

Article

The Influence of Selected Autochthonous *Saccharomyces cerevisiae* Strains on the Physicochemical and Sensory Properties of Narince Wines

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Abstract: *Vitis vinifera* cv. Narince is a Turkish native white grape variety. In this study, volatile and sensory properties of Narince wines that are produced with autochthonous *Saccharomyces cerevisiae* (*S. cerevisiae*) strains and commercial strain were compared. Autochthonous yeast strains 1044 (MG017575), 1088 (MG017577), and 1281 (MG017581) were previously isolated from spontaneous fermentations of Narince grapes. Volatile compounds formed in wines were extracted using a liquid–liquid extraction method and determined by GC-MS-FID. All yeast strains fermented Narince grape juice to dryness. The differences between the volatile profiles of the yeast strains were determined. Wines fermented with autochthonous strains 1281 and 1044 produced a higher amount of acetates and ethyl esters. While the highest concentrations of ethyl hexanoate and hexyl acetate were found in wine fermented with 1044, the highest concentrations of ethyl octanoate, ethyl decanoate, isoamyl acetate, and 2-phenylethyl acetate were found in wine fermented with strain 1281. Also, the highest contents of 2-phenyl ethanol and linalool were found in wine fermented with strain 1281. According to sensory analysis, the wine fermented with 1281 achieved the best scores in floral and fruity attributes, as well as balance and global impression. The data obtained in the present study showed that autochthonous yeast strains affect the final physicochemical composition and sensory profile of Narince wines.

Keywords: narince; autochthonous; *Saccharomyces cerevisiae*; aroma; white wine

1. Introduction

Wine quality is influenced, in part, by the composition of the grape juice and by the microbial communities present during the fermentation process. Aroma is one of the main characteristics that determine the quality and value of wine, especially white wines. The aroma of wine is a unique mixture of volatile compounds originating from grapes (varietal compounds), secondary products formed during the wine fermentation (fermentative compounds), and aging (post-fermentative compounds) [1–3]. Alcoholic fermentation is carried out by yeasts that convert sugars not only into ethanol and carbon dioxide but also into different secondary metabolites, such as higher alcohols, esters, and fatty acids [4,5]. The ability to produce these secondary compounds depends on the yeast species and yeast strains. Therefore, it is important to determine the dynamics of fermentation populations during fermentation, since the metabolism of yeasts has an effect on the chemical and sensory properties of the wine [4,6].

At present, commercial *S. cerevisiae* strains are widely used in winemaking, and in Turkey, most of them are imported. This practice usually guarantees fermentation control and quality of wines.

However, in some cases, the commercial inoculated *S. cerevisiae* strains cannot compete successfully with indigenous strains, and therefore, cannot dominate the fermentation as expected. Local selected strains of *S. cerevisiae*, which are better adapted to micro-area conditions of the wine production region and easily dominate the natural biota, are rather advisable as starters, and contribute to the regional characteristics of the wine. Recently, there has been an increase in the use of autochthonous or locally selected yeasts to carry out must fermentation [4,7–10]. In Turkey, Narince wine production is generally carried out by commercial *S. cerevisiae* strains imported from abroad.

Vitis vinifera cv. Narince is one of the most important native white grape varieties grown in the mid-southern Anatolia Region (Tokat and Cappadocia) of Turkey. Narince makes straw-yellow colored wines with floral notes, yellow fruit, and citrus aromas on the nose. On the palate, it produces round, medium to full-bodied wines. Because of their balanced acidity, these wines are suitable for aging and acquire a rich and complex bouquet over time [11,12].

The aim of this work was to monitor the effect of three selected autochthonous yeast cultures previously isolated from spontaneous fermentation of Narince grapes on the volatile and sensory profiles of wine samples and compare these experimental variants with a control sample produced by a commercial starter strain widely used for the production of Narince wines.

2. Materials and Methods

2.1. Yeast Strains

The autochthonous *S. cerevisiae* strains 1044, 1088, and 1281 used in this study were previously isolated from spontaneous fermentations of Narince grapes. These strains were chosen due to their good technological properties (Table 1). Commercial yeast strain X5 (Laffort, Bordeaux, France) was used as a control. Among autochthonous yeasts, technological properties of strain 1088 were previously explained by Çelik et al. [12].

Table 1. Technological properties of autochthonous *S. cerevisiae* strains previously isolated from spontaneous fermentations of Narince grapes.

Technological Properties	Strain 1044	Strain 1088	Strain 1281
Resistance to 12% (v/v) ethanol	**	***	**
Resistance to 200 mg/L SO ₂	**	***	**
Growth at low temperature 15 °C	**	**	**
H ₂ S Production	3	4	2
Killer activity	+	+	+
Growth at Brix 30°	***	***	***
Foam production (15/20 °C)	F1/F2	F0/F1	F1/F2
Fermentation rate (g CO ₂ /L.h)	1.27 ± 0.0	0.99 ± 0.0	2.47 ± 0.2
Fermentation vigor (% h/h)	9.9 ± 0.0	10 ± 0.1	10.12 ± 0.1
Volatile acidity (g/L)	0.74 ± 0.0	0.85 ± 0.0	0.58 ± 0.0
Flocculation (%)	98	98	95
Esterase (C4)	2	1	3
Esterase Lipase (C8)	3	3	3
Ethyl acetate (mg/L)	29.9 ± 0.5	27.26 ± 0.1	24.26 ± 0.04 ± 0.2
Acetaldehyde (mg/L)	8.39 ± 0.4	18.4 ± 0.2	12.49 ± 0.05 ± 0.2
Higher alcohols (mg/L)	263.6 ± 0.6	252.96 ± 0.5	241.05 ± 0.5

Note: ** = medium growth rate; *** = high growth rate, F1: 2–4 mm, F2: 4 mm, and higher; 1 = very low activity; 2 = low activity; 3 = medium activity; 4 = high activity; 5 = very high activity, + = positive activity.

2.2. Culture Media and Chemical Standards

Yeast peptone dextrose agar (YPD) and YPD broth were purchased from Sigma Aldrich (St Louis, MO, USA) and L-lysine agar was purchased from Oxoid (Basingstoke, UK). Dichloromethane (≥99.9% purity), sodium sulfate anhydrous (99%), internal standard (4-nonanol), and a mixture

of n-alkane standards ranging from C₈–C₄₀ were purchased from Merck (Darmstadt, Germany). Standard volatile compounds, glucose, fructose, glycerol, tartaric acid, and lactic acid used in the study were obtained from Sigma Aldrich (St Louis, MO, USA).

2.3. Fermentations

Grapes from *Vitis vinifera* L. Narince were harvested at optimum maturity during the 2015 vintage in the commercial vineyard of Kavaklıdere (Cappadocia Region, Nevşehir, Turkey). The grape juice had the following main analytical composition: pH 3.32; initial sugar content 214 g/L; total acidity 5.62 g/L; and free amino nitrogen (FAN) content 131 mg/L. Grapes were crushed and pressed, and 50 mg/L of SO₂ were added. After pressing, the juice was allowed to settle at 10 °C for 12 h, then separated from the lees and randomly distributed into twelve 1L glass bottles. The fermentation trials were carried out in bottles containing 750 mL of Narince grape juice. Each fermentation experiment was performed in triplicate using standard protocols for white wines. Autochthonous strains 1044, 1088, and 1281 were previously grown in YPD medium at 28 °C for 24 h on an orbital shaker (rotation, 150 rpm); following this, the cells were recovered by centrifugation and washed with sterile water. Yeasts were counted by using Thoma counting chamber by light microscopy (Olympus CX22, Olympus Optical Co Ltd., Tokyo, Japan) before inoculation. The final concentration of each yeast was adjusted to 1 × 10⁶ cells mL/L and added to the must. Control strain was added as suggested by the manufacturer. The bottles were locked with a fermentation airlock containing water and sulphuric acid to allow only CO₂ to escape from the system. All fermentations were conducted at 18 °C in a temperature-controlled room. The development of alcoholic fermentation (density and temperature) was monitored daily with a digital densimeter (Mettler Toledo, Inc., Columbus, OH, USA) until the end of alcoholic fermentation. The final wines were analyzed for residual sugars (glucose and fructose) using HPLC method, which is explained below. At the end of the alcoholic fermentation, all wines were racked off lees and 50 mg/L sulfur dioxide was added. After this, the wines were bottled and stored at 13–15 °C for 3 months until analysis.

2.4. Chemical Analysis and Microbial Enumeration

Density, alcohol, titrable acidity, pH, volatile acidity, reducing sugar, free SO₂, and total SO₂ were measured according to the methods outlined by International Organization of Vine and Wine (OIV) [13], while free amino nitrogen (FAN) was measured according to Ough and Amerine [14]. Glucose, fructose, tartaric acid, malic acid, and glycerol were quantified using HPLC LC-10A (Shimadzu, Kyoto, Japan) equipped with a refractive index detector (RID-10A) for the analysis of sugar and glycerol, and a UV/Vis detector (SPD-20A) for the analysis of organic acids monitored at 210 nm. Sugars, glycerol, and organic acids were simultaneously analyzed using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, Hercules, CA, USA). The column was eluted with 0.5 mM sulfuric acid at 50 °C at a flow rate of 0.5 mL/min. Before HPLC, wine samples were filtered through a membrane (0.45 µm) and passed through a C18 Sep-Pak. Quantification of glucose, fructose, tartaric acid, malic acid, and glycerol were done by external standard method [13,15].

The enumeration of culturable yeasts was performed during the first day after the initiation of fermentation, in the middle (when about 50% of total sugar was fermented), and at the end of fermentation (stabilization of the density). Samples of must and wine (1 mL) diluted in 0.1% peptone-water (decimal dilutions) were inoculated onto plates of yeast peptone dextrose YPD agar for total yeast count. Lysine agar was used for non-*Saccharomyces* yeast count, and modified YPD agar (% 10 ethanol *v/v* and 2 g/L potassium metabisulphite) was used for *S. cerevisiae* count. All agars were supplemented with chloramphenicol and sodium propionate to inhibit bacteria and filamentous fungi, respectively, and plates were incubated at 28 °C for 48 h [16].

2.5. Volatile Compounds Analysis

A liquid–liquid extraction method was used for the isolation of volatile compounds [17,18]. The extraction of volatile compounds was performed using dichloromethane. Then, 100 mL wine samples containing 40 mL of dichloromethane and 34 mg/L of 4-nonanol (5 µL, as an internal standard) were poured into a 500 mL flask, which was stirred at 4 °C, 700 rpm, for 30 min under nitrogen gas. The mixture was then centrifuged at 4 °C (9000 rpm, 15 min). After the dehydration process, using anhydrous sodium sulfate, the pooled organic extract was concentrated to a volume of 0.5 mL with a Vigreux distillation column prior to gas chromatography/mass spectrometry (GC/MS) analysis. Each sample was extracted in triplicate. The concentration of volatile compounds was quantified from the flame ionization detector FID peaks areas and the internal standard, 4-nonanol. The response factor was set to 1 for all compounds. The analytical methods for GC/MS-FID were well explained by Arslan et al. [11].

The determination of acetaldehyde and ethyl acetate was carried out by direct injection into gas chromatography using Agilent 6890 N equipped with FID. Each sample was prepared and analyzed as reported by Arslan et al. [11].

2.6. Sensory Analysis

The sensory characteristics of the final wines were evaluated according to Lawless and Heymann [19]. The sensory panel comprised 6 females and 4 males, 25–55 years of age, all belonging to the laboratory staff and having substantial experience with sensory analysis. The panelist used a 15-point scale, from 0 (no intensity) to 15 (very strong intensity). Each panelist smelled and then tasted the wines in a tasting glass to detect the intensity of the 8 attributes (floral, fruity, honey, herbaceous, acidity, persistence, balance, global impression). Sensory analysis was done in five-booth sensory panel room at 22 °C equipped with white fluorescent lighting. Wines were served (50 mL at 12 °C) in a tulip-shaped wine glasses covered by glass Petri dishes. The tasting glasses were coded with different three-digit numbers.

2.7. Statistical Analysis

The results were compared by the analysis of variance (ANOVA) using SPSS (for Windows version 16.0). Duncan's multiple-range tests were used to compare the significant differences of the mean values with $p < 0.05$. Principal component analysis (PCA) was used as a tool for screening, extraction, and compression of volatile compounds using XLStat Pro (Addisonsoft).

3. Results and Discussion

3.1. Yeast Growth and Fermentation Kinetic

The yeast counts (log CFU/mL) are shown in Figure 1. On the lysine agar no count was obtained. For this reason, only a modified agar count has been given. The initial yeast level was similar in all of the samples (6.4–6.9 Log CFU/mL). In general, a yeast population ranging from 8.0 Log CFU/mL to 8.5 Log CFU/mL was found in samples analyzed in the middle of fermentation, while values from 7.0 Log CFU/mL to 8.3 Log CFU/mL were found at the end of the fermentation.

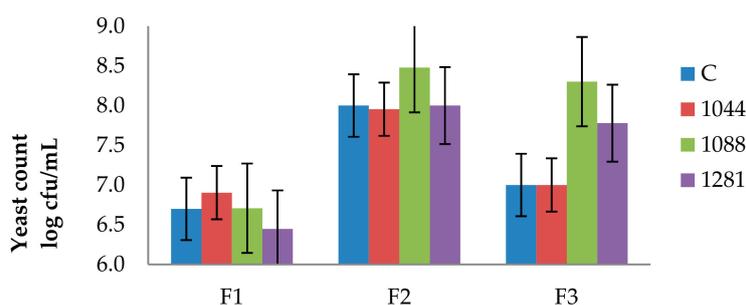


Figure 1. The growth of yeasts during fermentation. F1 is the beginning of fermentation, F2 is middle of fermentation, and F3 is end of fermentation. C represents wine inoculated with control strain, 1044 represents wine inoculated with 1044, 1088 is wine inoculated with 1088, and 1281 is wine inoculated with 1281.

Alcoholic fermentation of must started one day after inoculation in all Narince musts (Figure 2). The duration of alcoholic fermentation with control strain, autochthonous 1088, and 1281 strains were shorter (11 days) compared to the autochthonous 1044 strain (13 days). All musts were fermented to dryness.

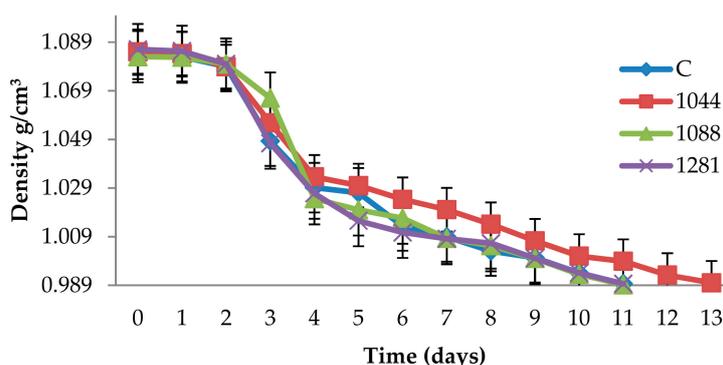


Figure 2. Daily fermentation monitoring in Narince must be fermented with different yeasts. C represents wine inoculated with control strain, 1044 is wine inoculated with 1044, 1088 is wine inoculated with 1088, and 1281 is: wine inoculated with 1281.

3.2. General Composition of Wines

The physicochemical compositions of Narince wines are summarized in Table 2. Glycerol and tartaric acid did not show significant differences among the wines made with autochthonous and commercial wine strains. Wines obtained by autochthonous strain have slightly higher ethanol strength compared to control. Acetic acid belongs to the group of volatile acids and is undesirable in wine. This acid is produced predominantly by oxidation of ethanol. However, it can also be imported into wine with grapes and small amounts of acetic acid may be produced by yeasts under anaerobic conditions [20]. Three autochthonous *S. cerevisiae* strains showed significant differences in volatile acid production. The 1088 strain produced the lowest amount of volatile acid. The concentration of residual sugar was lower than 4 g/L in all wines.

Table 2. General composition of Narince wines.

General Composition	Control	1044	1088	1281	F
Alcohol (% <i>v/v</i>)	11.40 ± 0.30 ^b	11.50 ± 0.50 ^{a,b}	11.65 ± 0.25 ^a	11.63 ± 0.18 ^a	*
Total acidity (g/L) **	5.95 ± 0.21 ^c	6.40 ± 0.14 ^a	6.04 ± 0.06 ^b	6.71 ± 0.10 ^a	*
pH	3.35 ± 0.22 ^b	3.35 ± 0.15 ^b	3.63 ± 0.04 ^a	3.37 ± 0.12 ^b	*
Volatile acidity (g/L) ***	0.56 ± 0.02 ^b	0.65 ± 0.06 ^a	0.41 ± 0.01 ^c	0.57 ± 0.02 ^b	*
Residual sugar (g/L)	3.10 ± 0.10 ^a	2.85 ± 0.16 ^b	2.65 ± 0.07 ^c	2.45 ± 0.02 ^d	*
Glycerol (g/L)	5.35 ± 0.34	5.40 ± 0.20	5.25 ± 0.26	5.35 ± 0.16	ns
Total SO ₂ (mg/L)	43.50 ± 0.60 ^b	39.06 ± 0.65 ^b	51.00 ± 2.10 ^a	32.66 ± 1.50 ^c	*
Sugars (g/L)					
Glucose	1.10 ± 0.10 ^b	1.5 ± 0.11 ^a	1.65 ± 0.02 ^a	1.6 ± 0.12 ^a	*
Fructose	2.00 ± 1.27 ^a	1.27 ± 0.11 ^b	0.95 ± 0.10 ^c	0.8 ± 0.02 ^c	*
Organic acids (g/L)					
Tartaric acid	3.15 ± 0.21	3.05 ± 0.04	3.18 ± 0.20	3.2 ± 0.40	ns
Malic acid	2.75 ± 0.11 ^a	2.45 ± 0.10 ^b	2.25 ± 0.04 ^c	2.45 ± 0.02 ^b	*

Note: ** = as tartaric acid; *** = as acetic acid. Data are means ± standard deviations. Data with different superscript letters (^{a,b,c}) within each line are significantly different (Duncan test; $p < 0.05$); ns = not significant; * = $p < 0.05$ level.

3.3. Volatile Compositions of Wines

GC/MS analysis of Narince wines produced with control strain, autochthonous 1044, 1088, and 1281 strains, which allowed the identification and quantification of 50 volatile compounds belonging to seven different groups, namely higher alcohols, esters, volatile acids, terpenes, lactones, volatile phenols, and carbonyl compounds (Table 3). Major volatile compounds of ethyl acetate and acetaldehyde concentrations were calculated by GC/FID. The volatile compounds detected in higher amounts in the present study were higher alcohols (isoamyl alcohol, 2-phenyl ethanol), esters (ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl-4-hydroxybutanoate), volatile acids (hexanoic acid, octanoic acid, and decanoic acid), as well as acetaldehyde.

Table 3. Aroma composition of Narince wines produced with different *S. cerevisiae* yeast strains.

Aroma Compounds (µg/L)								
	Higher alcohols	RI	ID	Control	1044	1088	1281	F
1	1-Propanol	1037	RI, MS, Std	682.44 ± 24 ^a	716.23 ± 7 ^a	294.86 ± 17 ^c	558.46 ± 44 ^b	*
2	Isobutyl alcohol	1085	RI, MS, Std	11,036.17 ± 157 ^a	11,628.40 ± 359 ^a	5849.93 ± 394 ^c	9567.90 ± 843 ^b	*
3	1-Butanol	1165	RI, MS, Std	339.02 ± 64 ^b	443.88 ± 32 ^a	28.73 ± 0 ^c	366.42 ± 25 ^b	*
4	Isoamyl alcohol	1210	RI, MS, Std	144,604.80 ± 497 ^b	165,956.55 ± 2805 ^a	111,408.47 ± 1245 ^c	144,826.57 ± 2537 ^b	*
5	2-Hexanol	1226	RI, MS, Std	259.13 ± 16 ^a	188.43 ± 91 ^{a,b}	24.66 ± 2 ^c	130.09 ± 1 ^b	*
6	4-Methyl-1-pentanol	1301	RI, MS, Std	318.98 ± 12 ^a	185.07 ± 11 ^c	153.23 ± 11 ^d	253.94 ± 20 ^b	*
7	1-Hexanol	1370	RI, MS, Std	1375.73 ± 135 ^b	1518.18 ± 155 ^a	1126.27 ± 73 ^b	1326.59 ± 70 ^{a,b}	*
8	(Z)-3-Hexen-1-ol	1401	RI, MS, Std	157.93 ± 12 ^a	113.92 ± 15 ^b	15.86 ± 1 ^c	32.65 ± 7 ^c	*
9	2,3-Butanediol	1495	RI, MS, Std	694.55 ± 96 ^b	942.93 ± 12 ^a	712.03 ± 82 ^b	811.68 ± 6 ^b	*
10	Methionol	1737	RI, MS, Std	44.92 ± 6 ^c	34.35 ± 2 ^c	298.61 ± 27 ^a	252.94 ± 22 ^b	*
11	Benzylalcohol	1804	RI, MS, Std	57.49 ± 8 ^a	47.31 ± 0 ^{a,b}	38.22 ± 5 ^b	55.83 ± 3 ^a	*
12	2-Phenyl ethanol	1916	RI, MS, Std	28,519.52 ± 674 ^b	28,202.12 ± 72 ^b	15,580.23 ± 468 ^c	33,597.08 ± 476 ^a	*
	Sum			188,090	209,977	135,526	191,780	
Esters								
13	Ethyl acetate **	895	RI, MS, Std	27,727.45 ± 753 ^a	26,303 ± 455 ^c	24,254.05 ± 200 ^d	27,127.40 ± 350 ^b	*
14	Ethyl-2-methyl propionate	960	RI, MS, Std	ND	ND	ND	287.14 ± 4	*
15	Ethyl butyrate	1037	RI, MS, Std	359.31 ± 79 ^c	682.37 ± 29 ^a	173.51 ± 10 ^c	513.92 ± 63 ^b	*
16	Isoamyl acetate	1119	RI, MS, Std	1635.03 ± 85 ^c	1815.57 ± 158 ^{a,b}	1951.53 ± 50 ^b	2249.17 ± 91 ^a	*
17	Ethyl hexanoate	1241	RI, MS, Std	1534.30 ± 137 ^b	1896.27 ± 47 ^a	441.37 ± 10 ^c	1680.05 ± 131 ^b	*
18	Hexyl acetate	1250	RI, MS, Std	280.00 ± 22 ^a	406.39 ± 31 ^a	40.36 ± 2 ^b	305.22 ± 3 ^a	*
19	Ethyl lactate	1353	RI, MS, Std	465.64 ± 15 ^a	604.12 ± 46 ^a	251.27 ± 21 ^b	465.78 ± 9 ^a	*
20	Ethyl octanoate	1430	RI, MS, Std	669.08 ± 44 ^b	739.52 ± 3 ^{a,b}	775.27 ± 56 ^a	792.90 ± 56 ^a	*
22	Ethyl decanoate	1635	RI, MS, Std	270.67 ± 20 ^a	276.17 ± 7 ^a	211.0 ± 53 ^b	301.94 ± 16 ^a	*
21	Diethyl succinate	1690	RI, MS, Std	71.99 ± 13 ^b	86.77 ± 4 ^a	31.29 ± 3 ^c	68.76 ± 2 ^b	*
23	Ethyl-9-decenoate	1709	RI, MS	106.17 ± 20 ^a	119.66 ± 3 ^a	42.83 ± 5 ^b	115.46 ± 14 ^a	*
24	2-Phenylethyl acetate	1785	RI, MS, Std	295.57 ± 20 ^b	315.03 ± 2 ^b	236.42 ± 18 ^c	429.71 ± 36 ^a	*
25	Ethyl-4-hydroxybutyrate	1819	RI, MS	3282.75 ± 145 ^c	5632.62 ± 98 ^b	2615.24 ± 234 ^b	6025.05 ± 107 ^a	*
26	Diethyl -DL-malate	2041	RI, MS, Std	124.31 ± 15 ^a	60.68 ± 5 ^b	12.35 ± 1 ^c	71.28 ± 5 ^b	*
27	Ethyl-2-hydroxy-3-phenyl propionate	2246	RI, MS	94.87 ± 10 ^a	62.531 ± 7 ^b	72.59 ± 6 ^b	69.57 ± 1 ^b	*
28	Ethyl hydrogen succinate	2331	RI, MS	845.07 ± 57 ^a	639.56 ± 42 ^b	460.80 ± 82 ^c	930.71 ± 82 ^a	*
	Sum			37,762	39,640	31,569	41,434	

Table 3. Cont.

Aroma Compounds (µg/L)								
	Volatile acids	RI	ID	Control	1044	1088	1281	F
29	Propanoic acid	1538	RI, MS, Std	70.94 ± 25 ^a	68.11 ± 6 ^a	32.96 ± 1 ^b	48.68 ± 1 ^{a,b}	*
30	Isobutyric acid	1584	RI, MS, Std	432.14 ± 26 ^c	634.92 ± 51 ^a	202.86 ± 18 ^d	470.32 ± 15 ^b	*
31	Butyric acid	1628	RI, MS, Std	260.79 ± 13 ^a	270.36 ± 11 ^a	149.68 ± 15 ^b	250.86 ± 19 ^a	*
32	Isovaleric acid	1608	RI, MS, Std	488.71 ± 17 ^c	832.67 ± 12 ^a	439.62 ± 38 ^d	668.45 ± 28 ^b	*
33	Hexanoic acid	1840	RI, MS, Std	1822.76 ± 14 ^c	2135.87 ± 22 ^b	857.19 ± 40 ^d	2356.65 ± 193 ^a	*
34	(E)-2-Hexanoic acid	1962	RI, MS	169.01 ± 6 ^b	138.97 ± 6 ^c	57.49 ± 4 ^d	195.55 ± 6 ^a	*
35	Octanoic acid	2060	RI, MS, Std	1638.37 ± 151 ^b	129.83 ± 4 ^c	4180.85 ± 75 ^a	3878.85 ± 282 ^a	*
36	Decanoic acid	2183	RI, MS, Std	993.61 ± 70 ^b	1086.43 ± 58 ^b	1525.73 ± 145 ^a	1104.96 ± 163 ^b	*
37	9-Decenoic acid	2237	RI, MS	253.46 ± 14 ^c	324.50 ± 38 ^b	452.11 ± 46 ^a	284.82 ± 27 ^{b,c}	*
38	Hexadecanoic acid	2910	RI, MS, Std	465.79 ± 32 ^a	94.52 ± 5 ^c	125.19 ± 16 ^c	181.54 ± 12 ^b	*
	Sum			6595	5716	8023	9440	
Terpenes								
39	Linalool	1551	RI, MS, Std	1.88 ± 0 ^b	ND	ND	37.60 ± 3 ^a	*
40	cis-Farnesol	1648	RI, MS, Std	208.13 ± 4 ^a	149.20 ± 10 ^b	26.12 ± 1 ^c	145.89 ± 2 ^b	*
	Sum			210	149	26	183	
Lactones								
41	γ-Butyrolactone	1635	RI, MS, Std	1172.93 ± 64 ^a	1244.98 ± 19 ^a	503.63 ± 55 ^c	1000.72 ± 15 ^b	*
42	γ-Caprolactone	1694	RI, MS, Std	87.19 ± 4 ^a	23.12 ± 2 ^c	32.85 ± 6 ^b	87.97 ± 2 ^a	*
43	Pantolactone	2414	RI, MS, Std	161.30 ± 6 ^a	79.92 ± 3 ^b	9.57 ± 1 ^c	80.63 ± 3 ^b	*
44	4-Ethoxycarbonyl-γ-butyrolactone	2673	RI, MS	202.80 ± 4 ^a	96.37 ± 2 ^b	37.65 ± 2 ^d	90.76 ± 1 ^c	*
	Sum			1624	1444	583	1260	
Volatile phenols								
45	4-Vinyguaiaicol	2091	RI, MS, Std	259.02 ± 11 ^a	138.65 ± 2 ^c	166.66 ± 10 ^b	142.61 ± 8 ^c	*
46	4-Vinylphenol	2415	RI, MS, Std	273.60 ± 9 ^a	156.90 ± 3 ^b	29.84 ± 4 ^d	45.38 ± 5 ^c	*
47	Propiovanillone	2693	RI, MS	91.18 ± 13 ^a	37.38 ± 3 ^b	29.31 ± 2 ^b	33.04 ± 13 ^b	*
48	Acetovanillone	2995	RI, MS, Std	78.52 ± 20 ^b	25.58 ± 2 ^d	57.36 ± 4 ^c	164.12 ± 11 ^a	*
	Sum			702	358	283	385	
Carbonyl compounds								
49	Acetoin	1291	RI, MS, Std	538.94 ± 17 ^c	825.60 ± 37 ^a	49.36 ± 13 ^d	592.73 ± 18 ^b	*
50	Acetaldehyde **	500	RI, MS, Std	4556.10 ± 150 ^d	22,071.3 ± 85 ^a	12,321.65 ± 200 ^b	10,203.35 ± 130 ^c	*
	Sum			5095	22,896	12,371	10,796	
	TOTAL SUM			240,078	280,180	188,381	255,278	

Note: Control = wine fermented with control strain; 1044 = wine fermented with autochthonous 1044 yeast; 1088 = wine fermented with autochthonous 1088 yeast; 1281 = wine fermented with autochthonous 1281 yeast; RI = retention index calculated on DB-Wax capillary column; ID = identification; MS = mass spectrometry; Std = chemical standard; ± = standard deviation of triplicate analysis of three wines for each strain; ND = not detected; F = significance at which means differ as shown using analysis of variance; * = $p < 0.05$ level. Data with different superscript letters (^{a,b,c,d}) within each line are significantly different ($p < 0.05$ level); ** = determined by direct injection to GC.

Higher alcohols were found in the quantitatively largest group of volatile compounds in Narince wines. Major higher alcohols, isobutyl alcohol, 1-butanol, isoamyl alcohol, benzyl alcohol, and methionol can be distinguished by their strong and pungent odor and taste. During alcoholic fermentation, the use of different yeast strains significantly contributes to the concentrations and variations of higher alcohol profiles [21]. In this study, the total amount of higher alcohols showed differences between autochthonous and commercial strains used and their concentrations ranged from 135.5 mg/L to 209.9 mg/L in Narince wines. 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 [1]. The wines produced during this study show the optimal values of these compounds. Isoamyl alcohol (3-methyl-1-butanol) was the most abundant compound in all of the wines. Strain 1044 produced the highest amount of total higher alcohols and isoamyl alcohol (165.9 mg/L) compared to other autochthonous and control strains. In contrast, strain 1088 produced the lowest amount of total higher alcohols and isoamyl alcohol (111.4 mg/L). Among the alcohols identified, 2-phenyl ethanol, contributing to wine aroma with sweet and flowery notes, was the second most abundant alcohol. While the strain 1281 (33.5 mg/L) produced the highest amount of 2-phenyl ethanol, strain 1088 (15.5 mg/L) produced the lowest amount. However, all three autochthonous strains and commercial strains produced 2-phenyl ethanol, higher than its threshold value of 10 mg/L [1]. Isobutyl alcohol and 1-propanol were also produced by all yeasts. The higher alcohols with six carbon atoms, which provide “vegetal” and “herbaceous” notes to wine, usually have a negative effect on wine quality when their concentration is above their odor threshold values [22,23]. However, these compounds (1-hexanol, Z-3-hexen-1-ol) produced concentrations lower than their threshold value by autochthonous and commercial strains. Methionol is generally described as an off-flavor with cauliflower or baked cabbage odor [24]. In Narince wines, strain 1088 produced the highest amount of methionol, followed by strain 1281. However, it did not exceed its threshold value of 1 mg/L [25] in all Narince wines. Torrens et al. [24] reported that the amount of methionol in Cava sparkling wines was influenced by the yeast strain used.

The majority of esters are produced by yeast during alcoholic fermentation and they have an important effect on the fruity characteristics of the wine. The important contribution of ethyl esters of fatty acids and acetates of higher alcohols to the sensory composition of young wine has been known for some time [1,21,26]. In terms of the number of components quantified, esters and acetates represent the largest group (16 individual compounds) of volatiles in Narince wines. Ethyl acetate was the main ester produced by autochthonous and commercial strains in the production of Narince wines. The highest amount of ethyl acetate was produced by control strain (27.7 mg/L), while the lowest was produced by 1088 (24.2 mg/L). The odor threshold value of ethyl acetate is 7.5 mg/L [1] and all strains used in this study produced this compound in concentrations higher than its odor threshold value. This compound may contribute a pleasant, fruity fragrance to the general wine aroma at concentrations lower than 150 mg/L. Contrary to this, when its concentration is greater than 150–200 mg/L, it may spoil the character of the wine [1]. Autochthonous strains and commercial strain used during this study produced ethyl acetate at optimal values. Other important acetate esters are isoamyl acetate and 2-phenylethyl acetate, which give wine banana and flowery rose aromas, respectively. They were produced by all yeasts, but strain 1281 produced a higher amount of isoamyl acetate (2.2 mg/L) and 2-phenylethyl acetate (0.42 mg/L) than the others. All strains used in this study produced a higher amount of isoamyl acetate than the threshold value of 0.03 mg/L. The 2-Phenyl acetate produced in concentrations higher than its threshold value of 0.25 mg/L by all strains used (except by the strain 1088). Strain 1044 produced the highest amount of ethyl hexanoate and ethyl octanoate. These compounds are ethyl esters of C₆ and C₈ fatty acids and they are responsible for fruity, floral, wine-like aroma [1]. Ethyl hexanoate and ethyl octanoate produced in concentrations higher than their threshold values of 0.05 mg/L and 0.02 mg/L [1] by all yeasts.

Volatile fatty acids are related to negative properties, such as rancid, fatty, cheesy notes, but also they are important for the aromatic equilibrium and complexity of wine [4]. As seen in Table 3,

the production of volatile fatty acids in the wine analyzed in the present study was dependant on the yeast strains inoculated. While autochthonous strain 1044 produced the highest concentration of isobutyric, butyric, and isovaleric acids, control strain produced the highest concentration of propionic acid. The strains 1044 and 1281 stand out for their levels of hexanoic acid, while the highest concentrations of octanoic and decanoic acids were produced by the strain 1088. Hexanoic acid was produced in concentrations higher than its threshold value of 420 µg/L by all yeast strains used in this study. All strains (except 1044) produced octanoic acid in concentrations higher than its threshold value of 500 µg/L. In addition, decanoic acid was produced by all strains but it was only produced in concentrations higher than its threshold value (1000 µg/L) by autochthonous strains.

Terpenes are responsible for some of the most characteristic and important aromas in grapes and wines. It has been reported that besides grapes, yeasts are also capable of producing terpenes [24,27]. Two terpene compounds, linalool and cis-farnesol, were produced. Between two terpene compounds, linalool was produced only by control strain and autochthonous 1281. Strain 1281 produced (37.60 µg/L) linalool at a higher concentration than its threshold value of 25 µg/L. Linalool has a rose-like floral aroma and contributes positively to wine aroma. It is generally accepted that linalool, the most powerful odorant in monoterpene compounds, is an important component in the aroma of many white wines [26]. Cis-farnesol was produced by all strains, ranging from 26 µg/L to 208 µg/L

Four lactones were identified in Narince wines. The most abundant lactone was γ -butyrolactone. This compound is associated with fruity, buttery, and rubbery descriptors [28]. However, the concentration of γ -butyrolactone was found to be lower than its threshold value of 35 mg/L [29] in all Narince wines.

Among the volatile phenols in white wines, vinyl phenols play the most important role [30]. In Narince wines, five volatile phenols were identified. Among them, 4-vinylguaicol and 4-vinylphenol were produced in highest concentrations by control strain, and 4-vinylphenol exceeded its threshold value of 180 µg/L in control wine; 4-Vinylguaiacol and 4-vinylphenol produce a pharmaceutical odor, particularly in white wines [1].

Two carbonyl compounds were detected in Narince wines and acetaldehyde was found to be the most abundant carbonyl compound in this study. Yeast strains show differences in their ability to produce acetaldehyde depending on the activity of the enzyme (alcoholic dehydrogenase) involved in the synthesis [24,31]. In the present study, acetaldehyde showed significant differences related to yeast. While strain 1044 produced a higher concentration (22 mg/L) of acetaldehyde, control strain produced a lower amount (4 mg/L). The aroma threshold value of acetaldehyde is 100 mg/L and at low levels, acetaldehyde contributes fruity flavors, while high levels (200 mg/L) cause flatness in wines [1,11]. In Narince wines it did not exceed its threshold value.

The principal component analysis was carried out to separate wines fermented with different yeasts. The first two components, PC1 and PC2, explained 81.79% of the variance (Figure 3). The distribution of samples in the PC1 and PC2 components displayed a clear separation among wines from different yeast strains. Autochthonous 1088 was characterized by the presence of octanoic acid (V35), decanoic acid (V36), 9-decenoic acid (V37), and methionol (V10) (Table 3), and plotted on the negative side of PC1. Wines obtained with autochthonous 1044 and 1281 grouped together in the positive portion of PC1 and negative portion of PC2. Those yeasts characterized by the highest amount of some important volatiles (e.g., isoamyl alcohol (V4), 2-phenylethanol (V12), ethyl hexanoate (V17), 2-phenylethyl acetate (V24) and isovaleric acid (V32), and 2,3-butanediol (V9)). Control strain was plotted on the positive side of both PC1 and PC2 and was characterized by the highest amounts of 2-hexanol (V5) and (Z)-3-hexen-1-ol (V8), which provide “vegetal” and “herbaceous” notes to wine, volatile phenols (V45, V46, V47) which are usually considered as off-flavors, and some volatile acids (such as propionic acid (V29), hexadecanoic acid (V38)), lactones (pantolactone (V43), 4-ethoxycarbonyl- γ -butyrolactone (V44)), and cis-farnesol (V40). Isoamyl acetate (V16) and ethyl octanoate (V20) were negatively correlated with hexadecanoic acid, 4-vinylguaiacol, propiovanillone, and also control strain. The wine fermented with control strain presented lower contents of isoamyl acetate and ethyl octanoate.

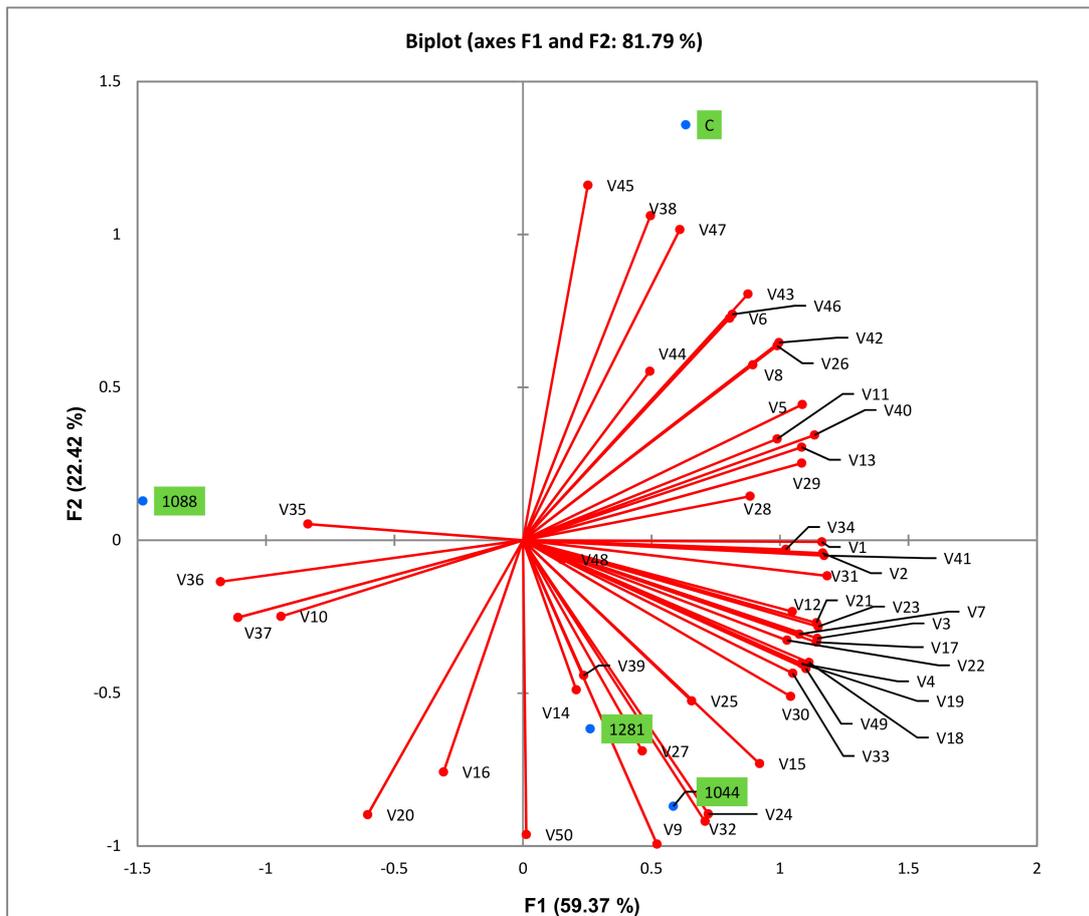


Figure 3. Principle component analysis according to yeast strain, based on volatile compositions of wines. Note: C = wine fermented with control strain; 1044 = wine fermented with autochthonous 1044 yeast; 1088 = wine fermented with autochthonous 1088 yeast; 1281 = wine fermented with autochthonous 1281 yeast; V = variable—the numbers that correspond to each compound are shown in Table 3.

The differentiation of volatile profiles of wines according to yeast strain has been widely reported [4,21,24,32]. The results of this study for Narince wines confirm those findings. Furthermore, autochthonous yeasts 1044 and 1281 produced the highest levels of important sensory volatile compounds, such as 2-phenylethanol, ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, ethyl hexanoate, and ethyl octanoate, compared to control strain. Also, the wines obtained with these yeasts were clearly separated by PCA. In addition, PC1 allowed us to distinguish between wines made with autochthonous strains.

3.4. Sensory Evaluations of Wines

The sensory properties of the four experimental wines considered in this study were performed by a sensory panel using eight attributes: floral, fruity, honey, herbaceous, acidity, persistence, balance, and global impression (Figure 4). Regarding fruity and floral properties, wine 1281 achieved the best score for both attributes, followed by 1044 and control, respectively. The wine fermented with autochthonous 1088 strain achieved the lowest score for those attributes. This result was in agreement with the aroma composition of this wine, because the wine fermented with 1088 contains the lowest amount of acetate and ethyl esters (except ethyl octanoate and isoamyl acetate), which contribute fruity and floral characteristics. Further, 1044 strain achieved the best score for honey attribute, while 1088 achieved the best for the acidity attribute. Wine produced with 1281 also achieved the best score for

both balance and global impression. These results were in agreement with the differences found at the chemical level. Wine fermented with autochthonous 1281 and 1044 contain higher amounts of acetates (isoamyl acetate (fruity), hexyl acetate (sweet, perfume), 2-phenylethyl acetate (floral)) and most of the ethyl esters (ethyl butyrate (fruity), ethyl hexanoate (green apple), ethyl lactate (lactic, fruity), ethyl decanoate (floral, soapy)), (Table 3), followed by control strain. However, the chemical composition of the wine and the interaction between the compounds and their effects on the sensory properties are still very complex and not well known [4].

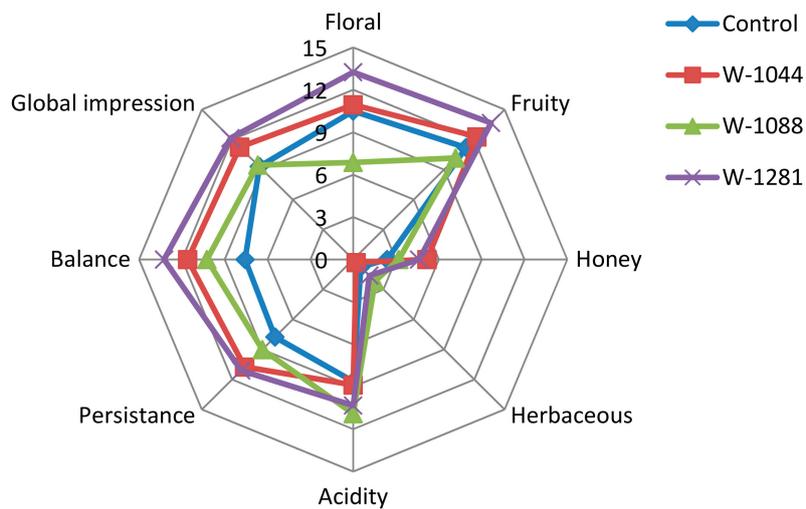


Figure 4. Sensory profile of Narince wines made with autochthonous and commercial *S. cerevisiae* yeasts. Note: C = wine fermented with control strain; 1044 = wine fermented with autochthonous 1044 yeast; 1088 = wine fermented with autochthonous 1088 yeast; 1281 = wine fermented with autochthonous 1281 yeast.

4. Conclusions

Winemaking is a highly industrialized process and different *S. cerevisiae* starter cultures are commercially available for its control. However, several investigations have underlined that using autochthonous yeasts during fermentation is able to give unique organoleptic properties to the produced wines. The present study investigated the effects of three different autochthonous yeast strains on the physicochemical and sensory properties of Narince wines. The autochthonous *S. cerevisiae* strains used in this study presented good fermentative ability. From a chemical point of view, certain trends were detected among strains used, as follows. Aroma compound analyses showed that autochthonous yeast strains 1044 and 1281 were able to produce a higher concentration of ethyl esters and acetates, which are responsible for fresh/fruit attributes. Sensory data were in agreement with chemical compositions. The discrimination analysis allowed the autochthonous strains 1281 and 1044 to be clearly distinguished by their volatile composition. To our knowledge, this is the first study on the effects of autochthonous *S. cerevisiae* yeast strains on the volatile and sensory properties of Narince wines. However, it would be best to confirm these results with industrial large-scale fermentation.

Author Contributions: All authors participated in the design and discussion of the research. Z.D.Ç. carried out the experimental part of work and analysis of the data. Z.D.Ç. wrote the original draft. The work was supervised by T.C. All the authors have read and approved the final manuscript.

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