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Suitability of indigenous *Saccharomyces cerevisiae* strains isolated from spontaneous fermentations as wine fermentation starter cultures in the Okanagan Valley wine region in Canada

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ABSTRACT

Winemaking from grape juice is typically carried out using the *Saccharomyces cerevisiae (S. cerevisiae)* yeast species as a starter culture for ethanolic fermentation. The specific *S. cerevisiae* strain performing the fermentation plays a key role in shaping the aroma profile of the wine. Commercially available strains of *S. cerevisiae* improve the reproducibility and predictability of wine by establishing well-controlled fermentations. However, indigenous *S. cerevisiae* strains may be better acclimated to the conditions of a particular wine producing region and may enhance the sensory properties and characteristic profiles of the wine. This work assesses the oenological performance of *S. cerevisiae* strains isolated from the Okanagan Valley wine region in Canada in laboratory and pilot-scale fermentations. Six indigenous *S. cerevisiae* strains isolated from spontaneous fermentations at Okanagan Crush Pad (OCP) winery were selected for this research. Pinot gris and Pinot noir grape juice was used to perform 250 mL lab-scale fermentations carried out at 20 °C and 25 °C, respectively. Primary metabolites were quantified by high pressure liquid chromatography coupled to a refractive index detector (HPLC-RID) and volatile compounds were quantified by solid phase microextraction coupled to gas chromatography and mass spectrometry (SPME-GC/MS). Lab-scale Pinot gris wines produced with OCP indigenous strains had higher concentrations of the ester ethyl butanoate (apple, pineapple aroma) than lab-scale wines fermented with a commercial strain. OCP-088 and OCP-125 strains were selected for OCP winery pilot-scale fermentations in 250 L stainless steel barrels with Pinot gris and Pinot noir juice during the 2022 harvest. Both OCP strains persisted throughout the fermentations. Residual sugars were identified in both wines, primarily consisting of fructose at a range of 16-22 g/L and the alcohol by volume of the finished wine ranged from 10.82-11.58 %. Pilot-scale wines produced with OCP-088 and OCP-125 strains had a similar aroma composition, producing an off-dry wine with a desirable fruity aroma bouquet that had a higher concentration of the ester ethyl decanoate (sweet, fruity aroma) than commercial wines fermented in the same vintage. This study indicates that Okanagan indigenous strains ferment wines with desirable aromatic profiles and may be suitable as starter-cultures for producing wine reflective of the region.

KEYWORDS: *Saccharomyces cerevisiae*, wine, fermentation, indigenous yeasts, fermentation metabolite, volatile organic compounds, starter culture, Pinot gris, Pinot noir

INTRODUCTION

Winemaking, a centuries-old practice, involves traditional and modern techniques to transform the grapes into wine. Factors like vineyard location, climate, soil, grape varietal, and winemaking practice all contribute to shape the unique flavour and aromas of wine (Bokulich *et al.,* 2016; Zang *et al.,* 2022). *Terroir* encompasses environmental factors at specific geographic locations, influencing the development of regional-specific grape metabolites and microbial strains and impacting the quality and typicity of the wines produced (Cheng *et al.,* 2020; Knight *et al.,* 2015). To impart region-specific attributes and connect the wine to its unique *terroir*, winemakers prefer spontaneous fermentations, utilizing indigenous yeasts present naturally in the vineyards and winery environment (Lappa *et al.,* 2020). Spontaneous, or natural fermentations aim to achieve a more complex wine with a distinctive sensory profile compared to inoculated fermentations. While desired regional characteristics may be enhanced in natural fermentations, there is also an increased risk of stuck fermentation and the challenge of controlling and reproducing the fermentation process (Diaz *et al.,* 2013; Zabukovec *et al.,* 2020). Inoculated fermentations, alternatively, offer more control of the winemaking process and allow for time-efficient fermentations with an expectation of quality wine. Commercial *S. cerevisiae* strains, which are genetically redundant, are typically used to inoculate grape juice fermentations, resulting in a reduction of overall wine complexity (Borneman *et al.,* 2016). As a consequence, inoculated fermentations can cause wine standardization and may mask the distinctive characteristic aromas of region-specific strains (Barrajon *et al.,* 2011; Cheng *et al.,* 2020; Mendes *et al.,* 2022; Tristezza *et al.,* 2014).

S. cerevisiae is widely used for food and beverage production and is the most commonly used microorganism for wine production. The *S. cerevisiae* yeast species has a great fermentative capacity and the ability to tolerate stressful conditions such as high ethanol levels, low pH and antimicrobial and antioxidant additives (Bauer & Pretorius, 2000). Not only is *S. cerevisiae* responsible for converting glucose and fructose sugars into ethanol and carbon dioxide (CO_2), but it also plays an important role in the formation of secondary metabolites which shape the organoleptic profile of the wine (Buglass, 2022; Maicas & Mateo, 2016; Molinet & Cubillos, 2020). *S. cerevisiae* produces small molecules such as fusel alcohols, esters, carbonyls, fatty acids and terpenoids through different metabolic pathways. These secondary metabolites contribute to the organoleptic properties of the wines, including its aroma, flavour and mouthfeel (Erten *et al.,* 2006; Hirst & Richter, 2016). Studies have previously shown that indigenous *S. cerevisiae* strains isolated from wine producing regions can be successful starter cultures and produce wines with desirable and unique organoleptic characteristics. These indigenous strains are better adapted to their specific environments by enhancing the efficiency of the fermentation process and leading to the development of regional-specific starter cultures (Aponte *et al.,* 2020; Lopes *et al.,* 2007a; Lopes *et al.,* 2007b; Zabukovec *et al.,* 2020). Striking a balance between using commercial strains and exploring the potential of indigenous yeast strains incorporates the best of both approaches. This balance enables winemakers to produce wines that achieve a blend of quality, consistency, and the distinctiveness of their wine-producing regions.

The Okanagan Valley wine region in British Columbia (BC), Canada is a narrow valley spanning from the United States border with Washington State to 250 km north (Senese *et al.,* 2012). The Okanagan Valley climate is cool and arid, however there are many microclimates and diverse soil types with 12 sub-regions (https://winebc.com). The suitability of Okanagan Valley yeast strains for wine fermentation has been explored for multiple indigenous *Saccharomyces uvarum* strains and one indigenous *S. cerevisiae* strain in small-scale lab conditions but not in larger scale winery conditions (Lyons *et al.,* 2021; Morgan *et al.,* 2020). In this study, we collaborated with Okanagan Crush Pad (OCP) winery in Summerland – an Okanagan Valley sub-region that specializes in Pinot gris (PG) and Pinot noir (PN) varietals. We assessed the enological capability of six indigenous *S. cerevisiae* strains, previously isolated from OCP lab-scale PG and PN natural fermentations. The six strains that we tested (OCP-041, OCP-080, OCP-088, OCP-090, OCP-095 and OCP-125) were characterized as indigenous based on whole genome sequence comparison to commercial *S. cerevisiae* strains (Marr *et al.,* 2023). *S. cerevisiae* population studies have shown that commercial wine strains belong to the 'Wine/European' clade and lack genetic diversity whereas the OCP strains that we tested belong to the 'Pacific West Coast Wine' clade that is genetically diverse (Borneman *et al.,* 2016; Duan *et al.,* 2018a; Legras *et al.,* 2018; Marr *et al.,* 2023; Peter *et al.,* 2018). Two OCP strains, OCP-088 and OCP-125, were selected for winery pilot-scale PG and PN (vin gris) fermentations and assessed for soluble and volatile metabolite production.

MATERIALS AND METHODS

1. Experimental design

1.1. Laboratory scale fermentations

Six indigenous *S. cerevisiae* strains (OCP-041, OCP-080, OCP-088, OCP-090, OCP-095 and OCP-125) were tested as fermentation starter cultures. OCP-080, OCP-088, and OCP-125 were previously isolated from spontaneous PG fermentations in the OCP winery whereas OCP-041, OCP-090, and OCP-095 were previously isolated from spontaneous PG fermentations carried out in the lab after the grapes were harvested from an OCP PG vineyard (Marr *et al.,* 2023).

For this study, PG and PN juice from the 2019 harvest were provided by OCP winery and frozen at -20 °C prior to use (Summerland, BC). After thawing, the juice was prefiltered using 2.0 µm glass fiber prefilters (AP2004700, Merck Millipore Ltd., USA). The grape must was adjusted to 10 parts per million (ppm) of SO_2 and 150 ppm of yeast-assimilable

nitrogen (YAN) by adding potassium metabisulfite (439008, Fisher Scientific) and Fermaid K™ (Lallemand, USA), respectively, followed by sterile filtration using a 0.22 µm vacuum-driven top-up filter (Millipore, USA).

The *S. cerevisiae* M2 strain (Lallemand Oenology, ENOFERM M2) was used as a reference commercial strain control. Prior to inoculation in grape juice, each strain was inoculated into Yeast extract Peptone Dextrose (YPD) liquid media and incubated overnight at 25 °C. To ensure that all fermentations received the same amount of yeast, each fermentation was standardized to an Optical Density at 600 nanometers (OD_{600}) of 0.1 (~1.5×10^6 cells/mL). Each 250 mL fermentation flask was inoculated with a single strain. The flasks were sealed with rubber bungs and an airlock containing 4 mL of distilled sterile water. Fermentations were carried out in triplicate (3*n*) for each strain in both PG and PN juice. PG fermentations were conducted at 20 °C and PN fermentations at 25 °C. A negative control of uninoculated juice was included in both PG and PN juice to confirm the absence of native microflora or contamination. Fermentations were monitored by weight loss every 24 hours. Fermentation was considered complete when the 24-hour interval weight loss was ≤ 0.05 g for three consecutive days.

1.2. Pilot-scale winery fermentations

Two indigenous *S. cerevisiae* strains, OCP-088 and OCP-125, were sent to the Siebel Institute of Technology, USA for propagation in dextrose liquid media. Pilot scale fermentations were performed at OCP winery, in Summerland, BC, Canada during the 2022 harvest. PG and PN grapes were individually processed and pressed. Before inoculation of yeast, 10 ppm of SO_2 was added and the PG must was settled for a week whereas the PN must was settled for one day. Both PG and PN grape varietals were processed following white winemaking practices and utilized stainless steel barrels for fermentation and a grape must volume of 250 L. The PG and PN must was inoculated individually with *S. cerevisiae* strain OCP-088 or OCP-125 at a concentration of 1.25×10^{12} cells/barrel. Fermentations were carried out in triplicate for each strain and grape must combination. Initial YAN was measured using an OenoFoss™ wine analyzer (FOSS Analytics, Denmark). Prior to yeast inoculation the PG must had a YAN of 139.6 ppm and the PN must had a YAN of 106.2 ppm. Nutrient supplementation was done 24 hours after inoculation, with 100 g/barrel of FermControl™ BIO (2B Ferm Control, Germany). Fermentations were monitored daily by measuring °Brix using a Densy Meter DMA 35 (Anton Paar, USA). Fermentation activity was considered to have stopped when there was no further decrease of sugar concentration or increase in ethanol production. To compare aroma profiles of OCP-088 and OCP-125 bottled wine to commercial wine, three OCP commercial wines were used; PG from Switchback vineyard (PG-SB), PG from King Family Vineyard (PG-KF), and Freeform Vin Gris 2022 (Freeform VG). PG-KF was fermented with X5 Laffort yeast whereas PG-SB and Freeform VG were naturally fermented at OCP winery. The commercial wines were fermented with PG and PN grapes sourced from the same vineyard as the juice used for the OCP strain winery fermentations.

1.3. Microsatellite analysis

OCP-088 and OCP-125 pilot-scale fermentations were sampled at post-inoculation, and at early (15-17 °Brix), mid (8-10 °Brix), and late (< 1 °Brix) fermentation. Samples were serially diluted in 0.1 % peptone and 10^{-3} to 10^{-5} dilutions were plated for single colonies on YPD agar supplemented with 100 mg/mL chloramphenicol and 150 mg/mL biphenyl to inhibit bacteria and mould. Twenty colonies from each sample were arrayed in 96-well PCR plates containing 1.25 mg/mL zymolyase and DNA extraction was performed as previously described (Ling *et al.,* 1995; Martiniuk *et al.,* 2016). Simultaneous amplification of 10 microsatellite loci (Cheng *et al.,* 2020; Legras *et al.,* 2005; Martiniuk *et al.,* 2016; Richards *et al.,* 2009) was achieved in 10 µL reactions using a Qiagen MultiPlex PCR kit (Cat. 206143) and 20-50 ng of DNA, and an annealing temperature of 54 °C. Primer sequences, their respective fluorescent tags, as well as concentrations, can be found in Cheng *et al.* (2020). PCR products were analysed using an Applied Biosystems 3730XL 96-capillary DNA Analyzer at the UBC Sequencing and Bioinformatics Consortium. Multi-locus genotypes (MLGs) were determined for each isolated colony using GeneMapper software (Thermo Fisher Scientific, Massachusetts, USA), and MLG profiles of isolates from this study were compared to previously determined profiles of OCP-088 and OCP-125 to confirm that the inoculated indigenous *S. cerevisiae* strains remained dominant during fermentation (Marr *et al.,* 2023).

2. Chemical analysis

2.1. Primary metabolites

Primary wine metabolites (citric acid, tartaric acid, malic acid, acetic acid, glucose, fructose, glycerol, and ethanol) were measured by High Pressure Liquid Chromatography (HPLC) with a refractive index detector (RID) using an Agilent 1260 (HPLC) and 1290 (RID) Infinity II LC system (Agilent Technologies, California, USA). A 1.5 mL volume of wine was filtered using 0.22 µm nylon filters (VWR, USA) into 2 mL HPLC vials. An ion exchange column [NUCLEOGEL ION OA 300 × 7.8 mm 8 µm 300 A (Macherey-Nagel, Düren, Germany)] with a NUCLEOGEL ION 300 OA 10 µm 21 × 4mm guard column, was used to elute the metabolites, with 5 mM H_2SO_4 as the mobile phase, following isocratic mode and a 0.5 mL/min flow rate, for a total run time of 35 min. RID purge-valve was set to automatic purge, the column and RID temperatures were set to 71 °C, and 55 °C, respectively and the injection volume was 20 µL for each sample. A six-point calibration curve using high-purity (< 98 %) ethanol (0-15 % v/v), glucose (0-30 g/L), fructose (0-40 g/L), acetic acid (0-1.25 % v/v), glycerol (0-2.5 % w/v), citric acid (0-1 g/L), and tartaric acid (0-7.5 g/L) from Sigma-Aldrich (Missouri, USA), dissolved in distilled water, was prepared to confirm metabolite identity, retention times (RT), and metabolite concentrations. A calibration standard was injected at the beginning, middle and end of each run to monitor instrument performance.

2.2. Secondary metabolites

Free volatiles were analyzed by solid-phase micro extraction followed by gas chromatography-mass spectrometry (SPME-GC/MS) with protocol and instrument conditions reported in Pico *et al.* (2022) using a 7890A GC coupled to a 597C single quadrupole MS detector, equipped with a GC Sampler 80 (SPME autosampler) and Chemstation software (version E.02.02.1431, Agilent Technologies, California, USA). An aliquot of 5 mL of fermented must was transferred into a 20 mL headspace vial containing 1.5 g of NaCl, and each biological replicate was injected in triplicate. D3-Ethyl butyrate and D3-linalool (CDN ISOTOPES, Canada) were added as internal standards to achieve a final concentration of 2 ppm into each vial. Wine samples were preincubated for 5 min at 45 °C with an agitation speed of 250 rpm, followed by exposure to a 1 cm and 50/30 µm DVB/CAR/PDMS SPME fiber (Sigma Aldrich, Ontario, Canada) for 30 min at 250 rpm. Volatile compounds were desorbed for 3 min in the injector at 250 °C, then separated by a polar DB Wax column (30 m length × 0.25 mm inner diameter × 0.25 µm film) (J&W Scientific, California, USA). The GC was operated under programmed temperature conditions: from 35 °C (4.36 min) to 65 °C (3 min) at 1.5 °C/min, up to 138 °C (0 min) at 3 °C/min, and up to 23 °C (5 min) at 60 °C/min for a total run time of 58.23 min. The interface, ion source, and quadrupole temperatures were 250 °C, 230 °C, and 150 °C, respectively. Electron impact ionization was performed with an energy of 70 eV and the quadrupole was simultaneously operated in SCAN (including a mass range of 35–500 *m/z*) and selected ion monitoring (SIM) mode. Identification of compounds was achieved comparing the RTs and accurate mass spectra of the volatiles in the samples with pure standards and confirmed using their Kovats Index (KI) and the Mass Spectra Library (NIST MS Search 2.2 & MS Interpreter). For those cases in which the standard was not available, tentative identification was claimed using KI and the Mass Spectra Library (Table S1). Quantification was performed using a 6-point calibration curve, prepared in 12 % ethanol (*i.e.*, external calibration curve). The limits of detection (LOD) and limits of quantification (LOQ) were calculated to ensure the proper identification and quantification for each volatile (Table S1). Matrix effect factors were calculated as described (Pico *et al.,* 2022).

RESULTS AND DISCUSSION

1. Lab-scale fermentations

1.1. Primary metabolites

The initial sugar concentration in the PG juice for the 2019 vintage (25.3 °Brix) was above average for this grape varietal, normally harvested at ~22 °Brix (Morgan *et al.,* 2019). Neither OCP indigenous strains nor the commercial M2 control strain were able to complete the PG fermentation to dryness (< 5 g/L of residual sugars) (Table 1). The PG wines fermented with OCP-090, OCP-125, OCP-088, OCP-095, and OCP-080 showed no significant difference ($p \leq 0.05$) in residual glucose and fructose content in comparison to the commercial control M2 which had 6.07 ± 2.83 g/L and 43.45 ± 6.56 g/L, respectively, of each sugar (Table 1).

TABLE 1. Primary metabolites quantified by HPLC-RID on PG and PN lab-scale fermentations. Samples were analyzed in triplicate, results show mean ± SD. Data was analysed using a one-way ANOVA followed by a Tukey HSD *post hoc* test. Different letters indicate that strains are significantly different at $p \leq 0.05$ for each compound.

		Residual glucose (g/L)	Residual fructose (g/L)	Ethanol (% v/v)	Glycerol (% v/v)	Citric acid (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Acetic acid (% v/v)
Pinot gris	OCP-041	16.04 ± 2.83^{a}	57.65 ± 3.01^{a}	10.88 ± 0.35^{b}	0.56 ± 0.01^{a}	0.57 ± 0.01^{c}	2.01 ± 0.10^{a}	1.61 ± 0.09^{a}	0.09 ± 0.01^{a}
	OCP-090	7.34 ± 0.45^{b}	47.56 ± 1.72^{ab}	11.99 ± 0.22^{a}	0.46 ± 0.02^{b}	0.68 ± 0.03^{ab}	1.88 ± 0.06^{a}	1.80 ± 0.03^{a}	0.05 ± 0.01^{b}
	OCP-095	12.32 ± 2.10^{ab}	53.45 ± 3.57^{ab}	11.30 ± 0.33^{ab}	0.45 ± 0.01^{b}	0.68 ± 0.04^{ab}	2.01 ± 0.06^{a}	1.80 ± 0.29^{a}	0.07 ± 0.01^{b}
	OCP-125	8.26 ± 0.63^{b}	48.23 ± 0.52^{ab}	11.80 ± 0.07^{ab}	0.48 ± 0.02^{b}	0.66 ± 0.04^{b}	2.04 ± 0.15^{a}	1.72 ± 0.11^{a}	0.05 ± 0.01^{b}
	OCP-080	9.29 ± 2.98^{b}	51.15 ± 5.00^{ab}	11.68 ± 0.48^{ab}	0.51 ± 0.03^{a}	0.67 ± 0.02^{b}	1.77 ± 0.10^{a}	1.67 ± 0.05^{a}	0.05 ± 0.01^{b}
	OCP-088	7.59 ± 2.34^{b}	50.13 ± 4.25^{ab}	11.78 ± 0.40^{ab}	0.50 ± 0.01^{b}	0.68 ± 0.06^{ab}	1.78 ± 0.16^{a}	1.64 ± 0.15^{a}	0.06 ± 0.00^{b}
	M2	6.07 ± 2.83^{b}	43.45 ± 6.56^{b}	12.08 ± 0.05^{a}	0.45 ± 0.02^{b}	0.75 ± 0.04^{a}	1.99 ± 0.21^{a}	1.81 ± 0.06^{a}	0.06 ± 0.00^{b}
Pinot noir	OCP-041	5.87 ± 1.40^{B}	34.95 ± 3.36^{A}	10.53 ± 0.25^{C}	0.67 ± 0.00^{A}	1.03 ± 0.01^{A}	2.95 ± 0.32^{A}	2.83 ± 0.06^{B}	0.11 ± 0.00^{A}
	OCP-090	2.72 ± 1.47^{C}	28.58 ± 5.41^{AB}	11.22 ± 0.40^{B}	0.53 ± 0.00^{BC}	1.01 ± 0.01^{AB}	2.90 ± 0.43^{A}	2.97 ± 0.04^{AB}	0.07 ± 0.00^{BC}
	OCP-095	8.10 ± 0.83^{A}	40.69 ± 1.07^{A}	10.25 ± 0.13^{C}	0.54 ± 0.00^{BC}	1.00 ± 0.01^{AB}	2.80 ± 0.20^{A}	2.84 ± 0.09^{B}	0.10 ± 0.00^{A}
	OCP-125	1.94 ± 0.75^{CD}	21.89 ± 4.31^{B}	11.48 ± 0.01^{B}	0.54 ± 0.00^{BC}	1.03 ± 0.01^{A}	2.50 ± 0.04^{A}	3.30 ± 0.13^{AB}	0.07 ± 0.00^{BC}
	OCP-080	5.85 ± 1.80^{B}	39.25 ± 3.06^{A}	10.46 ± 0.26^{C}	0.59 ± 0.01^{B}	1.04 ± 0.00^{A}	2.74 ± 0.11^{A}	3.13 ± 0.27^{AB}	0.09 ± 0.00^{AB}
	OCP-088	5.07 ± 0.75^{B}	37.87 ± 1.96^{A}	10.57 ± 0.15^{C}	0.58 ± 0.01^{B}	1.02 ± 0.00^{A}	2.77 ± 0.02^{A}	3.15 ± 0.23^{AB}	0.0 9± 0.01^{AB}
	M2	0.12 ± 0.02^{D}	2.55 ± 1.13^{C}	12.77 ± 0.03^{A}	0.46 ± 0.07^{C}	1.02 ± 0.01^{A}	2.55 ± 0.34^{A}	3.55 ± 0.40^{A}	0.06 ± 0.02^{C}

However, the PG wine fermented with OCP-041 had significantly higher concentrations with 16.04 ± 2.83 g/L of residual glucose and 57.65 ± 3.01 g/L of residual fructose (Table 1). The high residual sugar in all PG fermentations is likely due to a combination of high initial °Brix and low fermentation temperature (20 °C). The PN juice alternatively, had a lower initial sugar concentration of 22.5 °Brix, falling within the typical range for this varietal (Cheng *et al.,* 2020). Only the M2 commercial strain was able to ferment to dryness (Table 1). Fermentations were deemed complete when the interval weight loss was ≤ 0.05 g for three consecutive days. The time to complete fermentation of PG juice (64 days) was double that of PN juice (32 days), which is likely due to the different fermentation temperatures (20 °C for PG and 25 °C for PN) (Figures 1 and S1). The OCP-041 PG fermentation stopped the earliest at 52-54 days, whereas the remaining strains continued to ferment for 58-60 days (Figures 1A and S1A). For the majority of OCP strains, PN fermentation activity stopped by 32 days except for OCP-041 and OCP-095 which finished at 37 days and 42 days, respectively (Figures 1B and S1B). Overall, PN fermentations had a higher percentage of sugar consumed relative to the PG fermentations. The PN commercial M2 fermentation had the lowest levels of residual sugar, with 0.12 ± 0.02 g/L of glucose and 2.55 ± 1.13 g/L of fructose

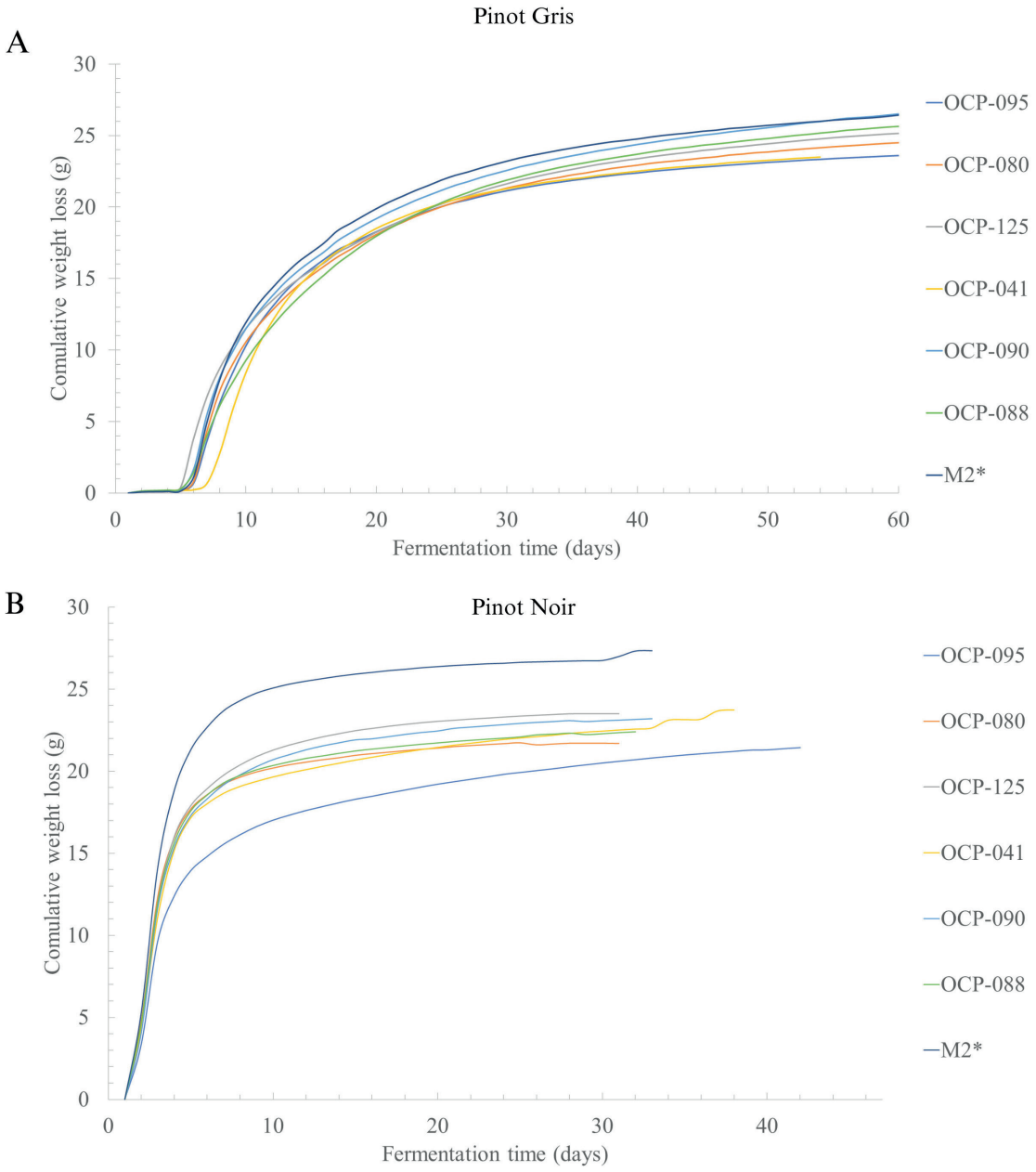


FIGURE 1. Cumulative weight loss of (A) PG 20 °C lab-scale fermentations and (B) PN 25 °C lab-scale fermentations. Fermentation progression was measured by weight loss every 24 hours. Fermentations were carried out in triplicate for each of the strains. *Indicates the commercial M2 control strain. The data points are the average weight loss for each timepoint. Standard deviation is not shown for presentation purposes but is shown in Figure S1.

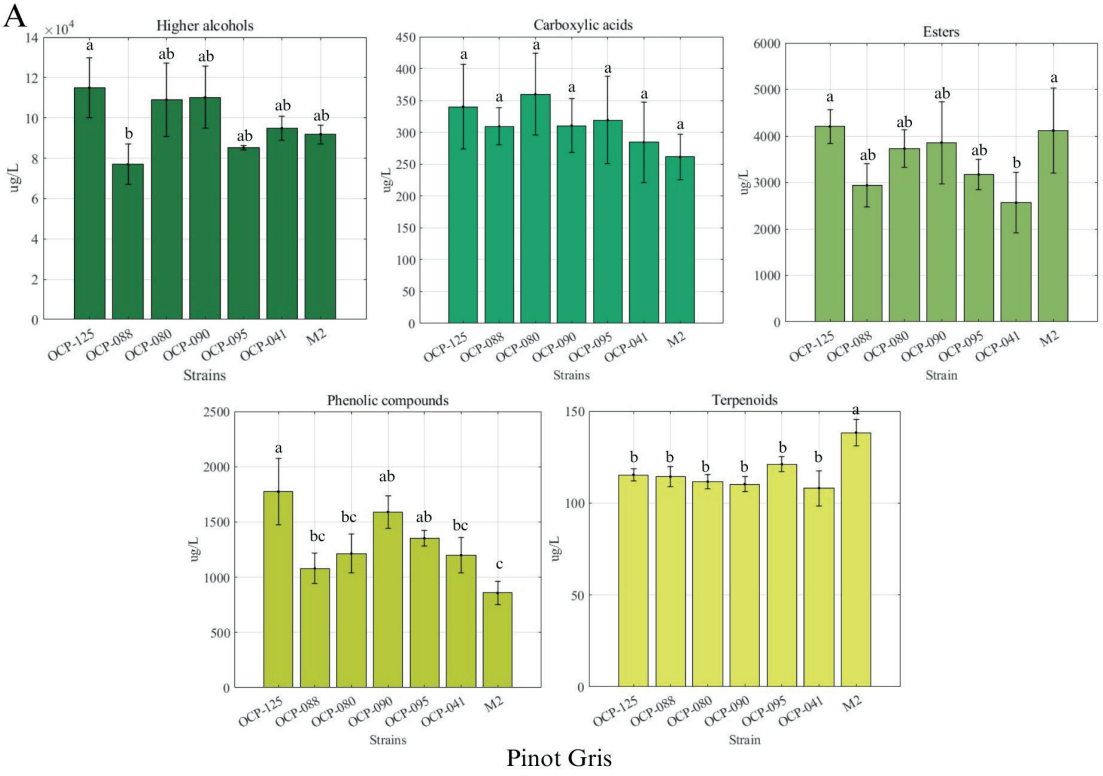
(> 98 % of sugars consumed) (Table 1). The level of residual glucose in the OCP-125 PN fermentation was not statistically different from M2, with 1.94 ± 0.75 g/L compared to 0.12 ± 0.02 g/L, respectively (Table 1). Except for the M2 strain, the OCP-125 strain also had the lowest levels of residual fructose in PN fermentations with 21.89 ± 4.31 g/L for OCP-125 compared to 28.58 ± 5.41 g/L for OCP-090 and even higher residual fructose levels for the other OCP strains (Table 1). The remaining OCP strain PN fermentations had residual glucose and fructose levels that were statistically higher than the M2 strain (Table 1). Other research has explored how various intrinsic factors, not considered in this study, such as the micronutrient composition of the juice and dissolved O_2 can positively influence the fermentation process (Berthels *et al.,* 2004; Mendes-Ferreira *et al.,* 2004; Zabukovec *et al.,* 2020). These factors as well as a more optimal fermentation temperature and lower initial sugar concentration likely explain why the strains performed better in the PN fermentation compared to the PG fermentation (Alonso-del-Real *et al.,* 2017; Lip *et al.,* 2020; Valentine *et al.,* 2019). In the PN fermentation, OCP-041 produced more glycerol than the six other strains, which suggest that the carbon flux of this strain has shifted towards glycerol production. Furthermore, the OCP-041 strain also produced significantly higher amounts of acetic acid than all other strains in the PG fermentation (Table 1). In the PN fermentation, the OCP-041 strain produced significantly higher amounts of acetic acid than OCP-090, OCP-125, and M2 (Table 1). The production of glycerol and acetic acid have a direct relationship, particularly when the initial sugar concentration is high. When producing glycerol, the yeast can produce excess NAD+ and disrupt the redox balance in the cell. Utilization of NAD+ via acetic acid production from acetaldehyde can restore the redox balance (Chidi *et al.,* 2018). All six OCP indigenous strains produced over 10 % alcohol by volume (ABV) in both PG and PN fermentations (Table 1). In the PG fermentation, ethanol production of the OCP-041 strain (10.88 ± 0.35 % v/v) was significantly lower than M2 (12.08 ± 0.05 % v/v) and OCP-090 (11.99 ± 0.22 % v/v) strains. The remaining strains (OCP-080, OCP-088, OCP-095, and OCP-125) had no significant differences in ethanol production when compared to each other, OCP-090 or M2 strains (Table 1). PN wines fermented with the commercial M2 control strain had the highest ethanol production (12.77 ± 0.03 % v/v) followed by OCP-090 and OCP-125 which had the highest ethanol production of the six indigenous strains (11.22 ± 0.40 % v/v and 11.48 ± 0.01 % v/v, respectively) (Table 1).

1.2. Secondary metabolites

For all seven strains, in both PG and PN fermentations, higher alcohols accounted for the most significant proportion of aromas, followed by esters and phenolic compounds (Figure S2). Differences between strains can be observed when visualizing the total concentrations for each of the chemical groups. For higher alcohol production in the PG fermentations, only OCP-125 and OCP-088 strains had a statistical difference, with OCP-125 fermentations containing the highest quantity of total higher alcohols (114,860.98 ± 14,947.07 μg/L) and OCP-088 fermentations containing the lowest quantity (76,944.56 ± 9,981.16 μg/L) (Figure 2A and Table S2). The total levels of carboxylic acids were similar for all seven strains tested in PG fermentations (Figure 2A and Table S2). M2 and OCP-125 strains produced the highest levels of total esters (4,116.15 ± 913.86 μg/L and 4,203.74 ± 368.62 μg/L, respectively) whereas the OCP-041 strain produced the lowest level of total esters (2,569.03 ± 654.05 μg/L) in PG fermentations (Figure 2A and Table S2). M2 produced the lowest level of total phenolic compounds (857.42 ± 104.37 μg/L) whereas OCP-125 (1,774.23 ± 302.79 μg/L) produced the highest levels in PG fermentations (Figure 2A and Table S2). Finally, M2 produced the highest levels of total terpenoid production in PG fermentations (138.30 ± 7.34 μg/L) compared to all the OCP strains (Figure 2A and Table S2). In conclusion, even though all the OCP strains belong to the same *S. cerevisiae* population clade (Pacific West Coast Wine), they differed in PG fermentation chemistry with OCP-125 producing the highest amount of total higher alcohols, total esters and total phenolic compounds when compared to the rest of the OCP strains (Figure 2A and Table S2).

In the PN fermentations, M2, OCP-090, and OCP-125 strains had the highest production of total higher alcohols (154,275.29 ± 25,534.86 μg/L, 144,883.88 ± 13,961.12 μg/L, and 155,646.19 ± 22,107.44 μg/L, respectively) with the lowest production of total higher alcohols for the OCP-095 strain (107,959.05 ± 10,614.89 μg/L) (Figure 2B and Table S3). The concentration of total esters was notably higher in M2 fermentations (6,580.43 ± 1,686.19 μg/L) compared to OCP-095 (3,545.95 ± 1,022.89 μg/L) (Figure 2B and Table S3). Total carboxylic acids, total phenolic compounds and total terpenoids were not significantly different amongst all strains in the PN fermentations (Figure 2B and Table S3).

There were some similarities in strain fermentation chemistry between the PG and PN fermentations with OCP125 producing the highest level of total alcohols and M2 producing the highest levels of esters in both fermentations. (Figure 2, Tables S2 and S3). A principal component analysis (PCA) plot was used to visualize how OCP indigenous strain lab fermentations differed in volatile aroma composition from the commercial M2 control strain lab fermentation. For the PCA plot of volatile organic compounds (VOCs) detected in PG fermentations, the PC1 axis, which explains 35.4 % of variation in the data, shows a pronounced separation between the commercial M2 strain, and the OCP strains (Figure 3A). The separation on the PC2 axis, which explains 28.9 % of the variation, also separates the OCP strains, with the OCP-041 and OCP-125 strains contributing greatly to the variation in comparison to the other four OCP strains (Figure 3A). For the PCA plot of VOCs detected in the PN fermentations, the PC1 axis explains 43.7 % of variation in the data (Figure 3C). The separation between the commercial M2 control strain and the indigenous OCP strains in the PN PCA plot is displayed along the PC1 axis. The PC2 axis, which explains 18.5 % of the variation, separates the OCP strains (Figure 3C).



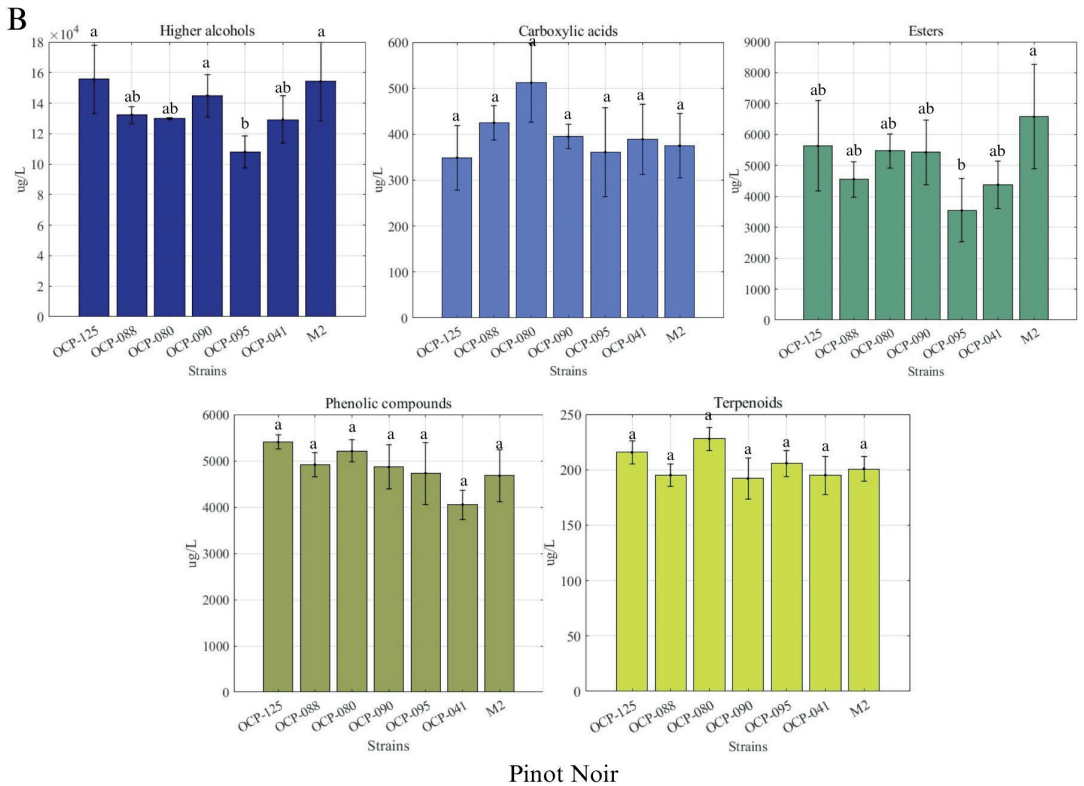


FIGURE 2. Total concentration of volatile higher alcohols, carboxylic acids, esters, phenolic compounds and terpenoids in (A) PG and (B) PN lab-scale wines fermented by the seven indicated *S. cerevisiae* yeast strains (3*n* per strain). Samples were analysed in triplicate; error bars indicate SD. Data was analysed using a one-way ANOVA followed by a Tukey HSD *post hoc* test. Different letters indicate that strains are significantly different at $p \leq 0.05$ for each group.

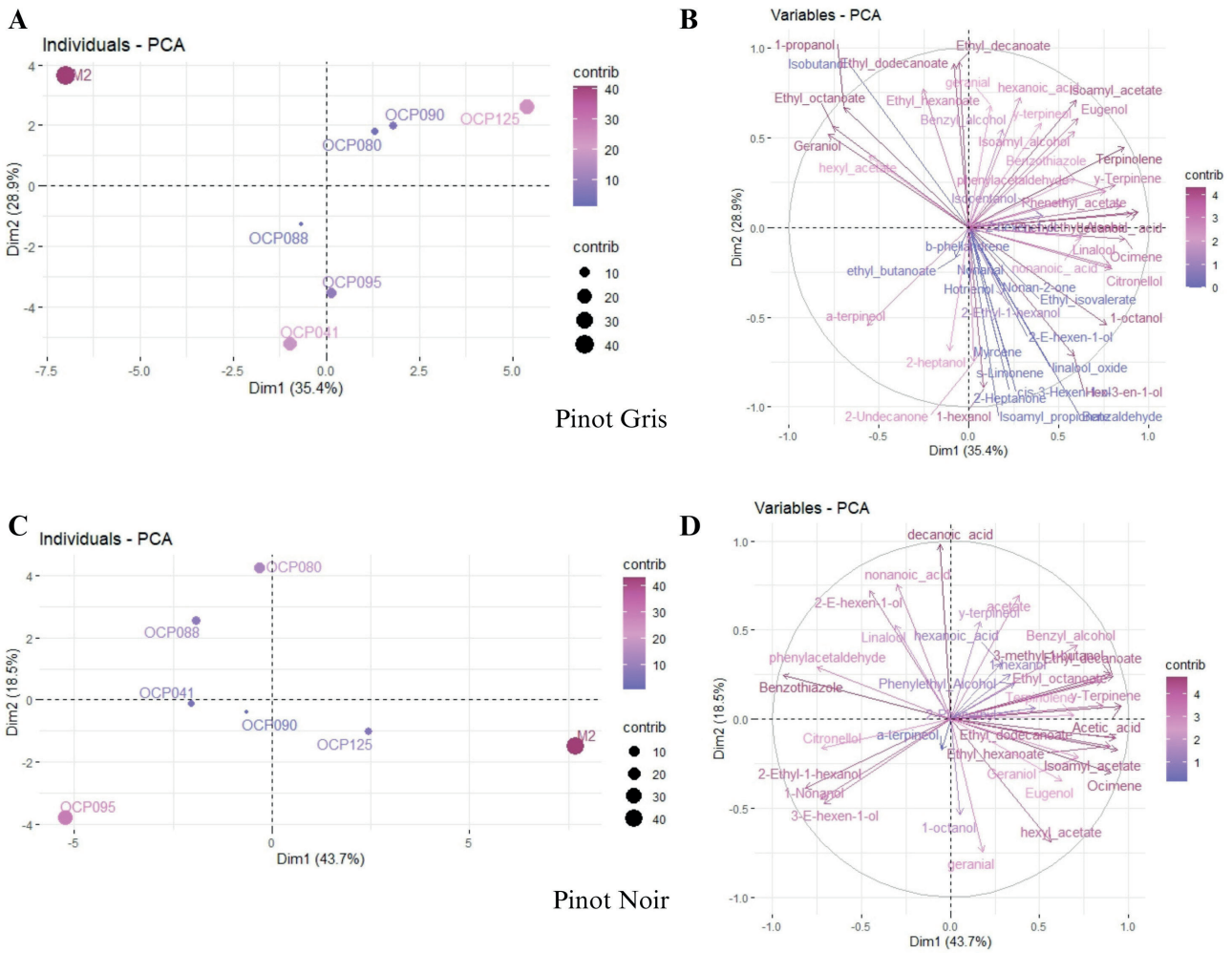


FIGURE 3. PCA plots of the distribution of wine made with *S. cerevisiae* strains and their contribution to the variation of aroma compound production in (A, B) PG and (C, D) PN lab-scale fermentations. (A, C) Individuals-PCA with circle size representing the quality of representation (cos2) of individuals and the colour gradient representing each individual's contribution to the total variance. (B, D) Variables-only PCA of the aroma compound distribution in lab-scale fermentations. The colour gradient represents each variable's contribution to the total variance.

In this analysis, OCP-080 and OCP-095 strains contributed greatly to the aroma variation when compared to the other OCP strains (Figure 3C).

Major differences between M2 and OCP strains can be observed for a few volatile compounds in the PG lab fermentations. For example, the higher alcohol 1-propanol ("alcohol" aroma) is almost two-fold more abundant in M2 than OCP strains whereas the concentrations of the ester ethyl butanoate ("apple", "pineapple" aroma) are 10-fold higher in all OCP strain PG fermentations when compared to the M2 fermentation (Table S2). The association of 1-propanol with the M2 strain and ethyl butanoate with OCP strains is evident from the PCA analysis (Figure 3B). Furthermore, the ester 2-phenethyl acetate, known for its "rose", "honey", and "floral" aroma descriptors, was detected in higher concentrations in PG fermentations with OCP-041, OCP-080, OCP-090, OCP-095, and OCP-125 strains when compared to the commercial control M2 (Table S2 and Figure 3B). OCP-088 produced more of the higher alcohol 2-E-hexen-1-ol ("fruity" aroma) in the PN lab fermentations than any other strain and the association between OCP-088 and this aroma can be seen on the PCA plot (Figure 3D and Table S3).

Overall, the proportionally similar aroma compositions between the M2 commercial control and the six OCP indigenous strains demonstrate the ability of OCP strains to produce wines with desirable organoleptic characteristics (Figures S2A and S2B). OCP-088 and OCP-125 were selected for pilot-scale winery fermentations. OCP-125 was chosen for its performance during lab-scale PG and PN fermentations, consuming a comparable amount of glucose to the commercial M2 strain and producing higher alcohols and fruity aroma as discussed above. OCP-088 was selected because of its fruity aromas production and because it was the dominant strain when originally isolated from spontaneous PG fermentations in the OCP winery.

2. Pilot-scale fermentations

2.1. Microbial

OCP-125 and OCP-088 strains were inoculated at a concentration of 1.25×10^{12} cells/barrel at OCP winery in triplicate using 250 L tanks containing either PN or PG must from the 2022 harvest for a total of 12 fermentation tanks. The must had an initial sugar concentration of 20.4 °Brix for PG and 21.5 °Brix for PN. Fermentation activity was observed within the first day post-inoculation for both OCP-125 and OCP-088 strains in PN juice (Figure 4B). In PG juice however, the OCP-088 strain was unable to start the fermentation after seven days even though the OCP-125 strain was able to start the fermentation in the first 24 hours (Figure 4A). The OCP-088 strain was therefore subsequently re-inoculated at 1×10^{12} cells/tank on day 7 (Figure 4A).

We suspected that the low temperature of the grape must at inoculation (13 °C for PG and 18 °C for PN), the low ambient temperature in the winery (below 10 °C) and the lack of fermentation temperature control impacted the fermentative activity of OCP-088. While OCP-125 and OCP-088 were the sole yeast inoculums, resident commercial strains in the winery environment may contaminate the juice and, in some cases, dominate a fermentation inoculated with a different strain (Lange *et al.,* 2014). To examine both the prevalence of the indigenous OCP strains and the absence of commercial strains in the respective fermentations, microsatellite analysis

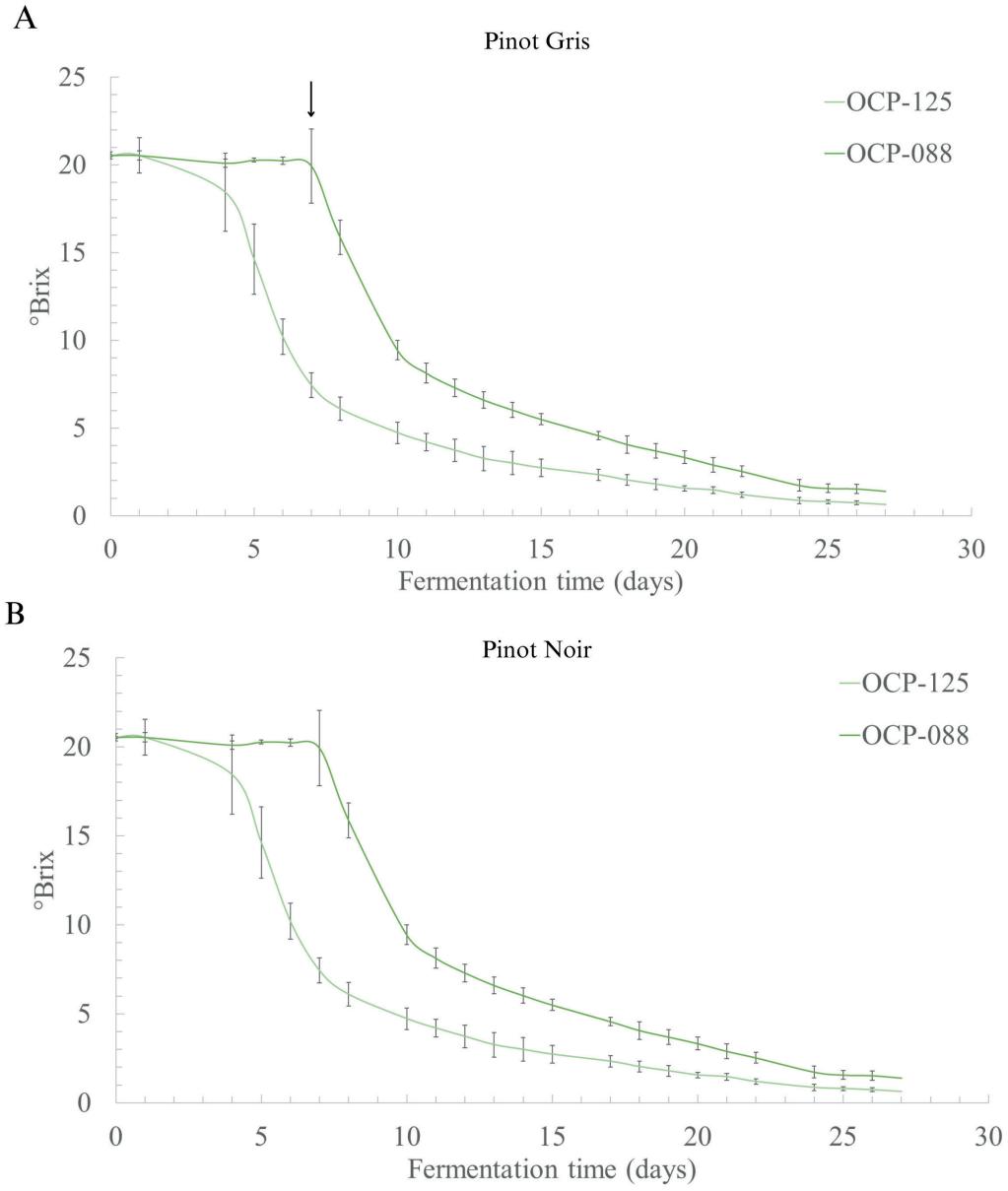


FIGURE 4. Fermentation kinetics of *S. cerevisiae* OCP-088 and OCP-125 strains in (A) PG and (B) PN pilot-scale winery fermentations. Fermentation was monitored by measuring °Brix ± SD (n = 3). The arrow in (A) indicates re-inoculation of OCP-088 on fermentation day 7.

was performed on colonies isolated after inoculation, and at early, middle, and late-stage fermentation. Microsatellite profiles were then compared to previously obtained microsatellite profiles of OCP-088 and OCP-125, as well as a database of commercial *S. cerevisiae* strain profiles (Marr *et al.,* 2023; Martiniuk *et al.,* 2016). Both OCP-088 and OCP-125 strains were found to be dominant in their respective fermentations, with 233/240 colonies isolated from middle or late-stage fermentation matching to their expected profiles (Table S4). One of the seven mismatched colonies was isolated at middle-stage fermentation from an OCP-088 PG tank and contained a unique microsatellite profile not matching any commercial strain. The remaining six colonies did not yield complete amplified microsatellite profiles, suggesting either PCR error or the presence of another species of *Saccharomyces* or non-*Saccharomyces* yeast. We also did not observe any colony microsatellite profiles that were identical or similar to commercial *S. cerevisiae* at any stage of fermentation, indicating the absence of contamination by winery resident commercial strains. The OCP-088 strain was poorly isolated in PG juice after inoculation, with zero colonies isolated from one of the replicate tanks and very few colonies isolated from the other two tanks. The poor isolation of OCP-088 after inoculation is consistent with the initially poor fermentative activity of OCP-088 in PG juice, further suggesting that external factors (such as temperature) negatively impacted strain viability and thus fermentation activity. However, following re-inoculation on day 7, we were able to isolate the OCP-088 strain at expected dilution concentrations (factors of 10^{-3} – 10^{-5}). We also observed that 5 colonies isolated after-inoculation of OCP-088 in PN juice and 16 colonies isolated during the early stages of OCP-088 PG fermentation had microsatellite profiles matching OCP-125; however, the absence of cross-contamination in middle and late-stage fermentation samples suggests this contamination occurred during PCR amplification and not during fermentation (Table S4). In summary, both OCP-088 and OCP-125 persisted and dominated PG and PN pilot-scale fermentations.

2.2. Primary metabolites

Once there were no changes in the daily °Brix monitoring, and no fermentation activity was observed, fermentations were deemed complete (fresh wine). Due to tank space limitations in the winery, each corresponding triplicate fermentation was blended in equal parts to fill up one tank for each strain and each juice. The wines remained in the barrels until bottling, seven months later (bottled wine). Both fresh wine (after completion of fermentation) and bottled wine samples were analysed. In PG fresh wine, OCP-125 had a lower concentration of residual sugar with 1.23 ± 0.17 g/L of residual glucose and 22.45 ± 0.88 g/L of residual fructose in comparison with OCP-088 (2.17 ± 0.56 g/L of residual glucose and 28.28 ± 2.32 g/L of residual fructose) (Table 2). The OCP-125 strain also produced fresh PG wines with a higher ethanol concentration than the OCP-088 strain (10.78 ± 0.10 % v/v compared to 10.37 ± 0.14 % v/v) (Table 2). In PN fresh wines both strains had similar ethanol production (11.27 ± 0.10 % v/v for OCP-125 and 11.40 ± 0.11 % v/v for OCP-088) and residual sugar (Table 2). The improved performance by OCP-125 compared to OCP-088 in the PG fermentation is likely due to the stuck OCP-088 fermentation that required re-inoculation (Figure 4A). The overall improved fermentation performance of both strains in both juices when compared to lab-scale fermentations can be attributed to several factors including a 5-fold increase in the concentration of yeast strain inoculum, less initial sugar concentration in the PG must and possibly nutrient availability. The triplicate 250 L tank fermentations for each strain were very consistent, as shown by the low standard deviation (Table 2). Our data demonstrates that

TABLE 2. Concentration of primary metabolites identified in OCP-088 and OCP-125 fermented PG and vin gris/PN pilot scale fresh wine (3*n* biological reps per strain). Samples were analysed in triplicate, results show mean ± SD. Data was analysed using a one-way ANOVA followed by a Tukey HSD *post hoc* test. Different letters indicate that strains are significantly different at $p \leq 0.05$ for each compound.

	Pinot gris		**Pinot noir**	
	OCP125	OCP088	OCP125	OCP088
Glucose (g/L)	1.23 ± 0.17^{a}	2.17 ± 0.56^{a}	2.08 ± 0.22^{A}	1.38 ± 0.55^{A}
Fructose (g/L)	22.45 ± 0.88^{b}	28.28 ± 2.32^{a}	26.22 ± 0.34^{A}	23.72 ± 2.51^{A}
Citric acid (g/L)	0.27 ± 0.02^{a}	0.29 ± 0.05^{a}	0.53 ± 0.04^{A}	0.57 ± 0.09^{A}
Tartaric acid (g/L)	3.08 ± 0.07^{a}	3.03 ± 0.12^{a}	1.57 ± 0.06^{A}	1.55 ± 0.10^{A}
Malic acid (g/L)	3.26 ± 0.26^{a}	2.95 ± 0.25^{a}	5.71 ± 0.35^{A}	5.47 ± 0.34^{B}
Acetic acid (% v/v)	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{A}	0.01 ± 0.01^{A}
Glycerol (% v/v)	0.50 ± 0.01^{a}	0.52 ± 0.02^{a}	0.45 ± 0.02^{B}	0.52 ± 0.02^{A}
Ethanol (% v/v)	10.78 ± 0.10^{a}	10.37 ± 0.14^{b}	11.27 ± 0.10^{A}	11.44 ± 0.11^{A}

inoculated fermentations with indigenous strains helps to improve reproducibility and predictability. When measuring primary metabolites in the bottled wine, both OCP-088 and OCP-125 strains had a decrease in sugar content as well as an increase in ethanol percentage (~0.5 %) when compared to the fresh wines (Tables 2 and 3). It is likely that when the triplicate fermentations were blended, sufficient oxygen was introduced to allow the remaining viable yeast to metabolize more sugar.

2.3. Secondary metabolites

Similar to the lab-scale fermentations, the highest quantity of VOCs for PG fresh wines fermented with OCP strains were higher alcohols, followed by esters, carboxylic acids, phenolic compounds and terpenoids (Figure 5A). The PN fresh wines had a similar order with VOCs dominated by higher alcohols, followed by esters, phenolic compounds, carboxylic acids and terpenoids (Figure 5B). Overall, we conclude that the OCP-088 and OCP-125 strains were able to produce wines with desired organoleptic profiles (Figure 5). Significant differences between the PG OCP strains winery fermentations were observed for the carboxylic acid nonanoic acid, with a "cheese", "fat", "waxy" aroma descriptor, which was significantly higher in OCP-088 *versus* OCP-125 fresh PG wines (2,635.89 ± 376.19 µg/L and 2,006.47 ± 112.81 µg/L, respectively) (Table S5). On the other hand, the ester isoamyl acetate, which imparts a "fruity", "banana", and "pear" aroma was significantly higher in OCP-125 *versus* OCP-088 in fresh PG wines (2,436.19 ± 279.43 µg/L and 1,950.43 ± 148.40 µg/L respectively) (Table S5). In the PN fresh wines, significant differences between strains were observed for the ester 2-phenethyl acetate (aroma descriptors of "floral" and "honey") with a greater concentration in OCP-088 compared to OCP-125 (303.01 ± 33.94 µg/L and 235.01 ± 47.62 µg/L, respectively) (Table S6). Concentrations of the ester ethyl dodecanoate were significantly higher in OCP-125 than OCP-088 PN fresh wines (401.56 ± 32.38 µg/L and 306.12 ± 31.01 µg/L, respectively) as was the ester ethyl decanoate (5,626.14 ± 291.88 µg/L and 4,650.44 ± 359.71 µg/L, respectively). To the contrary, the ester ethyl hexanoate was significantly higher in OCP-088 than OCP-125 fresh PN wines (914.18 ± 47.67 µg/L and 736.11 ± 38.29 µg/L, respectively) (Table S6). These three ethyl esters are responsible for fruity aromas in wine.

When comparing the fresh wines with the bottled wines, there were significant differences between VOCs. There was a clear increase in the total higher alcohols and phenolic compounds in the bottled PG wines for both OCP strains (Figure 5A). The increase in higher alcohols for both OCP-088 and OCP-125 strains was primarily due to a greater than 8-fold increase in 3-methyl-1-butanol ("banana" aroma) in bottled compared to fresh PG wines (Figure S3 and Table S5). To the contrary, higher alcohols decreased in the bottled compared to fresh PN wines (Figure 5B). The concentration of 3-methyl-1-butanol once again dictated the change in total higher alcohols with a 1.4-fold and 1.2-fold reduction in OCP-088 and OCP-125 bottled wines, respectively (Figure S4 and Table S6). Despite the decrease in total higher alcohols in PN bottled wines, there was a statistically significant increase in 1-hexanol ("herbaceous" and "green" aroma) and 3-e-hexen-1-ol ("grass" aroma) for both OCP strains (Figure S4 and Table S6). As well, the ester isoamyl acetate, which has a banana aroma, increased for both OCP-strains in the PN bottled wines (Figure S4 and Table S6). Other changes in the chemical composition of wine can occur during and after bottling. For instance, oxidation of the phenol compound phenylethyl alcohol, promotes the presence of phenylacetaldehyde (with an aroma described as "floral" and "honey") that was detected only in bottled PG and PN wines (Figures S3 and S4, Tables S5 and S6). Similarly, some acetate esters, such as isoamyl acetate

TABLE 3. Concentration of primary metabolites identified in PG and PN pilot scale bottled wines, fermented by the OCP-088 and OCP-125 strains and commercial OCP wine (PG-SB, PG-KF, VG) (3*n* technical reps per strain). The analysis was carried out three weeks after bottling. Samples were analysed in triplicate, results show mean ± SD. Data was analysed using a one-way ANOVA followed by a Tukey HSD *post hoc* test. Different letters indicate that strains are significantly different at $p \leq 0.05$ for each compound.

	Pinot gris		**Pinot noir**		**Commercial**		
	OCP125	OCP088	OCP125	OCP088	PG-SB	PG-KF	Freeform VG
Glucose (g/L)	0.50 ± 0.01^{b}	1.17 ± 0.01^{a}	1.39 ± 0.02^{A}	1.01 ± 0.04^{B}	-	-	-
Fructose (g/L)	15.58 ± 0.01^{b}	20.54 ± 0.01^{a}	22.12 ± 0.01^{A}	20.69 ± 0.29^{B}	1.45 ± 0.02	3.13 ± 0.001	1.90 ± 0.10
Citric acid (g/L)	0.26 ± 0.001^{a}	0.25 ± 0.001^{b}	0.80 ± 0.001^{B}	0.83 ± 0.001^{A}	0.33 ± 0.001	0.62 ± 0.001	0.61 ± 0.001
Tartaric acid (g/L)	4.04 ± 0.002^{b}	4.32 ± 0.02^{a}	2.63 ± 0.003^{A}	2.59 ± 0.005^{B}	2.62 ± 0.003	2.79 ± 0.05	1.65 ± 0.08
Malic acid (g/L)	2.95 ± 0.01^{a}	2.78 ± 0.01^{b}	5.21 ± 0.04^{A}	4.82 ± 0.09^{B}	3.03 ± 0.02	3.73 ± 0.07	4.15 ± 0.16
Glycerol (% v/v)	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.004^{A}	0.02 ± 0.003^{A}	0.43 ± 0.001	0.64 ± 0.02	0.51 ± 0.01
Ethanol (% v/v)	11.18 ± 0.04^{a}	10.82 ± 0.04^{b}	11.48 ± 0.08^{A}	11.58 ± 0.03^{A}	12.86 ± 0.55	13.21 ± 0.10	12.52 ± 0.32



FIGURE 5. (A) PG and (B) PN wine composition of VOC chemical groups in the fresh wines (Fresh OCP-125 and Fresh OCP-088) and the bottled wines (Bottled-OCP-125 and Bottled-OCP-088).

("banana" and "pear" aroma) in PG bottled wine and hexyl acetate in PN bottled wine, decreased post-fermentation during wine storage as consequence of hydrolysis (Figures S3 and S4, Tables S5 and S6).

To compare the aromatic profile of the wines with commercially produced wine during the same vintage in OCP winery, we quantified the concentration of VOCs for commercial wines produced using PG and PN from the 2022 harvest. To analyse the variation between commercial wines, fresh OCP-088 and OCP-125 wines and bottled OCP-088 and OCP-125 wines, a PCA analysis was performed using the concentration of VOCs. The OCP-088 and OCP-125 fresh wines clearly clustered together, as did the OCP-088 and OCP-125 bottled wines and the commercial PG-KF and PG-SB wines (Figures 6A and 6C). The differences between the PG commercial and OCP-strain fermented PG fresh and bottled wines are indicated along the X axis, which explains 53.1 % of the variation (Figure 6A). Differences between PG bottled and fresh OCP wines are also observed, as expected, mainly on the Y axis which explains only 26.8 % of the variation (Figure 6A). For PN wines, the clustering is similar to the PG wines, where PC1 explains 58.4 % of the variation and shows the separation between the Freeform VG wine and the wines fermented with OCP-strains (Figure 6C). PC2, which accounts for 27.7 % of the variation, separates the fresh wine and the bottled OCP wine (Figure 6C). Interestingly, both fresh and bottled PG and PN wines produced with the OCP indigenous strains had significantly higher concentrations of ethyl decanoate than the bottled commercial wines (Figures 6B and 6D, Tables S5 and S6). Ethyl decanoate is a fatty acid ester that contributes "sweet" and "fruity" aromas to wine. OCP-strain bottled PG wines had a higher concentration (over 2-fold) of the phenolic compound phenylethyl alcohol, which contributes to a "floral" aroma, in comparison to the commercial PG-SB and PG-KF wines (Figure 6B and Table S5). However, ethyl butanoate, associated with a "fruity" aroma, was significantly

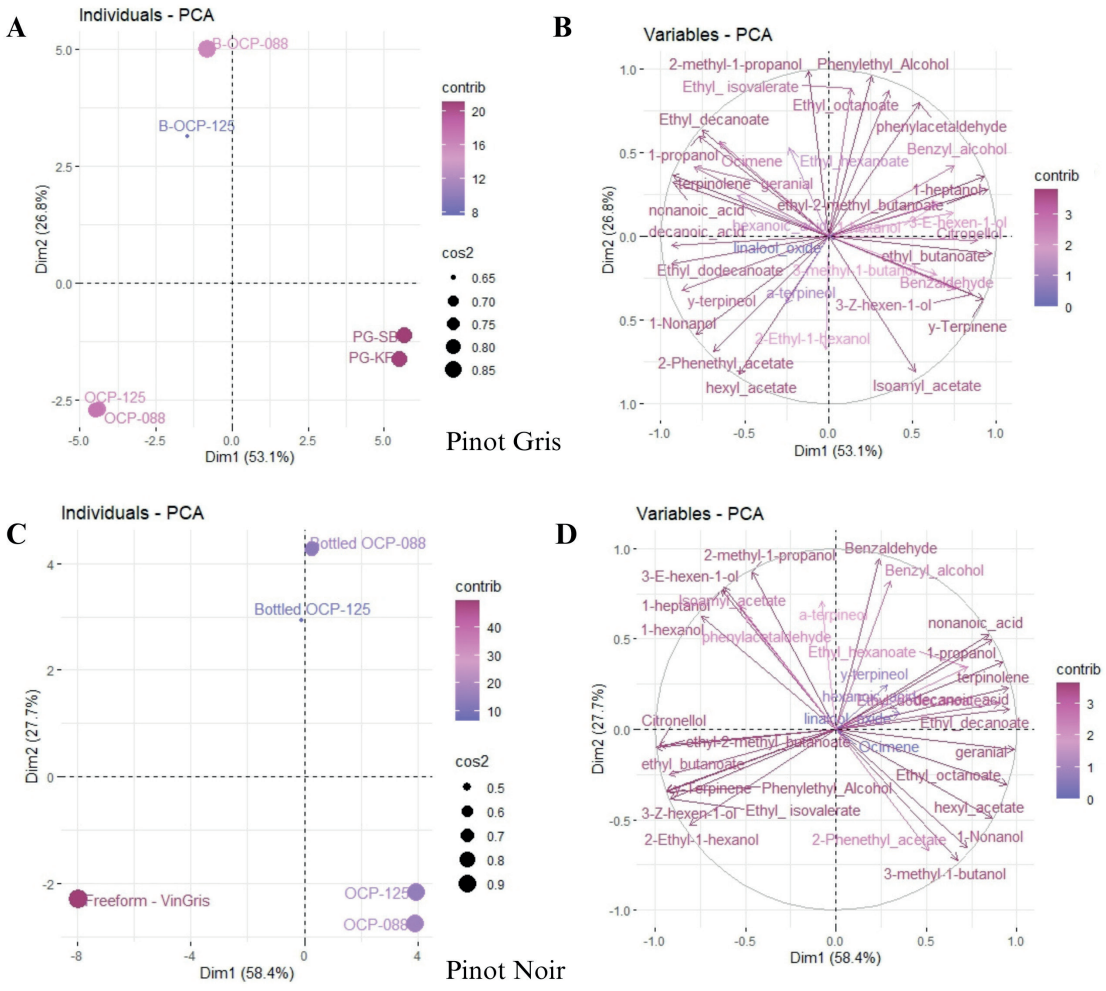


FIGURE 6. PCA plots of the distribution of wine made with either OCP-088, OCP-125, a commercial strain or naturally and their contribution to the variation of aroma compound production in (A, B) PG and (C, D) PN pilot scale and commercial wines. (A, C) Individuals-PCA with circle size representing the quality of representation (cos2) of individuals and the colour gradient representing each individual's contribution to the total variance. (B, D) Variables-only PCA of the aroma compound distribution in pilot scale and commercial wines. The colour gradient represents each variable's contribution to the total variance.

higher in both PG and PN commercial wines (Figures 6B and 6D, Tables S5 and S6). In comparison to a database of French wines, the levels of ethyl octanoate, ethyl decanoate and ethyl dodecanoate are much higher in the PG wines than young dry wines or sweet white wines from France (Antalick *et al.,* 2014). Therefore, Okanagan wine *terroir* may be associated with a fruity aroma due to higher levels of ethyl esters.

Odor Activity Values (OAVs) quantify the perceived odour intensity of VOCs which can determine the contribution of VOCs to the aroma perception of a product (du Plessis *et al.,* 2017; Duan *et al.,* 2018b; Vilanova *et al.,* 2013; Zhao *et al.,* 2019). To derive OAVs, the ratios between the concentration of each aroma compound and its odour detection threshold (ODT) were calculated (Tables S7 and S8) (du Plessis *et al.,* 2017; Zhao *et al.,* 2019). Compounds with an OAV < 1 enhance the complexity of the aroma profile without significantly affecting odor perception whereas compounds with an OAV > 1 indicate an active aroma (Tables S7 and S8). The OAVs of the wines were grouped into four attributes: fruity, green grass, floral, and rancid, according to their aroma descriptors (Pulcini *et al.,* 2022; Vilanova *et al.,* 2010). The log10 was calculated from the sum of OAVs for each group to better estimate the aroma profile of the wines (Figure 7). For fresh PG and PN wines, both OCP-125 and OCP-088 strains have very similar aroma characteristics (Figures 7A and 7C). The esters responsible for fruity aromas are the principal component of the aroma bouquet, followed by fusel alcohols that add some green grass notes. The green grass and floral attributes increased for both OCP-125 and OCP-088 strains in PG and PN bottled wines (Figures 7B and 7D). The estimated aroma profile of the OCP bottled wines is very similar to the estimated aroma profiles of both the PG and PN commercial wines produced by OCP (Figures 7B, 7D, 7E, and 7F).

Minimal differences in ester concentration can make a difference in aroma perception since the odor detection threshold of esters is very low (Parapouli *et al.,* 2020). The theoretical OAVs calculated to estimate the aroma profiles for the wines show "green grass", "fruity" (apple, pear, pineapple, banana) and floral as the main odor attributes, which are three common descriptors for white wines (Figure 7). The concentration of carboxylic acids, which impart waxy, fat, cheese-like aromas, is below the ODT for both PN and PG, which indicates that these strains do not produce off-flavours that would impact the wine quality (Tables S6 and S7).

Other studies have isolated *S. cerevisiae* strains from specific wine-making regions and have also shown the capability of these strains for producing desirable organoleptic characteristics. These studies have also shown that selecting and using a region-specific strain as a starter culture can differentiate the wines produced from commercial yeast strains compared to wines produced by spontaneous fermentations (Cus *et al.,* 2017; Elmaci *et al.,* 2014; Suzzi *et al.,* 2012; Vazquez *et al.,* 2023).

CONCLUSION

The use of OCP-088 and OCP-125 indigenous yeast strains from the Okanagan Valley wine region in BC, Canada has proven to be a promising approach for producing positive aroma attributes and positively influencing the organoleptic profile of pilot-scale wines. The aromatic data supports the hypothesis that the phylogenetic origin of an *S. cerevisiae* starter culture plays an important role in shaping the aroma characteristics of a wine. Indigenous *S. cerevisiae* strains can produce a wine that is distinctive from commercial strains, as evidenced in the PCAs for both lab-scale and pilot-scale fermentations. For instance, the six OCP indigenous strains produced more ethyl butanoate (apple, pineapple aroma) than the M2 commercial strain during lab-scale PG fermentations. In the pilot-scale winery fermentations the PG and PN wine produced by the OCP-088 and OCP-125 indigenous strains had more ethyl decanoate (sweet, fruity aroma). Our data suggests that the indigenous OCP strains are able to introduce regional attributes, such as a fruity and floral aroma, that may be reflective of the Okanagan wine region *terroir*. However, the OCP strains were not able to ferment to dryness, and struggled in low temperature conditions, requiring re-inoculation of OCP-088 in the PG pilot-scale fermentation. Further exploration of indigenous strain requirements such as optimal fermentation temperatures and nutrient requirements should help improve their fermentation performance. Alternatively, the use of two, or more, indigenous strains as a co-inoculum, or inoculation of an indigenous strain followed by a commercial strain to ensure that all sugars are consumed, can be explored as novel options for winemakers. Having access to indigenous strain starter cultures can also mitigate concerns about spontaneous fermentations becoming stuck. This study highlights the potential for indigenous *S. cerevisiae* strains to showcase the *terroir* of a wine region, the selection process for strains to trial in a winery and the need for strain optimization in winemaking.

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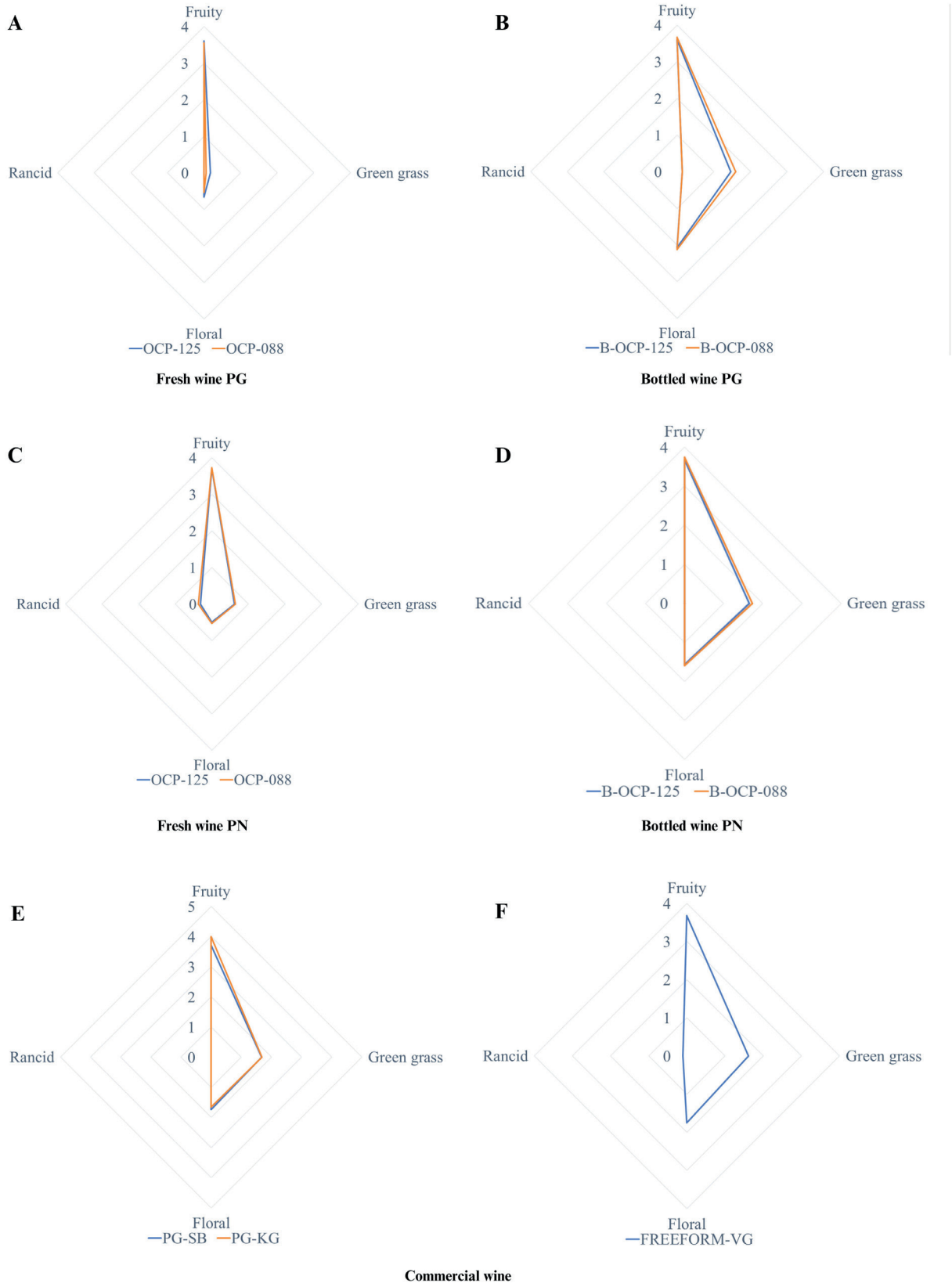


FIGURE 7. Estimated aroma sensory profile of the pilot-scale and commercial OCP wines. OAV of volatile compounds calculated as the log of the ratio of compound concentration to odour threshold for each wine (A) PG fresh OCP-125 and OCP-088 wine (B) PG bottled OCP-125 and OCP-088 wine (C) PN fresh OCP-125 and OCP-088 wine (D) PN bottled OCP-125 and OCP-088 (E) PG commercial wines (F) PN commercial wine.

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