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ORIGINAL ARTICLE

Monitoring volatile compounds production throughout fermentation by *Saccharomyces* and non-*Saccharomyces* strains using headspace sorptive extraction

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Abstract Currently, there is a growing interest in the use of non-*Saccharomyces* yeast to enhance the aromatic quality of wine, with pure or mixed cultures, as well as sequential inoculation. Volatile components of wines were closely related to their sensory quality. Hence, to study the evolution of volatile compounds during fermentation was of great interest. For this, sampling methods that did not alter the volume of fermentation media were the most suitable. This work reports the usefulness of headspace sorptive extraction as non-invasive method to monitor the changes in volatile compounds during fermentation. This method allowed monitoring of 141 compounds throughout the process of fermentation by *Saccharomyces cerevisiae* and *Lachancea thermotolerans* strains. Both strains showed a similar ability to ferment a must with high sugar content. The *S. cerevisiae* strain produced higher amount of volatile compounds especially esters that constitutes fruity aroma than *L. thermotorelans*.

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Introduction

Wine is a complex solution containing abundant volatile compounds which contribute to wine aroma (Boss et al. 2015). These aromatic components of wine are closely related to its sensory quality, which is determined by the consumer's acceptability (Vilanova 2006). Compounds that constitute the volatile profile of wine have different origins. Primary aromas are grape-derived volatiles that pass through fermentation often unchanged, and are largely responsible for "varietal" aromas. Secondary aromas, which are by far the greatest pool of volatile molecules, are produced through the winemaking process, the great majority produced by yeast as metabolism by-products (Robinson et al. 2014). Tertiary aromas develop in finished wine through storage and maturation, and result from intermolecular chemical interactions and equilibrium effects as the wine matrix changes (Boss et al. 2015). Therefore, volatile profile of wine depends on primary the quality and variety of grape employed, fermentation process (yeast, temperature…) and maturation (in bottle or wood barrel), if it takes place.

One of the most important factors in the alcoholic fermentation process is the yeast strain involved. The choice of yeast strain is also a determinant of the final concentration of these volatile compounds (Callejón et al. 2010). For this reason, one of the new yeast selection criteria that have emerged is the appropriate enhancement of aroma via the production of volatile compounds such as esters and higher alcohols, along with the scant production of off-flavours (Suárez-Lepe and Morata 2012).

Some authors call "yeast bouquet" to the set of volatile compounds produced by yeast as secondary metabolites. Among them there are ethyl esters, acetate esters, fusel alcohols, carbonyls, and volatile fatty acids synthesized by a wide range of microbial species (Cordente et al. 2012).

It is well known that in the fermentation of grape must there is a sequential development of *Saccharomyces* and non-*Saccharomyces* species (Renault et al. 2015). The conditions of alcoholic fermentation favour the development of *Saccharomyces cerevisiae*, being these yeasts predominant during the latter stages of fermentation. Moreover, because non-*Sacharomyces* has been related to negative aromatic notes and off-flavour in wines (Benito et al. 2015), to ensure proper development of the alcoholic fermentation, winemakers commonly inoculate the grape must with *Saccharomyces* commercial strains.

Currently, conversely, different research have revealed that certain non-*Saccharomyces* yeasts can enhance the aroma and improve the wine quality (Benito et al. 2015; Gobbi et al. 2013; Jolly et al. 2014; Renault et al. 2015). This has led to a new perspective on the use of non-*Saccharomyces* strains in winemaking.

To perform an exhaustive study of volatile compounds, these have to be analysed by gas chromatography–mass spectrometry which requires a previous sample extraction process. Presently, the most extensively used extraction technique for volatile compounds in wines is the solid phase microextraction (SPME) (Boss et al. 2015; Renault et al. 2015). The other extraction technique that has showed successful results in volatile profile analysis of wine is stir bar sorptive extraction (SBSE). Although, it has been used in lesser extent, it has major extraction capacity (Lancas et al. 2009). In wine analysis, the device with the polymeric extraction phase has been used in immersion, SBSE (Martinez-Gil et al. 2013), as well as in headspace, named headspace sorptive extraction (HSSE) (Callejón et al. 2010) with satisfactory results.

The study of the changes of volatile compounds produced in alcoholic fermentation are performed primarily in two ways, analysing samples at the end of process (Romano et al. 2015; Synos et al. 2015) or sampling at different stages of fermentation (Concejero et al. 2016). The former is the most widely used. However, and to our understanding, the possibility of studying the evolution of volatile compounds during fermentation, using sampling methods that not alter the volume of fermentation media, is of great interest. In spite of this, non-invasive methods to monitoring the evolution of volatile profile during must fermentation or wine maturation have been seldom used. Among them, we can mention the recent monitoring study of fermentative aromas produced by evolved *Saccharomyces cerevisiae* strain (pilot scale) using an on-line gas chromatography (GC) special device in the headspace (HS) (Mouret et al. 2015). Callejón et al. (2012) monitored the effects of skin contact time on the partitioning, release, and formation of volatile compounds during fermentation of Cabernet Sauvignon grapes (laboratory scale), using a polydimethylsiloxane (PDMS) SPME fiber in HS. Silva Ferreira et al. (2014) carried out a study of the changes of volatile profile at microscale fermentation (5 mL) in fermentation media with different sources of assimilable nitrogen using again HS-SPME.

This work had two aims; one of them was to test the use of HSSE as non-invasive method to monitor online the volatile compounds changes during fermentation. The other one was to study the influence of two types of yeast, *Saccharomyces cerevisiae* and *Lachancea thermotolerans*, on volatile profile throughout fermentation of a must with high sugar content.

Materials and methods

Yeast strains and media

Two different autochthonous yeast strains belonging to the collection housed in the *Area de Edafología y Química Agricola* (Univesity of Seville) were used for the fermentation assays. One corresponding to a *Saccharomyces cerevisiae* strain (coded as G263), and the other one to a *Lachancea thermotolerans* strain (coded as G234). Both of them were isolated from previous laboratory-scale fermentations with sun-dried Pedro Ximénez grape must and were identified at species level by PCR–RFLP of the 5.8S ribosomal region as described by Guillamón et al. (1998). Identification was corroborated by sequencing the D1/D2 variable domains of 26S rRNA gene according to Clavijo et al. (2011). In addition, isolates of *S. cerevisiae* were characterized at strain level by mitochondrial DNA restriction analysis following Querol et al. (1992). Yeast strains G234 and G263 were selected in order to their ability to ferment high sugar content grape must which was previously tested in laboratory assays.

Grape must for fermentation assays was kindly provided by local winery (Montalbán, Córdoba, Spain). It was obtained from sun-dried grapes of the "Pedro Ximénez" variety during 2014 vintage. Physical and chemical must parameters were the following: pH 4.51 ± 0.02, total acidity (g/L tartaric acid) 4.3 ± 0.1, and reducing sugar content 487 ± 20 g/L.

Fermentation assays

Duplicate fermentations were carried out under static conditions at 22 °C in 500 mL Erlenmeyer flasks containing 350 mL of sun-dried Pedro Ximénez must, previously pasteurized by 20 min heating at 100 °C. Erlenmeyer flasks were inoculated at a density of approximately 5.5×10^6 cell/mL from 48 h pure yeast cultures that were grown in the same grape must. Fermentation progress was monitored through measuring of turbidity at optical density of 660 nm (OD_{660}), using a spectrophotometer Beckman DU 640, and sugar consumption control. Data of cell per millilitre was determined using polynomial function previously calculated, which relates OD_{660} values to cell/mL. Residual fermentable sugars were determined according to Rebelein procedure involving reaction of reducing sugars with copper(II) in alkaline solution (MAPA 1993). For this purpose, aliquot samples were taken from each flask, after extraction of volatile compounds, throughout the fermentation process. End of the fermentation was established when no sugar consumption was detected.

Online extraction of volatile compounds

The online sampling procedure was performed in headspace by PDMS Twisters (HSSE). A special device made of stainless wire was designed to maintain the Twister in the headspace, in the centre of the Erlenmeyer flask at 2.5 cm above the liquid surface.

Twister was exposed to headspace of must during 2 h at 22 °C of temperature (fermentation temperature). The extraction time was established in previous assays. After extraction, the stir bar was removed with tweezers and introduced in a 2 mL vial to be transported to the analysis laboratory where they were thermally desorbed in a gas chromatograph/mass spectrometer (GC/MS). The stainless wire devices, the tweezers and the vials to transport Twister were autoclaved to avoid contamination of flasks. Moreover, the insertion of Twisters into the flasks and their removal were performed in a laminal flow chamber.

A total of six extractions were accomplished for each replicate of the fermentation assay as follows: before inoculation (MT0), every 24 h after inoculation (T24, T48 and T72) and at 144 and 192 h after inoculation (T144 and T192, respectively).

Thermal desorption and GC conditions

Gas chromatography analysis was carried out using a 6890 Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5975 inert and was equipped with a thermo desorption system (TDS2) and a cryo-focusing CIS-4 PTV injector (Gerstel). The thermal desorption was performed in splitless mode with a flow rate of 70 mL/min. The desorption temperature program was the following: the temperature was held at 35 °C for 0.1 min, was ramped at 60 °C/min to 210 °C and held for 5 min. The temperature of the CIS-4 PTV injector, with a Tenax TA inlet liner, was held at –35 °C using liquid nitrogen for the total desorption time and was then raised at 10 °C/s to 260 °C and held for 4 min. The solvent vent mode was used to transfer the sample to the analytical column. A CPWax-57CB column with dimensions 50 m × 0.25 mm and a 0.20 µm film thickness (Varian, Middelburg, Netherlands) was used, and the carrier gas was He at a flow rate of 1 mL/min. The oven temperature program was the following: the temperature was 35 °C for 4 min and was then raised to 220 °C at 2.5 °C/min (held 15 min). The quadrupole, source and transfer line temperatures were maintained at 150, 230 and 280 °C, respectively. The electron ionization mass spectra in the full-scan mode were recorded at 70 eV with the electron energy in the range of 29–300 amu.

Compound identification was based on mass spectra matching using the standard NIST 98 library and the retention index (LRI) of authentic reference standards.

Statistical analyses

One-way ANOVA was performed to evaluate significant differences among yeast strains and among different sampling points for each strain (significance levels $p < 0.05$). A principal component analysis (PCA) was carried out as an unsupervised method in order to ascertain the degree of differentiation between samples and which compounds were involved. ANOVA and PCA were performed using the Statistica (version 7.0) software package (Statsoft, Tulsa, USA).

Results and discussion

Fermentation kinetics and sugar consumption

Fermentations progress was monitored by measuring changes in OD_{660} and sugar consumption. In relation to yeast population, despite the fact that both yeasts strains were inoculated to reach the same final population, statistically significant differences were observed between *S. cerevisiae* G263 and *L. thermotolerans* G234 strain population during the fermentation process (Table 1). *L. thermotolerans* showed significant higher population than *S. cerevisiae* strain. In both strains, number of cells per mL significantly increased during the first 72 h of the assay, to keep more or less constant from T144 sampling point onwards.

Table 1 Sugar consumption (%) and yeast population (cell/mL) in fermentation assays (results are average and standard deviations of two fermentations conducted by *S. cerevisiae* (G263) strain and *L. thermotolerans* (G234) strain

		T0	T24	T48	T72	T144	T192
S. cerevisiae	% sugar consumption	0	9.1 ± 1.6a,A	9.3 ± 1.2a,A	13.4 ± 1.3a,A	28.2 ± 1.4a,B	32.7 ± 0.1b,B
L. thermotolerans		0	9.3 ± 0.1a,A	10.6 ± 2.5a,A	15.0 ± 0.9a,B	28.4 ± 0.1a,C	30.9 ± 0.1a,C
S. cerevisiae	cell/mL x 10^7	0.6	2.2 ± 0.1a,A	5.6 ± 0.0a,B	11.1 ± 0.3a,C	21.2 ± 0.6a,D	22.8 ± 0.0a,D
L. thermotolerans		0.6	4.8 ± 0.3b,A	8.3 ± 0.5b,B	13.5 ± 0.0b,C	26.7 ± 0.6b,D	28.6 ± 0.6b,E

Similar small letter in the same column indicates, for each parameter, no significant statistically differences ($p < 0.05$) between both yeast strains

Similar capital letter in the same row indicates no significant differences among sampling points for each yeast strain

Traditionally, non-*Saccharomyces* yeasts were described as weaker fermentative and less ethanol tolerant than *S. cerevisiae* strains (Fleet and Heard 1993); the latter together with added SO_2 toxicity contribute to explain their early disappearance during the fermentation. Recently, Jolly et al. (2014) have reviewed other effects to explain this, as the low oxygen level, especially for *L. thermotolerans*.

Regarding sugar consumption, due to the high initial sugar content of the must, none of the strains was able to consume total fermentable sugars (Table 1). No statistically significant differences in sugar consumption between both strains was observed until the last sampling point (T192); however *L. thermotolerans* exhibited a slightly faster sugar consumption than *S. cerevisiae* during first 72 h. Finally, percentage of sugar consumption by *S. cerevisiae* was 32.7%. This was in agreement with results reported by López de Lerma et al. (2012) for *Saccharomyces* strains in partially fermented Pedro Ximénez sun-dried grape musts. For the non-*Saccharomyces* strain sugar consumption was slightly lower (30.9%).

In this context, it should be taken into account that *L. thermotolerans* was isolated during the partial fermentation of sun-dried high sugar content Pedro Ximénez grape must, and afterwards tested for its ability to ferment high sugar content media with successful results. Thus, we consider that its high adaptation at such specific media, gave this autochthonous strain a competitive edge, as already described by Cray et al. (2013) for other indigenous non-*Saccharomyces* strains.

Production of volatile compounds during fermentation assays

HSSE-PDMS extraction method was observed to be useful for determining volatile composition in different foodstuffs. In this work, HSSE-PDMS non-invasive method was observed to be adequate for monitoring the changes of volatile compounds during the alcoholic fermentation. With this technique, the evolution of 141 volatile compounds throughout alcoholic fermentations could be monitored. Eighty-four of them were positively identified and twenty-eight tentatively identified (TI) (Tables 2, 3).

The extraction method was highly reproducible, among the 11 extractions performed in duplicate only in 6 of them, RSDs next to 15% were obtained for just 12–16 volatile compounds, that is, 9–11% of compounds determined. These compounds were primary acids followed by ketones and aldehydes.

Regarding the volatile profile of the substrate stood out alcohols, ketones and aldehydes as chemical groups with high values of total peak area (Tables 2, 3). In comparison with the other sampling points, we observed that the substrate presented the lowest values of total peak area for alcohols, ethyl and acetic esters, and the highest for aldehydes and C13-norisoprenoids. Some compounds were only detected in the substrate such as *cis*-2-hexen-1-ol, several aldehydes, ethyl 2-methylbutyrate, 3-penten-2-one, *trans*-linalool oxide, α-calacorene (TI), guaiacol, whilst isoamyl and others esters were not detected in it.

Figure 1, which groups the compounds according to their chemical classes, shows clearly the change in volatile profile throughout fermentation processes studied. The primary change is the importance acquired by ethyl esters during fermentation carried out by *Saccharomyces* strain, which implied a decrease of the proportion of alcohols and acetates. Whereas fermentation carried out by *L. thermotolerans* strain did not reveal a pronounced increase in ethyl esters, for this reason, in this case, alcohols continued to be the group of compounds that contributed more to volatile profile. Moreover, the percentage of ketones decreased during both types of fermentations.

In general, the two strains used in this study provided different volatile profile. Thus, the higher numbers of compounds with peak area values significantly different between strains were observed in the last sampling points (T144 and T192), 83 and 78 respectively. For most of these compounds, the values were higher when fermentation was carried out by *S. cerevisiae* than *L. thermotolerans*.

Table 2 Evolution of volatile compounds monitored online along alcoholic fermentation carried out by non-*Saccharomyces* yeast strain

Volatile compounds	ID[d]	LRI	Peak area ± sd[e]					
			MT0	NST24	NST48	NST72	NST144	NST192
Acetals								
Acetaldehyde diethylacetal	A	876	nd	253 ± 33^{a,b}	2488 ± 265^{a,b,c}	3971 ± 565^{b,c}	11,171 ± 1250^{a,b,c}	10,594 ± 1152^{b,c}
2,4,5-Trimethyl-1,3-dioxolane	C	911	764 ± 74	514 ± 52	230 ± 26^{a,b,c}	286 ± 22^{b,c}	253 ± 19^{b,c}	270 ± 21^{b,c}
Acetaldehyde ethyl amyl acetal	C	1069	nd	nd	291 ± 36^{a,b,c}	557 ± 4^{a,b,c}	3817 ± 375^{a,b,c}	3572 ± 120^{b,c}
Total of acetals			764	767	3010^{a,b,c}	4814^{b,c}	15,240^{a,b,c}	14,473^{b,c}
Acids								
Acetic acid	A	1444	7194 ± 1073	6464 ± 726	5564 ± 768	4921 ± 363	3703 ± 378^{b,c}	3247 ± 390^{b,c}
Propanoic acid	A	1536	598 ± 52	796 ± 89	603 ± 60	560 ± 66	485 ± 68	620 ± 37
Isovaleric acid[g]	A	1670	491 ± 68	495 ± 53	393.9 ± 0.9^{b}	413 ± 21^{b}	257 ± 35^{a,b,c}	284 ± 15^{b}
Pentanoic acid[g]	A	1739	562 ± 76	463 ± 30	263 ± 35^{a,c}	241 ± 33^{c}	237 ± 31^{c}	197 ± 17^{c}
Hexanoic acid	A	1847	3871 ± 526	2931 ± 164	1718 ± 236^{a,b,c}	1662 ± 208^{b,c}	1306 ± 180^{b,c}	1215 ± 24^{b,c}
Heptanoic acid[g]	A	1958	666 ± 85	749 ± 101	558 ± 35^{b}	609 ± 78	480 ± 36	550 ± 54
Octanoic acid	A	2066	1171 ± 165	2421 ± 331^{a}	2750 ± 51^{b,c}	1632 ± 235^{a,b}	1041 ± 112^{b}	1337 ± 180^{b}
Nonanoic acid[h]	A	2176	788 ± 108	1362 ± 180	1743 ± 255^{c}	988 ± 87	611 ± 71^{a}	1266 ± 174^{a}
Decanoic acid	A	2283	237 ± 26	1721 ± 204^{a,b}	2182 ± 176^{c}	1860 ± 28^{b,c}	612 ± 69^{a,b,c}	628 ± 87^{b,c}
Total of acids			15,577	17,400	15,775^{b}	12,886^{b}	8733^{a,b,c}	9344^{b,c}
Alcohols								
Ethanol[f,h]	A	922	3441 ± 355	3905 ± 556	6258 ± 14^{a,c}	6099 ± 268^{c}	8078 ± 135^{a,c}	8531 ± 598^{c}
1-Propanol	A	1017	1590 ± 114	2717 ± 347^{a}	7523 ± 943^{a,c}	5372 ± 251^{b,c}	6498 ± 141^{a,b,c}	6794 ± 512^{c}
Isobutanol[h]	A	1077	885 ± 97	1138 ± 75	2110 ± 152^{a,b,c}	1804 ± 104^{b,c}	2041 ± 125^{b,c}	2754 ± 236^{b,c}
1-Butanol[h]	A	1134	1099 ± 36	968 ± 34	821 ± 17^{a,b,c}	770 ± 23^{b,c}	1209 ± 18^{a,b}	1445 ± 14^{a,b,c}
2-Methyl-1-butanol[h]	A	1201	9851 ± 1237	22,981 ± 2052^{a,b}	33,800 ± 889^{a,c}	44,924 ± 2545^{a,c}	53,950 ± 2587^{c}	52,117 ± 3299^{c}
3-Methyl-1-butanol[f,h]	A	1206	444 ± 45	799 ± 85^{a}	1818 ± 231^{a,c}	1598 ± 11^{b,c}	3320 ± 64^{a,b,c}	3595 ± 28^{a,b,c}
1-Pentanol[g]	A	1245	3084 ± 171	2159 ± 319	1029 ± 31^{a,b,c}	865 ± 77^{c}	605 ± 30^{a,b,c}	422 ± 53^{c}
1-Hexanol[g]	A	1351	42,527 ± 2847	32,803 ± 1103^{a}	14,269 ± 1384^{a,c}	13,318 ± 237^{b,c}	6181 ± 240^{a,c}	4765 ± 37^{a,b,c}
cis-2-Hexen-1-ol[g]	C	1401	3560 ± 351	nd	nd	nd	nd	nd
1-Octen-3-ol[g]	A	1445	26,032 ± 1262	16,571 ± 2338^{a}	8669 ± 1266^{c}	8671 ± 532^{b,c}	3809 ± 19^{a,b,c}	2701 ± 167^{a,c}
1-Heptanol[g]	A	1456	8412 ± 84	7850 ± 833	4027 ± 437^{a,c}	3554 ± 172^{b,c}	1380 ± 33^{a,c}	1059 ± 6^{a,c}
6-Methyl-5-hepten-2-ol	C	1462	nd	651 ± 76^{a,b}	1917 ± 95^{a,b,c}	2111 ± 29^{b,c}	1627.6 ± 1.7^{a,b,c}	1236 ± 63^{a,b,c}
2-Ethyl-1-hexanol[g]	A	1488	1198 ± 176	1147 ± 156	899 ± 125	768 ± 96	451 ± 31^{a,c}	454 ± 57^{c}
2-Hepten-1-ol[g]	C	1509	1338 ± 37	nd	nd	nd	nd	nd
1-Octanol[g]	A	1558	4780 ± 233	3514 ± 430	2034 ± 134^{a,b,c}	1735 ± 46^{b,c}	621 ± 15^{a,b,c}	490 ± 50^{b,c}
cis-2-Octen-1-ol[g]	B[1]	1614	2070 ± 77	1004 ± 128^{a}	215 ± 22^{a,b,c}	332 ± 47^{c}	nq	nq
Furfuryl alcohol	A	1659	3040 ± 414	3789 ± 430	3123 ± 466	2759 ± 125	2646 ± 199	3276 ± 408
1-Nonanol[g]	A	1663	1998 ± 187	1509 ± 49	274 ± 16^{a,b,c}	nd	nd	nd
3-Methylthio-1-propanol[h]	B[2]	1723	nd	nd	245 ± 31^{a,b,c}	213 ± 18^{b,c}	197 ± 25^{b,c}	nq
4-Ethylbenzyl alcohol	C	1762	nd	nd	nd	nd	nd	nd

Table 2 continued

Volatile compounds	ID[d]	LRI	Peak area ± sd[e]					
			MT0	NST24	NST48	NST72	NST144	NST192
1-Decanol	A	1764	nd	nd	nd	nd	nd	nd
Benzyl alcohol[g]	A	1883	693 ± 46	579 ± 24	356 ± 15[a,c]	313.8 ± 1.0[c]	254 ± 8[a,b,c]	230 ± 5[b,c]
2-Phenylethanol[f,h]	A	1920	188 ± 12	206,2 ± 1.7	918 ± 133[a,c]	706 ± 11[b,c]	1885 ± 34[a,b,c]	1994 ± 181[b,c]
Total of alcohols[f]			5195	5904	9833[a,c]	9292[c]	14,068[a,b,c]	14,898[b,c]
Aldehydes								
3-Methyl-butanal[g]	C	890	3903 ± 577	nd	nd	nd	nd	nd
Hexanal[g]	A	1040	2483 ± 331	nd	nd	nd	nd	nd
Heptanal[g]	C	1149	343 ± 6	nd	nd	nd	nd	nd
trans-2-Heptenal[g]	B[3]	1306	3289 ± 282	nd	nd	nd	nd	nd
Nonanal[g]	A	1375	1514 ± 104	nd	nd	nd	nd	nd
2-Furfuraldehyde[g]	A	1448	18,968 ± 1883	12,614 ± 1413	5590 ± 826[a,c]	4272 ± 589[c]	4149 ± 597[c]	4919 ± 13[c]
trans–trans-2,4-Heptadienal[g]	C	1483	3156.4 ± 2.3	nd	nd	nd	nd	nd
Benzaldehyde[g]	A	1508	5507 ± 243	378 ± 13[a]	289 ± 35[c]	253 ± 32[c]	254 ± 19[c]	267 ± 7[c]
trans-2-Nonenal[g]	B[4]	1525	924 ± 114	nd	nd	nd	nd	nd
5-Methyl-2-furfuraldehyde[g]	A	1563	861 ± 9	844 ± 93	745 ± 101[b]	407 ± 53[c]	402 ± 49[c]	404 ± 51[c]
Cinnamaldehyde[g]	C	1574	309 ± 42	405 ± 4	263.6 ± 1.3[a]	292 ± 37	246 ± 33	252 ± 22[b]
trans-2-Decenal[g]	B[5]	1634	961 ± 64	nd	nd	nd	nd	nd
Safranal[g]	C	1635	791 ± 84	369 ± 26[a]	nd	nd	nd	nd
trans–trans-2,4-Nonadienal[g]	B[5]	1696	725 ± 77	nd	nd	nd	nd	nd
trans–trans-2,4-Decadienal[g]	B[5]	1804	5168 ± 266	nd	nd	nd	nd	nd
5-Hydroxymethylfurfural	A	2489	673 ± 39	2462 ± 317[a,b]	1216 ± 159[a,b,c]	515 ± 71[a]	564 ± 70[b]	642 ± 83
Total of aldehydes			49,657	16,703[a]	8104[a,c]	5739[c]	5616[c]	6485[b,c]
Acetic esters								
Ethyl acetate[h]	A	871	9404 ± 1315	8741 ± 687	18,255 ± 105[a,b,c]	23,923 ± 317[a,c]	65,748 ± 1463[a,b,c]	71,432 ± 1161[a,b,c]
Propyl acetate	A	934	nd	nd	160 ± 11[a,b,c]	195 ± 8[b,c]	434 ± 27[a,b,c]	504 ± 9[c]
Isobutyl acetate[h]	A	971	166 ± 17	248.5 ± 1.2[a]	1420 ± 80[a,b,c]	2037 ± 33[a,b,c]	7025 ± 177[a,b,c]	6958 ± 19[b,a]
Isoamyl acetate[f]	A	1081	8.2 ± 0.7	27.7 ± 1.3[a,b]	233 ± 7[a,b,c]	342 ± 18[a,b,c]	1004.8 ± 0.5[a,b,c]	992 ± 10[c]
Amyl acetate	A	1136	nd	nd	nd	223 ± 31[a,b,c]	nd	nd
Hexyl acetate	A	1252	398 ± 39	578 ± 74[b]	633 ± 75[b]	797 ± 4[b,c]	474 ± 6[a,b]	262 ± 16[a,b,c]
Heptyl acetate	B[3]	1356	nd	nd	nd	nd	nd	nd
Octyl acetate	A	1464	nd	nd	nd	nd	nd	nd
Nonyl acetate	B[3]	1565	nd	nd	nd	nd	nd	nd
Decyl acetate	B[3]	1672	nd	nd	nd	nd	nd	nd
Benzyl acetate	A	1718	nd	nd	nd	nd	nd	nd
2-Phenylethanol acetate	A	1806	517 ± 68	196 ± 8[a,b]	820 ± 110[a,b]	789 ± 106[b]	2507 ± 47[a,b,c]	2577 ± 330[b,c]
Nerolidol acetate	C	2257	nd	nd	nd	nd	nd	nd
Total of acetic esters			11,306	12,539[b]	44,614[a,b,c]	62,164[a,b,c]	176,670[a,b,c]	180,925[b,c]

Table 2 continued

Volatile compounds	ID[d]	LRI	Peak area ± sd[e]					
			MT0	NST24	NST48	NST72	NST144	NST192
Ethyl esters								
Ethyl propanoate[h]	A	924	182 ± 23	236 ± 27	1274 ± 167[a,b,c]	2617 ± 134[a,b,c]	8655 ± 142[a,b,c]	6812 ± 307[a,b,c]
Ethyl 2-methylpropanoate[h]	A	928	nd	nd	179.9 ± 2.2[a,b,c]	184 ± 9[b,c]	426 ± 17[a,b,c]	437 ± 17[b,c]
Ethyl butyrate	A	997	256 ± 31	270 ± 37[b]	887 ± 21[a,b,c]	1427 ± 21[a,b,c]	6209 ± 207[a,b,c]	7807 ± 282[a,c]
Ethyl 2-methylbutyrate[g]	A	1012	198 ± 10	nd	nd	nd	nd	nd
Ethyl valerate[h]	A	1092	259 ± 38	186 ± 23	268 ± 9[a,b]	445 ± 21[a,b,c]	1667 ± 6[a,c]	1570 ± 188[c]
Ethyl hexanoate[f]	A	1210	28.3 ± 1.0	37 ± 3	179 ± 8[a,b,c]	333 ± 11[a,b,c]	435 ± 31[a,b,c]	363 ± 14[b,c]
Ethyl heptanoate	A	1315	705 ± 83	1246 ± 110[a]	5344 ± 60[a,b,c]	7207 ± 230[a,b,c]	6873 ± 199[b,c]	4701 ± 434[a,b,c]
Ethyl 2-hexenoate[h]	B[6]	1325	nd	nd	nd	nd	361 ± 16[a,b,c]	408.5 ± 1.2[c]
Ethyl octanoate[f]	A	1435	29 ± 4	159 ± 7[a]	738 ± 74[a,b,c]	522.4 ± 0.3[b,c]	490 ± 36[b,c]	447 ± 61[b,c]
Ethyl 7-octenoate	A	1473	nd	nd	315 ± 23[a,c]	376 ± 6[b,c]	393 ± 25[b,c]	515 ± 72[b,c]
Ethyl nonanoate	A	1525	1673 ± 133	2494 ± 254[b]	4305 ± 194[a,b,c]	3945 ± 283[b,c]	2482 ± 196[a,b,c]	2135 ± 310[b]
Ethyl decanoate[f]	A	1641	7.4 ± 0.8	278 ± 18[a,b]	509 ± 37[a,b,c]	560 ± 39[b,c]	436 ± 34[b,c]	536 ± 73[b,c]
Ethyl 9-decenoate	B[6]	1686	nd	nd	3149 ± 432[a,c]	2216 ± 15[b,c]	1835 ± 234[b,c]	3174 ± 413[b,c]
Ethyl undecanoate	A	1730	nq	177 ± 3[a,b]	nq	177 ± 20[a,b,c]	nq	nq
Ethyl phenylacetate[h]	A	1774	221 ± 14	173 ± 8[b]	620 ± 89[a,b,c]	342.6 ± 2.2[a,b,c]	628 ± 38[a,b,c]	584 ± 80[b,c]
Ethyl dodecanoate[f]	A	1838	1.90 ± 0.21	3.32 ± 0.22[a,b]	18.5 ± 2.1[a,b,c]	50.58 ± 0.18[a,b,c]	87 ± 3[a,b,c]	77 ± 11[b,c]
Ethyl tetradecanoate[h]	A	2041	nq	nq	402 ± 54[a,c]	812 ± 28[a,c]	2077 ± 182[a,c]	2074 ± 104[b,c]
Ethyl hexadecanoate	A	2250	nq	nq	nq	281 ± 3[a,b]	253 ± 23[b,c]	287 ± 42[b,c]
Total of ethyl esters			10,169	52,538[a,b]	161,211[a,b,c]	166,612[b,c]	176,679[b,c]	172,858[b,c]
Isoamyl esters								
Isoamyl propionate[h]	C	1155	nd	nd	197 ± 18[a,c]	307 ± 20[a,c]	1506 ± 14[a,b,c]	1349 ± 61[b,c]
Isoamyl hexanoate	A	1450	nd	nd	nd	nd	nd	nd
Isoamyl octanoate	A	1654	nd	nd	1897 ± 204[a,b,c]	2246 ± 158[b,c]	2443 ± 179[b,c]	1620 ± 215[b,c]
Isoamyl decanoate	B[7]	1854	nd	nd	1018 ± 126[a,c]	1564 ± 53[a,c]	1963 ± 27[a,b,c]	2057 ± 307[b,c]
Total of isoamyl esters			–	–	3112[a,b,c]	4117[b,c]	5911[a,b,c]	5026[b,c]
Methyl esters								
Methyl hexanoate	A	1151	nd	162.0 ± 0.3[a,b]	162.3 ± 1.5[b,c]	215 ± 8[a,b,c]	nq	nq
Methyl octanoate	A	1371	234 ± 32	477 ± 66[a]	428 ± 60[b]	285 ± 7[b]	nq	nq
Methyl decanoate	A	1581	nd	908 ± 101[a,b]	308 ± 40[a,b,c]	247.82 ± 0.15[b,c]	nq	nq
Methyl salicylate	A	1762	244 ± 14	175 ± 22[b]	nq	nq	194 ± 24[a,b]	200 ± 27[b]
Total of methyl esters			479	1723[a,b]	898[a,b,c]	748[b,c]	194[a,b,c]	200[b,c]
Others esters								
Propyl hexanoate	B[8]	1299	nd	nd	nq	nq	nq	nq
Propyl octanoate	A	1509	nd	nd	nd	nd	nd	nd
Propyl decanoate	B[9]	1712	nd	nd	nd	nd	nd	nd
Isobutyl hexanoate	B[9]	1335	nd	nd	nd	nd	nq	nq
Isobutyl octanoate	A	1542	nd	nd	nd	nd	nd	nd

Table 2 continued

Volatile compounds	ID[d]	LRI	Peak area ± sd[e]					
			MT0	NST24	NST48	NST72	NST144	NST192
Isobutyl decanoate	B[9]	1746	nd	nd	nd	203 ± 28[a,c]	171 ± 11[b,c]	184 ± 15[b,c]
Total of other ester			–	–	–	203[a,b,c]	171[b,c]	184[c]
Ketones								
2-Pentanone[g]	A	939	473 ± 23	262 ± 10[a]	nd	nd	nd	nd
3-Penten-2-one[g]	C	1094	1242 ± 140	nd	nd	nd	nd	nd
2-Heptanone[g]	C	1152	744 ± 14	291 ± 24[a,b]	nq	nq	nq	nq
2-Pentylfuran[g]	B[3]	1196	29,133 ± 3173	29,364 ± 439	17,699 ± 2009[a,c]	16,158 ± 2314[c]	1404 ± 45[a,c]	484 ± 14[a,c]
3-Octanone[g]	B[3]	1233	1512 ± 143	1740 ± 144	854 ± 102[a,b,c]	940 ± 91[b,c]	484 ± 8[a,b,c]	367 ± 38[b,c]
2-Octanone[g]	B[3]	1266	4291 ± 458	1618 ± 223[a,b]	492 ± 69[a,b,c]	567 ± 25[c]	273 ± 17[a,c]	250 ± 33[c]
trans-2,2-Pentenyl-furan[g]	C	1271	1516 ± 134	1378 ± 38	460 ± 55[a,c]	637 ± 81[c]	nd	nd
Acetoin[f]	A	1273	711 ± 88	721 ± 83	1210 ± 10[a,b,c]	815 ± 39[a,b]	816 ± 12[b]	702 ± 25[a]
1-Hydroxy-2-propanone	A	1286	2265 ± 296	4120 ± 533	2373 ± 291	2686 ± 76	2310 ± 144	2459 ± 48
2-Hexylfuran[g]	C	1303	389 ± 42	394 ± 17[b]	274 ± 39	341 ± 30	nq	nq
6-Methyl-5-hepten-2-one[g]	A	1319	4095 ± 195	3988 ± 45	2230 ± 283[a,b,c]	2326 ± 143[b,c]	742 ± 6[a,b,c]	464 ± 11[a,b,c]
1-Hydroxy-2-butanone	B[3]	1363	1097 ± 150	1442 ± 155	1096 ± 62	1583 ± 21[a,b,c]	1449 ± 104	1467 ± 200
2-Nonanone[h]	A	1374	1657 ± 225	1072 ± 151[b]	2216 ± 92[a,b]	2054 ± 66[b]	1535 ± 86[a]	1464 ± 84[b]
2-Acetilfuran[g]	A	1493	881 ± 16	1123 ± 131	721 ± 95	579 ± 70[c]	487 ± 32[c]	580 ± 54[c]
Dihydro-3(2H)-thiophenone[h]	C	1515	nd	nd	281 ± 38[a,b,c]	268 ± 35[b,c]	nd	nd
6-Methyl-3,5-heptadiene-2-one[g]	B[3]	1588	11,941 ± 59	6343 ± 470[a]	2377 ± 6[a,b,c]	1714 ± 208[a,b,c]	454 ± 35[a,b,c]	271 ± 14[a,b,c]
Acetofenone[h]	A	1641	654 ± 77	711 ± 106	490 ± 67	434 ± 45	348 ± 49[b,c]	297 ± 41[b,c]
1,2-Cyclopentanedione	C	1775	2224 ± 270	3415 ± 260[a]	2645 ± 381	2238 ± 107	2029 ± 179	2763 ± 358
Cyclotene[h]	B[10]	1841	317 ± 47	397 ± 49	352 ± 47	312 ± 20	261 ± 14	403 ± 55[b]
Total of ketones			135,497	129,723	155,601[b]	114,387[a,b]	93,391[a,b,c]	81,515[a,c]
Lactones								
γ-Butyrolactone[g]	A	1625	2903 ± 35	2666 ± 252	1752 ± 31[a,c]	1603 ± 58[c]	1352 ± 10[a,c]	1483 ± 70[b,c]
γ-Nonalactona[g]	B[3]	2039	1506 ± 217	1966 ± 62[b]	1404 ± 209	1404 ± 33	1020 ± 20[a]	838 ± 20[a,b,c]
Total of lactones			4409	4632	3156[a,c]	3007[c]	2372[a,c]	2321[c]
C_{13}-Norisoprenoids								
TDN	A	1727	793 ± 88	740 ± 70	737 ± 22[b]	891 ± 17[a]	708 ± 42[a,b]	439 ± 60[a,b,c]
β-Damascenone[g]	A	1813	1595 ± 59	1125 ± 115[a]	790 ± 27[b,c]	863 ± 17[c]	562 ± 25[a,c]	633 ± 26[c]
β-Ionone[g]	A	1941	174 ± 22	129 ± 14[b]	nq	nq	nd	nd
Total of C_{13}-Norisoprenoids			2562	1994	1527[b,c]	1754[a,c]	1270[a,b,c]	1073[b,c]
Terpenes								
1R-α-Pinene[g]	C	976	1502 ± 210	983 ± 25[b]	780 ± 97[b,c]	987 ± 131[b]	350 ± 35[a,b,c]	219 ± 12[a,b,c]
Roseoxide[h]	C	1119	nd	nd	nd	525 ± 62[a,b,c]	916 ± 41[a,b,c]	671 ± 16[a,b,c]
Myrtenal	C	1123	663 ± 69	620 ± 44	774 ± 76	1075 ± 96[c]	568 ± 29[a]	252 ± 29[a,c]
Limonene	A	1149	426 ± 52	332 ± 22	798 ± 42[a,c]	355 ± 7[a]	nq	nq

Table 2 continued

Volatile compounds	ID[d]	LRI	Peak area ± sd[e]					
			MT0	NST24	NST48	NST72	NST144	NST192
Cymene[g]	B[11]	1237	604 ± 35	491 ± 30	280 ± 40[a,c]	298 ± 41[c]	nd	nd
trans-Linalool oxide[g]	B[12]	1468	219 ± 31	nq	nd	nd	nd	nd
2-Bornene[g]	C	1517	846 ± 32	619 ± 67[a]	417 ± 33[c]	388 ± 50[c]	275 ± 9[c]	186 ± 21[a,c]
Linalool[g]	A	1542	439 ± 35	246 ± 12[a]	186.6 ± 2.1[a,b,c]	181 ± 7[b,c]	nq	nq
Citronellol	A	1767	nd	nd	271 ± 33[a,b,c]	nd	286 ± 33[a,b,c]	296 ± 36[b,c]
α-Calacorene[g]	B[13]	1901	307 ± 24	nq	nq	nd	nd	nd
Nerolidol	A	2037	nd	nd	nd	nd	nq	nq
n.i. (m/z 69, 93, 121)	–	1743	nd	nd	nd	nd	nd	nd
Total of Terpenes			5005	3291[a]	3508[b,c]	3809[b,c]	2396[a,b,c]	1624[a,b,c]
Volatile phenols								
Guaiacol[g]	A	1858	273 ± 31	nq	nq	nq	nq	nq
4-Vinylguaiacol	A	2203	nq	nq	nq	nq	nd	nd
Coumaran[g]	C	2406	238 ± 32	194 ± 28	170 ± 21	nq	nq	nq
Total of volatile phenols			511	194[a]	170[b]	–	–	–
Others compounds								
2-Methylpyrazine[g]	A	1256	343 ± 46	415 ± 6	283 ± 32[a]	255 ± 31	nq	nq
Indole[h]	B[14]	2436	375 ± 56	455 ± 20[b]	714 ± 100	1073 ± 40[a,c]	470 ± 21[a,b]	205 ± 7[a,b]
Unidentified compounds								
n.i. (m/z 59, 43)[h]	–	1328	nq	nq	320 ± 22[a,b,c]	466 ± 5[a,c]	804 ± 13[a,c]	743 ± 78[c]
n.i. (m/z 67, 85, 151)[g]	–	1395	1332 ± 97	1076 ± 16	590 ± 85[a,b,c]	790 ± 70[b,c]	314 ± 9[a,b,c]	163 ± 18[a,b,c]
n.i. (m/z 55,88, 101)	–	1887	nd	nd	nd	nd	nd	nd
n.i. (m/z 126, 73)[g]	–	2106	4188 ± 85	394 ± 49[a,b]	nq	382 ± 37[a,c]	628 ± 56[a,b,c]	403.8 ± 1.3[a,b,c]

ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data; C, mass spectrum agreed with mass spectral data base

nd: peak not detected or lower than detection limit (a signal-to-noise ratio higher than or equal to 3); nq: lower than quantification limit (a signal-to-noise ratio higher than or equal to 10)

[a] There is significant different ($p < 0.05$) with previous sample

[b] There is significant different ($p < 0.05$) with wine produced by Saccharomyces cerevisiae strain

[c] There is significant different ($p < 0.05$) with substrate, only for samples from 48 to 192 h

[d] Literature reference agreed with experimental LRI data:(1) Weckerle et al. (2001), (2) Miranda-Lopez et al. (1992), (3) National Center for Biotechnology Information (2015), (4) Schnermann and Schieberle (1997), (5) Rychlik et al. (1998), (6) Ferrari et al. (2004), (7) Riu-Aumatell et al. (2006), (8) Girard and Lau (1995), (9) Sun et al. (2013), (10) Natali et al. (2006), (11) Olivera et al. (2007), (12) Losco et al. (2007), (13) Bicchi et al. (2003), (14) Shiratsuchi et al. (1994) (complete reference provided as supplementary material)

[e] Value of peak area and sd have been divided per 1000

[f] Value of peak area and sd have been divided per 100,000

[g] Variable highly correlated with substrate and T24 (non-*Saccharomyces* and *Saccharomyces*)

[h] Variable highly correlated with samples from T48 to T192 *Saccharomyces*

Table 3 Evolution of volatile compounds monitored online along alcoholic fermentation carried out by *Saccharomyces* yeast strain

Volatile compounds	ID	LRI	Peak area ± sd[c]				
			ST24	ST48	ST72	ST144	ST192
Acetals							
Acetaldehyde diethylacetal[f]	A	876	nd	6193 ± 219[a,b]	10,607 ± 1467[b]	54,828 ± 3506[a,b]	42,198 ± 1594[a,b]
2,4,5-Trimethyl-1,3-dioxolane[f]	C	911	614 ± 4	491 ± 13[b]	956 ± 56[a]	6902 ± 757[a,b]	8819 ± 683[b]
Acetaldehyde ethyl amyl acetal[f]	C	1069	nd	601 ± 3[a,b]	1449 ± 189[a,b]	8091 ± 89[a,b]	5651 ± 369[a,b]
Total of acetals			614	7285[a,b]	13,012[a,b]	69,821[a,b]	56,668[a,b]
Acids							
Acetic acid[f]	A	1444	7022 ± 906	6905 ± 888	5396 ± 775	11,031 ± 1146[a]	9543 ± 1354
Propanoic acid[f]	A	1536	742 ± 96	656 ± 80	648 ± 94	831 ± 102	683 ± 5
Isovaleric acid[e]	A	1670	769 ± 90	648 ± 56	567.1 ± 1.0	665 ± 43	527 ± 16
Pentanoic acid[e]	A	1739	496 ± 66	297.6 ± 1.0[b]	293 ± 10[b]	286 ± 5[b]	255 ± 28[b]
Hexanoic acid[f]	A	1847	3129 ± 446	3136 ± 263	4392 ± 176[a]	6094 ± 561	5551 ± 374
Heptanoic acid[e]	A	1958	687 ± 93	386 ± 39	564 ± 77	389 ± 31[b]	565 ± 81
Octanoic acid[f]	A	2066	2042 ± 291	8433 ± 449[b]	11,450 ± 392[a,b]	10,697 ± 1544[b]	11,299 ± 242[b]
Nonanoic acid	A	2176	844 ± 117	1219.83 ± 0.19[b]	907 ± 123	662 ± 89	1449 ± 203[a]
Decanoic acid[f]	A	2283	995 ± 110[a]	2655 ± 113[a,b]	4961 ± 111[a,b]	5065 ± 679[b]	5026 ± 75[b]
Total of acids			16,753[a]	24,336[a,b]	29,179[a,b]	35,719[a,b]	34,897[b]
Alcohols							
Ethanol[d]	A	922	3215 ± 30	6537 ± 605[b]	6785 ± 365[b]	8696 ± 305[a,b]	8212 ± 76[b]
1-Propanol[f]	A	1017	1675 ± 29	7867 ± 122[a,b]	7959 ± 123[b]	10,132 ± 303[a,b]	8346 ± 866[b]
Isobutanol	A	1077	891 ± 58	1213 ± 81	1287 ± 33[b]	1346 ± 178	1025 ± 53
1-Butanol	A	1134	988 ± 20	1549 ± 68[b]	1250.8 ± 1.1[a,b]	825 ± 92[a]	622 ± 71[b]
2-Methyl-1-butanol	A	1201	15,576 ± 632[a]	34,803 ± 1522[a,b]	37,770 ± 1883[b]	50,969 ± 1812[a,b]	45,303 ± 2586[b]
3-Methyl-1-butanol[d]	A	1206	871 ± 58[a]	1636 ± 72[a,b]	1421 ± 23[b]	1387 ± 41[b]	1272 ± 42[b]
1-Pentanol[e]	A	1245	2045 ± 40[a]	866 ± 39[a,b]	792 ± 17[b]	417 ± 3[a,b]	381.8 ± 2.3[a,b]
1-Hexanol[e]	A	1351	34,943 ± 296	11,934 ± 715[b]	9992 ± 315[b]	6106 ± 25[a,b]	4225 ± 31[a,b]
cis-2-Hexen-1-ol[e]	C	1401	nd	nd	nd	nd	nd
1-Octen-3-ol[e]	A	1445	17,768 ± 597[a]	7084 ± 198[a,b]	6010 ± 175[a,b]	3094 ± 86[a,b]	2227 ± 115[a,b]
1-Heptanol[e]	A	1456	8549 ± 652	3555 ± 68[b]	2610 ± 77[a,b]	1417 ± 25[a,b]	1201 ± 50[a,b]
6-Methyl-5-hepten-2-ol	C	1462	nd	658.7 ± 1.4[a,b]	815 ± 7[a,b]	899 ± 67[b]	667 ± 60[b]
2-Ethyl-1-hexanol[e]	A	1488	1228 ± 120	600 ± 30c	578 ± 77c	640 ± 75	637 ± 86
2-Hepten-1-ol[e]	C	1509	nd	nd	nd	nd	nd
1-Octanol[e]	A	1558	3700 ± 397	3413 ± 60[b]	3317 ± 106[b]	1710 ± 186[a,b]	1186 ± 23[b]
cis-2-Octen-1-ol[e]	B	1614	1455 ± 165[a]	427 ± 20[a,b]	303 ± 15[a,b]	nd	nd
Furfuryl alcohol	A	1659	4425 ± 628	3372 ± 386	3262 ± 444	3348 ± 482	2838 ± 277
1-Nonanol[e]	A	1663	1742 ± 223	672 ± 71[b]	735 ± 110[b]	331 ± 49[a,b]	326 ± 4[b]
3-Methylthio-1-propanol	B	1723	nd	nd	nd	nd	nd
4-Ethylbenzyl alcohol[f]	C	1762	nd	310 ± 8[a,b]	556 ± 49[a,b]	363 ± 3[a,b]	254 ± 12[a,b]
1-Decanol[f]	A	1764	nd	607 ± 35[a,b]	1083 ± 15[a,b]	765 ± 54[a,b]	432 ± 19[a,b]
Benzyl alcohol[e]	A	1883	501 ± 45	347 ± 4[b]	318 ± 4[b]	208 ± 3[a,b]	186 ± 11[b]
2-Phenylethanol[d]	A	1920	227 ± 31	1261 ± 78[b]	919 ± 13[a,b]	946 ± 47[b]	1067 ± 6[b]
Total of alcohols[d]			5268	10,227[b]	9911[b]	11,855[a,b]	11,249[b]
Aldehydes							
3-Methyl-butanal[e]	C	890	nd	nd	nd	nd	nd
Hexanal[e]	A	1040	nd	nd	nd	nd	nd
Heptanal[e]	C	1149	nd	nd	nd	nd	nd
trans-2-Heptenal[e]	B	1306	nd	nd	nd	nd	nd
Nonanal[e]	A	1375	nd	nd	nd	nd	nd

Table 3 continued

Volatile compounds	ID	LRI	Peak area ± sd[c]				
			ST24	ST48	ST72	ST144	ST192
2-Furfuraldehyde[e]	A	1448	14,496 ± 2084	6664 ± 755[b]	5260 ± 704[b]	4553 ± 544[b]	4245 ± 420[b]
trans–trans-2,4-Heptadienal[e]	C	1483	1286 ± 41[a]	nd	nd	nd	nd
Benzaldehyde[e]	A	1508	542 ± 74[a]	233 ± 5[a,b]	260 ± 27[b]	285 ± 35[b]	274 ± 35[b]
trans-2-Nonenal[e]	B	1525	nd	nd	nd	nd	nd
5-Methyl-2-furfuraldehyde[e]	A	1563	652 ± 93	407 ± 8[b]	399 ± 56[b]	529 ± 75[b]	314 ± 26[b]
Cinnamaldehyde[e]	C	1574	448 ± 66	253 ± 34	266 ± 33	173 ± 3[b]	175 ± 14[b]
trans-2-Decenal[e]	B	1634	nd	nd	nd	nd	nd
Safranal[e]	C	1635	nd	nd	nd	nd	nd
trans–trans-2,4-Nonadienal[e]	B	1696	nd	nd	nd	nd	nd
trans–trans-2,4-Decadienal[e]	B	1804	nd	nd	nd	nd	nd
5-Hydroxymethylfurfural	A	2489	909 ± 106	672.9 ± 0.7	805 ± 103	1154 ± 45[a,b]	465 ± 57[a]
Total of aldehydes			18,333[a]	8230[a,b]	6990[b]	6693[b]	5473[b]
Acetic esters							
Ethyl acetate	A	871	8359 ± 519	16,234 ± 104[b]	23,209 ± 519[a,b]	41, 238 ± 4762[a,b]	37, 890 ± 2344[b]
Propyl acetate[f]	A	934	nd	467 ± 40[a,b]	610 ± 27[b]	756 ± 88[b]	565 ± 60[b]
Isobutyl acetate	A	971	237 ± 20	2644 ± 203[b]	3872 ± 41[a,b]	3847 ± 410[b]	2320 ± 254[a,b]
Isoamyl acetate[d,f]	A	1081	48.2 ± 0.6[a]	1151 ± 73[a,b]	1608 ± 81[a,b]	1705 ± 199[b]	1153 ± 62[b]
Amyl acetate[f]	A	1136	nd	1969 ± 98[a,b]	3095 ± 45[a,b]	2368 ± 167[a,b]	1149 ± 101[a,b]
Hexyl acetate[f]	A	1252	4806 ± 36[a]	53,965 ± 3425[a,b]	78,336 ± 485[a,b]	46,441 ± 2127[a,b]	23,961 ± 826[a,b]
Heptyl acetate	B	1356	1212 ± 30[a,b]	9352 ± 405[a,b]	13,844 ± 142[a,b]	6651 ± 32[a,b]	3247 ± 47[a,b]
Octyl acetate[f]	A	1464	nd	5540 ± 185[a,b]	11,319 ± 287[a,b]	7289 ± 26[a,b]	4205 ± 4[a,b]
Nonyl acetate[f]	B	1565	nd	1204 ± 23[a,b]	2644 ± 130[a,b]	1558 ± 164[a,b]	1036 ± 112[b]
Decyl acetate[f]	B	1672	nd	556 ± 41[a,b]	1716 ± 126[a,b]	2414 ± 115[a,b]	2281 ± 83[b]
Benzyl acetate	A	1718	nd	230 ± 3[a,b]	215 ± 3[a,b]	164.2 ± 2.2[a,b]	nq
2-Phenylethanol acetate[f]	A	1806	1000 ± 128[a]	89,451 ± 2541[a,b]	61,507 ± 5304[a,b]	50,422 ± 872[b]	51,838 ± 461[b]
Nerolidol acetate[f]	C	2257	nd	nd	298 ± 25[a,b]	691 ± 97[a,b]	1023 ± 48[a,b]
Total of acetic esters			20,433[a]	296,712[a,b]	361,498[a,b]	334,354[b]	244,861[a,b]
Ethyl esters							
Ethyl propanoate	A	924	209 ± 5	362 ± 6[b]	626 ± 34[a,b]	860 ± 74[b]	866 ± 37[b]
Ethyl 2-methylpropanoate	A	928	nd	nd	nd	nd	nd
Ethyl butyrate[f]	A	997	426 ± 20[a]	2423 ± 3[a,b]	5636 ± 91[a,b]	12,466 ± 1600[a,b]	11,076 ± 1394[b]
Ethyl 2-methylbutyrate[e]	A	1012	nd	nd	nd	nd	nd
Ethyl valerate	A	1092	230,4 ± 1,3	352 ± 10	642 ± 16[a,b]	1664 ± 178[a,b]	1887 ± 109[b]
Ethyl hexanoate[d,f]	A	1210	41.86 ± 0.14[a]	1603 ± 112[a,b]	2989 ± 114[a,b]	4317 ± 182[a,b]	3620 ± 156[b]
Ethyl heptanoate[f]	A	1315	943 ± 49	12,828 ± 507[a,b]	13,765 ± 280[b]	15,435 ± 220[a,b]	16,275 ± 276[b]
Ethyl 2-hexenoate	B	1325	nd	nd	nd	226 ± 4[b]	394 ± 10[a,b]
Ethyl octanoate[d,f]	A	1435	136,3 ± 2,2[a]	4567 ± 121[a,b]	8601 ± 893[a,b]	14,101 ± 183[a,b]	15,920 ± 19[a,b]
Ethyl 7-octenoate[f]	A	1473	nd	642 ± 30[b]	779 ± 6[a,b]	1339 ± 26[a,b]	2431 ± 158[a,b]
Ethyl nonanoate[f]	A	1525	1065 ± 126[a]	5573 ± 53[a,b]	10,019 ± 63[a,b]	13,327 ± 595[a,b]	15,005 ± 87[b]
Ethyl decanoate[d,f]	A	1641	24.5 ± 1.8[a]	1353 ± 52[a,b]	3708 ± 589[a,b]	9668 ± 32[a,b]	10,261 ± 295[b]
Ethyl 9-decenoate[f]	B	1686	nd	14,756 ± 415[a,b]	36,581 ± 4997[a,b]	67,374 ± 15[a,b]	107,580 ± 2435[a,b]
Ethyl undecanoate[f]	A	1730	nq	479 ± 9[a,b]	886 ± 88[a,b]	2397 ± 59[a,b]	2402 ± 35[b]
Ethyl phenylacetate	A	1774	nq	319 ± 26[a,b]	246 ± 8	282 ± 12[b]	310 ± 11[b]
Ethyl dodecanoate[d,f]	A	1838	1.69 ± 0.23	99 ± 4[b]	325 ± 44[a,b]	1627 ± 23[a,b]	1657 ± 79[b]
Ethyl tetradecanoate	A	2041	nq	256 ± 20[a,b]	737 ± 71[a,b]	2957 ± 399[a,b]	3302 ± 129[b]
Ethyl hexadecanoate[f]	A	2250	nq	nq	411.2 ± 1.7[a,b]	1330 ± 179[a,b]	1717 ± 63[b]
Total of ethyl esters			23,309[a]	800,155[a,b]	1632,542[a,b]	3091,015[a,b]	3308,983[a,b]

Table 3 continued

Volatile compounds	ID	LRI	Peak area ± sd[c]				
			ST24	ST48	ST72	ST144	ST192
Isoamyl esters							
Isoamyl propionate	C	1155	nd	220 ± 21[a,b]	349 ± 7[a,b]	554 ± 20[a,b]	454.9 ± 0.3[a,b]
Isoamyl hexanoate[f]	A	1450	nd	2439 ± 90[a,b]	6615 ± 724[a,b]	11,509 ± 392[a,b]	8916 ± 76[a,b]
Isoamyl octanoate[f]	A	1654	nd	7176 ± 184[a,b]	15,667 ± 1742[a,b]	32,624 ± 816[a,b]	31,538 ± 616[b]
Isoamyl decanoate[f]	B	1854	nd	700 ± 72[a,b]	2282 ± 324[a,b]	10,581 ± 572[a,b]	15,170 ± 904[a,b]
Total of isoamyl esters			–	10,535[a,b]	24,913[a,b]	55,268[a,b]	56,079[b]
Methyl Esters							
Methyl hexanoate[f]	A	1151	204 ± 3[a]	797 ± 33[a,b]	1392 ± 42[a,b]	983 ± 106[a,b]	526 ± 12[a,b]
Methyl octanoate[f]	A	1371	623 ± 50[a]	2932 ± 124[a,b]	3304 ± 127[b]	2691 ± 76[a,b]	2023 ± 73[a,b]
Methyl decanoate[f]	A	1581	164 ± 3[a]	981 ± 77[a,v]	2066 ± 261[a,b]	2260 ± 143[b]	1617 ± 12[a,b]
Methyl salicylate[f]	A	1762	nq	nq	nq	297 ± 13[a]	374 ± 24[b]
Total of methyl esters			991[a]	4711[a,b]	6761[a,b]	6231[v]	4540[a,b]
Others esters							
Propyl hexanoate[f]	B	1299	nd	425 ± 13[a,b]	627 ± 31[a,b]	836 ± 33[a,b]	625 ± 7[a,b]
Propyl octanoate[f]	A	1509	nd	406 ± 5[a,b]	778 ± 85[a,b]	1301 ± 45[a,b]	1148 ± 50[b]
Propyl decanoate[f]	B	1712	nd	nq	257 ± 38[a,b]	858 ± 32[a,b]	750 ± 41[b]
Isobutyl hexanoate[f]	B	1335	nd	388 ± 15[a,b]	662 ± 5[a,b]	745 ± 73[b]	465 ± 7[a,b]
Isobutyl octanoate[f]	A	1542	nd	899 ± 19[a,b]	1690 ± 171[a,b]	3624 ± 136[a,b]	3091 ± 74[a,b]
Isobutyl decanoate	B	1746	nd	nd	277 ± 12[a,b]	1364 ± 111[a,b]	1570 ± 64[b]
Total of other ester			–	2188[a,b]	4291[a,b]	8728[a,b]	7650[a,b]
Ketones							
2-Pentanone[e]	A	939	385 ± 45	nd	nd	nd	nd
3-Penten-2-one[e]	C	1094	nd	nd	nd	nd	nd
2-Heptanone[e]	C	1152	691 ± 44	234 ± 6[b]	221 ± 14[b]	205 ± 7[b]	nd
2-Pentylfuran[e]	B	1196	29,825 ± 275	14,917 ± 489[b]	13,853 ± 1139[b]	1546 ± 71[a,b]	571 ± 34[a,b]
3-Octanone[e]	B	1233	1845 ± 76	489 ± 7[b]	540 ± 69[b]	225 ± 1[a,b]	nq
2-Octanone[e]	B	1266	4118 ± 234	748.6 ± 1.4[b]	564 ± 49[a,b]	228 ± 14[a,b]	180.7 ± 2.1[a,b]
trans-2,2-Pentenyl-furan[e]	C	1271	1456 ± 40	421 ± 3[b]	392 ± 12[b]	nd	nd
Acetoin[d]	A	1273	650 ± 38	1522 ± 90[b]	1664 ± 40[b]	1477 ± 115[b]	654.2 ± 0.6[a]
1-Hydroxy-2-propanone	A	1286	3346 ± 493	2970 ± 420	3160 ± 446	2883 ± 150	2782 ± 124
2-Hexylfuran[e]	C	1303	312.37 ± 0.17	355 ± 22	324 ± 6	nq	nq
6-Methyl-5-hepten-2-one[e]	A	1319	4016 ± 71	5999 ± 292[b]	5618 ± 205[b]	3493 ± 152[a]	2645 ± 259[b]
1-Hydroxy-2-butanone	B	1363	1442 ± 209	1339 ± 190	1572 ± 208	1271 ± 18	1427 ± 111
2-Nonanone	A	1374	1628 ± 40	841 ± 27[b]	793 ± 72[b]	1517 ± 12[a]	1023 ± 47[a]
2-Acetilfuran[e]	A	1493	1033 ± 137	610 ± 9[b]	637 ± 80	576 ± 79[b]	427 ± 58[b]
Dihydro-3(2H)-thiophenone	C	1515	nd	nd	nd	nd	nd
6-Methyl-3,5-heptadiene-2-one[e]	B	1588	6926 ± 913[a]	1677 ± 43[a,b]	697 ± 65[a,b]	nq	nq
Acetofenone	A	1641	750 ± 66	431 ± 10	356 ± 34[b]	nd	nd
1,2-Cyclopentanedione	C	1775	2838 ± 357	2861 ± 319	2912 ± 345	3460 ± 479	2619 ± 335
Cyclotene	B	1841	440 ± 47	369 ± 23	373 ± 49	321 ± 36	215 ± 3
Total of ketones			126,097	186,469[b]	198,450[b]	163,455	77,256[a,b]
Lactones							
γ-Butyrolactone[e]	A	1625	2743 ± 33[a]	1837 ± 26[a,b]	1607 ± 124[b]	1344 ± 79[b]	1223 ± 6[b]
γ-Nonalactona[e]	B	2039	1208 ± 180	1502 ± 38	1432 ± 95	1025 ± 124	999 ± 20
Total of lactones			3951	3339[b]	3039[a,b]	2370[a,b]	2222[b]

Table 3 continued

Volatile compounds	ID	LRI	Peak area ± sd[c]				
			ST24	ST48	ST72	ST144	ST192
C_{13}-Norisoprenoids							
TDN[f]	A	1727	718 ± 54	887 ± 25	1006 ± 88	1067.4 ± 0.3	1017 ± 37
β-Damascenone[e]	A	1813	1071 ± 88[a]	906 ± 3[a,b]	819 ± 7[a,b]	588 ± 39[a,b]	600 ± 69[b]
β-Ionone[e]	A	1941	nq	nq	nq	nd	nd
Total of C_{13}-Norisoprenoids			1789[a]	1793[b]	1825[b]	1656[b]	1616[b]
Terpenes							
1R-α-Pinene[e]	C	976	1572 ± 190	1311 ± 74	1512 ± 22	878 ± 61[a]	395 ± 32[a,b]
Roseoxide	C	1119	nd	nd	nd	316 ± 26[b]	331 ± 22[b]
Myrtenal	C	1123	486 ± 19	893 ± 4[b]	1148 ± 74[a,b]	583 ± 14[a]	330 ± 18[a,b]
Limonene	A	1149	379 ± 23	880 ± 18[b]	354 ± 8[a]	nq	nq
Cymene[e]	B	1237	563 ± 26	317 ± 5[b]	315 ± 9[b]	nd	nd
trans-Linalool oxide[e]	B	1468	nq	nq	nd	nd	nd
2-Bornene[e]	C	1517	616 ± 25[a]	392 ± 5[a,b]	364 ± 24[b]	233 ± 32[a,b]	189 ± 4[b]
Linalool[e]	A	1542	219 ± 28[a]	227 ± 5[a,b]	238 ± 11	nd	nd
Citronellol[h]	A	1767	nd	488 ± 15[a,b]	535 ± 31[b]	615 ± 58[b]	673 ± 41[b]
α-Calacorene[e]	B	1901	nd	nd	nd	nd	nd
Nerolidol[f]	A	2037	nd	nd	nd	370 ± 40[a,b]	403 ± 26[b]
n.i. (m/z 69, 93, 121)[f]	–	1743	nd	1029 ± 48[a,b]	1326 ± 109[b]	830 ± 99[a,b]	693 ± 68[b]
Total of terpenes			3833[a]	5536[a,b]	5793[b]	3825[a,b]	3015[a,b]
Volatile phenols							
Guaiacol[e]	A	1858	nq	nq	nq	nq	nq
4-Vinylguaiacol[f]	A	2203	nq	274 ± 32[a,b]	465 ± 47[a,b]	890 ± 7[a,b]	1976 ± 110[a,b]
Coumaran[e]	C	2406	174 ± 10	187 ± 7	214 ± 29	176 ± 11	229 ± 6a
Total of volatile phenols			174	460[a]	679[a]	1066[a,b]	2204[a,b]
Others compounds							
2-Methylpyrazine[e]	A	1256	619 ± 75[a]	327 ± 7[a]	233 ± 15[a]	nq	nq
Indole	B	2436	182.2 ± 0.9[a]	806 ± 29[a,b]	1196 ± 34[a,b]	316 ± 26[a]	nq
Unidentified compounds							
n.i. (m/z 59, 43)	–	1328	nq	461 ± 14[a,b]	456 ± 25[b]	720 ± 35[a,b]	506 ± 11[a,b]
n.i. (m/z 67, 85, 151)[e]	–	1395	1180 ± 38	1249 ± 19	1403 ± 13[a]	755 ± 5[a,b]	419 ± 24[a,b]
n.i. (m/z 55,88, 101)[f]	–	1887	nd	192 ± 3[a,b]	774 ± 97[a,b]	4071 ± 330[a,b]	5952 ± 17[a,b]
n.i. (m/z 126, 73)[e]	–	2106	788 ± 49[a]	nq	317 ± 34[a,b]	nq	nq

ID: reliability of identification: A, mass spectrum and LRI agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data; C, mass spectrum agreed with mass spectral data base

nd: peak not detected or lower than detection limit (a signal-to-noise ratio higher than or equal to 3); nq: lower than quantification limit (a signal-to-noise ratio higher than or equal to 10)

[a] There is significant different ($p < 0.05$) with previous sample

[b] There is significant different ($p < 0.05$) with substrate, only for samples from 48 to 192 h

[c] Value of peak area and sd have been divided per 1000

[d] Value of peak area and sd have been divided per 100,000

[e] Variable highly correlated with substrate and T24 (non-*Saccharomyces* and *Saccharomyces*)

[f] Variable highly correlated with samples from T48 to T192 *Saccharomyces*

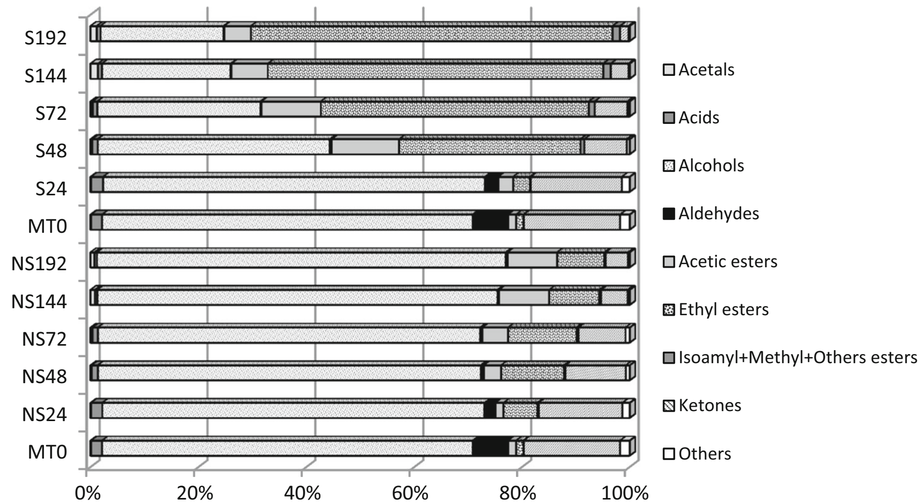


Fig. 1 Contribution (%) of different chemical groups of volatile compounds to the volatile profile of samples(S = *S. cerevisiae* and NS = *L. thermotolerans*)

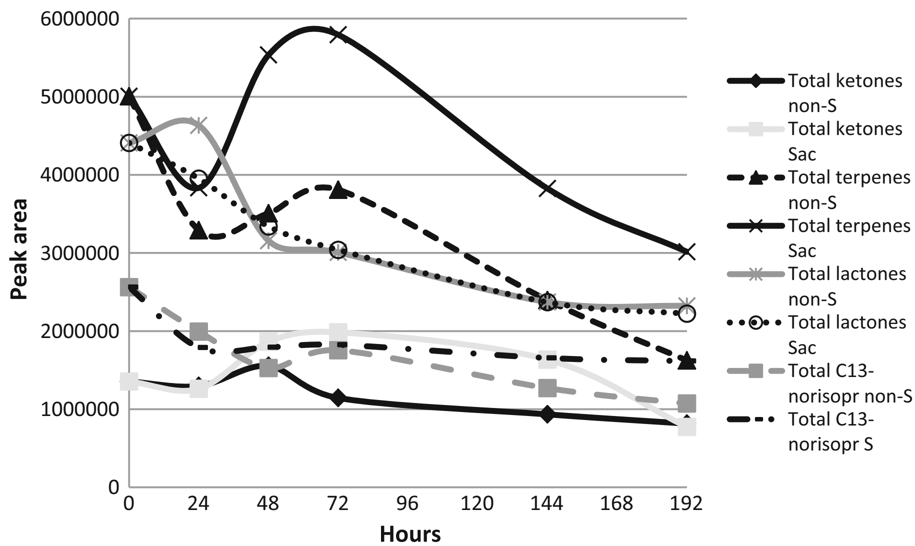


Fig. 2 Evolution of total area of ketones, terpenes and lactones in fermentation processes with *L. thermotolerans* and *S. cerevisiae*: *diamond* ketone *L. thermotolerans*, *square* ketone *S. cerevisiae*, *traingle* terpenes *L. thermotolerans*, *cross* terpenes *S. cerevisiae*, *asterisks* lactones *L. thermotolerans*, *circle* lactones *S. cerevisiae*, *grey square* C_{13}-norisoprenoids *L. thermotolerans*, *dots* C_{13}-norisoprenoids *S. cerevisiae*

Acetals

The total content of acetals increased along fermentation. This increase was higher for *S. cerevisiae* than *L. thermotolerans* strain, reaching significant different values at 48 h after inoculation. Three different acetals were determined. Acetaldehyde diethyl acetal and acetaldehyde ethyl amyl acetal increased in both fermentation processes reaching to maximum area values at 144 h after inoculation. *Saccharomyces* strain produced considerable increased being the highest amount for the first compound four times than that produced by the other strain at the final sampling point.

On the other hand, an opposite trend between both strains was observed for 2,4,5-trimethyl-1,3-dioxolane that decreased with the use of non-*Saccharomyces* strain and increased with the *Saccharomyces* one.

Acids

Regarding acids, after 192 h of fermentation the overall balance was increased for of acidity for wines produced by *Saccharomyces* strain and a decrease for wines produced by non-*Saccharomyces*. Although these compounds have unpleasant aromas (Beckner et al. 2015), they are precursors of esters which provided fruity aromas to wines. Saerens et al. (2006) verified that the addition of hexanoic or octanoic acid to the fermentation medium caused a strong increase in the formation of the corresponding ethyl ester.

The evolution of each acid throughout fermentation was very different among compounds and between strains. Pentanoic acid clearly diminished in both cases. However, contrary trends between strains were observed specially for octanoic, decanoic and hexanoic acids. In the case of

Saccharomyces strain, the highest increase was for octanoic acid.

Similar results were reported by Gobbi et al. (2013) and Beckner et al. (2015), who found higher volatile acidity and total amount of carboxylic acids in wines produced by *S. cerevisiae* than those by *L. thermotolerans*.

Alcohols

During fermentation, an increase in alcohols was observed. As expected, the alcohol that underwent the highest augmentation was ethanol, with the most important change between 24 and 48 h. The use of non-*Saccharomyces* yeast to produce wine with reduced alcohol content was reported earlier (Contreras et al. 2015; Quiros et al. 2014). Gobbi et al. (2013) reported that *L. thermotolerans* as little ethanol producers. However, in our study the same rate of ethanol production for both yeasts was observed and there were no significant differences between any of the stage analysed between strains. This agreed with the above stated relation of sugar consumption and the origin of both autochthonous yeast which were isolated during the spontaneous fermentation of sun-dried grape must and, thus, have developed a great adaptation to high osmotic pressure media. In addition to ethanol, among 23 alcohols determined, other 6 alcohols increased, standing out 3-methyl-1-butanol and 2-phenyletanol. Most of these were higher alcohols which were produced by yeast involving degradation of an amino via the Ehrlich pathway (Ugliano and Henschke 2009).

The alcohol global augmentation were significantly higher when the fermentation was carried out by non-*Saccharomyces* strain (significant different at T144 and T192). It seemed to be due to the higher increase of 3-methyl-1-butanol in this process. Moreover, some authors have reported higher production of 2-phenyletanol by *L. thermotolerans* (Gobby et al. 2013), in our case, it was observed in last fermentation stages (6 and 8 days), where the peak areas were two time higher in wine produced by aforesaid strain.

On the contrary, some alcohols decreased, especially, 1-hexenol and 1-octen-3-ol. The decrease was more pronounced between 24 and 48 h.

Aldehydes

Regarding total sum of aldehydes, the values followed a similar trend in both types of fermentations, decreasing significantly until 48 h.

Most aldehydes reached relative peak area under detection limits at 24 h from inoculation. Only furanic aldehydes, cinnamaldehyde and benzaldehyde presented quantifiable values at all sampling points throughout the fermentation process.

Acetic esters

The acetic esters are compounds where the acyl group is derived from acetate (in the form of acetyl-CoA), and the alcohol group is ethanol or a complex alcohol (Cordente et al. 2012). During alcoholic fermentation, these are synthesised by different alcohol acetyltransferases (Ugliano and Henschke 2009).

In present study, these compounds increased especially during fermentation by *Saccaromyces* strain. The changes were significant until 72 h from inoculation, after that, a decrease was observed. Non-*Saccharomyces* strain showed less pronounced increase which continued until the sampling point of 144 h, thus a good correlation between relative area values and the time was observed (0.949). Overall, acetic esters content in all the stages were significant higher for *Saccharomyces* strain. The difference observed between strains may be probably due to the high values of relative area accounted for compounds such as hexyl and 2-phenylethanol acetate and to the six acetic esters that were formed by *Saccharomyces* strain only. Among all the acetic esters determined, the highest increase was accounted by isoamyl acetate for both strains, the most relevant acetates of the wines.

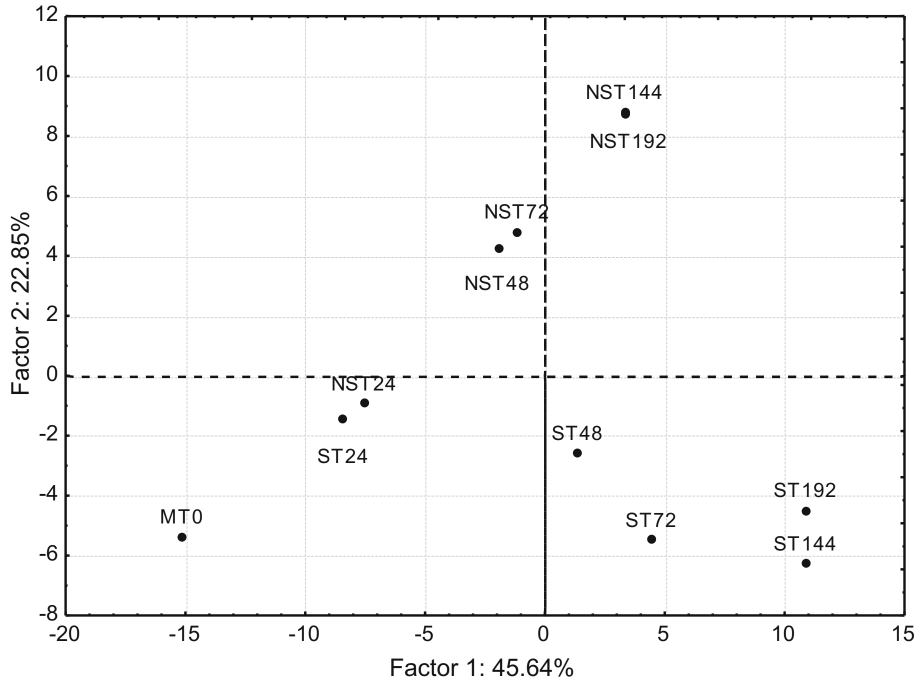
Most acetic esters have pleasant fruity and flower aromas (Lilly et al. 2006), however, ethyl acetate provides solvent and glue odour (Callejón et al. 2008). This compound presented area values significantly higher after 144 and 192 h of fermentation by *L. thermotolerans,* as observed by Gobbi et al. (2013). Although non-*Saccharomyces* strain produced higher amount of 2-phenylethanol (approximately two-fold), the values of the corresponding acetate reached area values 20 times higher in wines obtained by *Saccharomyces* at the final stages of alcoholic fermentation.

Ethyl esters

Ethyl esters are formed by ethanol and an acyl group derived from activated medium-chain fatty acids (Cordente et al. 2012). During *Saccharomyces cerevisiae* fermentation, the formation of the ethyl esters has been attributed to two acyl-CoA:ethanol O-acyltransferase enzymes (Saerens et al. 2008).

As mentioned above, the primary difference between the two yeast strains studied was the different rate of production of ethyl esters. After 48 h from inoculation, the values of total area of ethyl esters were significantly higher for *Saccharomyces* strain, being more than 15 times higher at the two last sampling points (T144 and T192). Beckner

Fig. 3 Data scores of all samples plot on the plan made up of the first two principal components (PC1 against PC2)



et al. (2015) also observed a considerable difference between the amount of ethyl esters produced by *Sacharomyces* and *Lachancea* yeast strains.

Thus, it led us to think that *S. cerevisiae* probably produced more amount of ethanol than *L. thermotolerans* but it was in form of ethyl ester, so that no differences were observed in ethanol production between strains.

Moreover, the evolution of these values was different for both yeast strains, during alcoholic fermentation by *Lachancea* strain a significant increase was observed until 48 h from inoculation. However, *Saccharomyces cerevisiae* produced ethyl esters continuously throughout the fermentation, for that, the correlation coefficient between total area of ethyl esters and the fermentation time was 0.954. The increase observed between each sampling point were statistically significant.

Thus, the values of peak area were higher for *Sacharomyces* yeast for the most of these compounds except to ethyl propanoate or ethyl 2-methylpropanoate.

During alcoholic fermentation carried out by *S. cerevisiae*, the highest increase was observed for ethyl octanoate and ethyl decanoate. Other remarkable increments were observed for ethyl hexanoate, ethyl dodecanoate and ethyl 9-decanoate.

In the case of *L. thermotolerans*, the ethyl decanoate was the ester that showed a greater increase during the fermentation, but in a much lesser extent than in fermentation by *S. cerevisiae*.

Since most of determined esters in this study have fruity aromas, probably wines produced using *Saccharomyces* strain may have more fruity aroma than those produced with *Lachancea* strain.

Others esters

In this study, we have also determined others esters formed by alcohols such as methanol, isoamyl alcohol, propanol and isobutanol, previously reported in wines (Beckner et al. 2015; Suklje et al. 2016). Different behaviour with respect to these compounds was also observed between both yeast strains tested. The total areas of these esters were significantly higher for *S. cerevisiae* than for *L. thermotolerans* in all sampling points from 48 h. On the contrary, isoamyl esters did not increase to a greater extent during fermentation carried out by *Lachancea*, methyl esters became non detectable in most of cases and, the only isobutyl ester determined was isobutyl decanoate.

Within this group of esters, *S. cerevisiae* caused the most considerable increase in isoamyl esters, being the total areas changed significant from 48 to 144 h. Moreover, for *S. cerevisiae*, the formation of esters derived from octanoic acid was more clearly over the others (isoamyl, methyl, propyl and isobutyl octanoate).

Ketones, lactones, C_{13}-norisoprenoids and terpenes

Most of compounds included in this section came from the grapes (Ribéreau-Gayon et al. 2006). They may be present as glycosylated flavourless precursors, such as terpenes and

Table 4 Variables with high contribution to factor 1 and 2 in PCA, their loading values and sample groups with which these are correlated

Volatile compounds	Sample group	Loading values	
		F1	F2
Isovaleric acid	MT0, NST24 and ST24	−0.073760	−0.772098
1-Pentanol[g]	MT0, NST24 and ST24	−0.937031	−0.296206
1-Hexanol	MT0, NST24 and ST24	−0.931785	−0.297974
1-Octen-3-ol	MT0, NST24 and ST24	−0.947265	−0.289980
1-Heptanol	MT0, NST24 and ST24	−0.918735	−0.279695
2-Ethyl-1-hexanol	MT0, NST24 and ST24	−0.833305	−0.327027
1-Octanol	MT0, NST24 and ST24	−0.722133	−0.633091
cis-2-Octen-1-ol	MT0, NST24 and ST24	−0.903878	−0.366522
Benzyl alcohol	MT0, NST24 and ST24	−0.948099	−0.270730
2-Furfuraldehyde	MT0, NST24 and ST24	−0.881613	−0.358810
Cinnamaldehyde	MT0, NST24 and ST24	−0.917130	−0.040580
2-Pentanone	MT0, NST24 and ST24	−0.855324	−0.324833
2-Pentylfuran	MT0, NST24 and ST24	−0.899011	−0.271896
3-Octanone	MT0, NST24 and ST24	−0.915904	−0.067750
2-Octanone	MT0, NST24 and ST24	−0.845447	−0.325230
trans-2,2-Pentenyl-furan	MT0, NST24 and ST24	−0.921479	−0.281489
6-Methyl-5-hepten-2-one	MT0, NST24 and ST24	−0.204803	−0.798575
6-Methyl-3,5-heptadiene-2-one	MT0, NST24 and ST24	−0.928428	−0.290765
γ-Butyrolactone	MT0, NST24 and ST24	−0.928418	−0.274416
β-Damascenone	MT0, NST24 and ST24	−0.909969	−0.366544
1R-α-Pinene	MT0, NST24 and ST24	−0.558796	−0.619010
Cymene	MT0, NST24 and ST24	−0.892236	−0.335194
2-Bornene	MT0, NST24 and ST24	−0.944324	−0.309547
Linalool	MT0, NST24 and ST24	−0.836326	−0.387011
Coumaran	MT0, NST24 and ST24	−0.165912	−0.885934
n.i. (m/z 67, 85, 151)	MT0, NST24 and ST24	−0.517886	−0.685602
Acetaldehyde ethyl amyl acetal	ST48, ST72, ST144 and ST192	0.819661	−0.109366
Acetic acid	ST48, ST72, ST144 and ST192	0.252113	−0.805874
Propanoic acid	ST48, ST72, ST144 and ST192	0.094181	−0.623983
Hexanoic acid	ST48, ST72, ST144 and ST192	0.346509	−0.905755
Octanoic acid	ST48, ST72, ST144 and ST192	0.692898	−0.681746
Decanoic acid	ST48, ST72, ST144 and ST192	0.727791	−0.604349
1-Propanol	ST48, ST72, ST144 and ST192	0.920658	−0.026868
4-Ethylbenzyl alcohol	ST48, ST72, ST144 and ST192	0.597740	−0.638649
1-Decanol	ST48, ST72, ST144 and ST192	0.590222	−0.636173
Propyl acetate	ST48, ST72, ST144 and ST192	0.925594	−0.128528
Isoamyl acetate	ST48, ST72, ST144 and ST192	0.874614	−0.211771
Amyl acetate	ST48, ST72, ST144 and ST192	0.586742	−0.624018
Hexyl acetate	ST48, ST72, ST144 and ST192	0.508613	−0.609353
Heptyl acetate	ST48, ST72, ST144 and ST192	0.448069	−0.582699
Octyl acetate	ST48, ST72, ST144 and ST192	0.576516	−0.627550
Nonyl acetate	ST48, ST72, ST144 and ST192	0.574227	−0.624231
Decyl acetate	ST48, ST72, ST144 and ST192	0.746226	−0.642165
2-Phenylethanol acetate	ST48, ST72, ST144 and ST192	0.570674	−0.573411
Ethyl butyrate	ST48, ST72, ST144 and ST192	0.884764	−0.133885
Ethyl hexanoate	ST48, ST72, ST144 and ST192	0.793592	−0.597680
Ethyl heptanoate	ST48, ST72, ST144 and ST192	0.878184	−0.376239
Ethyl octanoate	ST48, ST72, ST144 and ST192	0.769181	−0.604613

Table 4 continued

Volatile compounds	Sample group	Loading values	
		F1	F2
Ethyl 7-octenoate	ST48, ST72, ST144 and ST192	0.819209	−0.346015
Ethyl undecanoate	ST48, ST72, ST144 and ST192	0.737432	−0.588533
Isoamyl hexanoate	ST48, ST72, ST144 and ST192	0.743734	−0.637847
Methyl hexanoate	ST48, ST72, ST144 and ST192	0.541028	−0.641413
Methyl decanoate	ST48, ST72, ST144 and ST192	0.635654	−0.697817
Propyl hexanoate	ST48, ST72, ST144 and ST192	0.727725	−0.671690
Propyl octanoate	ST48, ST72, ST144 and ST192	0.752368	−0.645882
Isobutyl hexanoate	ST48, ST72, ST144 and ST192	0.693044	−0.670428
Isobutyl octanoate	ST48, ST72, ST144 and ST192	0.750106	−0.624371
TDN	ST48, ST72, ST144 and ST192	0.418331	−0.738714
Citronellol	ST48, ST72, ST144 and ST192	0.877780	−0.324558
n.i. (m/z 69, 93, 121)	ST48, ST72, ST144 and ST192	0.581783	−0.628157
3-Methylthio-1-propanol	NST48 and NST72	−0.007238	0.652629
Acetofenone	NST48 and NST72	−0.931971	0.104946
Ethanol	NST144 and NST192	0.923472	0.251117
Isobutanol	NST144 and NST192	0.280307	0.860025
2-Methyl-1-butanol	NST144 and NST192	0.868649	0.436190
3-Methyl-1-butanol	NST144 and NST192	0.444480	0.808629
6-Methyl-5-hepten-2-ol	NST144 and NST192	0.357365	0.703500
2-Phenylethanol	NST144 and NST192	0.644828	0.588573
Ethyl acetate	NST144 and NST192	0.617928	0.574295
Ethyl propanoate	NST144 and NST192	0.264100	0.835185
Ethyl 2-methylpropanoate	NST144 and NST192	0.166192	0.932632
Ethyl phenylacetate	NST144 and NST192	0.364038	0.729613
Ethyl tetradecanoate	NST144 and NST192	0.847124	0.055234
Isoamyl propionate	NST144 and NST192	0.519286	0.655810
Roseoxide	NST144 and NST192	0.444037	0.694096
n.i. (m/z 59, 43)	NST144 and NST192	0.834766	0.393159

C_{13}-norisoprenoids and they were released by enzymatic hydrolysis during alcoholic fermentation.

Nevertheless, several authors have reported that neither *Sacharomyces cerevisiae* (Van Rensburg et al. 2005) nor *Lachancea thermotolerans* (Comitini et al. 2011) seemed to have glycosidase activity.

In our assays, the overall changes in total areas of these groups of compounds were significantly decreased between initial and final values (192 h). The evolution of total area for each group was fluctuating for both strains and only a similar trend was observed for terpenes (Fig. 2).

Despite the downward trend of terpenes, we observed that three of them, roseoxide, 3,7-dimethyl-6-octen-1-ol and nerolidol, increased using both yeasts. The first one especially in the case of *L. thermotolerans* and the last two when fermentations was carried out by *S. cerevisae.*

Volatile phenols

Regarding volatile phenols, the behaviour of these strains was also different, especially for 4-vinylguaiacol. This compound increased significantly from 48 h onwards when alcoholic fermentation was carried out by *Saccharomyces*. This yeast can synthesize 4-vinylguicacol during fermentation (Coghe et al. 2004).

Principal component analysis

Principal component analysis (PCA) was applied to data. The first three principal components explained 81.76% of cumulative variance. Figure 3 shows how the samples are separated into the plan formed by two first components. In this Figure, it can clearly be seen that the differences in volatile profile of samples produced by the two yeasts are considerably different from 48 h of inoculation. Thus, the initial and at 24 h samples for *S. cerevisiae* as well as for *L. thermotolerans* are together in the same quadrant (second one). The PC1 separates these samples from the rest of those obtained using *S. cerevisiae*, placed all in the third quadrant. Finally, the samples belonging to fermentations carried out by *L. thermotolerans,* from 48 to 192 h, are separated from *S. cerevisiae* by PC2. Table 2 showed the variables that are more correlated with these three groups according to their loadings. For instance, initial samples are correlated with most of aldehydes, terpenes and ketones and samples from *Saccharomyces* fermentations with most of acids and all kind of esters. Moreover, the variables that contributed more to the two first components with their loading values are shown in Table 4.

Conclusions

HSSE method allows for monitoring a large number of compounds throughout fermentation. Thus, these results point out the HSSE as useful non-invasive method to study the evolution of volatile compounds during fermentation processes. It could be used to establish the optimal point to stop the fermentation according to volatile profile and moreover, it could be very useful to study the aroma evolution in co-inoculation assays and sequential inoculation, which are of great interest currently.

In this study, considerable changes in volatile compounds were observed from substrate to final sampling point. The two strains used had a similar capacity to ferment a must with high sugar content. However, they resulted into the wines with different aroma. *S. cerevisiae* produced higher amount of volatile compounds than *L. thermotorelans*. Moreover, wines produced by *S. cerevisiae* strain were richer in esters imparted fruity aroma. This showed that this strain could produce wines with better aromatic and volatile profile than those produced by non-*Saccharomyces* strain.

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