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ORIGINAL ARTICLE

Untangling the wine metabolome by combining untargeted SPME–GCxGC-TOF-MS and sensory analysis to profile Sauvignon blanc co-fermented with seven different yeasts

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Abstract *Saccharomyces cerevisiae* (*SC*) is the main driver of alcoholic fermentation, however for aroma and flavor formation in wine, non-*Saccharomyces* species can have a powerful effect. This study aimed to compare untargeted volatile compound profiles from SPME–GCxGC-TOF-MS and sensory analysis data of Sauvignon blanc wine inoculated with six different non-*Saccharomyces* yeasts followed by *SC*. *Torulaspora delbrueckii* (*TD*), *Lachancea thermotolerans* (*LT*), *Pichia kluyveri* (*PK*) and *Metschnikowia pulcherrima* (*MP*) where commercial starter strains, while *Candida zemplinina* (*CZ*) and *Kazachstania aerobia* (*KA*), were isolated from wine grape environments. Each wine showed a distinct profile both sensorially and chemically. *SC* and *CZ* wines were the most distinct in both of these cases. *SC* wine had guava, grapefruit, banana, and pineapple aromas while *CZ* wine was driven by fermented apple, dried peach/apricot, and stewed fruit as well as sour flavor. Chemically over 300 unique features were identified as significantly different across the fermentations. *SC* wine had the highest number of esters in the highest relative concentration but all the yeasts had distinct ester profiles. *CZ* wine displayed the highest number of terpenes in high concentration but also produced a large amount of acetic acid. *KA* wine was high in ethyl acetate. *TD* wine had fewer esters but three distinctly higher thiol compounds. *LT* wine showed a relatively high number of increased acetate esters and certain terpenes. *PK* wine had some off odor compounds while the *MP* wine had high levels of methyl butyl-, methyl propyl-, and phenethyl esters. Overall, this study gives a more detailed profile of these yeasts contribution to Sauvignon blanc wine than previously reported.

Keywords Non-*Saccharomyces* · SPME–GCxGC-TOF-MS · Sensory · Sauvignon blanc

1 Introduction

Wine has been consumed by humans for thousands of years and for the majority of that time it was produced by crushing grapes and allowing them to ferment using the organisms present on the grapes and in the surrounding environment. There was relatively little a winemaker could do to control the quality of the final product. However, after Louis Pasteur discovered that yeasts were responsible for the conversion of sugars into ethanol more than 150 years ago, the wine industry slowly began to move away from its more unpredictable production methods (Hutkins 2006). The use of spontaneous fermentation for example slowly gave way to intentional inoculation of meticulously selected and maintained *Saccharomyces cerevisiae* starter cultures to ensure a more consistent and predictable product vintage to vintage. We now understand that wine is the result of a complex biological process that takes place

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between grapes, microorganisms (yeasts, bacteria and fungi), vinification and the wine cellar environment (Fleet 2003). Of all the yeasts found to be associated with the winemaking process, *S. cerevisiae* is indeed by far the most capable and reliable ethanol producer. When it is inoculated at high cell density, it can drastically reduce the chances of stuck fermentation or the production of off-flavors that can come from the unwanted growth of other organisms (Fleet 1993). This simply owes to the fact that it can rapidly outcompete other yeast and bacterial species as well as quickly produce an environment inhospitable to most other organisms primarily through the production of ethanol. However, while this may reduce sources of microbial spoilage, some winemakers feel that this has resulted in a lack of organoleptic complexity. It has been shown in recent years that certain indigenous non-*Saccharomyces* yeasts can contribute to distinct regional and desirable characteristics of wine when inoculated at high concentrations (Jolly et al. 2006).

This has prompted an interest in beginning to understand the specific influences of non-*Saccharomyces* yeasts in winemaking (Andorrà et al. 2010, 2012; Benito et al. 2015; Ciani and Comitini 2010a; Comitini et al. 2011; Dashko et al. 2015; Jolly et al. 2014; Sadoudi et al. 2012; Sun et al. 2014; Zott et al. 2011). Even though the impact of non-*Saccharomyces* yeasts is usually limited because of the fast fermentative metabolism of *S. cerevisiae*, research has shown that this impact may be enhanced when non-*Saccharomyces* yeasts are inoculated at high cell density. However, because most non-*Saccharomyces* yeasts cannot ferment to dryness, *S. cerevisiae* must also be inoculated along with the non-*Saccharomyces* yeast when they are used intentionally. Two modes of inoculation are usually envisaged: staged (sometimes called sequential) and co-inoculations. In co-inoculation, all yeasts are added to the must at the same time while in staged inoculation, the non-*Saccharomyces* yeasts are added first, allowed to ferment for a given amount of time, and the *Saccharomyces* yeasts are added to finish the fermentation. Staged inoculations are of particular interest since they can ostensibly allow for even greater control over the species fermentation progress and thus the aroma and flavor profile of a fermentation. Both strategies have been shown to mimic the results of natural fermentations in having more complex aromas (Ciani and Maccarelli 1998; Romano et al. 2003a). The principal outcomes of fermentations conducted with the aid of non-*Saccharomyces* yeasts have been documented in literature already mentioned here. Nevertheless, the description of the impact of these yeasts is usually restricted to a few specific attributes such as enzyme, acetic acid, glycerol, ethyl acetate, and higher alcohol production (Andorrà et al. 2012; Charoenchai et al. 1997; Clemente-Jimenez et al. 2004; Gobbi et al. 2013; Pina et al. 2004;

Rojas et al. 2001; Romano et al. 2003b; van Breda et al. 2013; Villena et al. 2007; Wang et al. 2015).

There are between 9 and 15 different yeast genera that are typically reported to be associated with the winemaking process (Johnson and Echavarri-Erasun 2011). Many of these were originally studied in the context of spoilage but this work slowly began to shed light on some potentially beneficial aspects of these yeasts. For example, early work showed that the *Candida, Debaryomyces, Hanseniaspora, Hansenula, Kloeckera, Metschnikowia, Pichia, Saccharomyces* and *Torulaspora* genera isolated from wines could produce extracellular enzymes such as pectinases, amylases, lipases, proteases and glucosidases (Charoenchai et al. 1997). *β*-glucosidases are of particular interest for their ability to liberate otherwise bound terpenes and thus have a direct impact on wine aroma. This work was expanded on and complemented by investigations of the specific behaviors of certain species in grape must (Ciani and Maccarelli 1998; Esteve-Zarzoso et al. 1998). Studies began to characterize the macronutrient consumption as well as macromolecule production in single and mixed fermentations. This in turn gave way to more targeted studies of the potential impact of specific yeast (Andorrà et al. 2012; Anfang et al. 2009; Azzolini et al. 2012; Ciani et al. 2006; Clemente-Jimenez et al. 2004, 2005; Dias et al. 2003; Gobbi et al. 2013; Pina et al. 2004; Romano et al. 2003a, b; Wang et al. 2015; Zott et al. 2008). Based on this research, commercial non-*Saccharomyces* starter cultures have recently been developed for use in wine production and are comprised of the following yeast species: *Torulaspora delbrueckii, Lachancea thermotolerans, Pichia kluyveri* and *Metschnikowia pulcherrima*. Nevertheless, compared to *S. cerevisiae*, little research has been conducted that can indicate specifically what metabolic profiles to expect from these yeasts under various fermentation conditions. Indeed, though the mounting evidence supports the use of these yeasts to help improve wine aroma, the majority of the previously mentioned studies are somewhat limited in scope. They focus either on enzyme production or target ester and alcohol production and only Gobbi et al. (2013) complemented their targeted chemical analysis of *L. thermotolerans* and *S. cerevisiae* co-fermentation with sensory work. Therefore there is still a knowledge gap on the impact of these yeasts during wine fermentations.

In this study we specifically compared untargeted volatile compound profiles and sensory analysis data of Sauvignon blanc wine fermented sequentially with six different non-*Saccharomyces* yeasts. Of the six non-*Saccharomyces* species used, four were commercial starter strains, *Torulaspora delbrueckii, Lachancea thermotolerans, Pichia kluyveri*, and *Metschnikowia pulcherrima,* while the other two, *Candida zemplinina,* and *Kazachstania aerobia,* are laboratory strains. The goal of this study

was to expand on previous work where only the profile of the non-*Saccharomyces* yeasts in single fermentation were characterized (Beckner Whitener et al. 2015). This study completed the wine fermentations through the addition of *S. cerevisiae* in order to gain a better understanding of the aroma compounds present in the final wine following the use of the selected non-*Saccharomyces* yeasts in sequential inoculation. The potential metabolic implications, as well as how these compounds might contribute to the perceived sensory attributes of the finished wine product were assessed.

2 Materials and methods

2.1 Grapes, yeasts, and chemicals

Sauvignon blanc grapes (vintage 2014) were obtained from the vineyards at Welgevallen Experimental Farm, Stellenbosch University, Stellenbosch, South Africa. The clone was SB 316 and rootstock was R110, the vineyard was planted in 1991. The trellis system used was a seven wire hedge trellis with moveable foliage wires and grapevines were spaced at 2.7 × 1.5 m with a east–west row direction. Grapevines were unilateral cordon-trained and spur pruning was applied. The grapevines were not irrigated and the vineyard was established on a duplex Hutton/Glenrosa soil according to the 1992 South African Binomial Soil Classification system. *S. cerevisiae* (Enoferm M2®, Lallemand Inc., Quebec, Canada), *T. delbrueckii* (Biodiva®, Lallemand Inc., Quebec, Canada), *M. pulcherrima* (Flavia®, Lallemand Inc., Quebec, Canada), *P. kluyveri* (Viniflora® FROOTZEN™, Chr. Hansen, Horsholm, Denmark), *L. thermotolerans* (Viniflora® CONCERTO™, Chr. Hansen, Horsholm, Denmark), *C. zemplinina* (Institute of Wine Biotechnology (IWBT)-Y1082) and *K. aerobia* (IWBT-Y845) were used. Twenty-milliliter glass screw cap vials, sodium chloride (ACS grade), sodium azide, internal standard 2-octanol, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coating 50/30 μm, 2-cm length SPME fiber was purchased from Supelco by Sigma-Aldrich S.r.l., Milan, Italy.

2.2 Winemaking procedure

Fermentations were carried out using Sauvignon blanc grape must. The must was evaluated for initial sugar (21.7 Brix), titratable acidity (5.8 g/L) and yeast assimilable nitrogen (YAN) (170 mg/L) content, as well as pH (3.39). YAN was adjusted by adding 40 mg/L of diammonium phosphate (DAP) to the must. The yeasts were grown in yeast extract peptone dextrose (YPD) medium (Biolab-Merck, Wadeville, South Africa). They were shaken to ensure aerobic conditions at 30 °C in successively larger batches using a 1 % transfer rate starting from 10 mL and ending at 1 L at which point necessary cell concentrations for wine inoculation were obtained via centrifugation. The 11 L stainless steel fermentation vessels containing 10 L of must were inoculated with a volume of yeast determined from the pre-culture by plate count and optical density to obtain a level of 10^6 cfu/mL. The inoculation levels were confirmed and yeast growth monitored via plate count on WL Nutrient agar (Fluka, Sigma-Aldrich) which allows for visual differentiation of the yeast strains. Fermentations were carried out in triplicate at 15 °C. The non-*Saccharomyces* yeasts were allowed to ferment until approximately 2 % ethanol concentration was reached. At this point, *S. cerevisiae* was added at 10^6 cfu/mL concentration to finish the fermentations after being grown up in the same manner as the non-*Saccharomyces* yeasts. Samples were taken daily to track fermentation progress via plate count and by Fourier-transform mid-infrared spectroscopy (FOSS WineScan FT120, Foss Analytical, Denmark) in accordance with the protocol outlined in Nieuwoudt et al. (2006). The apparatus measured levels of glucose, fructose, titratable acidity, volatile acidity, pH, acetic acid and malic acid. The final wines were bottled after clarification via cold rest for 1 week at −4 °C in 750 mL glass bottles with screw caps. Wines were then transported to the laboratory of the Department of Food Quality and Nutrition, Research and Innovation Center, Fondazione Edmund Mach (FEM) for chemical analysis. Sensory analysis was performed at the Department of Viticulture and Oenology, Stellenbosch University.

2.3 Sensory evaluation

General Descriptive Analysis was used as the method to evaluate the experimental wines. A panel of 10 judges was selected; all had moderate to good experience in wine evaluation, in particular Sauvignon blanc. The panel was composed of eight females ranging in age from 25 to 55; and two males (aged about 25). A session was completely dedicated to the taste component of the wines and the panel was trained on sweetness, acidity, bitterness and astringency intensities. For this purpose, a commercial Sauvignon blanc wine was spiked with increasing levels of sugar, tartaric acid, quinine and alum respectively. All were over the counter items purchased at a local grocery store. To score the intensity of the attributes of the experimental wines a 100-mm unstructured scale was used, demarked with 'None' and 'Intense' at the extreme left and right sides, respectively. Panel performance was evaluated using Panel Check (Tomic et al. 2009). The descriptive study was performed in two sessions. Panelists were asked to taste in isolated booths and each treatment was presented

to them covered in ISO black glasses and marked with three-digit codes. A complete Block Design was used to randomise the distribution of the wines presented to the panellists (Lawless and Heymann 2010). Each judge evaluated each treatment in triplicate.

2.4 SPME extraction and GCxGC-TOF-MS analysis

Vials were prepared as follows: 5 mL of wine and 50 µL of 0.5 mg/L 2-octanol were added to 20 mL screw cap vials containing 1.5 g NaCl. A Gerstel MPS autosampler (GERSTEL GmbH & Co. KG) equipped with the standard sample agitator and SPME fiber conditioning station was used to extract the volatiles from the sample vial head-space. GCxGC-TOF-MS analysis of the extracts was performed using a LECO Pegasus-4D system consisting of an Agilent 6890N (Agilent Technologies) coupled to a LECO Pegasus 4D detector. The system employed a consumables free modulation system. The samples were incubated for 5 min at 35 °C under 500 rpm rotation at 10 s intervals. Extraction took place for either 10 s, 5 min, or 30 min prior to desorption in the inlet for 180 s at 250 °C. Quality control (QC) vials containing an equal mix of all wines were spaced at the beginning and every third sample thereafter within each time batch. Each extraction time consisted of only one batch as all samples and spaced QCs fit into a single cooling tray. Helium carrier gas was used with a flow set at 1.2 mL/min and a splitless time of 180 s. The oven was equipped with a 30 m x 0.25 mm × 0.25 µm VF-WAX MS primary column (Agilent Technologies) and a 1.5 m × 0.15 mm × 0.15 µm RXI 17Sil MS secondary column (Restek Corporation, Bellefonte, PA, USA). The GC oven parameters were as follows: initial temperature was 40 °C held for 2 min, followed by an increase to 250 °C at a rate of 6 °C/min, the oven was then held at 250 °C for 5 min before returning to the initial temperature (40 °C). The total cycle time, was 42 min. The modulation period was set to 7 s with a hot pulse time of 1.4 s. The modulator was offset by 15 °C. The MS protocol consisted of electron ionization at 70 eV with ion source temperature at 230 °C, a detector voltage of 1543 V with a voltage offset of 200 V, mass range of m/z 35–350, and acquisition rate of 200 spectra per second. There was an acquisition delay of 120 s.

2.5 Data processing and alignment

ChromaTOF software version 4.32 was used to perform baseline correction, deconvolution and peak picking of the raw data. The baseline offset was set to 1, just above the noise level. The first dimension peak width was set to 43 s

while the second dimension peak width was set to 0.1 s. A factor of 500 was set as the match required to combine peaks in the second dimension. A signal to noise (S/N) of 10 was used for the 10 s and 5 min extraction times data with a minimum S/N of 6 for sub peak retention. A S/N of 100 was used for the 30 min extraction time data with a minimum S/N of 60 for sub peak retention. Traditional, not adaptive, integration was used. Forward library searching was used with the following parameters: Hits to return were set to 10, minimum molecular weight was set to 40, maximum molecular weight was set to 350, the mass threshold was set to 50 and the minimum similarity match was set to 700. The NIST and Wiley libraries were used to achieve level II identification as defined by Sumner et al. 2007. For alignment the following parameters were used: a mass threshold of 10, a minimum similarity match of 600, the maximum number of modulation periods matching peaks could be apart was set to 1, a maximum retention time difference was set to 7 s, for peaks not found by initial peak finding the signal to noise ratio was set to 5 for the 10 s and 5 min extractions and to 50 for the 30 min extractions, for analytes to be kept they had to be found in all biological replicates within a class. Each yeast species was given its own class.

2.6 Statistical analysis

Each extraction time, 10 s, 5 and 30 min, was treated as a separate data set in the following way. First, to avoid underestimation of the variance of the data, zero intensity values (undetected features) were replaced feature-wise by a random number between the lowest detected intensity and zero. Following this, for each feature, a fixed effects linear model was fitted with yeast strain as the fixed effect. This model was used for pairwise comparisons between all wines without correction for multiple testing. Subsequently, the collection of p-values for all comparisons were corrected for multiple testing by controlling the false discovery rate (FDR) and q-values calculated (Strimmer 2008a, b).

To select the compounds of interest (COI) a filter with three requirements was applied to the data. Compounds were selected if the q-values for any of the comparisons between any of the wines were below 0.05 and at least one comparison had a fold change greater than 2.5. In addition, the QC samples were used to calculate the relative coefficient of variance (%CV) for each feature across the whole analysis. Only features with %CV lower than 50 % in the QC samples were selected. A venn diagram was generated to illustrate this filtering process for each extraction time Fig. 1. The features that fell into the center of these diagrams were considered significant COIs for each extraction time. The peak area values for each of these compounds
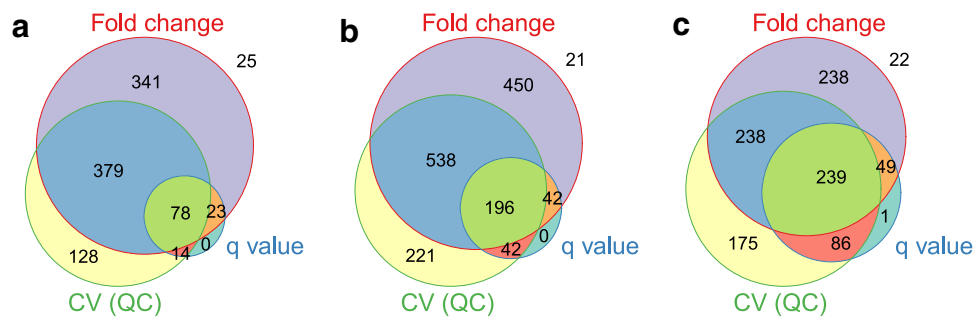
Fig. 1 Venn diagrams depicting the three extraction times **a** 10 s, **b** 5 min and **c** 30 min. The total number of features identified in each extraction time was 988 for (**a**), 1510 for (**b**), and 1048 for (**c**). The *center values* in each diagram represent the significant compounds of interest with q-values below 0.05, a fold change of 2.5 or higher and %CV in quality control samples lower than 50 %

were used to generate heat maps and PCA plots to better illustrate the data (Figs. 2, 3). Unit variance scaling was used for PCA and heat map generation as well as the values seen in Table 1 and Table 1S. Values outside the range of 3 standard deviations were reassigned to 3 in the case of the heat maps. The Pearson correlation coefficient and Ward's minimum variance method were used for hierarchical clustering (Murtagh and Legendre 2014). The PCA bi-plots from the sensory data were generated from the analysis performed using panel check (Fig. 3d).

3 Results and discussion

3.1 Fermentation progress and primary metabolite production

All fermentations progressed at slightly different rates. *S. cerevisiae* was the quickest fermenter, reaching 2 % ethanol in 3 days (Fig. 4). The first of the non-*Saccharomyces* fermentations to reach 2 % ethanol was *L. thermotolerans,* 4 days after inoculation. Next was *T. delbrueckii* 1 day later followed by *C. zemplinina* on day six. *K. aerobia* and *M. pulcherrima* each took 7 days and the *P. kluyveri* was the slowest at 8 days. The order of fermentation speed is comparable to results in our previous study for *L. thermotolerans, T. delbrueckii,* and *M. pulcherrima* (Beckner Whitener et al. 2015). Once the fermentations reached 2 % ethanol, *S. cerevisiae* was added to complete the fermentations. The musts inoculated with only *S. cerevisiae* fermented to dryness in 12 days while the rest of the fermentations took between 19 and 24 days with *L. thermotolerans* again finishing first among the non-*Saccharomyces* fermentations. Almost all of the non-*Saccharomyces* fermentations showed a similar pattern of sugar consumption in which glucose was consumed faster than fructose. The *C. zemplinina* fermentation stood out in that it was the only fermentation in which fructose was consumed more rapidly than glucose even after *S.*

cerevisiae addition. This was not surprising since *C. zemplinina* is known to be fructophilic and able to survive to the end of fermentation due to its high ethanol tolerance (Rantsiou et al. 2012). It has also been reported that sequential inoculation of *C. zemplinina* produced a wine lower in acetic acid compared to a *S. cerevisiae* pure culture but this was not the case in our study (Englezos et al. 2015). Of all the fermentations conducted, the *C. zemplinina* fermentations produced the most acetic acid (1.37 g/L while the *T. delbrueckii* produced the least (0.07 g/L) (Fig. 5). Despite the relatively large amount of acetic acid in the *C. zemplinina* fermentations the sensory panel did not note an acetic acid fault in the wine. It is worth mentioning however, that the two fermentations that showed the highest amounts of acetic acid did score the closest to the 'sour' descriptor, those being *C. zemplinina* and *K. aerobia* (Fig. 3d). *L. thermotolerans* fermentations were characterized by the least amount of overall titratable acidity as well as the least amount of malic acid at the end of the fermentation. In fact, all of the co-fermentations had lower overall levels of malic acid than the *S. cerevisiae* control (Fig. 5). This confirms previous findings that *S. cerevisiae* is characterized as a poor metabolizer of L-malate (Salmon 1987). The other yeasts in this study have not been investigated for their L-malate metabolism or their ability to metabolize other TCA cycle intermediates as a sole carbon source (Saayman and Viljoen-Bloom 2006). Given the results in this study however it is likely that all of the non-*Saccharomyces* yeasts used here are able to transport and metabolize L-malate as has been shown for *Candida sphaerica*, *Candida utilis*, *Hansenula anomala*, *Pichia stipitis* and *Kluyveromyces marxianus* (Saayman and Viljoen-Bloom 2006).

Even though there were obvious differences in growth patterns and macro metabolite production, all wines did eventually reach approximately the same ethanol concentration of 14 % v/v. The presence of the non-*Saccharomyces* yeasts was monitored during the fermentations and it should be noted that the non-*Saccharomyces* yeast
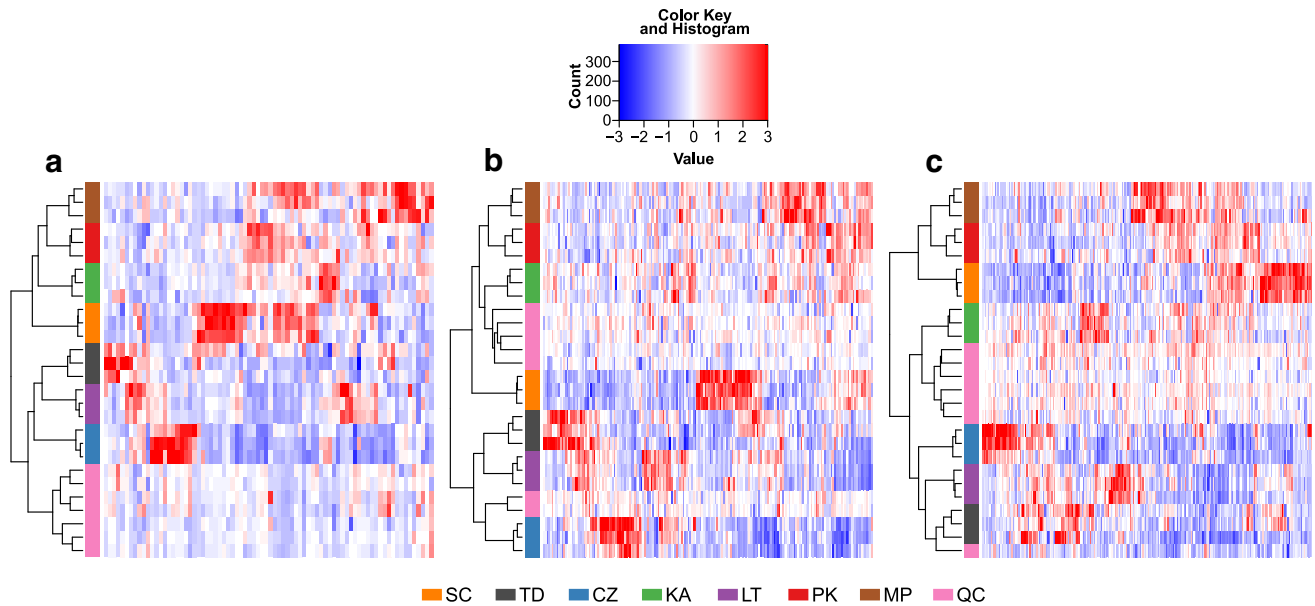
Fig. 2 Heat map of the Venn diagram center metabolites for the **a** 10 s, **b** 5 min, and **c** 30 min extraction time data. Ward's minimum variance was used for hierarchical clustering. *SC S. cerevisiae* fermentations, *TD T. delbrueckii* fermentations, *CZ C. zemplinina* fermentations, *KA K. aerobia* fermentations, *LT L. thermotolerans* fermentations, *PK P. kluyveri* fermentations, *MP M. pulcherrima* fermentations and *QC* quality control samples

Fig. 3 PCA after unit variance scaling of the Venn diagram center features for the **a** 10 s, **b** 5 min, and **c** 30 min extraction time data. **d** PCA bi-plots of the sensory data. *SC S. cerevisiae* fermentations, *TD T. delbrueckii* fermentations, *CZ C. zemplinina* fermentations, *KA K. aerobia* fermentations, *LT L. thermotolerans* fermentations, *PK P. kluyveri* fermentations, *MP M. pulcherrima* fermentations and *QC* quality control samples
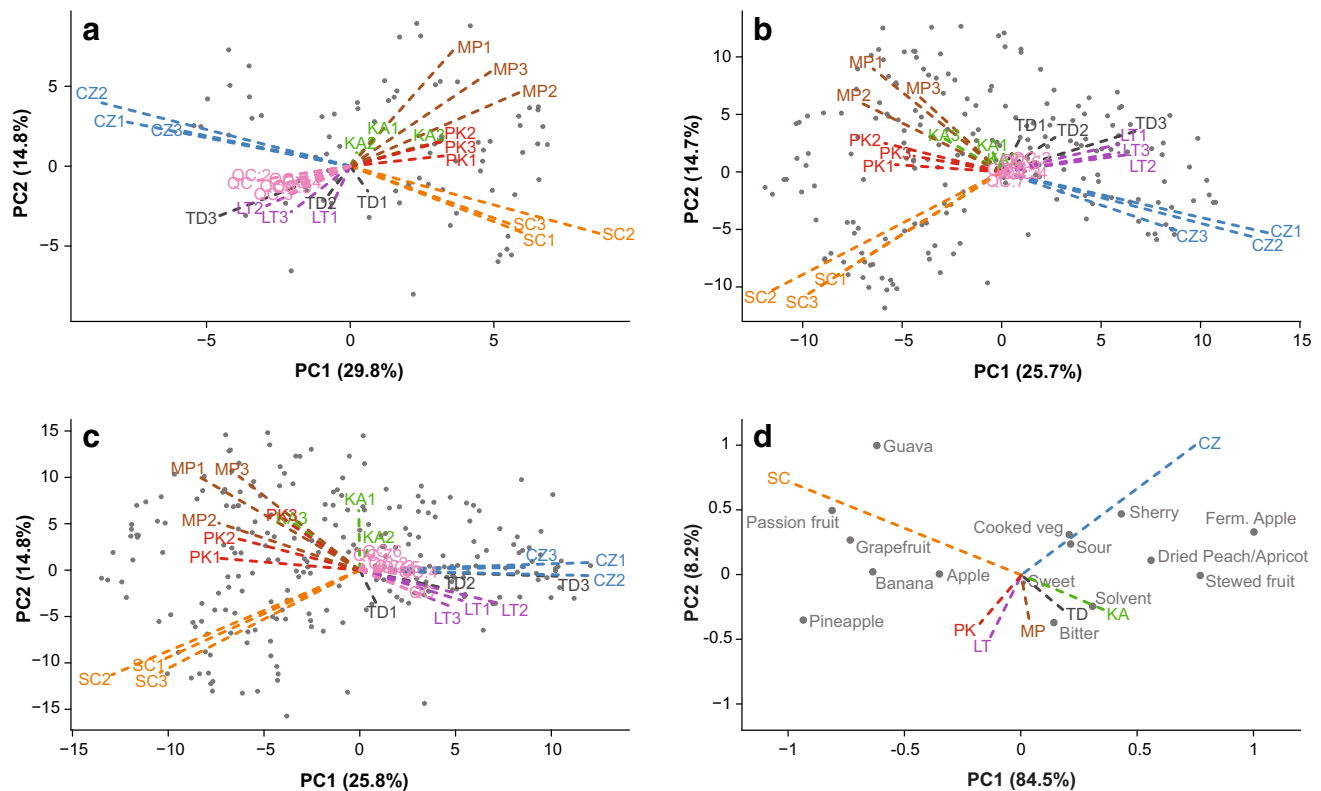
Table 1 Average peak areas after unit variance scaling for each metabolite found to be in highest relative concetration among the treatments across all extraction times

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT[i] (s)	2D RT[i] (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
CZ[a]	Acid	3-Methyl-butanoic acid	503-74-2	74	1156	1.35	Sour stinky feet sweaty cheese tropical	High	NF[h]	NF[h]	1.54 ± 0.30
	Acid	Cyclopentaneundecanoic acid	6053-49-2	124	911	2.73	NF[h]	NF[h]	NF[h]	NF[h]	1.41 ± 0.50
	Aldehyde	Octanal	124-13-0	57	673	2.47	Aldehydic waxy citrus orange peel green fatty	High	NF[h]	2.05 ± 1.31	NF[h]
	Aldehyde	α,4-Dimethyl-3-cyclohexene-1-acetaldehyde	29548-14-9	94	1247	3.39	Spicy herbal	High	NF[h]	1.64 ± 0.35	NF[h]
	Aldehyde	Decanal	112-31-2	57	953	2.69	Sweet aldehydic waxy orange peel citrus floral	High	NF[h]	1.99 ± 1.76	NF[h]
	Aldehyde	Nonanal	124-19-6	57	813	2.62	Waxy aldehydic rose fresh orris orange peel fatty peely	High	NF[h]	2.02 ± 1.49	NF[h]
	Amine	Phenethylamine	64-04-0	114	1317	2.23	Fishy	High	NF[h]	NF[h]	1.60 ± 0.22
	Ester	Ethyl 3-methylbutanoate	108-64-5	85	1009	1.80	Fruity sweet apple pineapple tutti frutti	High	NF[h]	NF[h]	2.27 ± 0.18
	Ester	3-(Methylthio)propyl acetate	16630-55-0	88	1107	2.10	Herbal mushroom cabbage asparagus potato	High	NF[h]	NF[h]	1.38 ± 0.46
	Ester	Octanoic acid, hexyl ester	1117-55-1	145	1310	3.35	Fruity green waxy berry apple ester	Medium	NF[h]	NF[h]	1.41 ± 0.16
	Ester	3-Methylbutyl octanoate	2035-99-6	127	1142	3.39	Sweet oily fruity green soapy pineapple coconut	Medium	1.73 ± 0.12	1.78 ± 0.51	1.35 ± 0.28
	Ester	Acetic acid, 2-phenylethyl ester	103-45-7	43	1317	2.18	Sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel	Medium	1.97 ± 0.54	1.59 ± 0.20	NF[h]
	Ester	3-Methylbutyl dodecanoate	6309-51-9	70	1366	3.50	Winey, alcoholic, fatty, creamy, yeasty and fusel	Medium	1.68 ± 1.01	1.66 ± 0.65	NF[h]
	Ester	S-ethyl octanethioate	2432-84-0	57	982	2.45	NF[h]	NF[h]	1.62 ± 0.61	NF[h]	NF[h]
	Ester	Ethyl 4-t-butylbenzoate	5406-57-5	191	1499	2.62	NF[h]	NF[h]	NF[h]	2.09 ± 0.77	2.07 ± 0.75
	Ester	4-Butyl 1,2-dimethyl 1,2,4-benzenetricarboxylate	54699-35-3	207	2042	1.50	NF[h]	NF[h]	2.00 ± 0.17	NF[h]	NF[h]
	Ester	2-Methylbutyl decanoate	68067-33-4	70	1399	3.53	NF[h]	NF[h]	NF[h]	NF[h]	1.44 ± 0.29
	Furan	2,3-Dihydrobenzofuran	496-16-2	120	1485	2.06	NF[h]	NF[h]	NF[h]	NF[h]	1.45 ± 0.35
	Ketone	3,4-Dihydroxy-3-cyclobutene-1,2-dione	2892-51-5	114	1149	1.98	NF[h]	NF[h]	NF[h]	1.49 ± 0.18	1.41 ± 0.47
	Terpene	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (γ-Terpinene)	99-86-5	93	610	3.25	Woody terpene lemon herbal medicinal citrus	Medium	NF[h]	NF[h]	1.45 ± 0.38
	Unknown	Analyte 1203	NF[h]	120	1457	2.05	NF[h]	NF[h]	NF[h]	NF[h]	1.36 ± 0.25
	Unknown	Analyte 1053	NF[h]	72	463	2.82	NF[h]	NF[h]	NF[h]	1.53 ± 0.37	NF[h]
	Unknown	Analyte 2070	NF[h]	58	1149	1.98	NF[h]	NF[h]	1.42 ± 0.33	NF[h]	NF[h]

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT[i] (s)	2D RT[i] (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
KA[b]	Acid	9-Decenoic acid	14436-32-9	110	1815	1.55	Waxy, green, fatty, soapy with a slight creamy cheese type nuance	Medium	NF[h]	NF[h]	1.26 ± 1.11
	Alcohol	2-Pentanol	6032-29-7	45	1030	1.79	Alcoholic, fusel, fermented, chocking and musty with sweet white wine top notes with over ripe banana and yellow apple nuances	Medium	0.84 ± 0.34	NF[h]	NF[h]
	Alcohol	2-Heptanol, (s)-	6033-23-4	45	708	1.70	Mushroom oily fatty blue cheese mouldy	Medium	NF[h]	NF[h]	1.68 ± 0.21
	Aldehyde	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	2111-75-3	150	1317	2.62	Fresh green herbal grassy sweet mint cumin	High	NF[h]	NF[h]	1.52 ± 0.68
	Alkene	1-Hexene, 4,5-dimethyl-	16106-59-5	71	1422	3.21	NF[h]	NF[h]	NF[h]	NF[h]	1.59 ± 0.69
	Alkene	1-Hexene, 4-ethyl-	16746-85-3	71	1422	3.21	NF[h]	NF[h]	NF[h]	1.34 ± 0.49	NF[h]
	Alkene	Bicyclo[4.2.0]octa-1,3,5-triene	694-87-1	78	624	2.12	NF[h]	NF[h]	1.83 ± 0.86	NF[h]	NF[h]
	Ester	Ethyl acetate	141-78-6	43	253	5.12	Ethereal fruity sweet weedy green	High	1.90 ± 0.27	1.88 ± 0.75	1.69 ± 0.09
	Ester	e-11-Hexadecenoic acid, ethyl ester	PubChemCID: 5364484	138	1396	3.08	Waxy, fruity, creamy and milky with a balsamic nuance	Low	NF[h]	NF[h]	1.50 ± 0.53
	Ester	10-Undecenoic acid, methyl ester	111-81-9	74	1138	2.36	Fatty waxy citrus earthy fungal rose flora	Medium	NF[h]	NF[h]	2.17 ± 0.97
	Ester	Isoamyl lactate	19329-89-6	70	1033	1.79	Fruity creamy nutty	Medium	NF[h]	NF[h]	1.68 ± 0.62
	Ester	Octyl formate	112-14-1	56	1016	1.76	Fruity rose orange waxy cucumber	Medium	NF[h]	NF[h]	1.46 ± 0.80
	Ester	9-Decenoic acid, ethyl ester	67233-91-4	69	1177	2.90	Fruity, fatty	Medium	1.21 ± 0.45	1.27 ± 0.56	NF[h]
	Ester	Ethyl e-2-octenoate	7367-82-0	125	1016	2.78	Fruity, pineapple, green with a fatty waxy nuance	Medium	NF[h]	NF[h]	1.43 ± 0.51
	Ester	2-Hexen-1-ol, propanoate, (e)-	53398-80-4	57	1238	1.99	Green, fruity apple and pear pulp with creamy and powdery nuances	Medium	NF[h]	1.68 ± 0.69	NF[h]
	Ester	2-Phenethyl acetate	103-45-7	104	1403	1.95	Sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel	Medium	NF[h]	NF[h]	1.34 ± 0.16
	Ester	Tridecanoic acid, 3-hydroxy-, ethyl ester	107141-15-1	117	1604	2.12	NF[h]	NF[h]	NF[h]	1.24 ± 0.17	NF[h]
	Ester	6-Methyl-2-heptanol, acetate	67952-57-2	87	904	2.89	NF[h]	NF[h]	NF[h]	NF[h]	1.62 ± 0.61
	Ether	1,4-Dioxane	123-91-1	59	736	1.94	Faint sweet	Low	NF[h]	1.46 ± 0.36	NF[h]

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT[i] (s)	2D RT[i] (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Terpene	Benzene, 1-methyl-4-(1-methylethyl)-	99-87-6	119	778	2.77	Fresh citrus terpene woody spice	High	NF[h]	NF[h]	1.92 ± 0.89
	Terpene	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5	121	967	3.30	Citrusy, woody, terpy with camphoraceous and thymol notes	Medium	NF[h]	1.22 ± 0.38	NF[h]
	Terpene	3,6-Octadien-1-ol, 3,7-dimethyl-, (z)-	106-25-2	121	1310	1.86	Fresh, citrus, floral, green, sweet, lemon/lime and waxy with a spicy depth	Medium	NF[h]	NF[h]	1.40 ± 0.19
	Terpene	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (z,e)-	26560-14-5	119	1555	3.47	Gardinia, floral	Medium	NF[h]	NF[h]	1.98 ± 0.28
	Terpene	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	586-62-9	68	934	3.20	Sweet, fresh, piney citrus with a woody old lemon peel nuance	Medium	NF[h]	1.31 ± 0.19	NF[h]
	Unknown	Analyte 551	NF[h]	113	907	2.31	NF[h]	NF[h]	NF[h]	NF[h]	1.73 ± 0.45
	Unknown	Analyte 703	NF[h]	73	1036	1.84	NF[h]	NF[h]	NF[h]	NF[h]	1.85 ± 0.97
	Unknown	Analyte 74	NF[h]	70	239	1.85	NF[h]	NF[h]	NF[h]	NF[h]	2.17 ± 0.38
	Unknown	Analyte 1697	NF[h]	113	904	2.32	NF[h]	NF[h]	NF[h]	1.47 ± 0.32	NF[h]
	Unknown	Analyte 1935	NF[h]	209	965	4.66	NF[h]	NF[h]	NF[h]	1.79 ± 0.73	NF[h]
	Unknown	Analyte 3784	NF[h]	60	2049	1.24	NF[h]	NF[h]	NF[h]	1.82 ± 0.26	NF[h]
LT[c]	Alcohol	1-Propanol	71-23-8	59	341	1.35	Alcoholic fermented fusel musty	Medium	NF[h]	1.80 ± 0.18	NF[h]
	Alcohol	2-Ethyl-1-hexanol	104-76-7	57	932	1.78	Citrus fresh floral oily sweet	Medium	NF[h]	NF[h]	1.86 ± 0.80
	Alcohol	3-Ethoxy-1-propanol	111-35-3	59	785	1.57	NF[h]	NF[h]	1.66 ± 0.23	1.64 ± 0.30	NF[h]
	Aldehyde	2-Furan carboxaldehyde	98-01-1	95	904	1.57	Sweet woody almond fragrant baked bread	Medium	NF[h]	NF[h]	1.58 ± 0.28
	Ester	Isoamyl acetate	123-92-2	70	1033	1.79	Sweet, banana, fruity with a ripe estry nuance	High	NF[h]	NF[h]	2.43 ± 1.24
	Ester	Citronellol acetate	150-84-5	123	1148	2.75	Floral green rose fruity citrus woody tropical fruit	Medium	NF[h]	2.01 ± 0.40	NF[h]
	Ester	Isobutyl acetate	110-19-0	43	309	2.03	Sweet fruity ethereal banana tropical	Medium	NF[h]	1.79 ± 0.40	NF[h]
	Ester	Ethyl 3-hydroxy tridecanoate	107141-15-1	117	1604	2.12	NF[h]	NF[h]	NF[h]	NF[h]	2.05 ± 1.47
	Ester	2-Heptenoic acid, ethyl ester, (e)-	54340-72-6	99	750	2.57	NF[h]	NF[h]	NF[h]	NF[h]	2.25 ± 0.72
	Ester	3-Methyl heptyl acetate	72218-58-7	70	799	2.76	NF[h]	NF[h]	2.10 ± 1.41	2.13 ± 1.36	NF[h]
	Ester	Ethyl 3,3,4-trimethyl pentanoate	80246-74-8	87	1212	2.04	NF[h]	NF[h]	2.05 ± 0.37	NF[h]	NF[h]
	Ester	5-Chlorooctylacetate	NF[h]	81	1142	2.78	NF[h]	NF[h]	1.83 ± 0.06	NF[h]	NF[h]
	Ester	Decanoic acid, propyl ester	30673-60-0	61	1219	3.26	Waxy, fruity, fatty, green vegetable, woody, oily, fruity	NF[h]	1.56 ± 0.22	2.08 ± 0.07	2.05 ± 0.65
	Ketone	2-Octanone	111-13-7	58	1142	2.47	Musty, ketonic, bleu and parmesan cheese-like with earthy and dairy nuances	Medium	1.57 ± 1.09	NF[h]	1.61 ± 0.97

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT[i] (s)	2D RT[i] (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Ketone	3-Acetoxy-2-butanone	4906-24-5	87	1996	2.09	Pungent sweet creamy buttery	Medium	NF[h]	2.39 ± 0.63	NF[h]
	Ketone	2-Undecanone	112-12-9	58	1072	2.73	Waxy, fruity, ketonic with fatty pineapple nuances	Medium	NF[h]	NF[h]	1.62 ± 0.79
	Ketone	7-Octen-2-one	3664-60-6	58	1135	2.52	NF[h]	NF[h]	NF[h]	NF[h]	1.64 ± 1.32	NF[h]
	Terpene	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (Farnesol)	4602-84-0	93	1317	3.65	Mild fresh sweet floral	Low	NF[h]	1.77 ± 0.23	NF[h]
	Terpene	2,6-Octadien-1-ol, 3,7-dimethyl-, (e)- (Geraniol)	106-24-1	69	1345	1.88	Sweet floral fruity rose waxy citrus	Medium	NF[h]	NF[h]	1.58 ± 0.17
	Terpene	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- (alpha ionene)	475-03-6	159	889	3.56	Violets, floral	Medium	NF[h]	NF[h]	2.48 ± 0.59
	Terpene	2,6-Dimethyl-1,3,5,7-octatetraene, e,e- (Cosemene)	460-01-5	117	894	2.45	NF[h]	NF[h]	NF[h]	NF[h]	1.81 ± 0.32
	Unknown	Analyte 530	NF[h]	151	897	1.65	NF[h]	NF[h]	NF[h]	NF[h]	1.64 ± 0.24
	Unknown	Analyte 276	NF[h]	151	533	4.14	NF[h]	NF[h]	NF[h]	NF[h]	1.95 ± 0.29
	Unknown	Analyte 925	NF[h]	87	1212	2.04	NF[h]	NF[h]	NF[h]	NF[h]	1.96 ± 0.92
	Unknown	Analyte 880	NF[h]	267	1188	2.41	NF[h]	NF[h]	NF[h]	NF[h]	2.02 ± 0.93
	Unknown	Analyte 518	NF[h]	151	890	1.44	NF[h]	NF[h]	NF[h]	NF[h]	2.12 ± 0.18
	Unknown	Analyte 586	NF[h]	81	953	1.65	NF[h]	NF[h]	NF[h]	NF[h]	2.26 ± 0.13
	Unknown	Analyte 594	NF[h]	47	959	1.88	NF[h]	NF[h]	NF[h]	NF[h]	2.38 ± 0.47
	Unknown	Analyte 1895	NF[h]	47	955	1.89	NF[h]	NF[h]	NF[h]	1.64 ± 0.55	NF[h]
	Unknown	Analyte 3856	NF[h]	54	2083	1.41	NF[h]	NF[h]	NF[h]	1.92 ± 1.72	NF[h]
	Unknown	Analyte 1650	NF[h]	111	891	2.97	NF[h]	NF[h]	NF[h]	1.97 ± 0.04	NF[h]
	Unknown	Analyte 1780	NF[h]	135	925	1.45	NF[h]	NF[h]	1.44 ± 0.85	NF[h]	NF[h]
	Unknown	Analyte 3317	NF[h]	64	2218	1.04	NF[h]	NF[h]	2.31 ± 0.72	NF[h]	NF[h]
MP[d]	Alcohol	1-Undecanol	112-42-5	69	1417	2.12	Fresh waxy rose soapy clean clothes floral citrus	NF[h]	NF[h]	NF[h]	1.76 ± 0.75
	Alcohol	Benzeneethanol	60-12-8	91	1415	1.66	Sweet, floral, fresh and bready with a rosey honey nuance	NF[h]	1.54 ± 1.36	NF[h]	NF[h]
	Alkane	(trans)-3,4-Oxa-2,5-dimethylhexane	NF[h]	56	1254	3.40	NF[h]	NF[h]	2.07 ± 0.51	NF[h]	NF[h]
	Alkene	(4E)-2,3-Dimethyl-1,4-hexadiene	18669-52-8	95	827	2.62	NF[h]	NF[h]	NF[h]	NF[h]	1.88 ± 0.30
	Amino Acid	Tyrosine	60-18-4	107	2346	1.56	NF[h]	NF[h]	NF[h]	NF[h]	1.80 ± 0.61
	Ester	Dibutyl phthalate	88-99-3	149	1989	2.56	Faint odor	Low	NF[h]	1.81 ± 0.72	1.96 ± 0.59
	Ester	Butyl octanoate	589-75-3	127	1016	3.30	Butter ether herbal dank	Medium	2.33 ± 0.35	2.22 ± 0.87	1.79 ± 0.52
	Ester	Isoamyl salicylate	PubChemCID:91695386	101	1408	2.39	Floral herbal woody orchid metallic	Medium	1.87 ± 0.76	NF[h]	NF[h]

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT (s)	2D RT (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Ester	Isobutyric acid, phenethyl ester	103-48-0	104	1471	2.47	Heavy fruity, honey and yeasty, with balsamic nuances and waxy rosy floral notes on dry out	Medium	NF[h]	1.91 ± 1.68	NF[h]
	Ester	2-Methylpropyl decanoate	30673-38-2	56	1254	3.41	Oily sweet brandy apricot cognac	Medium	NF[h]	2.10 ± 0.81	1.79 ± 1.13
	Ester	2-Methylpropyl benzoate	120-50-3	105	1289	2.48	Sweet fruity musty powdery balsam	Medium	NF[h]	2.28 ± 0.41	2.04 ± 0.82
	Ester	2-Phenethyl pentanoate	7460-74-4	104	1856	2.80	Fruity rose leaf	NA	NF[h]	NF[h]	1.85 ± 1.38
	Ester	2-Methylpropyl hexanoate	105-79-3	99	757	3.14	NF[h]	NF[h]	2.25 ± 0.75	2.37 ± 0.59	2.43 ± 0.18
	Ester	(E)-Ethyl-3-hexenoate	26553-46-8	69	693	2.45	NF[h]	NF[h]	2.49 ± 0.68	2.69 ± 0.26	2.64 ± 0.25
	Ester	Methyl 2-aminoacetate	616-34-2	89	443	2.72	NF[h]	NF[h]	NF[h]	1.59 ± 0.11	1.59 ± 0.64
	Ester	Ethyl glutarate	818-38-2	114	1275	2.21	NF[h]	NF[h]	NF[h]	1.85 ± 0.67	NF[h]
	Ester	2,6-Pyridinedicarboxylic acid, isobutyl phenethyl ester	PubChemCID:91703257	104	1667	2.66	NF[h]	NF[h]	NF[h]	NF[h]	1.99 ± 1.36
	Ester	Dimethylmalonic acid, ethyl 2-phenethyl ester	PubChemCID:91703267	104	1856	2.80	NF[h]	NF[h]	NF[h]	1.86 ± 0.83	NF[h]
	Ester	Ethyl 3-methylbutyl butanedioate	PubChemCID:91750109	101	1408	2.40	NF[h]	NF[h]	NF[h]	1.95 ± 0.59	1.87 ± 0.68
	Ester	β-Phenylethyl butyrate	103-52-7	104	1471	2.47	NF[h]	NF[h]	NF[h]	NF[h]	1.92 ± 1.76
	Ester	3-Methylbutyl dodecanoate	6309-51-9	70	1366	3.51	Winey, alcoholic, fatty, creamy, yeasty and fusel	NF[h]	NF[h]	1.96 ± 0.27	NF[h]
	Ketone	3-Ethoxy-2-butanone	1679-38-5	45	400	2.00	NF[h]	NF[h]	1.84 ± 0.79	NF[h]	NF[h]
	Lactone	4-(1-Hydroxy-ethyl) γ butanolactone	PubChemCID:12664706	86	1849	1.55	NF[h]	NF[h]	NF[h]	NF[h]	1.90 ± 0.67
	Unknown	Analyte 1411	NF[h]	101	1931	1.35	NF[h]	NF[h]	NF[h]	NF[h]	1.73 ± 0.78
	Unknown	Analyte 1030	NF[h]	129	1290	2.32	NF[h]	NF[h]	NF[h]	NF[h]	1.97 ± 0.99
	Unknown	Analyte 2936	NF[h]	151	1443	3.06	NF[h]	NF[h]	NF[h]	1.90 ± 0.66	NF[h]
	Unknown	Analyte 3184	NF[h]	55	1591	3.15	NF[h]	NF[h]	NF[h]	2.12 ± 0.29	NF[h]
	Unknown	Analyte 2651	NF[h]	129	1289	2.33	NF[h]	NF[h]	NF[h]	2.22 ± 0.83	NF[h]
	Unknown	Analyte 2898	NF[h]	103	1986	2.01	NF[h]	NF[h]	2.26 ± 0.28	NF[h]	NF[h]
PK[e]	Acid	3-Methyl-butanoic acid	503-74-2	74	1156	1.35	Sour stinky feet sweaty cheese tropical	High	NF[h]	NF[h]	1.54 ± 0.30
	Acid	Cyclopentaneundecanoic acid	6053-49-2	124	911	2.73	NF[h]	NF[h]	NF[h]	NF[h]	1.41 ± 0.50
	Aldehyde	Octanal	124-13-0	57	673	2.47	Aldehydic waxy citrus orange peel green fatty	High	NF[h]	2.05 ± 1.31	NF[h]
	Aldehyde	α,4-Dimethyl-3-cyclohexene-1-acetaldehyde	29548-14-9	94	1247	3.39	Spicy herbal	High	NF[h]	1.64 ± 0.35	NF[h]
	Aldehyde	Decanal	112-31-2	57	953	2.69	Sweet aldehydic waxy orange peel citrus floral	High	NF[h]	1.99 ± 1.76	NF[h]

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT^i (s)	2D RT^i (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Aldehyde	Nonanal	124-19-6	57	813	2.62	Waxy aldehydic rose fresh orris orange peel fatty peely	High	NF^h	2.02 ± 1.49	NF^h
	Amine	Phenethylamine	64-04-0	114	1317	2.23	Fishy	High	NF^h	NF^h	1.60 ± 0.22
	Ester	Ethyl 3-methylbutanoate	108-64-5	85	1009	1.80	Fruity sweet apple pineapple tutti frutti	High	NF^h	NF^h	2.27 ± 0.18
	Ester	3-(Methylthio)propyl acetate	16630-55-0	88	1107	2.10	Herbal mushroom cabbage asparagus potato	High	NF^h	NF^h	1.38 ± 0.46
	Ester	Octanoic acid, hexyl ester	1117-55-1	145	1310	3.35	Fruity green waxy berry apple	Medium	NF^h	NF^h	1.41 ± 0.16
	Ester	3-Methylbutyl octanoate	2035-99-6	127	1142	3.39	Sweet oily fruity green soapy pineapple coconut	Medium	1.73 ± 0.12	1.78 ± 0.51	1.35 ± 0.28
	Ester	Acetic acid, 2-phenylethyl ester	103-45-7	43	1317	2.18	Sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel	Medium	1.97 ± 0.54	1.59 ± 0.20	
	Ester	3-Methylbutyl dodecanoate	6309-51-9	70	1366	3.50	Winey, alcoholic, fatty, creamy, yeasty and fusel	Medium	1.68 ± 1.01	1.66 ± 0.65	
	Ester	S-ethyl octanethioate	2432-84-0	57	982	2.45	NF^h	NF^h	1.62 ± 0.61	NF^h	NF^h
	Ester	Ethyl 4-t-butylbenzoate	5406-57-5	191	1499	2.62	NF^h	NF^h	NF^h	2.09 ± 0.77	2.07 ± 0.75
	Ester	4-Butyl 1,2-dimethyl 1,2,4-benzenetricarboxylate	54699-35-3	207	2042	1.50	NF^h	NF^h	2.00 ± 0.17	NF^h	NF^h
	Ester	2-Methylbutyl decanoate	68067-33-4	70	1366	3.53	NF^h	NF^h	NF^h	NF^h	1.44 ± 0.29
	Furan	2,3-Dihydrobenzofuran	496-16-2	120	1485	2.06	NF^h	NF^h	NF^h	NF^h	1.45 ± 0.35
	Ketone	3,4-Dihydroxy-3-cyclobutene-1,2-dione	2892-51-5	114	1149	1.98	NF^h	NF^h	NF^h	1.49 ± 0.18	1.41 ± 0.47
	Terpene	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (γ-terpinene)	99-86-5	93	610	3.25	Woody terpene lemon herbal medicinal citrus	Medium	NF^h	NF^h	1.45 ± 0.38
	Unknown	Analyte 1203	NF^h	120	1457	2.05	NF^h	NF^h	NF^h	NF^h	1.36 ± 0.25
	Unknown	Analyte 1053	NF^h	72	463	2.82	NF^h	NF^h	1.42 ± 0.33	1.53 ± 0.37	NF^h
	Unknown	Analyte 2070	NF^h	58	1149	1.98	NF^h	NF^h	NF^h	NF^h	NF^h
SC^f	Acid	Octanoic acid	124-07-2	60	1562	1.54	Fatty waxy rancid oily vegetable cheesy	Medium	NF^h	1.02 ± 0.20	NF^h
	Alcohol	1-Hexanol	111-27-3	151	757	3.08	Pungent, etherial, fusel oil, fruity and alcoholic, sweet with a green top note	High	1.98 ± 0.94	NF^h	NF^h
	Alcohol	1,2-Propanediol	57-55-6	45	1051	1.34	Faint sweetness	Low	NF^h	1.84 ± 0.56	NF^h
	Alcohol	7-Tridecanol	927-45-7	97	1296	2.02	Musty	Low	NF^h	1.88 ± 0.99	1.84 ± 0.79
	Alcohol	1-Butanol	71-36-3	56	470	1.42	Fusel oil sweet balsam	Medium	2.56 ± 0.25	NF^h	NF^h
	Alcohol	1-Pentanol, 4-methyl-	626-89-1	56	701	1.56	Nutty	Medium	1.49 ± 0.54	NF^h	NF^h
	Alcohol	1-Pentanol, 3-methyl-	589-35-5	56	716	1.58	Pungent, fusel, cognac and wine, cocoa, with green fruity	Medium	2.11 ± 0.50	1.64 ± 0.43	1.70 ± 0.28

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT^i (s)	2D RT^i (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Aldehyde	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	432-25-7	109	1100	2.65	NF^h	NF^h	NF^h	1.24 ± 0.15	NF^h
	Aldehyde	Pentanal, 4-oxo-	626-96-0	85	981	2.14	NF^h	NF^h	NF^h	2.19 ± 0.31	NF^h
	Alkane	Propanal, 2-(acetyloxy)-, (r)-	NF^h	87	1324	2.10	NF^h	NF^h	NF^h	1.42 ± 0.85	NF^h
	Alkane	1-Iodotetradecane	19218-94-1	183	1443	2.70	NF^h	NF^h	NF^h	NF^h	1.99 ± 0.55
	Alkyne	1-Undecyne	2243-98-3	81	842	2.43	NF^h	NF^h	NF^h	NF^h	2.39 ± 0.23
	Alkyne	1-Decyne	764-93-2	81	841	2.45	NF^h	NF^h	NF^h	2.32 ± 0.43	NF^h
	Ester	Acetic acid, pentyl ester	628-63-7	43	512	2.37	Ethereal fruity banana pear banana apple	High	2.53 ± 0.56	2.41 ± 0.61	NF^h
	Ester	4-Penten-1-ol, acetate	1576-85-8	68	540	2.13	Green plastic weedy acrylate vegetable metallic cooked meat sulfide	High	2.62 ± 0.32	2.45 ± 0.28	NF^h
	Ester	Acetic acid, butyl ester	123-86-4	43	372	5.92	Sweet, ripe banana, tutti frutti, tropical and candy-like with green	High	NF^h	2.28 ± 0.66	2.52 ± 0.84
	Ester	3-Phenyl-1-propanol, acetate	122-72-5	117	1450	2.26	Balsamic, spicy, cinnamic and fruity with honey and hay-like nuances	Medium	NF^h	NF^h	1.99 ± 0.20
	Ester	Propyl octanoate	624-13-5	61	974	3.16	Coconut caco gin	Medium	1.98 ± 0.45	1.53 ± 0.51	NF^h
	Ester	Acetic acid, propyl ester	109-60-4	61	1373	1.57	Estry, fruity, etherial, tutti-frutti, banana and honey	Medium	NF^h	2.35 ± 0.52	1.95 ± 0.76
	Ester	Undecanoic acid, methyl ester	1731-86-8	74	1065	2.90	Fatty waxy fruity	Medium	1.77 ± 0.32	NF^h	NF^h
	Ester	2,4-Octadien-1-ol, acetate, (e,e)-	30361-34-3	79	1037	2.41	Fatty, chicken fat, with a creamy waxy nuance	Medium	NF^h	NF^h	2.16 ± 0.24
	Ester	Octanoic acid, ethyl ester	106-32-1	104	869	3.18	Fruity wine waxy sweet apricot banana brandy pear	Medium	1.69 ± 0.54	NF^h	NF^h
	Ester	Acetic acid, phenylmethyl ester	140-11-4	108	1219	2.07	Fruity, sweet, with balsamic and jasmin floral undernote	Medium	NF^h	2.63 ± 0.47	2.24 ± 0.73
	Ester	Acetic acid, octyl ester	112-14-1	82	918	2.78	Green earthy mushroom herbal waxy	Medium	1.50 ± 0.87	NF^h	NF^h
	Ester	3-Hepten-1-ol, acetate	3681-71-8	81	841	2.49	Green tropical banana vegetable fatty	Medium	2.56 ± 0.38	NF^h	NF^h
	Ester	Butanoic acid, hexyl ester	2639-63-6	71	842	3.08	Green, fruity, estry and vegetative with a waxy nuance	Medium	NF^h	2.45 ± 1.12	2.40 ± 1.04
	Ester	Acetic acid, hexyl ester	142-92-7	61	589	2.50	Green, fruity, sweet, fatty, fresh, apple and pear	Medium	2.56 ± 0.47	NF^h	NF^h
	Ester	Propanoic acid, hexyl ester	2445-76-3	57	743	2.85	Pear green fruity musty rotting fruit	Medium	NF^h	NF^h	2.29 ± 0.95
	Ester	2-Butenoic acid, ethyl ester, (-z)	6776-19-8	69	498	2.42	Pungent, sharp, rum- and cognac-like, wth tinny, pineapple, fruity and meaty nuances	Medium	NF^h	1.16 ± 0.43	1.59 ± 0.37

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT^i (s)	2D RT^i (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Ester	Hexanoic acid, propyl ester	626-77-7	99	715	2.93	Sweet fruity juicy pineapple green tropical	Medium	1.90 ± 0.20	NF^h	NF^h
	Ester	2-Methylbutyl acetate	624-41-9	70	463	2.22	Sweet, banana, fruity, ripe, estry and tropical with a juicy, fruit-like note	Medium	NF^h	NF^h	2.07 ± 0.68
	Ester	Butanoic acid, butyl ester	109-21-7	71	575	2.93	Sweet, fruity, fresh, diffusive and ripe	Medium	NF^h	2.40 ± 1.14	NF^h
	Ester	Octanoic acid, methyl ester	111-11-5	74	806	2.72	Waxy, green, sweet, orange and aldehydic with vegetative and herbal nuances	Medium	NF^h	1.18 ± 0.40	1.48 ± 0.26
	Ester	Butanedioic acid, hydroxy-, diethyl ester, (±)-	626-11-9	71	1541	1.71	Wine fruity apple skin	Medium	NF^h	2.24 ± 0.70	NF^h
	Ester	3-Ethoxypropyl acetate	NF^h	58	764	2.19	NF^h	NF^h	NF^h	1.44 ± 0.31	1.45 ± 0.27
	Ester	Diethyle 2-hydroxypentanedioate	NF^h	85	1660	1.78	NF^h	NF^h	NF^h	2.33 ± 0.65	NF^h
	Ester	2-Buten-1-ol, 1,4-dimethoxy-, acetate, (e)-	NF^h	71	960	2.06	NF^h	NF^h	2.70 ± 0.51	NF^h	NF^h
	Ester	Hexanoic acid, methyl ester	106-70-7	74	526	2.42	NF^h	NF^h	NF^h	1.03 ± 0.78	NF^h
	Ester	1,3-Propanediol, diacetate	628-66-0	43	1226	1.49	NF^h	NF^h	NF^h	1.56 ± 1.30	NF^h
	Ester	1,4-Butanediol, diacetate	628-67-1	43	1275	1.98	NF^h	NF^h	NF^h	NF^h	1.89 ± 0.88
	Ester	Ethanol, 2,2'-oxybis-, diacetate	628-68-2	87	1996	2.08	NF^h	NF^h	NF^h	NF^h	2.29 ± 0.58
	Ester	Butanoic acid, 1-methylpropyl ester	819-97-6	71	1541	1.71	NF^h	NF^h	NF^h	NF^h	2.49 ± 1.20
	Furan	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	NF^h	126	1610	2.16	NF^h	NF^h	NF^h	NF^h	1.28 ± 0.31
	Heterocycle	1,3-Dioxolane, 4,5-dimethyl-2-pentadecyl-	56599-61-2	101	708	3.11	NF^h	NF^h	1.78 ± 0.31	2.13 ± 0.34	2.24 ± 0.41
	Ketone	Acetophenone	98-86-2	105	1135	1.96	Sweet, cherry pit, marzipan and coumarinic. It has a slight almond nutty and heliotropin-like vanilla nuance	High	NF^h	NF^h	1.31 ± 1.10
	Ketone	2,3-Hexanedione	3848-24-6	71	960	2.05	Sweet, creamy, caramellic, buttery with a fruity jammy nuance	High	NF^h	2.58 ± 0.10	NF^h
	Ketone	3-Pentanone, 2,4-dimethyl-	565-80-0	71	967	2.03	Acetone	Medium	NF^h	NF^h	2.65 ± 0.33
	Ketone	2-Butanone, 3-(acetyloxy)-	10150-87-5	87	1996	2.09	NF^h	NF^h	NF^h	2.21 ± 0.77	NF^h
	Ketone	4-Penten-2-one	13891-87-7	61	701	2.34	NF^h	NF^h	NF^h	NF^h	2.47 ± 0.48
	Ketone	4-Hepten-2-one, (e)-	36678-43-0	43	1597	2.57	NF^h	NF^h	NF^h	2.45 ± 0.18	NF^h
	Ketone	2,5-Furandione, dihydro-3-methyl-	4100-80-5	42	1436	1.56	NF^h	NF^h	NF^h	NF^h	1.33 ± 0.16
	Ketone	2-Propanone, 1-(1-methylethoxy)-	42781-12-4	58	764	2.19	NF^h	NF^h	1.71 ± 0.21	NF^h	NF^h
	Phenol	Phenol, 4-octyl-	1806-26-4	74	1156	1.35	NF^h	NF^h	NF^h	NF^h	1.57 ± 0.63

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT[i] (s)	2D RT[i] (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Terpene	2,6-Octadien-1-ol, 3,7-dimethyl-, (e)-	106-24-1	93	1254	2.64	Floral, rosy, waxy, herbal and green with a slight cooling nuance	Medium	NF[h]	1.85 ± 0.90	NF[h]
	Thiol	2-Thiophenecarboxaldehyde	98-03-3	112	1184	1.76	NF[h]	NF[h]	NF[h]	1.77 ± 0.18	NF[h]
	Unknown	Analyte 363	NF[h]	85	659	3.27	NF[h]	NF[h]	NF[h]	NF[h]	1.62 ± 0.23
	Unknown	Analyte 966	NF[h]	61	1240	2.21	NF[h]	NF[h]	NF[h]	NF[h]	2.12 ± 0.75
	Unknown	Analyte 355	NF[h]	47	652	2.70	NF[h]	NF[h]	NF[h]	NF[h]	2.13 ± 0.28
	Unknown	Analyte 1158	NF[h]	125	1407	2.42	NF[h]	NF[h]	NF[h]	NF[h]	2.46 ± 0.06
	Unknown	Analyte 1411	NF[h]	77	743	1.31	NF[h]	NF[h]	NF[h]	1.12 ± 0.77	NF[h]
	Unknown	Analyte 1034	NF[h]	48	443	2.55	NF[h]	NF[h]	NF[h]	1.61 ± 1.51	NF[h]
	Unknown	Analyte 2547	NF[h]	61	1240	2.21	NF[h]	NF[h]	NF[h]	2.30 ± 0.71	NF[h]
	Unknown	Analyte 1887	NF[h]	55	972	2.69	NF[h]	NF[h]	1.63 ± 1.61	NF[h]	NF[h]
TD[g]	Acetal	1-Ethoxy-1-pentyloxyethane	13442-89-2	73	407	3.17	NF[h]	NF[h]	1.69 ± 1.48	NF[h]	NF[h]
	Acid	Benzoylformic acid	611-73-4	77	652	1.36	NF[h]	NF[h]	NF[h]	1.74 ± 0.83	2.02 ± 2.02
	Alcohol	2-Undecanol	1653-30-1	45	1205	2.12	Fresh waxy clean cloth cotton sarsaparilla	Medium	NF[h]	2.09 ± 1.20	2.00 ± 2.00
	Alcohol	(2S)-2-Heptanol	6033-23-4	45	708	1.71	Mushroom oily fatty blue cheese mouldy	Medium	NF[h]	1.80 ± 1.33	NF[h]
	Alcohol	2-Nonanol	628-99-9	45	967	1.93	Waxy green creamy citrus orange cheese fruity	Medium	1.95 ± 1.39	1.90 ± 1.50	2.05 ± 2.05
	Alcohol	7-Octen-2-ol	39546-75-3	67	1268	2.01	NF[h]	NF[h]	NF[h]	2.02 ± 1.35	NF[h]
	Alcohol	1-Chloro-4-hydroxybutane	928-51-8	45	1205	2.12	NF[h]	NF[h]	2.29 ± 1.34	NF[h]	NF[h]
	Alcohol-Thiol	3-(Methylthio)-1-propanol	505-10-2	106	1205	1.57	Raw potato, sulfurous, onion, vegetable soup	High	2.58 ± 0.80	2.50 ± 0.95	1.69 ± 1.69
	Alcohol-Thiol	3-[(2-Hydroxyethyl)thio]-1-propanol	5323-60-4	118	1261	1.70	Sulfurous onion sweet soup vegetable	High	NF[h]	1.68 ± 0.68	1.61 ± 1.61
	Alkane-Thiol	Trimethylene sulfide	287-27-4	46	776	2.10	NF[h]	NF[h]	NF[h]	2.00 ± 1.42	NF[h]
	Alkene	4,6,8-Trimethylazulene	941-81-1	155	1674	2.63	NF[h]	NF[h]	NF[h]	1.84 ± 0.20	NF[h]
	Benzene	1,1'-(1-Methyl-1,2-ethanediyl)bis-benzene	5814-85-7	105	1079	2.28	NF[h]	NF[h]	NF[h]	1.87 ± 1.89	1.69 ± 1.69
	Benzene	(1,2,3-Trimethyl-2-cyclopropen-1-yl)-benzene	6393-13-1	128	1184	2.60	NF[h]	NF[h]	NF[h]	1.64 ± 1.21	NF[h]
	Ester	2-(1-Pentyloxy)-ethyl acetate	NF[h]	87	1247	1.84	NF[h]	NF[h]	NF[h]	1.88 ± 0.52	NF[h]
	Ester	Butanoic acid, 3-hydroxy-, ethyl ester	5405-41-4	71	967	1.61	NF[h]	NF[h]	1.52 ± 0.77	1.87 ± 0.48	2.07 ± 2.07
	Ester	Propanoic acid, ethyl ester	105-37-3	57	260	2.44	NF[h]	NF[h]	NF[h]	1.81 ± 0.82	NF[h]
	Ester	3-Methylbutyl propionate	105-68-0	57	533	2.68	NF[h]	NF[h]	NF[h]	NF[h]	1.51 ± 1.51
	Ester	Acetic acid, nonyl ester	143-13-5	43	1044	2.87	NF[h]	NF[h]	NF[h]	2.17 ± 1.15	NF[h]
	Ester	4-Tert-butylcyclohexyl acetate	5451-55-8	80	1113	3.04	NF[h]	NF[h]	NF[h]	2.09 ± 0.79	1.50 ± 1.50

Table 1 continued

Yeast	Class	Metabolites	CAS#	Quant mass	1D RT[i] (s)	2D RT[i] (s)	Aroma and flavor	Odor strength	Extraction time		
									10 s	5 min	30 min
	Ester	Amylpropionate	624-54-4	57	533	2.69	NF[h]	NF[h]	NF[h]	1.87 ± 1.62	NF[h]
	Ester	Propanoic acid, 2,2-dimethyl-, 2-phenylethyl ester	67662-96-8	104	1387	2.36	NF[h]	NF[h]	NF[h]	2.49 ± 0.33	2.46 ± 2.46
	Ester	β-Phenylethyl butyrate	103-52-6	104	1471	2.47	Musty sweet floral yeast strawberry	NF[h]	NF[h]	NF[h]	2.06 ± 2.06
	Ester-Thiol	3-(Methylthio)propanoic acid ethyl ester	13327-56-5	74	1030	2.19	Sulfury metallic pineapple fruity ripe pulpy tomato	high	NF[h]	2.04 ± 0.50	NF[h]
	Ethane	1-Ethoxy-1-pentoxy-ethane	NF[h]	71	418	3.11	NF[h]	NF[h]	NF[h]	1.95 ± 1.92	NF[h]
	Ketone	2-Undecanone	112-12-9	58	1072	2.73	NF[h]	NF[h]	NF[h]	1.75 ± 1.42	NF[h]
	Norisoprenoid	2 h-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2α,4aα,8aα)-	41678-32-4	179	981	3.56	NF[h]	NF[h]	NF[h]	NF[h]	1.86 ± 1.86
	Thiane	1,3-Oxathiane	646-12-8	46	772	2.11	NF[h]	NF[h]	NF[h]	NF[h]	2.07 ± 2.07
	Unknown	Analyte 2703	NF[h]	118	1317	118	NF[h]	NF[h]	NF[h]	1.77 ± 0.71	NF[h]
	Unknown	Analyte 4390	NF[h]	72	2373	1.70	NF[h]	NF[h]	NF[h]	2.57 ± 1.07	NF[h]

[a] C. zemplinina
[b] K. aerobia
[c] L. thermotolerans
[d] M. pulcherrima
[e] P. kluyveri
[f] S. cerevisiae
[g] T. delbrueckii
[h] Not found
[i] Retention time

populations began to decline as soon as the *S. cerevisiae* was added but that they remained detectable via plate count for between 6 and 10 days (Fig. 4). This indicates that the non-*Saccharomyces* yeasts remained viable and detectable for over half of the total fermentation time. For *C. zemplinina*, *L. thermotolerans*, and *T. delbrueckii* this is in agreement with literature (Azzolini et al. 2012; Kapsopoulou et al. 2006; Maio et al. 2012). For *M. pulcherrima*, *P. kluyveri*, and *K. aerobia* this has not been previously reported in a sequential wine fermentation. In all likelihood, the yeasts remained metabolically active and thus able to contribute to the organoleptic profile for even longer than this since the non-*Saccharomyces* colonies became difficult to count due to the overcrowding of the *S. cerevisiae*.

3.2 Aroma compound presence in Sauvignon blanc due to specific species: chemistry and sensory analysis

GCxGC-TOF-MS is becoming more common in the field of metabolomics as it has proven to be a powerful tool that

can increase separation, detection and identification of a wide variety of metabolic analytes compared to 1D GC (Zhang et al. 2012). When Solid-Phase-Microextraction (SPME) is used as an extraction method it is possible to study, with great chemical selectivity and sensitivity, the volatile profile of samples. The different compounds that make up the headspace of wine samples will be present in a broad concentration range, with varying vapor pressures and have different adsorption affinities to the SPME fiber. Therefore, this study employed three separate extraction times to increase compound coverage while limiting chromatographic and detector saturation. The 10 s extraction time proved useful for obtaining peak shapes conducive to consistent integration for the most highly concentrated analytes such as esters and alcohols. The 30 min extraction time was used to characterize the smaller but no less important peaks that represent aroma compounds such as terpenes, volatile phenols, thiols and some of the less concentrated esters and alcohols. The 5 min extraction severed as a good average between compounds found in saturation at 30 min but not detected by the 10 s extraction time. Figure 1S illustrates this finding by
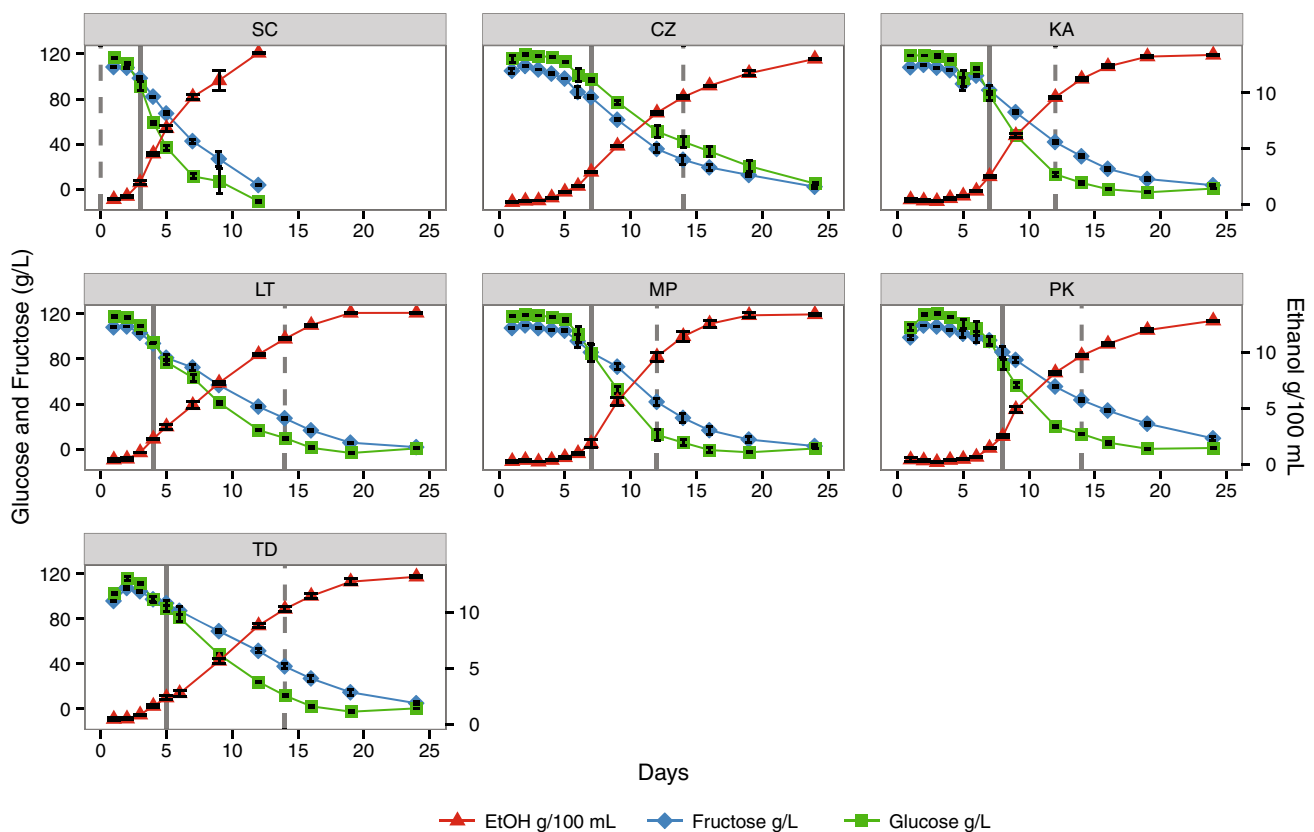
Fig. 4 Each graph indicates the progress of the fermentations by each species. *SC S. cerevisiae*, *CZ C. zemplinina*, *KA K. aerobia*, *LT L. thermotolerans*, *MP M. pulcherrima*, *PK P. kluyveri*, *TD T. delbrueckii*. Each *graph* shows glucose consumption (*square shape*), fructose consumption (*diamond shape*), and ethanol production (*triangle shape*). All of these *lines* are an average of the three biological replicates and the standard error of the mean is shown by *error bars*. The *solid vertical line* indicates where the ethanol concentration reached 2 % and in the case of the non-*Saccharomyces* fermentations *S. cerevisiae* was added. The *dashed vertical line* indicates where the non-*Saccharomyces* yeast was no longer detectable by plate count

showing two compounds. Compound 1 is in saturation at 30 min (Fig. 1Sc) but measurable in both the 5 min (Fig. 1Sb) and the 10 s (Fig. 1Sa) extraction times. The peak shape is however best for measurement in the 10 s chromatogram. Peak 2 in this figure shows the opposite trend. Some compounds were only measurable at 30 min and too small in 5 min and 10 s to be reliable. However, there were some compounds that were reliably measurable at two or all three extraction times and in this case the data was combined and represented as such in Table 1 and Table 1S. Each extraction time consisted of only one batch and the intra-batch reproducibility was assessed by comparing the peak area of the internal standard in each sample. Figure 2S shows the normalized mean peak area of the internal standard of each sample from each extraction time batch. Together, these three data sets, along with the sensory analysis, provided a highly detailed volatile compound and aroma profile of Sauvignon blanc wines generated in this study.

It is well known that certain sulfur compounds such as 4-mercapto-4-methylpentan-2-one, 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexanol, and 3-mercaptohexyl acetate give Sauvignon blanc its characteristic tropical and green aromas (Tominaga et al. 1998). Though these compounds were not reliably detectable with the analytical method used in this study, likely due to their relatively low concentration, the compounds have a very low sensory detection threshold and thus are easily distinguished by the human olfactory sense at much lower concentrations, the parts per trillion range, than the SPME fiber is capable of detecting (Dubourdieu et al. 2006). It is for this reason that tropical aromas such as guava and passion fruit were a critical part of the sensory panel evaluation as can be seen in Table 2S. The sensory analysis not only mirrors the untargeted volatile profile but complements and expands it.

Both the sensory and the analytical methods were able to show a distinct separation of the wines co-fermented sequentially with the different yeasts based on their detectable aroma features; this can be seen clearly in the principal component analyses (PCAs) (Fig. 3). The sensory analysis focused on 16 typical Sauvignon blanc aromas. Only 12 of these (guava, passion fruit, grapefruit, banana, apple, pineapple, cooked vegetable, solvent, sherry, fermented apple, dried peach and stewed fruit) proved to be consistently evaluated and significantly different across all samples according to ANOVA analysis ($p < 0.01$). Thus it is not surprising that, with so few parameters, the sensory PCA is able to account for more than 90 % of the total variance. The first principal component axis is largely defined by a difference in the fruity aroma profiles (Fig. 3d). Esters are primarily responsible for the bulk of fruity aromas and flavors in wine and this result could indicate a significant difference in ester production between the yeast species. In fact, the analytical method

showed significantly different ester profiles for each fermentation. The basic flavors of sweet, bitter and sour were also found to be significantly different across all samples and were distributed more along the second component. The SPME method on the other hand was able to detect thousands of volatile aroma compounds which after our feature selection was applied to cut the number of features down from over 1000 total identified features to 336 compounds found to be statistically significantly different across the fermentations. The breakdown according to extraction time is as follows: 78 compounds for the 10 s extractions, 196 for the 5 min extractions and 239 for the 30 min extractions. Some compounds were reliably extracted by more than one extraction time and their unit variance scaled values were combined and are shown in Table S1. It is clear from the PCA plots that like the sensory analysis the yeasts showed distinct profiles with strong grouping of the biological replicates. This same result is also confirmed by the hierarchical clustering (Fig. 2a–c) in which it is clearly shown that the yeast replicates grouped with themselves and each grouping had a distinct chemical signature. It should be noted that with so many chemical compounds it was only natural that the yeasts grouped together so well and showed such distinct profiles in the chemical data versus the sensory data. There are two possible explanations for this: either by focusing on only 16 compounds, the tasters "missed" significant odors in the wine, or the compounds produced (or not) by the different yeasts contribute to the overall wine complexity but were not distinguishable as individual aromas because they remained below detection threshold. In all likelihood the explanation lies somewhere between the two. To put this into perspective the most prominent details of these profiles of each fermentation are discussed below on a yeast by yeast basis.

3.2.1 Saccharomyces cerevisiae

The panel associated the *S. cerevisiae* fermentations most closely with guava, passion fruit, grapefruit, banana, pineapple and apple. It was least associated with fermented apple, dried peach and stewed fruit (Fig. 3d). Chemically, the *S. cerevisiae* fermentations were distinguished mostly by a group of 65 compounds found to be in the highest relative concentration across all fermentations. These compounds are seen in red in the heat maps (Fig. 2) meaning they consistently showed the highest relative concentration among those samples. Of these, the majority were alcohols and esters associated with green, fruity, and tropical notes (Table 1). This correlates well with the previously mentioned panel findings, specifically the banana, pineapple and apple aromas. A large portion of the most significant compounds have currently no documented

aromas or flavors. Some compounds were wholly unidentified features and all of them represent an area of possible future study. Out of all the fermentations, the *S. cerevisiae* showed the highest number of distinguishing esters, alcohols, and other compounds and this is in agreement with the literature (Dubourdieu et al. 2006; Lambrechts and Pretorius 2000; Majdak and Herjavec 2002; Zalacain et al. 2007). Metabolically speaking, there was nothing out of the ordinary for these fermentations and they served well as a control.

3.2.2 Candida zemplinina

The sensory panel found that the *C. zemplinina* fermentations had the most distinct aroma profile next to the *S. cerevisiae* fermentations. They were characterized by the guava, fermented apple, sherry, dried peach/apricot, and stewed fruit descriptors. This is not surprising given its profile of compounds found to be significantly higher, which can be seen in Table 1, and are represented in red in the heat maps in Fig. 3. There were 49 features with statistically significantly larger relative peak areas that separated the *C. zemplinina* fermentations from the rest. Of these, 12 were esters, one of which, 2-methyl-propanoic acid ethyl ester, has a very high odor strength and is characterized as sweet, ethereal and fruity with pungent, alcoholic, fusel and rummy descriptors as well. This is likely one of the main contributors to the 'fermented apple' aroma described by the panel. All other yeast fermentations showed almost none of this compound comparatively. Also worth noting is the statistically significant presence of relatively large acetic acid and hydroxyl acetic acid peaks in the SPME–GCxGC-TOF-MS analysis. As previously mentioned, the Fourier-transform mid-infrared spectroscopy analysis revealed a relatively high level of acetic acid (Fig. 5), and the sensory panel noted this fermentation to be more sour than others. This shows all three analysis methods to be both cohesive and complimentary to one another. It is however, in direct contrast to previously published work which indicates that *C. zemplinina* had the capacity to reduce the amount of acetic acid in a wine fermentation especially when used in conjunction with *S. cerevisiae* (Englezos et al. 2015; Rantsiou et al. 2012; Sadoudi et al. 2012). These differences however, could be due to biological variability between different strains used. Indeed, as noted by Englezos et al. (2015), within this species the strain diversity is significant.

The *C. zemplinina* fermentations were also characterized by the largest number of terpenes and sesquiterpenes. Of the 49 significant compounds, 11 were either a terpene or sesquiterpene including geraniol, nerol, α-pinene, α-farnesene, ocimene, and linalool (Table 1). In general, these compounds are responsible for floral, pine and citrus aromas. In wine, rather than being produced directly by the yeast through a metabolic pathway, terpenes are released when glucosidases such as *β*-glucosidase free bound glycosylated precursors (Carrau et al. 2005). Two previous studies, Englezos et al. 2015 and Sadoudi et al. (2012), looked specifically at terpene content in single and mixed culture fermentations of *C. zemplinina* and *S. cerevisiae*. Englezos et al. (2015) tested 63 different strains and found that only 5 % of the isolates showed *β*-glucosidase activity. Sadoudi et al. (2012) found that, in monoculture, *C. zemplinina* produced more norisoprenoids and terpenols but this trend did not hold in mixed fermentation with *S. cerevisiae*. Our results however, indicate that the strain of *C. zemplinina* used in this study may produce relatively high amounts of *β*-glucosidase even in the presence of *S. cerevisiae* resulting in a wine richer in terpenes. Further screening should be carried out on this strain to confirm and quantitate enzyme production.

In summary, the *C. zemplinina* and *S. cerevisiae* fermentations were both the most sensorially and chemically distinct with *C. zemplinina* displaying the highest number of terpenes and sesquiterpenes as well as some more uncommon esters and presenting more dried fruit rather than fresh fruit aromas. Unfortunately, of all the fermentations it also produced the largest amount of acetic acid.

3.2.3 Kazachstania aerobia

The strain of *K. aerobia* used for these fermentations was isolated from Cabernet Sauvignon grape must at the IWBT and here for the first time we outline the chemical and organoleptic properties that this yeast is capable of producing in a finished wine product. Chemically speaking, the *K. aerobia* only showed 30 compounds to be statistically significantly different from the other fermentations. Though less than *C. zemplinina*'s 49, they still provide an interesting picture of what this yeast can bring to a wine fermentation.

The sensory panel agreed that the *K. aerobia* fermentations were driven more by solvent and bitter characteristics and slightly by the dried or stewed fruit aromas than the fresh ones (Fig. 3d). The chemical analysis revealed that the bulk of the compounds, 12 out of the 30, found to be positively different from the other fermentations were ethyl and acetate esters including ethyl acetate. This is most likely the cause of the solvent aroma. This correlates well with the fermentation data which revealed that *K. aerobia* fermentations had the second highest volatile acidity level of which ethyl acetate is a contributor (Fig. 5). 2-phenethyl acetate and 6-methyl-2-heptanol acetate were two other acetate esters found to be in higher relative concentration. It is interesting to note that the higher alcohols corresponding to the acetate esters in these
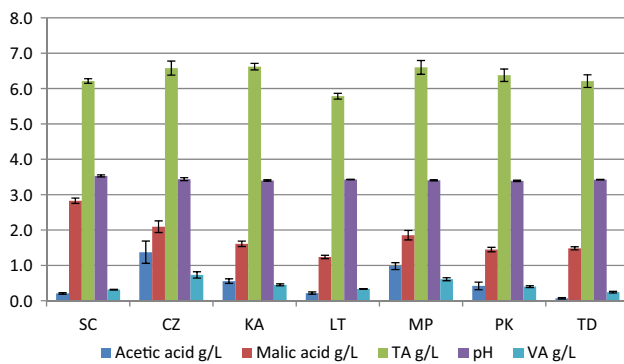
Fig. 5 Bar graph indicating the final average acidity and pH levels of each fermentation. TA titratable acidity while VA volatile acidity. *SC S. cerevisiae*, *CZ C. zemplinina*, *KA K. aerobia*, *LT L. Thermotolerans*, *MP M. pulcherrima*, *PK P. kluyveri*, *TD T. delbrueckii*

fermentations were not shown to be significantly higher. The next largest group of compounds found to be significantly positively different was terpenes. α-farnesene, γ-terpinene, nerol, *m*-cymene, and terpinolene all showed only trace peaks in the *S. cerevisiae* fermentations but much more substantial peaks in the *K. aerobia* fermentations. Though not responsible for the majority of a Sauvignon blanc flavor profile, terpenes are beneficial in their ability to provide complexity via subtle earthy, woody, citrus and floral undertones. They enhance and complement the more known fruity and floral notes provided by the esters. Besides a few alcohols, acids, aldehydes, and alkenes the rest of the *K. aerobia's* chemical profile was made up of six compounds which could not be identified based on their mass spectra.

Since *K. aerobia's* genome has yet to be fully sequenced, it is difficult to point to a specific cause for the abundant presence of these compounds relatively to the other fermentations. However, *Kazachstania's* nearest genetic relative is the *Saccharomyces* genus. It stands to reason that they share many of the same genes and thus regulatory pathways (Kurtzman 2003).

To conclude, the *K. aerobia* fermentations showed relatively high ethyl acetate, ester and terpene production and a few compounds that could not be identified. No major off-flavors were noted either chemically or sensorially.

3.2.4 Torulaspora delbrueckii

T. delbrueckii has been used in winemaking for years and is one of a few non-*Saccharomyces* species commercially available for use in wine and beer production. While it may be the best studied species of the genus, like all wine-related non-*Saccharomyces* species, it remains poorly understood. Of the studies that have been conducted, it has

been reported that wine fermented with *T. delbrueckii* in co-culture with *S. cerevisiae* were typically characterized by low volatile acidity, higher terpenols, 2-phenylethanol and C6 compound production (Ciani and Maccarelli 1998; Renault et al. 2009; Sadoudi et al. 2012; van Breda et al. 2013). Further metabolic and sensory evaluation of this yeast has yet to be done however.

Our study showed that sensorially *T. delbrueckii* fermentations were similar to the *L. thermotolerans*, *P. kluyveri* and *M. pulcherrima* all of which were most significantly characterized by the bitter attribute and equidistant from the fresh and dried fruit aromas (Fig. 3d). Fermentation data confirms previous reports in that *T. delbrueckii* produced the least amount to acetic acid and volatile acidity (Fig. 2) (Sadoudi et al. 2012). Chemically, its unique profile was most closely related to the *L. thermotolerans* across all extraction times (Fig. 2). Where it differed from *L. thermotolerans* and in fact all of the other fermentations was that it showed relatively higher concentrations of the sulfur containing compounds 3-(methylthio)-1-propanol, 3-[(2-hydroxyethyl)thio]-1-propanol, thietane, 3-(methylthio)-propanoic acid ethyl ester, and 1,3-oxathiane. Moreira et al. (2002) showed that increased amounts of methionine in grape must lead to increase in 3-(methylthio)-1-propanol and 3-(methylthio) propanoic acid ethyl ester among other unidentified sulfur compounds. They also showed that wines made from must generally low in amino acids had the highest total amount of sulfur compounds. As such, there are two likely causes of the increased sulfur compounds seen in our *T. delbrueckii* fermentations. Either *T. delbrueckii* itself assimilates and catabolizes methionine more readily than *S. cerevisiae* or *T. delbrueckii* creates an amino acid poor environment and facilitates the formation of these compounds by *S. cerevisiae*. As already stated, sulfur containing compounds have generally very low sensory thresholds and these are no exception. 3-(methylthio)-1-propanol has been described as having a raw potato, sulfurous, onion, soup, vegetable odor and 3-(methylthio)propanoic acid ethyl ester has been described as sulfurous, metallic, pineapple, fruity, and ripe pulpy tomato. They both have very high odor strengths and in too high a concentration would undoubtedly contribute to a wine fault. Given that the sensory panel did not identify a sulfurous fault in the *T. delbrueckii* fermentations, it is likely that though they were identified in the chemical analysis as significantly different these compounds were not in high enough concentration to be detected by the human palate. This however, does indicate the need for further study of amino acid catabolism by non-*Saccharomyces* yeasts with a specific focus on how differences may affect the metabolism and volatile compound production of *S. cerevisiae*. Besides these findings, it should be noted that like the *K. aerobia* fermentations there were two analytes

found to be significantly higher in the *T. delbrueckii* fermentations that could not be identified.

To summarize, while *T. delbrueckii* may reduce acetic acid in the final fermentation, it does little else to positively enhance the overall aroma profile. The wine showed higher levels of off-odor causing thiol compounds compared to the other fermentations which, while not noted by the sensory panel, could be detrimental to a final product if concentrations become too high.

3.2.5 Lachancea thermotolerans

Various studies have investigated its potential use in winemaking with regards to acetaldehyde, lactic acid, glycerol, 2-phenylethanol, and polysaccharide production as well as β-glucosidase activity. It is well established that this strain is capable of producing lactic acid and increasing the pH of wine while reducing its volatile acidity. It has also been shown to increase glycerol and 2-phenylethanol concentrations while being a low acetaldehyde producer (Ciani and Comitini 2010b; Ciani et al. 2006; Comitini et al. 2011; Cordero-Bueso et al. 2012; Kapsopoulou et al. 2006). Gobbi et al. (2013) is the most extensive study of this species in wine to date. They report that even in sequential inoculation, *L. thermotolerans* was the dominant species during fermentation and that these fermentations showed reduced 2-methyl-1-propanol and 3-methyl-1-butanol, higher 2-phenylethanol, reduced acetate esters but higher ethyl acetate. The ethyl acetate was below the sensory threshold, however. Some of this is in direct contrast to our findings where our results indicate that the *L. thermotolerans* population was slowly over taken by *S. cerevisiae* after its addition. Another difference was that over half of the esters found to be higher in the *L. thermotolerans* fermentations in our case were acetate esters. The *L. thermotolerans* fermentations were also characterized in our case by the lowest amount of both titratable acidity and malic acid out of all the fermentations (Fig. 5). Sensorially, these fermentations were mostly characterized along PC2 in the PCA, specifically the pineapple and bitter descriptor and as previously mentioned grouped closely with *T. delbrueckii, P. kluyveri,* and *M. pulcherrima* (Fig. 3d). Chemically, the *L. thermotolerans* and *T. delbrueckii* showed the most similar profiles according to the PCAs and hierarchical clusters. Of the 34 compounds shown to be significantly higher in the *L. thermotolerans* fermentations, 12 of them had no suitable matches in the NIST library. Many of these were small peaks that were only found in the 30 min extractions. The *L. thermotolerans* fermentations contained the largest number of unknown analytes. Only eight esters were shown to be higher, four of those were acetate esters one of which was citronellol acetate. Farnesol, geraniol, α-ionene,

and cosmene were found to be highest in the *L. thermotolerans* fermentations. This is supported by previous research which has shown that certain strains of *L. thermotolerans* can have high β-glucosidase activity (Cordero-Bueso et al. 2012).

In short, the *L. thermotolerans* fermentations showed a relatively high number of acetate esters and certain terpenes as well as the lowest amount of both titratable acidity and malic acid out of all the fermentations. There were no notable off-flavors in high relative concentration but there were 12 unidentified compounds, the highest number out of all the fermentations.

3.2.6 Pichia kluyveri

Despite the fact that this species is commercially available, comparatively even less research than on the other non-*Saccharomyces* yeasts has been published on its specific contributions to the winemaking process. Anfang et al. (2009) co-fermented Sauvignon blanc with a specific *P. kluyveri* isolate from New Zealand and showed that the resulting wines had elevated levels of 3-mercaptohexyl acetate (3MHA), indicating that the specific isolate was capable of releasing more favorable volatiles thiols from the Sauvignon blanc must. By contrast, the isolate used in this study did not show a sensorially significant increase in the tropical fruity aromas characterized by 3MHA. In fact, the *P. kluyveri* fermentations fell close to the center of PC1 being equally defined by both fresh and dried fruit aromas (Fig. 3d). Chemically, previous research had shown that *Pichia membraenifaciens* was a good acetate ester producer (Viana et al. 2008). However, this trait does not seem to carry over to *P. kluyveri* when compared to the other yeast in this study. This is unsurprising given the high amount of biodiversity observed in the *Pichia* genus (Domizio et al. 2011). Our study shows for the first time an in depth chemical profile of *P. kluyveri*. In both the PCAs and heat maps the *P. kluyveri* grouped most closely with the *M. pulcherrima* (Figs. 2, 3a–c). There were only 23 compounds found to be significantly higher in the *P. kluyveri* over all of the other fermentations. Eight of these were esters with significantly fruity aromas, three of which were 3-methylbutyl esters of three different organic acids (Table 1). 3-methyl-butanoic acid (isovaleric acid) was also relatively high. This compound is associated with an off-putting sour, sweaty, and cheesy aroma and in too high a concentration is considered a wine fault. It is a product of L-leucine catabolism and can undergo esterification to create 3-methyl-butanoic acid ethyl ester which has a much more pleasant, fruity aroma. This compound was one of the esters present in relatively high concentration in the *P. kluyveri* fermentations. Another potentially fault inducing compound found to be higher was phenethylamine.

Metabolically, there are two enzymes responsible for the conversion of the amino acid phenylalanine to phenethylamine: Aromatic-L-amino-acid decarboxylase and phenylalanine decarboxylase, either of which could have been up-regulated in either the *P. kluyveri* or the *S. cerevisiae*. Ultimately, neither of these potential fault compounds was in high enough concentration to have a sensory impact as the sensory panel did not note an off aroma in the wine. However, given these issues, combined with the lack of notable positive sensory attributes, this particular strain of *P. kluyveri* is conceivably not as good a candidate for Sauvignon blanc production as others covered by this study.

3.2.7 *Metschnikowia pulcherrima*

The *M. pulcherrima* fermentations were, sensorially, closest to the *P. kluyveri* fermentations and similarly not strongly associated with either the fresh or dried fruit aromas but fell closer to the sweet, bitter and solvent traits. Unlike the *P. kluyveri* fermentations however, chemically, there were no discernible off-aromas. A common isolate in vineyards and from grape must, *M. pulcherrima* has long been associated with grapes and wine and early research into the potential of this species showed that certain isolates displayed a high *β*-glucosidase activity (Fernández et al. 2000). Our study indicates that while some terpenes were higher in the *M. pulcherrima* fermentations when compared strictly to the control other yeasts showed higher amounts (Table 1S). Clemente-Jimenez et al. (2004) reported that *M. pulcherrima* produced high amounts of 2-phenyl ethanol and our findings support that as well. Of the thirty compounds found to be relatively higher in the *M. pulcherrima* fermentations, over half were esters most of which being either methyl butyl, methyl propyl, or phenethyl esters. Most of these however, have no recorded aroma. Similarly, there were six compounds that could not be identified, making the *M. pulcherrima* fermentations difficult to characterize both from a sensory and a metabolic standpoint.

Sadoudi et al. (2012) is, to date, the most comprehensive study of *M. pulcherrima* in co-culture with *S. cerevisiae*. They observed that fructose was consumed more slowly over the course of co-culture fermentation. This was not the case in our study but *S. cerevisiae* was added much later in our fermentations than the reported 48 h post *M. pulcherrima* inoculation of Sadoudi et al. (2012). They also reported that the co-cultures showed lower acetic production compared to the *S. cerevisiae* mono-culture. In our case, the opposite was true though in the *M. pulcherrima* fermentation the acetic acid level, though higher, remained below the sensory threshold (Fig. 5). These differences could be due to any number of variables such as yeast–yeast interactions, or changes in regulation of acetic acid metabolism in one or both species as a result of different fermentation stresses, to name a few. Like many of the other yeasts in this study, *M. pulcherrima* strain differences might be a possible reason for the discrepancies observed between studies.

4 Concluding remarks

In conclusion, of all the yeasts used in this study, *S. cerevisiae* and *C. zemplinina* had the most distinct and remarkable fermentation profiles. However each of the six non-*Saccharomyces* yeast co-fermentations displayed a unique sensory and metabolic profile. We were able to show that the sensory and chemistry methods complemented each other well and gave a much more detailed profile of these yeasts than any previously published work. Overall, our results would suggest that while the non-*Saccharomyces* yeasts produced wines that were unique, *S. cerevisiae* in single culture produced a product with the strongest positive sensory components thanks to high ester production. While it is true that our results are not fully in line with previously published results, this study was strongly dependent on the wine matrix composition, especially amino acids, terpene and thiol precursors, and thus is not 100 % reflective of the non-*Saccharomyces* capabilities. Given how little is currently known about these yeasts in wine and their contribution to wine aroma this study served to greatly increase the body of knowledge and understanding of these yeasts and their metabolism in the wine matrix used.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human or animal subjects.

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