POST PRINT

https://www.sciencedirect.com/science/article/pii/S0023643818307990

DOI: https://doi.org/10.1016/j.lwt.2018.09.067

LWT – Food Science and Technology 99 (2019) 188-196

Selection of an autochthonous yeast starter culture for industrial production of Primitivo "Gioia del Colle" PDO/DOC in Apulia (Southern Italy)

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Abstract

The aim of the present study was to isolate and characterize yeast strains as good candidates for driving the industrial fermentation process, from natural must fermentations of "Primitivo" grape cultivar, grown in the PDO/DOC "Gioia del Colle" (Apulia, Southern Italy). The selection protocol was based on parameters such as low production of acetic acid and hydrogen sulphide, complete sugar consumption during fermentation, significant production of some classes of volatile molecules responsible for wine aroma. Three *Saccharomyces cerevisiae* strains, named ITEM14088, ITEM14090 and ITEM14093, successfully dominated the fermentation process and contributed to increase organoleptic quality of the produced wines. The best performing strain, namely ITEM14093, was used as fermentation starter for three different industrial vinifications. The wines obtained were characterized by high levels of esters, associated to fruity nuances, as well as of alcohols responsible for vinous, sweet and floral notes. Furthermore, from a sensory point of view, all wines were positively judged, being characterized by frankness, gustatory persistence and intensity, good balance and body wine.

Keywords
Primitivo grape Alcoholic fermentation Saccharomyces cerevisiae Oenological selectionYeast starter

1. Introduction

Apulia (Southern Italy) is the second Italian area for wine production (ISMEA, 2017). The Apulian wines detain several peculiarities because of pedologic features of the production area, climatic conditions of this region and the specific adopted technologies, all contributing to the definition of a unique "terroir". The International Organization of Vine and Wine established in 2010 that "terroir" pertains to "an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied viticulture and oenological practices develops, providing distinctive characteristics for the products originating from this area" (Capozzi, Russo, & Spano, 2012; Capozzi & Spano, 2011). Several investigations have underlined the pivotal role of the microbiota associated with the "terroir" in which a particular grape cultivar is grown, able to give unique organoleptic properties to the produced wine (Di Maio et al., 2012). The "microbial terroir" associated to the grape/wine background has been recently studied and the obtained findings highlighted the close connection among microbial consortium, climate and production area (Bokulich et al., 2016; Bokulich, Thorngated, Richardsone, & Mills, 2014). A rising number of scientific surveys strongly focused on microbial biodiversity associated with spontaneous grape must fermentation, with the aim to identify autochthonous strains, characterized by optimal physiological and technological properties, to be used as fermentation starters in industrial production (Tristezza et al., 2013, 2014, 2012; Capozzi et al., 2010; Capozzi, Garofalo, Chiriatti, Grieco, & Spano, 2015; Cappello, Stefani, Grieco, Logrieco, & Zapparoli, 2008; Garofalo et al., 2015; Grieco et al., 2011).

As already reported, the diversity of indigenous yeast strains allows the production of wines denoted by high quality and peculiar flavour (Capozzi et al., 2015; Pérez-Coello, Briones Pérez, Ubeda Iranzo, & Martin Alvarez, 1999; Romano, Fiore, Paraggio, Caruso, & Capece, 2003; Tristezza et al., 2014). In contrast, the massive employment of commercial starters could affect the unique properties that differentiate typical regional wines (Cappello, Bleve, Grieco, Dellaglio, & Zacheo, 2004).

Primitivo is one of the most important vines grown in Southern Italy and, particularly, in the Apulia Region. Primitivo grapes produce wines with high alcohol levels and a ruby-purple colour denoted by the Protected Designation of Origin (PDO/DOC) in two different areas in Apulia, Manduria and Gioia del Colle (Southern Italy; Antonacci, 2004). Even though, the Gioia del Colle - Primitivo PDO/DOC wine consumer's appreciation has been recently increasing worldwide, scarce knowledge is available on the chemical and sensory characteristics of Primitivo wines and none studies give information on the yeast population associated to this area. (Baiano, Terracone, Gambacorta, & La Notte, 2009; Trani, Verrastro, Punzi, Faccia, & Gambacorta, 2016).

During a previous study, a population consisting of one thousand different isolates of *S. cerevisiae* was isolated, during the last step of the spontaneous alcoholic fermentation of Primitivo grape (collected in district of Gioia del Colle; Grieco et al., 2011) and subjected to oenological selection procedure (Tristezza et al., 2012). The genetic analysis of the rDNA region of 104 low H_2S-producers isolates confirmed that they all belonged to the species *S. cerevisiae* and it allowed the identification of 15 different strains, that were deposited in the International ISPA Collection (http://server.ispa.cnr.it/ITEM/Collection/).

The present investigation describes the genetic diversity of wild *Saccharomyces cerevisiae* strains in spontaneous fermentations of a Primitivo wine produced with grapes collected in the Gioia del Colle - Primitivo PDO/DOC area. A selection approach able to identify autochthonous yeast strains and providing significant oenological properties was performed and the selected strains tested in pilot- and industrial-scale vinification. To our knowledge, this study is the first investigation on the *S. cerevisiae* populations associated to the above PDO/DOC area grapes and of the employment of autochthonous starter cultures for the industrial production of this typical wine.

2. Materials and methods

2.1. Yeast strains genetic analysis

Yeast populations were sampled at the end of alcoholic fermentation. Yeast total genomic DNA was extracted according to De Benedictis et al. (2011) and isolates were genetically distinguished at strain level by inter-delta typing (Tristezza, Gerardi, Logrieco, & Grieco, 2009).

2.2. Lab-scale fermentations

Selected yeasts fermentation performances were evaluated by micro-fermentation trials. The must (sugars 215 g/L, pH 3.25, assimilable nitrogen 142.6 g/L) was centrifuged and sterilized by filtration (through 0.22 μm Ø membrane), then potassium metabisulphite (100 mg/L) was added. One liter of must was inoculated with a yeast culture (up to a concentration of 10^6 CFU/mL) grown in the same must. The lab-scale fermentations were carried in triplicate out at 20 °C. Samples were daily subjected to gravimetric analysis in order to record CO_2 production until the weight remained constant. A sample of fermented must (100 mL) was stored at −20 °C, the remaining was used for instrumental analysis. During fermentation, the hydrogen sulphide production was evaluated as described by Tufariello et al. (2014).

2.3. Pilot-scale fermentations

Pilot-scale fermentations were carried out in 100 L stainless steel vats. Primitivo must (3 L) was inoculated with 1.5×10^6 CFU/mL of yeast and left for 6 h at room temperature. After this period, the yeast-must mixture was added to 90 kg of Primitivo must (sugars 202 g/L, pH 3.2, assimilable

nitrogen 167.2 g/L). The fermentation process was carried out at 25 °C and its kinetics was followed daily by measuring the sugars consumption. At the end of alcoholic fermentation (0–1 °Babo), wine and residual lees were collected and yeast population was isolated for further molecular analyses.

2.4. Industrial-scale fermentations

Yeast biomass productions were carried out by employing a Biostat C fermenter (Sartorius, Germany) as previously described (Tristezza et al., 2012). The initial yeast inoculum (1.5×10^6 CFU/mL) was mixed with 300 L of Primitivo must and left for 6 h at room temperature. Then, the yeast-must mix was added to 15 tons of Primitivo must. The alcoholic fermentations were carried out at 25 °C and their kinetics were monitored daily by measuring the concentration of reducing sugars. At the end of alcoholic fermentation (0 °Babo), samples of wine and residual lees were collected for further analyses. The industrial test was conducted on Primitivo wines from three wineries located in the "Gioia del Colle" DOC area in Apulia Region (Southern Italy) specifically located in Cassano delle Murge (denoted as GT and LZ) and Locorotondo (denoted as LR).

2.5. Chemical analysis

Wines and musts were centrifuged at 8000 rpm for 10 min and then were analyzed by Fourier Transform Infrared Spectroscopy (FTIR), using the WineScan Flex (FOSS Analytical, DK). Acetaldehyde, ethyl acetate, 2-methyl-1-propanol, higher alcohols (3-methyl- and 2-methyl-1-butanol) and acetoin were determined by GC-FID system according to De Benedictis et al. (2011). Separation of wines from solids was performed, and then wines were bottled and stored at 16–19 °C. Volatile aroma compounds were extracted in triplicate by solid phase extraction (SPE) technique according to Tufariello, Capone, and Siciliano (2012).

2.6. Sensory analysis

The sensory analysis was performed by a panel composed of 15 professional experts, chosen among oenologists and producers involved in Primitivo wine production. The judges were asked to assign a score for different parameters of the wines, such as frankness, gustatory-intensity, balance, acidity, body, gustatory-persistence and aftertaste attributes, using a sensory analysis-tasting sheet with a scale ranging from 0 (absence of perception) to 10 (maximum perception). The mean scores of attributes were submitted to Quantitative Descriptive Analysis (QDA) according to Trani and Coworkers (2016).

2.7. Statistical analysis

The results were expressed as mean values ± standard deviations. Analysis of variance (ANOVA) of the mean values obtained for the volatiles concentrations was performed, followed by Tukey's post-hoc test when $P < 0.05$. In order to reveal any grouping of the wines based on the composition of

volatile compounds, as well as to identify the main components contained within each group, the data were subjected to principal component analysis (PCA).

3. Results and discussion

3.1. Oenological characterization of selected strains

The oenological selection of indigenous wine yeast strains is fundamental for wine producers in order to have starter cultures able either to control wine fermentations or to link wines to their productive area. Even tough, the employment of autochthonous yeast starters for industrial-scale wine production is, to date, scarcely adopted by local winemakers (Berbegal, Spano, Tristezza, Grieco, & Capozzi, 2017; Petruzzi et al., 2017). Yeasts play a substantial role in the transformation of grape must in wine (Howell, Cozzolino, Bartowsky, Fleet, & Henschke, 2006; Romano, Fiore, Paraggio, Carusi & Capece, 2003) and the use of selected autochthonous strains was employed to produce wines with peculiar aroma (Alves et al., 2015) or to enhance the aromatic properties of a specific grape cultivar (Garofalo et al., 2015; Garofalo, Tristezza, Grieco, Spano, & Capozzi, 2016; Ilieva, Veličkovska, Dimovska, Mirhosseini, & Spasov, 2017; Vigentini et al., 2016). Moreover, selected autochthonous strains were also used to make a linkage between wines and the culture and history of the production area (Capozzi et al., 2015).

Laboratory-scale fermentations with *S. cerevisiae* isolates, selected on the basis of biotype, revealed a significant impact of these strains on oenological and technological properties that affect fermentation process (Romano, 2005) and wine aroma (Swiegers & Pretorius, 2005; Tempère et al., 2018). The evaluation of the fermentative performances of the isolates was based on the analysis of some key parameters, such as acetic acid production (<0.6 g/L) (Fleet & Heard, 1993), total sugar consumption (>4 g/L) (Pérez-Coello et al., 1999) and the absence of H_2S production during fermentation. All the strains analyzed produced wines characterized by a high value of fermentation purity (FP) index (Table 1) and low values of acetic acid (<0.6 g/L) reported as volatile acidity (Table 2). Moreover, ten strains (14088-14090-14091-14093-14094-14095-14096-14098-14099, 14102) were unable to produce H_2S during fermentation process and only three of them (14091, 14094, 14096) produced detectable foam (Table 1).

Table 1. Main oenological and technological properties determined in one commercial (CM) and 15 autochthonous *S. cerevisiae* strains.

Strain	ITEM nr.	H_2S[a]	Foam[a]	FP
CM	–	+	+	0.03
P32A	14088	–	–	0.02
PR43A	14089	+	–	0.02

Strain	ITEM nr.	H_2S[a]	Foam[a]	FP
PR49A	14090	–	–	0.02
PR6A	14091	–	+	0.02
PR22B	14092	+	–	0.02
PR12A	14093	–	–	0.02
PR16B	14094	–	+	0.02
P13A	14095	–	–	0.02
PR 51B	14096	–	+	0.02
PR25A	14096	+	–	0.02
PR32B	14098	–	–	0.02
PR 16B	14099	–	–	0.02
PR8A	14100	+	–	0.02
PR45B	14101	+	–	0.02
PR 1A	14102	–	–	0.02

Data, measured at the end of fermentation, represent the average of three replicates.
ITEM, ISPA Agro-Food Toxigenic Fungi Culture Collection.
FP, fermentation purity [volatile acidity (g/L)/ethanol (% v/v)].
[a]H_2S and foam production: absent (−); low (+), high (++), very high (+++).

Table 2. Concentration of major chemical compounds in fermented musts obtained by 15 autochthonous and one commercial (CM) strain of *S. cerevisiae*.

ITEM nr.	Ethanol	Residual sugar	Volatile acidity[a]	pH	Malic acid	Lactic acid	Total acidity[b]	Citric acid	Glycerol
	g/100 mL	g/L	g/L	Empty Cell	g/L	g/L	g/L	g/L	g/L
CM	11.81 ± 2.45	2.20 ± 0.65	0.56 ± 0.05	3.35 ± 0.66	2.80 ± 0.66	nd	2.26 ± 0.38	0.29 ± 0.05	6.89 ± 1.10
14088	12.94 ± 4.05	3.15 ± 0.26	0.45 ± 0.06	3.40 ± 0.48	3.31 ± 0.66	nd	2.34 ± 0.38	0.36 ± 0.06	8.16 ± 2.06
14089	11.60 ± 3.80	2.10 ± 3.60	0.36 ± 0.06	3.20 ± 0.56	2.58 ± 0.68	nd	2.15 ± 0.55	0.35 ± 0.05	6.30 ± 1.60
14090	12.53 ± 3.10	1.84 ± 0.55	0.30 ± 0.06	3.23 ± 0.56	2.56 ± 0.50	nd	2.11 ± 0.22	0.34 ± 0.05	6.85 ± 1.66
14091	12.14 ± 3.66	3.25 ± 0.60	0.33 ± 0.10	3.32 ± 0.43	3.06 ± 0.54	nd	2.41 ± 0.30	0.41 ± 0.66	6.40 ± 2.66
14092	10.86 ± 2.35	3.62 ± 0.32	0.35 ± 0.10	3.40 ± 0.60	2.88 ± 0.66	nd	2.42 ± 0.66	0.35 ± 0.06	6.68 ± 2.05
14093	12.86 ± 3.60	1.96 ± 0.22	0.32 ± 0.06	3.20 ± 0.44	2.56 ± 0.40	nd	2.63 ± 0.11	0.29 ± 0.05	6.63 ± 1.90
14094	12.09 ± 3.60	4.06 ± 0.40	0.34 ± 0.06	3.30 ± 0.66	3.09 ± 0.66	nd	2.45 ± 1.15	0.40 ± 0.05	6.43 ± 1.10
14095	11.64 ± 4.10	3.36 ± 0.55	0.35 ± 0.06	3.33 ± 0.58	3.09 ± 0.40	nd	2.46 ± 0.49	0.39 ± 0.11	6.18 ± 2.05
14096	12.03 ± 4.10	3.03 ± 0.64	0.34 ± 0.04	3.33 ± 0.38	3.19 ± 0.40	nd	2.62 ± 0.19	0.36 ± 0.06	6.46 ± 1.53
14096	12.±3	3.56 ± 0.40	0.34 ± 0.05	3.32 ± 0.66	3.24 ± 0.48	nd	2.54 ± 0.84	0.36 ± 0.04	6.61 ± 2.11
14098	12.66 ± 4.66	1.65 ± 0.12	0.31 ± 0.05	3.22 ± 0.55	2.58 ± 0.60	nd	2.03 ± 0.20	0.31 ± 0.06	6.85 ± 2.80
14099	12.31 ± 4.55	3.58 ± 0.26	0.33 ± 0.06	3.33 ± 0.45	3.20 ± 0.30	nd	2.55 ± 0.45	0.38 ± 0.11	6.66 ± 1.68
14100	11.84 ± 4.10	3.85 ± 0.48	0.35 ± 0.05	3.31 ± 0.83	3.16 ± 0.84	nd	2.49 ± 0.28	0.35 ± 0.36	6.35 ± 1.85

ITEM nr.	Ethanol	Residual sugar	Volatile acidity[a]	pH	Malic acid	Lactic acid	Total acidity[b]	Citric acid	Glycerol
	g/100 mL	g/L	g/L	Empty Cell	g/L	g/L	g/L	g/L	g/L
14101	12.03 ± 3.65	3.11 ± 4.05	0.34 ± 0.11	3.32 ± 0.22	3.21 ± 1.10	nd	2.60 ± 0.48	0.36 ± 0.11	6.32 ± 1.11
14102	12.10 ± 3.06	3.52 ± 0.23	0.35 ± 0.06	3.33 ± 0.35	3.16 ± 0.60	nd	2.66 ± 0.66	0.38 ± 0.66	6.41 ± 2.05

Values are the mean of three injections of each replicate ($n = 9$); the standard deviation values (±) are indicated; nd: not detected.
[a] Measured as acetic acid.
[b] Measured as tartaric acid.

Produced wines were analyzed for residual sugars, ethanol, volatile and total acidity, malic and lactic acids, glycerol and pH (Table 2) following the method reported by Tristezza et al. (2012). The primary screening indicates that only three strains (14090, 14093, 14098) produce musts with very low values of residual sugars (1.84, 1.96, 1.75 g/L). In all the obtained fermented musts, alcohol was present at high concentrations (up to 12.94) while volatile acidity, expressed as acetic acid, was quite low ranging from 0.30 to 0.45 g/L.

No lactic acid was detected in any of the samples, while malic acid concentrations among the different wines, were also significantly different and ranged from 2.57 g/L (in 14090 and 14093 strains) and 3.31 g/L (14088). Total acidity, expressed as tartaric acid, ranged from 2.03 to 2.67 g/L. Glycerol produced by yeast during fermentation is one of the main components of wine (Goold et al., 2017), where usually it is found in concentrations ranging from 2 to 11 g/L (Remize, Cambon, Barnavon, & Dequin, 2003). No significant pH values variation was detected in all the produced wines (Table 2). The results indicated that all micro-fermentations took place properly and that all wines had a composition considered normal for this winemaking scale. However, relevant differences were observed among some wines compounds produced by different yeast strains. Among the chemical parameters indicated to evaluate the good fermentation performance of the strains, secondary fermentation products such as higher alcohols concentrations were observed (Table 3). Acetaldehyde is the dominating aldehyde in the wine. It is associated with fruity aromas and notes of dried fruits when present at concentrations below its odor threshold (100 mg/L). All the 15 selected strains were characterized by a low production of acetaldehyde and total higher alcohols. These results suggest a good performance for all strains because elevated concentrations of both acetic acid (more than 0.8 g/L) and higher alcohols (more than 300 g/L) are related to defective wines (Swiegers, Bartowsky, Henschke, & Pretorius, 2005), whereas optimal levels impart fruity characters (Swiegers & Pretorius, 2005). The class of higher alcohols includes 1-propanol, 2-methyl-1-propanol, isoamyl alcohols, and 2-phenylethanol. In particular 14088, 14091, 14100 and 14102 show significant amounts s of 2-phenylethanol, above its odor threshold

(30 mg/L), contributing with fine rose's notes to wine aroma and general complexity (Tufariello et al., 2012).

Table 3. Concentration of major volatile compounds, determined by GC-FID, in wines obtained by 15 autochthonous and one commercial (CM) strain of *S. cerevisiae*.

Strain	Acetaldehyde	1-Propanol	2-Methyl-1-propanol	Isoamyl alcohols	2-Phenylethanol	Ethyl acetate
CM	1.21 ± 0.60	8.83 ± 1.66	16.56 ± 4.05	54.31 ± 8.10	30.02 ± 5.44	56.81 ± 6.60
14088	3.82 ± 0.66	9.26 ± 1.58	14.66 ± 5.66	58.06 ± 6.10	31.86 ± 6.46	66.20 ± 6.05
14089	12 ± 0.2	10.26 ± 2.10	5.40 ± 0.35	60.98 ± 6.61	21.96 ± 6.44	16.24 ± 2.10
14090	2.05 ± 0.30	8.81 ± 1.66	13.80 ± 3.50	59.88 ± 6.05	12.52 ± 5.66	56.65 ± 5.80
14091	11.13 ± 2.20	9.05 ± 2.30	5.10 ± 0.55	56.04 ± 4.20	35.09 ± 6.15	13.22 ± 3.41
14092	12.10 ± 0.30	8.84 ± 1.65	4.36 ± 0.66	46.60 ± 4.81	22.14 ± 4.35	13.83 ± 2.20
14093	1.68 ± 0.54	8.60 ± 1.66	14 ± 3	59.86 ± 6.48	21.26 ± 4.66	56.03 ± 8.16
14094	11.55 ± 2.34	9.25 ± 1.65	5.94 ± 0.16	41.61 ± 4.60	25.36 ± 5.21	6.38 ± 1.95
14095	10.90 ± 2.10	6.55 ± 1.10	4.15 ± 0.35	43.13 ± 5.66	22.11 ± 5.11	12.84 ± 3.81
14096	9.43 ± 0.22	6.43 ± 0.20	3.18 ± 0.04	34.20 ± 0.21	12 ± 4.4	6.56 ± 0.64
14096	11.94 ± 3.50	9.44 ± 2.20	5.03 ± 0.50	55.64 ± 4.65	22 ± 5.10	11.42 ± 3.41
14098	21.83 ± 4.10	8.96 ± 0.50	9.26 ± 2.06	61.46 ± 6.30	16.40 ± 4.40	16.51 ± 1.12
14099	11.56 ± 3.10	9.22 ± 0.06	5.20 ± 0.20	51.44 ± 0.23	19.62 ± 5.35	38.84 ± 2.13
14100	10.94 ± 1.40	8.68 ± 1.10	4.34 ± 0.55	46.34 ± 5.11	36.66 ± 4.20	11.08 ± 1.35
14101	11.36 ± 0.31	8.63 ± 0.11	4.32 ± 0.12	46.44 ± 0.32	22.25 ± 5.21	28 ± 1.82
14102	13.59 ± 4.33	9.10 ± 2.10	4.36 ± 0.36	46.01 ± 5.11	32.61 ± 6.20	11.94 ± 3.10

Values expressed in mg/L are the mean of three injections of each replicate ($n = 9$); the standard deviation values (±) are indicated.

Moreover, all strains were characterized by high production of the major ester (ethyl acetate) that ranged from 7.38 to 67.20 mg/L and of the isoamyl alcohols that ranged from 34.20 to 61.46 mg/L (Table 3). However, the production of these compounds unaffected the analytical profiles of the wines, because they were below the sensory threshold.

In order to identify yeast strains producing wines with the best oenological and chemical characteristics, the principal component analysis (PCA) was performed on the concentrations of molecules detected by GC-FID and the principal oenological parameters (volatile and total acidity as well as alcohol degree). Two bi-plots displaying PC1 vs. PC2 are illustrated in Fig. 1 which shows the projection of the considered variables on the plane defined by the first and second

principal component. The first PCA dimension (31.47% of explained variance) discriminates three selected yeast strains (14088, 14090, 14093), which lies on the positive semi-axis of the first component, from the other nine isolates (14091, 14092, 14094, 14095, 14096, 14097, 14100, 14101, 14102) and the control (CM). Differences relied on high content, besides other variables, of ethyl acetate, glycerol, 2 methyl-1-propanol and isoamyl alcohols. However, the second dimension (26.25% of explained variance) discriminates the two remaining isolates: 14098 and 14099, lying on the positive semi-axis. Acetaldehyde and 1-propanol contributes to this discrimination. In conclusions the three isolates, 14088, 14090 and 14093 exhibit the best fermentative performances and seem to produce better wines.

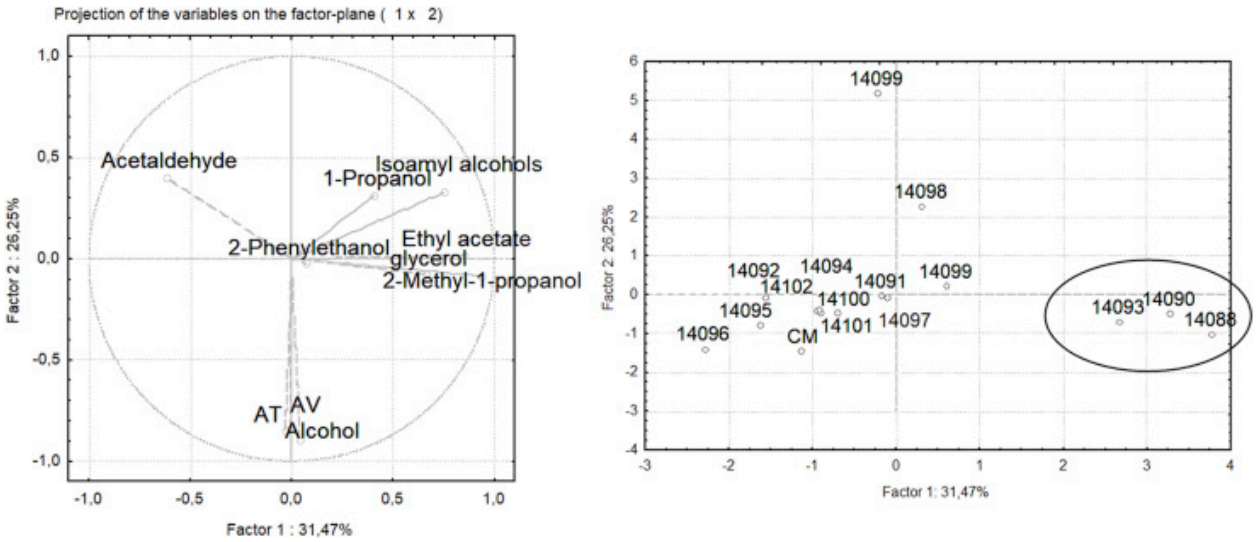

Fig. 1. Principal Component Analysis (PCA) performed employing the data obtained by the chemical analysis of must fermented with the selected strains as variables.

3.2. Pilot-scale vinification

On the basis of the performances in the micro-vinification trials, the strains 14088, 14090 and 14093 were selected to be tested in pilot-scale fermentations.

Table 4 shows the values of the major chemical compounds identified and quantified by FT-IR and GC-FID. The analysis of the principal oenological characters of pilot-scale fermentations (Table 4) confirms that the strains 14088, 14090 and 14093 produce wines with low values of volatile acidity (0.33, 0.31, 0.20 g/L) compared to commercial control (0.57 g/L) and low values of residual sugars (<2.10 g/L) indicating the correct evolution of fermentations. Taken together, the above results indicated that the strain ITEM14093 produced the wine with the lowest residual concentrations of both, fermenting sugars and acetic acid (Table 4).

Table 4. Main parameters characterizing the chemical properties and concentrations of major volatile compounds in wines obtained by selected yeast strains and one commercial (CM) strain of *S. cerevisiae* in pilot-scale.

Empty Cell	**Strain**			
	14088	**14090**	**14093**	**CM**
Ethanol (mL/100 mL)	11.85 ± 0.44	11.84 ± 0.90	11.90 ± 0.05	10.68 ± 1.15
Residual sugars (g/L)	2.03 ± 0.26	2.06 ± 0.24	1.66 ± 0.26	2.50 ± 0.60
Volatile acidity[a] (g/L)	0.33 ± 0.10	0.31 ± 0.08	0.20 ± 0.06	0.56 ± 0.10
Total acidity (g/L)	8.35 ± 2.04	8.96 ± 2.16	9.45 ± 1.60	8.02 ± 1.45
Glycerol (g/L)	8.33 ± 2.55	8.45 ± 2.05	8.46 ± 1.10	9.60 ± 2.45
Malic acid (g/L)	1.65 ± 0.44	1.66 ± 0.06	1.80 ± 0.06	1.63 ± 0.65
Lactic acid (g/L)	0.16 ± 0.06	0.10 ± 0.05	0.12 ± 0.08	0.13 ± 0.11
Tartaric acid (g/L)	3.51 ± 0.94	3.42 ± 0.60	3.33 ± 0.55	3.26 ± 0.66
Citric acid (g/L)	0.28 ± 0.05	0.30 ± 0.06	0.30 ± 0.06	0.28 ± 0.05
Total polyphenols (mg/L)	1304 ± 60	1280 ± 66	1362 ± 55	1366 ± 130
Anthocyanins (mg/L)	365 ± 16	314 ± 30	224 ± 22	205 ± 34
Acetaldehyde	12.15 ± 4.66	31.25 ± 4.30	13.42 ± 3.54	21.88 ± 3.60
Ethyl acetate	46.11 ± 4.25	66.11 ± 5.80	35.10 ± 4.16	46.13 ± 4.60
1-Propanol	18.50 ± 3.18	31.11 ± 4.16	26.13 ± 4.66	11.60 ± 3.66
2-Methyl-1-propanol	43.10 ± 5.66	25.60 ± 3.50	44.66 ± 3.60	34 ± 4
Isoamyl alcohols	65.20 ± 6.10	56.30 ± 6.05	65.14 ± 6.48	69.11 ± 8.10
2-Phenylethanol	53.80 ± 6.46	32.12 ± 5.66	51.86 ± 4.66	51.26 ± 5.44

The standard deviation values (±) are indicated.
[a]Measured as acetic acid.

The four fermentations show different chemical profiles (Table 4), all wines obtained by the selected yeast strains, were characterized by high ethanol content (ranging from 11.84 to 11.90) in comparison to control (10.78) and satisfactory levels of glycerol ranging from 8.33 to 8.46 g/L.

The amount of higher alcohols produced was influenced by the strain of yeast, composition of the juice and conditions of fermentation. Higher alcohols and esters, produced during alcoholic fermentation, play an important role in determining the flavor of wines, depending on the types of compounds and their concentrations (Valero, Moyano, Millan, Medina, & Ortega, 2002). At concentrations above 250–300 mg/L, they are regarded as negative quality factors (de la Fuente Blanco, Sáenz Navajas, & Ferreira, 2017). The acetaldehyde is one of the most important carbonyl compound produced during fermentation; at low levels it contributes to fruity flavour, while high

concentrations (>200 mg/L) confer flatness to wines. The three selected strains produced this compound in quantities ranging from 12.15 mg/L (strain 14088) to 31.25 mg/L (14090). Ethyl acetate may contribute to the wine aroma with pleasant, fruity fragrance if present at concentrations lower than 150 mg/L; the wines produced by the yeast strains selected show good levels of this molecule, ranging from 47.11 mg/L (14088) to 66.11 mg/L (14090). As far as higher alcohols are concerned, the amount of 2-methyl-1-propanol produced ranged from 25.70 mg/L (14090) to 44.67 mg/L (14093), isoamyl alcohols concentration ranged from 57.30 mg/L (14090) to 75.20 mg/L (14088). All the strains under study produced amounts of 2-phenylethanol, responsible for rose-floral notes in wine, ranging from 32.12 to 53.80 mg/L.

The dominance of inoculated strains was confirmed by the analysis of the interdelta region polymorphism, that highlighted the strains 14088, 14090 and 14093 were able to dominate the yeasts naturally present in the must (Fig. 2).

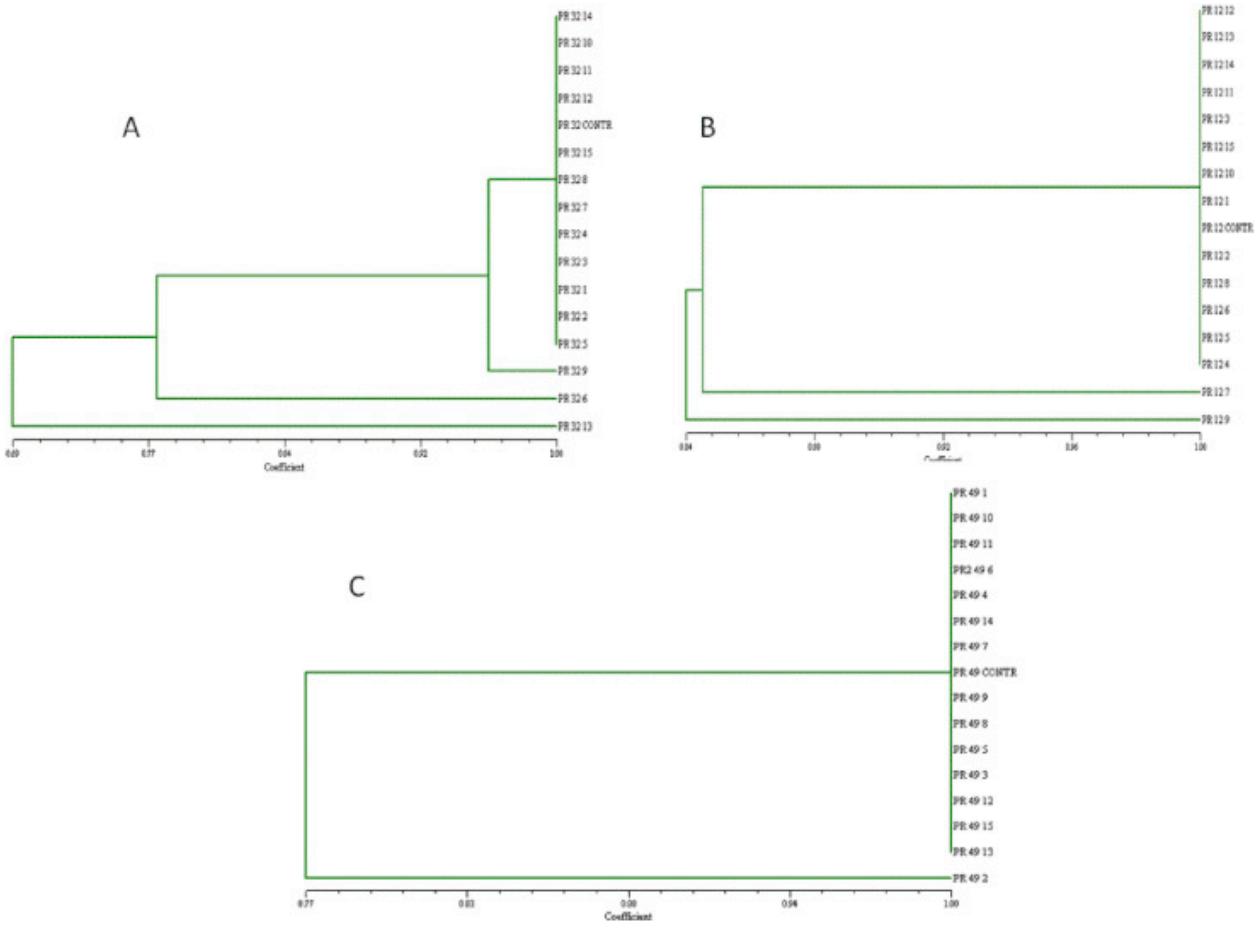

Fig. 2. UPGMA dendrograms generated by cluster analysis of inter-δ region patterns obtained from the *Saccharomyces cerevisiae* strains isolated during the later stages of pilot scale vinifications of Primitivo grape must, respectively inoculated with the 14088 (A), 14093 (B) and 14090 (C) strains. The genomic DNA extracted from pure cultures of the inoculated strain has been used as control (CONTR).

The wines obtained were also subjected to sensory analysis (Fig. 3). In order to define the best attributes describing the sensory characteristics of wines, the panellists evaluated commercial wines prior the formal sessions. The sensory analysis carried out by the panel of experienced wine tasters revealed that the most important descriptors were *fruity, floral, herbaceous, sweet, acids* and *vinous* notes. Wines produced by selected yeast strains presented higher values of these odor notes compared to control. The mean aroma-intensity scores were reported in a radar plot (Fig. 3). The *fruity* and *vinous* attributes mainly associated to ethyl acetate and isoamyl alcohols, were most intense in wine fermented by 14093 strain (Fig. 3). The *floral* note, linked to high content of 2-phenylethanol, characterized in particular the wine fermented by 14090 yeast and finally the *acids* note, associated to ethyl acetate content responsible of freshness of the wine, was higher in the aroma profile of wine obtained by 14088 yeast strain. The results of the sensorial evaluation, taken together with the outcome of the chemical analyses of the above three wines, indicated that the three selected strains, and in particular the strain ITEM14093, detained the technological, chemical and aromatic properties required for their possible use as industrial starter for "Primitivo di Gioia" wine production.

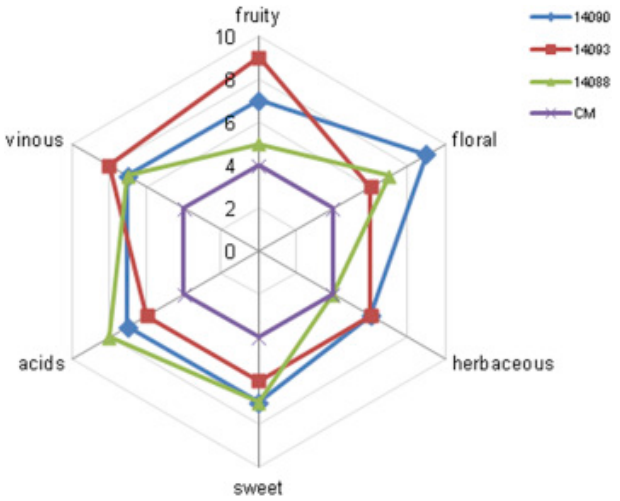

Fig. 3. The mean aroma-intensity scores of panellists for Primitivo wines produced by the three selected yeast strains and control strain in the pilot-scale fermentations.

3.3. Industrial-scale vinification

The strain ITEM14093 was furthermore used as starter culture in the industrial-scale vinifications, in three different industrial cellar (GT, LZ and LR) located in the Gioia del Colle area.

The main chemical parameters, determined by GC-FID, of the wines obtained in the different industrial cellars are reported in Table 5.

Table 5. Main parameters characterizing the chemical properties and concentrations of major volatile compounds in wines obtained using the selected ITEM14093 strain in three vinifications carried out at the industrial-scale.

Empty Cell	**WINES**		
	GT	**LZ**	**LR**
Alcohol (mL/100 mL)	14.86 ± 4.11	13.40 ± 4.11	15.86 ± 5.05
Residual sugars (g/L)	2.36 ± 0.55	1.91 ± 0.25	9.66 ± 2.44
Total acidity (g/L)	6.93 ± 1.60	5.86 ± 0.66	5.53 ± 0.84
Volatile acidity (g/L)	0.44 ± 0.06	0.50 ± 0.05	0.60 ± 0.06
Glycerol (g/L)	10.01 ± 2.94	9.69 ± 1.60	8.90 ± 2.10
Malic acid (g/L)	1.42 ± 0.55	1.28 ± 0.05	0.88 ± 0.11
Lactic acid (g/L)	ND	ND	ND
Tartaric acid (g/L)	3.95 ± 0.66	2.65 ± 0.64	2.41 ± 0.55
Citric acid (g/L)	0.28 ± 0.05	0.23 ± 0.05	0.22 ± 0.06
Total polyphenols (mg/L)	2845.44 ± 100	2339.2 ± 110	1909 ± 120
Anthocyanins (mg/L)	539 ± 43	296 ± 22	315 ± 34

The standard deviation values (±) are indicated; ND: not detected.

The dominance of ITEM 14093 strains was confirmed by the analysis of the inter-δ region polymorphism (Fig. 4). Data show that this strain was able to overcome the indigenous yeast population, with a high proportion (ranging from 67 to 87%) at the end of fermentation.

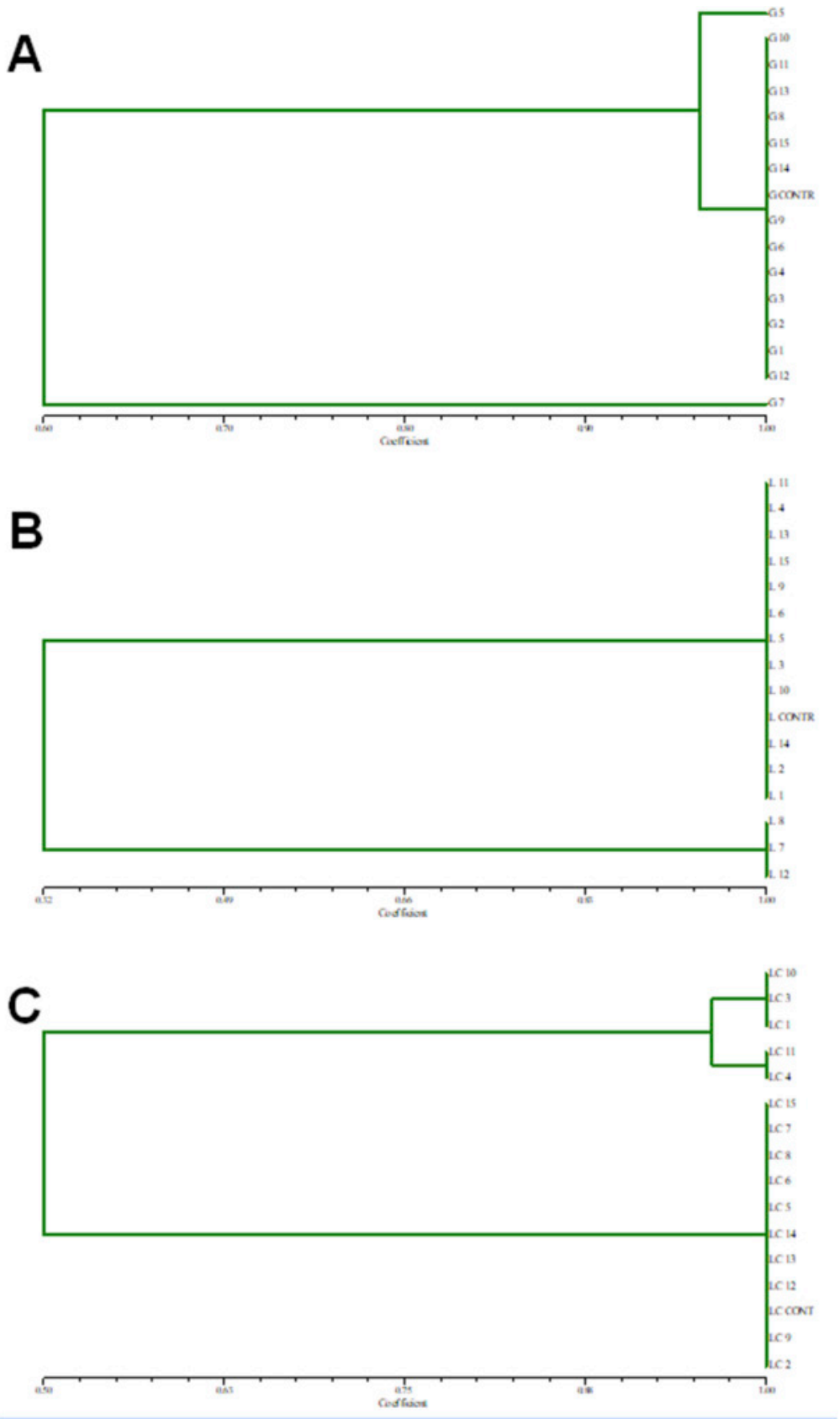

Fig. 4. UPGMA dendrograms generated by cluster analysis of inter-δ region patterns obtained from the *Saccharomyces cerevisiae* strains isolated during the later stages of three different large-scale vinifications of Primitivo grape must, respectively inoculated with the 14093 strain in the GT (A), LZ (B) and LR (C) industrial cellars. The genomic DNA extracted from a pure culture of the 14093 strain has been used as control (CONTR).

In order to characterize a complete volatile profile of the obtained wines, the gas-cromatographic coupled to mass-spectrometric (SPE/GC-MS) analysis was applied and the results are reported in Table 6. The volatile compounds of the wines, grouped according to the chemical classes are reported. Higher alcohols, indicated in bold in Table 6, were evaluated by GC-FID. The SPE/GC-MS analysis allowed the identification of a total of 37 volatile compounds in GT wine and 38 in LZ and LR. Our results are in good accordance with those reported by Tufariello et al. (2012). Among the volatile compounds, the esters and alcohols were the most abundant in all samples, with 10 esters identified in GT and 13 in LZ and LR. As far as alcohols are concerned, they are 11 in GT and LZ, and 12 in LR wine. Ethyl esters of fatty acids and acetates have long been considered important contributors to wine aroma (Etievant, 1991). Ethyl esters are synthesized mainly during yeast fermentation; it is well known that their concentrations are influenced by yeast strain, fermentation temperature, aeration degree and sugar content. Ethyl butanoate, responsible for fruity flavour, and ethyl decanoate were detectable only in LZ and LR wines, on the contrary isoamyl acetate, ethyl acetate, ethyl octanoate, diethyl succinate, phenyl acetate, diethyl malate and monoethyl succinate were identified in all wines. All the esters contribute with fruity notes to the wine aroma (Swiegers et al., 2005).

Table 6. SPE-GC/MS quantitative data, including concentrations (µg/L) with standard deviation (SD) of all the volatile compounds identified in the wines produced using the selected ITEM14093 strain in three vinifications carried out at the industrial scale.

Volatiles	**GT**	**LZ**	**LR**
Esters	µg/L	µg/L	µg/L
Ethyl butanoate	nd	168 a±23	163 a±34
Isoamyl acetate	830 c±60	655 b ± 40	426 a±30
Ethyl hexanoate	260 a±31	335 a±43	219 a±34
Ethyl acetate[a]	**50.65 a±6.30**	**66.36 a±9.10**	**60.82 a±9.53**
Ethyl lactate	260b ± 20	328b ± 21	148a±25
Butanoico acid-2-hydroxy-3-methyl-ethyl ester	nd	51 ± 4.5	nd
Ethyl octanoate	146a±40	314b ± 23	141a±51
3-hydroxy-ethyl butanoate	49 a±10	nd	40 a±11
Hydroxy-ethyl hexanoate	nd	166 ± 12	nd
Ethyl decanoate	nd	169 ± 33	125 ± 36
Diethyl succinate	859 b ± 51	919 b ± 45	482 a±90

Volatiles	**GT**	**LZ**	**LR**
Phenyl acetate	323 b ± 21	348 b ± 22	134 a±31
Diethyl malate	528 a±65	342 a±16	384 a±40
Mono ethyl succinate	2602 b ± 165	2461b ± 146	1650 a±114
Ethyl vanillate	nd	nd	321 ± 16
Alcohols			
1 Propanol[a]	**11.36 a±2.15**	**10.38 a±2.35**	**19.6 a±3.50**
2-Methyl-1-propanol[a]	**13.66 b ± 3.10**	**15.60 b ± 3.25**	**6.56 a±1.10**
1-butanol	102 a±30	nd	100 a±25
Isoamyl alcohols[a]	**68.48 b ± 6.50**	**59.56 a±4.50**	**54.48 a±5.20**
3-Metil-1-pentanol	184 b ± 16	141 a±15	119 a±13
1-Hexanol	1446b ± 66	1442 b ± 112	863 a±26
3-Hexen-1-ol (Z)	136 b ± 16	116 b ± 18	61 a±9
2 -Hexen-1-ol (E)	nd	68 a±9	46 a±6
1-Heptanol	169 a±16	160 a±15	182 a±21
Methyl-tio-1-propanol	262 a±25	203 a±18	205 a±16
Benzyl alcohol	162 a±14	196 a±25	166 a±13
Phenylethyl alcohol[a]	**34.61 a±6.10**	**36.44 a±5.66**	**36 a±5**
Acids			
Isobutanoic acid	111b ± 16	63a±9	nd
Butanoic acid	66 a±6	nd	60 a±5
3-Methyl butanoic acid	426b ± 16	240a±21	249a±25
Hexanoic acid	913 a±65	888 a±33	680 a±35
Octanoic acid	1616 a±142	1485 a±120	1404 a±180
Decanoic acid	546 a±56	413 a±33	688 a±116
Aldehydes-Ketons			
acetaldehyde[a]	**0.84 a±0.20**	**0.66 a±0.20**	**0.85 a±0.24**
acetoin [a]	**1.53 a±0.15**	**5.01 b ± 0.30**	**1.16 a±0.12**
Benzaldehyde	32 a±5	66 b ± 6	Nd
Terpenes			
Linalol	nd	nd	94 ± 6
Terpineol	14a±4	13a±5	22b ± 4

Volatiles	GT	LZ	LR
Citronellol	63.10 a±8.11	nd	80 a±5
3,6-Dimethyl-2,6-octadien-1-ol (E)	nd	nd	66 ± 8
Geranial	180 ± 16	nd	nd
Lactone			
Butyrolactone	140 a±21	95 a±9	124 a±10
Volatile phenols			
Guaiacol	69 ± 8	nd	nd
4-Ethyl-guaiacol	nd	266 ± 11	nd
4-Ethylphenol	nd	663 ± 22	nd
2-Metoxy-4-vinilphenol	234 a±22	218 a±24	163 a±18
Siringol	196 a±15	226 a±26	nd

[a]In bold, volatile components quantified by GC-FID, whose concentrations are expressed as mg/L; nd: not detected; according to the result of the Anova test, values that do not share a common superscript are significantly different ($p < 0.05$).

Alcohols are produced either from yeasts, as secondary fermentation products (Swiegers et al., 2005), or by catabolism of the corresponding amino acids. Higher alcohols positively affect the wine aroma, when present in concentrations below 300 mg/L, whereas concentrations that exceed 400 mg/L have a detrimental effect (de la Fuente Blanco, Sáenz Navajas, & Ferreira, 2017). The wines produced during this study show optimal values of these molecules. Isoamyl alcohols (1-butanol, 3-methyl) were the most abundant compounds in all the wines, ranging from 54.48 mg/L (LR) to 78.48 mg/L (GT). Among the alcohols identified, 2-phenylethanol, contributing with fine rose's notes to wine aroma, was the second most abundant alcohol at concentrations ranging from 34.61 mg/L (GT) to 37.44 mg/L (LZ) higher than its threshold, i.e.10 mg/L, in all samples. 2-Methyl-1-propanol and 1-propanol were also present in all samples, although this had no sensory significance, due to their concentration below odor thresholds (40 and 306 mg/L respectively). Fatty acids, produced during fermentation, constitute an important group of aromatic compounds that can contribute with fruity, cheese, fatty and rancid notes. In this case, the quantified fatty acids, showed levels lower than their perception threshold. In all the wines concentrations of aldheydes and ketons are definitely below their odor threshold values. As regard terpenes, that contribute to the floral aroma, only terpineol was detected in all wines, ranging from 13 μg/L to 22 μg/L. Among the five volatile phenols identified, the 4-ethylphenol was present only in LZ wine at concentration of 673 μg/L, much higher than the odor threshold (110 μg/L).

In summary, the autochthonous ITEM14093 yeast strain, selected in this investigation, was able to produce wines with a variegated pattern of volatile compounds responsible for a complex aroma profile.

Sensory analysis was performed involving the panel of experts and the results were subjected to QDA (Fig. 5). Similar odor profiles were identified in the wines produced by using ITEM 14093 as the starter strain either in the pilot or at industrial scale. However, the three wines produced at the industrial scale showed an improvement in the sensorial quality associated to fruity and floral notes and a decrease of herbaceous, vinous and acidity descriptors.

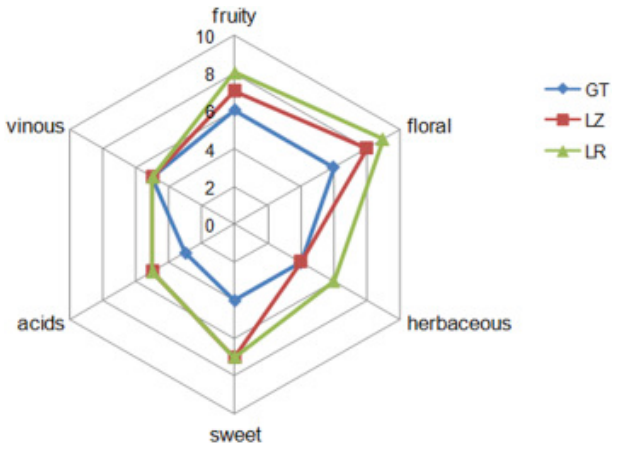

Fig. 5. Sensory profile of Primitivo wine obtained using the strain ITEM14093 as starter at industrial scale in three different industrial cellars (GT, LZ an LR).

4. Conclusions

This work represents the first phase of a wider project for the qualitative improvement of Primitivo wine. Some yeast strains were characterized for their ability to be used as microbial starter for Primitivo wine fermentation and, based on the results reported, the selected starter cultures could be produced on demand in the imminence of the vintage season by employing low-cost plants (Maqueda et al., 2011) and dispensed in a liquid concentrate form to the wineries. Furthermore, they may be usefull to investigate the use of mixed industrial starters, composed of a blend of *Saccharomyces* and non-*Saccharomyces* mixed strains (Tristezza et al., 2016), as strategy to further exalt the aromatic complexity of Primitivo wine.

Acknowledgments

This research was partially supported by the Apulia Region in the framework of the Project DOMINA APULIAE (POR Puglia FESR – FSE 2014-2020-Azione 1.6. – InnoNetwork; Project code AGBGUK2). Vittorio Capozzi was supported by Fondo di Sviluppo e Coesione 2007-

2013 - APQ Ricerca Regione Puglia "Programma regionale a sostegno della specializzazione intelligente e della sostenibilità sociale ed ambientale-FutureInResearch". The authors wish to thank Mr. Giovanni Colella for his valuable technical assistance and Prof. H. Smith for proofreading and providing valuable linguistic advice.

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