CHEMICAL ENGINEERING TRANSACTIONS
VOL. 32, 2013
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-23-5; **ISSN** 1974-9791
DOI: 10.3303/CET1332307

Characterization of Free Volatile Compounds in Fiano Wine Produced by Different Selected Autochthonous Yeasts

Alida Sorrentino[a], Floriana Boscaino[a], Rosaria Cozzolino[a], MariaG. Volpe[a], Elena Ionata[b], Sabato Guerriero[c], Teresa Picariello[c], Francesco La Cara[b]

[a]Istituto di Scienze dell'Alimentazione, CNR, Via Roma 64, 83100 Avellino, Italia
[b]Istituto di Biochimica delle Proteine, CNR, Via Pietro Castellino 111, 80131 Napoli, Italia
[c] IstitutoTecnico Agrario "F.de Sanctis", Via Tuoro Cappuccini 1, 83100 Avellino, Italia
f.lacara@ibp.cnr.it

Fiano cultivar is widespread in the South of Italy and is the most representative white wine variety in the Campania region designated as DOCG (Denomination of Controlled and Guarenteed Origin). For many years, wines have been produced by natural fermentation carried out by non-*Saccharomyces* and *Saccharomyces* yeasts originate from both the grapes and the cellar. Recently, non-*Saccharomyces* yeasts role in the wine production has been reassessed, consequently to the contribution that they give in the first part of the fermentation process which positively influences the qualitative features of the wine flavor, that is notoriously ''the backbone'' of the wine quality. It is therefore critical to increase and improve such aromas in order to characterize the typicalness of the native vineyards. The aim of this study was the selection of both non-*Saccharomyces* and *Saccharomyces* yeasts, native of Fiano grape and must. Among them, two indigenous strains, *Hanseniaspora guilliermondii* and *Saccharomyces cerevisiae,* were selected to prepare new different combination of autochthonous selected yeast strains to compare with commercial yeast *S. cerevisiae*. These combinations were applied for experimental cellar wine productions and the new starters showed some peculiar aroma production compared to commercial yeast. Moreover, in order to evaluate the aromatic profiles, the different wine samples were characterized by Solid Phase Micro Extraction–Gas Chromatography/Mass Spectrometry (SPME-GC/MS) technique. The results showed that the two native strains successfully dominated the fermentation process and contributed to improve the wines aromatic profiles.

1. Introduction

Many studies have been carried out on the ecology of wine yeasts and established the complexity of alcoholic fermentation, whether spontaneous or inoculated. It is now accepted that wine fermentation involve the growth of non-*Saccharomyces* and *Saccharomyces* species, and that the former play a relevant role in the organoleptic characteristics of a wine (Fleet, 2008). Today, the use of selected commercial yeasts from *Saccharomyces* species "sensu stricto" is widespread, because it allows to minimize risks in the production process, to standardize the winemaking procedure and ensure the quality of the final product. On the other hand, the widespread use of starter cultures in the wine industry has resulted in a loss of wine sensory characteristics and in a flattening of those differences crucial to distinguish wines of different cultivars. For this reason, research in the wine industry is focused on studying new yeast cultures that can enrich the final product, especially with those aromatic compounds which enhance the character of the wine. It is known that the production of many of this aromatic molecules occurs due to the presence of non-*Saccharomyces* yeasts, found on the grapes and the must. These non-*Saccharomyces* strains that predominate in the first phase of the alcoholic fermentation are able to release metabolites and enzymes, responsible of the aromatic complexity of the wine (Romano et al., 2003; Viana et al., 2011). In this regard, several researchers have promoted the utilization of such selected yeasts strains in order to get mixed fermentation inocula (Ciani et al., 2010) and many studies revealed significant positive differences in the qualitative and quantitative volatile compounds profile of the wines obtained with a guided fermentations compared to those produced with spontaneous yeasts (Calabretti et al., 2011). In the literature, a lot examples of analytical methods for studying

Please cite this article as: Sorrentino A., Boscaino F., Cozzolino R., Volpe M.G., Ionata E., Guerriero S., Picariello T., La Cara F., 2013, Characterization of free volatile compounds in fiano wine produced by different selected autochthonous yeasts, Chemical Engineering Transactions, 32, 1837-1842 DOI: 10.3303/CET1332307

the composition of volatile compounds of the wine are present (Canuti et al., 2009) and some of them have been carried out by SPME-GC/MS (Pawliszyn J. 2009). The aim of our work was the exploitation of new combinations of selected yeasts, obtained from the native microflora of Fiano grapes and musts, not only to improve the quality and organoleptic characteristics of the final product, also to emphasizeing its link with the territory and the autochthonous wine cultivar.

2.Materials and Methods

2.1 Chemicals

The culture media for the isolation, the reactivation and the growth of yeast strains were purchased from Oxoid (Hampshire, UK). All the enzymes and nutrients was obtained from Erbslöh Geisenheim AG, Geisenheim,Germany. Chemical reagents and solvents are all of analytical grade and were from Sigma-Aldrich (St.Louis, MO, USA). The SPME fibers PDMS- 100μm (polydimethylsiloxane), was from Supelco (Bellefonte, PA, USA).

2.2 Isolation and molecular identification of yeasts

To isolate the different yeasts populations, serial dilutions of Fiano grape and must samples, previously homogenized in sterile Ringer solution, were plated on two different solid agar media: WL-nutrient and Lysine-agar. On WL medium, yeast species have been distinguished by different colony morphologies and colours. The isolation of non-*Saccharomyces* species has been carried out on Lysine medium, that does not allow the growth *Saccharomyces spp.* The yeast species identification was performed by the analysis of the D1/D2 domain of 26S rDNA sequence. The genetic region was PCR amplified directly from individual yeast colony, following the protocol described by Arroyo-Lopez et al. (2006). The standard primers utilized were the commonly referred as NL1 and NL4 in the literature (O'Donnell 1993).

2.3 Determination of the yeast fermentative power

The must (100ml) after pasteurization (100 °C for 30 min) was inoculated with a 1 % (v/v) microbial biomass suspension (24h growth). The must fermentation tests were carried out in shake-flasks incubated at 28 °C. The flasks weight decrease was measured every day, until a constant weight has been reached and maintained for three days.

2.4 Yeasts selection for the resistance to sulphur dioxide

The tests were carried out on Fiano must which was pasteurized and inoculated with a 1 % microbial biomass suspension (24h growth). Potassium metabisulfite (MBK) was used as the source of sulphur dioxide and the yeasts resistance at concentrations of 100 and 250 mg/L was checked. The tests were performed at 28 °C for 7 days.

2.5 Growth of yeast strains

The selected yeast strains were inoculated in 100 mL YPD broth (2 % pepton+2 % dextrose+1 % yeast extract + 2 % agar). The growth was carried out at 28 °C in shake-flasks at 200 rev min^{-1} (rpm) in an orbital shaker (New Brunswick Scientific Co., Inc., USA) for 24 h. The obtained yeast suspension was used to inoculate, at (1 % v/v) 10 L of YPD broth, in a pilot plant fermenter. The obtained biomass was stored at 4 °C.

2.6 Winemaking process

Fiano grapes were harvested at 21.1 °Brix in a vineyard located in the area surrounding Avellino a town of the Campania region. The winemaking process was carried out in the cellar of ''Istituto Tecnico Agrario, F. de Sanctis'' in Avellino. The Fiano grapes were crushed, destemmed and separated from the skins by pressing. One hundred milligrams of potassium metabisulfite per liter of obtained juice were added, which was subsequently clarified by cold settling at 10 °C for 18 h, with the addition of 20 mg/L Trenolin® Opti DF pectolitic enzyme. The juice was then racked, divided in five 50 L tanks, warmed up to 16 °C, one of which inoculated with 0.15 g/L ($4.8x10^6$ cells/mL) of dry *S. cerevisiae* (var. *bayanus*) (ST5), Oenoferm® Freddo ($3.2 x 10^{10}$ CFU/g) (Erbslöh Geisenheim AG, Geisenheim, Germany), previously rehydrated according to manufacturer instructions. The other four tanks were inoculated with $2x10^6$ cells/mL of different selected yeast (commercial and autochthonous) combination: FWL66 (*H.guillermondii)*+FYP69 (*S. cerevisiae)* (ST1); FYP69 (ST3); ST5+ FWL66 + FYP69 (ST2); ST5+FWL66 (ST4). A quantity of 0.2 g/L of a biological mobilisator and nutrient (VitaDrive®, Vitamon® Combi), respectively, was added at the beginning of fermentation. Moreover, 0.2 g/L of a nutrient (Vitamon® Combi) were added in the middle of fermentation and the process was completed in 21 days, when the residual sugars concentration was<2 g/L. The wine was racked and cold settled for 4 months at 10 °C after the addition of 60 mg/L of potassium metabisulfite. On the Fiano wine obtained, following OIV methods (www.oiv.int), chemical-physical analyses were performed.

2.7 Monitoring of yeast populations during winemaking process

In order to monitor the alcoholic fermentation, samples of must were taken before the addition of potassium metabisulfite, after the inoculum with the different selected yeast strain (autochthonous and commercial) and at different intervals of times during the process. The samples were withdrawn in sterile containers and maintained at 4 °C, until the laboratory analysis was performed. Serial dilutions of the samples were plated onto WL Nutrient (WL) to evaluate the total yeast populations and to analyze the different colony morphologies and on Lysine Medium to assess the presence of non-*Saccharomyces* yeasts. The plates were incubated at 28 °C for 5 days to allow the development of the colonies and viable counts.

2.8 Sample preparation of HS-SPME-GC analysis

The SPME fiber (PDMS-100μm, polydimethylsiloxane) was conditioned according to the manufacturer's recommendations prior to its first use. To a 20 mL Headspace vial was added 5 mL of wine samples, together to 3g of NaCl and octan-3-ol, in hydro-alcoholic solution (1/1, v/v) at 100 μg/L, as Internal Standard. The solution was homogenized with a vortex shaker and then loaded onto a Gerstel autosampling device. The program consisted of swirling the vial at 250 rpm for 5 min at 40 °C, then inserting the fiber into the headspace for 30 min at 40 °C as the solution was swirled again, then transferring the fiber to the injector for desorption at 240 °C for 30 min.

2.9 GasCromatography-MassSpectrometry

Gas chromatography analysis were carried out using a 7890 Agilent GC system coupled to an Agilent 5975 inert quadrupole mass spectrometer equipped with a Gerstel MPS2 autosampler. The capillary column employed was a HP-Innowax (Agilent technologies) (30 m x 0,25 mm id. 0,50 μm film thickness) and the carrier gas was Helium. Splitless injections were used. The initial oven temperature was set to 40 °C for 1 min. The temperature was increased in four steps: 40–60 °C at 2 °C/min; 60–150 °C at 3 °C/min, 150–200 °C at 10 °C/min and 200-240 °C at 25 °C/min; the final temperature was maintened for 7 min. The injector, the quadrupole, the source and the transfer line temperature were maintained at 240 °C, 150 °C, 230 °C and 200 °C, respectively. Electron ionization mass spectra in full-scan mode were recorded at 70eV electron energy in the range 40–300 amu. Peaks were identified using both the NIST 98 and Wiley libraries. Quantification was performed by using the relative concentration in μg/L of the Internal Standard, calculated as the ratio between each compound area and the internal standard area. The samples were analyzed in triplicate and blank runs were made by using an empty vial every two analysis.

3.Results and discussion

3.2 Chemical-physical analyses

The chemical-physical analyses conducted on the Fiano wine, obtained employing the different winemaking starters selected in this study, are reported in Table 1

Table 1. Chemical-physical analyses of Fiano wine obtained with different winemaking starters(OIV met)

	ST1	ST2	ST3	ST4	ST5
Total acidity	6,53 g/L	6,37 g/L	5,55 g/L	7,58 g/L	7,73 g/L
Volatile acidity	0,30 g/L	0,30 g/L	0,30 g/L	0,42 g/L	0,39 g/L
pH	3,24	3,27	3,40	3,16	3,13
Sulfur dioxide	38,4 mg/L	44,8 mg/L	38,4 mg/L	57,6 mg/L	38,4 mg/L
Density	0,99275	0,99275	0,99195	0,99315	0,99325
% Alcohol	11,76 %vol	11,75 %vol	11,88 %vol	11,80 %vol	11,71 %vol
Total dry matter	21,7 g/L	21,6 g/L	19,5 g/L	22,8 g/L	22,7 g/L

In the Table 1, the quantities of free sulfur dioxide, pH, volatile acidity, density, % alcohol, total dry matter and total acidity in the experimental wines are presented. All values are the average of two duplicate experiments and were within the ranges acceptable for the wine industry. No significant differences in the levels of these items among the wines were observed.

3.1 Monitoring of yeast populations during the winemaking process

Microbiological analysis of Fiano grape and must microflora revealed the presence of species belonging mainly to the *Hanseniaspora, Kloeckera, Candida* and *Saccharomyces* genera, according to Beltran et al. (2002). In particular, *Hanseniaspora* and *Kloeckera* were predominant in the must before the addition of MBK, after the inoculation and in the first days of the fermentative process. These non-*Saccharomyces* yeast species were usually present, together with *Saccharomyces* spp., during the early stages of the alcoholic fermentation, due to their ability to tolerate alcoholic concentrations up to 3 or 4 % alcohol by volume(Fleet 2008). From the results obtained from technological experiments, such as resistance to sulphur dioxide and the alcohologenic and fermentative power (data not shown), two strains of non-*Saccharomyces* (FWL66) and *Saccharomyces* (FYP69) were selected. These two strains, by the analysis of the D1/D2 domain of 26S rDNA

sequence, have been identified as belonging to *H. guilliermondii* and *S. cerevisiae* species. The experimental winemaking processes carried out with the Fiano must inoculated with the starters obtained from a combination of the isolated yeasts and the commercial *S. cerevisiae* showed that the autochtonous yeasts were able to trigger and complete the alcoholic fermentation, leaving a sugar residue smaller than 2 g/L, as reported in Figure 1.

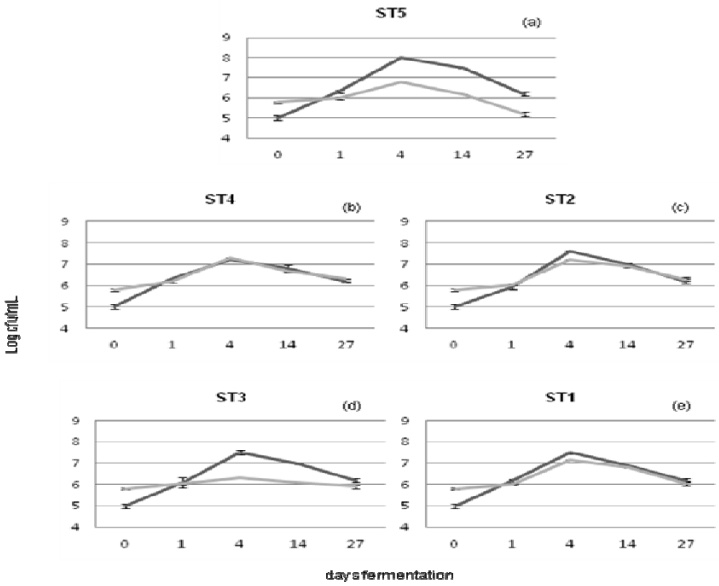

Figure1: Evolution of yeast population during winemaking process of must inoculated with selected yeast mix (□) Saccharomyces; (□) non-Saccharomyces:ST1 (S.cerevisiae FYP69 + H.guilliermondii FWL66);ST2 (Oenoferm® Freddo + S.cerevisiae FYP69 + H.guilliermondii FWL66); ST3 (S.cerevisiae FYP69); ST4(Oenoferm® Freddo + H.guilliermondii FWL66); ST5 (Oenoferm® Freddo)

Figure 1 shows the kinetics of fermentations obtained by employing the 5 starters used in the present trial. From the curve profile of fermentation conducted by commercial yeast (ST5), used as control (Figure 1.a), it is possible to noted that the population of the *Saccharomyces* yeast reaches an order of magnitude of about 10*8 cfu/mL during the fourth day of the process, remaining high for the entire winemaking process. Furthermore, the population of non-*Saccharomyces* yeasts is higher at the beginning of the winemaking process and then decreases during the other phases of the process, as already reported (Ciani et al.,2010; Prakitchaiwattana et al., 2004). The growth curves of native selected cultures, ST3 and ST1 (Figure 1.d and 1.e), show that native *S.cerevisiae* yeast (FYP69) is able to start and complete the winemaking process reaching, after 4 days, a population higher than 10*7 cfu/mL and also to counteract the growth of non-*Saccharomyces* microflora, naturally present in the must (Figure 1.d). On the other side, Figure 1.e, related to the starter ST1, we can note that the yeast *H.guilliermondii* (FWL66) was able to grow and to show a growth trend similar to that of *S.cerevisiae* (FYP69). The Figure 1.b and 1.c show the behavior of the yeast mixtures containing the commercial yeast. In Figure 1.b we can see that both the curves relating to commercial yeast and non-*Saccharomyces* yeasts are very similar, presenting a comparable kinetics of growth. This behavior can be explained by the fact that the selected non-*Saccharomyces* yeast is able to compete both with the commercial yeast and with the autochthonous *S.cerevisiae* yeast (Figure 1.d). This result, strengthening what is observed in Figure 1.a and 1.d, related to cultures in which the yeast FWL66 is absent, allows to conclude that *S. cerevisiae* yeasts (both commercial and autochthonous) are able to predominate and limit the growth of the non-*Saccharomyces* component.

3.3 Volatile compounds detected by SPME-GC/MS analysis

The data about the volatile compounds identified in Fiano wine samples by SPME-GC/MS, reported in Table2, are in general agreement with those reported in literature (Ugliano et al., 2008; Genovese et al.,2007). In particular, according to Romano et al. (2003), it is to underline that the differences in the volatile molecules composition of wine samples, obtained utilizing different yeast species, appear to be quantitative rather than qualitative. Ethyl esters of straight-chain fatty acids and acetates of higher alcohols are the dominating esters in wine samples and they are formed during the alcoholic fermentation process. The aroma compounds isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate and diethyl succinate were determined as the major esters. Also, other types of acetates such as ethyl acetate, hexyl acetate and phenylethyl acetate were

identified. Hexanoic, octanoic and decanoic acids, which also contribute to the aroma of the wine, were detected as the most abundant acids. Acetaldehyde and benzaldehyde were identified in the aldehydes class, while, in the alcohol group, isobutyl alcohol, 1-butanol, isoamyl alcohol, 1-hexanol, 1-pentanol-4-methyl, 1-pentanol-3-methyl, (Z)-3-hexen-1-ol and 2-phenylethanol were detected. Terpenes have been found to play an important role in the aroma composition of wine and have been used to differentiate different grape cultivar (Komes et al., 2006). Specifically, among this class of compounds, in the Fiano wine samples, linalool, 4-terpineol, α-terpineol, geraniol, nerol and β-citronellol were identified, whereas in the class of nor-isoprenoids, α-ionone, β-damascenone, TDN and vitispiranes were determinated. Furthermore, the volatile phenol and 4-vinylguaiacol was identified. Among the esters, ethyl caproate, ethyl caprate and ethyl caprylate, responsible for the fruity, green apple and soap flavor, appear to be present in higher concentration. In particular, the amounts of these compounds seem to be comparable in wines produced by using commercial *S. cerevisiae* (ST5) or autochthonous *S. cerevisiae*, both alone than mixed with other yeast (ST3, ST1) (see also table 3). Even the amount of diethylsuccinate, which mainly contributes to create the body of the wine (Viana et al., 2011); appears to be present in a slightly higher quantity in wines obtained by using ST1 starter, compared to wine samples produced by other starters. Among terpenes, linalol (floral aroma), α-terpineol (lilac aroma), geraniol (floral and orange citrus flavor) and β-citronellol (citrus and grapefruits flavor) (Genovese et al., 2007), are present in higher amount in wines produced by ST3 and ST1 starters, compared to control starter (ST5).

Table 2. Volatile compounds detected on wines obtained by using different starters in winemaking process

	ST3	ST1	ST4	ST2	ST5
	µg/L ± SD	µg/L ± SD	µg/L ± SD	µg/L ± SD	µg/L ± SD
aldehydes					
acetaldehyde	20,16 ±1,81	12,4±0,10	6,7±0,14	9,44±0,08	15,04±0,11
benzaldehyde	57,58±0,83	80,94±0,78	37,36±1,57	76,00±1,09	46,39±1,46
alcohols					
isobutyl alcohol	60,99±1,4	77,27±0,37	85,83±0,03	68,57±0,45	113,65±2,31
1-butanol	1,35±0,04	1,29±0,06	3,55±0,07	1,33±0,04	6,48±0,33
isoamyl alcohol	2017,11±6,79	2409,24±11,65	2109,58±12,73	1906,4±1,39	2672,75±26,52
1-pentanol,4-methyl	3,57±0,11	4,07±0,32	3,56±0,09	3,41±0,21	3,2±0,66
1-pentanol,3-methyl	4,73±0,25	5,95±0,22	5,71±0,29	4,89±0,43	5,01±0,37
1-hexanol	82,29±0,42	94,81±1,00	86,38±4,02	86,34±0,48	91,72±0,67
3-hexen-1-ol cis	4,44±0,27	5,04±0,21	3,68±0,57	3,73±0,25	4,56±0,43
2-phenylethanol	743,54±2,18	1156,66±7,16	638,54±2,76	779,3±0,43	511,16±1,64
esters					
ethyl acetate	865,88±4,08	867,06±9,91	908,26±2,82	728,45±4,88	943,23±1,73
isobutyl acetate	4,55±0,07	4,00±0,81	2,96±0,07	4,2±0,07	7,39±0,20
ethyl butanoate	92,66±1,17	87,66±0,51	85,74±1,42	81,02±0,02	96,81±1,42
ethyl 2methylbutyrate	15,39±0,56	11,66±0,9	7,14±0,19	15,39±0,22	16,43±1,42
ethyl isovalerate	21,35±0,39	22,06±0,56	9,44±0,02	19,59±0,49	7,03±0,04
isoamyl acetate	105,73±2,45	84,28±0,05	79,34±0,80	101,17±1,44	137,08±1,18
ethyl caproate	1645,6±7,91	1128,15±14,35	1504,27±4,62	1334,77±6,75	1435,93±18,28
hexylacetate	3,4±0,15	2,72±0,36	3,92±0,92	3,47±0,31	3,58±0,38
ethyl lactate	66,39±0,55	99,38±0,38	43,74±0,33	56,28±0,23	72,3±1,41
ethyl caprylate	7746,58±9,31	5500,6±1,52	8981,06±12,65	6724,19±17,24	7855,11±7,23
ethyl caprate	3865,54±5,01	2788,33±11,38	5541,41±33,36	4014,02±2,85	3631,37±9,00
diethyl succinate	264,92±4,13	294,12±5,66	205,1±2,83	343,81±2,56	269,99±14,13
phenethyl acetate	16,58±0,53	19,72±0,97	13,88±0,35	83,18±0,25	9,83±0,43
acids					
hexanoic acid	88,43±0,60	114,17±1,39	60,6±2,27	167,43±0,60	81,42±0,27
octanoic acid	1230,83±1,18	1336,51±7,05	995,44±1,59	1198,73±3,85	1017,64±3,74
decanoic acid	883,43±2,02	822,57±9,9	901,58±10,49	844,72±1,01	584,97±4,20
terpenes					
linalol	35,58±1,59	30,99±1,51	36,24±0,84	32,78±3,02	27,57±0,06
4-terpineol	5,99±0,05	5,6±0,23	5,26±0,16	5,97±0,33	5,43±1,15
α-terpineol	21,55±1,27	19,07±1,30	13,28±1,56	18,4±0,85	18,24±0,71
geraniol	6,05±0,31	5,74±0,03	6,37±0,67	3,63±1,70	5,4±0,55
nerol	3,97±0,21	3,95±0,41	5,47±2,5	3,00±0,97	3,67±0,81
β-citronellol	7,45±7,45	6,71±0,01	3,61±0,54	8,27±0,19	4,13±0,03
Nor-isoprenoids					
α-ionone	1,71±0,42	1,2±0,28	0,51±0,04	0,32±0,10	0,18±0,01
β-damascenone	11,7±1,83	15,06±1,14	14,94±3,00	14,38±4,40	9,8±2,00
vitispirane	28,14±0,89	16,05±0,16	16,24±0,30	39,20±0,68	18,53±0,81
TDN	7,64±0,91	3,01±0,28	6,12±0,58	6,78±0,27	6,76±0,33
other					
4-vinylguaiacol	8,71±0,66	6,86±0,09	9,23±0,09	4,87±1,01	12,76±0,57

Vitispirane and β-damascenone are the most abundant norisoprenoids found in the present study, but, it is very reasonably that, between them, the contribution to the flavor is mostly given by β-damascenone (roses and honey aroma), which is present in concentrations higher than the threshold of perception (0.002 μg/L) (Fan et al., 2010). Furthermore, wine samples produced by using ST1 and ST3 contain a higher amount of β-damascenone compared to that produced by ST5 starter. The concentrations of TDN and the 4-vinylguaiacol are similar in all the experimental wines obtained and, being below the threshold of perception, their contribution to the aroma can be considered negligible (Fan et al., 2010). Benzaldehyde, which gives a characteristic sweety and fruity aroma, is more abundant in wines obtained by employing ST1 and ST3 starters compared to ST5. Among the alcohols, 2-phenylethanol (rose fragrance) presents a higher concentration in wines produced by ST3 and ST1 compared to that derived by commercial yeast (ST5). Finally, wines produced using ST2 and ST4 starters have showed a volatile compounds content intermediate, compared to those of the products obtained by ST3, ST1 and ST5 starters. This result can be probably due to the presence in ST2 and ST4 mixtures of commercial yeast, which did not confer a relevant qualitative and quantitative difference to the wine, in comparison with the products obtained utilizing autochthonous starter.

4.Conclusions

In conclusion, many non-*Saccharomyces* yeasts showed interesting oenological properties, in terms of production of ethanol, secondary metabolites and fermentation purity. When used in mixed cultures with *S. cerevisiae*, these non-*Saccharomyces* strains can contribute to increase the production of volatile compounds, enhance the characteristics of the cultivar and then strengthen the concept of "terroir" of a wine.

References

Arroyo-Lòpez F.N., Duràn-Quintana M.C., Ruiz-Barba J.L., Querol A., Garrido-Fernàndez A., 2006, Use of molecular methods for the identification of yeast associated with table olives. Food Microbiol. 23, 791-796.

Beltran G., Torija M.J., Novo M., Ferrer N., Montserrat P., Guillamòn J.M., Rozès N., Mas A., 2002, Analysis of yeast populations during alcoholic fermentation: A six year follow-up study. System. Appl. Microbiol., 25, 287-293.

Calabretti A., Volpe M.G., Sorrentino A., Ionata E., Santomauro F., La Cara F., 2011, Aglianico and Fiano wines obtained with an autochthonous non-*Saccharomyces* yeast. Ann. Microbiol., 61, 131-136.

Canuti A., Conversano M., Li Calzi M., Heymann H., Matthews M.A., Ebeler S.E., 2009, Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. J. Chrom. A, 1216, 3012–22.

Ciani M., Comitini F., Mannazzu I., Domizio P., 2010, Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. FEMS Yeast Res., 10, 123-133.

Fan W., Xu Y., Jiang W., Li J., 2010, Identification and Quantification of Impact Aroma Compounds in 4 nonfloral, J. Food Science. 75, S81-S88.

Fleet G.H., 2008, Wine yeasts for the future, FEMS Yeast Res., 8, 979-95.

Genovese A., Gambuti A., Piombino P., Moio L., 2007, Sensory properties and aroma compounds of sweet Fiano wine, Food Chem.,103, 1228-36.

Komes D., Ulrich D., Lovric T.,2006, Characterization of odor-active compounds in Croatian Rhine Riesling wine, subregion Zagorje. Eur. Food Res. Technol., 222, 1–7.

O'Donnel K., 1993, *Fusarium* and its near relatives, in: The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. Eds Reynold D.R., and Taylor J.W., CAB International Wallingford, England, pp. 225–233.

Pawliszyn J., 2009, Handbook of Solid Phase Microextraction. Ed. Chemical Industry Press, Beijing, China.

Prakitchaiwattana C.J., Fleet G.H., Heard G.M., 2004, Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. FEMS Yeast Res., 4, 865-877.

Romano P., Fiore C., Paraggio M., Caruso M., Capece A., 2003, Function of yeast species and strains in wine flavor. Int. J. Food Microbiol., 86, 169-180.

Ugliano A., Moio L., 2008, Free and hydrolytically released volatile compounds of Vitis vinifera L. cv. Fiano grapes as odour-active constituents of Fiano wine. Anal. Chim. Acta, 621, 79-85.

Viana F., Belloch C., Vallés S., Manzanares P., 2011, Monitoring a mixed starter of *Hanseniaspora vineae*–*Saccharomyces cerevisiae* in natural must: Impact on 2-phenylethyl acetate production. Int. J. Food Microbiol. 151, 235-240.