Molecular characterization and technological properties of wine yeasts isolated during spontaneous fermentation of *Vitis vinifera* L.cv. Narince grape must grown in ancient wine making area Tokat, Anatolia

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Abstract. Narince is a native white grape variety of *Vitis vinifera* L grown in Tokat and produces rich and balanced wines often with a greenish yellow tint and delicate fruity flavour. Fermentation by indigenous yeasts may produce wines with complex oenological properties that are unique to specific region. In this study yeast population during alcoholic fermentation of Narince was investigated. Yeasts were identified by PCR-RFLP analysis of the 5.8 ITS rRNA region and sequence information for the D1/D2 domains of the 26S gene. Eight different species belonging to nine genera were identified as: *Hanseniaspora uvarum*, *Hansenispora guilliermondii*, *Pichia kluyveri*, *Metschnikowia* spp., *Pichia occidentalis*, *Torulaspora delbrueckii*, *Candida zemplinina*, *Lachancea thermotolerance* and *Saccharomyces cerevisiae*. *Hanseniaspora guilliermondii, Metschnikowia* spp., *Pichia occidentalis* and *Pichia kluyveri* were identified only in the early stage of fermentation. Selected yeasts tested for their physiological traits, ethanol, SO_2, temperature, pH tolerance, H_2S production, killer and enzymatic activity, fermentation rate, flocculation characteristic, foam, volatile acid and volatile compounds production. Among the yeasts, one,*Lachancea thermotolerance* and four *Saccharomyces cerevisiae* strain showed remarkable technological properties and results were compared with those obtained by using commercial starter culture.

1. Introduction

Vitis vinifera cv. Narince grape is one of the most important native white varieties grown in North-east Anatolia Region of Turkey. Narince produces rich and balanced wines that often have a greenish yellow tint and delicate, fruity aromas. Because of their balanced acidity, these wines are suitable for ageing and acquire a rich and complex bouquet over time [1,2].

One of the important factors affecting wine quality is yeast. Grape must harbour indigenous Non-*Saccharomyces* spp. and *Saccharoyces* yeasts. Many studies state that production of wines by indigenous yeasts isolated from its *terroir* can contribute to the regional character of wines, especially wine flavour [3–5]. On the other hand, winemakers usually prefer to use *Saccharomyces* (*S.*) *cerevisiae* strains unique to the region because those strains adapt easily to regional conditions and can readily dominate must during alcoholic fermentation [6].

Cv. Narince is the commercially important unique white wine grape variety of the Tokat region. Narince vineyards of Tokat region has remarkable historical value dating back to Hittie times civilized 4000 years ago. The wineries produced wines from cv. Narince, unfortunately, use the commercial dry yeasts instead of autochthonous indigenous yeasts. As far as it is known, there is no previous study on the molecular characterization of yeasts isolated from Narince spontaneous wine fermentations and then determination of their technological properties.

The aim of this study was to identify yeasts during spontaneous fermentation of cv. Narince and then to determine technological properties of some selected *S. cerevisiae* and Non-*Saccharomyces* spp. yeasts.

2. Materials and methods

2.1. Chemical analysis of Narince must

Density, brix, titrable acidity, $OD_4$20, FAN (free amino nitrogen), reducing sugar and pH were measured according to the methods outlined by OIV, 2015 [7]; glucose and fructose were quantified using HPLC (LC-20AD, Shimadzu, Kyoto, Japan) [8,9].

2.2. Sampling and Yeast Isolation

The study was conducted in commercial vineyard located in the Tokat Region, Turkey. White grapes of cv. Narince from Tokat hand harvested, was randomly collected (undamaged and healthy grapes) in sterile plastic bags. From the sampling points approximately 10 kg grapes were collected and transported to the laboratory

Indigenous strains belonging to *Saccharomyces* and non-*Saccharomyces* genera were isolated from Narince grape berries and also from different stage of the

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spontaneous fermentation. Yeasts on Narince grape berries were isolated according to Combine (2005) [10]. At the laboratory the samples were crushed under aseptic conditions in the original plastic bags. The juice obtained (1 L) was transferred into sterile bottles and spontaneous fermentation was performed under controlled temperature at 18 °C. During first day after the initiation of fermentation, in the middle and at the end of fermentation, samples of must and wine, diluted in 0.1% peptone-water (decimal dilutions), were inoculated onto plates of YPD, Lysine and modified YPD agar (% 10 ethanol, v/v and 2 g/L photassium metabisulphite) supplemented with chloramphenicol and sodium propionate to inhibit bacteria and filamentous fungi, respectively. Plates were incubated at 28 °C for 48 h and about 30 yeast colonies per plate must were collected, then purified and kept at −40 °C in 50% glycerol.

2.3. DNA extraction and RFLP-PCR analysis

Cell lysis for DNA extraction was performed using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's instruction. The primers used for amplification of 5.8S ITS rDNA region were ITS1 (5′- TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′- TCCTCCGCTTATTGATATGC-3′) [11]. The DNA amplifications were carried out in a final volume of 50 μL containing 5 U Taq DNA polymerase (MARKA), 1 X PCR reaction buffer, 25 μM dNTP, 25 mM$MgCl_2$, from each primers 100 μM, DNA (50–100 mg/μL). The PCR reaction was performed on a TC-PLUS thermal cycler (Techne, Germany). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturating at 94 °C for 1 min; annealing 55 °C for 2 min; an extension at 72 °C for 2 min; and a final extension step of 10 min at 72 °C. The PCR products were subjected to restriction analysis with the restriction endonucleases Hae III, HinfI and Hha I (Thermo, USA), following the manufacturer's instructions. Amplified products and their restriction fragments were separated on % 1.5 (w/v) agarose gels at 130 V constant voltages for 100 min.

2.4. Partial 26S rRNA gene sequence analysis

Isolates sharing identical restriction patterns were classified into groups and one or two samples were chosen as representative of each group for sequence analysis of the D1/D2 domains of the 26S rRNA gene. Amplification of the D1/D2 domains of 26S rRNA was carried out using NL1 (5′ GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers (Invitrogen, Milan, Italy), according to Kurtzman and Robnett (1998) [12], and the resulting products were commercially sequenced. The sequences obtained in FASTA format were compared with those deposited at the National Center for Biotechnology Information (NCBI), using BLAST to determine their closest known relatives. In order to confirm the identification of isolates at the species level, the sequences of the D1/D2 domain of the 26S rRNA gene were further investigated. To this purpose, the multisequence alignments among our sequences and those of type strains of their closest relatives were performed using ClustalW (Bioedit v. 7.0.9) [13,14]. The number of nucleotide differences between D1/D2 sequences of our isolates and those of their closest relative were also analysed.

2.5. Determination of oenological properties of strains

The ability to grow at different temperature [15,16], to test the resistance to different concentration of SO_2 and ethanol [17], the H_2S production [18], the killer activity and sensitivity of the strains to K_2 killer toxin [14,20,21], enzymatic activity (BioMerieux), foam production [16,22], fermentation rate [3,23], Helm sedimentation test used to determine strains flocculation capacity [24], and the volatile acidity [7] were determined.

2.5.1. Aroma compounds analysis

In 15 mL screw-capped centrifuge tubes, containing, 4.1 g Ammonium sulphate, 2.7 Ml of wine, 20 μL internal standard (140 μg/ml of 4-methyl −2 pentanol and 2-octanol), 6.3 Ml water, 250 μL of dichloromethane were added. The tube was shaken for 90 min and then centrifuged at 2500 rpm, for 10 min, at 20 °C. Once the phases had been separated, the dichloromethane phases was recovered with a 0.5 mL syringe and transferred to a 0.3 mL viale. The extract was injected into a GC-MS under the conditions listed below. Each sample was extracted in duplicate [25,26]. The extracted were analysed by GC-MS-FID. The gas chromatography (GC) system consisted of an Agilent 6890 chromatograph equipped with a flame ionization detector (FID) (Wilmington, DE, USA), and an Agilent 5973-Network mass selective detector (MSD). Volatile compounds were separated on DB-Wax (30 m length ×0.25 mm i.d. ×0.5 μm thickness; J&W Scientific Folsom, CA, USA) column. 3 μL sample of extract was injected. Injector and FID detectors were set at 250 °C. The flow rate of carrier gas (helium) was 3.3 mL/min. The oven temperature of the DB-Wax column was increased from 40 °C (after 3 min holding) to 90 °C at a rate of 2 °C/min, then at a rate of 3 °C/min to 130 °C and at a rate of 4 °C/min to 240 °C with a final hold, at 240 °C for 12 min. The same oven temperature programs were used for the mass selective detector. The MS (electronic impact ionization)(Agilent 5975B VL MSD) conditions were as follows: ionization energy of 70 eV, mass range m/z of 29–350 a.m.u., scan rate of 1.0 scan s−1, interface temperature of 250 °C, and source temperature of 120 °C. The volatile compounds were identified by comparing their retention index and their mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, NBS 75 k) and of the instrument's internal library created from the previous laboratory studies. Some of the identifications were confirmed by the injection of the chemical standards into the GC-MS system. Retention indices of the compounds were calculated by using an n-alkane series [27,28].

The determination of acetaldehyde and ethyl acetate was carried out by direct injection of 1 μL samples into a gas chromatograph, Agilent 6890 N equipped with FID. Acetaldehyde and ethyl acetate separated using a Chrompack CP-WAX-57CB capillary column (0.25 mm i.d. × 60, m × 0.4 μm film thickness) (Middelburg, The Netherlands). GC settings were as follows: injection temperature: 160 °C; oven temperature: 5 min at 40 °C,

Table 1. General composition of Narince must.

General Composition	Must
Density (g/cm^3, 20 °C)	1.087 ± 0.0
°Brix	20.06 ± 0.3
pH	3.18 ± 0.0
Titratable acidity (g/L)*	5.44 ± 0.0
Reducing sugar (g/L)	189.9 ± 2
OD_{420}	0.06 ± 0.0
FAN (mg/L)	205 ± 0.3
Sugars (g/L)	
Glucose	97.1 ± 1.4
Fructose	92.8 ± 2.0

*As tartaric acid.

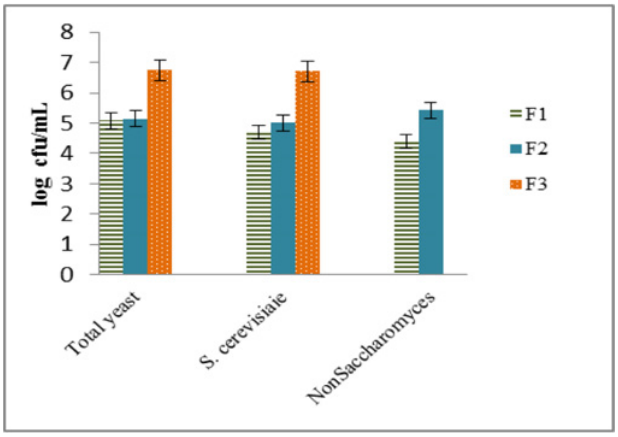

Figure 1. The viable counts from the selective and non-selective agars. F1: beginning of fermentation, F2: middle of fermentation, F3: end of fermentation.

then increased by 4 °C per minute up to 102 °C and 2 °C per minute up to 125 °C and hold for 5 minute and then 3 °C per minute up to 160 °C, 6 °C per minute up to 200 and finally hold 5 minute at 200 °C; carrier gas: He (1.3 mL/min); split rate: 1:50. The quantification was performed by using equation stated reference of Cabaroğlu et al. (2011) [29] with internal standard (4-methyl-2-pentanol) method. Analysis was done in duplicate.

2.6. Statistical analysis

The results of major aroma compounds were compared by the analysis of variance (ANOVA) and principal component analysis (PCA) was carried out using XLSTAT (2015, Addinsoft, New York, USA). PCA tests were used to determine the significant or differences between strains.

3. Results and discussion

Proximate composition of Narince must is given in Table 1.

3.1. Enumeration of yeast population

The viable counts from the selective and non-selective agars are shown in Fig. 1. At the beginning of the spontaneous fermentation a total yeast count on YPD medium was 5 log cfu/mL and they increased to 6.7 log cfu/mL at the middle of fermentation. Initial levels of total non-*Saccharomyces* spp. yeasts were 4.7 log cfu/mL at the beginning and then number increased to 6 cfu/mL at the middle of fermentation and end of fermentation it was not counted.

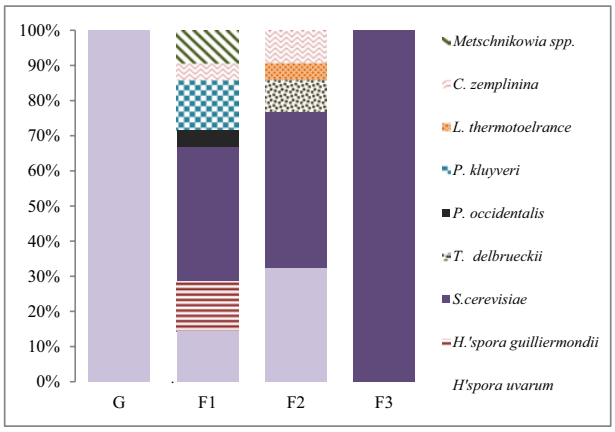

Figure 2. The diversity of yeast at grape and different fermentation stages. G: grape, F: beginning of fermentation, F2: middle of fermentation, F3: end of fermentation, *L. thermotolerance* (*Lachancea thermotolerance*), *T. delbrueckii* (*Torulaspora delbrueckii*), *P. kluyveri* (*Pichia kluyveri*), *C. zemplinina* (*Candida zemplinina*), *P. occidentalis* (*Pichia occidentalis*), *S. cerevisiae* (*Saccharomyces cerevisiae*), *H'spora guilliermondii* (*Hanseniospora guilliermondii*), *H'spora uvarum* (*Hanseniaspora uvarum*).

3.2. Strain identification and genetic characterization

The diversity of yeast at grape and different fermentation stages are shown in Fig. 2. *Hanseniaspora uvarum* was the only yeast isolated from Narince grapes before fermentation. During fermentation, however, a diversity of *S. cerevisiae* and non-*Saccharomyces* spp was observed. *S. cerevisiae* and *Hanseniaspora uvarum* were the dominant species at the beginning and *Metschnikowia* spp, *Pichia kluyveri, Hansenispora guilliermondii, Pichia occidentalis and Candida zemplinina* were isolated with less extend at the beginning of fermentation. At the middle of fermentation, *S. cerevisiae*, *Hanseniaspora uvarum*, *Candida zemplinina* started to overcome yeasts and others were not isolated from middle of fermentation with the exception of *Lachancea thermotolerance* and *Torulaspora delbrueckii* which were not observed at the beginning. *S. cerevisiae* was the only isolated yeast at the end of fermentation.

The total yeasts diversion is shown in Fig. 3. Five different genera and nine different yeast species were identified. The *S. cerevisiae* yeasts represented most of the total yeast population (49%). *S. cerevisiae*, followed by *Hanseniaspora uvarum* (23%), *Candida zemplinina* and *Pichia kluyveri* (6%), *Torulaspora delbrueckii* (5%), *Hanseniaspora guilliermondii* (4%)*Lachancea thermotolerance* (3%), *Metschnikowia* spp. (3%), and *Pichia occidentalis* (1%).

3.3. Technological properties of yeasts

Eighty two yeast strains were isolated from spontaneous fermentation of Narince must. Strains were grouped according to their phylogenetic mapping (results not shown). Some of the strains belonging to different groups were examined to determine their technological properties according to the Perez-Coello et al., (1999); Labagnara (2016) [3,30]. The results are shown in Table 2. Twenty three strains were eliminated because of high production

Table 2. Technological properties of remaining yeasts.

	884	1088	1120	1333	1378
Resistance to 12% (v/v) ethanol	+	+++	++	++	+++
Resistance to 200 mg/L SO_2	+++	+++	+++	+++	+++
Growth at low temperature 15 °C	++	++	++	++	++
H_2S Production	3:light brown	4:brown	4:brown	3:light brown	4:brown
Killer activity	+	+	+	+	+
Growth at Brix 30°	+++	+++	+++	+++	+++
Foam production (20 °C)	F2	F1	F0	F1	F2
Foam production (15 °C)	F0	F0	F0	F1	F1
Fermentation rate (g CO_2/L.h)	0.45± 0.01	0.99± 0.02	1.7± 0.01	0.8± 0.02	1.6± 0.1
Volatile acidity (g/L)	1	0.72	1.02	1	0.8
Flocculation (%)	69	98	97	86	82
Alkaline phospotase	1	2	2	1	2
Esterase (C4)	3	1	3	3	3
Esterase Lipase (C8)	2	3	4	2	3
Arylamidase	5	5	5	5	4
Valine arylamidase	1	3	4	2	3
Cysteine arylamidase	3	2	3	2	2
Acid phospotase	4	5	2	4	5
Naphthol-AS-BI-phosphohydrolase	3	3	3	2	3
$\beta-$glucosidase	2	2	4	-	3

+: low growth, ++: medium growth, +++: high growth, F0:0-2 mm, F1: 2-4 mm, F2: 4 and higher, 1: very low activity, 2: low activity, 3: medium activity, 4 high activity, 5: very high activity.

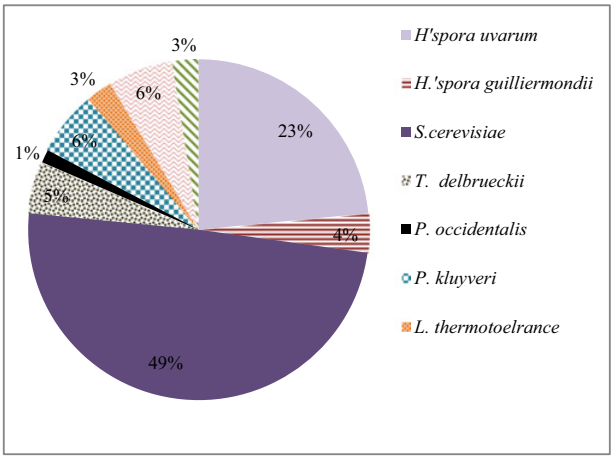

Figure 3. Total Yeast diversion. *L. thermotolerance* (*Lachancea thermotolerance*), *T. delbrueckii* (*Torulaspora delbrueckii*), *P. kluyveri* (*Pichia kluyveri*), *C. zemplinina* (*Candida zemplinina*), *P. occidentalis* (*Pichia occidentalis*), *S. cerevisiae* (*Saccharomyces cerevisiae*), *H'spora guilliermondii* (*Hansenispora guilliermondii*), *H'spora uvarum* (*Hanseniaspora uvarum*).

of the H_2S, weak growth at different levels of ethanol, SO_2 content and temperature. Of the remaining strains were subjected to determine their capacity to initiate fermentation in YPD broth containing 200 ppm of SO_2 and 12% (v/v) alcohol at 15 °C. According to their growth, five strains were eliminated. In the next stage, killer activity, foam production, enzyme profile of the remaining strains was determined and six of them were eliminated. Remaining 4 strains of *S. cerevisiae* and 1 strain of *Lachancea thermotolerance* (Non-*Saccharomyces*) were examined for their volatile acid production, flocculation and seconder metabolites.

Table 2 shows technological properties of studied yeasts. All remaining strains showed high growth rate at 200 mg/L SO_2 and at 30° Brix. Only strain 884 was the more sensitive to the presence of ethanol at 12% (v/v). All strains showed medium growth rate at low temperature (15 °C). All yeasts showed killer activity and their foam production decreased by decreasing temperature. All yeasts were produced more or less H_2S. Fermentation rate of remaining yeasts were found between 0.45–1.7 g CO_2/L.h. Volatile acid production of strains were found between 0.7 g/L-1.02 g/L. The 1120 strain produced high level of volatile acidity. The flocculation capacity was found between 68%–98% and the *S. cerevisiae* strain 884 was the less flocculants one. Enzymatic activities of yeasts depend on strain.

3.4. Seconder metabolites of wines

The results of the analysis of volatile compounds of wines fermented with the 5 selected strains and one commercial strain are shown in Table 3. Principal Component Analysis (PCA) was applied on the data set of the concentration of aroma compounds (Fig. 4).

A total of 30 aroma compounds were identified in Narince wines by GC-MS and GC/FID including 7 higher alcohols, 12 esters, 8 volatile acids, 1 lactone and 1 carbonyl compounds. Major aroma compounds ethyl acetate and acetaldehyde was calculated by GC-FID. The wines fermented with commercial strain, 884, 1088, 1120, 1333 and 1378 contained 328 mg/L, 276 mg/L, 309 mg/L, 381 mg/L, 289 mg/L and 415 mg/L of aroma compounds respectively.

The fermentation of Narince must with the all the isolated yeasts gave different volatile profiles. Among aroma compounds higher alcohols were the most abundance compounds of wines. These compounds are formed during alcoholic fermentation. At concentrations below 300 mg/l, they contribute to the desirable complexity of wine. When their concentration exceeds 400 mg/l, higher alcohols are regarded as a negative quality factor [31,32]. Isoamyl

Table 3. Aroma compounds of Narince wines fermented with different autochthonous yeasts.

	Aroma Compounds (mg/L)						
Higher Alcohols	C	**884****	**1088****	**1120****	**1333****	**1378****	F
1-propanol	2.36 ± 0.02^{b}	1.83 ± 0.02^{c}	2.35 ± 0.04^{b}	2.85 ± 0.08^{a}	1.91 ± 0.01^{c}	1.29 ± 0.05^{d}	*
Isobutyl alcohol	13.14 ± 0.09^{b}	9.37 ± 0.13^{d}	13.50 ± 0.15^{b}	9.65 ± 0.08^{c,d}	9.82 ± 0.04^{c}	14.31 ± 0.4^{a}	*
Isoamyl alcohol	190.27 ± 3.7^{b}	114.31 ± 2.5^{d}	188.84 ± 0.01^{b}	112.08 ± 4.9^{d}	133.63 ± 0.5^{c}	253.51 ± 7.5^{a}	*
2,3-Butanediol	0.56 ± 0.04^{b}	0.12 ± 0.01^{d}	0.40 ± 0.01^{c}	0.61 ± 0.02^{b}	1.52 ± 0.06^{a}	0.15 ± 0.01^{d}	*
Methionol	0.81 ± 0.02^{b}	0.25 ± 0.01^{e}	0.34 ± 0.01^{d}	0.21 ± 0.01^{f}	0.47 ± 0.01^{c}	0.89 ± 0.05^{a}	*
2-Phenylethyl alcohol	63.28 ± 0.7^{b}	24.99 ± 0.7^{e}	47.53 ± 0.01^{c}	30.62 ± 2.9^{d}	48.63 ± 3^{c}	102.52 ± 3.5^{a}	*
Tyrosol	1.69 ± 0.07^{a}	0.32 ± 0.01^{e}	0.94 ± 0.05^{c}	0.5 ± 0.01^{d}	0.52 ± 0.04^{d}	1.12 ± 0.07^{b}	*
Total	272.11	151.19	253.9	156.52	196.5	373.9	
Esters							
Ethyl acetate	30.3 ± 1.7^{d}	83.39 ± 1.4^{b}	27.26 ± 0.11^{e}	184.22 ± 0.05^{a}	62.39 ± 1.5^{c}	24.14 ± 0.7^{f}	*
Isoamyl acetate	1.67 ± 0.1^{b}	1.37 ± 0.06^{c}	0.16 ± 0.01^{e}	0.54 ± 0.03^{d}	1.68 ± 0.01^{b}	2.42 ± 0.07^{a}	*
Hexyl acetate	0.24 ± 0.03^{a}	0.18 ± 0.01^{c}	0.05 ± 0^{d}	nd	0.21 ± 0.01^{b}	0.21 ± 0.01^{b}	*
Ethyl hexaonate	0.35 ± 0.01^{a}	0.33 ± 0.01^{a}	0.12 ± 0.01^{b}	0.15 ± 0.0^{b}	0.32 ± 0.05^{a}	0.35 ± 0.02^{a}	*
Ethyl lactate	0.20 ± 0.03^{d}	0.15 ± 0.01^{e}	0.22 ± 0.03^{d}	0.49 ± 0.06^{a}	0.3 ± 0.01^{c}	0.33 ± 0.01^{b}	*
Ethyl octaonate	0.12 ± 0.01^{c}	0.18 ± 0.01^{a}	0.09 ± 0^{d}	nd	0.13 ± 0.01^{c}	0.16 ± 0.01^{b}	*
Ethyl-3-hydroxybutanoate	0.22 ± 0.04^{a}	0.15 ± 0.01^{c}	0.16 ± 0.01^{b,c}	0.10 ± 0.01^{d}	0.18 ± 0.01^{b}	0.18 ± 0.01^{b}	*
Ethyl decanoate	0.07 ± 0.02^{c}	0.02 ± 0.01^{d}	0.40 ± 0.02^{a}	0.13 ± 0.01^{b}	0.07 ± 0.00^{c}	0.06 ± 0.01^{c}	*
Propanediol diacetate	0.44 ± 0.01^{b}	0.21 ± 0.01^{e}	0.34 ± 0.01^{c}	0.55 ± 0.0^{a}	0.29 ± 0.01^{d}	0.15 ± 0.01^{f}	*
2-Phenylethyl ester	0.27 ± 0.04^{b}	0.25 ± ^{b}	0.27 ± 0.02^{b}	0.06 ± 0.0^{c}	0.28 ± 0.01^{b}	0.86 ± 0.07^{a}	*
Ethyl-4-hydroxybutanoate	3.06 ± 0.06^{c}	1.15 ± 0.02^{e}	2.53 ± 0.3^{d}	5.17 ± 0.4^{a}	3.80 ± 0.3^{b}	2.46 ± 0.09^{d}	*
Ethyl hydrogen succinate	0.13 ± 0.01^{c}	0.11 ± 0.01^{d}	0.07 ± 0.00^{e}	0.09 ± 0.0^{d}	0.21 ± 0.01^{a}	0.19 ± 0.03^{b}	*
Total	36.8	87.49	31.67	191.50	69.86	31.51	
Volatile acids							
Acetic acid	0.54 ± 0.02^{c}	0.62 ± 0.01^{c}	0.37 ± 0.02^{d}	1.25 ± 0.08^{a}	1.12 ± 0.5^{b}	0.60 ± 0.02^{c}	*
Isobutyric acid	0.23 ± 0.02^{d}	0.25 ± 0.02^{c}	0.32 ± 0.01^{b}	0.2 ± 0.01^{e}	0.19 ± 0.01^{e}	0.42 ± 0.02^{a}	*
Butyric acid	0.12 ± 0.01^{b,c}	0.11 ± 0.01^{c}	0.12 ± 0.01^{c}	0.14 ± 0.01^{a,b}	0.15 ± 0.01^{a}	0.13 ± 0.01^{b,c}	*
Isovaleric acid	0.62 ± 0.01^{c}	0.27 ± 0.01^{e}	0.69 ± 0.03^{b}	0.24 ± 0.0^{f}	0.34 ± 0.01^{d}	0.85 ± 0.01^{a}	*
Hexanoic acid	1.87 ± 0.02^{d}	1.86 ± 0.02^{d}	1.93 ± 0.02^{c}	0.76 ± 0.01^{d}	3.03 ± 0.2^{a}	2.31 ± 0.04^{b}	*
Octanoic acid	2.74 ± 0.1^{b}	2.42 ± 0.02^{c}	1.56 ± 0.03^{d}	0.89 ± 0.03^{e}	2.95 ± 0.4^{a}	2.85 ± 0.01^{a,b}	*
Decanoic acid	1.38 ± 0.1^{a}	0.47 ± 0.01^{c}	0.07 ± 0.00^{d}	0.50 ± 0.01^{c}	0.74 ± 0.02^{b}	0.57 ± 0.01^{c}	*
Hexadecanoic acid	0.39 ± 0.01^{c}	0.15 ± 0.02^{e}	0.1 ± 0.01^{e}	0.55 ± 0.03^{b}	0.28 ± 0.01^{d}	0.90 ± 0.08^{a}	*
Total	7.89	6.45	5.16	4.53	8.80	8.63	
Lactones							
gamma-Butyrolactone	0.40 ± 0.04^{e}	0.49 ± 0.01^{d}	0.34 ± 0.01^{f}	0.87 ± 0.03^{a}	0.67 ± 0.02^{c}	0.81 ± 0.01^{b}	*
Total	0.40	0.49	0.34	0.87	0.67	0.81	
Aldehydes							
Acetaldehyde	11.12 ± 0.5^{e}	21.35 ± 0.7^{b}	18.43 ± 0.2^{c}	27.9 ± 0.06^{a}	14.06 ± 1^{d}	9.26 ± 0.7^{f}	*
Total	11.12	21.35	18.43	27.9	14.06	9.26	
TOTAL	**328.30**	**266.97**	**309.5**	**381.32**	**289.89**	**424.11**	

C: wine fermented with commercial yeast strain, ± Standard deviation, nd: not detected, F: significance at which means differ as shown using analysis of variance,*p<0.05 level, **: 1120 is *Lachancea thermotolerance* and others are *S. cerevisiae*

alcohol and 2-phenylethyl alcohol were the most abundant higher alcohols in all wines and they both were found highest in wine with inoculated 1378. Odour threshold values of isoamyl alcohol and 2-phenylethyl alcohol are 30 mg/L and 10 mg/L, respectively. Concentration of these compounds exceeded their threshold values in all Narince wines. 2-phenylethyl alcohol has a floral and rose like aroma and contributes positively to wine aroma [1].

Esters are an important group of volatile compounds produced by yeasts during alcoholic fermentation [31]. Ethyl acetate was the major ester compound produced in Narince wines. Aroma threshold value of ethyl acetate is 7.5 mg/L [31], and it exceeded its odour threshold value in all wines. Ethyl acetate may contribute with pleasant fruity fragrance to the general wine aroma at low concentration, whereas it contributes significantly do defect aroma at a concentration of 150–200 mg/L [32]. The wine inoculated with 1120, the ethyl acetate exceeded this value, which makes this strain unsuitable for making quality wine. Other important volatile esters, isoamyl acetate, 2-phenylethyl acetate, ethyl hexaonate and ethyl octaonate were found above their odour threshold values [31].

As indicated in Table 3, the most abundant volatile acids in Narince wines were octanoic and hexanoic acids. Similar results were found by Selli et al. (2006) [1]. The contribution of volatile acids on Narince wines cannot be considered important because their concentrations were much lower than their odour threshold values [1,31].

γ-Butyrolactone was the only lactone was found in all Narince wines. This compound is found in fermented products and probably some from glutamic acid and related compounds (succinic, 2-oxoglutaric or γ-aminobutyric acids) [1,31].

The acetaldehyde is one of the most important carbonyl compound produced during fermentation and at low levels it contributes to fruity flavours, while high concentrations (200 mg/L) cause flatness in wines [32]. The concentration of acetaldehyde in Narince wines were found between

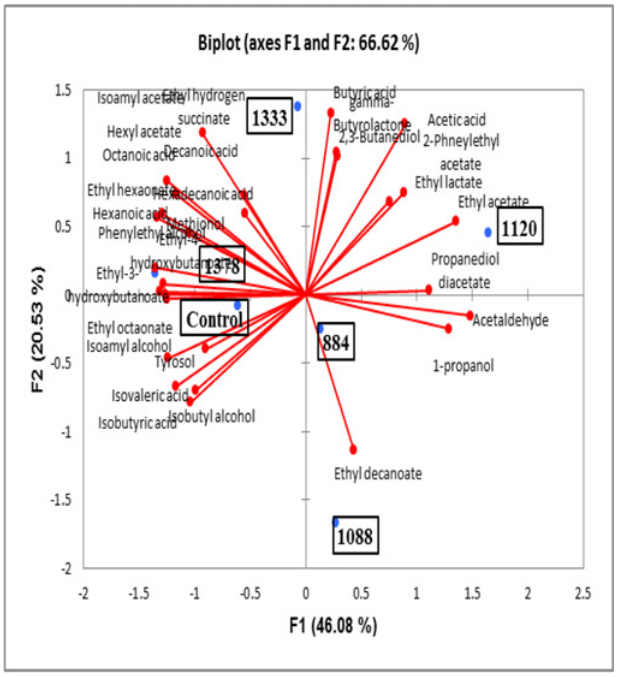

Figure 4. Principal Component Analysis of aroma compounds produced by selected yeasts.

11 mg/L to 27 mg/L. Nevertheless its concentration did not exceed its threshold values of 100 mg/L [31].

The PCA bi-plots (Fig. 4) showed the relationship between better and poorer performing yeast with regards to the aroma compounds. The PCA of aroma compounds showed that most of data explained by the first (F1) and second (F2) factors which are 46.08 and 20.53% respectively. The F1 explained 46% data variation and showed correlation 1120 and 1378. The F2 explained 20.53% of data variation and correlated with 1088 and 1333. The F3 explained 15.4% data variation and correlated with 884 and F4 explained 11% data variation and correlated with the control. As shown in F1, 1088 well differentiated from the other yeasts due to its high content of ethyl decanoate.

4. Conclusion

In this study PCR-RFLP analysis of the 5.8 ITS rRNA region and sequence information for the D1/D2 domains of the 26 S gene were used for yeasts identification. Only one Non-*Saccharomyces* spp. (*Hanseniaspora uvarum*) identified on Narince grape surface. During spontaneous fermentation of Narince musts eight different species belonging to seven genera were identified. Of the 69 isolated native strains of *S. cerevisiae* and Non-*Saccharomyces* from fermenting musts of Narince, only 4 *S. cerevisiae* and 1 *Lachancea thermotoelerance* (Non-*Saccharomyces* spp.) were considered to have good enough technological properties. Moreover 1088 was the most suitable strain to make quality wine with low volatile acid and foam production, high flocculation capacity, high resistance to 12% (v/v) ethanol and producing well balanced aroma compounds.

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