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Unique volatile chemical profiles produced by indigenous and commercial strains of *Saccharomyces uvarum* and *Saccharomyces cerevisiae* during laboratory-scale Chardonnay fermentations.

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ABSTRACT

Each wine growing region hosts unique communities of indigenous yeast species, which may enter fermentation and contribute to the final flavour profile of wines. One of these species, *Saccharomyces uvarum*, is typically described as a cryotolerant yeast that produces relatively high levels of glycerol and rose-scented volatile compounds as compared with *Saccharomyces cerevisiae*, the main yeast in winemaking. Comparisons of fermentative and chemical properties between *S. uvarum* and *S. cerevisiae* at the species level are relatively common; however, a paucity of information has been collected on the potential variability present among *S. uvarum* strains. The objective of this study was to compare the fermentation kinetics and production of volatile compounds between indigenous and commercial *Saccharomyces* strains at different temperatures. We compared laboratory-scale fermentation of Chardonnay juice at 15 °C and 25 °C for 11 *Saccharomyces* yeast strains (six indigenous *S. uvarum*, one commercial *S. uvarum*, one indigenous *S. cerevisiae* and three commercial *S. cerevisiae*). Fermentation kinetics and the production of volatile compounds known to affect the organoleptic properties of wine were determined. The indigenous *S. uvarum* strains showed comparable kinetics to commercially sourced strains at both temperatures. Volatile compound production among the strains was more variable at 15 °C and resulted in unique chemical profiles at 15 °C as compared with 25 °C. Indigenous *S. uvarum* strains produced relatively high levels of 2-phenylethyl acetate and 2-phenylethanol, whereas these compounds were found at much lower levels in fermentations conducted by commercial strains of both *S. cerevisiae* and *S. uvarum*. Production of glycerol by indigenous *S. uvarum* strains did not differ from commercial strains in this study. Our findings demonstrate that indigenous strains of *S. uvarum* show functional variation among themselves. However, when compared with commercial *S. cerevisiae* and *S. uvarum* strains, they have comparable fermentation kinetics but unique volatile compound profiles, especially at low fermentation temperatures.

KEYWORDS

Headspace GCMS, Aroma-producing compounds, Low-temperature fermentation, *Saccharomyces uvarum*, Indigenous yeasts

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INTRODUCTION

While many factors affect the final aromas and flavours of wine, including grape varietal, available sugars, assimilable nitrogen, pH and fermentation temperature, different yeast strains can also impact wine quality by producing different concentrations of volatile compounds (Fleet, 2003; Romano *et al.,* 2003). *Saccharomyces cerevisiae* is the most commonly used yeast in winemaking because of its high ethanol tolerance, fast fermentation kinetics and competitive abilities over other yeasts; these characters make it ideal for industrial wine production. Nevertheless, grapes contain a variety of yeast and bacterial species at harvest that are brought into the winery environment and are transferred to the grape must during crushing/pressing (Cray *et al.,* 2013). These local yeasts can be an important factor in developing the *terroir* of a wine (Capozzi *et al.,* 2015; Jara *et al.,* 2016). The unique consortia of microorganisms on the grapes can help start the process of alcoholic fermentation, sometimes persisting well into the fermentation and may even take over both spontaneous and inoculated fermentations, adding character to wines (Bokulich *et al.,* 2013; Lange *et al.,* 2014; Mas *et al.,* 2016; Morgan *et al.,* 2019a).

One such yeast species is *Saccharomyces uvarum*, which is typically described as a cryotolerant yeast that produces more fruity aromas, adds body (Alonso-del-Real *et al.,* 2017; Hu *et al.,* 2018; Varela *et al.,* 2017) and reduces the amount of acetic acid in wine (Kelly *et al.,* 2020). Due to its noted cryotolerance, the use of *S. uvarum* strains in fermentation can be of use to winemakers who wish to conduct reduced-temperature fermentations or wineries located in cooler climates. Recent findings show that *S. uvarum* can compete with or even dominate over *S. cerevisiae* in controlled mixed cultures (Cheraiti *et al.,* 2005; Morgan *et al.,* 2020; Su *et al.,* 2019) and can dominate uninoculated fermentations at commercial wineries (Contreras *et al.,* 2014; Demuyter *et al.,* 2004; Morgan *et al.,* 2019a). The functional diversity of *S. uvarum* strains has not been as exhaustively characterised as *S. cerevisiae* in terms of oenological potential. The majority of studies on the fermentative characteristics of *S. uvarum* have included relatively few strains in each study, generally fewer than three (Gamero *et al.,* 2013; Stribny *et al.,* 2015; Varela *et al.,* 2017). Some previous studies did include a larger number of strains to better characterise the diversity of *S. uvarum* (Magyar *et al.,* 2008; Masneuf-Pomarède *et al.,* 2010), but these studies did not pre-screen for aggressive fermentation traits or utilise modern mass-spectrometric techniques to analyse the volatile compounds produced during fermentation. In this study we have chosen indigenous *S. uvarum* strains that were identified in commercial fermentations, some of which have been observed to persist in the winery environment over multiple vintages and/or have been observed as particularly competitive and identified as dominant strains. There is a paucity of research regarding the diversity of indigenous *S. uvarum* strains, particularly in North America. An *S. uvarum* population with high genetic diversity was recently identified at a winery in the Okanagan Valley of Canada, which appeared to competitively exclude *S. cerevisiae* in uninoculated winery fermentations in two separate vintages (McCarthy *et al.,* 2021; Morgan *et al.,* 2019b). The high genetic diversity of *S. uvarum* found in this region provides an opportunity to explore the potentially wide range of metabolic functionality in this species.

Previous studies have demonstrated that there is a strong effect of temperature on the final flavour profile of fermentation (Alonso-del-Real *et al.,* 2017; Beltran *et al.,* 2008; Molina *et al.,* 2007a). Additionally, low-temperature fermentations have been explored to increase the production and retention of aroma compounds (Gamero *et al.,* 2013). However, this potential benefit does not come without risk, since low temperatures can cause stuck or sluggish fermentations (Alonso-del-Real *et al.,* 2017). Many studies have demonstrated that *S. uvarum* species show greater cryotolerance relative to other fermentative yeasts (Gamero *et al.,* 2013; Moreira *et al.,* 2008; Su *et al.,* 2019; Tosi *et al.,* 2009).

Microbially derived compounds, including higher alcohols, ethyl esters, acetate esters, ethanol and glycerol, are important in forming the aroma and flavour characteristics of wine. Some of these compounds are dependent on precursor availability in must, while others are limited by the metabolic capabilities of different yeast strains. Higher alcohols are the main source of flavour characteristics in wine and include any alcohols with more than two carbons. They are formed from amino acid precursors as by-products of sugar metabolic pathways, anabolic reactions and the Ehrlich pathway and

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their production varies greatly between yeast strains (Dickinson *et al.,* 2003; Pires *et al.,* 2014). Ethyl esters impart fruity characteristics to the wine and are produced by yeast metabolism of medium-chain fatty acid precursors. Esters are produced via enzymatic condensation reactions and their levels are affected by many factors including fermentation temperature, initial concentrations of medium-chain fatty acid precursors and unsaturated fatty acid concentrations in the grapes (Saerens *et al.,* 2008). Acetate esters are another important compound group for wine, imparting fruity or solvent flavours and are produced by the enzymatic activity of yeast-produced alcohol acetyl-transferases, which are usually limited by the expression of the *ATF1* gene, as opposed to the available substrate in grape must (Rojas *et al.,* 2001). Glycerol is vital for giving body and texture to wine and is produced by yeasts through the glycolytic pathway. Glycerol production is also limited by the expression of genes that produce the enzymes in this pathway and not by substrate (Remize *et al.,* 2001; Wang *et al.,* 2001).

In addition to producing glycerol, indigenous yeast strains such as *S. uvarum* have the potential to make wines more complex with better mouthfeel through the production of higher concentrations of beneficial secondary metabolites and through the secretion of extracellular enzymes that alter wine flavour (Jolly *et al.,* 2014; Padilla *et al.,* 2016). This can also result in lower levels of ethanol being produced, which is advantageous in the face of climate change; warmer weather in many winemaking regions is increasing the sugar content in grapes, thereby potentially increasing the ethanol content of these wines above legal limits and consumer sensibilities (Ciani *et al.,* 2016). While alcoholic fermentation is the primary reaction necessary to produce wine, residual (i.e., unfermented) sugars in finished fermentations are a major driver of wine flavour profiles. Although grape sugar concentrations are generally evenly split between glucose and fructose, many yeast strains metabolise glucose more efficiently than fructose (Berthels *et al.,* 2004; D'Amore *et al.,* 1989). In addition, fructose also tastes sweeter than glucose, so residual fructose has a more pronounced effect on the sweetness of the wine than glucose. While increased sweetness may be desirable in some wines and to some consumers, the presence of this residual sugar in commercial wines reduces its microbial stability and can pose an increased risk of spoiling (Loureiro and Malfeito-Ferreira, 2003).

The objective of this study was to investigate, under laboratory conditions, the fermentative potential of seven indigenous *Saccharomyces* strains isolated in the Okanagan Valley wine region of Canada (six *S. uvarum* and one *S. cerevisiae*), as compared with four commercial *Saccharomyces* strains (one *S. uvarum* and three *S. cerevisiae*) at two temperatures. Each indigenous strain used in this experiment was included due to their competitive abilities and unique origins, to use these strains in future inoculated fermentations. We conducted controlled Chardonnay fermentations of these 11 *Saccharomyces* strains at two temperatures, 15 °C and 25 °C and monitored the rate of fermentation, the production of glycerol and ethanol and the relative abundances of yeast-derived volatile compounds produced after 25 days of fermentation. We expected that at lower temperatures, the *S. uvarum* strains would ferment faster than the *S. cerevisiae* strains and that the volatile chemical profiles of the resulting wines would differ with species, strain and temperature.

MATERIALS AND METHODS

1. Yeast strains selected

Eleven *Saccharomyces* yeast strains were chosen for this study: seven *S. uvarum* strains (six indigenous and one commercial) and four *S. cerevisiae* strains (one indigenous and three commercial) (Table 1). The *S. uvarum* and *S. cerevisiae* strains of indigenous origin were isolated from uninoculated fermentations conducted at commercial wineries in the Okanagan Valley wine region of Canada between the 2013 and 2017 vintages. Each strain was typed using eleven hypervariable microsatellite loci, as described previously (Morgan *et al.,* 2019a). The microsatellite profiles of all strains are provided in the supplementary material (Table S1, S2). Additionally, PCR-RFLP (restriction fragment length polymorphism) analysis was performed by amplifying the ITS1 region of the yeast rRNA gene, followed by a digest with the restriction enzyme *Hae*III, to confirm that the indigenous strains in this study were pure strains and not hybrids (Figure S1).

All indigenous *S. uvarum* strains used in this study except for P01H01 were isolated from uninoculated Chardonnay fermentations conducted at a single commercial winery during the 2015 and/or 2017 vintages. The indigenous *S. uvarum* strains '2015 Strain 1' and '2015 Strain 4' were both dominant strains in 2015 and were identified in 2017 as non-dominant strains (McCarthy *et al.,* 2021; Morgan *et al.,* 2019b).

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TABLE 1. *Saccharomyces* strains used in laboratory-scale Chardonnay fermentations.

Strain name	Species	Yeast type	Source
Velluto BMV58™	*S. uvarum*	Commercial	(Lallemand, Edwardstown, SA, Australia)
P01H01	*S. uvarum*	Indigenous	(unpublished data)
2015 Strain 1	*S. uvarum*	Indigenous	(McCarthy *et al.,* 2021)
2015 Strain 3	*S. uvarum*	Indigenous	(McCarthy *et al.,* 2021; Morgan *et al.,* 2019a)
2015 Strain 4	*S. uvarum*	Indigenous	(McCarthy *et al.,* 2021; Morgan *et al.,* 2019a)
2017 Strain 197	*S. uvarum*	Indigenous	(McCarthy *et al.,* 2021)
2017 Strain 151	*S. uvarum*	Indigenous	(McCarthy *et al.,* 2021)
Fermol Mediterranée	*S. cerevisiae*	Commercial	(AEB Group, Lodi, CA, USA)
Premier Classique ǂ	*S. cerevisiae*	Commercial	(Red Star Yeasts, Milwaukee, WI, USA)
Lalvin EC-1118	*S. cerevisiae*	Commercial	(Lallemand, Montréal, QC, Canada)
UBC47	*S. cerevisiae*	Indigenous	(unpublished data)

ǂ Formerly known as Red Star Montrachet

During previous laboratory-controlled experiments, '2015 Strain 1' was able to compete with a commercial *S. cerevisiae* strain at low temperatures and produced a unique volatile compound profile (Morgan *et al.,* 2020). The indigenous *S. uvarum* yeast strain '2015 Strain 3' was a dominant strain in both 2015 and 2017 at the same commercial winery (McCarthy *et al.,* 2021; Morgan *et al.,* 2019a). The indigenous strains '2017 Strain 151' and '2017 Strain 197' were identified for the first time in the 2017 vintage, but only '2017 strain 151' presented as a dominant strain (McCarthy *et al.,* 2021). The indigenous *S. uvarum* strain P01H01 was originally isolated in 2013 and dominated Pinot gris fermentations at a different Okanagan winery. The commercial *S. uvarum* strain Velluto BMV58 (Lallemand Inc, Edwardstown, SA, Australia) was used as a commercially sourced comparison. This strain was originally isolated in Spain and was the only *S. uvarum* strain available commercially for wine fermentations when this study was conducted.

The *S. cerevisiae* strain UBC47 is a putative indigenous strain originally isolated from uninoculated fermenting musts in the Okanagan Valley. The *S. cerevisiae* strains EC-1118 (Lallemand Inc., Petaluma, CA, USA), Fermol Mediterranée (AEB Group, Lodi, CA, USA) and Montrachet Premier Classique (Red Star Yeasts, Milwaukee, WI, USA) are commercially available strains. Multiple commercial comparisons were included to better interpret the variation in indigenous strains because there is a wide range of commercial yeasts available. EC-1118 is a very commonly used yeast strain in wine research and has been well-studied (Callejón *et al.,* 2012; Egli *et al.,* 1998; Lee *et al.,* 2012). However, it is considered one of the most aggressive fermenters; therefore, we also included Fermol Mediterranée and Montrachet Premier Classique as additional comparison datapoints.

2. Experimental Design and Sampling

The 11 *Saccharomyces* strains were each inoculated into 450 mL sterile-filtered Chardonnay juice for single-strain fermentations under controlled laboratory conditions. The volume chosen was equal to or greater than similar studies that also conducted micro-fermentations in the lab (Alonso-del-Real *et al.,* 2017; Gamero *et al.,* 2013; Masneuf-Pomarède *et al.,* 2010). The fermentations were conducted at both 15 °C and 25 °C, for a total of 22 treatments (n = 3 per treatment, for a total of 66 experimental fermentations). To maintain as much consistency between batches as possible, we used juice from concentrated Chardonnay winemaking kits (Cellarmaster Wines, Surry Hills, NSW, Australia). The concentrated juice was diluted to 23 °Brix with water (as measured by a refractometer) and sterile filtered by successive filtration beginning with 11 µm pore nitrocellulose filters and ending with 0.22 µm pore nitrocellulose filters (GSWP04700 Millipore® Sigma, Burlington MA, USA). Chemical adjustments to the juice were not made prior to or during the fermentation.

Commercial strains were rehydrated by adding a small amount of active dry yeast to a 2.0 mL microcentrifuge tube containing sterile water pre-heated to approximately 40 °C on a heating block:

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the sample tube was left for approximately 15 minutes to allow the yeasts to rehydrate. The rehydrated yeasts were then diluted, spread-plated onto Yeast Extract Peptone Dextrose (YEPD) agar (10 g/L yeast extract, 10 g/L agar, 20 g/L bacteriological peptone, 20 g/L dextrose) and incubated at 28 °C for 48 h. Indigenous strains were isolated from their original fermentations by aseptically sampling active commercial fermentations, Indigenous strains were isolated from their original fermentations by aseptically sampling active commercial fermentations, diluting and spread-plating samples onto YEPD agar and then isolating individual colonies by streak-plating onto new YEPD agar plates. These pure isolates were strain-typed using microsatellite loci analysis (outlined in Morgan *et al.,* 2019a) and added to an in-house library of indigenous strains. The yeast isolates were stored in 20 % glycerol at -80 °C until further use. For this study, yeast isolates were propagated from the glycerol stocks by diluting and spread-plating the samples onto YEPD agar and incubating at 28 °C for 48 h. Individual yeast colonies were then transferred to their own YEPD storage plates, incubated and kept at 4 °C. Prior to inoculation, a single colony of each strain was aseptically transferred to a flask containing liquid YEPD medium and incubated overnight at 25 °C. The yeast cell concentration was standardised after measuring the absorbance (600nm) for each starter culture immediately before inoculation so that each fermentation received the same amount of yeast. This method has been used previously for similar experiments (Ciani *et al.,* 2016; Minebois *et al.,* 2020). These starter cultures were used to inoculate 450 mL of sterile Chardonnay juice at 10^6 cells/mL. The 500 mL fermentation flasks (GL45, Schott) were sealed with custom 3D-printed airlocks made from cyanate ester (CE) 220 carbon resin with aluminium tops, filled with 5 mL of sterile water and containing a central sampling port closed with a silicone septum (https://www.carbon3d.com/case-studies/tthandadelaide/, accessed 2020-06-29).

Fermentations were conducted with constant agitation inside incubators at 80 RPM. Negative control of a flask of the same Chardonnay juice left uninoculated was included in each round of fermentation at both temperatures for chemical composition comparisons and to confirm the absence of contaminants. Fermentation progression was measured via weight loss of CO_2 as described previously (Ergun and Ferda Mutlu, 2000). Due to the limited space in each incubator and a large number of strains, it was not feasible to conduct every replicate fermentation at once. To address this issue, this experiment was conducted in three batches, with one replicate from each strain represented in each batch. To keep the duration of fermentations consistent, all fermentations of each batch were ended when the EC-1118 replicate fermentation at 15 °C reached completion as determined by weight loss of CO_2. This was determined to be 25 days during the first round of fermentation and was kept constant for subsequent replicate batches. Due to the non-commercial nature of the experimental strains, many of the fermentations were not expected to reach completion based on previous studies involving some of these strains (McCarthy *et al.,* 2021; Morgan *et al.,* 2019b). Therefore, we chose to conduct all fermentations for the same amount of time, regardless of whether they reached dryness. While this approach precluded the direct comparison of finished wines, it was a controlled method that allowed for a preliminary investigation into the fermentative potential of these non-commercial and non-traditional yeasts. Future follow-up studies involving these strains will not limit fermentation time.

3. Chemical analysis

An aliquot of 15 mL of fermented Chardonnay wine was sampled from each flask at the termination of the fermentation and stored at –80 °C for chemical analysis using both GC-MS and enzyme kits. Solution preparations are described in Section 3.1 for GC-MS and Section 3.2 for enzyme kit analysis. A comprehensive table of properties for all of the chemical compounds analysed in this study can be found in the supplemental materials (Table S3).

Standards for compounds were run for retention time and mass spectral matching (Table 2). Standard solutions were prepared from high purity solutions obtained from Alfa Aesar (Ward Hill, MA, USA; 99 %) 3-methylbutanoic acid, 2-methylbutanoic acid, octanoic acid, 2-methylpropanol, hexanol, benzalcohol, 2-methyl butanol, 3-methyl butanol, ethyl-2-methyl propanoate, ethyl butanoate, ethyl-2-methyl butanoate, ethyl-3-methyl butanoate; Aldrich (Milwaukee, WI, USA; ≥ 99 %) hexyl acetate, 2-methylbutyl acetate , 2-phenylethyl acetate, hexanoic acid, methionol, 2-phenylethanol, ethyl hexanoate, ethyl octanoate, ethyl decanoate; Fluka (Buchs, SG, Switzerland; ≥ 99 %) ethyl acetate; Acros Organics (Fair Lawn, New Jersey, USA) ethyl propanoate.

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TABLE 2. Volatile compounds analysed in laboratory-scale Chardonnay fermentations using Headspace-GC-MS.

Compound Category	Compound	Standard Run	Retention Time (min)	Kovat's Retention Index (polar/TempRamp)	Quantitation Ion	Confirmation Ions
Acetates	ethyl acetate	Yes	3.57	880–900	43	61, 29
	hexyl acetate	Yes	10.14	1255–1301	88	228, 101
	2-methylbutyl acetate	Yes	5.71	1132	72	57, 69, 74
	2-phenylethyl acetate	Yes	27.92	1785–1835	104	65, 91
Acids	hexanoic acid	Yes	29.78	1857	60	73, 87, 157
	3-methylbutanoic acid	Yes	22.69	1660	60	61, 74, 87
	2-methylbutanoic acid	Yes	22.72	1660	74	60, 73, 87
	acetic acid	No	16.28	1400–1465	60	43
	octanoic acid	Yes	34.59	2070	60	73, 115
Alcohols	2-methylpropanol	Yes	4.75	1075–1122	74	41, 43
	hexanol	Yes	12.76	1320–1362	56	45, 55
	methionol	Yes	24.20	1708–1738	106	61, 73
	benzalcohol	Yes	30.73	1834–1895	79	51, 77, 108
	2-phenylethanol	Yes	9.19	1874–1931	55	70
	2-methyl butanol	Yes	7.11	1194–1202	57	55, 56, 70
	3-methyl butanol	Yes	7.74	1190–1240	55	42, 56, 57, 70
Ethyl Esters	ethyl-2-methyl propanoate	Yes	3.77	961	116	43
	ethyl butanoate	Yes	4.55	1008–1035	88	101, 116
	ethyl-2-methyl butanoate	Yes	4.37	1053	102	87, 115
	ethyl-3-methyl butanoate	Yes	4.67	1067	88	115, 130
	ethyl hexanoate	Yes	8.49	1220–1267	88	99, 115
	ethyl octanoate	Yes	15.04	1442–1477	88	101, 172
	ethyl decanoate	Yes	20.75	1639–1678	101	157, 200
	ethyl propanoate	Yes	3.92	80–170	57	43, 102
Formates	ethyl formate	No	3.37	822–848	28	45.1, 32
Aldehydes	acetaldehyde	No	3.14	689–744	44	29
Amines	ethanolamine	No	2.97	1402–1427	28	43, 44
Flavenoids	5,7 Dihydroxy 3,3',4',5',6,8 hexamethoxyflavone	No	29.96	1880	28	32, 44

If more than one value for Kovat's Retention Index was available in the NIST database, the range is displayed.

3.1. GC-MS analysis

A 6 mL aliquot of fermented Chardonnay juice was mixed with 2 g NaCl into borosilicate glass headspace vials. A pooled quality control (QC) mixture was created using equal volumes from all samples and injected between every 10 sample injections to monitor instrument stability. Each sample was injected once. Prior to injection, samples were incubated at 70 °C for 10 minutes, pressurised to 19 kPa and equilibrated for 0.2 minutes. A 1 mL portion of headspace gas was collected using a headspace autosampler attached to a Thermo Scientific Trace 1300 gas chromatograph. Compound separation was performed on a Zebron™ ZB-WAX GC capillary column (60 m × 0.25 mm × 0.25 µm, cat. 7KG-G007-11). The chromatography conditions were as described by Haggerty *et al.* (2016). In short, a constant flow rate of 1.7 mL/min was used with an inlet temperature of 250 °C. The temperature program started at 80 °C for 10 minutes, increased to 130 °C at a rate of 10 °C/minute, was held constant for 5 minutes, increased to 151 °C at a rate of 2 °C/minute, increased to a final temperature of 240 °C at a rate of 20 °C/minute and was held for 5 minutes. The chromatograph was coupled to a Thermo ISQ mass spectrometer. The transfer line temperature was set to 250 °C. The MS source temperature was set to 230 °C and an ionization energy of 70 eV. The eluting compounds were analysed using full scan mode with a range of 25–500 m/z with a 0.2 second dwell time. Peaks were identified using a combination of comparison to standards injected separately, as well as putative identifications based on spectral matches in the NIST database and Kovat's Retention indices. Each peak area was normalised to the total ion current (TIC) of the injection to produce relative abundance values used in downstream statistical analysis. Relative abundance in this study was used to allow comparison of all compounds we were able to detect regardless of whether or not we ran an analytical standard. Additionally, as we were comparing the relative production of volatile compounds between indigenous and commercial yeast strains. Instead of internal standards, the peak areas were normalised to the total ion current of the injection to allow for non-targeted data acquisition.

Data was collected in a non-targeted manner to allow for the detection and comparison of as many compounds as possible. This produced relative quantitative data, not absolute quantitation. Relative quantitation was used to determine what percentage of the total signal for that injection was produced by a given compound. This technique is a non-targeted approach to data collection, which allows for a better determination of overall chemical profiles and generate hypotheses to advise further experiments.

3.2. Enzyme kit analysis

Residual sugars (glucose and fructose) were quantified using the Megazyme® D-Fructose/D-Glucose Assay Kit (K-FRUGL, Wicklow, Ireland) following the manufacturer's specifications with the following modification: to increase the accuracy and precision of the measurements, instead of sequential addition of enzymes with measurements taken in between the additions, we recorded separate absorbance measurements in three different wells as follows. The first well contained 200 µL of HPLC grade water, 10 µL of sample diluted 1:100 in HPLC grade water, 10 µl of Solution 1 (buffer) and 10 µL of Solution 2 (NADP+/ATP). The second well contained the same as the first well plus 2 µL of Solution 3 (HK/G6P-DH). The third well contained the same as the second well plus 2 µL of suspension 4 (PGI). As such, for each individual sample, there were 3 wells: a blank well with buffer reagents; a second well measuring the absorbance for glucose and fructose; and a third well measuring the absorbance for fructose alone. A six-point standard curve ranging from 200 µg/mL to 6.25 µg/mL was made using the standards provided with the kit. Two technical replicate measurements were performed on each sample. Absorbance data were collected using a plate reader (Varioskan Lux, Thermo Scientific Waltham, MA USA).

Glycerol was quantified using the Megazyme® Glycerol Kit (K-GCROL). Samples were diluted at 1:100 in HPLC grade water. A six-point standard curve ranging from 6.25 µg/mL to 200 µg/mL was made using the standards provided with the kit. Two technical replicate measurements were performed on each sample.

Ethanol was quantified using the Megazyme® Ethanol Kit (K-ETOH). Samples were diluted 1:1000 in HPLC grade water. A six-point standard curve was made using the standard provided in the kit ranging from 20 µg/mL to 150 µg/mL. Two technical replicate measurements were performed on each sample and averaged for analysis.

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Yeast Assimilable Nitrogen (YAN) was not measured during this experiment and no chemical adjustments of any kind were made before or during alcoholic fermentation, with the exception of the initial dilution of the grape juice to achieve an acceptable and consistent sugar concentration.

4. Statistical analysis

All statistical analysis was performed in R 3.6.1 (R Core Team 2019). All tests were considered significant at $p \leq 0.05$. The reproducible code is provided in full in the supplementary materials. Figures were made in R 3.6.1 or GraphPad Prism version 9.0 (La Jolla, CA, USA). The growthcurver R package (Sprouffske, 2018) was used to fit a logistical curve and calculate the area under this logistical curve for each sample by taking the integral of the logistic equation (Liccioli *et al.,* 2011). This value provides a metric, which includes all parameters of the growth curve including initial population size, maximum growth rate and carrying capacity into a single value. A one-way analysis of variance (ANOVA) was used to analyse the differences in the logistical curve area between strains within each temperature group (Table S4, S5). A principal component analysis (PCA) ordination was used to visualise the distribution of strains within each temperature treatment, in terms of their production of volatile compounds. Differences in the concentrations of non-volatile (Table 3) and volatile (Tables 4 and S5) fermentation-related compounds among strains were tested by performing one-way ANOVA for each compound, within an individual temperature treatment. Whenever the main statistical test (ANOVA) returned a significant result, a Tukey HSD *post hoc* test was performed to determine differences among treatments using the agricolae R package (de Mendiburu and Yaseen, 2020). Differences in the concentrations of non-volatile (Table 3) and volatile (Tables 4 and S6) fermentation-related compounds between the two temperature treatments for each strain were tested by performing Welch's two-sample *t*-tests using the R base package. The assumption of equal variances was accepted for all ANOVA and *t*-tests using Levene's homogeneity of variance test (R base package). Full datasets, as well as scripts for data analysis and visualization used in this publication, are made available at https://osf.io/p765x/.

RESULTS

1. Fermentation properties

Fermentation progression was monitored by CO_2 weight loss of each laboratory-scale fermentation; all fermentations in this study were terminated at 25 days, regardless of completion (Figure 1). All strains conducted alcoholic fermentation more slowly at 15 °C than at 25 °C and most *S. uvarum* strains had comparable kinetics to most *S. cerevisiae* strains at 25 °C (Figure 1, ANOVA results in Table S4, S5). *S. cerevisiae* strain EC-1118 had a statistically significantly larger area under the logistical curve (AUC) than most other strains at both temperatures, except for *S. uvarum* '2017 strain 151' at 15 °C and BMV58 at 25 °C, where the AUC metric did not differ significantly from EC-1118 (Figure 1). At 15 °C, however, *S. uvarum* '2017 strain 151' exhibited a significantly larger AUC value than the other *S. cerevisiae* strains, including the indigenous strain UBC47 and the commercial strains Fermol Mediterranée and Premier Classique. There were no differences found in the residual sugar concentrations among strains except for the commercial *S. cerevisiae* strain Premier Classique, which consumed less glucose than EC-1118 at 15 °C (Table 3). Additionally, there were no significant differences found in glycerol concentrations among strains within each temperature treatment. The indigenous *S. cerevisiae* strain UBC47, however, produced less glycerol at 15 °C than at 25 °C (Table 3). Lastly, strains did not differ significantly in the final concentrations of ethanol within temperature treatments.

2. Yeast production of volatile compounds

All four *S. cerevisiae* strains, as well as the commercial *S. uvarum* strain BMV58, had produced similar concentrations of volatile aroma compounds at the time of sampling. For example, at both temperatures, the *S. cerevisiae* strains and BMV58 all produced lower levels of 2-phenyl ethyl acetate than the six indigenous *S. uvarum* strains (Figure 2, Figure 3). Similarly, at 15 °C BMV58 produced the most 2-methyl-butyl acetate, followed by two commercial *S. cerevisiae* strains, Premier Classique and EC-1118 (Figure 2). Additionally, at 15 °C Fermol Mediterranée and EC-1118 produced higher levels of ethyl octanoate than the other strains, followed by Premier Classique, BMV58 and UBC47.

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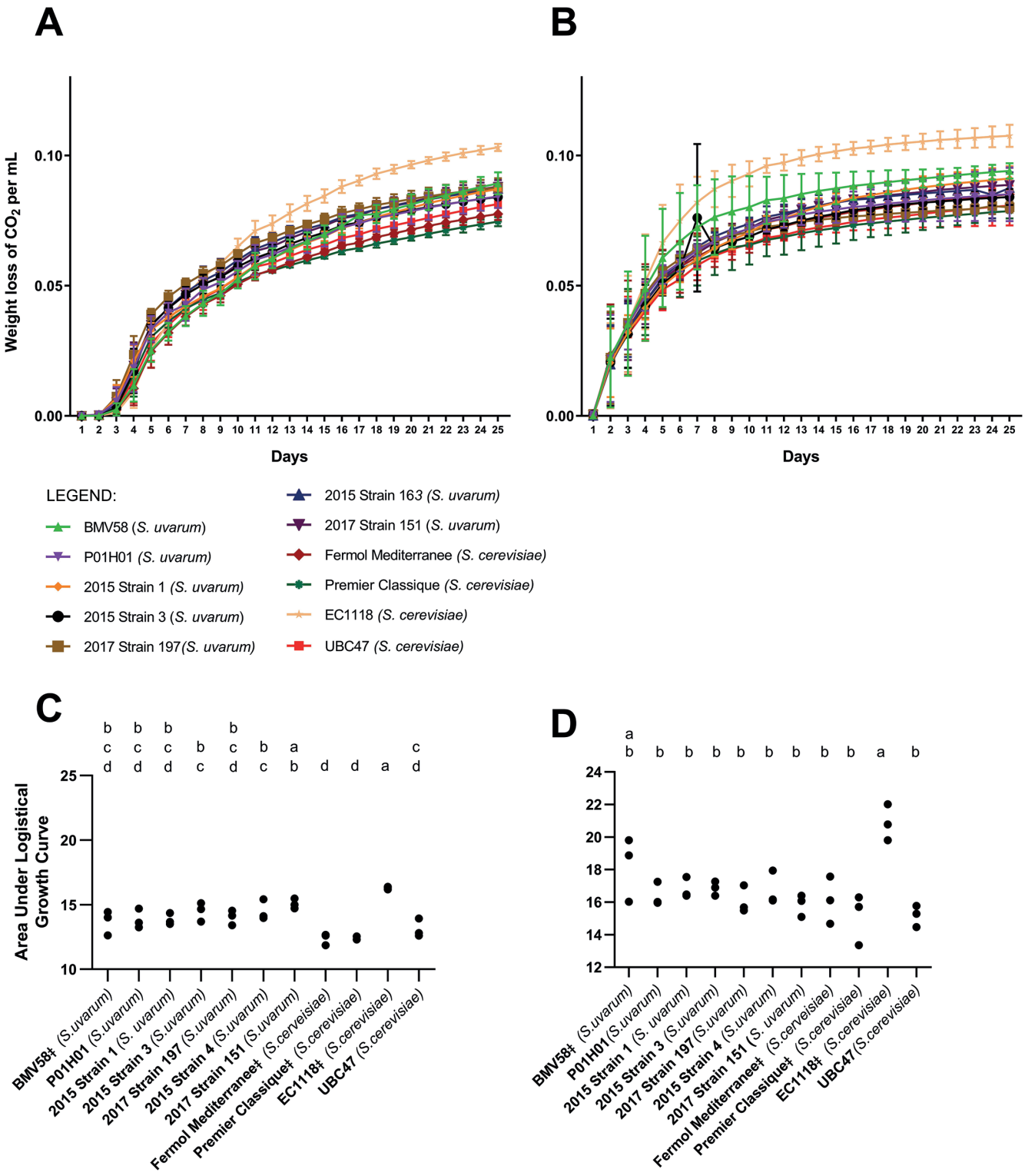


FIGURE 1. Fermentation kinetics of different *S. cerevisiae* and *S. uvarum* strains incubated at two different temperatures.

Weight loss in g CO_2 per mL of grape must ± SD (n = 3 per treatment) at A) 15 °C and B) 25 °C. Scatterplots of the area under the calculated logistical curves for each replicate fermentation were calculated for the fermentations at C) 15 °C and D) 25 °C. Fermentation progression was measured by weight loss as a function of CO_2 production during alcoholic fermentation. Fermentation vessels were weighed daily. Differences in the areas were analysed using one-way ANOVA followed by a Tukey HSD *post hoc* test. If letter codes for any given strains do not share a letter, then the strains are significantly different at $p \leq 0.05$. An asterisk (*) indicates that the strain is of commercial origin.

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TABLE 3. Concentration of fermentation-related non-volatile compounds in Chardonnay wines fermented by 11 *Saccharomyces* yeast strains at two different temperatures (15 °C and 25 °C) (n = 3 per treatment).

Strain Name	Residual Glucose (g/L)		Residual Fructose (g/L)		Glycerol (g/L)		Ethanol (% v/v)	
	15 °C	25 °C	15 °C	25 °C	15 °C	25 °C	15 °C	25 °C
				S. uvarum				
BMV58 ǂ	3.7 ± 2.3^{ab}	3.6 ± 2.6^{a}	25.2 ± 15.9^{a}	29.3 ± 18.6^{a}	6.5 ± 5.1^{a}	9.4 ± 2.6^{a}	8.38 ± 1.27^{a}	10.55 ± 3.71^{a}
P01H01	5.8 ± 2.5^{ab}	9 ± 6.0^{a}	34.3 ± 16.5^{a}	39.5 ± 25.2^{a}	9.2 ± 1.3^{a}	10.6 ± 1.6^{a}	9.30 ± 2.99^{a}	8.92 ± 2.46^{a}
2015 Strain 1	4.1 ± 1.1^{ab}	4.5 ± 2.2^{a}	25.9 ± 11.5^{a}	31.9 ± 14.4^{a}	10.5 ± 1.9^{a}	11 ± 0.6^{a}	9.03 ± 0.98^{a}	10.66 ± 4.21^{a}
2015 Strain 3	5 ± 1.2^{ab}	6.5 ± 2.5^{a}	22.1 ± 15.8^{a}	41.5 ± 19.6^{a}	10 ± 1.1^{a}	10.1 ± 0.8^{a}	8.94 ± 1.28^{a}	8.76 ± 1.23^{a}
2017 Strain 197	4.8 ± 1.6^{ab}	7.7 ± 2.7^{a}	29.1 ± 11.6^{a}	42.2 ± 14.4^{a}	11.9 ± 0.9^{a}	11.1 ± 1.0^{a}	8.42 ± 1.30^{a}	8.67 ± 1.27^{a}
2015 Strain 4	3.4 ± 1.4^{ab}	8 ± 6.1^{a}	29.8 ± 16.5^{a}	45.1 ± 26.6^{a}	9 ± 2.0^{a}	9.5 ± 1.3^{a}	8.56 ± 0.50^{a}	9.27 ± 1.97^{a}
2017 Strain 151	3.8 ± 0.4^{ab}	8 ± 3.4^{a}	29.9 ± 13.4^{a}	35.8 ± 10.9^{a}	9 ± 1.2^{a}	8.8 ± 0.9^{a}	8.14 ± 1.41^{a}	10.18 ± 3.58^{a}
				S. cerevisiae				
Fermol Med. ǂ	7.9 ± 3.2^{ab}	7.9 ± 1.9^{a}	35.1 ± 16.5^{a}	47.8 ± 20.3^{a}	7.5 ± 1.5^{a}	8.1 ± 0.9^{a}	6.83 ± 3.27^{a}	9.51 ± 2.60^{a}
Premier Classique ǂ	8.9 ± 2.8^{a}	9 ± 3.2^{a}	38.6 ± 14.5^{a}	39.3 ± 17.1^{a}	7.8 ± 0.6^{a}	8.1 ± 0.7^{a}	7.29 ± 1.96^{a}	8.36 ± 1.74^{a}
EC-1118 ǂ	1.5 ± 0.9^{b}	1.4 ± 1.1^{a}	9.1 ± 5.9^{a}	13.6 ± 9.8^{a}	7.2 ± 0.9^{a}	8.5 ± 1.2^{a}	9.53 ± 1.73^{a}	9.43 ± 0.28^{a}
UBC47	8.1 ± 5.1^{ab}	9.3 ± 4.7^{a}	38.3 ± 18.2^{a}	40.9 ± 21.9^{a}	6.9 ± 0.9^{a}	9.1 ± 0.9^{a}	7.64 ± 2.49^{a}	6.62 ± 0.75^{a}

Samples were taken at the termination of fermentation and presented as the mean ± standard deviation. For comparisons among strains within a single temperature treatment, data were analysed by performing one-way ANOVA (separate tests for each compound and temperature), followed by Tukey HSD *post hoc* tests if the ANOVA returned a significant result. Within each temperature column for each compound, unique superscript letters indicate significant differences among strains ($p \leq 0.05$). For comparisons between temperature treatments for a single strain, data were analysed by performing Welch's two-sample t-tests (separate tests for each compound and each strain). Bolded results indicate significant differences in compound concentration between temperatures for a given strain ($p \leq 0.05$).

ǂ Indicates commercially sourced strains

There was high variation among the six indigenous *S. uvarum* strains with respect to the production of volatile aroma compounds (Figure 2, Figure 3). For example, 2-phenylethanol, a compound often reported as characteristic of *S. uvarum*, was produced in higher concentrations by the indigenous *S. uvarum* strains '2017 strain 197' and '2017 strain 151' than by the other *S. uvarum* indigenous strains. In addition, the indigenous *S. uvarum* strains '2015 strain 3', '2017 strain 197', '2015 strain 4' and '2017 strain 151' also produced more methionol than all other strains at 15 °C, but not at 25 °C. Furthermore, '2017 strain 197', '2015 strain 4' and '2015 strain 1' produced more acetaldehyde than all other strains at 15 °C. At colder temperatures, the unique chemical profiles of the indigenous *S. uvarum* strains were more apparent, with higher variation in the production of ethyl esters and higher alcohols (Figure 2); at warmer temperatures, the chemical profiles were more homogenous (Figure 3).

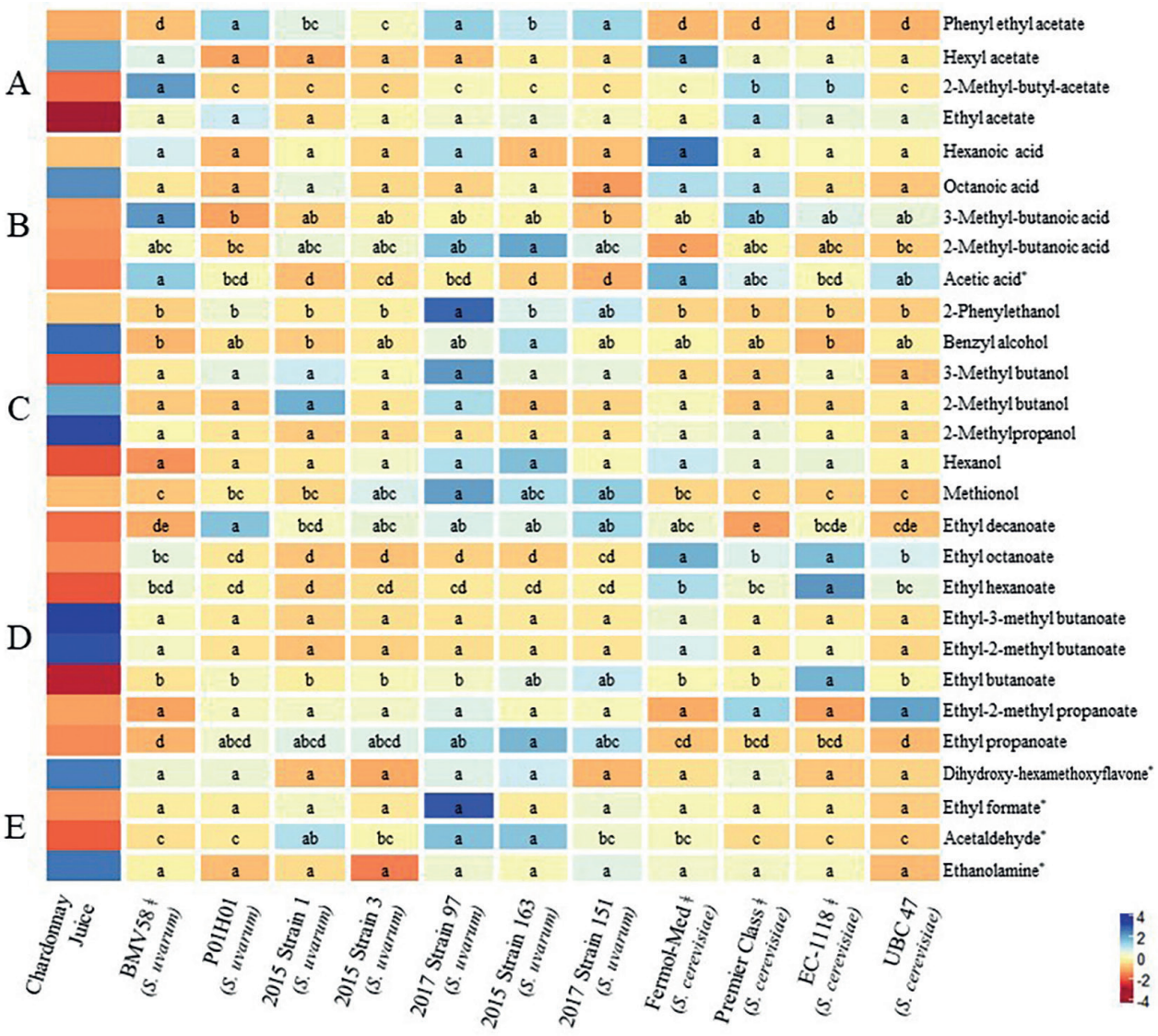


FIGURE 2. Heatmap showing the relative abundances of analysed compounds relative to unfermented Chardonnay juice, organised by class of compound, for fermentations conducted at 15 °C by different strains of *S. uvarum* and *S. cerevisiae* (n = 3 per treatment).

Group A, acetates; group B, acids; group C, alcohols; group D, ethyl esters; and group E, all other compounds. If letter codes for any given strains within a row do not share a letter, then those strains are significantly different at $p \leq 0.05$ for that compound, as indicated by a Tukey HSD *post hoc* test.

* Indicates putative identifications. ǂ Indicates commercially sourced yeast strains.

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TABLE 4. Percent change in the relative abundance of analytes in wines fermented by different *Saccharomyces* strains at 15 °C as compared to 25 °C.

	S. uvarum BMV58 ǂ	*S. uvarum* P01H01	*S. uvarum* 2015 Strain 1	*S. uvarum* 2015 Strain 3	*S. uvarum* 2017 Strain 197
Phenylethyl acetate	–32 % (0.06)	**32 % (0.01)**	–23 % (0.36)	–17 % (0.38)	10 % (0.29)
Hexyl acetate	24 % (0.68)	–35 % (0.44)	–125 % (0.22)	–20 % (0.76)	–47 % (0.36)
2-methyl-butyl-acetate	29 % (0.15)	21 % (0.07)	7 % (0.71)	14 % (0.43)	**30 % (0.03)**
Ethyl acetate	–34 % (0.07)	0 % (0.96)	–40 % (0.1)	–18 % (0.4)	–7 % (0.49)
Hexanoic acid	97 % (0.19)	38 % (0.4)	88 % (0.16)	80 % (0.39)	65 % (0.31)
Octanoic acid	–40 % (0.57)	–60 % (0.44)	**74 % (0.04)**	24 % (0.46)	–40 % (0.65)
2-methyl-butanoic acid	61 % (0.16)	–60 % (0.44)	–2 % (0.9)	–17 % (0.59)	46 % (0.27)
3-methyl-butanoic acid	71 % (0.13)	–4 % (0.83)	12 % (0.61)	–8 % (0.71)	37 % (0.34)
Acetic acid*	–28 % (0.31)	–31 % (0.15)	**–83 % (0.05)**	–57 % (0.1)	17 % (0.54)
2-phenylethanol	–55 % (0.41)	24 % (0.61)	–119 % (0.47)	–109 % (0.15)	41 % (0.08)
Benzyl alcohol	–19 % (0.78)	–119 % (0.24)	–40 % (0.41)	18 % (0.8)	48 % (0.07)
3-methyl butanol	–7 % (0.7)	59 % (0.11)	48 % (0.51)	27 % (0.7)	63 % (0.07)
2-methyl butanol	5 % (0.93)	–99 % (0.4)	83 % (0.43)	60 % (0.27)	59 % (0.53)
2-methylpropanol	32 % (0.45)	7 % (0.93)	–41 % (0.66)	–17 % (0.84)	–5 % (0.95)
Hexanol	–50 % (0.07)	–46 % (0.25)	–21 % (0.45)	–32 % (0.18)	8 % (0.79)
Methionol	–29 % (0.73)	–24 % (0.82)	–60 % (0.63)	76 % (0.27)	**73 % (0.05)**
Ethyl decanoate	–78 % (0.22)	14 % (0.24)	–52 % (0.08)	–12 % (0.68)	–17 % (0.48)
Ethyl octanoate	16 % (0.1)	–33 % (0.22)	**–152 % (< 0.01)**	**–72 % (0.03)**	–87 % (0.02)
Ethyl hexanoate	28 % (0.19)	16 % (0.24)	–29 % (0.13)	–3 % (0.87)	15 % (0.07)
Ethyl-3-methyl butanoate	31 % (0.46)	7 % (0.93)	–42 % (0.65)	–17 % (0.84)	–4 % (0.96)
Ethyl-2-methyl butanoate	27 % (0.49)	36 % (0.6)	–45 % (0.56)	–15 % (0.83)	–9 % (0.89)
Ethyl butanoate	19 % (0.35)	39 % (0.07)	24 % (0.38)	34 % (0.15)	40 % (0.06)
Ethyl-2-methyl propanoate	–283 % (0.41)	–22 % (0.85)	2 % (0.99)	–92 % (0.67)	–22 % (0.88)
Ethyl propanoate	–156 % (0.12)	–5 % (0.82)	–51 % (0.29)	–26 % (0.46)	15 % (0.6)
Dihydroxy-hexamethoxyflavone*	66 % (0.16)	20 % (0.73)	2 % (0.97)	–5 % (0.91)	0 % (0.99)
Ethyl formate*	-6 % (0.92)	7 % (0.9)	17 % (0.68)	24 % (0.49)	70 % (0.34)
Acetaldehyde*	–57 % (0.43)	–39 % (0.14)	10 % (0.35)	–5 % (0.84)	32 % (0.11)
Ethanolamine*	25 % (0.31)	15 % (0.8)	36 % (0.1)	–17 % (0.79)	14 % (0.45)
Glucose	2 % (0.98)	–55 % (0.46)	–12 % (0.76)	–31 % (0.41)	–61 % (0.19)
Fructose	–16 % (0.79)	–15 % (0.78)	–23 % (0.6)	–88 % (0.26)	–45 % (0.3)
Ethanol	–26 % (0.42)	4 % (0.88)	–18 % (0.57)	2 % (0.87)	–8 % (0.6)
Glycerol	–46 % (0.44)	–15 % (0.32)	–5 % (0.68)	–1 % (0.93)	6 % (0.41)

Positive values indicate a higher abundance in the 15 °C fermentations compared with the 25 °C fermentations and negative values indicate a lower relative abundance. Values are the average of three biological replicates per treatment. The TIC normalised abundance values were compared between the two temperatures for each strain using Welch's two-sample t-test, the results of which are shown in brackets (p values). Significant results are in bold ($p \leq 0.05$).

ǂ indicates commercially sourced strains; *indicates putatively identified compounds.

(Table 4 continues on next page)

S. uvarum 2015 Strain 4	*S. uvarum* 2017 Strain 151	*S. cerevisiae* Fermol Mediterranee ǂ	*S. cerevisiae* Premier Classique ǂ	*S. cerevisiae* EC-1118 ǂ	*S. cerevisiae* UBC47
–10 % (0.3)	8 % (0.29)	–16 % (0.54)	15 % (0.08)	–7 % (0.63)	–23 % (0.14)
18 % (0.64)	14 % (0.74)	4 % (0.96)	49 % (0.21)	–8 % (0.92)	–48 % (0.25)
22 % (0.07)	11 % (0.18)	22 % (0.21)	**37 % (0.02)**	25 % (0.29)	12 % (0.49)
–2 % (0.84)	–11 % (0.27)	–23 % (0.35)	2 % (0.95)	**–30 % (0.03)**	–34 % (0.18)
60 % (0.17)	48 % (0.19)	69 % (0.33)	38 % (0.76)	94 % (0.44)	93 % (0.41)
61 % (0.14)	3 % (0.89)	66 % (0.22)	33 % (0.53)	36 % (0.56)	15 % (0.68)
24 % (0.26)	30 % (0.15)	–11 % (0.6)	52 % (0.07)	41 % (0.41)	**36 % (0.02)**
–4 % (0.88)	29 % (0.06)	17 % (0.59)	**76 % (0.03)**	69 % (0.28)	**61 % (0.03)**
–118 % (0.13)	–61 % (0.25)	34 % (0.04)	–34 % (0.45)	–20 % (0.48)	–49 % (0.34)
–91 % (0.37)	68 % (0.36)	8 % (0.92)	–63 % (0.57)	72 % (0.11)	–55 % (0.61)
47 % (0.15)	21 % (0.28)	36 % (0.17)	34 % (0.4)	–61 % (0.4)	7 % (0.85)
9 % (0.88)	70 % (0.31)	–39 % (0.57)	6 % (0.93)	61 % (0.25)	14 % (0.85)
–137 % (0.49)	27 % (0.52)	56 % (0.16)	–2 % (0.97)	29 % (0.69)	45 % (0.23)
6 % (0.94)	–73 % (0.28)	–16 % (0.7)	45 % (0.26)	27 % (0.53)	–21 % (0.81)
–14 % (0.47)	–20 % (0.42)	–1 % (0.98)	1 % (0.98)	–49 % (0.13)	–38 % (0.06)
2 % (0.98)	58 % (0.06)	37 % (0.5)	5 % (0.92)	–18 % (0.75)	–104 % (0.32)
–19 % (0.11)	3 % (0.87)	18 % (0.52)	**–166 % (0.02)**	**–48 % (0.03)**	–55 % (0.23)
–80 % (0.03)	–49 % (0.13)	35 % (0.15)	21 % (0.11)	–6 % (0.8)	15 % (0.33)
7 % (0.35)	–2 % (0.77)	35 % (0.07)	**29 % (0.01)**	16 % (0.37)	26 % (0.16)
8 % (0.91)	–13 % (0.88)	46 % (0.21)	17 % (0.81)	18 % (0.71)	–23 % (0.79)
10 % (0.86)	–14 % (0.84)	42 % (0.24)	48 % (0.5)	27 % (0.51)	–18 % (0.82)
44 % (0.04)	39 % (0.09)	38 % (0.06)	**40 % (0.01)**	27 % (0.23)	32 % (0.16)
–72 % (0.7)	–101 % (0.46)	–190 % (0.42)	5 % (0.97)	–361 % (0.19)	10 % (0.93)
4 % (0.84)	2 % (0.86)	–148 % (0.21)	–20 % (0.66)	–111 % (0.09)	–118 % (0.08)
52 % (0.06)	–44 % (0.3)	19 % (0.58)	16 % (0.79)	–36 % (0.52)	–41 % (0.63)
–54 % (0.07)	–1 % (0.97)	–53 % (0.4)	4 % (0.9)	–99 % (0.14)	**–173 % (0.03)**
23 % (0.19)	6 % (0.77)	–76 % (0.35)	–19 % (0.65)	22 % (0.25)	–1 % (0.96)
30 % (0.19)	**49 % (0)**	39 % (0.07)	39 % (0.06)	**44 % (0.01)**	–13 % (0.82)
–137 % (0.32)	–113 % (0.16)	1 % (0.97)	0 % (1)	6 % (0.9)	–15 % (0.78)
–51 % (0.45)	–20 % (0.59)	–36 % (0.45)	–2 % (0.96)	–49 % (0.54)	–7 % (0.88)
–25 % (0.44)	–3 % (0.82)	–39 % (0.33)	–15 % (0.52)	1 % (0.93)	13 % (0.56)
–5 % (0.75)	3 % (0.79)	–8 % (0.6)	–4 % (0.61)	–19 % (0.2)	**–32 % (0.05)**

(Table 4 continued from previous page)

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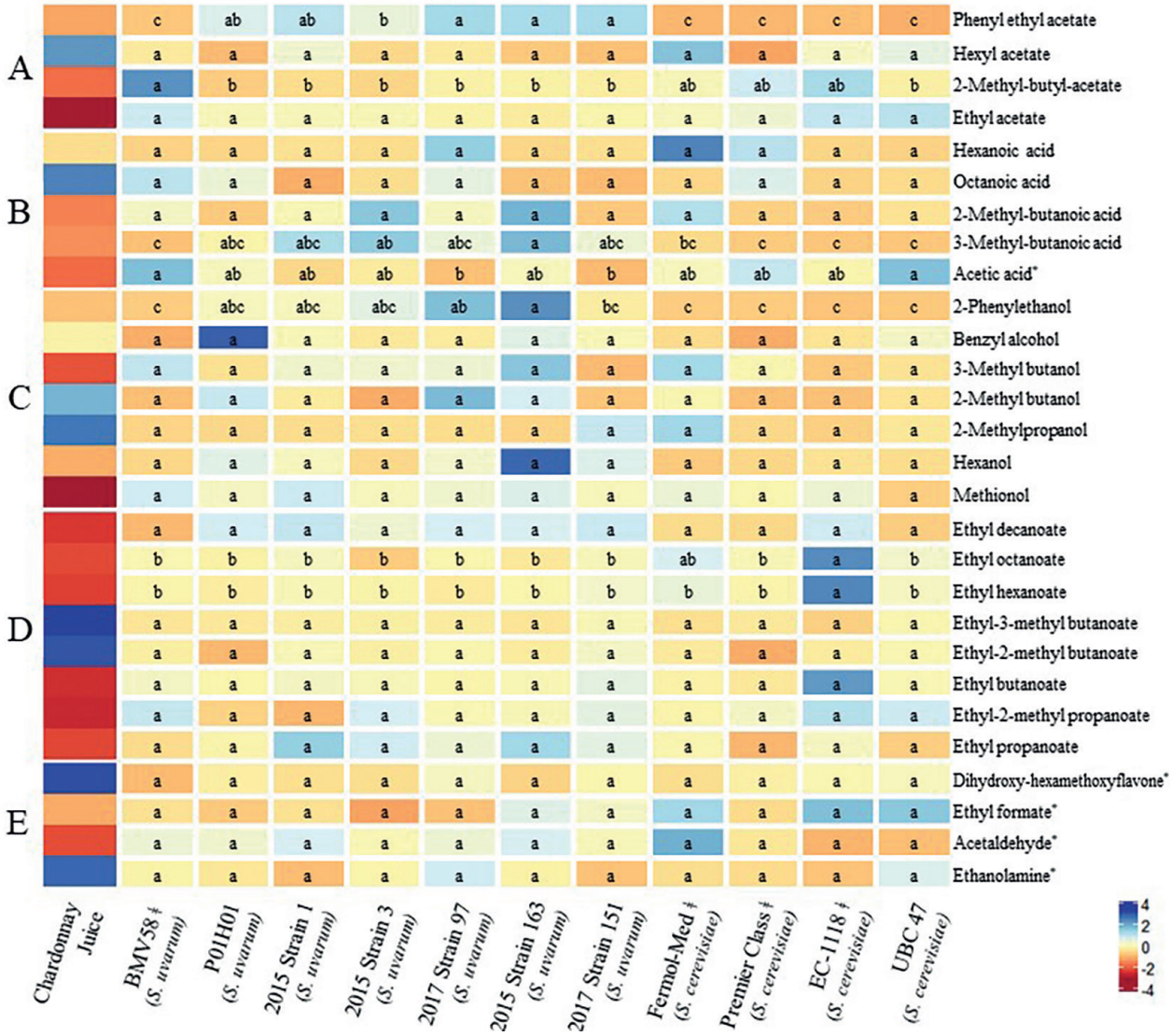


FIGURE 3. Heatmap showing the relative abundances of analysed compounds relative to unfermented Chardonnay juice, organised by class of compound, for fermentations conducted at 25 °C by different strains of *S. uvarum* and *S. cerevisiae* (n = 3 per treatment).

Group A, acetates; group B, acids; group C, alcohols; group D, ethyl esters; and group E, all other compounds. If letter codes for any given strains within a row do not share a letter, then those strains are significantly different at $p \leq 0.05$ for that compound, as indicated by a Tukey HSD *post hoc* test.

* Indicates putative identifications. ǂ Indicates commercially sourced yeast strains.

All the strains in this study tended toward increasing 2-methyl-butyl acetate production at 15 °C as compared to 25 °C, although this was only significant for '2017 strain 197' and Premier Classique. There was also a trend towards decreased ethyl acetate production at 15 °C compared to 25 °C for most strains, although it was only significant for EC-1118 (Table 4).

3. Overall characteristics

Principal Component Analysis (PCA) plots were used to visualise the overall variation in the dataset including relative abundance of volatile compounds and the area under the logistical curve (Figure 4). There were strong trends showing separation of temperature treatments along the PC2 axis (Figure 4A), explaining 14.62 % of the variation in the data. The PC1 axis of the full dataset, which explains 20.68 % of the variation in the data, showed a pronounced separation when coloured by species (Figure 4B). When including only the fermentations conducted at 15 °C, there was the separation of yeast species along the PC1 axis (Figure 4D) with the exception of three datapoints. When coloured by strain (Figure 4C), we observed that these three outliers

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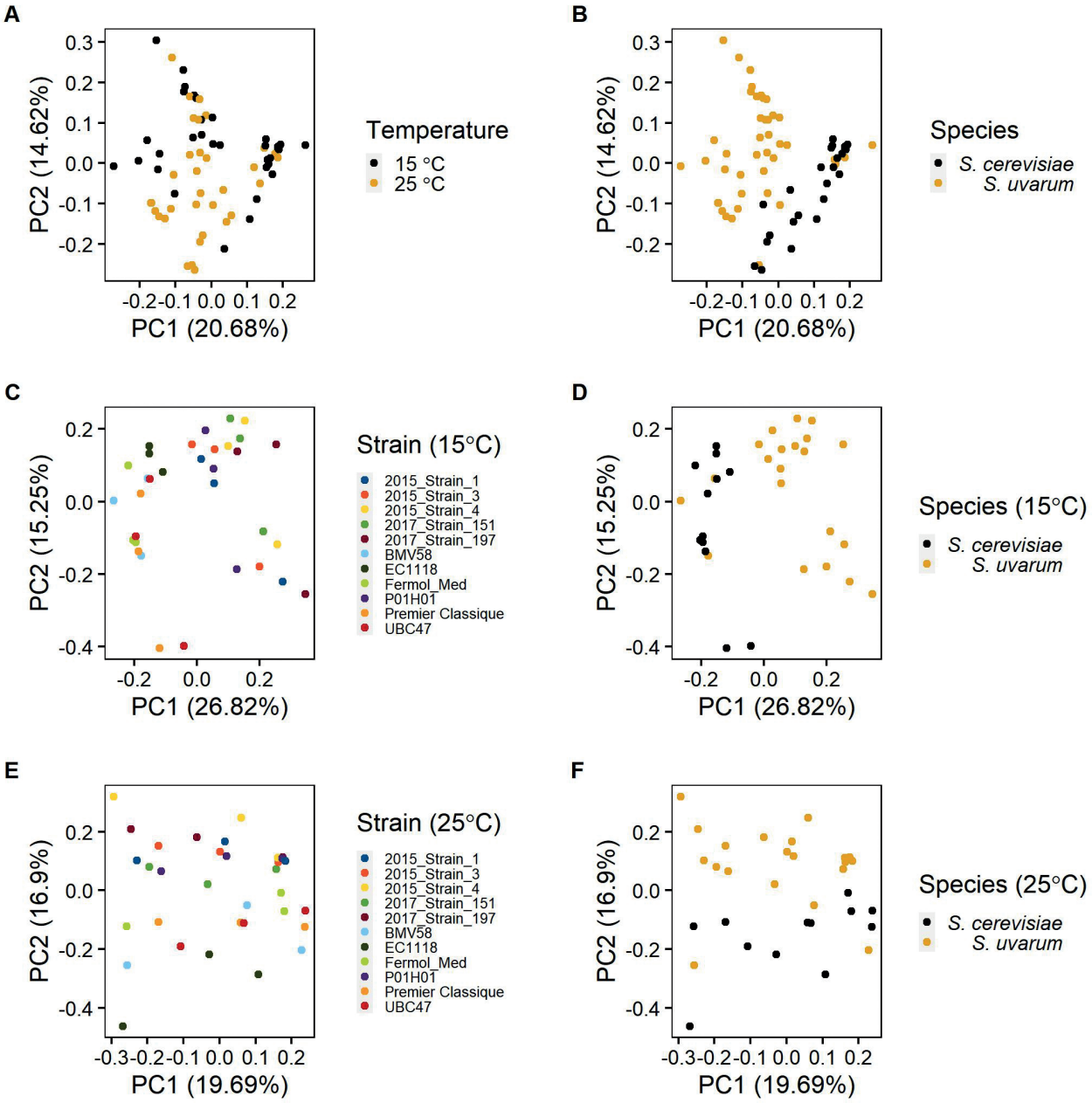


FIGURE 4. Principle component analysis (PCA) ordinations where each individual point represents all chemicals analysed and area under logistical growth curves from Chardonnay juice fermented with different strains of *S. cerevisiae* and *S. uvarum*.

A) All fermentations plotted, in which fermentation temperature is distinguished by colour.
B) All fermentations plotted, in which yeast species are distinguished by colour.
C) Only the 15 °C fermentations, distinguishing points by strains.
D) Only the 15 °C fermentations, distinguishing points by species.
E) Only the 25 °C fermentations, distinguishing points by strains.
F) Only the 25 °C fermentations, distinguishing points by species.

belonged to the commercial *S. uvarum* strain BMV58, which grouped with the *S. cerevisiae* strains. The axis of PC1 covered a wider range of variation in the 15 °C treatment with 26.82 % of the variation compared to 19.69 % of the variation in the data for the 25 °C treatment. At 25 °C, there was a vertical separation between most *S. cerevisiae* and *S. uvarum* strains along the PC2 axis, which explained 16.9 % of the variation in the data (Figure 4E, Figure 4F). As observed in the 15 °C fermentations, BMV58 again grouped closer to the *S. cerevisiae* strains than with the other *S. uvarum* strains at 25 °C.

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DISCUSSION

1. Fermentation properties

The importance of fermentation temperature has been emphasised in the literature (Alonso-del-Real *et al.,* 2017; Deed *et al.,* 2017; Demuyter *et al.,* 2004; Naumov *et al.,* 2000). *Saccharomyces uvarum* has been described as a cryotolerant or cryophilic strain, with its kinetic performance usually favoured over *Saccharomyces cerevisiae* at lower temperatures (< 15 °C), but not at higher temperatures (> 20 °C) (Alonso-del-Real *et al.,* 2017; Masneuf-Pomarède *et al.,* 2010; Stribny *et al.,* 2015). Thus, our expectation from these previous studies was that *S. uvarum* would conduct fermentations more quickly at 15 °C than *S. cerevisiae* and that it would have slower overall fermentation kinetics at 25 °C as compared with *S. cerevisiae*. In contrast to the findings of these previous studies, the *S. uvarum* strains in our study had comparable kinetics to most *S. cerevisiae* strains at 25 °C. Similar to our findings, a recent study published by Morgan *et al.* (2020) investigated the fermentative performance of an indigenous *S. uvarum* strain ('2015 strain 1' from this current study) and a commercial *S. cerevisiae* strain (Lalvin QA23) and found no difference in fermentation rate between the two strains at different fermentation temperatures; specifically, both strains fermented well at a higher temperature (24 °C) and both exhibited sluggish fermentation at a lower temperature (15 °C). In our study, the commercial *S. cerevisiae* strain EC-1118 finished alcoholic fermentation more quickly than most other strains at both temperatures, indicating it may have a broader temperature range than indicated in the literature (Binati *et al.,* 2020; Gao *et al.,* 2019; Lu *et al.,* 2017; Trinh *et al.,* 2011). There were a number of strains belonging to both *S. cerevisiae* and *S. uvarum* that did not reach dryness (Figure 1), precluding the direct comparison of finished wines fermented by different yeast strains in this study. It is possible that the volatile profiles of these strains were still evolving when the fermentations were terminated at 25 days and that the profiles of the finished wines would differ from those characterised in this study; future follow-up studies involving these strains would not limit fermentation time to elucidate these true differences. In this current study, the residual sugar concentrations were similar among the different temperature and yeast strain treatments at the time of sampling, suggesting that the differences in volatile and non-volatile compounds measured in this study represent real differences among treatments, whether or not they represent the true profiles of the finished wines. We also note that while the same amount of yeast was inoculated into each fermentation, some strains may have possessed different growth rates which could have affected their fermentation performance. Yeast growth (CFU/mL) was not measured in this experiment, thus further research is needed to determine if the growth rate was indeed a factor in the different fermentation performances observed among these yeast strains. Differences in fermentation kinetics and growth rates among the different strains of *S. cerevisiae* and *S. uvarum* could potentially be attributed to differences in their abilities to use ammonia and different amino acids (Englezos *et al.,* 2021; Minebois *et al.,* 2020), but more research is needed to determine if this is the case.

Surprisingly, two *S. uvarum* strains, one at 15 °C and the other at 25 °C, had similar fermentation kinetics to that of EC-1118. One of these *S. uvarum* strains, BMV58, which had similar fermentation kinetics to EC-1118 at 25 °C, was the only commercially available *S. uvarum* strain at the time this study was conducted. It is marketed for red wine fermentation and is reported to produce high levels of glycerol and floral/fruity aromas. (https://www.lallemandwine.com/en/australia/products/catalogue/wine-yeasts/66/velluto-bmv58/). In this study, we did not find that BMV58 produced higher levels of glycerol nor did it produce a significantly higher abundance of the compounds we detected associated with floral and fruity aromas. These findings indicate that the generalization of fermentation performance at certain temperatures, based on yeast species alone, may not be appropriate and further studies should be conducted to determine different strain performances at different fermentation temperatures or with different grape varietals. These results also indicate that the indigenous *S. uvarum* strains in this study can conduct and complete alcoholic fermentations while still producing unique volatile compound profiles, particularly at temperatures < 20 °C. However, we acknowledge that the performance of these commercial strains may be different under operational conditions; therefore, more research is needed with fermentations being conducted at a larger scale to make more applicable conclusions about the relative fermentation performances of these strains.

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Although the temperature of fermentation did affect the rate at which strains fermented, overall, we did not find significant differences in the metabolism of sugars (specifically glucose and fructose) and the subsequent rate of ethanol production among strains, or between fermentation temperatures for a single strain. The only significant result we found was related to the commercial *S. cerevisiae* strain Premier Classique, which consumed less glucose than EC-1118 at 15 °C. The paucity of differences between *S. cervisiae* and *S. uvarum* strains in their fermentation kinetics, sugar use, ethanol production, glycerol production and the differences we found among strains in the production of unique volatile compounds, highlights the potential of our *S. uvarum* strains to be used as commercial strains in the future. Our ethanol result is contradictory to previous literature, which has found final levels of ethanol production to be higher at colder fermenting temperatures as compared to warmer fermentation temperatures (Gao *et al.,* 2019; Veloso *et al.,* 2019). It is unclear as to why these fermentations do not appear to show significant shifts in sugar and ethanol profiles between 15 °C and 25 °C. It may be that higher temperatures affect fermentation enzymes responsible for alcoholic output differently than those associated with the primary growth of yeasts (Samoticha *et al.,* 2019). In this study, we did not observe *S. uvarum* strains to be high glycerol producers, which is in contrast with previous studies that have shown *S. uvarum* to produce more glycerol than *S. cerevisiae* (Hu *et al.,* 2018; Magyar and Tóth, 2011; Moreira *et al.,* 2008). This discrepancy with current literature on *S. uvarum* traits again shows the need for the characterization of more strains to examine how indigenous yeasts can participate in commercial fermentations. The only significant result we recorded for glycerol was a difference in the production at different temperatures by the indigenous *S. cerevisiae* strain UBC47. Future research on the production of glycerol by this *S. cerevisiae* strain, which is indigenous to the Okanagan Valley wine region of Canada, may be warranted.

2. Yeast production of volatile compounds

Acetate esters, such as ethyl acetate and 2-phenyl ethyl acetate, can impart fruity, sweet and tropical flavours to wine, but at high concentrations can result in vinegar or acetone-like aromas and flavours. Similar to our results, others also found that *S. uvarum* strains produced higher levels of acetate esters, especially 2-phenylethyl acetate, compared with *S. cerevisiae* (Stribny *et al.,* 2015). At both fermentation temperatures, we observed a significant increase in the relative amounts of 2-phenylethyl acetate produced by the indigenous *S. uvarum* strains compared to the *S. cerevisiae* strains and the commercial *S. uvarum* strain BMV58. The indigenous strains also showed variation in the production of phenylethyl acetate, with '2015 Strain 3' producing significantly less than '2017 Strain 197,' '2015 Strain 4,' and '2017 Strain 151' at 15 °C (Table/Figure 2). Both 2-phenylethyl acetate and 2-phenylethanol (higher alcohol) are known to impart a rose-like scent to wine, which is an established characteristic of *S. uvarum* (Gamero *et al.,* 2013; Gangl *et al.,* 2009; Tosi *et al.,* 2009). Interestingly, the commercial *S. uvarum* did not exhibit a similar increased production of these compounds but instead produced a volatile chemical profile that was similar to the *S. cerevisiae* strains used in this study. Previous studies have observed a decrease in the concentrations of acetate esters when fermentations were conducted at lower temperatures (Gamero *et al.,* 2013). In this study, however, we observed a wide variation of acetate esters between temperature treatments. This is expected because the formation of esters relies on the interaction of numerous variables including fermentation temperature, type of yeast, alcohol content and nutrients present (Killian and Ough, 1979; Rojas *et al.,* 2001; Stribny *et al.,* 2015).

Higher alcohols are another major contributor to aromatic profile, especially 2-phenylethanol, which imparts a rose-like aroma, which can be desirable depending on the style of wine being produced, as described above. Indigenous *S. uvarum* strains produced more 2-phenylethanol than the commercial strains of both *S. cerevisiae* and *S. uvarum*, supporting results from previous studies (Masneuf-Pomarède *et al.,* 2010; Stribny *et al.,* 2015). This significant difference was observed at both temperatures. The commercial *S. uvarum* strain BMV58 did not share this potentially beneficial trait with the other indigenous *S. uvarum* strains. Differences in the production of rose-scented chemicals among the *S. uvarum* strains in this study could potentially be attributed to differing abilities to use different amino acids (Pérez *et al.,* 2021). In our study, we did not measure how our strains used individual amino acids and the nitrogen content of the grape juice prior to fermentation was not measured. Further research on the abilities of our *S. uvarum* strains to use different amino acids and how they compare with that of *S. cerevisiae* is warranted

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and future research of this nature should include the measurement of yeast assimilable nitrogen (YAN), an important component of grape must.

High levels of volatile acidity are undesirable in wines, adding a sharp nail polish-like smell and is a concerning trait exhibited by *S. uvarum* strains in previous studies (Loureiro and Malfeito-Ferreira, 2003; Tristezza *et al.,* 2013). Similar to our results, Molina *et al.* (2007) found that lower fermentation temperatures increased the production of octanoic acid, 2-methyl butanoic and acetic acid by *S. uvarum* strains; contrastingly, Kelly *et al.* (2020) observed a decrease in acetic acid production by some *S. uvarum* strains. However, as mentioned previously, the fermentations conducted in this study were terminated at 25 days, so the final concentrations of these compounds could differ in finished wines. Future additional experimentation on finished wines will be conducted to determine if the level of these compounds is above the aroma detection threshold.

We observed that across all yeast strains in this study, there was a trend towards increased production of 2-methyl-butyl acetate, an enhancer of fruity notes in wine (Cameleyre *et al.,* 2017) and decreased production of ethyl acetate at 15 °C. This trend supports mainly anecdotal reports that lower fermentation temperatures can enhance/preserve the fruity notes in wines. In this study, this trend was significant for only a few strains. However, we hypothesise that this trend would become even more apparent if the fermentation had been allowed to complete, but further experimentation will determine if this is the case.

We also note that this experiment was conducted at a laboratory scale using commercially purchased grape juice. Therefore, some of the characteristics of the yeast strains used in this experiment may change under operational conditions at a winery. To this point, we recommend future experiments be conducted at a larger scale using fresh grape must, if possible.

3. Overall characteristics

The PCA clustering of wines by temperature treatment was expected (Figure 4A), as lower fermentation temperatures have been shown to alter yeast metabolism, which in turn changes the final characteristics of the wine (Beltran *et al.,* 2008; Llauradó *et al.,* 2005). Additionally, colder fermentations retain volatile compounds better than warmer fermentations (Deed *et al.,* 2017). The clustering of fermentation by species is equally prevalent in the full dataset, indicating that species-related characteristics exist regardless of fermentation temperature (Figure 4B). As PC1 and PC2 from Figures 4A, 4B and 4C explain < 35 % of the total variation for each of the figures, there are likely other factors besides temperature, species and strain affecting the grouping of chemical profiles. Differences in juice chemistry (such as sugar concentration and nutrient status) at the time of sampling may be another factor to consider in the explanation of the variation we found in this data, particularly because the samples for chemical analysis were taken before alcoholic fermentation had completed for most strains.

Separation of fermentation profiles by species was more prevalent in the 15 °C fermentations as compared with the 25 °C fermentations. The 15 °C fermentations also exhibited greater variation along the PC1 axis than the 25 °C fermentations, as well as greater variation among replicates. The indigenous *S. uvarum* strains '2015 strain 4' and '2017 strain 151', as well as the indigenous *S. cerevisiae* strain UBC47, showed substantial within-strain biological replicate variation at both temperatures. This is in contrast to the commercial strain EC-1118, whose replicates grouped tightly together (Figure 4C). These results suggest that indigenous strains may produce less consistent results than commercial strains, but this may also be an artefact of EC-1118 coming closer to completing fermentation than most other strains in this study. To determine the true oenological potential of these indigenous strains, fermentations over multiple generations should be performed to determine if strain-specific profiles and traits stabilise over time.

The commercial *S. uvarum* strain BMV58 grouped more closely with the *S. cerevisiae* strains than the indigenous *S. uvarum* strains at both temperatures, meaning it produced a volatile chemical profile more similar to the *S. cerevisiae* strains than to the other strains of its own species (Figure 2C, 2E). This result is congruent with the fermentation kinetics observed in this study, where BMV58 performed more similarly to the *S. cerevisiae* strains in terms of fermentation properties and volatile composition. These similarities imply that the expected advantages of using an *S. uvarum* strain for fermentation are not necessarily actualised in this commercial version, as compared to the indigenous *S. uvarum* strains we tested. Further research on *S. uvarum* strains such as '2017 strain 151' is warranted, due to its ability

to perform alcoholic fermentation at a similar rate to EC-1118, but at the same time produce typical *S. uvarum* compounds such as 2-phenylethanol.

CONCLUSIONS

Our results reveal that under laboratory conditions the unique indigenous *S. uvarum* strains in this study, isolated from local wineries, were able to ferment at similar rates as commercial strains and produce unique volatile chemical profiles, particularly at a fermentation temperature of 15 °C. Indigenous *S. uvarum* strains favoured the production of 2-phenylethyl acetate and 2-phenylethanol and displayed increased variation among replicate fermentations, suggesting that indigenous strains may not be as stable or consistent as commercial strains across vintages. The production of glycerol in indigenous *S. uvarum* strains did not differ from the commercially sourced strains, which also contradicts the stereotype of *S. uvarum* strains as high glycerol producers. We observed variation among the indigenous strains in terms of their relative production of volatile compounds, indicating high metabolic heterogeneity across the different strains. The commercial *S. uvarum* strain BMV58 did not display traits consistent with the expectations of *S. uvarum* strains; its fermentation kinetics at lower temperatures did not differ from commercial *S. cerevisiae* strains and its profile of volatile compounds was more similar to the *S. cerevisiae* strains than it was to the indigenous *S. uvarum* strains in this study. These results highlight the need to further investigate the fermentative potential of other indigenous strains as well as better characterise commercial strains under fermentation conditions. We have shown that these indigenous *S. uvarum* strains warrant further investigation to determine their oenological potential at a commercial scale. Future studies will be conducted using fermentations that are more in line with those typically conducted in wineries (e.g., an operational volume and a fermentation duration allowing for completeness). These studies should also include a sensory evaluation to determine whether differences in secondary metabolite production leads to significant differences in the sensory profiles of these wines. Additional studies will also assess if these strains produce consistent results over multiple generations. Finally, the main industrial takeaways from the findings of this study are that unique chemical profiles are produced from both *S. cerevisiae* and *S. uvarum* indigenous yeast strains at 1) different fermentation temperatures and 2) different residual sugar concentrations, both of which can be easily measured in a winery setting.

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