cells than to planktonic ones. However, they are still less resistant than the connected biofilm itself [13]. According to Bastard et al. [14], Tofalo et al. [15], and Pannella et al. [16], *O. oeni* biofilm-detached cells can improve the outcome of malolactic fermentation, showing similar kinetics to those observed in sessile cells. Moreover, Perpetuini et al. [17] compared the volatile compounds produced by different strains of *Kluyveromyces marxianus* under different aggregation states: planktonic, biofilm-detached, and MATS-forming cells. Higher alcohols, ketones, phenols, and terpenes were mainly produced by biofilm-detached cells. Moreover, the same authors showed that the co-inoculation of *S. cerevisiae* and biofilm-detached cells of *St. bacillaris* improved the red color of wines [18].

Therefore, in this study, the influence of biofilm-detached cells on the overall aroma characteristics of Montepulciano d'Abruzzo wine was investigated. In particular, the impact of different strains of *St. bacillaris* inoculated as planktonic or biofilm-detached cells on the volatilome and sensory profile of wines was compared.

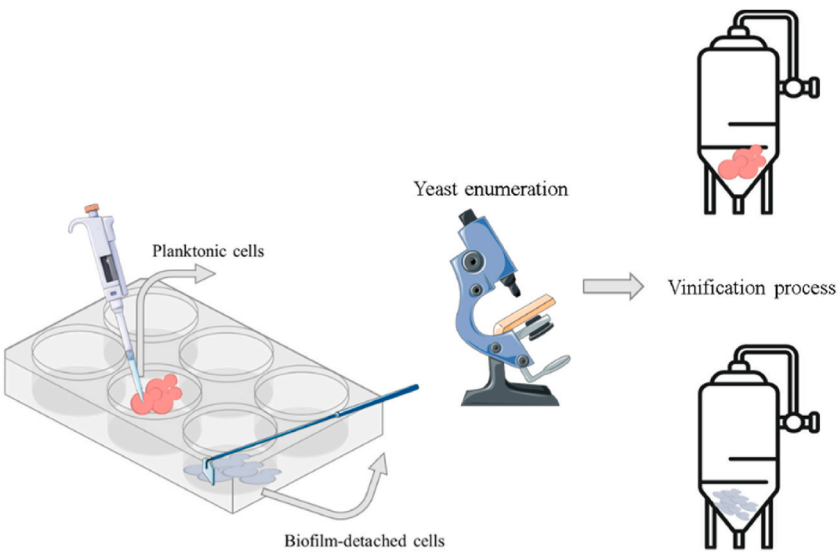

Fig. 1. Schematic representation of the experimental design of this study. Graphical elements were obtained from Servier Medical Art by Servier, available on https://smart.servier.com/(accessed on 12 June November 2024) under a Creative Commons Attribution 3.0 Unported License.

2. Materials and methods

2.1. Strains origin

Ten strains of *St. bacillaris* (SB1, SB3, SB5, SB7, SB8, SB9, SB10, FUC9, FUC16, and FUC17) and a strain of *S. cerevisiae* (SRS1) were used. The strains belong to the Culture Collection of the Microbial Biotechnology Laboratory (Department of BioScience and Technology for Food, Agriculture, and Environment at the University of Teramo, Italy) and were characterized in previous studies [9, 19–21]. FUC9, FUC16, and FUC17 were isolated from Nero Antico di Pretalucente grapes, while the other strains were from Montepulciano d'Abruzzo grapes. All strains were routinely grown on YPD medium (1 % w/v yeast extract, 2 % w/v peptone, and 2 % w/v glucose) and incubated at 28 °C for 48 h. The strains were stored at −80 °C in YPD broth supplemented with glycerol (20 % v/v, Sigma-Aldrich, Milan, Italy).

2.2. Cellar vinifications

The vinification process was conducted in tanks with a capacity of 50 L containing Montepulciano must (248 g/l of fermentable sugars, 7.67 titratable acidity, pH of 3.4). Both yeasts (*S. cerevisiae* and *St. bacillaris*) were co-inoculated at a final concentration of 6 Log CFU/mL. *Starmerella bacillaris* strains were inoculated both as planktonic and biofilm-detached cells. In order to obtain biofilm-detached cells, *St. bacillaris* cells were inoculated in flat-bottom 6-well cell culture plates (Costar, Corning, NY, USA) with 5 mL of YPD and incubated for 7 days at 28 °C. Planktonic and loosely attached cells were recovered by washing the wells with phosphate-buffered saline. Sessile cells were removed through a cell scraper (Fig. 1) [22].

The fermentations were carried out in triplicate at 25 °C ± 2 °C under static conditions. The yeast lees were allowed to settle for 7 days at the end of fermentation. Subsequently, the wines were transferred into glass bottles of 750 mL, sealed with crown caps, and kept at a temperature of 15–20 °C for a maximum duration of 30 days, during which sensory evaluations were conducted.

The main wine analytical parameters were evaluated through FOSS WineScan (FT-120) rapid scanning Fourier Transform Infrared Spectroscopy with FOSS WineScan software version 2.2.1 according to the manufacturer's instructions. Official OIV methods were used for the instrument calibration [23]. The pH was measured using an InoLab 730 pH meter (WTW, Weilheim, Germany).

2.3. Volatile organic compounds detection

Solid-phase microextraction (SPME) was used to extract volatile organic compounds (VOCs) according to Perpetuini et al. [9]. The gas chromatography-mass spectrometry (GC-MS) analysis was carried out using a gas chromatograph (Clarus 580; PerkinElmer, Waltham, MA, USA) coupled with a mass spectrometer (SQ8S; PerkinElmer). An 85 μm fiber coated with carboxen-polydimethylsiloxane (Sigma-Aldrich, Milan, Italy) was used. The subsequent program was implemented: 50°C for 2 min; first ramp: 1°C min to 65 C; second ramp: 10°C min to 150°C (10 min hold); third ramp: 10°C min to 200°C (1 min hold). Aroma components were identified by comparing the retention times of pure reference standards tested under the same conditions. 2-methyl-hexanol was employed as an internal standard. In order to provisionally identify the compounds, a minimum similarity threshold of 85 % was employed to match mass spectra with MS fragmentation patterns in the National Institute for Standards and Technology database (NIST version 2005). The volatile compounds were quantified using the calibration curve of standards (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, 1-octanol, 1-hexanol, 2-phenylethanol, octanoic acid, acetic acid, 2, 3-butanedione, 4-vinylguaiacol, ethyl phenol, benzaldehyde, acetaldehyde), plotting the relative peak areas (analyte versus the corresponding internal standard) as a function of the compound concentration. The concentration of the volatile compounds without a pure reference was determined by utilizing the calibration curve of a standard compound with a closely related chemical structure [24]. The analyses were conducted in triplicate.

2.4. Odor activity value (OAV) and relative odor contribution (ROC)

The OAV was calculated by dividing the concentration of each compound by its odor threshold value (OTS). ROC (%) is the ratio of OAV percentage of each individual compound and the sum of the OAV of compounds with OAV>1 [25].

2.5. Sensory analysis

Twenty tasters (12 women and 8 men with an age ranging from 25 to 62 years old) participated in the sensory analysis. The judges were trained according to the ISO 8586-2012 regulation [26] and evaluated the following descriptors: (persistence, body, astringency, mouth-feel, grassy, reduced, floral, fruity, spicy, cherry, raspberry, plum, jam). The scale ranged from zero, representing the absence of perception, to ten, representing the highest level of perception. The samples were assigned a 3-digit numerical code. The analysis was conducted in a sensory room, with separate booths illuminated by white lights, following the ISO 8589:2007 regulation [27].

2.6. Statistical analysis

Prism 7.0 program (GraphPad Software Inc., La Jolla, CA, USA) was used to analyse the data and prepare the graphs. XLStat 2014 software (Addinsoft, New York, NY, USA) was used to carry out the principal component analysis (PCA). The significant differences (p

< 0.05) of oenological parameters and volatile compounds were evaluated by the *t*-test. The hierarchical clustering heat map was graphed by ChiPlot (https://www.chiplot.online) before data normalization based on the Z-score.

3. Results and discussion

3.1. Oenological parameters

The sugars were exhausted in all trials, and the final alcohol content ranged from 13.3 % (vol/vol) to 14.1 % (vol/vol) and from 13.9 % (vol/vol) to 14.7 % (vol/vol) in wines obtained with biofilm-detached and planktonic cells, respectively (Table 1). The lower ethanol content in the presence of biofilm-detached cells could be related to a redirection of carbon fluxes, with the formation of acetaldehyde, acetate, and acetyl-CoA, and their integration into the glyoxylate cycle or Krebs cycle to produce energy, or into lipid and amino acid synthesis [28]. Moreover, Moreno-García et al. [29] compared the proteome between yeasts grown under biofilm formation conditions and no biofilm formation conditions. These authors found that Ald4p, which plays a role in ethanol degradation, resulting in the production of acetaldehyde and its conversion to acetate, was only present in cells grown as biofilm. The obtained data highlighted that wines obtained with biofilm-detached cells showed a higher amount of glycerol compared to planktonic ones. In fact, these wines contained about 2 g more glycerol than those made with planktonic cells. At these concentrations, glycerol enhances the viscosity and smoothness of the wine, resulting in a favorable impact on its flavor [30]. The occurrence of proteins involved in glycerol metabolism in biofilm-detached cells could explain this data. Desai et al. [31] found that the genes responsible for glycerol biosynthesis are up-regulated in biofilms, and the levels of glycerol are considerably greater in sessile cells than in planktonic ones [31]. It is not surprising, since glycerol metabolism has a significant role in activating the expression of genes related to biofilm formation. Based on the obtained results, it appears that biofilm-detached cells had a metabolism more similar to sessile cells than to planktonic ones in terms of glycerol production. The other parameters showed no significant differences.

3.2. Volatile composition of wines

Sessile cells and biofilm-detached cells show different cell growth, physiology, and metabolic activity from their planktonic counterparts. The majority of studies have been performed on sessile cells [9,10,29,32,33]. Few data are available concerning the impact of non-*Saccharomyces* biofilm-detached cells on wine aroma characteristics. Therefore, this study deserves special attention for examining the impact of *St. bacillaris* biofilm-detached cells on the volatile profile of Montepulciano d'Abruzzo wines. The non-*Saccharomyces* population has a wider range of extracellular enzymes compared to *S. cerevisiae*. In particular, *St. bacillaris* demonstrated the capacity to generate aroma compounds through the action of its excreted enzymes, including esterases, glycosidases, lipases, β-glucosidases, proteases, and cellulases. The protease activity breaks down proteins, promoting the availability of amminoacids, which are aroma compound precursors. The cellulolytic and hemicellulolytic enzymes and glycosidases have a crucial function in facilitating the extraction of volatile compounds from the grape berry [8].

A total of 71 compounds belonging to different chemical classes (higher alcohols, organic acids, esters, ketones, norisoprenoids, terpenes, phenols, and aldehydes) were detected. Wines obtained with planktonic or biofilm-detached cells contained a similar amount of ketones, phenols, and aldehydes. Their presence was primarily strain-dependent. In particular, 3 ketones were detected (2-butanone, 2,3-butanedione, and 2-nonanone), and their concentrations ranged from 0.64 mg/L to 1.95 mg/L and from 0.71 mg/L to 1.64 mg/L in wines obtained with planktonic and biofilm-detached cells, respectively. Aldehydes (benzaldehyde, 3-furaldehyde) content varied from 1.38 mg/L to 2.58 mg/L and from 2.26 mg/L to 3.32 mg/L in wines obtained with planktonic and biofilm-detached cells, respectively. In both wines, the phenol content (4-vinylguaiacol and 4-ethylguaiacol) was less than 2 mg/L. For the other compounds, quantitative differences were obtained.

The total content of higher alcohols ranged from 27.49 mg/L to 34.48 mg/L and from 30.32 mg/L to 36.84 mg/L in wines obtained with planktonic and biofilm-detached cells, respectively (Fig. 2 and Supplementary Table S1). The inoculation of *St. bacillaris* biofilm-detached cells induced an increase of about 10 % of their content (Fig. 2). In particular, this type of inoculation resulted in an increase of isoamyl alcohol (fusel, alcoholic, whiskey, fruity, banana), heptanol, and phenethyl alcohol (sweet, floral, fresh, bready, rose, honey), and a decrease of 1-butanol 2-methyl (Supplementary Table S1). The production of higher alcohols may help in the detoxification of any aldehydes that are formed during the breakdown of amino acids, or it may play a role in regulating the synthesis of amino acids [34].

The mechanisms involved in higher alcohol production are well-known in *S. cerevisiae*, while little information is available concerning *St. bacillaris*. A recent study performed by Russo et al. [8] tested the ability of different strains of *St. bacillaris* to produce aroma compounds during must fermentation as a single culture. The obtained results revealed that 3-methyl-1-butanol and phenylethanol were the main alcohols produced, ranging from 24 mg/L to 65.83 mg/L and from 11.97 mg/L to 46.69 mg/L. Moreover, these compounds showed an OAV >1, contributing a fine fruity and rose odor to the wines. According to Sadoudi et al. [35], *St. bacillaris* produces 2.5 times lower amounts of higher alcohols than *S. cerevisiae*. The main alcohols produced were isoamyl alcohol, isobutanol, butanol 3-methyl, and 2-phenylethyl alcohol.

The synthesis of higher alcohols in *S. cerevisiae* is mediated by a large number of genes and proteins involved in the Ehrlich pathway. According to Moreno-Garcia et al. [29], some aminotransferases, such as Aro8p, Sfa1p, Bat1p, and Bat2p, were detected in both sessile and planktonic cells of *S. cerevisiae*, even if they were more abundant in cells grown as biofilm. On the other hand, Thi3p, involved in leucine deamination, was only detected in sessile cells. Probably, the different metabolic activities of biofilm-detached cells and planktonic cells can explain this difference. These results suggested a reorientation of metabolic fluxes in biofilm-detached cells

Table 1
Main oenological parameters detected in wines obtained with *S. cerevisiae* and *St. bacillaris* biofilm-detached or planktonic cells. [a]

Trial	Ethanol (% v/v)		Residual sugars (g/L)		pH		Titratable acidity (g/L)*		Volatile acidity (g/L)**		Glycerol	
	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic	Biofilm-detached	Planktonic
SRS1+SB1	13.5 ± 0.32^{A}	14 ± 0.14^{B}	0.37 ± 0.03^{A4}	0.35 ± 0.03^{A}	3.33 ± 0.13^{A}	3.31 ± 0.05^{A}	5.53 ± 1.33^{A}	5.61 ± 1.43^{A}	0.45 ± 0.03^{A}	0.43 ± 0.08^{A}	8.12 ± 1.23^{B}	6.13 ± 0.44^{A}
SRS1+SB3	14.1 ± 0.13^{A}	14.5 ± 0.53^{A}	0.31 ± 0.08^{A}	0.32 ± 0.03^{A}	3.3 ± 0.27^{A}	3.32 ± 0.17^{A}	5.65 ± 0.37^{A}	5.71 ± 1.04^{A}	0.48 ± 0.03^{A}	0.51 ± 0.03^{A}	9.22 ± 0.44^{B}	7.65 ± 0.35^{A}
SRS1+SB5	14 ± 0.54^{A}	14.5 ± 0.99^{A}	0.44 ± 0.03^{A}	0.41 ± 0.02^{A}	3.33 ± 0.08^{A}	3.35 ± 0.14^{A}	5.76 ± 2.12^{A}	5.71 ± 0.32^{A}	0.51 ± 0.08^{A}	0.49 ± 0.04^{A}	7.82 ± 2.43^{B}	6.22 ± 1.93^{A}
SRS1+SB7	13.3 ± 1.78^{A}	13.9 ± 2.13^{B}	0.47 ± 0.04^{A}	0.44 ± 0.04^{A}	3.33 ± 0.15^{A}	3.34 ± 0.34^{A}	6.33 ± 1.93^{A}	6.37 ± 1.34^{A}	0.55 ± 0.07^{A}	0.53 ± 0.09^{A}	7.57 ± 1.22^{B}	$6.190.56^{A}$
SRS1+SB8	13.6 ± 1.23^{A}	14.3 ± 2.23^{B}	0.44 ± 0.06^{A}	0.39 ± 0.07^{A}	3.32 ± 0.07^{A}	3.31 ± 0.14^{A}	5.99 ± 1.23^{A}	5.83 ± 1.98^{A}	0.47 ± 0.03^{A}	0.51 ± 0.02^{A}	8.74 ± 2.89^{B}	6.55 ± 1.29^{A}
SRS1+SB9	13.4 ± 0.67^{A}	14.1 ± 0.43^{B}	0.32 ± 0.06^{A}	0.29 ± 0.03^{A}	3.34 ± 0.16^{A}	3.33 ± 0.04^{A}	6.51 ± 0.32^{A}	6.43 ± 0.67^{A}	0.53 ± 0.02^{A}	0.51 ± 0.06^{A}	9.55 ± 0.77^{B}	7.92 ± 0.93^{A}
SRS1+SB10	13.5 ± 0.37^{A}	14.5 ± 0.57^{B}	0.33 ± 0.03^{A}	0.31 ± 0.02^{A}	3.31 ± 0.21^{A}	3.33 ± 0.17^{A}	6.26 ± 0.53^{A}	6.32 ± 0.75^{A}	0.59 ± 0.13^{A}	0.61 ± 0.11^{A}	8.76 ± 1.98^{B}	7.31 ± 0.37^{A}
SRS1+FUC9	14.1 ± 0.22^{A}	14.6 ± 0.65^{B}	0.34 ± 0.05^{A}	0.29 ± 0.04^{A}	3.3 ± 0.05^{A}	3.3 ± 0.07^{A}	6.63 ± 2.32^{A}	6.59 ± 1.09^{A}	0.47 ± 0.05^{A}	0.51 ± 0.07^{A}	9.36 ± 2.32^{B}	7.88 ± 2.36^{A}
SRS1+FUC16	14.1 ± 0.12^{A}	14.7 ± 0.17^{B}	0.36 ± 0.06^{A}	0.28 ± 0.03^{A}	3.29 ± 0.05^{A}	3.31 ± 0.08^{A}	6.26 ± 1.98^{A}	6.33 ± 1.73^{A}	0.56 ± 0.08^{A}	0.52 ± 0.03^{A}	9.57 ± 1.66^{B}	78.37 ± 0.32^{A}
SRS1+FUC17	14.1 ± 0.43^{A}	14.5 ± 0.84^{B}	0.41 ± 0.05^{A}	0.31 ± 0.02^{A}	3.28 ± 0.03^{A}	3.31 ± 0.13^{A}	6.39 ± 0.32^{A}	6.36 ± 2.12^{A}	0.57 ± 0.03^{A}	0.61 ± 0.05^{A}	9.34 ± 0.43^{B}	7.98 ± 1.76^{A}

[a] * Expressed as tartaric acid. ** Expressed as acetic acid.

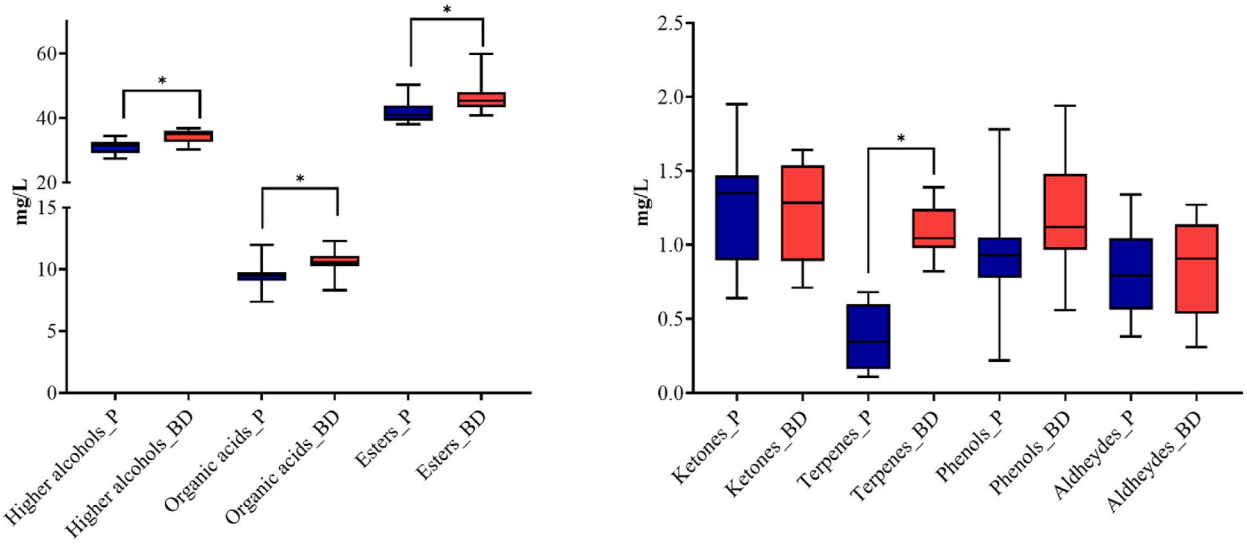

Fig. 2. Box plot showing the total amount of detected compounds grouped on the basis of chemical classes. BD: biofilm-detached cells, P: planktonic cells.

with an increased production of amino acids, which are then converted into higher alcohols. Moreover, an increased activity of amino acid transmembrane transporters in biofilm-detached cells could be postulated. A similar metabolic shift can be postulated in *St. bacillaris* biofilm-detached cells.

Esters confer fruity and floral notes to wines [36]. Several genes, including *ATF*1, Lg-*ATF*1, *ATF*2, *EEB*1, *EHT*1, and *IAH*1, mediate the formation of esters in *S. cerevisiae* [36]. The genes involved in ester synthesis in *St. bacillaris* are unknown. However, according to Russo et al. [8], *St. bacillaris* produced ethyl and acetate esters in a strain-dependent way, and the main ester released during must fermentation is ethyl octanoate. The ability to produce esters has also been revealed by Sadoudi et al. [35], who highlighted that the main esters produced by the tested strains were isoamyl acetate, 2-phenylethyl acetate, and ethyl hexanoate.

A total of 30 esters were detected, and isoamyl acetate, ethyl butanoate, ethyl decanoate, ethyl octanoate, and ethyl acetate were the main ones (Supplementary Table S1). Wines obtained with biofilm-detached cells showed a higher concentration of esters than those obtained with planktonic cells. In particular, their concentration ranged from 38.09 mg/L to 50.33 mg/L and from 40.82 mg/L to 59.87 mg/L in wines obtained with planktonic and biofilm-detached cells, respectively (Fig. 2). The main esters detected were isoamyl acetate, ethyl decanoate, ethyl octanoate, and ethyl acetate. The use of biofilm-detached cells allowed to obtain wines characterized by a higher content of phenethyl acetate (sweet, honey, floral rosy), diethyl succinate (fruity, cooked apple, ylang), butyl ethanoate (sweet, fruity), butyl butanoate (sweet, fruity, fresh, ripe), ethyl lactate (sweet, fruity, ethereal, buttery, butterscotch), butyl lactate (green, fruity, lactonic, waxy, winey, apple), geranyl phenylacetate (fruity, honey, geranium), and methyl decanoate (oily, wine, fruity, floral) than the others (Supplementary Table S1). Other authors have also reported an increase in ester formation in biofilm-forming cells [9,22]. For instance, Perpetuini et al. [9] highlighted that *St. bacillaris* adhering to oak chips induced an increase in ester concentration in red wines. Moreover, Moreno-Garcia et al. [29] revealed that even if the production and consumption of the ethyl esters of acetic, lactic, octanoic, and decanoic acids were similar in sessile and planktonic cells, some esters (e.g., ethyl acetate) were produced only by sessile cells. Two factors primarily influence the formation of esters: the concentration of acyl-CoA and fusel alcohols, the activity of the enzymes involved in their formation (alcohol acetyl transferases I and II, encoded by *ATF*1 and *ATF*2 genes, respectively), and degradation (esterases). Probably, *ATF*1 and *ATF*2 genes are upregulated in biofilm-detached cells since ester formation can be considered a sort of stress response involved in the detoxification mechanism for free medium-chain fatty acids (C6, C8, and C10). The occurrence of these fatty acids during must fermentation could cause stuck and sluggish alcoholic fermentations since they inhibit the growth of *S. cerevisiae* [37,38].

During wine fermentation, yeasts produce acetic acid, as well as short-, medium-, and long-chain fatty acids [39]. According to some studies, *St. bacillaris* is associated with low production of acetic acid. The main organic acids produced are methyl butanoic, methyl propanoic, octanoic, and dodecanoic acids [8,35]. A total of 13 volatile organic acids have been detected. The wines obtained with biofilm-detached cells showed a higher concentration of organic acids than the others. Their content ranged from 7.36 mg/L to 11.96 mg/L and from 8.3 mg/L to 12.29 mg/L in wines obtained with planktonic and biofilm-detached cells, respectively (Fig. 2 and Supplementary Table S1). Acetic acid, nonanoic acid, octadecanoic acid, and butanoic acid were mainly present in wines obtained with biofilm-detached cells. Isovaleric acid and 2-phenylacetic acids were detected only in these wines. Moreno-Garcia et al. [29] revealed an increase in organic acid content when yeast was grown under biofilm-forming conditions. In particular, the proteomic analysis of yeasts revealed that Fas2p and Fas3p, involved in the biosynthesis of saturated fatty acids, were only found under biofilm-forming conditions, while Pox1p, involved in the fatty acid oxidation pathway, was only detected under non-biofilm-forming conditions [29]. Probably, a differential expression of genes involved in organic acid biosynthesis and degradation can explain the obtained

results.

The grape is the primary source of terpenes and norisoprenoids in wines. They occur in low quantities in wines, but because of their low odor thresholds, they contribute to wine aroma, by imparting floral and fruity notes [40, 41].

St. bacillaris is able to produce β-damascenone, linalool, α-terpineol, citronellol, geraniol, nerolidol, and farnesol [8,35]. Extracellular enzymes, including pectinases, glycosidases, and glucanases, described in this species, contribute to this ability by cleaving terpenes and isoprenoids from their sugar-bound precursors [8]. The importance of *St. bacillaris* has been demonstrated by Sadoudi et al. [35], who revealed that the levels of terpenols and norisoprenoids were lower in wines obtained with a co-inoculation of *St. bacillaris* and *S. cerevisiae* than those inoculated with *St. bacillaris* in monoculture. Yeasts, like *S. cerevisiae*, do not efficiently release monoterpenes. They produce geranyl diphosphate, as a step in the synthesis of farnesyl diphosphate. This compound is crucial in the isoprenoid pathway, which ultimately leads to the production of dolicols, ubiquinones, and sterols [35]. Four terpenes (linalool, citronellol, nerol, and eugenol) and 2 norisoprenoides (β-damascenone and β-ionone) were detected (Supplementary Table S1). Under the conditions applied in this study, the production of norisoprenoids was strain-dependent and not influenced by the lifestyle. On the contrary, terpenes were mainly detected in wines obtained with *St. bacillaris* biofilm-detached cells. In fact, their concentration ranged from 0.11 mg/L to 0.68 mg/L and from 0.79 mg/L to 1.34 mg/L in wines obtained with planktonic and biofilm-detached cells, respectively. In particular, the wines produced with biofilm-detached cells were characterized by the presence of nerol, whose concentration was at least 2-fold higher in these wines than in the others. Terpenes confer flowery and fruity notes to the wine. Probably, biofilm-detached cells are characterized by a major presence of enzymes involved in terpene release and/or a different contribution of these enzymes in a planktonic or biofilm-detached state.

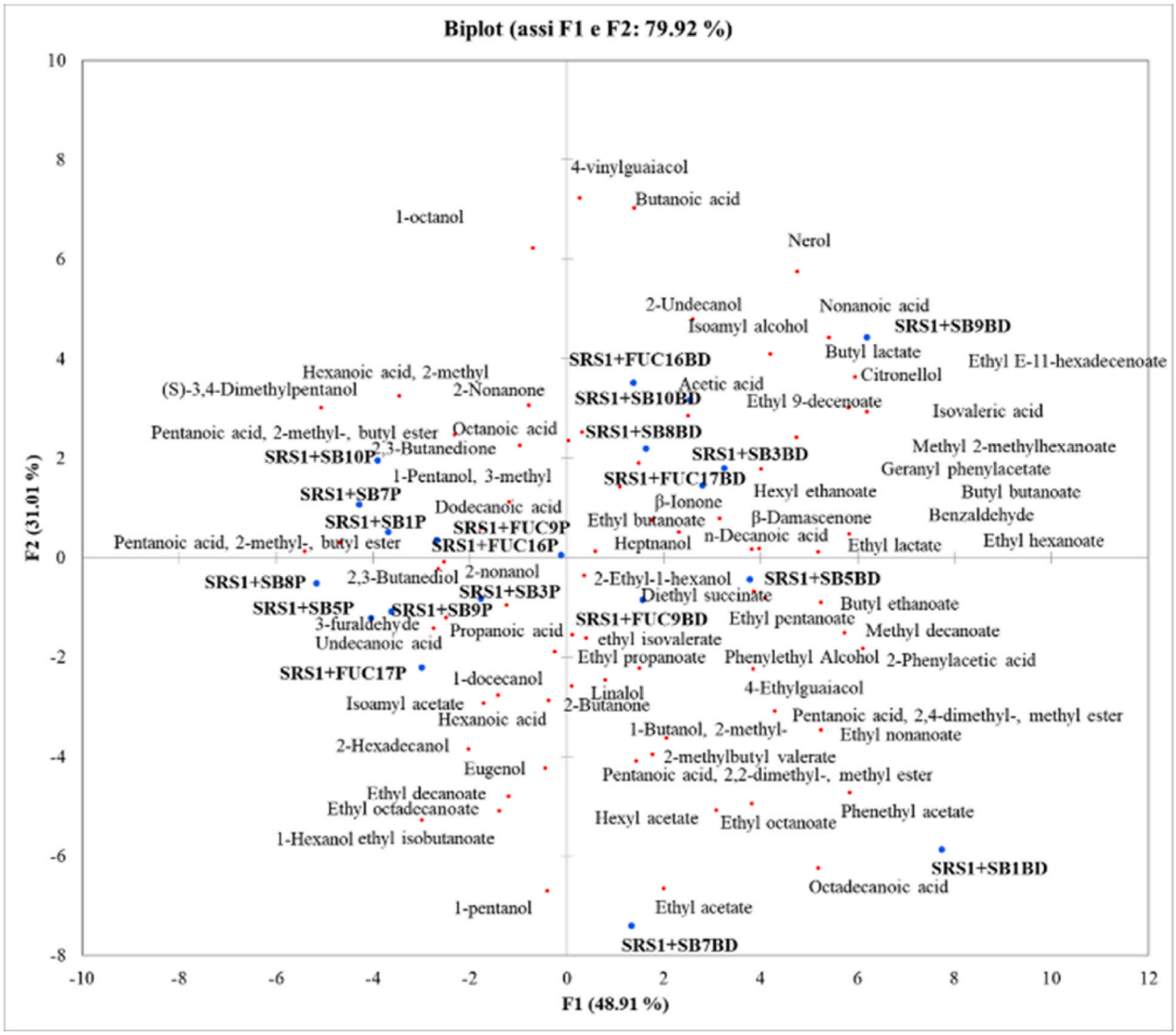

Fig. 3. Biplot of aroma compounds. BD: biofilm-detached cells, P: planktonic cells.

In order to better highlight the differences between wines obtained with planktonic and biofilm-detached cells, a PCA analysis was performed. PCA explained 79.92 % of the total variance (48.91 % and 31.01 % for F1 and F2, respectively). Wines were well differentiated based on the inoculation strategy used (Fig. 3). Wines obtained with biofilm-detached cells were present in the I and IV quadrants, while the others were in the II and III ones. Moreover, a strain-dependent effect can be highlighted (Fig. 3). In fact, wines obtained with SRS1+SB9BD, SRS1+FUC16BD, SRS1+SB10BD, SRS1+SB8BD, SRS1+SB3BD, and SRS1+FUC17BD clustered together and were differentiated from the others for 24 compounds (5 organic acids, 10 esters, 3 higher alcohols, 2 ketones, 4-vinyl-guiacol, and benzaldehyde). While wines obtained with SRS1+SB5BD, SRS1+FUC9BD, SRS1+SB1BD, and SRS1+SB7BD were characterized by 22 compounds (14 esters, 3 higher alcohols, 2 organic acids, 4-ethylguaiacol, linalol, and 2-butanone), Wines obtained with planktonic cells can be divided into two groups. The first is made up of wines obtained with SRS1+SB10P, SRS1+SB7P, SRS1+SB1P, SRS1+FUC9P, and SRS1+FUC19P, and the second is made up of those obtained with SRS1+SB8P, SRS1+SB3P, SRS1+SB5P, SRS1+SB9P, and SRS1+FUC17P. The wines belonging to the I group were characterized by 10 compounds (3 higher alcohols, 2 esters, 3 organic acids, and 2 ketones). While those of the second one has 14 compounds (3 organic acids, 5 higher alcohols, 3-furaldehyde, eugenol, and 4 esters).

3.3. OAV and ROC

In order to better understand the individual contribution of each quantified volatile compound to the overall wine aroma, OAV and ROC for compounds present in concentrations higher than their corresponding odor thresholds were evaluated (Fig. 4 and Table 2). Compounds with an OAV $\geq$1 are directly involved in the definition of wine aroma. However, it is crucial to identify the remaining volatile compounds with OAV <1 as they contribute to wine aroma through a synergistic effect.

There were 30 aroma compounds with OAV>1 in at least one wine sample, including 4 higher alcohols, 2 organic acids, 14 esters, 2 ketones, 2 norisoprenoids, 4 terpenes, a phenol, and an aldehyde. To visualize the main differences between wines obtained with *S. cerevisiae* and *St. bacillaris* grown as planktonic or biofilm-detached cells, a heatmap with hierarchical analysis was constructed. *Starmerella bacillaris* aggregation state influenced the aroma composition of wines; in fact, two main clusters were obtained. The first one contained the wines produced with planktonic cells, while the second one contained the wines obtained with biofilm-detached cells. These last wines were characterized by higher OAV than the others for the following compounds: phenethyl alcohol,

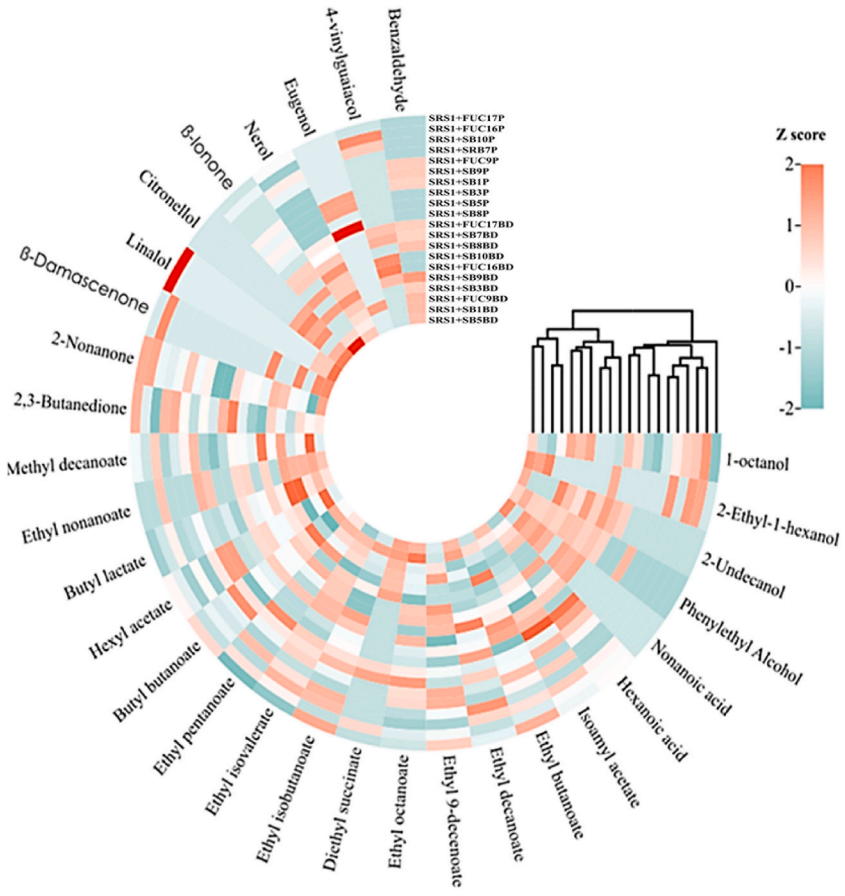

Fig. 4. Hierarchical heat map of aroma compounds with OAV>1. BD: biofilm-detached cells, P: planktonic cells.

Table 2
Odor description and ROC of compounds with an OAV >1. BD: biofilm-detached cells, P: planktonic cells.

Chemical classes	Odor description	OTS	SRS1+SB1		SRS1+SB3		SRS1+FUC9		SRS1+FUC16		SRS1+SB5		SRS1+SB7		SRS1+SB8		SRS1+FUC17		SRS1+SB9		SRS1+SB10	
Higher alcohols			P	Bd	P	Bd	P	Bd	P	Bd	P	Bd	P	Bd	P	Bd	P	Bd	P	Bd	P	Bd
1-octanol	Waxy, green, rose, mushroom,	0.12[c]	0.8	1.1	1.4	2.7	2.6	1.5	4.1	4.7	3.6	2.4	3.7	2.5	3.7	1.7	1.3	1.5	1.8	3.8	3.8	2.8
2-Ethyl-1-hexanol	Citrus, floral, sweet	8[b]	–	0.1	–	–	0.1	0.2	0.1	–	–	0.1	–	–	–	–	–	–	–	–	0.2	–
2-Undecanol	Fresh, waxy, laundered	0.041[b]	–	–	–	–	–	–	–	0.3	0.2	0.2	–	0.2	0.2	0.2	–	0.2	–	0.3	–	0.1
Phenylethyl Alcohol	Sweet, floral, fresh, bready	14[b]	0.1	0.1		0.1		0.1		0.2		0.1		0.1	0.1	0.1		0.1		0.1		0.1
Organic acids																						
Nonanoic acid	Fatty, waxy, cheesy	3[b]	–	–	–	0.1	–	0.2	–	0.2	–	0.1	–	0.1	–	0.1	–	–	–	0.2	–	–
Hexanoic acid	Sour, fatty, sweat	0.42[a]	0.1	0.1	0.3	0.2	–	0.2	0.1	–	0.4	–	–	0.2	–	–	0.2	0.2	0.1	–	0.2	0.2
Esters																						
Isoamyl acetate	Sweet, banana, estery	0.16[b]	7.1	5.8	8.3	8.4	9.1	8.8	9.7	12.0	13.6	7.9	9.8	9.1	11.1	7.7	10.5	8.0	7.2	8.8	11.5	7.2
Ethyl butanoate	Fruity, juicy, cognac	0.4[b]	0.4	0.4	0.5	0.3	0.6	0.5	0.5	0.5	0.4	0.4	0.6	0.8	0.9	0.5	0.9	0.7	0.6	0.9	0.8	0.3
Ethyl decanoate	Sweet, waxy, fruity, apple, grape, brandy	0.2[b]	1.6	1.7	1.3	1.6	0.9	0.8	1.2	1.5	3.3	2.0	3.4	3.7	1.8	1.4	1.7	1.2	1.2	0.9	2.5	0.7
Ethyl 9-decenoate	Fruity, fatty	0.1[a]	1.0	0.9	1.6	2.4	2.1	2.0	1.8	2.0	1.5	1.3	2.0	2.6	2.0	1.8	2.4	0.9	1.9	1.5	1.5	1.8
Ethyl octanoate	Fruity, waxy, apricot, banana, pear	0.005[a]	64.4	61.5	62.0	56.1	67.1	55.5	55.6	36.3	58.1	65.4	49.8	40.5	58.0	65.7	54.7	67.6	61.1	56.4	40.0	58.4
Diethyl succinate	Fruity, cooked apple, ylang	1.2[a]	0.1	0.1	0.1	0.2	–	0.2	0.1	0.3		0.1	–	–	–	–	–	–	–	0.2	–	–
Ethyl isobutanoate	Sweet, ethereal fruity, fusel	0.015[b]	4.4	4.3	4.9	3.4	2.2	3.7	2.9	6.7	5.4	2.6	7.7	8.5	4.5	1.1	8.0	4.9	3.4	1.7	7.3	0.9
Ethyl isovalerate	Fruity, apple, pineapple	0.01[b]	6.6	7.0	9.7	9.9	4.4	10.8	8.9	14.6	4.4	2.3	11.0	14.0	5.7	8.9	2.6	1.6	8.7	1.5	13.8	8.2
Ethyl pentanoate	Green, tropical	0.05[b]	1.3	1.3	2.2	1.7	1.2	2.0	2.0	1.7	1.6	1.4	2.4	1.6	1.4	1.3	1.1	1.7	1.4	2.3	1.5	1.6
Butyl butanoate	Sweet, fruity, fresh ripe	0.1[b]	1.2	1.3	0.5	1.0	1.2	0.4	1.1	2.4	1.8	1.1	1.4	1.7	1.4	1.2	2.0	1.5	2.0	2.6	1.5	1.4
Hexyl acetate	Fruity, green, apple, banana	0.67[b]	0.3	0.3	0.4	0.1	0.2	0.3	0.3	0.5	0.3	0.2	0.4	0.4	0.3	0.2	0.4	0.3	0.3	0.4	0.2	0.2
Butyl lactate	Green, lactonic	1[b]	0.1	0.2	0.1	0.4	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.4	0.3	0.2
Ethyl nonanoate	Fruity, rose, waxy, rum	1.3[b]	0.1	0.1	0.1	0.2	–	0.2	–	–	–	0.1	–	0.1	–	–	–	–	–	0.2	0.2	0.1
Methyl decanoate	Oily, fruity, floral	0.012[b]	9.3	10.8	5.0	7.8	7.1	10.2	7.4	13.6	4.3	6.3	6.2	11.0	6.9	6.9	10.3	8.0	8.7	14.9	14.1	12.5
Ketones																						
2.3-Butanedione	Buttery, creamy, pungent, caramellic	0.1[b]	0.3	0.4	0.6	0.4	0.9	0.8	0.5	1.1	0.6	0.6	1.1	0.6	1.0	0.2	1.1	0.9	0.6	0.6	0.2	0.1
2-Nonanone	Fresh, green, herbal	0.041[b]	0.1		0.2	0.2	0.1	0.2	0.3	0.3	–	0.2	0.2	0.2	–	0.1	0.3	0.2	0.2	0.2	0.2	0.1
β-damascenone	Sweet, fruity rose plum grape, raspberry	0.00005[b]	–	1.5	–	2.4	–	–	2.5	–	–	1.9	–	–	–	–	–	–	–	–	–	1.8
Terpenes																						
Linalol	Citrus, floral, woody, green, blueberry	0.0019[b]	–	0.4	–	–	–	0.7	–	–	–	1.0	–	–	–	–	2.2	–	–	–	–	–
Citronellol	Floral, leather, waxy, rose, bud, citrus	0.1[b]	–	0.1	–	0.2	–	–	–	0.2	–	0.1	–	–	–	–	–	–	–	0.1	–	0.1
β-ionone	Violet, floral, woody	0.005[b]	0.2	0.4	0.4	–	–	–	0.3	–	0.3	1.7	–	1.0	–	–	–	–	0.4	1.2	–	0.9
Nerol	Sweet, neroli, citrus	0.4[b]	–	0.1	–	0.3	–	0.2	–	0.4	–	0.2	0.2	0.3	0.2	0.3	0.2	0.1	–	0.4	0.2	0.2
Eugenol	Sweet, spicy, clove, woody	0.005[b]	0.2	–	0.2	–	–	0.2	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–
Phenols																						
4-vinylguaiacol	Spicy, clove, carnation, phenolic, peppery, smoky, woody	1.1[a]	–	–	–	–	–	–	0.2	0.3	–	–	–	0.2	–	–	–	0.1	–	0.1	0.1	0.1
Aldheydes																						
Benzaldehyde	Almond	2[b]	0.09	0.09		0.13	0.12	0.15	–	–	–	0.11	–	0.14	–	0.12	–	0.10	0.11	0.19	–	–

- compounds that showed OAV <1.
[a] [41].
[b] [18].
[c] [42].

nonanoic acid, ethyl octanoate, diethyl succinate, ethyl isovalerate, methyl decanoate, β-damascenone, citronellol, β-ionone, and nerol. Probably, these compounds could exert a strong effect on wine aroma. ROC values highlighted that the main aroma compounds that contributed to the wine bouquet were: isoamyl acetate, ethyl octanoate, ethyl isobutanoate, ethyl isovalerate, and methyl decanoate. Even if a strain-dependent effect on the aroma composition of wines should be considered, it is possible to notice some differences due to the aggregation state. The wines obtained with *St. bacillaris* planktonic cells showed the highest ROC values for isoamyl acetate, ethyl octanoate, ethyl isobutanoate, and methyl decanoate, while those produced with biofilm-detached cells showed higher ROC values than the others for phenetyl alcohol, β-damascenone, citronellol, β-ionone, and nerol. It seems that the inoculation of planktonic cells resulted in less distinguishable wines because of the dominance of fruity notes. Even if the wines obtained with biofilm-detached cells showed fruity notes, the role of varietal aromas to the wine bouquet was more evident.

3.4. Sensory analysis

In order to verify if the differences detected in the aroma profile resulted in wines with different sensory traits, a sensory analysis was performed. In order to facilitate the visualization of results the mean values obtained for wines obtained with biofilm-detached and planktonic cells, respectively were used for the construction of the radar map (Fig. 5). Wines inoculated with biofilm-detached cells were characterized by a higher mouth-feel in agreement with the content of glycerol detected in these wines. Moreover, they showed the highest scores for the following descriptors: spicy, floral, and raspberry. The floral attribute is probably correlated to the presence of nerol, β-ionone, and phenylethyl alcohol; their amount was higher than the odor threshold in all the samples. The raspberry nuances are probably related to the presence of β-damascenone with OAV>1 and higher ROC values in wines obtained with biofilm-detached cells. No significant differences were detected for the other descriptors. These results underlined that the inoculum of biofilm-detached can be useful in shaping the sensory characteristics of Montepulciano d'Abruzzo wines and eventually emphasize some of them.

4. Conclusion

This study highlighted that biofilm-detached and planktonic cells contributed to wine composition differently. The different lifestyles influenced the content of glycerol, higher alcohols, esters, organic acids, and terpenes. According to the OAV, wines obtained with biofilm-detached cells showed higher values than the others for 10 compounds with a positive impact on wine aroma. The different contribution of biofilm-detached and planktonic cells was also highlighted by the sensory analysis which revealed that wines obtained with biofilm-detached cells were characterized by spicy, floral, and raspberry notes.

This study showed that the use of biofilm-detached cells could represent a good strategy for modulating wine aroma characteristics and obtaining wines with specific traits (e.g., fruity, spicy, floral notes). In fact, the results obtained in this study revealed that the different lifestyles of *St. bacillaris* can influence the metabolic traits of *S. cerevisiae* generating wines with different aroma characteristics. The most interesting results were obtained with the strain SB1. In fact, the wines obtained with this yeast showed the highest scores for persistence, mouthfeel, floral, tropical, cherry, and plum notes. This sensory profile is in agreement with the high content of esters, terpenes, aldehydes, and glycerol found in these wines. Furthermore, it is interesting to note that the use of this strain allowed for a reduction in ethanol content in accordance with consumers' demand for healthier products.

Further transcriptomic and proteomic studies on biofilm-detached cells are necessary to better investigate the reasons underlying these differences and to obtain information to improve wine fermentation employing this kind of cells.

Data availability

Additional data will be made available on request.

Ethics statement

All subjects gave their informed oral consent for inclusion before they participated in the study. The panellists are researchers and professors of the viticulture and oenology degree of the University of Teramo. They participate in the activities of the study course and sensory analyses are among these. Therefore, a written consent was not necessary.

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CRediT authorship contribution statement

Alessio Pio Rossetti: Writing – original draft, Methodology, Formal analysis, Data curation. **Giorgia Perpetuini:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Rosanna Tofalo:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

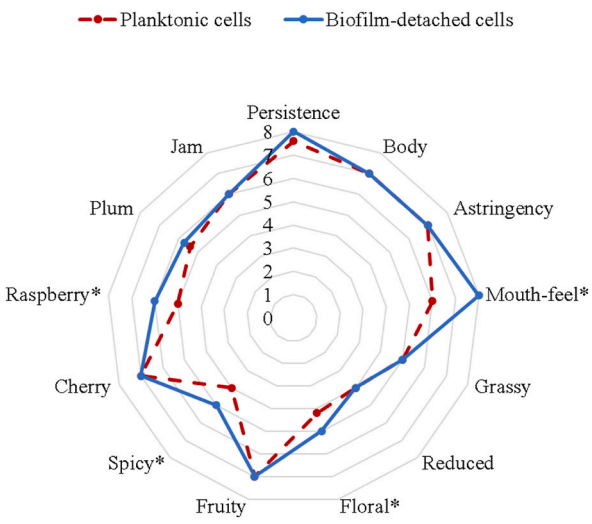

Fig. 5. Sensory analysis of obtained wines. $^{*}p < 0.05$.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35692.

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