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Influence of autochthonous *Saccharomyces cerevisiae* strains on volatile profile of Negroamaro wines

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ABSTRACT

Saccharomyces cerevisiae is the yeast species predominating the alcoholic fermentation of grape must. The aim of this research was to evaluate the impact of indigenous *S. cerevisiae* strains biodiversity on the aroma of wines from Negroamaro grapes. Grapes collected in two different Negroamaro producing micro districts in Salento (Southern Italy), were subjected to natural fermentation and two indigenous *S. cerevisiae* populations were isolated. Fifteen strains for each of the two populations were selected and tested by micro fermentation assay in order to evaluate their specific contribute to the volatiles composition and sensory impact of the produced wines. The aromatic profile of wines obtained by each selected strain was characterized by different contents of acetates, ethyl esters of fatty acids, higher alcohols, thus showing to be related to the strains geographical origin. The sensorial analysis of wines produced by the six best performing strains confirmed that they are good candidates as industrial starter cultures, This study indicates that the use of a "microarea-specific" starter culture is a powerful tool to enhance the peculiarity of wines deriving from specific areas.

1. Introduction

The conversion of grape must into wine is promoted by a fermentation process naturally carried out by indigenous yeasts, which give a noticeable contribute to the chemical and organoleptic features of the final product (Fleet, 2003). The main function of oenological yeasts is to guarantee the rapid and complete conversion of grape sugar into ethanol, carbon dioxide, and many secondary metabolites, avoiding the production of off-flavours (Bauer & Pretorius, 2000). Although many flavour components derive directly from the grapes, the essential part of a wine flavour is achieved during the alcoholic fermentation process (Torrens et al., 2008). It is well known that the diversity of native yeast strains is responsible for the production of wines with different qualities and peculiar flavours (Fleet, 2003; Pérez-Coello, Briones Pérez, Ubeda Iranzo, & Martin Alvarez, 1999). Yeast species and, within each species, different strains exhibit wide differences in volatile compound production (Bertolini, Zambonelli, Giudici, & Castellari, 1996; Riponi, Carnacini, Antonelli, Castellari, & Zambonelli, 1997), accounting for the differences in composition and in taste of wine. Hence, the analysis of wine volatile compounds allows the evaluation of yeast fermentative properties (Mateo, Jimenez, Huerta, & Pastor, 1992).

Among the many factors that contribute to the typicality and quality of wine, aroma is probably the most important organoleptic characteristic and it is also a key attribute for consumers. Several hundred chemically different flavour compounds such as higher alcohols, aldehydes, ethyl esters of fatty acids, fatty acids, ketones,

Abbreviations: OPT, Odour Perception Threshold; I, index; OAV, Odour Activity Value; PCA, Principal Component Analysis.

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monoterpenes and volatile phenols have been found in wines (Pérez-Coello et al., 1999). However, the aroma perceived by smelling can rarely be ascribed to a specific compound and any distinct volatile contributes with a different intensity to the wine aroma. The contribution of a given compound depends on different parameters such as the Odour Perception Threshold (OPT) and the so called aroma index (I), also known as Odour Activity Value (OAV), defined as the ratio between concentration and OPT; OAV is an useful measure to assess the relative importance of the individual chemical components present in a sample (Gomez-Mıguez, Cacho, Ferreira, Vicario, & Heredia, 2007). The role of each volatile compound as odorant in the multiplicity of wine aroma components can be described in terms of one or several sensory descriptors (Franco, Peinado, Medina, & Moreno, 2004). In addition, as reported by other authors (Gomez-Mıguez, Cacho, et al., 2007; Muñoz, Penaido, Medina, & Moreno, 2007; Peinado, Mauricio, & Moreno, 2006), odorant (or aromatic) series can be defined by grouping all the volatile compounds with similar sensory descriptor; a generalised Odour Activity Value (generalised OAV) for each aromatic series can be calculated by adding the OAV of each aromatic series component. In such a way an odour profile of wine can be established by plotting in a radar graph the generalised OAV of all the aromatic series; the resulting "OAVs' Aroma Wheel" allows to link quantitative information, obtained by chemical analysis, to sensory perceptions, and provides a valid tool to compare the wine aroma profiles (Capone, Tufariello, & Siciliano, 2013).

Saccharomyces cerevisiae is highly tolerant to ethanol and highly competitive for growth in media with high sugar concentration (Querol, Barrio, Huerta, & Ramón, 1992) and is the main species of yeast in winemaking. Different strains of *S. cerevisiae* can produce significantly different flavour profiles when fermenting the same must and this is a consequence of three factors: i) the difference in winemaking conditions; ii) the differential ability of wine yeast strains to release varietal volatile compounds from grape precursors; iii) the strain-specific capability to *de novo* synthesise yeast-derived volatile compounds (Vilanova & Sieiro, 2006).

The use of selected starter yeast cultures for winemaking provides technological advantages such as guaranteeing that the must ferments in the correct way (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2007). On the contrary the use of locally selected yeast strains with strain-specific metabolic characteristics could positively affect the final quality of wine (Capece et al., 2010) and ensure the maintenance of the typical sensory properties of wines deriving from any given region (Ribéreau-Gayon et al., 2007).

Salento is a district of Apulia, the second largest Italian wine-producing area in terms of volume, whose pedologic characteristics and climatic conditions contribute to improve the wine aromatic properties and to give a characteristic and intense taste to the finished product. Negroamaro is a non-aromatic red wine grape variety native to South-East Italy, which is almost exclusively grown in the Southern area of Apulia, denoted as Salento (Fanet, 2004). This grape cultivar produce wines very deep in colour, combining perfume with an earthy bitterness, with a pleasant organoleptic complexity appreciated by the consumers. Salento producers have increasingly put the accent on premium quality and, by now, Negroamaro wines are well acknowledged by the international experts as one of the best Italian red wines. To date, Negroamaro cultivar has a great economic importance since it is the basic grape variety in the production disciplinary of 14 controlled designation of origin and five typical geographic indication. Moreover, Negroamaro wine has beneficial effects on health, because of its high content of resveratrol, a compound known for its anti-inflammatory and anti-cancerogenous properties (Baur & Sinclair, 2006).

An increasing number of reports have recently described the Negroamaro volatile composition (Capone, Tufariello, Francioso, et al., 2013; Capone, Tufariello & Siciliano, 2013; Coletta et al., 2013; Toci et al., 2012; Tufariello, Capone, & Siciliano, 2012). In particular, Tufariello et al. (2012) have recently reported the volatile profile of Negroamaro wine based on the analysis of ten commercial wines, bottled after malolactic fermentation and characterized by at least 4 years ageing sequentially operated in steel, barrel and bottle. The authors found very little dissimilarities among different Negroamaro samples, produced in the Northern and Middle areas of Salento having similar characteristics. This finding is likely to be due to the use of the same commercial dry starter preparations by the different industrial plants (Cappello, Bleve, Grieco, Dellaglio, & Zacheo, 2004).

However, to date no data are available about the influence of autochthonous yeast strains on Negroamaro volatile and aromatic profiles. The aim of this research was to study biodiversity of autochthonous *S. cerevisiae* strains and their effect on the volatile flavour components of Negramaro wines deriving from the Salento district, in order to select candidate fermentation starters for industrial wine production. Moreover, this study represents the first investigation on the volatile profiles of wines obtained using different yeast strains selected in the two most relevant Negroamaro-producing areas of Salento, Southern Italy.

2. Materials and methods

2.1. Yeast selection

Negroamaro (*Vitis vinifera*) grapes (80 kg), were harvested in the Guagnano (North Salento) and Ugento (South Salento) areas. The samples were separately smashed after stalks removal thus producing two grape musts one from North (sugars 226 g/L, 19.5°Ba, pH 3.35, assimilable nitrogen concentration 164.7 g/L) and another from South (sugars 238 g/L, 19.8°Ba, pH 3.41, assimilable nitrogen concentration 182.4 g/L) grapes. Spontaneous fermentations were carried out in 100 L sterile steel tanks in an experimental cellar with a temperature ranging between 24 and 26 °C. Fermentations were daily monitored by measuring the Babo grade (1°Ba = 10 g of fermentable sugars per litre of must). Yeast populations were sampled at the end of alcoholic fermentation (0-1°Ba) and from the residual lees. The two yeast populations were characterized at strain level as already reported, and the best-performing strains were then selected according to their molecular, physiological and oenological characteristics (Grieco et al., 2011). The strains were denoted by a code indicating the provenience, i.e. NP = Northern Population and SP = Southern Population, followed by the serial number of the isolate.

The procedure to select *S. cerevisiae* strains, candidates as industrial starter cultures consisted of the following steps: i) strain evaluation by fermentation test; ii) chemical analysis of fermented must; iii) sensorial analysis of fermented must; iv) statistical analysis of the obtained data.

2.2. Micro-vinification assays

The identified *S. cerevisiae* strains were tested by micro-fermentation assays conducted in 2 L of Negroamaro grape must (sugars 223 g/L, 19.6°Babo, pH 3.4) as previously described by Grieco and coworkers (Grieco et al., 2011).

Two different commercial starter culture, denoted as CN and CS, routinely used by the local wineries of the two sampling areas, were respectively utilized as controls. Wines were analysed by applying the Fourier transform infrared spectroscopy (FTIR), by means of the WineScan Flex (FOSS Analytical, DK).

2.3. Analysis of volatile composition

Identification and quantification of the volatile compounds by GC–MS was obtained by using an internal standard. Standard compounds used as a reference for volatile composition determination were purchased from Sigma–Aldrich (USA) and their solutions prepared as described by Tufariello et al. (2012). For major volatile compounds analysis [acetaldehyde, ethyl acetate, 2-methyl-1-propanol, higher alcohols (3-methyl- and 2-methyl-1-butanol), acetoin], the samples were spiked with 4-methyl-1-pentanol as an internal standard (200 mg/L) and directly injected in a CP-3800 Varian Gas Chromatograph coupled to a flame ionisation detector, using a Zebron Wax Plus capillary column (30 m × 0.32 mm × 0.50 μm film thickness; Phenomenex, USA). The oven temperature was programmed as follows: 40 °C for 5 min and then raised to 120 °C at a rate of 5.0 °C/min, followed by an increase to 230 °C at a rate of 25 °C/min, at which it was held for 5 min. The injector temperature was maintained at 200 °C, whereas the detector temperature was at 240 °C, according to the method proposed by by Mallouchos et al. (2003). Minor volatile compounds of wines were extracted in triplicate by solid phase extraction (SPE) technique (Piñeiro et al., 2006). The samples were then injected into a DB-WAX capillary column (60 m × 0.25 mm I.D., 0.25 μm film thickness; Agilent, USA) and analysed with a 6890N series gas chromatograph (Agilent, USA) equipped with an Agilent 5973 mass spectrometer selective detector (MSD) as previously described (Tufariello et al., 2012). The carrier gas was helium at a flow rate of 1.0 mL/min. A split/splitless injector was used in the splitless mode, the injector temperature was 250 °C and the injected volume was 2 μL. The column oven temperature was initially held at 40 °C, then it was programmed to 200 °C at 4 °C/min, with a final holding time of 20 min. Spectra were recorded in the electron impact mode (ionisation energy, 70 eV) in a range of 30–500 amu at 3.2 scans/s. A solvent delay time of 10 min was used to avoid overloading the mass spectrometer with solvent. The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 98, $P > 90\%$), with the retention data of commercially available standards and MS data reported in the literature. Quantification analysis was based on the principle that the component peak area is proportional to the amount of the analyte present in the sample. The quantification was carried out following the internal standard quantification method. The different sets of standard mixtures, previously prepared and containing known concentrations of the chemical standard and the I.S. concentration (CI.S.), were analysed and their component areas recorded. For each chemical standard, a six points calibration graph of relative component area versus analyte concentration was drawn to confirm a linear detector response and from which the amount of the analyte can be determined.

2.4. Sensory evaluation

The wines were evaluated by a panel of 8 local oenologists of large experience in the production and estimation of Negroamaro wine quality. The evaluation board referred to a scale with score values (indicated for each parameter) for the senses of smell and taste, according to the Tasting Regulation of the Italian Oenologist Association. For the sense of sight, clarity (0–6), hue (0–6), intensity (0–6) were considered. For the sense of smell the following parameters were taken into account: frankness (0–6), intensity (0–8), finesse (0–8), harmony (0–8). For the sense of taste, frankness (0–6), intensity (0–8), body (0–8), harmony (0–8), persistence (0–8), aftertaste (0–6) were evaluated. A value of 94 was considered as 100% of acceptance.

2.5. Odour Activity Value

To evaluate the contribution of a volatile compound to the aroma, the Odour Activity Value (OAV) was calculated as the ratio between the concentration of each compound and the perception threshold in a specified matrix reported in literature (Swiegers, Bartowksy, Henschke, & Pretorius, 2005). An aromatic series was defined as a group of volatile compound with similar aroma descriptors. The value of every aromatic series was calculated as the sum of the OAVs of the compounds that integrate it; this procedure allowed to correlate the information derived by instrumental analysis to sensory perception.

2.6. Statistical analysis

Significant differences among selected strains were determined for each chemical and volatile compound by analysis of variance (Duncan's test, $\alpha = 0.05$). Statistical data processing was performed using the STATISTICA 7.0 software package. Principal Component Analysis (PCA) was carried out using the OriginLab software (OriginLab Corporation, USA).

3. Results and discussion

3.1. Yeast strains selection

To analyse the biodiversity of indigenous *S. cerevisiae* strains in function of their impact on the volatile composition of Negroamaro wine, grapes were collected from the two most representative Negroamaro producing areas in Salento, which clearly differ in soil and geographical composition. The district of Guagnano (North Salento) has a soil of red clay structure of medium fertility, distributed on a flat area: from 30 to 60 m above sea level. On the contrary, the very southern portion of Salento is characterized by arrays of small rocky hillock, locally called *serre*. Due to the nature of the rocks, limestone and very hard, the vine are cultivated in areas where it is possible to find a small surface layer of earth. The two areas also differ in climate. In fact, in the area of Guagnano the climate is typically Mediterranean, with mild winters and hot summers, rainfall is scarce, nearly 400 mm of water during the year but more conspicuous than that of Ugento, where summers are particularly sultry and winters are colder to due to the constant presence of wind.

From each area, 4000 different strains of *S. cerevisiae* were isolated from the grapes during the last steps of natural fermentation (Grieco et al., 2011). Among these, 104 isolates were selected as low H_2S-producers, being H_2S an undesired fermentation by-product compound. The molecular characterization, based on rDNA analysis, allowed the identification of 36 isolates belonging to the species *S. cerevisiae*. Characterization of these isolates was performed by inter-delta sequence profiling as reported by Tristezza et al. (2012). Strains selected from the North Salento (Guagnano) and South Salento (Ugento) yeast populations were respectively denoted as NP and SP. The selection procedure allowed the identification of 15 different strains in each population, which were further tested in micro-fermentation assays in order to evaluate the strain-specific impact on the volatile and sensory profile of the Negroamaro wines.

3.2. Chemical and physical analysis of wines

The main physical and chemical parameters of the wines obtained were analysed to determine the content of alcohol and sugar, total and volatile acidity, glycerol and SO_2. Density and pH values were also determined (Tables 1 and 2). In NP derived wines

Table 1
Primary composition of the wines produced by *S. cerevisiae* strains from the North Salento area.

Parameters	CN	NP1	NP8	NP11	NP13	NP20	NP22	NP27	NP33	NP38	NP45	NP50	NP55	NP81	NP94	NP103
Ethanol	12.5 ± 0.2	13.0 ± 0.2	13.4 ± 0.2	12.9 ± 0.3	13.2 ± 0.4	12.8 ± 0.3	12.8 ± 1.5	13.1 ± 1.1	13.1 ± 0.4	12.4 ± 0.3	12.1 ± 0.1	12.9 ± 0.2	12.8 ± 0.1	13.3 ± 0.3	12.4 ± 0.4	12.9 ± 0.2
Sugars	3.2 ± 0.5	2.4 ± 0.4	2.5 ± 0.5	2.9 ± 0.11	3.1 ± 0.4	2.93 ± 0.3	2.9 ± 0.2	2.3 ± 0.8	2.9 ± 0.3	2.9 ± 0.2	2.8 ± 0.5	2.78 ± 0.5	2.88 ± 0.3	2.41 ± 0.2	2.6 ± 0.3	2.9 ± 0.2
TA	6.45 ± 2.11	5.81 ± 2.21	5.64 ± 0.51	6.01 ± 1.01	5.71 ± 0.61	6.32 ± 1.11	6.25 ± 0.61	6.02 ± 1.11	5.44 ± 0.21	5.60 ± 0.51	5.64 ± 0.41	6.13 ± 2.22	6.13 ± 1.21	5.89 ± 1.13	5.74 ± 1.03	5.71 ± 2.03
VA	0.27 ± 0.02	0.22 ± 0.03	0.24 ± 0.04	0.26 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.03	0.23 ± 0.02	0.27 ± 0.04	0.23 ± 0.03	0.23 ± 0.02	0.25 ± 0.03	0.26 ± 0.02	0.26 ± 0.02	0.22 ± 0.02	0.26 ± 0.02
pH	3.51 ± 0.04	3.52 ± 0.03	3.41 ± 0.51	3.51 ± 0.32	3.51 ± 0.41	3.52 ± 0.11	3.53 ± 0.11	3.53 ± 0.32	3.50.4 ± 0.15	3.52 ± 0.45	3.51 ± 0.51	3.52 ± 0.11	3.52 ± 0.10	3.52 ± 0.03	3.53 ± 0.04	3.51 ± 0.05
Malic acid	2.44 ± 0.11	2.31 ± 0.41	2.21 ± 0.32	2.31 ± 0.21	2.31 ± 0.40	2.33 ± 0.22	2.40 ± 0.41	2.28 ± 0.11	2.22 ± 0.11	2.21 ± 0.11	2.28 ± 0.30	2.42 ± 0.41	2.35 ± 0.11	2.23 ± 0.12	2.18 ± 0.11	2.28 ± 0.52
Citric acid	0.23 ± 0.05	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.03	0.32 ± 0.20	0.23 ± 0.41	0.23 ± 0.32	0.24 ± 0.05	0.28 ± 0.04	0.24 ± 0.03	0.31 ± 0.02	0.25 ± 0.04	0.23 ± 0.02	0.22 ± 0.05	0.23 ± 0.02	0.26 ± 0.03
Glycerol	7.8 ± 1.1	7.2 ± 0.3	7.7 ± 0.1	7.3 ± 0.4	7.8 ± 0.1	7.5 ± 0.2	7.9 ± 1.2	6.9 ± 0.3	7.2 ± 0.1	7.1 ± 0.2	7.5 ± 0.1	8.1 ± 0.5	8.3 ± 0.1	7.0 ± 0.6	6.9 ± 0.1	7.8 ± 0.3

TA, total acidity. VA, volatile acidity. The ethanol concentration is expressed as g/100 mL. The other values are expressed as g/L. CN, commercial starter control.

Table 2
Primary composition of the wines produced by *S. cerevisiae* strains from the South Salento area.

Parameters	CS	SP 4	SP 16	SP 20	SP 24	SP 32	SP 33	SP 34	SP 43	SP 44	SP 52	SP 62	SP 66	SP 77	SP 86	SP_91
Ethanol	12.5 ± 1.5	12.7 ± 0.2	12.7 ± 0.5	12.9 ± 0.7	12.4 ± 0.5	13.3 ± 0.3	12.7 ± 0.2	12.7 ± 0.3	13.1 ± 0.1	12.58 ± 1.5	9.3 ± 0.1	9.1 ± 0.2	12.8 ± 1.5	13.2 ± 1.2	13.1 ± 1.8	12.6 ± 2.2
Sugar	2.35 ± 0.25	1.6 ± 0.2	2 ± 0.3	1.91 ± 0.4	1.73 ± 0.9	1.71 ± 0.4	1.90 ± 0.34	1.80 ± 0.54	1.64 ± 0.22	1.55 ± 0.24	1.57 ± 0.11	2 ± 0.03	1.4 ± 0.3	1.36 ± 0.2	1.64 ± 0.5	1.64 ± 0.35
TA	5.40 ± 0.31	5.01 ± 1.05	4.83 ± 1.10	5.01 ± 1.10	4.77 ± 0.55	4.71 ± 0.36	4.92 ± 0.45	4.91 ± 1.20	4.91 ± 1.10	5.18 ± 0.58	4.88 ± 0.35	5.01 ± 2.10	5.01 ± 1.02	5.04 ± 1.05	5.02 ± 1.10	4.88 ± 2.12
VA	0.28 ± 0.05	0.27 ± 0.03	0.31 ± 0.02	0.27 ± 0.05	0.27 ± 0.03	0.27 ± 0.02	0.27 ± 0.02	0.29 ± 0.04	0.27 ± 0.05	0.25 ± 0.05	0.25 ± 0.04	0.26 ± 0.02	0.29 ± 0.06	0.31 ± 0.04	0.27 ± 0.07	0.29 ± 0.06
pH	3.51 ± 0.03	3.54 ± 0.05	3.54 ± 0.02	3.55 ± 0.03	3.53 ± 0.03	3.54 ± 0.04	3.54 ± 0.01	3.54 ± 0.02	3.54 ± 0.02	3.54 ± 0.01	3.53 ± 0.04	3.54 ± 0.03	3.53 ± 0.02	3.53 ± 0.02	3.54 ± 0.01	3.54 ± 0.38
Malic acid	2.14 ± 0.52	2.01 ± 0.5	1.91 ± 0.36	1.94 ± 0.22	1.93 ± 0.23	1.91 ± 0.14	1.96 ± 0.15	1.94 ± 0.16	1.94 ± 0.14	2.05 ± 0.05	1.94 ± 0.6	1.93 ± 0.42	2.01 ± 0.11	2 ± 0.05	1.97 ± 0.03	1.92 ± 0.24
Citric acid	0.22 ± 0.02	0.23 ± 0.03	0.21 ± 0.01	0.22 ± 0.03	0.21 ± 0.04	0.2 ± 0.03	0.21 ± 0.05	0.20 ± 0.04	0.20 ± 0.03	0.22 ± 0.04	0.21 ± 0.04	0.22 ± 0.03	0.22 ± 0.03	0.20 ± 0.04	0.21 ± 0.04	0.20 ± 0.06
Glycerol	7.8 ± 0.2	7.1 ± 0.2	7.1 ± 0.2	6.9 ± 0.2	7.1 ± 0.2	7.5 ± 0.2	7.2 ± 0.27	7.4 ± 0.3	7.0 ± 0.3	7.1 ± 0.3	7.0 ± 0.3	6.8 ± 0.3	7.1 ± 0.3	7.1 ± 0.3	6.9 ± 0.3	7.4 ± 0.3

TA, total acidity. VA, volatile acidity. The ethanol concentration is expressed as g/100 mL. The other values are expressed as g/L. CS, commercial starter control.

Table 3
Quantification of volatile compounds identified in the wines produced by *S. cerevisiae* strains (NP) from the North Salento area.

No	Compounds	CN	NP1	NP8	NP11	NP13	NP20	NP22	NP27
	Alcohols								
3	1-Propanol	7.52a ± 2.54	14.20ab ± 0.32	24.82ab ± 1.84	17.10ab ± 2.03	17.52ab ± 0.20	20.17b ± 2.20	20.02b ± 5.11	15.00ab ± 2.04
4	2-Methyl-1-propanol	5.85ab ± 0.47	4.82a ± 0.12	9.03c ± 1.10	4.47ab ± 1.61	5.54ab ± 0.22	5.42ab ± 1.02	8.02bc ± 2.12	5.12ab ± 2.11
6	1-Butanol	0.34b ± 0.11	0.26ab ± 0.11	0.32b ± 0.06	0.31b ± 0.20	0.23ab ± 0.06	0.24ab ± 0.04	0.27ab ± 0.06	0.19ab ± 0.04
7	2+3-Methyl-1-butanol	48.72a ± 4.72	42.53a ± 4.44	68.51a ± 4.21	48.17a ± 2.41	48.47a ± 2.35	58.92a ± 5.51	67.62a ± 5.63	44.83a ± 4.18
13	1-Hexanol	3.40e ± 0.40	1.71cd ± 1.02	3.10de ± 0.52	2.63bc ± 0.26	1.42cd ± 0.12	2.76bc ± 1.33	3.04de ± 1.12	2.70bc ± 1.16
15	*Trans*-3-hexen-1-ol	ND	0.12b ± 0.05	ND	0.04ab ± 0.01	0.11bc ± 0.03	0.07abc ± 0.03	0.10bc ± 0.02	ND
14	*Cis*-3-hexen-1-ol	0.32bc ± 0.08	0.27bc ± 0.04	0.33c ± 0.13	0.21a ± 0.05	0.25bc ± 0.05	0.22bc ± 0.05	0.25ab ± 0.04	0.20a ± 0.10
18	1-Heptanol	0.50c ± 0.20	0.23ab ± 0.09	0.32ab ± 0.17	0.23ab ± 0.05	0.15a ± 0.06	0.26ab ± 0.04	0.31ab ± 0.10	0.22ab ± 0.07
21	(R,R) + (S,S) 2,3-Butanediol	3.99b ± 0.92	0.22a ± 0.10	0.22a ± 0.06	0.05a ± 0.02	ND	4.26b ± 1.96	1.11a ± 0.37	0.34a ± 0.10
23	(R,S) 2,3-Butanediol	0.81b ± 0.25	2.32c ± 1.12	ND	ND	ND	0.81b ± 0.23	0.25ab ± 0.12	ND
33	Benzyl alcohol	0.65a ± 0.14	0.34a ± 0.16	0.41a ± 0.12	0.56a ± 0.20	0.25a ± 0.04	0.38a ± 0.05	0.42a ± 0.12	0.22a ± 0.10
34	2-Phenylethanol	67.81a ± 15.91	75.12a ± 6.84	59.14a ± 10.50	72.31a ± 41.11	56.92a ± 21.10	63.85a ± 5.60	70.21a ± 14.21	57.91a ± 10.21
	Total Alcohols	139.82	142.14	166.20	146.08	130.86	157.36	172.0	126.74
	Esters								
2	Ethyl butyrate	0.17ab ± 0.06	0.10cde ± 0.02	0.51bc ± 0.07	0.52cde ± 0.12	0.70e ± 0.11	0.46a ± 0.05	0.68e ± 0.04	0.52cde ± 0.22
8	Ethyl hexanoate	0.11a ± 0.07	0.15ab ± 0.02	0.15ab ± 0.06	0.81cd ± 0.10	0.17ab ± 0.02	0.65bcd ± 0.20	1.16d ± 0.25	0.34ab ± 0.16
5	2+3-Methyl-1-butanol acetate	0.52c ± 0.32	0.40ab ± 0.08	0.22ab ± 0.07	0.20ab ± 0.01	0.46bc ± 0.21	0.13a ± 0.04	0.32ab ± 0.12	0.18a ± 0.07
9	Ethyl acetate	20.42bc ± 1.42	16.82ab ± 0.72	14.04ab ± 1.10	18.04b ± 1.96	20.12bc ± 0.92	22.39bc ± 2.12	14.40ab ± 3.60	18.07bc ± 0.72
10	Hexyl acetate	0.24a ± 0.08	0.20a ± 0.10	0.13a ± 0.04	ND	0.24a ± 0.09	ND	0.09a ± 0.02	0.10a ± 0.03
12	Ethyl lactate	8.20b ± 2.60	15.11bc ± 2.01	19.41c ± 9.51	12.07bc ± 7.43	17.13bc ± 6.10	12.19bc ± 1.34	18.73c ± 0.83	8.72b ± 2.42
16	Ethyl octanoate	0.65a ± 0.20	1.12a ± 0.62	1.08a ± 0.13	0.65a ± 0.30	1.32a ± 0.32	0.55a ± 0.11	0.82a ± 0.27	0.56a ± 0.05
19	Ethyl 3-hydroxybutyrate	0.22abcd ± 0.09	0.16abcd ± 0.13	0.22abcd ± 0.02	0.19bcd ± 0.11	0.18bcd ± 0.04	0.21bc ± 0.06	0.29d ± 0.04	0.04a ± 0.02
26	Ethyl decanoate	0.42b ± 0.23	0.15a ± 0.05	ND	0.09a ± 0.04	ND	0.09a ± 0.03	0.14a ± 0.03	0.10a ± 0.03
28	Diethyl succinate	1.45a ± 0.22	1.82a ± 0.05	2.15a ± 0.43	1.33a ± 0.50	2.34a ± 0.70	1.48a ± 0.50	1.27a ± 0.18	1.14a ± 0.55
29	Ethyl-9-decenoate	0.62b ± 0.22	0.32ab ± 0.20	0.32ab ± 0.04	0.35ab ± 0.14	0.26ab ± 0.11	0.27ab ± 0.03	0.48ab ± 0.24	0.16a ± 0.05
31	2-Phenylethyl acetate	0.82d ± 0.12	0.51abcd ± 0.05	0.48abcd ± 0.12	0.52abcd ± 0.12	0.72cd ± 0.09	0.50abcd ± 0.10	0.60abcd ± 0.1	0.40abc ± 0.20
40	Ethylhexadecanoate	2.85b ± 0.41	0.30a ± 0.09	0.31a ± 0.19	0.06a ± 0.02	0.08a ± 0.02	0.15a ± 0.05	0.11a ± 0.03	0.06a ± 0.02
41	Mono ethyl succinate	2.92ab ± 1.42	3.65ab ± 1.82	3.88ab ± 0.84	4.40ab ± 1.80	3.31ab ± 1.09	4.72b ± 1.82	3.96ab ± 1.30	2.62ab ± 0.52
	Total esters	39.61	40.81	42.92	39.24	47.03	44.34	43.05	33.01
	Fatty acids								
17	Acetic acid	6.03b ± 2.05	0.92a ± 0.12	1.12a ± 0.72	3.42a ± 1.12	2.08a ± 0.98	8.34b ± 3.96	1.53a ± 0.55	0.26a ± 0.05
22	2-Methyl-propanoic acid	0.82c ± 0.26	0.52b ± 0.22	0.58bc ± 0.02	0.42b ± 0.10	0.42ab ± 0.12	0.61bc ± 0.11	0.64bc ± 0.21	0.39ab ± 0.06
24	Butyric acid	0.42bc ± 0.28	0.32bc ± 0.12	0.43bc ± 0.13	0.25bc ± 0.12	0.26bc ± 0.12	0.34bc ± 0.11	0.50c ± 0.08	0.12a ± 0.04
27	2-Methyl butyric acid	2.36ab ± 0.12	2.14ab ± 0.34	2.27ab ± 0.22	2.24ab ± 1.11	2.27ab ± 1.12	2.77ab ± 1.10	3.19b ± 1.50	1.78a ± 0.25
32	Hexanoic acid	7.23b ± 1.13	6.50ab ± 0.12	5.93ab ± 0.84	5.15ab ± 1.50	4.67ab ± 2.05	5.53ab ± 2.05	5.93ab ± 2.43	5.85ab ± 3.15
36	2-Hexenoic acid	0.30bc ± 0.18	0.30bc ± 0.10	0.45c ± 0.11	ND	0.23abc ± 0.03	0.24abc ± 0.10	0.30bc ± 0.15	0.19ab ± 0.10
37	Octanoic acid	10.74b ± 1.34	10.20b ± 1.05	9.40ab ± 1.50	7.05ab ± 2.65	7.73ab ± 2.23	8.51ab ± 1.50	9.10ab ± 2.40	9.49ab ± 4.25
39	n-Decanoic acid	2.40ab ± 1.03	2.30ab ± 1.10	2.61b ± 1.64	1.05a ± 0.30	2.23ab ± 1.13	1.95 ± 0.56	1.38ab ± 0.40	1.77ab ± 0.35
42	Benzoic acid	2.93b ± 0.42	ND	0.67b ± 0.14	0.48ab ± 0.23	0.55ab ± 0.20	0.23ab ± 0.13	0.43ab ± 0.23	0.27ab ± 0.11
	Total fatty acids	33.23	23.20	23.46	20.06	20.44	28.52	23.0	20.12
	Aldehydes–Ketones								
1	Acetaldehyde	7.54ab ± 1.08	7.90ab ± 1.35	7.90ab ± 2.34	9.73bc ± 0.55	11.56bc ± 1.10	8.90ab ± 3.60	5.93a ± 0.43	9.66b ± 0.30
11	3-Hydroxy-2-butanone	2.13bc ± 0.20	1.74ab ± 0.13	1.74ab ± 0.13	1.96b ± 2.06	2.38bc ± 0.24	2.08bc ± 0.80	1.41a ± 0.33	1.93ab ± 0.93
20	Benzaldehyde	0.11cd ± 0.02	0.13bcd ± 0.03	0.08bcd ± 0.02	ND	0.08bcd ± 0.03	0.04ab ± 0.01	0.13d ± 0.05	0.06abc ± 0.02
	Total aldehydes–ketones	9.78	9.74	9.72	11.69	14.02	11.02	7.44	11.62
	Lactone								
25	g-Butyrolactone	0.67e ± 0.10	0.11abc ± 0.05	0.33d ± 0.10	0.21abc ± 0.11	0.16abc ± 0.03	0.27bcd ± 0.13	0.21abc ± 0.12	0.13ab ± 0.04

(*continued on next page*)

Table 3 (*continued*)

No	Compounds	CN	NP1	NP8	NP11	NP13	NP20	NP22	NP27
	Vanillin derivatives								
43	Methyl vanillate	0.15b ± 0.08	0.16b ± 0.09	0.08a ± 0.03	0.32b ± 0.20	ND	0.09a ± 0.02	ND	0.14b ± 0.04
44	Acetovanillon	0.87a ± 0.10	0.66a ± 0.05	0.87a ± 0.34	0.55a ± 0.20	ND	0.48a ± 0.12	ND	0.50a ± 0.20
	Total vanillin derivatives	1.02	0.76	0.95	0.87		0.57		0.19
	Sulphur compound								
30	Methil-thio-1-propanol	0.98b ± 0.28	0.42a ± 0.13	0.35a ± 0.08	0.25a ± 0.08	0.26a ± 0.12	0.33a ± 0.12	0.33a ± 0.11	0.27a ± 0.11
	Volatile phenols								
34	2-Methoxyphenol	0.14b	ND	ND	ND	ND	ND	ND	ND
38	2-Methoxy-4-vinylphenol	1.33c ± 0.13	0.93bc ± 0.31	0.94bc ± 0.18	0.78abc ± 0.33	0.91bc ± 0.10	0.77ab ± 0.35	0.77ab ± 0.14	0.55ab ± 0.17
	Total phenols	1.44	0.93	0.94	0.78	0.91	0.77	0.77	0.55

No	Compounds	CN	NP33	NP38	NP45	NP50	NP55	NP81	NP94	NP103
	Alcohols									
3	1-Propanol	7.53a ± 0.47	14.40ab ± 0.20	18.04ab ± 0.51	12.75ab ± 0.03	19.70ab ± 2.10	17.91ab ± 0.14	21.12b ± 2.50	12.01ab ± 0.25	18.96b ± 2.36
4	2-Methyl-1-propanol	5.85ab ± 0.47	4.73a ± 0.03	5.46a ± 0.20	4.60ab ± 0.10	6.16b ± 2.03	5.94ab ± 0.33	7.10b ± 0.20	4.10a ± 1.10	6.61ab ± 2.15
6	1-Butanol	0.34b ± 0.11	0.16ab ± 0.01	0.18ab ± 0.03	0.23ab ± 0.08	0.22ab ± 0.03	0.24ab ± 0.11	0.28ab ± 0.11	0.11ab ± 0.05	0.19a ± 0.08
7	2+3-Methyl-1-butanol	48.65a ± 0.69	42.56a ± 0.09	49.27a ± 4.24	45.74a ± 0.35	47.60a ± 0.60a	48.72 ± 4.33	59.21a ± 0.15	45.84a ± 11.05	48.40a ± 5.40
13	3-Methyl-1-pentanol	ND	ND	0.15a ± 0.03	0.21a ± 0.06	0.28a ± 0.05	ND	ND	ND	ND
15	1-Hexanol	3.41de ± 0.40	2.51bc ± 0.43	2.94de ± 1.11	2.20bc ± 0.20	2.63bc ± 1.02	2.94de ± 1.06	3.10de ± 0.50	2.80bc ± 0.20	2.73bc ± 0.13
14	*Trans*-3-hexen-1-ol	ND	0.07bc ± 0.01	0.09bc ± 0.01	0.11bc ± 0.02	0.04ab ± 0.02	0.10ab ± 0.02	0.05bc ± 0.02	ND	0.08bc ± 0.02
18	*Cis*-3-hexen-1-ol	0.32bc ± 0.08	0.18a ± 0.04	0.22ab ± 0.05	0.23bc ± 0.05	0.22ab ± 0.10	0.24bc ± 0.10	0.20a ± 0.06	0.21a ± 0.03	0.22ab ± 0.06
21	1-Heptanol	0.50ab ± 0.20	0.13a ± 0.01	0.16ab ± 0.03	0.18ab ± 0.02	0.23ab ± 0.11	0.28ab ± 0.09	0.30ab ± 0.10	0.13a ± 0.02	0.24ab ± 0.09
23	(R,R) + (S,S) 2,3-Butanediol	3.99b ± 0.92	0.21a ± 0.03	ND	ND	0.46a ± 0.23	ND	0.23a ± 0.06	1.12a ± 0.55	ND
33	(R,S) 2,3-Butanediol	0.81b ± 0.25	0.03a ± 0.01	ND	ND	ND	ND	0.04a ± 0.02	0.17ab ± 0.08	ND
34	Benzyl alcohol	0.55a ± 0.05	0.19a ± 0.02	0.33a ± 0.11	0.46a ± 0.20	0.50a ± 0.12	0.41a ± 0.09	0.24a ± 0.09	0.23a ± 0.05	0.32a ± 0.11
	2-Phenylethanol	67.70a ± 15.90	45.10a ± 7.50	56.70a ± 2.10	80.12a ± 40.46	56.20a ± 21.20	64.70a ± 5.60	65.96a ± 6.16	56.02a ± 6.11	55.90a ± 5.40
	Total alcohols	139.66	110.27	133.55	146.83	134.24	141.48	157.83	122.74	133.65
	Esters									
2	Ethyl butyrate	0.17ab ± 0.06	0.44cde ± 0.09	0.61de ± 0.04	0.15ab ± 0.04	0.56de ± 0.11	0.35bcd ± 0.04	0.50cde ± 0.05	0.57de ± 0.04	0.50cde ± 0.20
8	Ethyl hexanoate	0.11ab ± 0.09	1.05d ± 0.63	0.92d ± 0.06	0.16ab ± 0.05	0.22d ± 0.11	0.85d ± 0.08	0.92d ± 0.06	1.10d ± 0.40	0.12a ± 0.05
5	2+3-Methyl-1-butanol acetate	0.53bc ± 0.30	0.20ab ± 0.08	0.34ab ± 0.02	0.46bc ± 0.02	0.30a ± 0.05	0.17a ± 0.05	0.17a ± 0.06	0.28ab ± 0.03	0.27ab ± 0.11
9	Ethyl acetate	20.40bc ± 1.40	17.82ab ± 0.65	20.16bc ± 0.73	14.46ab ± 1.17	11.44a ± 0.62	17.00ab ± 0.61	23.70bc ± 2.4	16.25ab ± 1.03	16.80ab ± 5.60
10	Hexyl acetate	0.24a ± 0.04	0.14a ± 0.09	0.18a ± 0.03	0.20a ± 0.07	0.07a ± 0.02	0.21a ± 0.02	0.07a ± 0.02	0.15a ± 0.06	0.13a ± 0.06
12	Ethyl lactate	8.20a ± 2.63	11.46ab ± 0.88	12.65ab ± 2.50	14.7bc ± 3.45	12.40ab ± 2.46	18.01b ± 3.02	17.90c ± 1.30	12.44ab ± 5.20	14.40bc ± 4.12
16	Ethyl octanoate	0.65a ± 0.09	0.98a ± 0.53	1.16a ± 0.3	0.82a ± 0.21	0.80a ± 0.23	0.90a ± 0.20	0.86a ± 0.06	0.82a ± 0.08	1.19a ± 0.27
19	Ethyl 3-hydroxybutyrate	0.22bc ± 0.09	0.06ab ± 0.0	0.09ab ± 0.04	0.08ab ± 0.03	0.22bc ± 0.02	0.21bc ± 0.04	0.10ab ± 0.04	0.07ab ± 0.02	0.12ab ± 0.05
26	Ethyl decanoate	0.42b ± 0.27	0.15a ± 0.05	0.41b ± 0.04	0.40b ± 0.1	0.04a ± 0.02	0.15a ± 0.05	NI	0.10a ± 0.06	NI
28	Diethyl succinate	1.45a ± 0.22	1.77a ± 0.06	1.26a ± 0.51	1.41a ± 0.33	1.97a ± 0.21	1.50a ± 0.40	1.83a ± 0.71	1.22a ± 0.07	1.78a ± 0.36
29	Ethyl-9-decenoate	0.56ab ± 0.19	0.26ab ± 0.14	0.40ab ± 0.05	0.35ab ± 0.10	0.30ab ± 0.10	0.46ab ± 0.04	0.31ab ± 0.06	0.30ab ± 0.12	0.44ab ± 0.11
31	2-Phenylethyl acetate	0.80d ± 0.10	0.29a ± 0.16	0.58abcd ± 0.01	0.78d ± 0.40	0.50abcd ± 0.25	0.70bcd ± 0.02	0.38ab ± 0.06	0.40abc ± 0.10	0.44abcd ± 0.04
40	Ethylhexadecanoate	2.85b ± 0.41	0.20a ± 0.03	0.18a ± 0.04	0.30a ± 0.06	0.13a ± 0.05	0.20a ± 0.05	0.16a ± 0.05	0.15a ± 0.07	0.13a ± 0.05
41	Mono ethyl succinate	2.91ab ± 1.35	1.46a ± 0.44	2.29ab ± 0.05	4.24ab ± 2.32	2.70ab ± 1.10	3.62ab ± 0.17	2.87ab ± 1.11	1.68ab ± 0.56	2.92ab ± 1.15
	Total esters	39.51	36.28	41.23	38.51	31.65	44.32	49.78	35.53	39.24
	Fatty acids									
17	Acetic acid	6.03b ± 2.03	3.64a ± 0.34	2.66a ± 1.05	3.11a ± 1.45	2.02a ± 0.20	2.10a ± 1.05	2.34a ± 1.15	1.71a ± 0.25	2.10a ± 1.10
22	2-Methyl-propanoic acid	0.80bc ± 0.03	0.42ab ± 0.09	0.40ab ± 0.01	0.46b ± 0.11	NI	0.60bc ± 0.10	0.44b ± 0.20	0.15a ± 0.02	0.40ab ± 0.10
24	Butyric acid	0.42bc ± 0.29	0.21ab ± 0.05	0.25bc ± 0.04	0.33bc ± 0.11	0.31bc ± 0.02	0.35bc ± 0.06	0.23ab ± 0.10	0.17ab ± 0.03	0.23ab ± 0.06
27	2-Methylbutyric acid	2.36ab ± 0.12	1.61a ± 0.31	1.95ab ± 0.08	2.55ab ± 0.70	2.20ab ± 0.50	2.70ab ± 0.50	2.34ab ± 1.12	1.50a ± 0.06	2.20ab ± 0.32
32	Hexanoic acid	7.20b ± 1.13	4.9ab ± 0.11	6.30ab ± 0.30	7.30b ± 3.80	3.94a ± 0.15	5.98ab ± 0.02	5.92ab ± 2.30	5.07ab ± 1.1	0.57ab ± 0.23
36	2-Hexenoic acid	0.30bc ± 0.18	0.19abc ± 0.06	0.25abc ± 0.04	0.30bc ± 0.14	0.14ab ± 0.05	0.33bc ± 0.04	0.24abc ± 0.04	0.15ab ± 0.02	0.21abc ± 0.02
37	Octanoic acid	10.74ab ± 3.64	7.87ab ± 3.32	9.78ab ± 2.18	11.26b ± 5.30	5.01a ± 0.21	9.92ab ± 1.15	8.87ab ± 3.60	9.30ab ± 2.53	8.70ab ± 0.90
39	n-Decanoic acid	2.40ab ± 1.03	2.30ab ± 1.05	2.41ab ± 1.10	2.75b ± 1.28	0.72a ± 0.33	1.80ab ± 0.40	1.10ab ± 0.30	1.21ab ± 0.06	1.80ab ± 1.03

42	Benzoic acid	2.90b ± 1.35	0.33ab ± 0.21	0.44ab ± 0.12	0.64ab ± 0.25	0.28ab ± 0.05	0.44ab ± 0.11	0.32ab ± 0.05	ND	0.38ab ± 0.02
	Total acids	33.15	21.47	24.44	28.70	14.59	24.22	21.8	19.26	16.59
	Aldehydes–ketones									
1	Acetaldehyde	7.54ab ± 0.08	10.67bc ± 0.32	14.06c ± 0.28	12.24bc ± 0.88	11.71bc ± 0.54	8.02ab ± 0.2	8.80ab ± 2.30	10.55bc ± 1.65	8.62ab ± 3.50
11	3-Hydroxy-2-butanone	2.13bc ± 0.19	1.77ab ± 0.16	2.83c ± 0.10	2.34bc ± 0.07	2.44bc ± 1.05	1.88ab ± 0.12	1.81ab ± 0.09	2.30bc ± 0.11	1.80ab ± 0.10
20	Benzaldehyde	0.11b ± 0.01	0.05ab ± 0.01	0.05ab ± 0.0	0.06ab ± 0.02	ND	0.09ab ± 0.04	0.06ab ± 0.03	0.05ab ± 0.002	0.04a ± 0.02
	Total aldehydes–ketones	9.78	12.49	16.94	14.64	14.15	9.99	10.67	12.9	10.46
	Lactone									
25	g-Butyrolactone	0.67b ± 0.10	0.12ab ± 0.03	ND	ND	0.22ab ± 0.11	0.22ab ± 0.08	0.15a ± 0.05	0.22ab ± 0.06	0.32ab ± 0.04
	Vanillin derivatives									
43	Methyl vanillate	0.15b ± 0.10	0.07a ± 0.02	0.04a ± 0.02	ND	ND	0.10b ± 0.02	0.23b ± 0.11	0.10b ± 0.04	0.08a ± 0.03
44	Acetovanillon	0.87a ± 0.01	ND	0.55a ± 0.09	3.02b ± 1.75	ND	0.51a ± 0.02	0.05a ± 0.02	0.44a ± 0.04	0.50a ± 0.20
	Total vanillin derivatives	1.02	0.07	0.59	3.02		0.61	0.28	0.54	0.58
	Sulphur compound									
30	Methil-thio-1-propanol	0.98b ± 0.28	0.20a ± 0.03	0.20a ± 0.02	0.36a ± 0.04	0.26a ± 0.08	0.33a ± 0.04	0.30a ± 0.01	0.26a ± 0.13	0.18a ± 0.08
	Volatile phenols									
34	2-Methoxyphenol	0.14b ± 0.02	ND	ND	ND	ND	ND	ND	ND	ND
38	2-Methoxy-4-vinylphenol	1.30b ± 0.03	0.34a ± 0.11	0.56ab ± 0.05	0.10a ± 0.35	0.77ab ± 0.09	0.74ab ± 0.05	0.60ab ± 0.20	0.65 ab ± 0.25	0.66 ab ± 0.15
	Total phenols	1.44	0.34	0.56	0.10	0.77	0.74	0.60	0.65	0.66

No: peak numbering in order of elution. Each value is expressed in mg/L and is the mean of 3 extraction replicates ± ds (standard deviation).
Values with different letters in the same row are significantly different according to the Duncan's test ($p < 0.05$).

the ethanol content (vol. %) ranged from 12.1 (NP45) to 13.4 (NP8) and in SP derived wines, a part from SP52 (9.3) and SP62 (9.1) characterized by an unusually low ethanol content level, ethanol content ranged from 12.4 (SP24) to 13.3 (SP32), thus within normal levels of a good quality Negroamaro wine (12–14%). Sugar content in the wines obtained using NP strains varied between 2 and 3 g/L while it varied from 1 to 2 g/L in the SP strains produced wines. In both cases the sugar content accounts for a completed fermentation (Pérez-Coello et al., 1999). The total acidity content ranged from 5.44 g/L to 6.32 g/L in wines produced by NP strains and from 4.71 g/L to 5.18 g/L in wines produced by SP strains.

The level of volatile acidity, with the principal contribution of acetic acid produced by yeast during fermentation, was from 0.22 g/L to 0.27 g/L in NP strains derived wines and from 0.25 g/L to 0.31 g/L in wines produced by SP strains. In both cases volatile acidity content is quite below the 0.6 g/L value considered as undesirable because as it increases above this critical level, it gradually gives a sour taste to the wine (Fleet & Heard, 1993). A small variation of pH values was observed (3.41–3.53 for NP strains produced wines; 3.53–3.55 for SP strains produced wines). In all cases, the pH of the wines was <3.6, that is the maximum value for wine correct conservation. The glycerol formed by yeast during fermentation is one of the main components of wine, in which it is usually found in concentrations ranging from 5 to 8 g/L. The glycerol has a key role in the formation of the bouquet of wine, as it improves the balance and structure of wine (Noble & Bursick, 1984). All the selected strains were found to produce satisfactory amounts of glycerol, with values ranging from 6.9 to 8.3 g/L for the NP strains wines and from 6.8 to 7.5 for SP strains produced wines.

3.3. Identification of volatile compounds

GC-FID and SPE/GC–MS analysis of the Negroamaro wines produced with NP and SP yeast strains allowed the identification and quantification of respectively a total of 43 and 41 key volatile compounds belonging to eight different groups that are by-products of yeast metabolism namely: alcohols, esters, fatty acids, aldehydes-ketones, lactone, vanillin derivates, sulphur compound, and volatile phenols (Tables 3 and 4). A part from the presence only in wines obtained from NP yeast strains of trans-3-hexen-1-ol and benzaldehyde, all the differences observed in the volatile composition of the wines obtained from the different yeast strains resulted to be quantitative rather than qualitative. In Negroamaro wines alcohols represent the most abundant volatile compounds and usually account for 80–90% of the wines total aromatic content (Usseglio-Tomasset, 1998). The content of higher alcohols of the wines from the two geographic areas were quite variable but in general values of wines produced with NP strains are higher. In particular 1-propanol, 2-methyl-1-propanol, 2+3-methyl-1-butanol, and 2-phenylethanol were determined in the analysed wines, and resulted to be quantitatively the most representative compounds in this group, and their concentrations varied according to the yeast strain used.

Concerning 2+3methyl-1-butanol, the wine produced using NP8 strain showed the highest concentration of this alcohol (68.51 mg/L); while the wines produced by NP1 and NP33 strains contained the lowest 2+3methyl-1-butanol quantity (42.53; 42.56 mg/L). 1-Propanol was detected in all wines produced by NP and SP strains, with concentration ranging from 12.01 mg/L (NP94) to 24.82 mg/L (NP8) in NP wines and from 5.62 mg/L (SP77) to 17.70 mg/L (SP34) in SP wines. Benzyl alcohol and 2-phenylethanol were also detected in Negroamaro wine samples. The content of these compounds was variable in all the wine

Table 4
Quantification of volatile compounds identified in the wines produced by *S. cerevisiae* strains (SP) from the South Salento area.

No.	Compounds	CS	SP4	SP16	SP20	SP24	SP32	SP33	SP34
	Alcohols								
3	1-Propanol	7.53 ab ± 0.05	13.71b ± 2.22	14.51bc ± 0.12	15.41bc ± 0.26	11.24 ab ± 0.18	13.72b ± 2.12	12.62 ab ± 1.72	17.70c ± 0.34
4	2-Methyl-1-propanol	7.36b ± 0.07	4.46a ± 1.11	4.12a ± 0.12	4.81a ± 0.03	4.60a ± 0.20	4.32a ± 0.22	4.75a ± 0.14	4.82a ± 0.22
6	1-Butanol	0.51b ± 0.12	0.31b ± 0.11	0.41b ± 0.03	0.30b ± 0.03	0.45b ± 0.20	0.52b ± 0.22	0.31b ± 0.11	0.26ab ± 0.010
7	2+3-Methyl-1-butanol	68.50c ± 2.14	49.95ab ± 0.50	45.12a ± 0.18	50.90ab ± 0.045	59.81bc ± 0.40	45.82a ± 2.31	54.52bc ± 2.31	51.84ab ± 2.22
13	1-Hexanol	2.32ab ± 0.14	2.13bc ± 0.70	2.28abc ± 0.03	2.21abc ± 0.14	3.53d ± 1.72	3.11cd ± 0.84	2.31abc ± 1.11	2.32abc ± 1.12
14	*Cis*-3-Hexen-1-ol	0.18a ± 0.06	0.16a ± 0.03	0.17a ± 0.02	0.17a ± 0.03	0.27a ± 0.14	0.23a ± 0.07	0.18a ± 0.06	0.17a ± 0.03
17	1-Heptanol	0.22abc ± 0.10	0.22± 0.06	0.22bcd ± 0.02	0.22bcd ± 0.04	0.37d ± 0.22	0.30cd ± 0.12	0.22cd ± 0.06	0.20bcd ± 0.10
19	(R,R) + (S,S) 2,3-Butanediol	0.27ab ± 0.10	1.31bcd ± 0.43	1.93d ± 0.35	1.03abcd ± 0.74	0.60abcd ± 0.11	1.71cd ± 0.51	0.40abc ± 0.20	ND
21	(R,S) 2,3-Butanediol	0.05ab ± 0.02	0.23ab ± 0.08	0.45bcd ± 0.15	0.41abcd ± 0.21	0.80de ± 0.40	0.44bcd ± 0.22	0.10ab ± 0.02	ND
30	Benzylalcohol	0.13abc ± 0.02	0.03ab ± 0.01	0.11abc ± 0.02	0.11abc ± 0.02	0.14bc ± 0.06	0.16bc ± 0.06	0.09abc ± 0.03	0.10bc ± 0.02
31	2-Phenylethanol	52.81bc ± 2.02	45.21abc ± 4.21	48.81bc ± 3.62	54.34bc ± 0.98	84.94c ± 55.66	32.52bc ± 7.61	43.18abc ± 22.15	49.41bc ± 12.51
	Total alcohols	139.88	117.72	118.13	129.91	166.75	102.85	118.68	126.82
	Esters								
2	Ethyl butyrate	0.66a ± 0.14	0.72ab ± 0.36	0.61ab ± 0.11	0.85b ± 0.06	0.62ab ± 0.30	NI	0.54ab ± 0.20	0.53ab ± 0.11
5	2+3-Methyl-1-butanol acetate	0.18a ± 0.02	1.07 ± 0.55	0.13a ± 0.02	0.80 ± 0.02	0.17 ± 0.03	0.51 ± 0.08	0.13a ± 0.05	0.13a ± 0.05
8	Ethyl hexanoate	0.47ab ± 0.13	1.01c ± 0.03	0.88c ± 0.05	0.87bc ± 0.12	0.63abc ± 0.11	0.18a ± 0.03	1.15c ± 0.22	1.13c ± 0.06
9	Ethyl acetate	23.66 ± 2.25	19.30 ± 0.30	18.36 ± 1.78	19.11 ± 0.64	18.84 ± 0.26	18.63 ± 2.20	21.91 ± 0.95	19.34 ± 1.12
10	Hexyl acetate	0.15abc ± 0.05	0.10abc ± 0.05	0.08abc ± 0.02	0.13abc ± 0.02	0.23abc ± 0.06	0.18bc ± 0.02	0.30c ± 0.05	0.11bc ± 0.02
12	Ethyl lactate	9.23a ± 2.30	11.62abcd ± 1.91	12.01bcde ± 2.03	14.52cde ± 1.11	8.96abc ± 3.45	13.01abcd ± 3.11	10.81abcd ± 1.11	7.42a ± 2.44
15	Ethyl octanoate	0.18ab ± 0.05	0.61abcde ± 0.11	0.78def ± 0.07	0.72cdef ± 0.18	0.13abc ± 0.03	0.13abc ± 0.05	0.54abcde ± 0.22	0.60bcdef ± 0.20
18	Ethyl 3-hydroxybutyrate	0.16a ± 0.03	0.13a ± 0.07	0.11a ± 0.02	0.15a ± 0.04	0.60ab ± 0.06	0.18a ± 0.05	0.10a ± 0.05	ND
24	Ethyl decanoate	0.41b ± 0.21	0.10a ± 0.04	0.41b ± 0.03	0.13ab ± 0.06	0.12ab ± 0.06	0.42b ± 0.12	0.09a ± 0.02	0.10a ± 0.04
25	Diethyl succinate	1.63a ± 0.35	1.31a ± 0.61	1.63a ± 0.45	1.51a ± 0.16	1.70a ± 0.42	1.41a ± 0.08	1.62a ± 0.26	1.40b ± 0.22
27	Ethyl-9-decenoate	0.32abc ± 0.11	0.18abc ± 0.02	0.40abc ± 0.10	0.24abc ± 0.07	0.11b ± 0.03	0.35abc ± 0.19	0.14abc ± 0.07	0.11abc ± 0.22
29	2-Phenylethyl acetate	0.96abc ± 0.22	0.71bc ± 0.03	0.35abc ± 0.05	0.83cd ± 0.21	1.72abc ± 0.82	0.92cd ± 0.25	0.50abc ± 0.12	0.50abc ± 0.12
36	Ethyl hexadecanoate	0.18b ± 0.10	ND	0.06a ± 0.04	0.04a ± 0.01	0.11a ± 0.04	0.05a ± 0.02	ND	0.11a ± 0.05
37	Mono ethyl succinate	6.01abc ± 2.35	4.81abc ± 2.71	6.06abc ± 0.90	5.88abc ± 2.10	6.5abc ± 4.5	8.81abc ± 4.11	2.84bc ± 1.10	2.31ab ± 1.12
	Total Esters	44.20	41.67	41.86	45.78	40.44	44.77	40.67	33.79
	Fatty acids								
16	Acetic acid	6.25abcde ± 2.25	0.81a ± 0.31	5.44bcde ± 1.30	3.72bcde ± 1.92	6.32de ± 3.22	6.42de ± 1.32	0.55a ± 0.10	0.45a ± 0.22
20	2 Methyl propanoic acid	0.07a ± 0.02	0.42abcd ± 0.33	0.53abc ± 0.11	0.48abcd ± 0.13	0.83e ± 0.41	0.73abc ± 0.30	0.30abc ± 0.06	0.32ab ± 0.12
22	Butyric acid	0.45abc ± 0.09	0.31abc ± 0.15	0.36abc ± 0.06	0.33abc ± 0.04	0.63c ± 0.23	0.54bc ± 0.20	0.23ab ± 0.11	0.20ab ± 0.05
25	3-Methyl butyric acid	0.24a ± 0.04	2.13abc ± 1.40	2.17abc ± 0.03	2.34abc ± 1.10	3.82c ± 1.44	2.95c ± 1.22	1.70abc ± 0.25	1.66abc ± 0.25
29	Hexanoic acid	7.17bc ± 3.15	5.72b ± 1.52	5.62b ± 0.42	6.30b ± 0.24	10.29c ± 5.03	0.90a ± 0.26	5.60b ± 2.30	5.56b ± 2.33
32	2-Hexenoic acid	0.12ab ± 0.03	0.15abcd ± 0.10	0.17bcd ± 0.05	0.18bcd ± 0.06	0.28d ± 0.03	0.28d ± 0.09	0.18bcd ± 0.09	0.15bcd ± 0.05
33	Octanoic acid	10.14abc ± 4.50	8.91abcd ± 2.51	7.51abcd ± 0.51	10.14bcd ± 2.06	15.74d ± 5.19	14.51cd ± 3.80	9.51ab ± 3.11	8.95abcd ± 3.60
35	Decanoic acid	1.21a ± 0.31	0.92a ± 0.56	0.64a ± 0.04	1.20a ± 0.16	1.45a ± 0.55	1.80b ± 0.40	1.52a ± 0.80	1.03a ± 0.20
38	Benzoic acid	0.35ab ± 0.15	0.23ab ± 0.11	0.36b ± 0.05	0.28ab ± 0.06	0.57c ± 0.21	0.54c ± 0.20	0.21ab ± 0.11	0.14ab ± 0.05
	Total acids	26.0	19.61	22.80	24.97	39.93	28.67	19.80	18.46
	Aldehydes–Ketones								
1	Acetaldehyde	13.75a ± 0.21	24.45ab ± 0.29	22.10ab ± 0.52	26.95b ± 2.36	22.64ab ± 2.75	22.62ab ± 2.12	22.82ab ± 0.72	17.77ab ± 0.75
11	3-Hydroxy-2-butanone	2.37a ± 0.07	4.34c ± 0.06	3.60b ± 0.20	4.80c ± 0.28	4.43c ± 1.70	3.96b ± 0.23	3.04b ± 0.30	4.01c ± 0.20
	Total Aldehydes–ketones	16.12	28.79	25.70	31.75	27.07	26.58	25.86	21.77
	Lactones								
23	g-Butyrolactone	0.39ab ± 0.05	0.30ab ± 0.06	0.33b ± 0.05	0.30ab ± 0.03	0.48b ± 0.20	0.48b ± 0.22	0.20ab ± 0.10	0.18a ± 0.08
	Vanillin derivatives								
39	Methyl vanillate	0.04a ± 0.01	0,04a ± 0.02	0.05ab ± 0.02	0.06ab ± 0.02	0.30b ± 0.15	0.05ab ± 0.02	0.05ab ± 0.02	0.04a ± 0.02
40	Acetovanillon	0.21ab ± 0.04	0.16a ± 0.12	0.19ab ± 0.03	0.20ab ± 0.03	0.35b ± 0.15	0.26ab ± 0.09	0.22ab ± 0.12	0.20ab ± 0.06
	Total vanillin derivatives	0.25	0.20	0.24	0.26	0.65	0.31	0.27	0.24

	Sulphur compound									
28	Methyl-thio-1-propanol	0.62ab ± 0.18	0.49abc ± 0.14	0.62bc ± 0.05	0.65bc ± 0.17	0.97bc ± 0.20	0.90bc ± 0.40	0.46bc ± 0.11	0.40bc ± 0.10	
	Volatile phenols									
34	2-Methoxy-4-vinylphenol	0.62ab ± 0.05	0.54ab ± 0.40	0.60abc ± 0.12	0.64abc ± 0.06	1.23c ± 0.40	0.85bc ± 0.22	0.67bc ± 0.08	0.56ab ± 0.20	

Saccharomyces cerevisiae strain										
No.	Compounds	CS	SP43	SP44	SP52	SP62	SP66	SP77	SP86	SP91
	Alcohols									
3	1-Propanol	7.53ab ± 0.05	16.12bc ± 0.20	13.95b ± 0.20	11.2ab ± 0.11	15.13bc ± 0.11	5.88a ± 0.16	5.62a ± 0.10	15.35bc ± 0.20	16.01bc ± 0.1
4	2-Methyl-1-propanol	7.36b ± 0.07	4.60a ± 0.22	4.60a ± 0.10	4.42a ± 0.20	4.30a ± 0.2	6.32ab ± 0.30	6.83ab ± 0.30	4.62a ± 0.06	4.37a ± 0.05
6	1-Butanol	0.50ab ± 0.10	0.40ab ± 0.15	0.50ab ± 0.30	0.33ab ± 0.06	0.03a ± 0.01	0.23ab ± 0.11	0.22ab ± 0.05	0.23ab ± 0.02	0.45ab ± 0.21
7	2+3-Methyl-1-butanol	68.50c ± 2.14	47.10a ± 0.35	51.71ab ± 0.20	58.40bc ± 0.10	48.48a ± 0.20	48.87a ± 1.10	56.80b ± 0.30	49.70a ± 2.06	52.84ab ± 0.22
13	1-Hexanol	2.32abc ± 0.14	2.60abc ± 0.7	2.80abc ± 1.1	2.21abc ± 0.18	0.22a ± 0.11	1.33ab ± 0.25	2.22abc ± 0.30	1.91bc ± 0.24	2.32abc ± 1.10
14	Cis-3-hexen-1-ol	0.18a ± 0.06	0.20a ± 0.06	0.20a ± 0.08	0.16a ± 0.06	0.02a ± 0.01	0.20a ± 0.05	0.18a ± 0.02	1.90b ± 0.40	0.18a ± 0.06
17	1-Heptanol	0.22cd ± 0.10	0.26cd ± 0.10	0.40d ± 0.11	0.20cd ± 0.06	0.02ab ± 0.01	0.08abc ± 0.03	0.21bcd ± 0.05	0.23cd ± 0.04	ND
19	(R,R) + (S,S) 2,3-Butanediol	0.27ab ± 0.10	3.42e ± 0.70	0.67abcd ± 0.33	5.75e ± 1.50	0.16ab ± 0.08	0.73abcd ± 0.22	0.70abcd ± 0.30	1.34abcd ± 0.73	3.70d ± 1.15
21	(R,S) 2,3-Butanediol	0.05ab ± 0.02	0.74cde ± 0.14	0.32abc ± 0.11	1.06e ± 0.42	0.03ab ± 0.01	0.20ab ± 0.05	0.18ab ± 0.02	0.33abc ± 0.08	0.98e ± 0.06
30	Benzylalcohol	0.13bc ± 0.02	0.10abc ± 0.05	1.16d ± 0.10	0.17 ± 0.02	NI	0.13bc ± 0.05	0.12bc ± 0.04	0.07abc ± 0.03	0.11abc ± 0.06
31	2-Phenylethanol	52.80bc ± 2.0	60.80bc ± 12.54	76.85c ± 35.88	64.75bc ± 7.9	5.01a ± 0.51	43.60abc ± 21.10	47.80bc ± 1.40	50.74bc ± 6.74	53.20bc ± 21.4
	Total alcohols	139.86	136.34	153.16	148.65	73.39	107.57	120.88	126.42	134.16
	Esters									
2	Ethyl butyrate	0.66ab ± 0.14	0.50a ± 0.10	0.72ab ± 0.10	0.51ab ± 0.11	0.06a ± 0.03	0.70ab ± 0.22	0.61ab ± 0.24	0.63ab ± 0.14	ND
5	2+3-Methyl-1-butanol acetate	0.18a ± 0.02	0.72b ± 0.23	0.16ab ± 0.02	0.92ab ± 0.08	0.33ab ± 0.13	0.18ab ± 0.06	0.85ab ± 0.30	0.15a ± 0.06	0.12a ± 0.03
8	Ethyl hexanoate	0.47abc ± 0.13	0.12a ± 0.07	1.15c ± 0.5	0.65abc ± 0.22	0.12a ± 0.02	1.01c ± 0.20	1.11c ± 0.04	1.11c ± 0.05	0.96c ± 0.15
9	Ethyl acetate	23.66b ± 2.25	18.63ab ± 0.80	19.54ab ± 2.30	18.45ab ± 0.30	18.01a ± 0.44	19.24ab ± 0.11	19.41ab ± 0.62	19.30ab ± 0.15	19.53ab ± 0.22
10	Hexyl acetate	0.15abc ± 0.05	0.11abc ± 0.05	0.07ab ± 0.02	0.12abc ± 0.03	ND	0.10abc ± 0.04	0.11abc ± 0.03	0.11abc ± 0.02	0.09ab ± 0.02
12	Ethyl lactate	9.23abc ± 2.30	17.25e ± 6.33	12.9abcd ± 2.9	10.70abcd ± 0.04	8.64abc ± 2.60	12.80abcd ± 2.2	15.62de ± 3.90	11.80abcd ± 2.55	11.86abcd ± 3.12
15	Ethyl octanoate	0.18ab ± 0.05	0.60abcdef ± 0.11	1.07fg ± 0.05	1.20f ± 0.50	0.08ab ± 0.02	1.90g ± 0.15	0.37abcd ± 0.08	0.64bcdef ± 0.18	ND
18	Ethyl 3-hydroxybutyrate	0.16a ± 0.03	0.15a ± 0.05	0.28a ± 0.06	0.15a ± 0.07	ND	3.18b ± 0.11	0.06a ± 0.03	0.13a ± 0.04	0.14a ± 0.06
24	Ethyl decanoate	0.40b ± 0.2	0.40b ± 0.22	0.12ab ± 0.05	0.44b ± 0.22	0.03a ± 0.01	0.09ab ± 0.02	0.40b ± 0.04	0.35ab ± 0.20	0.09ab ± 0.03
25	Diethyl succinate	1.63a ± 0.35	2.16a ± 0.60	1.30a ± 0.60	2.15a ± 0.20	1.98a ± 0.40	1.90a ± 0.12	1.77a ± 0.40	1.43a ± 0.16	1.64a ± 0.11
27	Ethyl-9-decenoate	0.32ab ± 0.11	0.43abc ± 0.11	0.70bc ± 0.05	0.54abc ± 0.18	0.03a ± 0.01	0.15ab ± 0.07	0.40abc ± 0.10	0.73c ± 0.25	0.20abc ± 0.08
29	2-Phenylethyl acetate	0.96cd ± 0.22	0.64abc ± 0.11	1.10cd ± 0.05	0.82bc ± 0.13	0.07a ± 0.02	0.52abc ± 0.20	0.71abc ± 0.20	0.76abc ± 0.20	0.50abc ± 0.12
36	Ethylhexadecanoate	0.18b ± 0.10	0.08a ± 0.02	ND	0.03a ± 0.02	0.02a ± 0.002	0.74a ± 0.15	0.10a ± 0.03	0.08a ± 0.02	0.09a ± 0.02
37	Mono ethyl succinate	6.01abc ± 2.35	6.65abc ± 2.25	10.80c ± 5.6	6.53abc ± 1.33	0.50a ± 0.14	6.30abc ± 2.10	4.10abc ± 1.85	4.95abc ± 2.50	6.61abc ± 2.35
	Total esters	44.19	48.44	49.91	43.21	29.87	48.81	45.62	42.17	41.83
	Fatty acids									
16	Acetic acid	6.25cde ± 2.25	6.93de ± 3.10	1.35abc ± 0.20	3.32abcd ± 1.45	0.20a ± 0.06	0.21a ± 0.11	4.40abcde ± 5.85	6.10cde ± 1.13	8.23e ± 3.10
20	2-Methyl-propanoic acid	0.07a ± 0.02	0.60abc ± 0.25	0.70abc ± 0.40	0.48abcd ± 0.05	0.04a ± 0.02	0.80cd ± 0.30	0.70abc ± 0.3	0.41abcd ± 0.10	0.60abc ± 0.23
22	Butyric acid	0.45abc ± 0.09	0.40abc ± 0.11	0.50bc ± 0.22	0.36abc ± 0.15	0.03a ± 0.01	0.40abc ± 0.12	0.33abc ± 0.15	0.32abc ± 0.10	0.40abc ± 0.12
25	3-Methyl butyric acid	0.24ab ± 0.04	2.52c ± 0.80	3.81c ± 2.25	2.46bc ± 0.15	0.21ab ± 0.04	2.14abc ± 1.06	2.13abc ± 0.4	2.21abc ± 0.65	2.44bc ± 1.15
29	Hexanoic acid	7.17bc ± 3.15	6.9bc ± 1.9	4.9b ± 1.10	6.20b ± 0.90	0.60a ± 0.03	5.84b ± 1.20	5.92b ± 0.65	6.33b ± 0.90	5.93b ± 2.32
32	2-Hexenoic acid	0.12ab ± 0.03	0.20abc ± 0.08	0.24cd ± 0.11	0.17abc ± 0.03	0.02a ± 0.01	0.10abc ± 0.03	0.08ab ± 0.03	0.20abc ± 0.04	0.18abc ± 0.05
33	Octanoic acid	10.14bcd ± 4.50	10.6bcd ± 2.5	11.8bcd ± 5.9	10bcd ± 1.2	0.96a ± 0.05	8.64abcd ± 2.36	8.43abcd ± 0.40	9.74bcd ± 2.02	8.36abcd ± 3.60
35	Decanoic acid	1.20ab ± 0.30	1.11ab ± 0.70	1.30b ± 0.60	1.02ab ± 0.10	0.13a ± 0.03	1.31b ± 0.25	0.76ab ± 0.13	1.12ab ± 0.40	0.90ab ± 0.30
38	Benzoic acid	0.35bc ± 0.15	0.38bc ± 0.20	0.46bc ± 0.22	0.32ab ± 0.10	0.03a ± 0.01	0.36b ± 0.11	0.20ab ± 0.06	0.30ab ± 0.10	0.42bc ± 0.21
	Total acids	25.99	29.64	20.16	24.33	2.22	19.80	22.95	26.73	27.46
	Aldehydes–Ketones									
1	Acetaldehyde	13.75a ± 0.21	18.7ab ± 0.65	24.43ab ± 1.10	18.55ab ± 0.25	20.32ab ± 0.25	21.4ab ± 0.73	17.93ab ± 0.81	21.87ab ± 0.40	20.38ab ± 0.51
11	3-Hydroxy-2-butanone	2.37a ± 0.07	3.55bc ± 0.15	4.29c ± 0.16	2.60a ± 1.10	3.50b ± 0.20	2.50a ± 0.20	2.44a ± 0.30	3.86bc ± 0.06	3.88cb ± 0.20
	Total aldehydes–ketones	16.12	22.25	28.72	21.15	23.82	23.90	20.37	25.73	24.26

(*continued on next page*)

Table 4 (*continued*)

	Saccharomyces cerevisiae strain										
No.	Compounds	CS	SP43	SP44	SP52	SP62	SP66	SP77	SP86	SP91
	Lactones									
23	g-Butyrolactone	0.39bc ± 0.05	0.36bc ± 0.14	0.45bc ± 0.15	0.32bc ± 0.06	0.02a ± 0.01	0.30abc ± 0.1	0.26abc ± 0.12	0.25abc ± 0.12	0.32bc ± 0.10
	Vanillin derivatives									
39	Methyl vanillate	ND	ND	ND	ND	ND	ND	ND	ND	ND
40	Acetovanillone	0.21ab ± 0.04	0.21ab ± 0.05	0.30b ± 0.12	0.21ab ± 0.09	0.02a ± 0.0	0.20ab ± 0.10	0.20ab ± 0.07	0.23ab ± 0.08	0.23ab ± 0.10
	Sulphur compound									
	Methyl-thio-1-Propanol	0.62bc ± 0.18	0.70bc ± 0.20	1.62d ± 0.64	0.70bc ± 0.03	0.06a ± 0.02	0.72bc ± 0.08	0.63bc ± 0.11	0.61bc ± 0.26	0.63bc ± 0.15
28	**Volatile phenols**									
	2-Methoxy-4-vinylphenol	0.62abc ± 0.05	0.75bc ± 0.15	0.88bc ± 0.40	0.63abc ± 0.20	0.06a ± 0.02	0.88bc ± 0.25	0.77bc ± 0.13	0.71bc ± 0.09	0.61abc ± 0.25

No: peak numbering in order of elution. Each value is expressed in mg/L and is the mean of 3 extraction replicates ± ds (standard deviation). ND: non detected.
Values with different letters in the same row are significantly different according to the Duncan's test ($p < 0.05$).

samples without correlation with strain origin. 2-Phenylethanol, that is an important yeast fermentation metabolic by-product responsible for rose flavour (Aznar, Lopez, Cacho, & Ferreira, 2001), was found at higher levels in wines fermented with NP1 (75.12 mg/L), NP45 (80.12 mg/L) and NP81 (65.96 mg/L) as well as SP24 (84.94 mg/L) and SP44 (76.85 mg/L) yeast strains. Benzyl alcohol, associated to floral like flavour (Torrens et al., 2008), was detected in all NP and SP obtained wines. Among NP strains, the highest benzyl alcohol content is obtained by NP11 (0.56 mg/L) and among SP strains the highest concentration is obtained by SP44 (1.16 mg/L).

Overall, NP22, NP81 and SP24 generated the highest content of the volatile alcohols respectively 171.60 mg/L, 157.83 mg/L, and 166.85 mg/L, due to the presence of the highest concentrations of 1-propanol, 2-methyl-1-propanol, 2+3-methyl-1-butanol, benzyl alcohol and 2-phenylethanol.

Higher alcohols production is an individual strain characteristic (Giudici, Romano, & Zambonelli, 1990) that can have both positive and negative impacts on the aroma of wine. In fact, higher alcohols concentrations exceeding 400 mg/L can have an unpleasant effect (Nikolaou, Soufleros, Bouloumpasi, & Tzanetakis, 2006), whereas levels below 400 mg/L confer fruity characters to the wines (Swiegers et al., 2005). 2+3methyl-1-butanol is the most abundant higher alcohol in wine and is responsible for disagreeable flavour (Romano, Fiore, Paraggio, Caruso, & Capece, 2003). The content of 1-propanol in wine is mainly influenced by the starter strain responsible for the fermentation (Giudici et al., 1990). The data reported indicate a good level of higher alcohol content and composition in some of NP and SP wines. Another large group of volatile compounds found in wines is represented by esters. Most of the analysed yeasts strains produced esters and, in particular, ethyl lactate and ethyl acetate. Ethyl lactate appears mainly in the malolactic fermentation during vinification (Fang & Qian, 2005), accounting for 45.23% (NP8) and 35.62% (SP43) of total esters. Ethyl acetate was another major ester in the Negroamaro wines. These compounds are responsible for fruity notes and play an important role in the flavour of young wines (Callejon et al., 2010). This compound adds complexity to the aroma of wine, with fruity notes at concentrations lower than 150 mg/L, while at higher concentrations it can donate a sour, vinegary off-odour (Clarke & Bakker, 2004). Its higher concentration was found in NP81 (23.7 mg/L) and SP33 (21.91 mg/L) derived wines. The other important esters detected in our samples were ethyl butanoate (strawberry, pineapple-like flavour), ethyl hexanoate (fruity-like flavour), hexyl acetate (sweet fruity-like flavour), ethyl octanoate (cooked fruity-like flavour), ethyl decanoate (fruity-like flavour) and diethyl succinate (fruity-like flavour) (Fang & Qian, 2005).

Among acetates, 2+3methyl 1 butanol acetate, ethyl acetate, and 2-phenyl ethyl acetate were markedly the most abundant esters in the wines produced with both NP and SP yeast strains. Acetates are considered indicative of the aromatic quality of young wines and desirable compounds giving fruity notes (Gomez-Míguez, Cacho, et al., 2007).

Ethyl acetate was produced in relative amounts by all yeast strains (Tables 3 and 4), ranging from 11.44 mg/L (NP50) to 23.7 mg/L (NP81) in NP wines, and from 18.01 (SP62) to 21.91 mg/L (SP33) in SP wines. Ethyl acetate adds complexity to the aroma of wines at low levels, but can confer an unpleasant odour (vinegary) to the wine at concentrations higher than 150 mg/L (Amerine & Roessler, 1983). Other esters, ethyl butyrate, ethyl hexanoate, ethyl octanoate, diethyl succinate were detected in all samples; ethyl esters of fatty acids and acetate esters of higher alcohols evolve fruity and floral odours (Lema, Garcia-Jares, Orriols, & Angulo, 1996), thus these *S. cerevisiae* strains produce very aromatic wines. The fatty

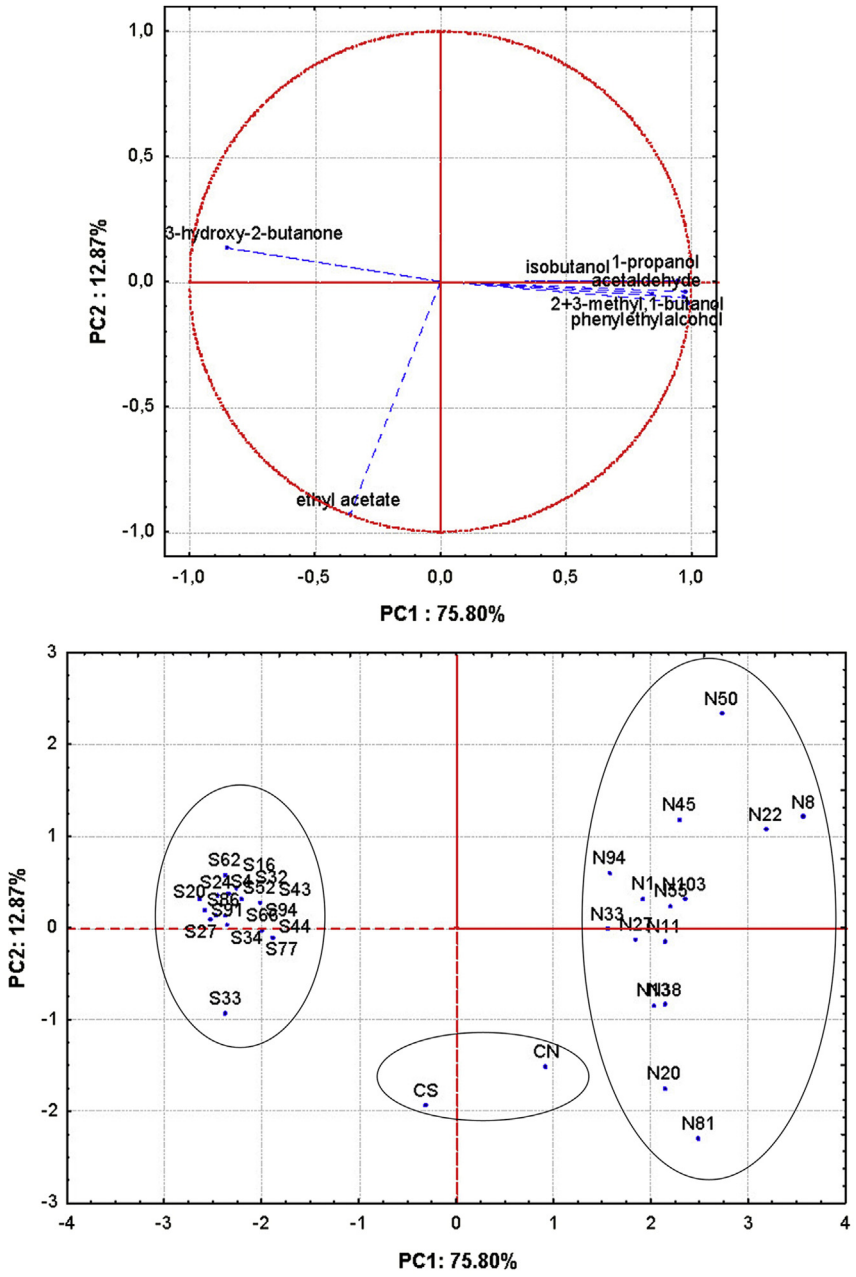

Fig. 1. Plots of the PCA analysis referred to major volatiles variables. PCA biplots in the PC1-PC2 plane combining score plots of major volatiles variables and wine samples fermented with different yeast strains. N, NP yeast population; S, SP yeast population; CN, Northern commercial control; CS, Southern commercial control.

acid content of the experimental wines was correlated to fruity, cheesy, fatty and rancid notes (Rocha, Rodrigues, Coutinho, Delgadillo, & Coimbra, 2004).

Based on the results of Duncan's multiple range tests, differences in the content of fatty acid identified and quantified were observed among all tested wines, with the exception of decanoic acid. Although the presence of C6 and C10 fatty acids is usually related to the appearance of negative odours, they are very important for the aromatic equilibrium of wines because they prevent the hydrolysis of the corresponding esters (Ferreira, Fernandez, Gracia, & Cacho, 1995).

The C4, C6, C8, and C10 fatty acids were synthesized by 8–9% of NP and 9–11% of SP strains. Among acid compounds, acetic acid is the main responsible for the volatile acidity of wine and its formation during the fermentation depends on the yeast strain (Garde-Cerdán & Ancín-Azpilicueta, 2006; Rojas, Gil, Piñaga, & Manzanares, 2003).

The highest concentration of acetaldehyde was found in NP38 (14.06 mg/L) and SP20 (26.95 mg/L) wines, while its lowest level was detected in NP22 (5.93 mg/L) and SP34 (17.77 mg/L) wines. The yeast strains analysed differ in their ability to produce acetaldehyde, which is known to be related to the strain-specific activity level of alcohol dehydrogenase (Pérez-Coello et al., 1999).

As regards to volatile phenols 2-methoxy-4-vinylphenol was found in all the analysed wines and its concentration ranged from 0.10 (NP45) to 0.94 (NP8) mg/L in wines derived from NP strains, and from 0.06 mg/L (SP62) to 1.23 mg/L (SP24) in SP derived wines. The volatile phenols can affect negatively the wine quality; vinylphenols are responsible for animal and smoky odours. No significant amount of phenolic volatiles was found in the analysed wines;

only a minimum concentration of 2-methoxy-4-vinylphenol, below its threshold perception (0.38 mg/L), was found in all the wine samples.

The analytical data evaluated by Principal Component Analysis (PCA) in order to identify the volatile compounds that best discriminated the wines produced by yeast strains selected in the two different areas. The PCA analysis of data concerning the concentrations of higher alcohols and minor volatiles are respectively shown in Figs. 1 and 2. When a two-dimensional plot (Fig. 1) was drawn, the two principal components accounted for 75.80% and 12.87% of the variance, respectively. Indeed, a natural separation of the wines according to strains origin area was achieved and the first PC was responsible for the separation between Negroamaro wines produced by yeast strains selected in the northern and southern areas. The southern samples were located in the first and second quadrant (positive PC1), and it is clear that these samples were characterized by variables associated to positive values of PC1, mainly esters while northern samples were grouped in two clusters on the left part of plot (negative PC1).

The data reported in Fig. 2, include all volatiles produced during yeast fermentation and the first principal component (PC1) accounted for 38.67% of the total variation, while PC2 explained 15.40% of the total variation. As indicated by PCA analysis (Fig. 2), it is confirmed the evidence that the wines are clearly separated

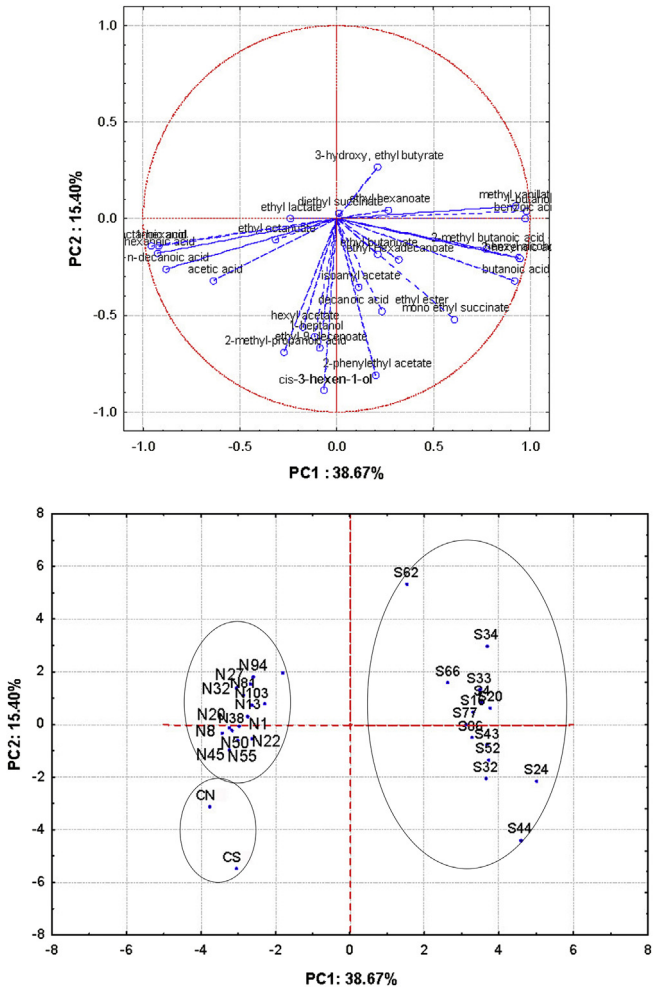

Fig. 2. Plots of the PCA analysis referred to minor volatiles variables. PCA biplots in the PC1-PC2 plane combining score plots of minor volatiles variables and wine samples fermented with different yeast strains. N, NP yeast population; S, SP yeast population; CN, Northern commercial control; CS, Southern commercial control.

according to the strain area of origin. Indeed, the wines fermented by the NP (negative PC1) and the SP strains (positive PC1) showed the highest differentiation according to function 1.

The cluster of NP wines was defined by fatty acids, ethyl octanoate and ethyl lactate, while the cluster of SP samples wine was defined by 3-hydroxy-ethyl butyrate, ethyl exanoate, ethyl butyrate, monoethyl succinate, methyl vanillate and 2-methyl butanoic acid. The fermentation inoculated with the strain SP62, located in a different quadrant of the PCA plot, was not associated with any of the molecules showing a neutral profile.

3.4. Sensorial analysis

According to the results obtained by the chemical analysis, three wines exhibiting the best aromatic features were selected for a sensory analysis among those produced using either NP and SP yeasts. The quality of wines was evaluated by using the Official Rating Evaluation Form provided by Italian Association of Oenologists in comparison with the wine produced using the commercial control strain.

The wines showed no defects, such as bad tastes and odours resulting from the action of yeasts during alcoholic fermentation; therefore, the overall final score of the sensory analysis was high for all samples (Table 5). All samples showed positive profiles with average score higher than that of the wine obtained using the commercial strain, thus confirming the results obtained by the instrumental analysis.

3.5. Odour Activity Value

In order to assess the influence of the compounds studied on overall wine aroma, odour activity value (OAV) was calculated by dividing the concentration of each compound by its perception threshold (OTh).

In Table 5 are reported the odour descriptors and odour threshold of compounds, by-products of yeast metabolism, such as fatty acids, alcohols and ethyl esters, which are largely responsible for the aroma characteristics of wine (Culleré, Escudero, Cacho, & Ferreira, 2004) taking into consideration the parameters already reported by other authors (Charles et al., 2000; Gómez García-Carpintero, Sánchez-Palomo, Gómez Gallego, & González-Viñas, 2012; Peinado, Mauricio, Medina, & Moreno, 2004). Only the compounds with OAV higher than 1 contribute individually to the wine aroma (Guth, 1997; Vilanova, Genisheva, Masa, & Oliveira, 2010). However also compounds exhibiting OAVs lower than one could contribute to the aroma character of wines, since they might improve some traits already present, because of the additive effect of similar compounds (similar structure or odour) as well as possible synergies with other odorant molecules (Francis & Newton, 2005; Lopez, Ferreira, Hernandez, & Cacho, 1999). To estimate overall wine aroma, the odour descriptors were grouped

Table 5
Sensory analysis of selected wines. Results are expressed as the average score obtained from eight judges (Mean ± SD).

Yeast strain	Score
N1	80 ± 5.0
N45	81 ± 6.2
N94	72 ± 6.5
CN	71 ± 4.2
S24	78 ± 4.5
S32	75 ± 6.0
S44	71 ± 3.5
CS	70 ± 2.5

into different aromatic series. The series used in this work group compounds with similar odour descriptors and they represent the main constituents of the aroma profile of wine: fruity, solvent, floral, fatty, spicy, herbaceous and caramelized (Zea, Moyano, Moreno, & Medina, 2007). All the NP and SP wines odour profiles are characterized by high intensity of fruity notes. In particular NP wines odour profiles exhibit high intensity ($4.05 \leq OAV \leq 38.59$) of the apple fruity nuances due to ethylhexanoate, on the contrary SP wines odour profiles are characterized by high intensity of the apple/banana fruity nuances due to isoamylacetate ($8.59 \leq OAV \leq 82.26$).

In addition, the odour profile of both NP and SP wines was characterized by high levels of floral ($1.52 \leq OAV \leq 5.7$ for NPs; $1 \leq OAV \leq 7$ for SPs), fatty ($0.49 \leq OAV \leq 2.76$ for NPs; $0.10 \leq OAV \leq 2.55$ for SPs), and spicy ($13 \leq OAV \leq 60$ for NPs; $8 \leq OAV \leq 24$ for SPs) odour.

These results confirm that the aroma of wines of non aromatic grape varieties, such as Negroamaro, are essentially dominated by the by-products of yeasts fruity, floral, fatty and spicy odours. The most volatile compounds associated with fruity notes are ethyl esters and acetate. Ethyl esters originate from the enzymatic or chemical esterification of acids with ethanol, and possess pleasant sweet and fruity notes. Acetates are produced by the reaction of acetylCoA with higher alcohols that are formed from degradation of amino acids or carbohydrates.

The overall aroma of wine produced with the two different yeast populations was estimated by grouping each odour descriptors and the related compound reported in Table 5 into different aromatic series. Total intensities for each aromatic series were calculated as the sum of the OAVs of each compound assigned to that series, detected in all wines produced with either NP and SP yeast populations (Fig. 3). This method allows to relate quantitative information obtained by chemical analysis to sensory perception and provides a valid tool to compare the wine aroma profiles. As shown in Fig. 3, in relation to the aroma series, the highest aroma contributions were those of the fruity, followed by spicy, floral and fatty.

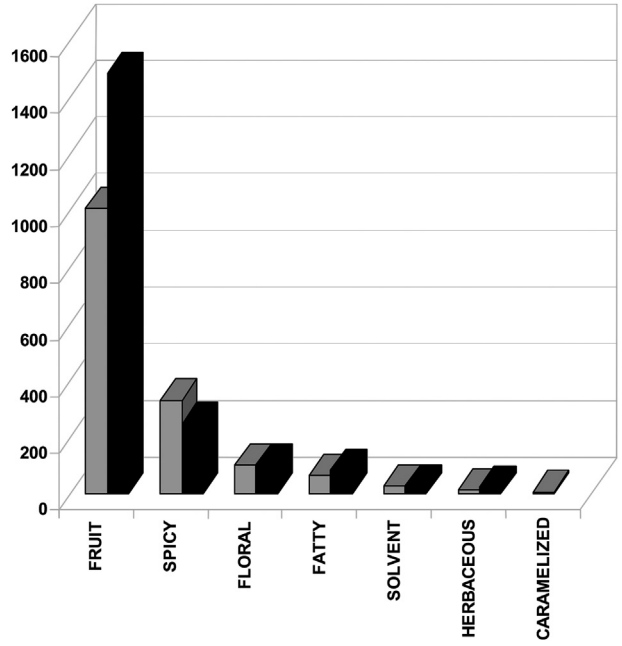

Fig. 3. Aromatic series values calculated by adding the odour activity values of the compounds grouped in each one of the wines produced using the Northern (grey bars) and the Southern Salento (black bars) selected yeast.

The wines produced with the SP yeast population exhibit higher fruity intensity with respect to those produced with the NP yeasts, in which, on the contrary, the aromatic series spicy intensity is higher.

4. Conclusions

The study of the chemical composition of wines produced with two yeast population isolates from two different micro districts in Salento, have shown that the volatile profile of the produced wines is strictly related to the geographical origin of the yeast employed for the fermentation process. The wines produced by SP yeast strains are characterized by a more complex chemical profile, exhibiting higher concentrations in esters (fruity notes) and fatty acids (fatty and sweet notes) compounds than wines produced by NP yeast strains. The latter however showed the highest OAVs for spicy aromatic series. The present study for the first time describes the impact of different autochthonous yeast strains on the aromatic composition of Negroamaro wine. Moreover, the results obtained highlight the considerable aroma potential of the yeast strains characterized and, as already indicated by other authors (Carrascosa et al., 2012), confirm the potential of "area-specific" yeast starter cultures in enhancing the peculiarity of distinguishing marks of regional productions, even in a limited wine-producing area. To confirm this hypothesis, further experiments are now under the way in order to complete the selection protocol by separately using the NP1, NP45, NP94, SP24, SP32 and SP44 strains as starter culture in real winemaking conditions.

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