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Effect of Indigenous *S. cerevisiae* Strains on Higher Alcohols, Volatile Acids, and Esters in Wine

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Abstract

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Higher alcohols, volatile fatty acids, and esters are the most important volatiles and their formation is closely related to yeast strains employed during fermentation. In the present work, the effect of indigenous yeast strains on selected wine volatiles was examined using a highly sophisticated analytical method – comprehensive two-dimensional gas chromatography. Results of the statistical analysis revealed that each strain could be characterised and differentiated according to its volatile composition: strain Y2 was characterised by 2-phenylethanol and 1-hexanol, strain Y1 was in close relationship with high amounts of 4-methyl-1-pentanol, iso-amyl alcohol, ethyl 3-hydroxypentanoate and 3-methylpentanoic acid and strain Y3 was associated with 1-heptanol, cis-3-hexen-1-ol, β-phenylethyl butyrate, octanoic, and decanoic acids. The selection of an appropriate yeast strain thus represents a critical variable affecting the analysed volatile compounds (wine flavour) not only in a qualitative but also in a quantitative way.

Keywords: volatile organic compounds; secondary aroma of wine; yeast; comprehensive gas chromatography

One of the most common procedures in a winemaking process is controlled fermentation in order to provide desired qualitative parameters (mainly flavour) of wine. This is ensured by the employment of commercially available *Saccharomyces cerevisiae* yeast strains. Nevertheless, the competitive character of the wine market leads to the tendency of wine producers to look for innovations in fermentation technologies (Fleet 2008). In recent years, the study of indigenous (autochthonous) yeast strains has seemed to be in the scope of scientists. Wines obtained using such microorganisms are closely related to a particular geographical region, known as 'terroir', and thus are characterised by uniqueness and originality (Cadot *et al.* 2012).

Wine flavour (including its odour and taste) is very complex and consists of a high number of different volatile organic compounds (VOCs) of various chemical and physical properties and concentrations. Among all of them, volatiles synthesised during fermentation (so called 'fermentative' flavour) are meant to be the most numerous group (Styger *et al.* 2011) and are mainly represented by esters, higher (fusel) alcohols, and volatile organic acids. Their formation is directly linked to a particular yeast strain (i.e. its metabolism) (Lambrechts & Pretorius 2000) and

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therefore, each strain produces a different profile of VOCs even when fermenting the same grape must (Molina *et al*. 2009).

According to Stribny *et al*. (2015), higher alcohols and esters are significant aroma contributors in alcoholic beverages. In the vast majority, conventional gas chromatography (1D-GC), and in the last years mainly comprehensive two-dimensional gas chromatography (GC×GC) has been applied to examine the composition of wine aroma (i.e. the profile of volatile organic compounds). The latter method is preferred because it disposes of several advantages: (I) enhanced peak capacity, (II) higher power signal/noise ratio, and thus resolution, and (III) obtaining of 'structured' chromatograms (Murray 2012). GCxGC was successfully employed for studying VOCs in different wines (Weldegergis *et al*. 2011a).

To the best knowledge of the Authors, there are no published works about the effect of indigenous yeast strains on the volatile profile of wines analysed by LLE-GCxGC-TOF-MS. The aim of the present study was to obtain a detailed profile of higher alcohols, volatile acids, and esters in wines to describe the effect of three indigenous *Saccharomyces cerevisiae* strains.

MATERIAL AND METHODS

Origin of grapes. In the present work, grapes of the wine variety Gewurztraminer (clone N20 with Kober 5BB rootstock) of 2011 vintage were used. Grapes originated from the production of the Slovak company from the Middle Slovak vineyard region, Modrý Kameň locality. The natural starting concentration of reducing sugars in pressed grape juice was 250 g/l, and no additional sugar was added. The pH value of original grape juice was 3.5.

Yeast strains. For the fermentation of Gewurztraminer juice, three indigenous yeast strains of *Saccharomyces cerevisiae* var. *cerevisiae* signed as Y1, Y2, Y3 were used. All strains were isolated from grapes in 2010 and originated from the same locality as the grape used in the experiment. Yeast strains were previously identified using diagnostic keys (Kurtzman & Fell 2000) and taxonomical classification was verified by PCR. Before the experiment, axenic *S. cerevisiae* cultures were characterised also in terms of their oenological properties and became part of the collection of microorganisms of the Faculty of Chemical and Food Technology (Slovak University of Technology, Slovakia). Axenic cultures of *S. cerevisiae var. cerevisiae* Y1, Y2, Y3 used in this experiment are characterised by low production of volatile acids, acetaldehyde, succinic acid, and foam; their osmotolerance is above 45% glucose in the medium and ethanol tolerance 12.5% (v/v) of ethanol, they have good sedimentation characteristics and are able to produce wines with residual sugars.

Yeast starters for grape juice inoculation were prepared from a yeast strain culture grown aerobically for 24 h in a 100 ml of liquid medium (20 g/l glucose, 10 g/l yeast extract; pH 6.5) in a 500 ml culture flask on an orbital shaker (2 Hz) at 28°C. After cultivation, the concentration of yeast biomass was determined by counting in a Bürker chamber. The calculated volume of biomass was withdrawn and centrifuged (10 min, 1370 *g*). Separated biomass was washed with distilled water, centrifuged again, and finally added to grape juice.

Winemaking procedure. Destemmed and crushed Gewurztraminer grapes were macerated for 4 hours and subsequently pressed. Clarification of pressed grape juice was performed statically using bentonite and must gelatine (the product Mostgelatine, Erbslöh, Germany) (dose 100 g/l). After 12 h, the clarified juice was treated with gaseous SO_2 (10 mg/l), filled into 50 l glass flasks and inoculated by an axenic yeast strain in the form of liquid yeast starter; the starting concentration of biomass in grape juice was 10^6 cells/ml. The main alcoholic fermentation had proceeded at the temperature of 17°C for 2 weeks. Then, the young wine was separated from rough yeast sediments, treated with SO_2 (80 mg/l), and left for another month. After the month, wines were racked again and underwent chemical and sensory analysis.

Basic analysis. Basic oenological parameters as well as detailed volatile profile of all wines were determined. Concentrations of reducing sugars were analysed by the Schoorl method (Schoorl & Regenbogen 1917), concentration of alcohol and extract pycnometrically (OIV-MA-AS312-01A, OIV-MA-AS2-03B). Total volatile acids (expressed as acetic acid) were separated from the sample by steam distillation and then total acids (expressed as tartaric acid) were determined by acid-base titration with 0.1 mol/l KOH (OIV-MA-AS313-01, OIV-MA-AS313-02).

Extraction and concentration of wine volatiles. Extraction of volatile compounds from grape juice and wine samples was performed using liquid-liquid extraction (LLE) into hexane. 100 ml of wine sample with the addition of 20 µl of ethanol solution of benzo-

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phenone (internal standard, 1.66×10^{-2} g/l) and 2.5 g of NaCl (p.a., dried at 250°C before use) was placed in a glass separatory funnel with stopcock. The mixture was extracted four times with 25 ml aliquots of hexane (overall 100 ml of hexane) at laboratory temperature. Collected hexane fractions were mixed, dried over anhydrous sodium sulphate, and concentrated using the Kuderna-Danish evaporator. Obtained extracts were placed in 2 ml volumetric flasks and filled with hexane to an exact volume of 2 ml.

Gas chromatography. For GC analysis, a Pegasus IV system consisting of an Agilent 6890N gas chromatograph (Agilent Technologies, USA), an MPS II multipurpose sampler (Gerstel GmbH, Germany), a TOF-MS detector (LECO, USA), and a four-jet cryogenic modulator was used. For GC×GC analysis, the following column set was used: a polar 30 m × 0.25 mm i.d. × 0.25 μm d_f DB-FFAP primary column coupled to a medium-polar 1.39 m × 0.10 mm i.d. × 0.10 μm d_f BPX-50 secondary column. Temperature programs used for separation were: (I) 1st column: initial temperature 40°C kept for 10 min, ramped at 2°C/min to 220°C kept for 5 min, (II) 2nd column: initial temperature 50°C kept for 10 min, ramped at 2°C/min to 230°C, and held for 5 minutes. Helium (99.996% purity, Merck) was used as a carrier gas at a constant flow of 1 ml/minute. Ions in the mass range of m/z 29–400 were acquired at a rate of 100 spectra/second. 1 μl of the concentrated hexane extract of each wine sample was injected into the GC system.

Data processing. Acquisition control and data processing were performed automatically using the LECO ChromaTOF™ software (Version 4.21). Automated peak finding and spectra deconvolution with a baseline offset of 0.8 and signal-to-noise ratio set to the value 50 were used for data treatment. Individual peaks were identified by a comparison of retention times and mass spectra with standards (when available), and data found in the library of the National Institute of Standards and Technology (NIST11 Mass Spectra library). Tentative identification of volatile organic compounds (VOCs) was performed only based on the mass spectra comparison with NIST11 library (with a minimal match factor 850). For determination of the experimental 1st dimension linear temperature programmed retention index (LTPRI) of each compound, the series of n-alkanes was analysed under the same conditions. Experimental LTPRIs ($\text{LTPRI}_{\text{exp}}$) were compared with LTPRIs of standards (LTPRI_{st}) and/or with reference LTPRI values ($\text{LTPRI}_{\text{litr}}$) obtained from the NIST WebBook Chemistry database. A compound was considered as identified if the difference between $\text{LTPRI}_{\text{exp}}$ and LTPRI_{st} was less than 20 units. In case of missing standards, compounds were considered only tentatively identified based on a comparison of $\text{LTPRI}_{\text{exp}}$ with $\text{LTPRI}_{\text{litr}}$. Relative peak areas of VOCs identified in samples (A_{rel}) were calculated based on Equation $A_{\text{rel}} = A_x/A_{\text{IS}}$, where: A_x – peak area of identified volatile organic compound; A_{IS} – peak area of internal standard (benzophenone).

Standards. Standard compounds and n-alkanes used in the experiment were purchased from Sigma-Aldrich/Fluka (Germany) in purity of ≥ 95%. Stock solutions of each standard compound were prepared by dilution in a solution of methanol (12%, v/v) and tartaric acid (6 g/l) in MilliQ deionised water and extracted, concentrated, and analysed in the same way as wine samples.

Statistical analysis. Each analysis was performed in triplicate. The experimental values of A_{rel} of all Gewurztraminer wines fermented with 3 autochthonous strains of *S. cerevisiae* were evaluated by the statistical dispersion method Analysis of Variance between Groups – ANOVA (STATGRAPHICS plus for Windows 3.0) and by Principal Component Analysis – PCA (Statistica® software, StatSoft, USA).

Sensory evaluation of wines. Quality of the aroma of Gewurztraminer wines fermented with 3 different strains of *S. cerevisiae* was evaluated by eight certified wine tasters in blind sensory evaluation (50 ml wine poured into ISO XL5 wine taster glasses). Tasters evaluated positive fruity and flower-like smells as well as green tones, and possible off-flavours by a ten-point scale test (zero representing non-existent and ten representing the extreme value of smell perception). Intensities of the following attributes were evaluated: rose, honey, lychee, white flowers, citruses, yellow fruits, elder flowers, mushrooms, herbal, and green tones. Aromagrams of individual wines were constructed based on average values of means obtained by the evaluation.

RESULTS AND DISCUSSION

Gewurztraminer juice and wines fermented with three different strains of *S. cerevisiae* var. *cerevisiae* were analysed by methods of basic chemical analysis, gas chromatography (LLE-GC×GC-TOF/MS), and sensory analysis to evaluate the influence of the yeast strain on the profile of higher alcohols, volatile acids, and esters in wine.

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Table 1. Basic oenological characteristics of Gewürztraminer wines fermented with autochthonous strains of *S. cerevisiae*

Strain *S. cerevisiae*	Y1	Y2	Y3
Reducing sugars (g/l)	14.1 ± 0.10	14.7 ± 0.06	31.1 ± 0.12
Ethanol (% v/v)	14.2 ± 0.10	13.9 ± 0.10	11.9 ± 0.06
Total extract (g/l)	33.6 ± 0.20	35.4 ± 0.21	56.4 ± 0.23
Volatile acids (g/l)	0.31 ± 0.01*	0.31 ± 0.01*	0.29 ± 0.01*
Total acids (g/l)	4.9 ± 0.06	5.5 ± 0.06	5.5 ± 0.12
SO_2 free (mg/l)	34	34	34
SO_2 total (mg/l)	152	157	153

Data were mean values of triplicate samples (maximum SD ± 5%); $P < 0.05$; *the same characters in the same row correspond to a not statistically significant difference ($P > 0.05$)

The results of the basic analysis of wines are shown in Table 1. The statistical analysis ANOVA performed for the basic analysis of Gewurztraminer wine samples showed significant differences ($P < 0.05$) in all descriptors except the means of volatile acid concentrations which were evaluated as not significantly different ($P > 0.05$). All wines contained a higher concentration of residual sugars and a lower concentration of total acids, which is typical of wines of the Gewurztraminer variety.

Using the above-mentioned gas chromatographic method, more than 900 VOCs were detected in analysed Gewurztraminer samples. In wines, 175 VOCs in total were identified (tentatively or based on comparisons with standards). Out of this number, 41 volatiles belonged to esters, 34 to higher alcohols, and 8 to volatile acids. On the contrary, in grape juice only 56 VOCs in total were identified and out of them only 17 higher alcohols, 6 esters, and 3 volatile acids. The remaining VOCs (30 in grape juice and 92 in wines) belonged to groups of terpenoids, furans, pyrans, carbonyls, volatile phenols, and sulphur compounds. For the illustration of the wine VOC profile complexity, higher alcohols, esters, and volatile acid profiles in wines and juice before fermentation are presented in Table 2. A comparison of A_{rel} of compounds identified in juice and in wines showed which VOCs are products of yeast metabolism and which originate naturally from the grape juice (and are produced by the vine plant).

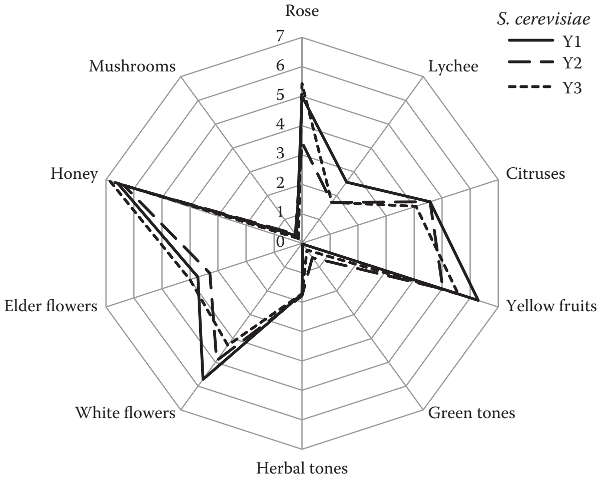

Figure 1. Aromagrams of Gewurztraminer wines fermented with 3 autochthonous strains of *S. cerevisiae*

Data were calculated as the arithmetic average of evaluations done by eight wine tasters; maximum SD ± 15%; $P < 0.05$

Higher alcohols. Among all identified higher alcohols, C6 and C8 alcohols were the most abundant in grape juice; the highest A_{rel} in juice was shown by 2-hexen-1-ol and 1-hexanol. Alcohols with minor abundance: 1-heptanol, 1-octanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, 1-octen-3-ol, 2-octanol, 3-octanol, 2-heptanol, 2-nonanol, and 2-undecanol bring predominantly green aroma tones, while higher saturated alcohols like 1-nonanol, 1-decanol, 1-undecanol, and 2-ethylhexanol are responsible for citrus-like smells. During fermentation, concentrations of all these fusel alcohols decreased and the most significant decrease was observed in 2-hexenol. The highest variance, according to the yeast strain used for fermentation, was observed in 1-hexanol. The relative peak area of this alcohol (responsible for ethereal and fruity aroma) decreased using *S. cerevisiae* Y2 from 21.40 to 18.18 compared with 0.40 (Y1) and 1.08 (Y3).

The presence of most fusel alcohols in wine is bound to the metabolism of yeasts participating in alcoholic fermentation. Their occurrence and concentration depend on the varietal amino acid profile of grapes, metabolic activity of fermenting microflora, and technological conditions (Hernandez-Orte

Table 2. Esters, higher alcohols, and volatile acids identified in Gewurztraminer wines fermented with three autochthonous strains of *S. cerevisiae*

$\text{LTPRI}_{\text{exp}}$	$\text{LTPRI}_{\text{lit}}$	ID	VOC identified	A_{rel}				Odour descriptor
				grape juice	Y1	Y2	Y3	
Higher alcohols								
1065	1064	ST	1-propanol	nd	10.93 ± 0.13	9.26 ± 0.44	12.86 ± 0.32	musty, yeasty, sweet
1100	1100	ST	iso-butanol (2-methyl-1-propanol)	0.91 ± 0.10	29.72 ± 1.05	13.20 ± 0.47	6.68 ± 0.24	ethereal, winey
1114	1100	ST	3-pentanol	nd	1.31 ± 0.05	0.92 ± 0.03	1.12 ± 0.04	sweet, herbal, oily, nutty
1125	1113	ST	2-pentanol	nd	4.47 ± 0.16*	3.23 ± 0.11	4.31 ± 0.15*	mild, green, oily, fermented
1144	1151	ST	1-butanol	nd	0.36 ± 0.01	0.31 ± 0.01	0.16 ± 0.01	fusel, oil, sweet, balsam, whiskey
1183	1201	ST	active amyl alcohol (2-methyl-1-butanol)	nd	0.02 ± 0.00	0.40 ± 0.01	0.66 ± 0.02	fermented, yeasty
1205	1213	ST	iso-amyl alcohol (3-methyl-1-butanol)	nd	42.42 ± 1.50	15.79 ± 0.56*	14.21 ± 0.50*	pungent, fruity, banana
1230	1248	ST	2-methyl-1-penten-3-ol	nd	0.04 ± 0.00*	0.04 ± 0.00*	0.01 ± 0.00	nf
1231	1248	ST	3-methyl-3-buten-1-ol	nd	nd	0.10 ± 0.00*	0.10 ± 0.00*	sweet, fruity
1233	1251	ST	1-pentanol	0.49 ± 0.11	nd	0.94 ± 0.03	0.88 ± 0.03	fermented, yeasty, winey
1312	1317	ST	2-heptanol	0.42 ± 0.08	nd	0.36 ± 0.01	0.28 ± 0.01	lemon grass, sweet, green
1317	1341	ST	3-methyl-1-pentanol	nd	0.34 ± 0.01	2.17 ± 0.08	1.41 ± 0.05	fusel, cognac, wine, fruity
1317	1324	ST	iso-hexanol (4-methyl-1-pentanol)	nd	6.17 ± 0.22	0.07 ± 0.00	nd	nutty
1336	1351	ST	1-hexanol	21.40 ± 0.25	0.40 ± 0.01*	18.18 ± 0.64	1.08 ± 0.04*	ethereal, fruity, alcoholic
1344	1334	ST	*trans*-3-hexen-1-ol	0.60 ± 0.04	2.00 ± 0.07	nd	nd	green, leafy
1358	1355	ST	*cis*-3-hexen-1-ol	0.31 ± 0.03	nd	nd	0.05 ± 0.00	green, grassy, melon rind
1365	1390	ST	3-octanol	0.62 ± 0.10	nd	0.02 ± 0.00*	0.02 ± 0.00*	earthy, mushroom, herbal, spicy
1410	1412	TI	2-hexen-1-ol, isomer	24.51 ± 0.91	0.03 ± 0.00	0.15 ± 0.01	0.11 ± 0.00	fresh, vegetative, slightly fatty
1413	1416	ST	2-octanol	0.10 ± 0.00	nd	0.08 ± 0.00	0.06 ± 0.00	fresh, spicy, green, woody
1438	1451	ST	1-octen-3-ol	3.50 ± 0.12	0.33 ± 0.01	0.26 ± 0.01	0.24 ± 0.01	earthy, green, oily, fungal
1442	1453	ST	1-heptanol	2.04 ± 0.09	0.28 ± 0.01	0.24 ± 0.01	0.65 ± 0.02	musty, leafy, vegetative
1448	1451	ST	sulcatol	nd	0.24 ± 0.01	0.15 ± 0.01	0.13 ± 0.00	sweet, oily, green, coriander
1467	1485	ST	2-ethyl-1-hexanol	1.10 ± 0.07	0.09 ± 0.00	0.08 ± 0.00	0.05 ± 0.00	citrus, fresh, floral, oily, sweet
1480	1509	TI	3-ethyl-4-methylpentanol, (*s*)-	nd	0.03 ± 0.00*	0.03 ± 0.001	0.03 ± 0.00*	nf
1512	1515	ST	2-nonanol	0.30 ± 0.01	0.02 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	waxy, green, creamy, orange
1531	1538	ST	2,3-butanediol	nd	2.52 ± 0.09*	0.17 ± 0.01	2.42 ± 0.09*	fruity, creamy, buttery
1544	1536	ST	1-octanol	3.72 ± 0.04	0.27 ± 0.01	0.41 ± 0.01	0.23 ± 0.01	waxy, green, citrus, floral
1645	1654	ST	1-nonanol	3.30 ± 0.05	0.06 ± 0.00*	0.06 ± 0.00*	0.18 ± 0.01	fresh, fatty, floral, rose, orange
1750	1759	ST	1-decanol	nd	nd	0.01 ± 0.00*	0.01 ± 0.00*	fatty, sweet, floral, orange

Table 2 to be continued

1865	1865	ST	benzyl alcohol	0.10 ± 0.00	0.58 ± 0.02	0.49 ± 0.02	0.16 ± 0.01	jasmine, hyacinth
1894	1903	ST	2-phenylethanol	3.02 ± 0.08	46.00 ± 0.21	13.30 ± 0.47	28.2 ± 0.45	sweet, floral, rose, honey, lilac
1956	1920	TI	*n*-dodecan-1-ol	nd	nd	nd	0.01 ± 0.00	soapy, waxy, aldehydic, fatty
2038	2047	TI	hydrocinnamic alcohol	nd	nd	0.04 ± 0.00	0.02 ± 0.00	spicy, cinnamon, fruity, floral, honey
2365	2366	TI	1-hexadecanol	nd	0.03 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	waxy floral
Esters								
904	896	ST	ethyl acetate	nd	15.99 ± 0.21	23.24 ± 0.71	33.46 ± 0.56	fruity, nail polish
1026	1000	TI	iso-butyl acetate (acetic acid, 2-methylpropyl ester)	nd	10.58 ± 0.30	6.08 ± 0.17	7.74 ± 0.22	sweet, ethereal, apple, banana
1050	1028	ST	ethyl butanoate	nd	13.19 ± 0.38	3.73 ± 0.11	7.35 ± 0.21	sweet, fruity, tutti-frutti
1081	1060	ST	ethyl iso-valerate (3-methylbutanoic acid, ethyl ester)	nd	0.09 ± 0.00	nd	nd	sweet, pineapple, apple, orange
1128	1117	ST	iso-amyl acetate (acetic acid,3-methylbutyl ester)	nd	8.24 ± 0.23	5.54 ± 0.16	27.84 ± 0.79	pear, banana (pear drops)
1158	1161	TI	ethyl 2-butenoate	nd	nd	0.11 ± 0.00	0.28 ± 0.01	pungent, rum- and cognac-like
1225	1226	ST	ethyl hexanoate	0.29 ± 0.09	20.01 ± 0.57	15.26 ± 0.43	4.37 ± 0.12	sweet, fatty, pineapple, banana
1247	1270	ST	hexyl acetate	19.38 ± 0.51	nd	0.16 ± 0.00	2.66 ± 0.08	green, fruity, sweet, fatty, fresh
1249	1259	TI	ethyl pyruvate (2-oxopropanoic acid, ethyl ester)	nd	0.07 ± 0.00	1.08 ± 0.03	0.30 ± 0.01	etherial, fruity, sweet, rum-like
1329	1358	ST	ethyl lactate (2-hydroxypropanoic acid, ethyl ester)	nd	0.07 ± 0.00	0.58 ± 0.02	0.43 ± 0.01	butter, cream
1330	1335	TI	ethyl 2-hexenoate	0.09 ± 0.00	nd	0.04 ± 0.00	0.03 ± 0.00	rum, fruity, green, sweet, juicy
1362	1388	ST	methyl octanoate	nd	0.07 ± 0.00	0.09 ± 0.00	0.27 ± 0.01	waxy, green, sweet, orange
1416	1405	TI	ethyl 2-hydroxy-iso-valerate (butanoic acid, 2-hydroxy-3-methyl-, ethyl ester)	nd	0.20 ± 0.01	0.12 ± 0.00	0.09 ± 0.00	pineapple, strawberry, tea, honey
1423	1427	ST	ethyl octanoate	0.19 ± 0.02	15.65 ± 0.45	12.96 ± 0.37	24.59 ± 0.70	tropical fruit, pineapple, apple
1443	1450	TI	iso-amyl hexanoate (hexanoic acid, 3-methylbutyl ester)	nd	nd	nd	0.04 ± 0.00	fruity, banana, apple, pineapple
1510	1490	TI	ethyl 3-hydroxybutyrate	nd	0.70 ± 0.02	0.34 ± 0.01	0.43 ± 0.01	fruity, green, grape, apple skin
1533	1538	TI	ethyl 2-hydroxy-4-methylvalerate	nd	0.29 ± 0.01*	0.28 ± 0.01*	0.28 ± 0.01*	fresh blackberry
1552	1552	TI	ethyl 3-hydroxypentanoate	nd	4.65 ± 0.13	2.55 ± 0.07	1.13 ± 0.03	nf
1565	1576	TI	diethyl malonate (propanedioic acid, diethyl ester)	nd	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	sweet, green, apple, fruity
1572	1581	ST	methyl decanoate	0.20 ± 0.04	nd	0.02 ± 0.00	0.06 ± 0.00	oily, wine, fruity, floral
1617	1599	TI	ethyl 2-furoate	nd	0.18 ± 0.01	0.10 ± 0.00	0.11 ± 0.00	ethyl, benzoate, fruity, floral
1625	1615	ST	ethyl decanoate	nd	3.87 ± 0.11*	3.59 ± 0.10*	6.96 ± 0.20	sweet, waxy, fruity, apple
1630	1631	TI	ethyl-, methyl succinate	nd	0.13 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	nf
1645	1648	TI	isoamyl octanoate (octanoic acid, 3-methylbutyl ester)	nd	0.04 ± 0.00	nd	nd	sweet, fruity, waxy, green, pineapple
1651	1650	ST	ethyl benzoate	nd	nd	nd	0.02 ± 0.00	sweet, cherry, grape

Table 2 to be continued

1661	1672	ST	ethyl 3-hydroxyhexanoate	nd	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	fruity, grape, green, sweet, hay like
1663	1670	ST	diethyl succinate (butanedioic acid, diethyl ester)	nd	0.59 ± 0.02	6.01 ± 0.17	2.82 ± 0.08	mild, fruity, cooked apple
1665	1671	ST	*n*-decyl acetate	0.22 ± 0.05	4.70 ± 0.13	nd	nd	waxy, clean, fresh, citrus, soapy
1671	1675	TI	ethyl 9-decenoate	nd	0.09 ± 0.00	0.07 ± 0.00	0.45 ± 0.01	fruity, fatty
1769	1773	TI	ethyl phenylacetate	nd	0.41 ± 0.01	0.39 ± 0.01	0.19 ± 0.01	sweet, floral, honey, rose, cocoa
1810	1810	TI	2-phenylethyl acetate	nd	3.63 ± 0.10	4.94 ± 0.14	4.42 ± 0.13	sweet, honey, floral, rosy
1833	1820	ST	ethyl dodecanoate	nd	0.15 ± 0.00	0.19 ± 0.01	0.61 ± 0.02	sweet, waxy, soapy, rummy
1869	1846	TI	ethyl hydrocinnamate (benzenepropanoic acid, ethyl ester)	nd	nd	0.01 ± 0.00	0.01 ± 0.00	hyacinth, rose, honey, fruity, rummy
1942	1938	TI	(*z*)-3-hexenyl nonanoate	nd	0.05 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	
1952	1958	TI	2-phenylethyl butanoate	nd	nd	0.09 ± 0.00	0.01 ± 0.00	musty, sweet, floral, yeasty, nuance
2038	2053	TI	diethyl *dl*-malate (butanedioic acid, hydroxy-, diethyl ester)	nd	0.02 ± 0.00*	0.05 ± 0.00	0.02 ± 0.00*	wine, fruity, apple skin
2040	2042	TI	ethyl myristate (tetradecanoic acid, ethyl ester)	nd	nd	0.02 ± 0.00	nd	sweet, waxy
2248	2250	TI	ethyl palmitate (hexadecanoic acid, ethyl ester)	nd	0.17 ± 0.00*	0.16 ± 0.00*	0.24 ± 0.01	mild, waxy, fruity, creamy, milky
2450	2467	TI	ethyl stearate (octadecanoic acid, ethyl ester)	nd	nd	0.03 ± 0.00	nd	mild, waxy
2599	2598	TI	methyl vanillate (benzoic acid, 4-hydroxy-3-methoxy-, methyl ester)	nd	0.14 ± 0.00*	0.15 ± 0.00*	0.14 ± 0.00*	spicy, vanilla, phenolic
2626	2612	TI	ethyl vanillate (benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester)	nd	0.15 ± 0.00	0.17 ± 0.00	0.18 ± 0.01	phenolic, burnt, smoky, powdery
Volatile acids								
1432	1434	TI	4-methyl-2-oxovaleric acid	nd	0.08 ± 0.00*	0.07 ± 0.00*	0.02 ± 0.00	fruity
1558	1563	TI	iso-butanoic acid (2-methylpropanoic acid)	nd	nd	0.04 ± 0.00	0.07 ± 0.00	acidic, cheese, dairy, buttery
1627	1619	ST	butanoic acid	nd	nd	nd	0.08 ± 0.00	sharp, cheesy, buttery
1659	1684	TI	3-methylvaleric acid (3-methylpentanoic acid)	nd	0.50 ± 0.03*	0.37 ± 0.02	0.48 ± 0.03*	animalic, sharp, acidic, green
1840	1837	ST	hexanoic acid	nd	0.74 ± 0.04	2.41 ± 0.14*	2.21 ± 0.13*	sour, fatty, sweat, cheese
1944	1969	ST	2-ethyl-hexanoic acid	0.17 ± 0.00	0.22 ± 0.01	0.17 ± 0.01	0.26 ± 0.01	herbaceous, earthy
2051	2059	ST	octanoic acid	0.59 ± 0.03	1.57 ± 0.09*	2.18 ± 0.13*	9.39 ± 0.54	fatty, waxy, vegetable, cheesy
2268	2274	ST	*n*-decanoic acid	0.17 ± 0.01	5.74 ± 0.33	2.08 ± 0.00	1.11 ± 0.06	unpleasant, sour, fatty, citrus

The data were mean values of triplicate samples ($P < 0.05$); LTPRI$_{exp}$ – experimental value of linear temperature programmed retention index (LTPRI); LTPRI$_{lib}$ – reference LTPRI value obtained from the NIST WebBook Chemistry database; ID – identification; VOC – volatile organic compound; ST – VOC confirmed by authentic standard; TI – tentatively identified VOC; A_{rel} – relative peak area of VOC; nf – not found; *values in the same row correspond to a not statistically significant difference ($P > 0.05$)

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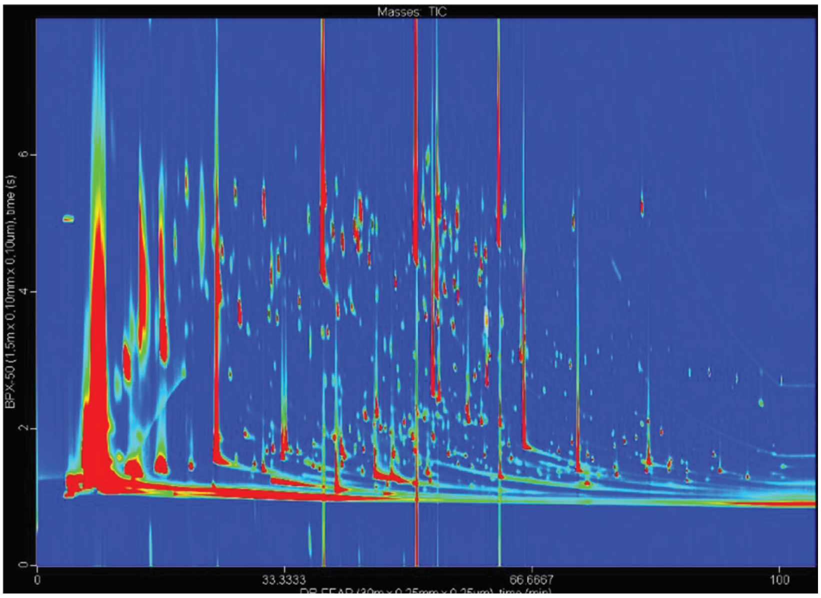

Figure 2. Colour plot of Gewurztraminer wine fermented with *S. cerevisiae* Y2 obtained by GC×GC-TOF-MS with the employment of DB-FFAP (polar) × BPX-50 (mid-polar) column configuration

The x-axis represents 1st dimension retention time expressed in min; the y-axis represents 2nd dimension retention time given in second

et al. 2002). The most abundant higher alcohols in the studied Gewurztraminer wines were 2-phenylethanol, 3-methylbutanol, 2-methyl-1-propanol, 1-propanol, and 2,3-butandiol. 2-Phenylethanol was identified in all samples at significantly higher A_{rel} than the other identified VOCs. It can be considered as the major yeast produced higher alcohol in tested wines in spite of the fact that the grape juice also contained it before fermentation (3.02). *S. cerevisiae* produces this rose-scent alcohol by bioconversion of L-phenylalanine via the Ehrlich pathway and its production strictly depends on the yeast strain (Stark *et al.* 2003). The highest A_{rel} of 2-phenylethanol was confirmed in wine fermented with the autochthonous strain of *S. cerevisiae* Y1 (46.0). 2-Phenylethanol is important since it can support the typical rose-like aroma of Gewurztraminers.

The second abundant alcohol identified in wine samples was 3-methylbutanol (iso-amyl alcohol). At an optimal concentration it brings the typical banana and pear-like aroma (Swiegers *et al.* 2005; Samappito & Butkhup 2010), excessive concentrations cause the nail polish odour of wines. Precursors of 3-methylbutanol are the amino acids leucine and valine, but it can be formed also by the metabolism of pyruvate (Swiegers *et al.* 2005). The highest A_{rel} of 3-methylbutanol reported wine fermented with *S. cerevisiae* Y1 (42.4), the lowest with *S. cerevisiae* Y3 (14.2). From a sensory point of view, grassy and green aromas are very important for the final flavour of each wine. Except of primary aroma alcohols (discussed previously) this type of smell is represented by higher alcohols produced by the yeast and detected in wine samples: 2-pentanol, 3-pentanol, and DL-6-methyl-5-hepten-2-ol (sulcatol).

Higher alcohols identified in wines fermented with three different yeast strains underwent statistical treatment using PCA (Figure 3). The correlation matrix was calculated in order to discriminate the variables. Finally, 20 higher alcohols were considered as products of the metabolism of tested yeast strains (the values of A_{rel} after fermentation were higher than before fermentation). The PC analysis explained 98.9% of total variance (60.9% for the PC1 and 38% for PC2).

The score plot of PCA displays three clearly differentiated groups occupying different quadrants of the plot. The profile of higher alcohols related to the strain Y1 was separated from strains Y2 and Y3 by a positive correlation with PC1. Y1 was characterised by the production of 4-methyl-1-pentanol (nutty aroma), 3-methylbutanol (banana descriptor), 6-methyl-5-hepten-2-ol (spiciness), 2-methylpropan-1-ol (winey), and 2-phenyl ethanol (roses). The strains Y2 and Y3 differ from Y1 by higher production of 3-methyl-3-buten-1-ol and 1-decanol (both sweet fruity smell). The PC2 allowed the differentiation of strains Y2 and Y3. 1-Propanol, n-dodecanol, and 2-methyl-1-butanol (active amyl alcohol) were positively correlated with *S. cerevisiae* Y3 and negatively with Y2. *S. cerevisiae* Y2 was characterised by higher production of 3-methyl-1-pentanol.

Esters. Most esters in wine are formed during fermentation enzymatically by the metabolism of yeasts or during wine maturation as a consequence of the slow non-enzymatic esterification of different

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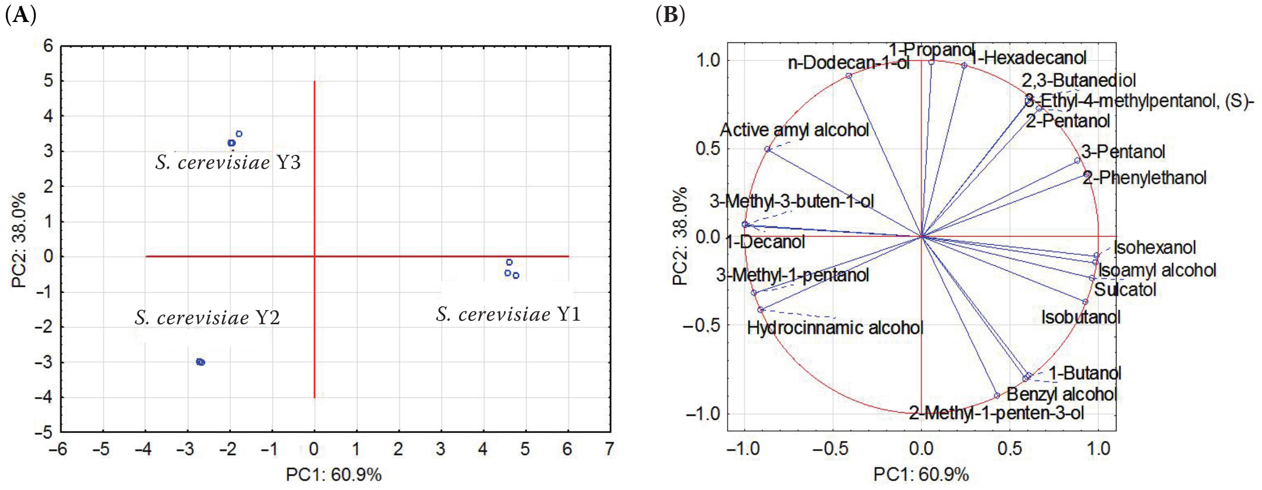

Figure 3. Score plot (**A**) and loading plot (**B**) of the first and second principal components after the PC analysis of higher alcohol profiles of Gewurztraminer wines fermented with three autochthonous strains of *S. cerevisiae* (Y1, Y2, and Y3)

organic acids with higher alcohols present in wine (Campo *et al.* 2007; Weldegergis *et al.* 2011b). Branched-chain aliphatic esters are the result of the enzymatic esterification of acids with alcohols produced by yeasts from the corresponding amino acids (Ehrlich pathway) or amino acid derivatives, such as α-ketoacids (Antalick *et al.* 2010). The production of esters depends on many factors including aeration, concentration of fatty acids, higher alcohols, and their precursors. The species and strain of yeasts plays an important role in the production of esters (Plata *et al.* 2003). In Gewurztraminer wines fermented with three autochthonous *S. cerevisiae* strains 41 esters were identified by the LLE-GC×GC-TOF/MS technique (Table 1). According to literature (Jackson 2008) the most frequent were ethyl esters (28), methyl (5), and isoamyl esters (3) and esters derived from acetic (7), hexanoic (3), octanoic (3), and decanoic acid (3). Based on A_{rel} of identified VOCs the most abundant were ethyl hexanoate, ethyl acetate, ethyl octanoate, ethyl butanoate, 2-methylpropyl acetate, and 3-methylbutyl acetate.

Esters have been studied very well in terms of the yeast and bacteria metabolism as well as winemaking technology. However, the profile of naturally occurring esters in grapes or grape juice has not been published so frequently. Jackson (2008) showed that the major esters in grapes are phenolic esters, methyl antranilate, and partially isoamyl acetate in Pinotage; ethyl 2-methylpropanoate, ethyl 2-butenoate, 3-methylbutyl butanoate, ethyl hexanoate, ethyl octanoate, hexyl acetate, 2-metylbutyl acetate were identified

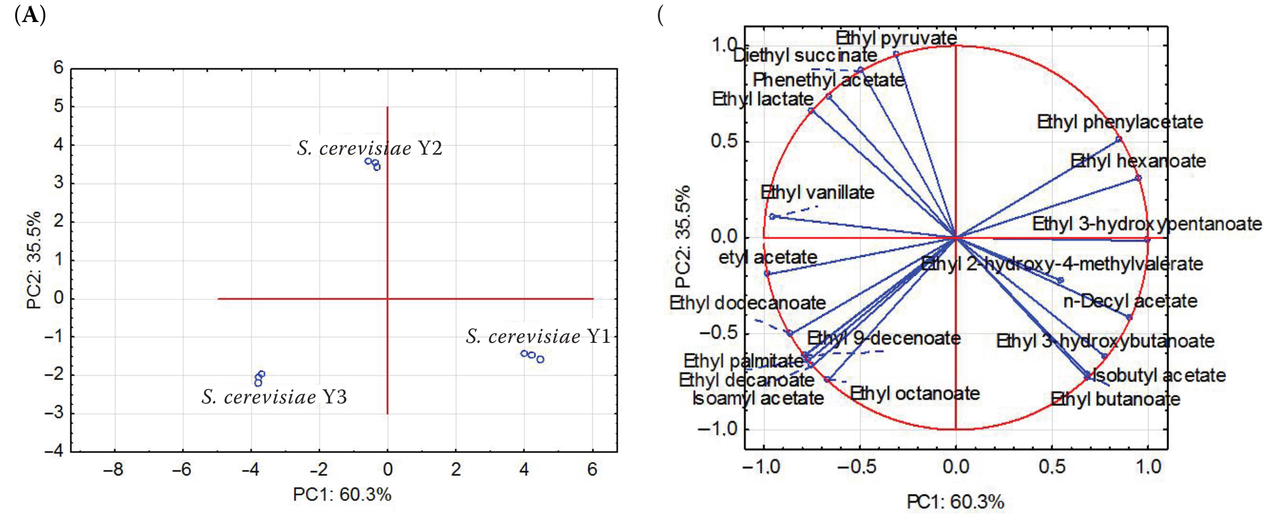

Figure 4. Score plot (**A**) and loading plot (**B**) of the first and second principal components after the PC analysis of ester profiles of Gewurztraminer wines fermented with three autochthonous strains of *S. cerevisiae* (Y1, Y2, and Y3)

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in Chardonnay juice and a significant concentration of isoamyl butanoate and ethyl hexanoate in Riesling (Swiegers *et al.* 2005). In the analysed Gewurztraminer grape juice 6 esters were identified: hexyl acetate, ethyl hexanoate, *n*-decyl acetate, methyl decanoate, ethyl octanoate, and ethyl 2-hexenoate. In terms of A_{rel} ethyl hexanoate (19.38) was the major ester. The relative peak area of other esters in juice was significantly lower (0.09–0.29). During fermentation, the concentration of most juice esters rapidly decreased; only ethyl octanoate, ethyl hexanoate, and *n*-decyl acetate (in case of *S. cerevisiae* Y1) increased.

The influence of the yeast strain on the profile of esters in wine was analysed using PCA (Figure 4) and the correlation matrix was calculated based on A_{rel} values of 20 esters with the highest average increase of the value in wine compared with grape juice. PCA explained 95.8% of total variance (60.3% for the PC1 and 35.8% for PC2). Similarly to higher alcohols, each yeast strain was located in an individual quadrant of the score plot. The ester profile related to *S. cerevisiae* Y1 was separated from the other two strains by a positive correlation with PC1 and *S. cerevisiae* Y2 and Y3 were separated by PC2. Strain Y1 was characterised by higher production of ethyl butanoate, 2-methylpropyl acetate, and ethyl hexanoate (typical fruity scent esters) and low production of ethyl acetate. Therefore, this strain can be useful in the production of wines characterised by sweet and fruity (apple, banana, pineapple) smell descriptors. This finding correlates also with the sensory evaluation of tested wine (Figure 1). Strains Y2 and Y3 could be distinguished from each other according to PC2. *S. cerevisiae* Y2 was characterised by higher production of ethyl pyruvate (sweet, rum-like), diethyl succinate (cooked apple), 2-phenylethyl acetate (honey, rose-like), and ethyl lactate (cream-like) and by the lowest production of fruity esters typical of the strain Y1. *S. cerevisiae* Y3 produced the highest concentration of isoamyl acetate (pear/nail polish) and of long-chain fatty acid esters (fatty and waxy aroma). Based on these facts, *S. cerevisiae* Y3 was considered too risky and not suitable for practical winemaking.

Volatile acids. The most abundant volatile acids in wines include short-chain acids (acetic, propanoic, and butanoic) and medium-chain saturated acids (hexanoic, octanoic, decanoic, and dodecanoic). Short-chain acids are formed as metabolic by-products of alcoholic fermentation, while medium-chain acids are considered as intermediates of the long-chain fatty acid biosynthesis (Lambrechts & Pretorius 2000). Beside acetic acid (Table 1), 8 volatile acids were identified in the analysed wine samples (Table 2), while 3 of them were also present in grape juice (octanoic acid, decanoic acid, and 2-ethylhexanoic acid). Generally, A_{rel} of all identified volatile acids increased during fermentation depending on the applied yeast strain. According to literature (Jascon 2008) the largest increase was recorded in octanoic and decanoic acid (both responsible for fatty and waxy odours).

To judge the influence of particular yeast strains on the profile of volatile organic acids in wine, the PC

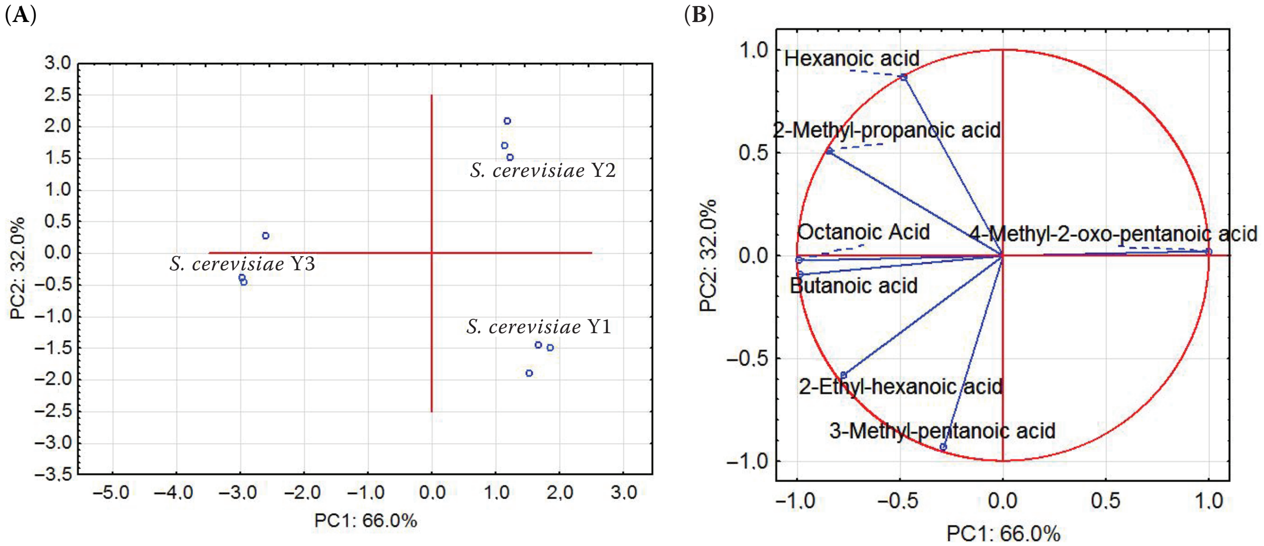

Figure 5. Score plot (**A**) and loading plot (**B**) of the first and second principal components after the PC analysis of volatile acid profiles of Gewurztraminer wines fermented with three autochthonous strains of *S. cerevisiae* (Y1, Y2, and Y3)

analysis was performed; the correlation matrix was calculated based on A_{rel} values of all 8 volatile acids identified in wine samples. PCA explained 98% of total variance (66% for PC1 and 32% for PC2). The profile of volatile acids related to *S. cerevisiae* Y3 was separated from the other two strains by a negative correlation with PC1; strains Y1 and Y2 were more similar and separated by PC2. Strain Y1 (compared with the other strains) was characterised by the lowest production of octanoic acid and by the highest production of decanoic acid; yeast strain Y2 was characterised by higher production of hexanoic acid. *S. cerevisiae* Y3 produced all 8 volatile acids. Compared to the other strains, the strain Y3 was characterised by higher production of octanoic, butanoic, 2-methylpropanoic, and 2-ethylhexanoic acid. Among all tested yeast strains *S. cerevisiae* Y3 produced the lowest concentration of *n*-decanoic acid but a high amount of decanoates. Because of the significantly different odour threshold, the production of volatile acids does not affect the aroma of wine as considerably as the production of their corresponding esters (Jiang & Zhang 2010): odour threshold of *n*-decanoic acid 15 mg/l versus ethyl decanoate 0.2 mg/l; odour threshold of octanoic acid 0.5 mg/l versus ethyl octanoate 2 μg/l. Nevertheless, the evaluation of the aroma profile by sensory analysis did not confirm any waxy, fatty, or soapy aroma in tested wines.

CONCLUSION

Gewurztraminer wines fermented with three different strains of *S. cerevisiae* var. *cerevisiae* as well as grape juice before fermentation were analysed by methods of basic chemical analysis, gas chromatography (LLE-GCxGC-TOF/MS), and sensory analysis to evaluate the influence of the yeast strain on the profile of higher alcohols, esters, and volatile acids in wine.

In the analysed wine samples, 175 volatile organic compounds (VOCs) were identified while in the initial grape juice only 56 VOCs belonging to higher alcohols (17), esters (6), and volatile acids (3) were present. From those VOCs found in wines; 41 belong to esters, 34 are higher alcohols, and 8 belong to volatile acids. A comparison of A_{rel} values of VOCs identified in juice with those in wines provided information on VOCs originating naturally from grapes and those produced by the yeast metabolism.

C6 and C8 alcohols identified in juice decreased during fermentation, probably as a result of stripping with CO_2 or esterification. The influence of the yeast strain was mostly obvious in case of 1-hexanol. The profile of higher alcohols associated with yeast metabolism was strictly yeast strain dependent and the most abundant alcohols were 2-phenylethanol, 3-methylbutanol, 2-methylpropanol, 1-propanol, and 2,3-butandiol. Based on A_{rel} the most abundant esters in analysed wines were ethyl hexanoate, ethyl acetate, ethyl octanoate, ethyl butanoate, 2-methylpropyl acetate, and 3-methylbutyl acetate; 6 esters were identified already in grape juice (hexyl acetate, ethyl hexanoate, *n*-decyl acetate, methyl decanoate, ethyl octanoate, and ethyl 2-hexenoate). During fermentation the A_{rel} of most 'juice esters' rapidly decreased; only ethyl octanoate and ethyl hexanoate increased. Eight volatile acids were identified in the analysed wine samples, while 3 of them were already present in grape juice (octanoic acid, decanoic acid, and 2-ethylhexanoic acid). The concentration of all identified volatile acids increased during fermentation and the increase depended on the yeast strain used for fermentation.

To consider the influence of particular yeast strains on the profile of higher alcohols, esters, and volatile acids in wine, the means of A_{rel} acquired by GC analysis were used as input values for the principal component analysis. Using PCA it was found that *S. cerevisiae* Y1 was characterised by higher production of 4-methyl-1-pentanol, 3-methylbutanol, 6-methyl-5-hepten-2-ol, 2-methylpropanol, and 2-phenyl ethanol. Among the esters, higher production of ethyl butanoate, 2-methylpropyl acetate, and ethyl hexanoate and low production of ethyl acetate was observed. This strain also produced the lowest concentration of octanoic acid and the highest concentration of decanoic acid. The profile of wine fermented with *S. cerevisiae* Y2 was characterised by higher concentration of 3-methyl-3-buten-1-ol and 1-decanol, 3-methyl-1-pentanol, ethyl pyruvate, diethyl succinate, 2-phenyletyl acetate, and ethyl lactate and by the lowest concentration of fruity esters typical of the strain Y1. Also, Y2 was characterised by higher production of hexanoic acid. *S. cerevisiae* Y3 was characterised by high production of 3-methyl-3-buten-1-ol, 1-decanol, 1-propanol, *n*-dodecanol, and 2-methylbutanol. It also differentiated by the highest production of ethyl acetate, 3-methylbutyl acetate, and of long-chain fatty acid esters.

The obtained GCxGC-TOF-MS, statistical data treatment as well as sensory evaluation of wines confirmed that *S. cerevisiae* Y1 is the most suitable yeast for the production of wines characterised by

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clean aroma and sweet, fruity (apple, banana, pineapple) smell descriptors. *S. cerevisiae* Y2 provided rather a neutral aroma profile with intense green tones and Y3 (in spite of the most intense rose-like aroma) was not considered suitable for practical winemaking because of the excessive production of ethyl and isoamyl acetate and waxy scents.

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