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Selection of indigenous yeast strains for the production of sparkling wines from native Apulian grape varieties

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ABSTRACT

We report the first polyphasic characterization of native *Saccharomyces cerevisiae* in order to select candidate strains for the design of starter cultures tailored for Apulian sparkling wines obtained from local grape variety. In addition, it is the first survey in our region that propose the selection of autochthonous starter cultures for sparkling wine i) including a preliminary tailored genotypic and technological screening, and ii) monitoring analytical contribution during secondary fermentation in terms of volatile compounds (VOCs). Furthermore, we exploit the potential contribute of autochthonous cultures throughout the productive chain, including the possible improvement of base wine. One representative strain from each cluster was characterized i) for tolerance to abiotic and biotic stressors peculiar of sparkling wine fermentation, ii) for the performances in base wine production, and iii) for the aptitudes to promote in-bottle secondary fermentation in white and rosé sparkling wines, both obtained from Apulian grape varieties. Genetic characterization led to group 164 *S. cerevisiae* in 16 genetic clusters based on interdelta profiles. Stress tolerance assays shown a certain correlation with fermentative attitude. Our evidences demonstrated a different fermentative behavior and release of VOCs of the different strains in association with primary and secondary fermentations and as function of wine and rosé sparkling wine. Furthermore, performances in white/rosé sparkling wines have been found to be strain-dependent characters. Overall, we propose different strains as biotechnological resources suitable to improve the quality of regional sparkling wines and to provide a driver of innovation/segmentation in the market.

1. Introduction

Sparkling wines belong to the category of “special wines”, and they can be defined as effervescent wines, since they contains a relevant concentration of carbon dioxide, about 3–7 atm of pressure in the bottle (Carrascosa et al., 2011). Sparkling wines may be produced by several technological procedures, in particular the traditional method also called *méthode champenoise* and *charmat* one (Garofalo et al., 2016). Both methods involve two fermentations steps, the primary and secondary fermentations. In the traditional method, secondary fermentation consist an in-bottle refermentation that occurs after the addition to the base wine of the so-called *tirage* solution (saccharose 20–25 g/l, Yeasts, grape must or wine, and bentonite). This step, also known as *prise de mousse*, is followed by an aging period. During this time, sparkling wine matures and acquires the several intracellular compounds released by the yeast cells as excretion at the end of secondary fermentation, furthermore aging on lees led to yeast autolysis, both contributing to the development of the final aromas (Di Gianvito et al., 2018; Pozo-Bayón et al., 2009).

For this reason, the yeast strain involved in sparkling wine production by traditional method has a considerable effect on the final quality of the wines (Martínez-Rodríguez et al., 2001a). In this light, several authors investigated their biological and technological properties, with the aim to select starter cultures able to tolerate the harsh base wine conditions and to produce sparkling wines (Borrull et al., 2015; Di Gianvito et al., 2018; Martí-Raga et al., 2016; Perpetuini et al., 2016). In fact, the chemical composition of grape must (primary fermentation) and base wine (secondary fermentation) represents a hostile environment for yeasts development and for their fermentation efficiency due to several stressing factors.

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In particular, fermenting must usually contains high amount of sugars (about 200 g/l), low pH (3–3.5), sulphites, growing alcohol and glycerol concentration and gradual exhaustion of nutrients (i.e. vitamins, proteins, amino acids and lipids). In contrast, the chemical composition of base wines usually is characterized by considerable amount of ethanol (about 10%–12% v/v), low pH (2.8–3.5), high total acidity (5–7 g/l H_2SO_4), and total SO_2 contents (50–80 mg/l). Furthermore, sparkling wine production involves other stressors such as low temperatures (10–15 °C) and high amount of CO_2 and the resulting high pressure (about 6 atm) (Borrull et al., 2015). Therefore, the starter cultures selected for sparkling wine production needs to possess several technological properties in addition to those suggested for yeast strains utilized in the primary fermentation (Kemp et al., 2015). The use of selected autochthonous yeasts for the secondary fermentation has been recently suggested to enhance the specific features of typical regional wines and prevent fermentative problems (Garofalo et al., 2016; Torresi et al., 2011). Autochthonous starter cultures have a potential important role on wine quality in reason of the possible different adaptation to specific environmental conditions and of the prospective contribution in differentiating organoleptic properties of final products (Capozzi et al., 2015; Martínez-Rodríguez et al., 2001a; Tofalo et al., 2016; Torresi et al., 2011). The work by Vigentini et al. (2017) testified the existing interest in the characterization and selection of autochthonous yeasts to improve diversification in sparkling wines. The authors proposed a screening function of oenological traits, such as fermenting power and vigor, SO_2 tolerance, alcohol tolerance, flocculence, production of acetic acid, glycerol, and H_2S, including a sensory analysis (Marcon et al., 2018; Vigentini et al., 2017). In the present study, we aim to confirm the suitability of autochthonous *S. cerevisiae* to improve the quality of regional sparkling wine, testing this biotechnological approach in a different region/*terroir* using different grape varieties. In addition, it was the first survey in our region to propose the selection of autochthonous starter cultures for sparkling wine including a preliminary tailored genotypic and technological screening, the performance in primary fermentation and the contribution during secondary fermentation in terms of volatile compounds.

2. Material and methods

2.1. Yeast isolation and culture media

Yeasts were isolated from grape berries directly collected in the vineyard. About 1 kg of grape berries were collected aseptically in six North Apulia vineyards, pressed for 20 min using a Bag Mixer (Interscience, France), then spontaneous fermentation of grape juices were carried out in laboratory at 28 °C. In the last phases of alcoholic fermentations (AF), aliquots of 0.1 mL from serially diluted samples in physiological solution were plated on Wallerstein Laboratory (WL) nutrient agar (Oxoid, USA), added with 10 mg/l chloramphenicol (Garofalo et al., 2016). *Saccharomyces cerevisiae* DV10 (Lallemand, USA) was used as control in all winemaking assays (both for primary and secondary fermentation).

2.2. Specie-specific PCR and identification of Saccharomyces cerevisiae strains

Yeast total genomic DNA was obtained by the UltraClean DNA Microbial Isolation Kit (MoBio, Milan, Italy), following the instructions provided by the manufacturer. Yeast strains were identified as *S. cerevisiae* by PCR specie-specific with the species-specific primer pair, SC1/SC2, designed on ITS-1 region and *LSU* gene of *S. cerevisiae* (Josepa et al., 2000).

2.3. Genetic characterization of Saccharomyces cerevisiae strains

The genetic variability of *S. cerevisiae* isolates was evaluated by amplification of interdelta region, using the primers d12-d21 (Legras and Karst, 2003), according to Capece et al. (2012). Electrophoresis gel was analyzed by using the BioNumerics software 7.6 version (Applied Maths, Belgiuolm). The electrophoresis patterns were grouped, and analyzed for the similarity and cophenetic correlations through the Dice coefficient. Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA). Cophenetic correlation was the measure of how faithfully the tree represents the dissimilarities among observations (similarity 70%).

2.4. Technological characterization of yeast strains

2.4.1. Stress tolerance analysis

Tolerance analyses were carried out, taking into account stress factors during base and sparkling wine production. We tested the effect of pH (3.5), different concentrations of ethanol (6%, 8%, 10% and 12% v/v) and total SO_2 (100, 150, and 200 mg/l). Moreover, some combined stress were evaluated, that is, pH 3.5 + ethanol (6, 8, 10 and 12% v/v), pH 3.5 + SO_2 (100, 150 and 200 mg/l) and pH 3.5 + 10% ethanol + 150 mg/l SO_2. General medium was prepared using YPD (10 g/l yeast extract, 20 g/l peptone and 20 g/l glucose), also adjusted to the desired pH using HCl 1 N. The synthetic wine medium was used according to Serpaggi et al. (2012). Growth was monitored at 600 nm in a Gen5 Microplate Reader (BioTek Instruments, USA) at 30 °C. All experiments were carried out in triplicate.

2.4.2. Technological analyses

The fermentative rate in grape juice was determined based on weight loss after 2 days at 30, 37, and 42 °C and after 8 days at 6 and 12 °C. Ethanol production (% v/v) was calculated by multiplying the weight loss (as gram of CO_2) by 1,36 (Tristezza et al., 2012). Production of hydrogen sulphide was evaluated on plates assay by the blackening of a yeast culture on BIGGY agar (Marullo et al., 2004).

Killer activity of selected strains was investigated on methylene blue plates (Kaiser et al., 1994). Strains *S. cerevisiae* 3+ (K1+R1+), 4+ (K2+ R2+), 1− (K1−R1−) and 2− (K2−R2−) were used as control strains, respectively K+R+ for killer activity and K−R− for sensitivity.

Calcium-induced flocculation assay was performed according to Penacho et al. (2012) and autolysis capacity assay was performed in accordance to Martínez-Rodríguez et al. (2001b).

2.5. Primary and secondary fermentation assay

2.5.1. Base wine production

Fermentation tests were carried out in triplicate on grape juice (Nero di Troia, pH 3.2, sugars 190 g/l, YAN 94 mgN/l), at room temperature (about 20 °C). Flasks containing 100 ml of sterilized grape juice (supplemented with 30 mg/l free SO_2) were aseptically inoculated with the different strains to obtain an initial population of 2×10^6 CFU/ml. The progress of fermentation was monitored determining the weight loss caused by release of CO_2.

2.5.2. Sparkling wine production

Production of sparkling wine was performed using the traditional methods, following the method reported by Fia and Rosi (2001), that consist in four steps: i) preculture in YPD, ii) activation in base wine/water (base wine 16 ml, water 16 ml, sugar 4 g, yeast extract 0.1 g), iii) acclimation in base wine/water (base wine 120 ml, water 40 ml, sugar 10 g, yeast extract 0.1 g) and iiii) inoculation in base wine with *liqueur de tirage* (sugar 25 g/l, yeast, fermentation activator 0.3 g/l) in 750 ml bottle for sparkling wine, closed with crown cap and the bidule. Nero di Troia and Bombino bianco base wines were used for *prise de mousse*. The internal pressure in the bottles was measured each day during the second fermentation using an aphrometer (Oenoitalia, Italy). Second fermentation was carried at 15 °C by horizontally keeping the bottles.

The commercial strain of *S. cerevisiae* DV10 (Lallemand, USA) was employed, besides as control was used base wine not inoculated. Each assay was performed in triplicate.

2.6. Chemical analysis

General grape juice and wine parameters (alcohol content, residual sugars, pH, titratable and volatile acidity, tartaric, citric, lactic acid, malic acid, glycerol, and total sulfur dioxide) were determined using WineScan FT120 (Foss, Hillerød, Denmark) instrument. The analyses were performed in triplicate.

2.7. Analysis of volatile composition

Identification and quantification of the volatile compounds by GC–MS were carried out using an internal standard as already described (Tufariello et al., 2012). Volatile compounds were extracted in triplicate by solid phase extraction (SPE) technique (Piñeiro et al., 2006). The samples were injected into a DB-WAX capillary column (60 m × 0.25 mm I.D., 0.25 μm film thickness; Agilent, USA) and then analyzed with a 6890N series gas chromatograph (Agilent, USA) equipped with an Agilent 5973 mass spectrometer selective detector (MSD). The analysis was performed as previously reported (Tufariello et al., 2014).

2.8. Statistical data analysis

Statistical analysis was performed using an analysis of variance (ANOVA) to determine statistically different values at a significance level of $P \leq 0.05$. Statistical analyses were carried out using the STATISTICA 7.0 software (StatSoft software package, Tulsa, OK, USA). Principal Component Analysis (PCA) was carried out using the OriginLab software (OriginLab Corporation, USA). Classical ecology indices, such as the Shannon-Wiener index of general diversity (H), the richness (S) of the microbial community, Simpson's diversity indices (D and 1 _ D) and Evenness (eˆH/S) were calculated using the free software package PAST (Palaeontology Statistics, http://folk.uio.no/ohammer/past/).

3. Results and discussions

3.1. Genetic characterization of Saccharomyces cerevisiae strains

The yeast populations associated with spontaneously alcoholic fermentation of autochthonous Apulian grape varieties ranged from 10^5 to 10^7 CFU/ml, with 240 putative *S. cerevisiae* isolates (elliptical shape, cream colonies on WL agar plates) selected. 164 out of 240 isolates were identified as *S. cerevisiae* by PCR specie-specific (Josepa et al., 2000) and subjected to intraspecific genotypic characterization by analysis of interdelta sequences.

Interdelta analysis shows a high discriminative power, leading to 127 different profiles (Table S1). According to the resulting dendrogram (Fig. 1), the strains were distributed in 16 (A-R) main groups (similarity 70%). In Table S2 classical ecology indices (Shannon index, H; richness, S; variability; Simpson index, D and 1-D; Evenness, $e^{H/S}$) and Berger-Parker dominance are reported. Our strains reported an elevated polymorphism, calculated as the ratio between the number of molecular patterns and the number of isolates (77.44%), with the total number of individuals are almost equally spread among the strains analyzed (Evenness index 0.88). The congruent richness of the most abundant type is very low (Berger-Parker index, 0.03). In contrast, higher indices of general biodiversity ($H = 4.71$) and low concentration of dominance ($D = 0.02$) was observed (Table S2). Overall, our results confirmed that interdelta analysis allow to detect a high polymorphism within *S. cerevisiae* populations as previously reported by other authors (Capece et al., 2012). Moreover, in comparison with other studies on *S. cerevisiae* diversity associated with Apulian spontaneous fermentation of autochthonous grape varieties (Tristezza et al., 2012, 2013), a higher variability (about 78%) was observed in the analyzed population.

3.2. Technological characterization of yeast strains for sparkling wine production

The results of the genetic analysis allowed to cluster the *S. cerevisiae* population in 16 groups (Fig. 1, Table S1) A single strain, representative for each group, was selected for technological characterization and to perform the alcoholic fermentation in the production of base wine and of sparkling wine. In order to analyze the fermentative performances of the selected strains, the *S. cerevisiae* isolates were investigated by carrying out fermentations test at different temperatures (6 °C and 12 °C) using a commercial starter yeast as control. After 2 days, all strains produced very low level of CO_2/day (about 0.1 g CO_2/day). The fermentation rate of all of the strains investigated was lower at 12 °C and 6 °C (Fig. S1). At 12 °C only strains 21, 114 and 158 produced level of CO_2 higher than those reported for commercial strain (about 0.8 g CO_2/day), while other strains released lower amount of CO_2 (between 0.2 and 0.6 g CO_2/day). In addition, strains tested at 6 °C shown very low fermentation rate, with level of CO_2 comparable to those reported for commercial strain (about 0.2 g CO_2/day, strains DV10, 229, 150, 114, 158, 1566 and 118). Production of hydrogen sulphide was evaluated on plates assay by the blackening of a yeast culture on BiGGY agar. All strain investigated resulted low hydrogen sulphide producers (data not shown). Moreover, assay performed to evaluate the autolytic capacity, led to conclude that all the strains investigated released small amounts of proteins after induced autolysis in the model wine system, both after 24 h, 7 and 30 days of incubation (about 0.2 and 0.35 mg Bovine Serum Albumin/l), without significant differences detected (data not shown). Concerning stress tolerance analyses, all 16 strains result to be resistant to pH 3.5 (see Table S3). The tolerance of yeasts to ethanol was assayed at concentrations ranging from 6% and 12%. A concentration of 6% of ethanol was well tolerated by all strain investigated, as they show a low inhibition level, instead higher alcohol amount (8%, 10% and 12%) affects yeast growth to different extents, with inhibition level between 20 and 80%. All the strains investigated were able to grow in presence of SO_2 (100, 150, and 200 mg/l). Moreover, combined stresses were evaluated, suggesting a different ability from strain tested to survive to hostile conditions that mimic wine environment. In particular, when low pH, SO_2 and high ethanol were tested together, only few strains (17, 229, 64, 21, 89 and 150) were able to survive in a synthetic medium, while only strains 17, 174, 89 and 156 were able to growth in a model wine. Killer activity of selected strains was investigated on plates with pH values of 4.7, 4.0 and 3.5 (Fig. S2 and Table S4). The strains 41, 21, 174, 89 and 150 showed inhibition halos against sensitive *S. cerevisiae* strains 1^- and 2^-. Strains 41 and 89 presented an inhibition halo of 3 mm for all considered pH values, while for other strains, inhibition halos depended from sensitive strain and pH value. Only strain 229 show inhibition halos against killer strains *S. cerevisiae* 3^+ and 4^+ (see Fig. S2B and Table S4). The strains 55, 64, 158 and 199 were able to flocculate in grape juice, (about 10–20% fermentation rate, Fig. S3). In particular, strain 21 showed a higher flocculation degree, about 40 and 70%, respectively in grape juice washed and supplemented with calcium and in grape juice. No flocculation aptitude was detectable for all the 16 yeast strains when they were grown in YPD. Flocculation is sometimes affected by environmental stress, chemical or physical and these features may explain the differences observed (Soares et al., 2015).

3.3. Base wine chemical analysis

We tested the potential exploitation of autochthonous cultures at different steps of the productive chain, starting with the differentiation of base wine. The main chemical and volatile compounds found in

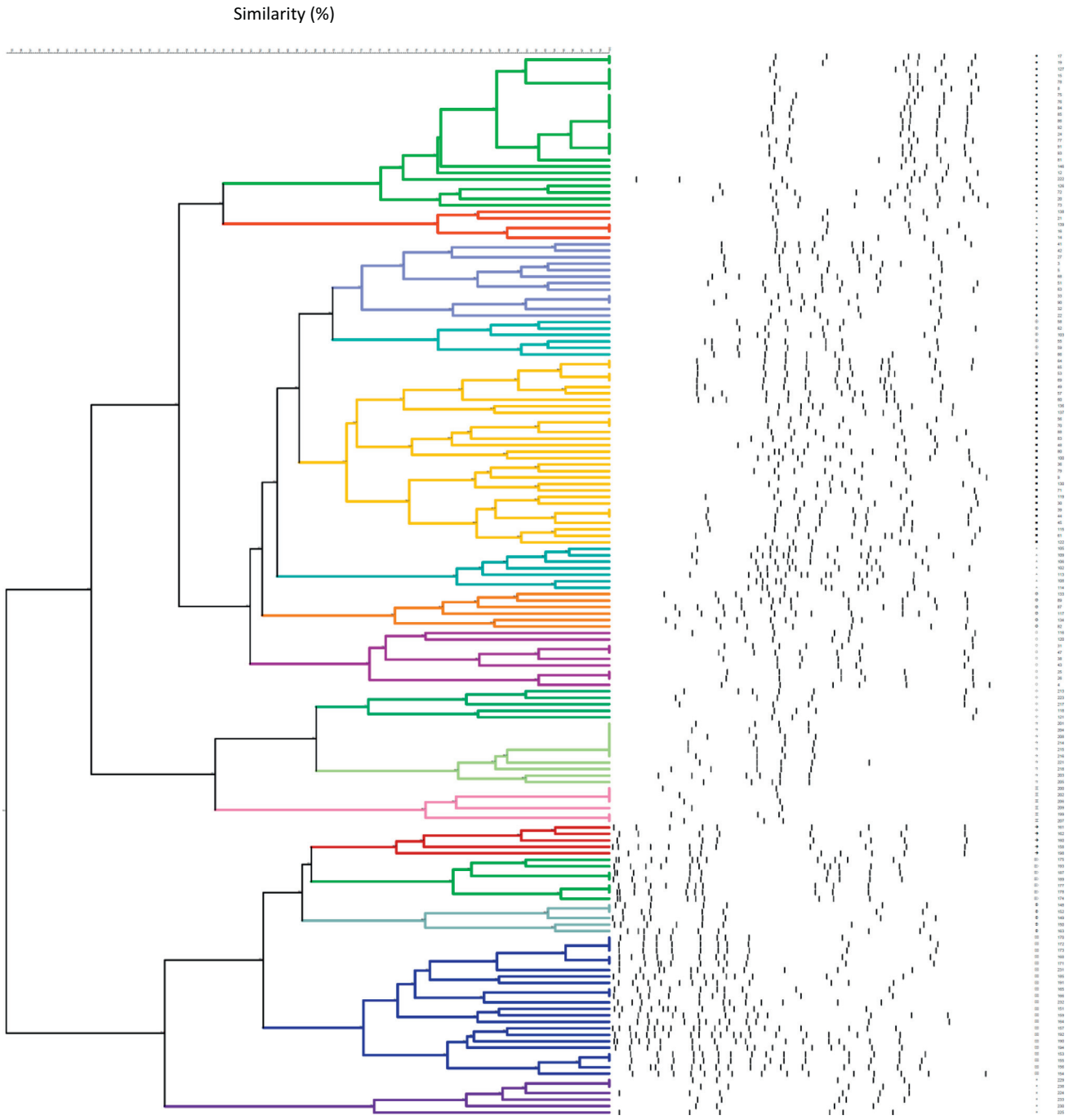

Fig. 1. UPGMA dendrogram generated by cluster analysis of interdelta region patterns obtained from the *Saccharomyces cerevisiae* strains isolated during the later stages of spontaneous fermentation of "Uva di Troia" grapes. Calculated percentages of similarity are given on the axis.

association with must samples separately fermented with each *S. cerevisiae* strains are reported in Tables S5 and 1, respectively. The production of ethanol (about 10–12% v/v) for the majority of the strains tested was comparable with that reported for control strain DV10. Only in strains 17, 118, a lower concentration of ethanol was observed (8 and 9%). Higher values of glycerol were detectable for strains 38, 55, 41, 208, 17, 229, 64, 21, 89 and 150 (Table S5). In contrast, the lowest glycerol concentration was reported for strains 174, 156, 114, 158, 199, 118, DV10. These findings are in accordance with those reported in literature, where glycerol concentration usually ranges between 5 and 8 g/l (Scanes et al., 1998). The mean value of acetic acid production was 0.39 g/l (see Table S5, volatile acidity), comparable to that reported in literature (Vigentini et al., 2017). The majority of the strains investigated presented volatile acidity values lower than sensitive threshold (about 0.8 g/l of acetic acid), except strain 17 that present the highest volatile acidity (1.26 g/l), and was therefore excluded from further analysis. Indeed, volatile acidity usually ranges among 0.6–0.9 g/l level. Concentrations higher than 1.2–1.3 g/l can result unpleasant, and that European Economic Community (EEC) legal limit of volatile acidity is around 1.5 g/l (Bely et al., 2008).

The volatile compounds involved in the wine flavor were also evaluated for each strains analyzed and the results are reported in Fig. S4 and Table 1. Regarding sparkling base wine aroma, different yeast strains were found to modulate the content of different volatile compounds.

For example, significant differences on esters production were

Table 1
Concentration of major volatile compounds in fermented musts obtained with autochthonous *S. cerevisiae* and one commercial starter (DV10).

Molecules	Odour threshold[A] (mg/l)	38	55	41	208	114	229	64	21	174	156	89	150	17	158	199	118	DV10
Alcohols																		
2-Methyl propanol	0.2 [B]	**0.58^{b}**	**0.77^{b}**	**1.20^{c}**	**0.21^{a}**	0.11^{a}	**0.32^{a}**	**1.20^{c}**	**2.30^{d}**	**0.32^{a}**	**0.21^{a}**	**1.63^{e}**	**0.21^{a}**	**1.63^{a}**	**0.66^{b}**	0.19^{a}	**0.42^{b}**	**0.29^{b}**
Isoamyl alcohol	30 [B]	29.74^{b}	24.71^{b}	**39.73^{c}**	22.88^{b}	7.49^{a}	**30.84^{b}**	**51.95^{d}**	**55.21^{d}**	19.92^{b}	22.41^{b}	**30.98^{b}**	6.42^{a}	0.23^{a}	24.12^{b}	26.36^{b}	17.98^{b}	19.32^{b}
Hexanol	8 [B]	0.21^{b}	0.19^{b}	0.33^{c}	0.32^{c}	0.04^{d}	0.42^{c}	0.61a	0.63^{a}	0.17^{b}	0.18^{b}	0.29^{c}	0.10^{b}	0.06^{d}	0.18^{b}	0.21^{b}	0.17^{b}	0.20^{b}
3-Hexenol	0.4 [C]	nd	0.04^{b}	0.07^{b}	0.06^{b}	**0.17^{c}**	0.08^{b}	**0.11a**	**0.13^{a}**	nd	0.04^{b}	0.06^{b}	0.03^{b}	0.32^{d}	0.04^{b}	0.05^{b}	0.05^{b}	0.04^{b}
Methionol	1 [B]	0.06^{a}	0.05^{a}	0.08^{a}	nd	0.04^{a}	0.09^{a}	nd	nd	nd	nd	nd	0.08^{a}	0.10^{a}	nd	0.09^{a}	0.06^{a}	0.03^{a}
Phenylethanol	10 [B]	**33.78^{b}**	**22.24^{c}**	**34.73^{b}**	**18.31^{c}**	0.31^{a}	**44.23^{d}**	**47.74^{d}**	**38.29^{b}**	**17.76^{c}**	**17.97^{c}**	**23.17^{c}**	nd	**33.10^{b}**	**20.07^{c}**	0.80^{a}	**24.24^{c}**	**19.02^{c}**
Total		64.37^{b}	48.01^{c}	76.13^{d}	41.79^{a}	8.16^{c}	75.98^{c}	101.61^{e}	96.57^{c}	38.17^{b}	40.80^{f}	56.13^{c}	6.83^{a}	35.44^{c}	45.06^{c}	27.70^{c}	42.92^{c}	38.90^{c}
Esters																		
Isoamyl acetate	0.03 [B]	**0.48^{b}**	**0.30^{c}**	**0.84^{d}**	**0.30^{c}**	**0.36^{c}**	**0.32^{c}**	**0.87^{d}**	**0.76^{d}**	**0.30^{c}**	**0.44^{b}**	**0.40^{b}**	**0.07^{a}**	**0.30^{c}**	**0.33^{c}**	**0.25^{c}**	**0.25^{c}**	**0.56^{b}**
Ethyl hexanoate	0.014 [B]	**0.22^{a}**	0.09^{b}	**0.22^{a}**	**0.30^{c}**	nd	**0.11^{b}**	**0.33^{c}**	**0.41^{d}**	**0.15^{b}**	**0.15^{b}**	**0.11^{b}**	0.04^{b}	0.06^{b}	**0.11^{b}**	**0.19^{a}**	**0.15^{b}**	**0.26^{a}**
Ethyl lactate	150 [B]	0.12^{a}	0.06^{b}	0.09^{b}	0.05^{b}	0.23^{c}	0.09^{b}	0.22^{c}	0.45^{d}	nd	0.03^{b}	nd	0.06^{b}	0.22^{c}	0.05^{b}	0.08^{b}	0.14^{a}	0.05^{b}
Ethyl octanoate	0.005 [B]	**0.37^{a}**	**0.20^{b}**	**0.41^{a}**	**0.48^{a}**	**0.02^{c}**	**0.10^{c}**	**0.27^{b}**	**0.29^{a}**	**0.13^{b}**	**0.29^{a}**	nd	**0.19^{b}**	**0.09^{c}**	**0.31^{a}**	**0.47^{a}**	**0.39^{a}**	**0.26^{a}**
Ethyl decanoate	0.2 [C]	**0.21^{a}**	0.14^{b}	**0.29^{c}**	**0.29^{c}**	**0.33^{c}**	0.05^{d}	0.19^{a}	**0.24^{a}**	nd	0.17^{a}	nd	0.10^{d}	nd	0.17^{a}	**0.20^{a}**	**0.20^{a}**	0.13^{b}
Diethyl succinate	200 [C]	0.26^{a}	0.26^{a}	0.30^{a}	0.37^{a}	0.13^{b}	nd	0.30^{a}	0.80^{c}	0.36^{a}	0.32^{a}	0.16^{b}	0.05^{d}	0.38^{a}	0.28^{a}	0.29^{a}	0.30^{a}	0.03^{d}
Ethyl 9-decenoate		nd	0.03^{a}	0.06^{b}	nd	0.05^{b}	0.04^{b}	nd	nd	nd	nd	nd	0.07^{b}	nd	0.05^{b}	nd	nd	nd
2-Phenyl acetate	0.25 [C]	**0.42^{a}**	**0.35^{a}**	**0.77^{b}**	**0.26^{a}**	0.03^{c}	**0.53^{a}**	**1.09^{d}**	**0.62^{b}**	**0.29^{a}**	**0.32^{a}**	**0.27^{a}**	**0.29^{a}**	**0.31^{a}**	**0.29^{a}**	0.06^{c}	**0.34^{a}**	**0.70^{b}**
Total		2.08^{b}	1.43^{c}	2.96^{c}	2.04^{d}	1.14^{d}	1.24^{e}	3.28^{b}	3.57^{c}	1.23^{b}	1.72^{d}	0.93^{d}	0.86^{f}	1.35^{d}	1.58^{d}	1.54^{f}	1.75^{d}	1.99^{e}
Terpenes/lactones																		
Linalool	0.025 [C]	nd	0.02^{a}	**0.03^{b}**	nd	nd	**0.05^{c}**	nd	nd	nd	0.02^{a}	nd	nd	nd	nd	nd	nd	0.02^{a}
Butyrolactone	35 [C]	nd	0.03^{a}	nd	nd	nd	0.05^{b}	nd	nd	nd	nd	nd	0.03^{a}	nd	nd	nd	nd	0.03^{a}
Ho-trienol	0.11 [E]	nd	0.04^{b}	nd	nd	nd	0.07c	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total		nd	0.09^{b}	0.03^{b}	nd	nd	0.17^{b}	nd	nd	nd	0.02^{a}	nd	0.03^{b}	nd	nd	nd	nd	0.05^{b}
Acids																		
2-Methyl propanoic acid	2.3 [D]	0.08^{a}	nd	0.09^{a}	nd	nd	nd	Nd	nd	nd	nd	nd	nd	0.04^{b}	nd	nd	nd	nd
Butanoic acid	2.2 [B]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.16^{a}	nd	nd	nd	nd
Hexanoic acid	0.42 [B]	**0.74^{a}**	**0.47a**	**0.84^{a}**	**0.62^{a}**	**0.45^{a}**	**0.64^{a}**	**1.49^{b}**	**1.67^{b}**	**0.46^{a}**	**0.73^{a}**	nd	nd	**1.30^{b}**	**0.44^{a}**	**0.80^{a}**	**0.61^{a}**	**1.02^{a}**
Octanoic acid	0.50 [B]	**1.21^{a}**	**0.64^{a}**	**1.48^{a}**	**0.89^{a}**	**12.33^{b}**	**0.94^{a}**	**2.45^{a}**	**1.89^{a}**	**0.57^{a}**	**0.79^{a}**	nd	**13.53^{b}**	**1.00^{a}**	nd	nd	**0.94^{a}**	**1.85^{a}**
Decanoic acid	1 [B]	nd	nd	0.56^{a}	nd	nd	nd	0.92^{b}	0.60^{a}	nd	nd	nd	0.43^{a}	nd	nd	nd	nd	0.65^{a}
Total		2.03^{a}	1.11^{a}	2.96^{a}	1.51^{a}	12.77^{b}	1.58^{a}	4.85^{c}	4.15^{c}	1.03^{a}	1.52^{a}	nd	13.95^{b}	2.50^{a}	0.44^{d}	0.80^{d}	1.55^{a}	3.51^{c}

[A]Reference from which the value has been taken is given in parentheses. [B]: Perestrelo et al., 2006; [C]: Campo et al., 2006; [D]: Capone et al., 2013; [E]: Vilanova and Oliveira, 2012. In bold, the concentrations of compounds having the Odour Activity Value (OAV) > 1, where OAV = compound concentration/odour threshold. The different letters in the same row mean significant differences at $p \leq 0.05$; nd, not detected.

observed within the selected strains. In effect, the composition of ethyl esters of fatty acids was observed to be a strain-dependent character as already suggested by Kemp et al. (2015). In particular, the highest values were reported for strains 21, 64 and 41 (3.57, 3.28 and 2.96 mg/l), while negative control showed the lowest one (0.36 g/l). Esters are the major chemical group of volatile compounds identified in base and sparkling wines and, particularly in base wines the most important esters that can contribute to wine aroma are ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl lactate and diethyl succinate (Welke et al., 2012), all molecules detected in our samples (Table 1). Higher alcohols are very important for wine quality and strains characterization, due to their correlation to yeast metabolism (Romano et al., 2003). In the samples analyzed, the values of higher alcohols varied significantly according to the yeast used. Higher alcohol concentration in wines was variable and ranged from 6.91 (negative control) to 101.61 mg/l (strain 64) (Table 1). Our results suggest also a strain-dependent correlation for terpenes (see Fig. S4 and Table 1), probably addressable to differences in glycosidases associated to yeast enzymatic systems (Fia et al., 2005). We also confirmed a linearly proportion of esters content with the concentrations of the corresponding higher alcohols, indicating that the availability of the precursors is the main limiting factor for the production of esters (Capozzi et al., 2016). The differences observed in the volatile composition of wines obtained from the different yeast strains appear to be a quantitative rather than a qualitative trait (Table 1), in agreement with previous studies (Mateo et al., 2001; Romano et al., 2003; Torrens et al., 2008). Based on volatile compounds such as higher alcohols, esters and volatile acids, three strains, named 21, 41 and 64, were selected as putative starter cultures for the fermentation of base wine.

3.4. Quality evaluation of sparkling wine samples

In Fig. 2 we report the evolution of pressure in the bottle during *prise de mousse* of four representative *S. cerevisiae* strains (38, 55, 156 and 229), using white and rosé base wines. For more details on overpressure released by all the strains investigated, see Fig. S5.

The kinetic of *prise de mousse* was described by a sigmoid curve (Fig. 2), in accordance to the evidences reported by Martí-Raga et al. (2016). Several differences among the strains tested and base wines were reported, in rosé sparkling wine, all strain reach high pressure values (about 7 bar, Fig. 2), except negative control (no pressure detected), while in white sparkling wine, only strains 38, 55, 41, 174, 118 and DV10 reach this level of pressure values. The other strains (i.e. 208, 64, 21, 158, 199, 229, 156, 89, 150 and 114) led to lower pressure (about 5–6 bar). Even in this case, pressure was undetectable for negative control. The differences observed can be correlated to the polyphenols content of rosé and white sparkling wine, that can affect yeast performances (Pozo-Bayón et al., 2003) suggesting the need to integrate stress conditions with polyphenols-related stressor. Our evidences indicated that the different technological prescreening trials based on biotic and abiotic stressors typical of sparkling wine secondary fermentation, were suitable to predict the performances in re-fermentation, with different trends within white and rosé sparkling wines. To select starter cultures for Apulian sparkling wines, low pH and high ethanol concentration resulted more effective stress as selector for white sparkling wine, while no clear indication is possible to address in the case of rosé sparkling wines, probably due to the absence of polyphenols among stressors.

The main chemical and volatiles compounds present in sparkling wine fermented by *S. cerevisiae* strains are reported in Tables S6-2 and S7-3 (respectively white and rosé sparkling wine). The average alcoholic strength in white sparkling wines is 10.39% v/v. Alcoholic strength and residual sugar contents were in agreement with results reported in literature (Martínez-Lapuente et al., 2016; Vigentini et al., 2017). Total acidity mean was about 7 g/l, only for strains 55, 150 and 158 lower values were observed (about 5–6 g/l). Volatile acidity resulted to be lower than sensory threshold in all sample analyzed (about 0.17 g/l). Similar results were reported for rosé sparkling wine (see Table S5). Several volatile compounds are identified and quantified by SPE-GC/MS (Tables 2 and 3), including alcohols, esters of fatty acid, acetic esters and acids. The volatile composition of the wines obtained from different yeast strains seem to be quantitative rather than qualitative, according to previous studies (Mateo et al., 2001; Romano et al., 2003). Hence, among white wines, samples 229, 21, 156, 158 and sevDV10 had the highest total content of alcohols, esters and acids. The highest total content of alcohols is due to 3-methyl butanol and phenylethanol, while, among esters ethyl lactate, diethyl succinate and monoethyl succinate were the prevalent esters. Hexanoic and octanoic acids, responsible for freshness notes, were more abundant in the acid class. Among rose sparkling wines, samples 55, 118, 229, 208, 158, 156 and 64 stands out for its high content in alcohols, in particular isoamyl alcohol and phenylethanol, esters, such as ethyl lactate, diethyl succinate and diethyl malate, and acids (hexanoic and octanoic acids). Among rose wines, samples 55, 229 and 118 had the highest total content of alcohols, esters and acids. The highest total content of alcohols is due to isoamyl alcohol and phenylethanol, while, among

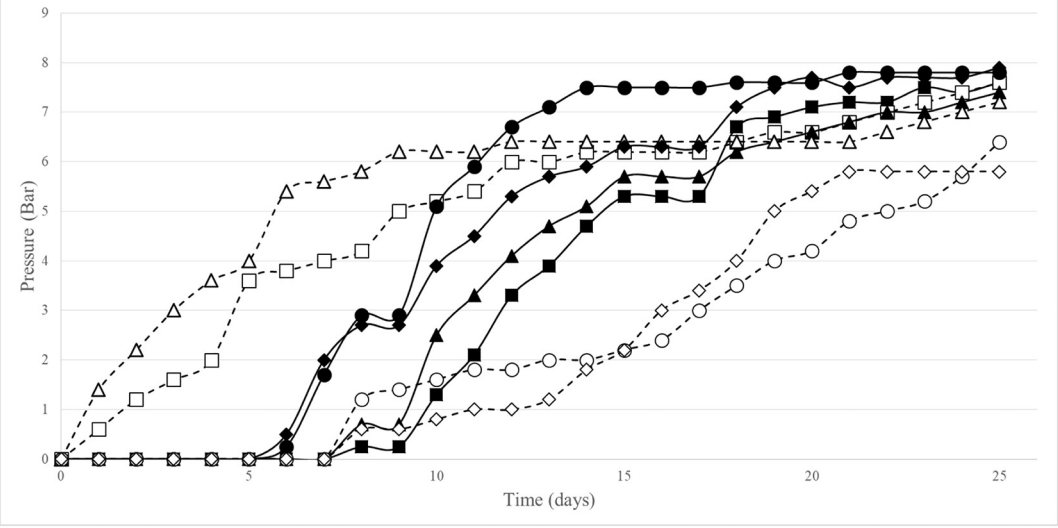

Fig. 2. Monitoring of CO_2 overpressure during sparkling wine production of representative selected strains in white (dotted line and white indicator) and rosé (full line and black indicator) sparkling wines. Square *S. cerevisiae* 38, triangle *S. cerevisiae* 55, round *S. cerevisiae* 156 and rhombus *S. cerevisiae* 229.

Table 2
Concentration of major volatile compounds in white sparkling wine obtained with autochthonous *S. cerevisiae* and one commercial starter (DV10).

Molecules	Odour threshold[A] (mg/l)	38	55	41	208	229	64	21	174	156	89	150	114	158	199	118	DV10
Alcohols																	
2-Methyl propanol	0.2 [B]	**0.25**^{a}	**0.33**^{a}	nd*	nd	**2.00**^{b}	nd	nd	nd	**2.59**^{b}	**0.38**^{a}	**0.28**^{a}	**0.27**^{a}	**3.79**^{b}	**0.27**^{a}	**0.51**^{a}	nd
Isoamyl alcohol	30 [B]	**38.03**^{a}	27.62^{a}	27.35^{a}	22.84^{a}	**116.88**^{b}	**42.04**^{a}	**197.17**^{c}	**46.05**^{a}	**146.16**^{b}	28.10^{a}	**31.24**^{a}	27.49^{a}	**175.11**^{c}	10.87^{d}	**33.99**^{a}	**137.08**^{b}
Hexanol	8 [B]	2.56^{a}	2.22^{a}	3.33^{a}	1.54^{a}	**9.29**^{b}	2.38^{a}	**17.47**^{c}	2.34^{a}	**11.69**^{b}	1.80^{a}	1.53^{a}	1.71^{a}	**14.08**^{d}	0.54^{a}	2.87^{a}	**8.48**^{b}
3-Hexenol	0.4 [C]	0.23^{a}	0.26^{a}	nd	0.17^{a}	**1.01**^{a}	0.25^{a}	**2.35**^{a}	nd	**1.32**^{a}	0.22^{a}	0.22^{a}	0.20^{a}	**1.73**^{a}	0.11^{a}	0.32^{a}	**1.00**^{a}
Phenylethanol	10 [B]	**16.55**^{a}	**16.69**^{a}	**44.39**^{b}	**18.37**^{a}	**48.89**^{b}	**16.11**^{a}	**80.35**^{c}	**87.61**^{c}	**54.43**^{b}	9.73^{d}	**10.92**^{d}	**11.44**^{d}	**72.71**^{c}	5.11^{d}	**23.73**^{a}	**50.20**^{b}
Total		57.62^{a}	47.12^{a}	75.07^{a}	42.93^{a}	178.08^{b}	60.78^{a}	297.34^{c}	136.00^{d}	216.20^{b}	40.23^{a}	44.18^{a}	41.12^{a}	267.42^{c}	16.90^{a}	61.42^{a}	196.76^{b}
Esters																	
Isoamyl acetate	0.03 [B]	**0.38**^{a}	**0.39**^{a}	nd	**0.17**^{a}	**7.41**^{b}	**0.48**^{a}	**14.15**^{c}	**0.16**^{a}	**8.59**^{b}	**1.34**^{d}	**1.16**^{d}	**1.34**^{d}	**13.72**^{c}	**0.11**^{a}	**0.18**^{a}	**6.11**^{b}
Ethyl hexanoate	0.014 [B]	**0.92**^{a}	**0.81**^{a}	nd	**0.43**^{a}	**7.43**^{b}	**0.95**^{a}	**13.74**^{b}	nd	**8.65**^{b}	**1.42**^{a}	**1.14**^{a}	**1.34**^{a}	**12.83**^{b}	**0.76**^{a}	**0.47**^{a}	**6.41**^{b}
Ethyl lactate	150 [B]	11.72^{a}	11.11^{a}	21.38^{a}	10.48^{a}	33.11^{b}	12.08^{a}	55.32^{b}	nd	38.09^{b}	7.50^{a}	8.55^{a}	8.11^{a}	47.59^{b}	6.06^{a}	15.14^{a}	38.16^{b}
Ethyl octanoate	0.005 [B]	**3.01**^{a}	**1.97**^{a}	**3.83**^{a}	**2.21**^{a}	**8.43**^{b}	**2.57**^{a}	**19.57**^{c}	nd	**12.18**^{b}	**2.11**^{a}	**1.60**^{a}	**2.25**^{a}	**13.44**^{b}	**3.07**^{a}	**2.33**^{a}	**6.35**^{a}
Ethyl decanoate	0.2 [C]	**0.41**^{a}	**0.21**^{a}	**0.97**^{a}	**0.35**^{a}	**0.89**^{a}	**0.28**^{a}	**2.29**^{a}	nd	**1.21**^{a}	**0.22**^{a}	0.11^{a}	**0.25**^{a}	**1.18**^{a}	**0.54**^{a}	**0.38**^{a}	**0.49**^{a}
Diethyl succinate	200 [C]	4.89^{a}	4.98^{a}	12.75^{b}	4.19^{a}	17.92^{b}	5.16^{a}	31.02^{c}	nd	18.13^{b}	3.26^{a}	2.71^{a}	3.46^{a}	26.75^{c}	3.54^{a}	7.98^{a}	16.30^{b}
2-Phenyl acetate	0.25 [C]	0.28^{a}	**0.31**^{a}	**0.70**^{a}	0.22^{a}	**1.43**^{a}	**0.35**^{a}	**1.99**^{a}	**2.70**^{a}	**1.07**^{a}	0.20^{a}	0.19^{a}	0.21^{a}	**1.90**^{a}	**0.43**^{a}	**0.47**^{a}	nd
Diethyl malate	10 [D]	2.03^{a}	2.41^{a}	6.03^{b}	2.50^{b}	6.81^{b}	1.83^{b}	**13.95**^{c}	1.97^{a}	7.33^{b}	1.20^{b}	1.17^{a}	1.41^{a}	**10.91**^{c}	0.87^{a}	3.30^{b}	5.85^{b}
Mono-ethyl succinate	nd	2.83^{a}	2.48^{a}	7.33^{a}	3.56^{a}	10.97^{b}	2.95^{a}	26.23^{c}	nd	15.93^{b}	2.56^{a}	2.29^{a}	2.08^{a}	16.41^{b}	0.95^{a}	3.53^{a}	6.82^{a}
Total		26.46^{a}	24.68^{a}	53.00^{b}	24.11^{a}	94.40^{c}	26.65^{a}	178.27^{d}	4.83^{e}	111.18^{c}	19.82^{a}	18.93^{a}	20.44^{a}	144.73^{f}	16.33^{a}	33.79^{a}	86.50^{c}
Acids																	
Hexanoic acid	0.42 [B]	**16.41**^{a}	**14.91**^{a}	**51.13**^{b}	**14.39**^{a}	**48.72**^{b}	**16.46**^{a}	**70.44**^{c}	**3.45**^{d}	**51.30**^{b}	**10.08**^{a}	**8.43**^{a}	**9.79**^{a}	**64.59**^{c}	**7.87**^{a}	**24.29**^{a}	**43.71**^{b}
Octanoic acid	0.50 [B]	**29.73**^{a}	**25.37**^{a}	**91.10**^{b}	**28.09**^{a}	**80.04**^{c}	**31.80**^{a}	**106.87**^{b}	**7.59**^{d}	**77.38**^{c}	**16.70**^{d}	**14.28**^{d}	**16.78**^{d}	**96.47**^{b}	**8.11**^{d}	**45.61**^{a}	**70.63**^{c}
Decanoic acid	1 [B]	**3.36**^{a}	**3.77**^{a}	**14.32**^{b}	**3.38**^{a}	**12.69**^{b}	**3.72**^{c}	**23.23**^{b}	nd	**13.60**^{b}	**2.31**^{a}	**1.69**^{d}	**2.13**^{a}	**16.60**^{b}	**1.10**^{d}	**7.91**^{c}	**6.80**^{c}
9-Decenoic acid	nd	0.69^{a}	0.75^{a}	3.00^{b}	0.71^{a}	2.88^{b}	0.88^{a}	5.15^{b}	3.30^{b}	3.19^{b}	0.60^{a}	0.33^{a}	0.47^{a}	3.76^{b}	0.67^{a}	1.71^{b}	1.88^{b}
Total		56.69^{a}	51.70^{a}	174.24^{b}	53.04^{a}	165.53^{b}	59.31^{a}	241.73^{c}	17.94^{d}	166.66^{b}	33.99^{a}	27.93^{a}	33.26^{a}	213.87^{c}	22.24^{a}	89.80^{a}	141.28^{b}

[A]Reference from which the value has been taken is given in parentheses. [B]: Perestrelo et al., 2006; [C]: Campo et al., 2006; [D]: Capone et al., 2013; [E]: Vilanova and Oliveira, 2012. In bold, the concentrations of compounds having the Odour Activity Value (OAV) > 1, where OAV = compound concentration/odour threshold. The different letters in the same row mean significant differences at $p \leq 0.05$; *nd, not detected.

Table 3
Concentration of major volatile compounds in rosé sparkling wine obtained with autochthonous *S. cerevisiae* and one commercial starter (DV10).

Molecules	Odour threshold[A] (mg/l)	38	55	41	208	229	64	21	174	156	89	150	114	158	199	118	DV10
Alcohols																	
2-Methyl propanol	0.2 [B]	nd*	nd	**0.23^{a}**	nd	**0.26^{a}**	nd	nd	nd	nd	**0.20^{a}**	nd	0.23^{a}	nd	nd	nd	nd
Isoamyl alcohol	30 [B]	16.10^{a}	**43.00^{b}**	21.85^{a}	24.87^{a}	**32.66^{a}**	24.20^{a}	1.47^{c}	7.22^{d}	25.10^{a}	8.16^{d}	3.13^{c}	16.10^{a}	26.75^{a}	0.13^{e}	**42.74^{b}**	1.25^{c}
Hexanol	8 [B]	1.00^{a}	1.40^{a}	1.32^{a}	1.75^{b}	1.91^{b}	1.40^{a}	0.31^{c}	0.21^{c}	1.14^{a}	0.80^{a}	0.51^{c}	1.30^{a}	1.56^{a}	nd	2.46^{b}	0.22^{c}
3-Hexenol	0.4 [C]	0.14^{a}	0.23^{a}	0.14^{a}	0.17^{a}	0.23^{a}	0.14^{a}	0.32^{a}	0.03^{a}	0.16^{a}	**0.66^{b}**	nd	0.06^{a}	nd	nd	**0.43^{b}**	nd
Phenylethanol	10 [B]	6.51^{a}	**18.99^{b}**	0.90^{c}	9.32^{a}	**15.52^{b}**	7.19^{a}	nd	2.19^{c}	9.83^{a}	**14.66^{b}**	8.45^{a}	6.70^{a}	8.89^{a}	0.14^{c}	**13.48^{b}**	8.69^{a}
Total		23.75^{a}	63.63^{b}	24.46^{a}	36.13^{c}	50.61^{d}	32.94^{c}	2.12^{e}	9.66^{e}	36.25^{c}	24.50^{a}	12.11^{e}	24.41^{a}	37.21^{c}	0.27^{f}	59.12^{b}	10.17^{e}
Esters																	
Isoamyl acetate	0.03 [B]	**0.50^{a}**	**0.27^{a}**	**0.55^{a}**	**1.21^{b}**	**0.50^{a}**	**0.72^{a}**	nd	**0.36^{a}**	**0.43^{a}**	**0.11^{a}**	nd	**0.70^{a}**	**0.96^{a}**	nd	**0.78^{b}**	nd
Ethyl hexanoate	0.014 [B]	**0.31^{a}**	**2.83^{b}**	**0.34^{a}**	**0.70^{a}**	**0.50^{a}**	**0.43^{a}**	**0.18^{a}**	**0.21^{a}**	**0.32^{a}**	**0.11^{a}**	nd	**0.49^{a}**	**0.51^{a}**	nd	**0.42^{a}**	nd
Ethyl lactate	150 [B]	4.34^{a}	14.07^{b}	6.26^{a}	6.20^{a}	17.86^{b}	3.78^{a}	1.70^{a}	1.22^{a}	11.35^{b}	6.12^{a}	3.42^{a}	4.47^{a}	5.30^{a}	0.05^{a}	13.48^{b}	3.38^{a}
Ethyl octanoate	0.005 [B]	**0.36^{a}**	nd	**0.31^{a}**	**0.67^{a}**	**0.62^{a}**	**0.31^{a}**	**1.67^{b}**	**0.34^{a}**	**0.37^{a}**	**4.68^{c}**	**0.45^{a}**	**0.14^{a}**	**0.41^{a}**	nd	**0.64^{a}**	**0.20^{a}**
Ethyl decanoate	0.2 [C]	0.11^{a}	nd	**2.72^{b}**	nd	nd	nd	nd	nd	nd	nd	nd	0.62^{a}	nd	nd	nd	nd
Diethyl succinate	200 [C]	1.88^{a}	3.30^{a}	0.11^{b}	3.03^{a}	4.44^{a}	2.16^{a}	3.15^{a}	0.41^{b}	2.40^{a}	nd	2.75^{a}	0.09^{b}	2.83^{a}	0.03^{b}	2.56^{a}	2.28^{a}
2-Phenyl acetate	0.25 [C]	0.05^{a}	0.08^{a}	**7.85^{b}**	0.14^{a}	nd	0.09^{a}	**8.71^{b}**	**0.28^{a}**	0.09^{a}	0.23^{a}	0.14^{a}	**2.52^{c}**	nd	nd	nd	nd
Diethyl malate	10 [D]	0.74^{a}	2.66^{a}	8.50^{b}	0.89^{a}	1.50^{a}	0.66^{a}	1.14^{a}	0.34^{a}	1.91^{a}	1.76^{a}	9.79^{b}	7.02^{b}	nd	nd	0.3^{a}	1.04^{a}
Mono-ethyl succinate	nd	1.82^{a}	nd	nd	nd	nd	nd	nd	0.68^{b}	nd	3.36^{c}	nd	1.53^{a}	nd	nd	nd	nd
Total		10.15^{a}	23.23^{b}	26.67^{b}	12.87^{a}	25.44^{b}	8.17^{a}	16.58^{a}	3.87^{c}	16.91^{a}	16.40^{a}	16.57^{a}	17.62^{a}	10.02^{a}	0.08^{e}	18.20^{a}	6.91^{a}
Acids																	
Acetic acid	200	nd	nd	nd	nd	1.10^{a}	nd	nd	nd	1.36^{a}	nd	nd	nd	nd	nd	0.82^{a}	nd
3-Methyl butanoic acid	0.25 [D]	nd	nd	nd	nd	nd	nd	nd	nd	0.15^{a}	nd	nd	nd	nd	nd	nd	nd
Hexanoic acid	0.42 [B]	**5.02^{a}**	**8.62^{b}**	**8.90^{b}**	**9.09^{b}**	**12.47^{b}**	**7.36^{a}**	**9.55^{b}**	**3.06^{a}**	**6.92^{a}**	**13.84^{b}**	**8.56^{b}**	0.10^{c}	**8.20^{b}**	nd	**7.04^{a}**	**6.88^{a}**
Octanoic acid	0.50 [B]	**5.71^{a}**	**9.39^{a}**	0.38^{a}	**9.65^{a}**	**13.35^{a}**	**7.28^{a}**	**10.30^{a}**	**1.41^{b}**	**7.66^{a}**	**17.90^{a}**	**1.19^{b}**	**0.82^{b}**	nd	nd	**2.56^{b}**	**9.22^{a}**
Decanoic acid	1 [B]	nd	**3.43^{a}**	**1.93^{a}**	nd	**4.37^{a}**	nd	nd	0.57^{b}	0.41^{b}	nd	nd	**8.14^{a}**	nd	nd	**3.66^{a}**	nd
Total		10.73^{a}	21.46^{b}	11.23^{a}	18.74^{b}	31.30^{c}	14.64^{a}	19.86^{b}	5.04^{d}	16.53^{b}	31.75^{c}	9.75^{a}	9.08^{a}	8.20^{a}	nd	14.10^{a}	16.10^{a}

[A]Reference from which the value has been taken is given in parentheses. [B]: Perestrelo et al., 2006; [C]: Campo et al., 2006; [D]: Capone et al., 2013; [E]: Vilanova and Oliveira, 2012. In bold, the concentrations of compounds having the Odour Activity Value (OAV) > 1, where OAV = compound concentration/odour threshold. The different letters in the same row mean significant differences at $p \leq 0.05$; *nd, not detected.

esters ethyl lactate, 2-phenyl acetate and diethyl malate were the prevalent esters. Finally, hexanoic and octanoic acids were more abundant in the acid class.

In general, isoamyl alcohol and phenylethanol were the most abundant volatile compounds in all the samples analyzed, followed by ethyl octanoate in white and rosé samples, 2-phenyl acetate in rosé samples and diethyl succinate in white samples. As reported in Tables 2 and 3, an increase of higher alcohols, esters and acids is apparently linked to the different yeast strain used.

In order to identify the most important wine odourants of produced base and sparkling wines, the aroma index (I), i.e. the Odour Activity Value (OAV), defined as the ratio between the concentration of the volatile compound (C_{odour}) and its odour perception threshold, was calculated for all the identified chemical species. Volatiles with OAV > 1, the concentrations of which are indicated in bold in Tables 1, 2 and 3 are commonly considered the compounds able to contribute to wine aroma (Gómez-Míguez et al., 2007; Romano et al., 1997; Styger et al., 2011; Capone et al., 2013). Among compounds with high odour activities, some alcohols (2-methyl-propanol, isoamyl alcohol, phenylethanol), esters (code: isoamyl acetate, ethyl hexanoate, ethy octanoate, ethyl decanoate, 2-phenyl acetate) and acids (exanoic, octanoic and decanoic acids), were identified in most of the produced base and sparkling wine.

Isoamyl alcohol and phenylethanol are fusel alcohols, which are usually present in wines because of yeast metabolism during alcoholic fermentation. Concentrations above 300 mg/l of these alcohols are responsible of a pungent smell and taste (Capone et al., 2013), while concentrations below 300 mg/l can have a positive impact by imparting the wine with fruity and floral notes. Among acetates and the ethyl ester family, isoamyl acetate and ethyl hexanoate or octanoate, were the major volatile and esters, originated from the yeasts fermentative metabolism. According to Ribéreau-Gayon et al. (2006), acetate esters of higher alcohols, contribute to the complex aroma of naturally neutral wines, but may mask some varietal aromas (Ribéreau-Gayon et al., 2006). Concerning the acids family, they can contribute to a balanced aroma in wine by hindering hydrolysis of their esters (Flanzy, 2003).

The presence of these potential aroma-contributing substances in the produced sparkling wines indicates fruity and floral main nuances. However, it should be underlined the importance of the interactions between aroma compounds in wine rather than the presence of specific impact odourants. Indeed, interactions between the different aroma components may occur. In fact, molecules with OAV > 1 may be covered up due to camouflage by other compounds (antagonistic effects), whereas compounds with OAV < 1 can be perceived due to additive or synergic effects of the other volatile components in the wine matrix (Gòmez-Miguez et al., 2007; Gómez García-Carpintero et al., 2011; Francis and Newton, 2005).

The PCA explains the relationship between the different sparkling wines based on their chemical composition by the interpretation of multivariate analysis, in order to identify the volatile compounds that discriminated best among wines. Two PCA were performed on the concentration of the 18 volatile compounds analyzed, respectively for the white and rosé sparkling wines (Fig. 3A–B). Fig. 3A shows that the first two principal components (PC1 and PC2) accounted for 87.98% of total variance (59.06% and 21.92% respectively). PC1 was characterized by major levels of isoamyl acetate, mono-ethyl succinate, hexanoic acid, phenylethanol, ethyl octanoate, ethyl lactate and 3-hexen-ol (Z). While, PC2 showed high values of ethyl decanoate, diethyl malate, 2-methylpropanol and ethyl hexanoate. These compounds were the principal components of volatile profile of negative control, located at positive values for PC2 and negative values of PC1. The samples of white sparkling wines were situated in two zones of the plot. The first group (S21, S158, S156, S229, DV10 and S41) were located at negative values of PC1 and PC2 and were characterized by volatiles located in

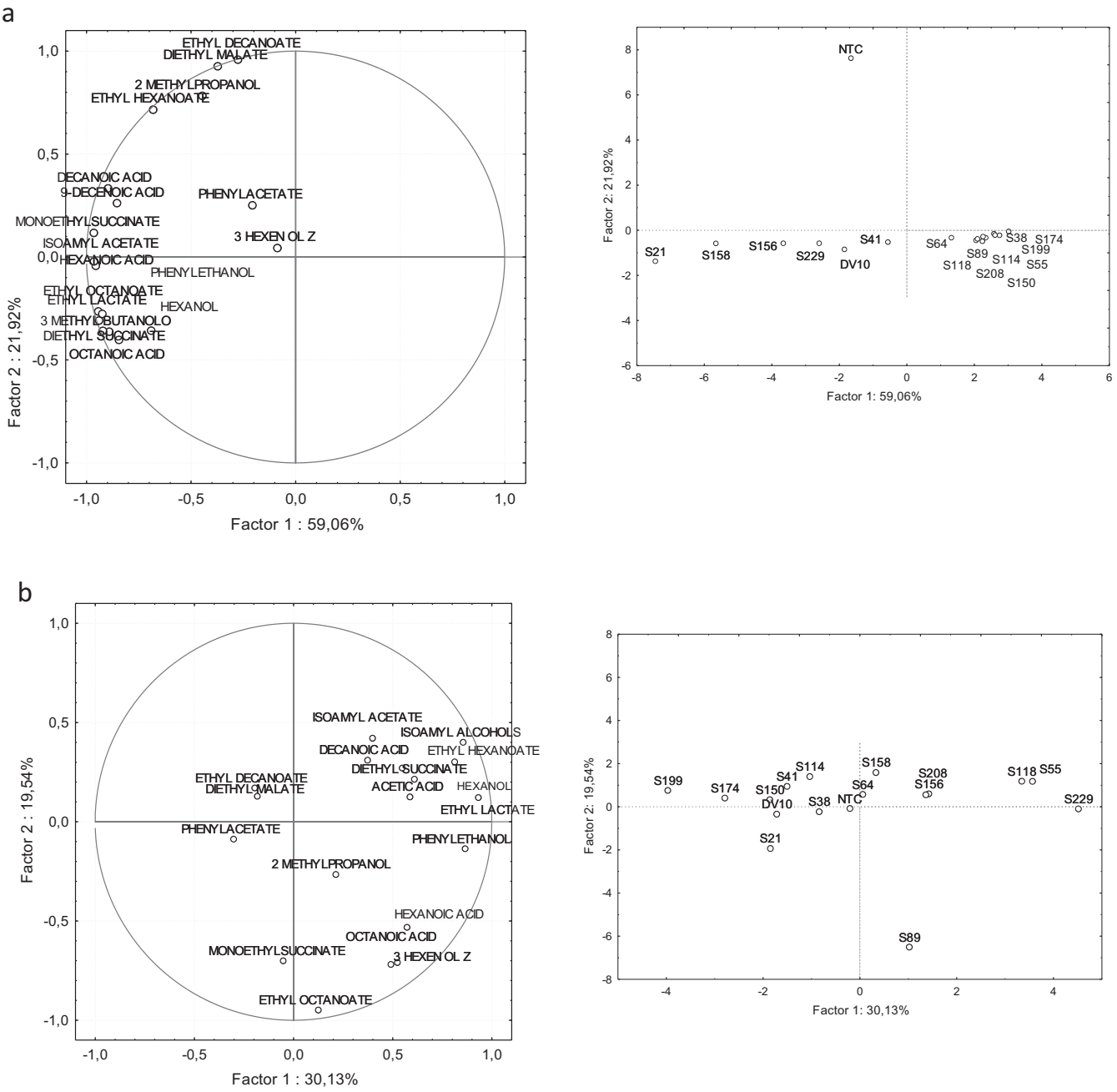

Fig. 3. (a) PCA analysis of white sparkling wines. (b) PCA of rosé sparkling wines. Samples 21. 156. 64. 158. DV10. 41. 229. 118. 89. 208. 114. 38. 199. 55. 150. 174 and negative control (NTC).

this zone, such as phenylethanol, ethyl octanoate, ethyl lactate, hexanol, 3-methylbutanol, diethyl succinate and octanoic acid. The second group (S64, S89, S118, S114, S38, S208, S150, S55, S199 and S174) was located in a region of the Cartesian plane denoted by the absence of volatiles compounds. In Fig. 3B, the first two principal components, PC1 and PC2, accounted for 49.67% of total variance (30.13% and 19.54% respectively). PC1 is positively correlated with ethyl lactate, phenylethanol, hexanol, diethyl succinate. These volatiles present high concentrations in S229, S118, S55 rosé sparkling wines, located at the positive component of PC1. PC2 show high and positive values for the attribute 2-phenyl acetate and diethyl, while mono-ethyl succinate, 2-methylpropanol and ethyl octanoate contributed to the negative side of same principal component. The positive component of PC2 separates two groups of samples. The PCA allowed the clusterization of the different sparkling wines in three groups. The first, located to the right of the positive PC2, consisted of S156, S158, S208, S118, S55 and S229. It was characterized by the isoamyl acetate, isoamyl alcohol, decanoic acid, ethyl hexanoate and diethyl succinate. The second group, positioned on the negative side of PC2, included S199, S174, S150, S41, S114, S38, and S21 and it was characterize by the diethyl malate, 2-phenyl acetate and ethyl decanoate. On the positive PC1 the S89 sample (third group) differed from the others since it was denoted by high values of hexanoic and octanoic acid, mono-ethyl succinate ethyl octanoate and 3-hexenol (z).

Intriguingly, taken together, the results indicate that each yeast biotype has diverse performances in base wine, white and rosé sparkling wines in terms of VOCs release, following strain-dependent trends (Table S8).

4. Conclusions

To our knowledge, this is the first report for a potential industrial employment of autochthonous starter cultures to enhance the characteristics of sparkling wine produced in the Apulia region. Furthermore, it was also the first study that includes a preliminary tailored genotypic and technological screening and the monitoring analytical contribution in terms of volatile compounds, in order to perform a selection of autochthonous starter cultures for base and sparkling wine. Our evidences demonstrated a different fermentative behavior of the tested strains to perform primary fermentation in base wine, and secondary fermentation in white and rosé sparkling wines respectively.

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