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Saccharomyces uvarum yeast isolate consumes acetic acid during fermentation of high sugar juice and juice with high starting volatile acidity

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ABSTRACT

Aim: A *Saccharomyces uvarum* isolate was assessed for its ability to metabolize acetic acid present in juice and during the fermentation of partially dehydrated grapes. The impact on other yeast metabolites was also compared using an *S. uvarum* isolate and an *S. cerevisiae* wine yeast. The upper limit of fruit concentration that allowed the *S. uvarum* isolate to ferment wines to < 5 g/L residual sugar was defined.
Methods and results: Cabernet franc grapes were partially dehydrated to three different post-harvest sugar targets (24.5 °Brix, 26.0 °Brix, and 27.5 °Brix) along with non-dehydrated grapes (21.5 °Brix control). Musts from all treatments were vinified with either the *S. uvarum* isolate CN1, formerly identified as *S. bayanus*, or *S. cerevisiae* EC1118. All wines were successfully vinified to less than 5 g/L residual sugar. Fermentation kinetics between the two yeasts were similar for all wines other than 27.5 °Brix, where CN1 took three days longer. During fermentation with CN1, acetic acid peaked on day two, then decreased in concentration, resulting in final wine acetic acid lower than that measured on day two. Wines fermented with EC1118 showed an increase in acetic acid over the time-course of fermentation. Significantly lower wine oxidative compounds (acetic acid, acetaldehyde and ethyl acetate) and higher glycerol resulted in wine produced with CN1 in comparison to EC1118. Both yeasts produced comparable ethanol at each Brix level tested. Further studies showed that CN1 lowered acetic acid seven-fold from 0.48 g/L in juice to 0.07 g/L in wine whereas EC1118 reduced acetic acid to 0.18 g/L.
Conclusions: The autochthonous *S. uvarum* yeast isolate successfully fermented partially dehydrated grapes to < 5 g/L sugar up to 27.5 ºBrix. The consumption rate of acetic acid was faster than its production during fermentation, resulting in low acetic acid, acetaldehyde and ethyl acetate in wine in comparison to a commercial *S. cerevisiae* yeast while consistently producing higher glycerol.
Significance and impact of the study: The *S. uvarum* yeast isolate can metabolize acetic acid during fermentation to significantly lower acetic acid, ethyl acetate and acetaldehyde in wine. It can also reduce acetic acid by seven-fold from the starting juice to the finished wine, which could have potential application for managing sour rot arising in the vineyard or during the dehydration process in making appassimento-style wines.

KEYWORDS

acetic acid, appassimento, climate change adaptation, Ontario, oxidation compounds, partially dehydrated grapes, *Saccharomyces uvarum*, winemaking, wine quality

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/2594

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INTRODUCTION

Of the many tools available to the winemaker in influencing final wine composition and quality, yeast strain choice can be particularly important (Lambrechts and Pretorius, 2000; Blanco *et al*., 2014; Petrovic *et al*., 2019). In the process of vinifying high sugar must, such as the methods used for appassimento-style wines, initiating and completing fermentation and minimizing production of oxidation compounds such as acetic acid or ethyl acetate are important factors informing yeast selection (Bellincontro *et al*., 2016; López de Lerma *et al.,* 2012).

Winemaking using the appassimento technique involves post-harvest dehydration of wine grapes, and subsequent processing when the required starting sugar concentrations are reached. Grape dehydration increases sugars, polyphenols and aromatic compounds (Paronetto and Dellaglio, 2011), resulting in a rich wine high in ethanol and with a unique sensory profile (Moreno *et al*., 2008).

Vinifying partially dehydrated grapes for high-quality wine production may be beneficial to cool climate viticultural regions such as Ontario, Canada, as it can potentially mitigate the challenges of a changing climate and erratic weather events, which threaten the sustainability and ongoing success of cool climate wine industries (Pickering *et al*., 2015). This strategy represents a method of adapting to vintage-to-vintage variation that may jeopardize the stability of grape yield, development and composition, as well as wine production and quality (Ashenfelter and Storchmann, 2010; Teixeira *et al.*, 2013; Shaw, 2017). Grapes are further ripened post-harvest in a protected environment, allowing them to achieve high levels of sugar and volatile constituents, despite ambient weather conditions in the vineyard (Paronetto and Dellaglio, 2011).

During post-harvest dehydration, berry volatile organic compounds (VOCs) increase as water is lost (Bellincontro *et al*., 2016). Oxidation compounds that are important for wine quality, such as acetaldehyde, acetic acid and ethyl acetate, also increase during dehydration of the grapes, which can negatively impact the organoleptic profile of the final wine when present at elevated concentrations (Bellincontro *et al*., 2004; López de Lerma *et al*., 2012). Fermenting must with high starting sugar concentrations can also pose quality challenges to winemakers due to the overproduction of potentially unfavourable compounds such as acetic acid caused by the increased osmotic stress placed on the yeast from the higher sugar concentrations (Nevoigt and Stahl, 1997; Pigeau and Inglis, 2005; Heit *et al*., 2018).

In Ontario, Canada, wine made from partially dehydrated grapes is regulated by the Vintners Quality Alliance of Ontario (VQAO), Ontario's governing wine authority, under the name Vin de Curé. Grapes are required to be dried to a minimum of 27.0 °Brix at time of transfer to the fermentation vessel (VQA, 2019). Further, VQA Ontario has outlined permissible limits of volatile acidity (VA) for Vin de Curé, based on starting Brix. Thus, adhering to legally imposed limits on such quality parameters are important considerations for this wine style. This wine style typically has less than 5 g/L residual sugar.

In the present study, an indigenous yeast isolate, CN1, from the skin of Riesling Icewine grapes (Nurgel *et al*., 2004; Kelly *et al*., 2018), was trialed for winemaking from partially dehydrated grapes over a range of dehydration levels. Our previous study of vinifying grapes dried to 28 °Brix with this yeast isolate left 15.8 ± 6.7 g/L residual sugar (Kelly *et al*., 2018). The yeast isolate was reclassified as *S. uvarum* from the previous classification of *S. bayanus*. The aim of the present study is to further define the sugar concentration range of the dehydrated grapes under which the yeast isolate can ferment to less than 5 g/L and assess its impact on reducing oxidation compounds during fermentation and in the final wine. This was assessed by comparing the fermentation kinetics, cell growth, final ethanol and additional yeast metabolites in wines fermented by the *S. uvarum* isolate to the commonly used commercial *S. cerevisiae* yeast EC1118 at varying levels of grape dehydration. In order to examine this, local Cabernet franc grapes were dehydrated to three starting sugar concentrations – 24.5 °Brix, 26.0 °Brix and 27.5 °Brix, and compared to control fruit not dehydrated but processed immediately after picking (21.5 °Brix). These grapes were vinified with either the *S. uvarum* isolate or a commercial strain, *S. cerevisiae* EC1118, and assessed chemically. In addition, grapes infected with sour rot containing a high concentration of acetic acid (0.48 g/L) were vinified with this yeast to determine its ability to moderate the acetic acid in the final wine. This work will further

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characterize the *S. uvarum* isolate and determine its upper fermentative limit for vinification of wine made from partially dehydrated grapes.

MATERIALS AND METHODS

1. Grapes

Cabernet franc grapes were selectively hand-picked from Mazza Vineyards donated by Pillitteri Estates Winery (Niagara-on-the-Lake, Ontario, Canada) and placed in perforated picking bins in a single layer. A total weight of 821 kg was harvested and divided into four batches. One batch represented the control treatment (183 kg, 21.5 °Brix), which was delivered to the Cool Climate Oenology and Viticulture Institute (CCOVI, Brock University, St. Catharines, Ontario, Canada) and processed the following day after temperature stabilization overnight at room temperature (18 °C). The other three batches were delivered to a drying barn (Cave Spring Winery, Beamsville, Ontario, Canada) dedicated to commercial winegrape drying to dehydrate grapes to three drying targets (24.5, 26.0 and 27.5 °Brix). The picking bins were stacked 14-layers high, with air space between each container to receive ventilation in the barn. The drying barn did not have internal temperature and humidity regulation. Stand up fans were placed strategically throughout the facility to promote airflow. Grapes were sampled weekly (15 randomly selected clusters), and 105 randomly selected berries from the 15 clusters were weighed and then processed for analysis. The clusters and berries were then crushed by hand in a plastic bag and strained through a metal strainer to collect must for immediate determination of soluble solids, pH and titratable acidity. When the fruit reached the drying target, the grapes were delivered to CCOVI for processing the following day, after temperature stabilization overnight at room temperature (18 °C). The drying time was 31 days to reach 24.5 °Brix (original weight 197 kg, post-drying weight 146 kg), 37 days to reach 26.0 °Brix (original weight 215 kg, post-drying weight 164 kg), and 61 days to reach 27.5 °Brix (original weight 226 kg, post-drying weight 158 kg). During drying, any fruit that showed infection with *B. cinerea* was removed, as is the practice of the winery (none for control fruit or 24.5 °Brix treatment; 12.6 kg total removed from 26.0°Brix treatment, and 10.2 kg total removed from 27.5 °Brix treatment).

Pinot noir must at 20.5 °Brix with high VA from sour rotted fruit (0.48 g/L) was donated by Hughes Vineyards in Beamsville, Ontario, Canada.

2. Yeast strains

Two yeast strains were used in this study for wine fermentations: the commercial yeast *S. cerevisiae* strain EC1118 was supplied by Lallemand Inc. (Montreal, Canada) and CN1, which was isolated at CCOVI (St. Catharines, Canada) from the bloom of local Riesling Icewine grapes (Kelly *et al*., 2018). CN1 was reclassified as an *S. uvarum* strain, formerly referenced as *S. bayanus*. To reclassify the yeast, additional gene sequencing comparisons for *GDH1* between the CN1 isolate and yeast sequences deposited in GenBank® were compared based on the methods of Nguyen and Gaillardin (2005).

The primers were selected and PCR amplification for regions of the *GDH1* gene was performed according to Nguyen and Gaillardin (2005). Three areas of the *GDH1* gene were analyzed to identify this yeast: open reading frame (ORF); promoter (Pro); and intergenic spacer (IGS) regions. The ORF region was amplified via PCR as follows: with the designed primer pairs SugdhU (5'- GTACTACTCTACTA TACCCG) and SugdhL (5' ACATCACCTTGG TCGAAC); with the primer pairs SugdhF1 (5'- TCAAGAACTCTTTGACCG) and SugdhR1 (5'- CAAACTTCACACCTTGAG), which are internal primers for *S. uvarum*; and with the primer pairs SbgdhF1 (5'- AACGGTAAGGA ATCCTTC) and SbgdhR1 (5'- CGGAAATGTA TTGGACTTTG), which are internal primers for *S. bayanus/pastorianus*. The Pro region was amplified with primer pairs SugPrU (5'- CCTCGATCTGCTTATCGC) and SugPrL (5'- GAAGAGACAACTTCATCGTAA). The IGS region was amplified with primer pairs SugIgU (5'- GCCAAGAAGTACACTAAGG) and SugIgL (5'- CTGTCGATGCTTTACAAAACT). PCR products were purified with QIAquick PCR Purification Kit 28104 (QIAGEN, Hilden, Germany). Direct sequencing of PCR fragments was carried out (at the Centre for Applied Genomics, The Hospital for Sick Children, Peter Gilgan Centre for Research and Learning, Toronto, Canada), and the results were compared with all of the available sequences in the GenBank® database using the Basic Local Alignment Search Tool (BLAST) (Table S1).

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3. Winemaking

For Cabernet franc, a total of eight fermentation treatments were carried out, each in triplicate: must vinified at 21.5, 24.5, 26.0 and 27.5 °Brix and inoculated with *S. cerevisiae* EC1118 or *S. uvarum* isolate CN1 at each sugar level. Before fruit processing, control and dried grapes were divided randomly and equally into three replicates based on weight (approximately 20 kg grapes/replicate), and each replicate was processed separately through the crusher/destemmer (model Gamma 50, Mori-TEM; Florence, Italy) into 20-L steel fermentation vessels with lids. After ensuring a homogenious mixture of the must, 100 mL of sample from each replicate was taken for chemical analysis. Musts were blanketed with carbon dioxide, the temperature was brought to 22 °C and 500 mg/L of diammonium phosphate (DAP) (Laffort, Bordeaux, France) was added to each fermentation prior to inoculation. A further 250 mg/L of DAP was added on the third day of fermentation. For Pinot noir, a total of two treatments were carried out, each in triplicate: must inoculated with *S. cerevisiae* EC1118 or *S. uvarum* isolate CN1.

4. Fermentation

The yeast culture build-up and acclimatization procedure was as described in Kelly *et al*. (2018) with the following modifications. Yeast cultures of EC1118 and CN1 were prepared by inoculating plated yeast colonies into 0.48 L of sterile-filtered Cabernet franc or Pinot noir must diluted to 10 °Brix and grown to 2 × 10^8 cell/mL. For the Pinot noir fermentations, 0.48 L of the starter culture was then inoculated into 20L fermentations, fermented at 25 ºC and punched down twice daily. For the appassimento fermentations, in the first step of acclimatization an equal volume of undiluted sterile-filtered control Cabernet franc must (0.48 L) was added to the starter cultures once the desired yeast concentration was achieved, and in the second step the must at respective drying targets (0.48 L) was used for fermentations at different sugar levels. This 1.44-L starter culture was inoculated into each fermentation to achieve an inoculum of 5.0 × 10^6cells m/L in a final volume of approximately 20 L. All fermentations were kept at 22 °C and punched down twice a day using a separate punch down tool for each yeast trial to prevent cross-contamination. All fermentations were monitored once a day by recording soluble solids (hydrometer, °Brix) and temperature (thermometer, °C), and viable cells were measured using methylene blue staining and cell counting with a hemocytometer (Zoecklein *et al*., 1995). Samples were collected daily (5 × 1 mL aliquot and 1 × 50 mL tube) and stored at -30 °C for metabolite analysis. As the cap started to fall, fermentations were blanketed with CO_2 to protect from oxidation. Fermentations were considered complete once the sugar dropped below 5 g/L as confirmed by WineScanTM (FOSS; Hillerød, Denmark). Once complete, fermentation replicates were pressed separately with a small bladder press (Enotecnica Pillan; Vicenza, Italy) at 1 bar for 2 min into 11 L glass carboys. Treatments were sulfited at 50 mg/L of sulfur dioxide (as potassium metabisulfite) and settled at room temperature. Wines were then racked and moved to a chamber for cold stabilization at -2 °C until bottling. Before bottling, an additional 50 mg/L of sulfur dioxide (as potassium metabisulfite) was added to each treatment. The wines were subsequently pad filtered through filter pads. Pinot noir wines were bottled as separate treatments into 375 mL green glass bottles and appassimento wines were bottled as separate treatments into 750 mL green glass bottles with a manual bottler (Criveller Group, Niagara Falls, Canada), closed with natural cork and automated corker (model ETSILON-R; Bertolaso, San Vito, Italy) then stored in the CCOVI wine cellar (15 °C and 74.5 % humidity).

5. Grape, must and fermentation analysis

Fermentation temperature was monitored with a thermometer (°C). Soluble solids were determined using an ABBE bench top refractometer (model 10450; American Optical, Buffalo, New York) for grape and must samples and using a degree Brix hydrometer for fermentation time-course samples. The pH was determined by pH meter (SympHony model SB70P, VWR Mississauga, Canada) and titratable acidity by titration with 0.1 mol/L NaOH to an endpoint of pH 8.2 (Zoecklein *et al*., 1995). The glucose and fructose, glycerol, acetaldehyde, amino acid nitrogen, ammonia nitrogen, and acetic acid were determined in the starting must and fermentation samples with Megazyme kits (K-FRUGL, K-GCROL, K-ACHD, K-PANOPA, K-AMIAR, K-ACET; Megazyme International Ireland, Limited, Wicklow, Ireland). Yeast assimilable nitrogen content (YANC) was the sum of ammonia

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TABLE 1. Must metabolites and basic physio-chemical characteristics for Cabernet franc grapes (mean ± standard deviation). The control and dehydrated grapes were divided randomly and equally into three replicates and each sample was tested in duplicate for all metabolites other than soluble solids.

Metabolite	Yeast	Control (21.5 °Brix) target	24.5 °Brix target	26.0 °Brix target	27.5 °Brix target
Soluble solids (°Brix)	*S. cerevisiae* EC1118	21.5±0.5	24.3±0.1	26.0±0.2	27.4±0.1
	S. uvarum CN1	21.7±0.5	24.4±0.1	25.9±0.1	27.5±0.0
Reducing sugar (g/L)	*S. cerevisiae* EC1118	236±7 d	267±8 c	293±4 b	313±3 a
	S. uvarum CN1	227±5 D	269±7 C	290±4 B	315±6 A
pH	*S. cerevisiae* EC1118	3.41±0.07 d	3.76±0.02 b	3.65±0.02 c	3.84±0.02 a
	S. uvarum CN1	3.43±0.01 C	3.71±0.03 B	3.69±0.03 B	3.85±0.01 A
Titratable acidity (g/L tartaric acid)	*S. cerevisiae* EC1118	5.1±0.3 a	4.8±0.1 b	4.4±0.1 c	4.7±0.0 b
	S. uvarum CN1	4.9±0.3 A	4.7±0.1 A,B	4.5±0.1 B	4.6±0.1 B
Ammonia (mg N/L)	*S. cerevisiae* EC1118	13.2±5.8 a	12.4±1.0 a	6.6±0.5 b	11.2±0.7 a,b
	S. uvarum CN1	15.2±3.8 A	11.2±1.0 A,B	8.5±0.7 B	13.6±2.3 A
Amino acid nitrogen (mg N/mL)	*S. cerevisiae* EC1118	82.3±19.3 b	98.1±19.6 a,b	87.1±5.1 b	115.3±8.3 a
	S. uvarum CN1	92.7±14.7 B	103.1±3.6 B	93.0±3.0 B	125.6±10.6 A
YANC (mg N/L)	*S. cerevisiae* EC1118	95.5±24.9 a	110.5±20.5 a	93.6±6.4 a	125.6±7.5 a
	S. uvarum CN1	108.0±18.5 A	114.3±4.5 A	101.5±2.3 A	139.2±11.3 A
Glycerol (g/L)	*S. cerevisiae* EC1118	0.03±0.00 d	0.64±0.09 a	0.35+0.01 c**	0.47±0.03 b
	S. uvarum CN1	0.05±0.03 B	0.50±0.10 A	0.41±0.01 A**	0.50±0.05 A
Acetaldehyde (mg/L)	*S. cerevisiae* EC1118	12±0 b	14±1 a	12±0 b	12±0 b
	S. uvarum CN1	12±0 B	13±1 A	12±0 B	12±0 B
Acetic acid (g/L)	*S. cerevisiae* EC1118	0.004±0.001 d	0.017±0.001 c	0.012±0.001 b	0.021±0.002 a
	S. uvarum CN1	0.003±0.001 C	0.015±0.001 B	0.015±0.003 B	0.019±0.002 A
Ethyl acetate (mg/L)	*S. cerevisiae* EC1118	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)
	S. uvarum CN1	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)

Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by ANOVA with mean separation by Fisher's Protected (LSD0.05). Asterisks (*p<0.05, **p<0.01, ***p<0.001) indicate significant differences between yeast strains at the indicated dehydration target (Student's *t*-test).

nitrogen and amino acid nitrogen. Ethyl acetate and final ethanol were determined by gas chromatography using a Hewlett-Packard 6890 series gas chromatograph equipped with a flame ionization detector (FID) and a split/split-less injector (Agilent Technologies Inc., Palo Alto, CA). Separations were carried out with a DB®-WAX (30 m, 0.25 mm, 0.25 µm) GC column (model 122–7032; Agilent Technologies Inc.) with helium as the carrier gas at a flow rate of 1.5 mL/min. The retention times were as follows: ethanol, 1.674 min; internal standard (99.4% 1-butanol), 2.706 min; ethyl acetate, 3.443 min; internal standard (99.9% 4-methyl 2-pentanol; Sigma Aldrich), 6.877 min. The analyzing software was Chemstation (version E.02.00.493; Aligent Technologies Inc., Mississauga, Canada).

6. Statistical analysis

Differences between variables were determined by XLSTAT statistical software package released by Addinsoft (Version 7.1; New York, New York). The statistical methods used were analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD) test ($p<0.05$) and Student's *t*-test ($p<0.05$, $p<0.01$, $p<0.001$). The normality of ANOVA conditions was tested with Jarque-Bera test.

RESULTS

1. Must analysis for partially dehydrated grapes

Cabernet franc grapes at harvest measured approximately 21.5 °Brix (227–236 g/L reducing sugar) and were dehydrated to targets of 24.5, 26.0 and 27.5 °Brix (Table 1). The grapes required 31 days to reach 24.5 °Brix, 37 days to reach 26 °Brix and 61 days to reach 27.5 °Brix. The results from must analysis once grapes reached target levels and before any DAP addition are shown in Table 1. The starting acetic acid and acetaldehyde concentrations were low, and no measurable ethyl acetate (limit of detection = 3.0 mg/L) was detected in grapes at any Brix level. The reducing sugars increased

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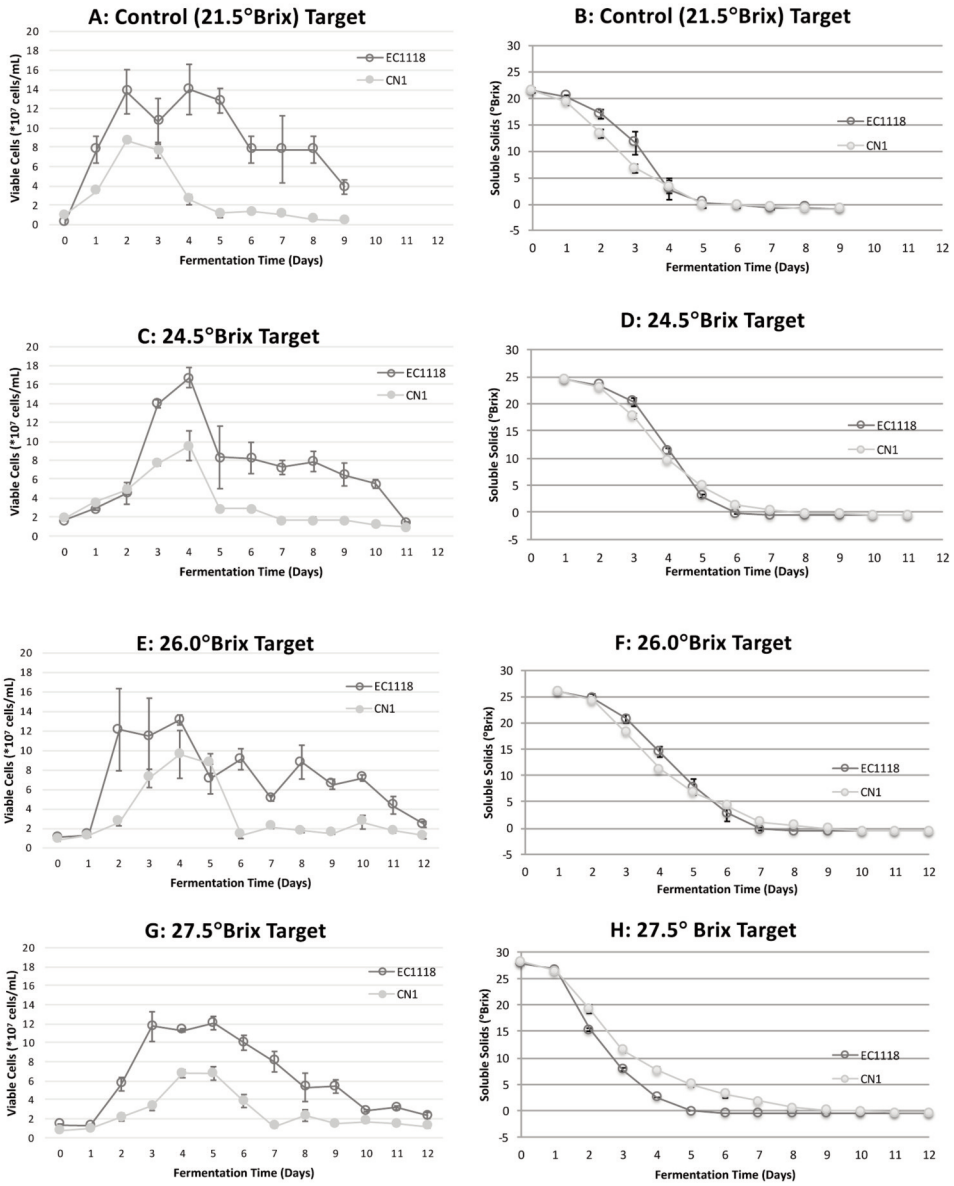


Figure 1. Total viable cell concentrations of Cabernet franc at (A) 21.5 °Brix (control), (C) 24.5 °Brix, (E) 26.0 °Brix and (G) 27.5 °Brix for *S. cerevisiae* EC1118 and *S. uvarum* CN1, and fermentation kinetics of Cabernet franc at (B) 21.5 °Brix (control), (D) 24.5 °Brix, (F) 26.0°Brix and (H) 27.5 °Brix for *S. cerevisiae* EC1118 and *S. uvarum* CN1.

with degree of drying. The titratable acidity decreased and pH increased with dehydration.

2. Yeast cell growth and fermentation

CN1 was reclassified from *S. bayanus* to *S. uvarum* through sequence comparison of three regions of the GDH1 gene using multiple primer sets for the open reading frame (ORF), promoter (Pro) and intergenic spacer (IGS) regions (see supplementary Table S1). Fermentations using *S. uvarum* isolate CN1 were compared to those using the commercially available EC1118 at four starting sugar concentrations. Although inoculated at the same starting cell concentration, viable cell concentrations differed between EC1118 and CN1 throughout the fermentations in all treatments, with fermentations inoculated with CN1 showing approximately half the peak cell concentration under the treatment conditions (Figure 1

TABLE 2. Basic physio-chemical characteristics of Cabernet franc control wine and wine made from partially dehydrated grapes (mean ± standard deviation). Fermentations were conducted in triplicate and each sample was tested in duplicate for all metabolites.

Basic physio-chemical characteristics	Yeast	Control (21.5 °Brix)	24.5 °Brix target	26.0 °Brix target	27.5 °Brix target
Reducing sugar (g/L)	*S. cerevisiae* EC1118	0.07±0.00 a	0.11±0.07 a	0.07±0.00 a	0.09±0.03 a*
	S. uvarum CN1	0.07±0.00 B	0.26±0.15 B	0.07±0.00 B	2.35±1.23 A*
pH	*S. cerevisiae* EC1118	3.82±0.05 b	3.87±0.01 b**	3.84±0.02 b**	4.02±0.01 a**
	S. uvarum CN1	3.78±0.07 B	3.77±0.03 B**	3.72±0.04 B**	3.9±0.03 A**
Titratable acidity (g/L tartaric acid)	*S. cerevisiae* EC1118	8.5±0.3 a***	6.5±0.1 b***	6.7±0.1 b***	6.4±0.1 b**
	S. uvarum CN1	6.7±0.1 C***	7.7±0.1 B***	7.8±0.1 A***	6.8±0.1 C**
Ammonia (mg N/L)	*S. cerevisiae* EC1118	8.8±4.9 a	6.3±0.6 a	9.3±4.6 a	6.5±1.5 a
	S. uvarum CN1	6.0±0.0 A	6.0±0.0 A	10.3±3.8 A	12.5±4.6 A
Amino acid nitrogen (mg N/mL)	*S. cerevisiae* EC1118	39.8±11.4 c*	52.3±0.7 b,c*	61.5±2.9 b	81.0±5.2 a
	S. uvarum CN1	65.8±1.3 B*	62.6±4.5 B*	66.9±3.9 C,B	75.4±1.4 A
YANC (mg N/L)	*S. cerevisiae* EC1118	48.7±18.9 c	58.6±0.7 b,c	70.8±6.2 a,b	87.5±7.8 a
	S. uvarum CN1	71.8±1.6 B	68.6±5.5 B	77.2±4.3 B	87.9±6.0 A

Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by ANOVA with mean separation by Fisher's Protected (LSD0.05). Asterisks (*p<0.05, **p<0.01, ***p<0.001) indicate significant differences between yeast strains at the indicated dehydration target (Student's *t*-test).

A,C,E,G). Despite these lower viable cell concentrations for fermentations inoculated with CN1, these fermentations consumed sugar at a comparable rate to those inoculated with EC1118 at 21.5, 24.5 and 26.0 °Brix treatments, fermenting to less than 5 g/L (Figure 1 B,D,F,H; Table 2). At the 27.5°Brix treatment, fermentations inoculated with CN1 showed a slower sugar consumption rate, requiring three additional days to complete the fermentations, and left 2.35 g/L residual sugar in the wine (Table 2).

3. Cabernet franc wine metabolites

CN1 inoculated fermentations for the control must consumed more YANC and produced lower titratable acidity in comparison to the control treatment inoculated with EC1118 (Table 2). For all treatments using dried grapes (24.5, 26 and 27.5 °Brix), fermentations inoculated with CN1 produced significantly more titratable acidity relative to those inoculated with EC1118. For each sugar level tested, except 24.5°Brix, fermentations inoculated with CN1 had the same wine ethanol concentration as found in wines resulting from EC1118 (Figure 2A). CN1 inoculated fermentations using the dehydrated grape treatments produced higher concentrations of glycerol in comparison to wines inoculated with EC1118 (Figure 2B). However, all CN1 fermentations produced significantly less acetic acid and ethyl acetate at each sugar level tested in comparison to EC1118 (Figure 2 D,E) and significantly less acetaldehyde (Figure 2C) at the 26 and 27.5 °Brix treatments.

Acetic acid was measured throughout the course of fermentation for all Cabernet franc treatments inoculated with both yeast strains (Figure 3 A–D). For all grape drying treatments, fermentations inoculated with *S. uvarum* isolate CN1 reached maximum acetic acid concentration on day two, followed by a decline on day three and then an increase that plateaued over the remainder of the fermentation, resulting in a final acetic acid concentration in the wine lower than that measured on day two of fermentation. A similar trend was observed in the control fermentation for CN1 with the peak on day one. In fermentations inoculated with EC1118, the acetic acid did not peak on day two but continued to increase throughout the fermentation except for the 27.5 °Brix treatment, where the acetic acid concentration peaked on day two and remained high for the rest of the fermentation, with no significant difference in the end concentration compared to that measured on day two.

To determine the impact the *S. uvarum* isolate would have on wine fermented with juice containing a high concentation of VA, Pinot noir must at 20.5 ºBrix from sour rotted grapes were fermented. When acetic acid was monitored during these table wine fermentations of Pinot noir (Figure 4, B) with a high starting concentration of acetic acid (0.48 g/L), the *S.*

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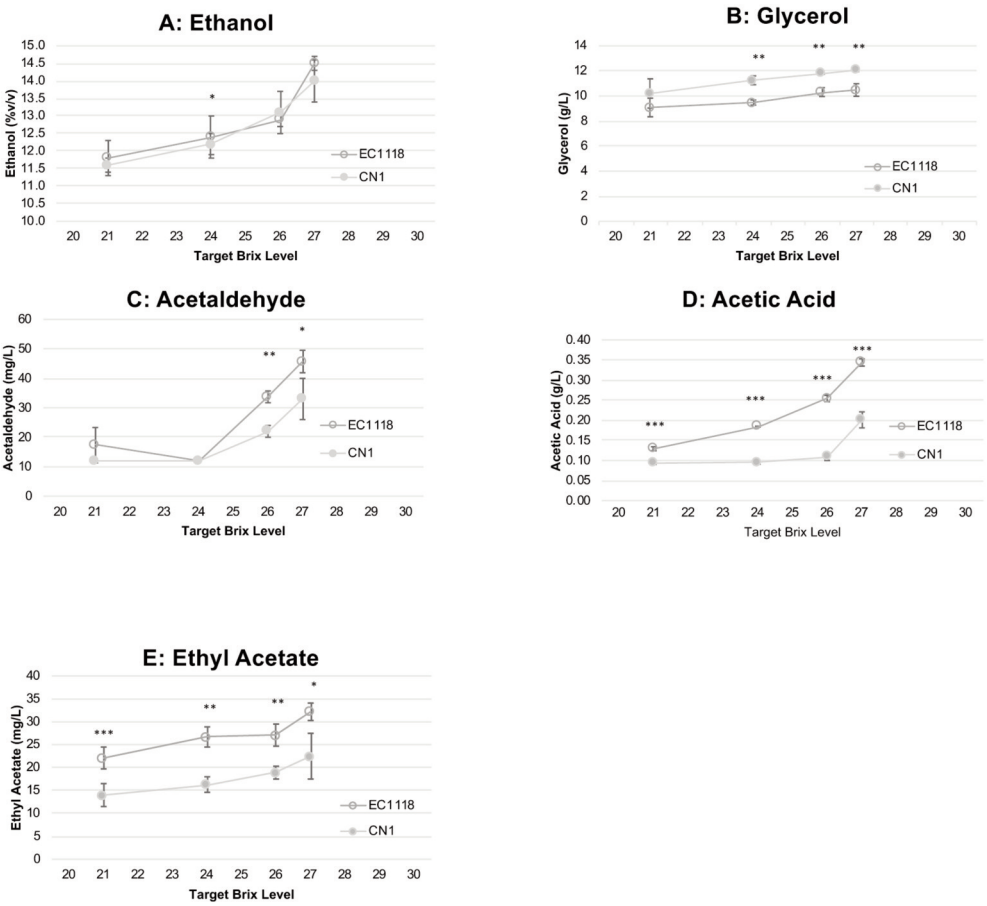


FIGURE 2. Yeast metabolites (A) ethanol, (B) glycerol, (C) acetaldehyde, (D) acetic acid, and (E) ethyl acetate concentrations of Cabernet franc control (21.5°Brix), 24.5°Brix, 26.0 °Brix and 27.5°Brix treatments.

Fermentations were conducted in triplicate and each replicate sample was tested in duplicate for all metabolites (mean ± SD). Asterisks (*p<0.05, **p<0.01, ***p<0.001) indicate significant differences between yeast strains at the indicated dehydration target (Student's *t*-Test).

uvarum isolate CN1 consumed acetic acid more rapidly than EC1118 during the first two days of fermentation, and by day two, significantly lower levels of acetic acid resulted from CN1 in comparison to EC1118. After day two, the concentrations of acetic acid plateaued and remained low for the remainder of the fermentations from both yeasts. The end concentration of acetic acid from the *S. uvarum* isolate CN1 was 0.07 g/L whereas for EC1118, it was 0.18 g/L (Figure 4 F). As with the Cabernet franc fermentations, wines vinified with the *S. uvarum* isolate also had lower ethyl acetate levels in comparison to EC1118 (Figure 4 G).

DISCUSSION

This study aimed to define the range of sugar concentrations that the indigenous *S. uvarum* isolate could ferment to less than 5 g/L in musts from partially dehydrated grapes and to assess the impact this yeast has on oxidative compounds in the final wines. Cabernet franc grapes in this study were dried in a commercial barn in Ontario, Canada, that was used for making Vin de Curé as part of a commercial winemaking operation. Based on the time required to dry the grapes to 27.5 °Brix (61 days), this method is considered a very slow dry time (Mencarelli and Bellincontro, 2013). Traditionally, dry time is defined as follows: very fast, five to ten days; fast, two to three weeks; slow, four to eight weeks; very slow, more than eight weeks (Mencarelli and Bellincontro, 2013). A slow dry time for appassimento wine style is considered beneficial because in a controlled environment (versus sun drying) it results in a reduction in the formation of potentially undesirable metabolites such as acetaldehyde and acetic acid (Bellincontro *et al*., 2004).

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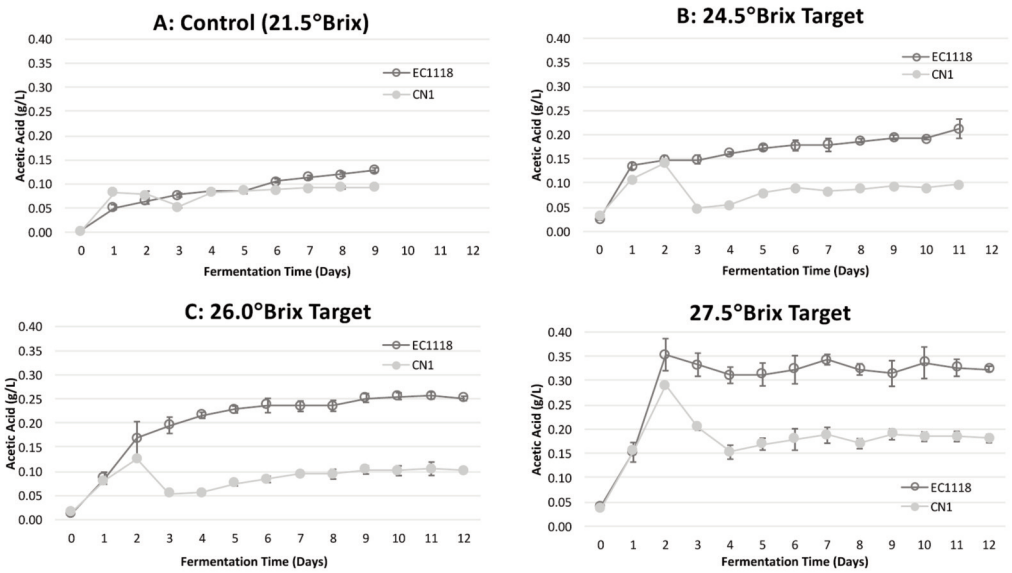


FIGURE 3. Acetic acid concentration during fermentation of musts at (A) 21.5 °Brix (control), (B) 24.5 °Brix, (C) 26.0 °Brix and (D) 27.5 °Brix.
Each data point represents the mean acetic acid concentration from triplicate fermentations, with each time point from each replicate measured in duplicate (± standard deviation).

The *S. uvarum* isolate, formerly identified as *S. bayanus*, was reclassified based on sequencing of three regions of the *GDH1* gene that have been published to differentiate between *S. uvarum* and *S. bayanus* (Nguyen and Gaillardin, 2005). The *S. uvarum* isolate produced similar ethanol values to EC1118 and left low residual sugar in the wines produced from partially dehydrated grapes. These traits of producing low oxidation compounds, high ethanol and leaving low residual sugar have been reported as advantageous for appassimento wine production (Accordini, 2013).

For the range of grape dehydration targeted, fermentations inoculated with the *S. uvarum* isolate only showed a slower sugar consumption rate compared to EC1118 for the 27.5 °Brix treatment, with all other fermentations showing a comparable fermentation rate or slightly faster rate relative to EC1118. Although at 27.5 °Brix the *S. uvarum* isolate required three additional days to complete the fermentation and left 2.4 g/L residual sugar in the wine, the ethanol levels were not significantly different between the two yeasts.

Previous work with this *S. uvarum* isolate demonstrated that when the starting must was at 28 °Brix, the yeast was not able to ferment to dryness in Cabernet franc, leaving 15.8 g/L residual sugar in the wine (Kelly *et al*., 2018). The present study has established the upper concentration limit of must at 27.5 °Brix from this vintage of Cabernet franc that the yeast can ferment to less than 5 g/L sugar. The application of this isolate to other cultivars and wine styles using high Brix juice would be valuable to define more broadly the upper limit it can ferment to dryness. The additional three days required for the *S. uvarum* isolate to reach dryness still allowed the fermentations to be completed within nine days, which is within acceptable ranges for this wine style as previously reported (Tosi *et al*., 2009).

Elevated concentrations of oxidation compounds have been reported to arise during the appassimento grape drying process (Bellincontro *et al*., 2016). Identifying yeast strains that generate low acetic acid, acetaldehyde and ethyl acetate during the fermentation of partially dehydrated grapes may be of commercial importance in making this wine style, as less oxidation compounds originating from the yeast would be released into the wine, and therefore, there would be less of a cumulative effect. Fermentations with the *S. uvarum* isolate produced significantly lower levels of these oxidation compounds in comparison to EC1118, in agreement with results previously reported with Cabernet franc from an earlier vintage

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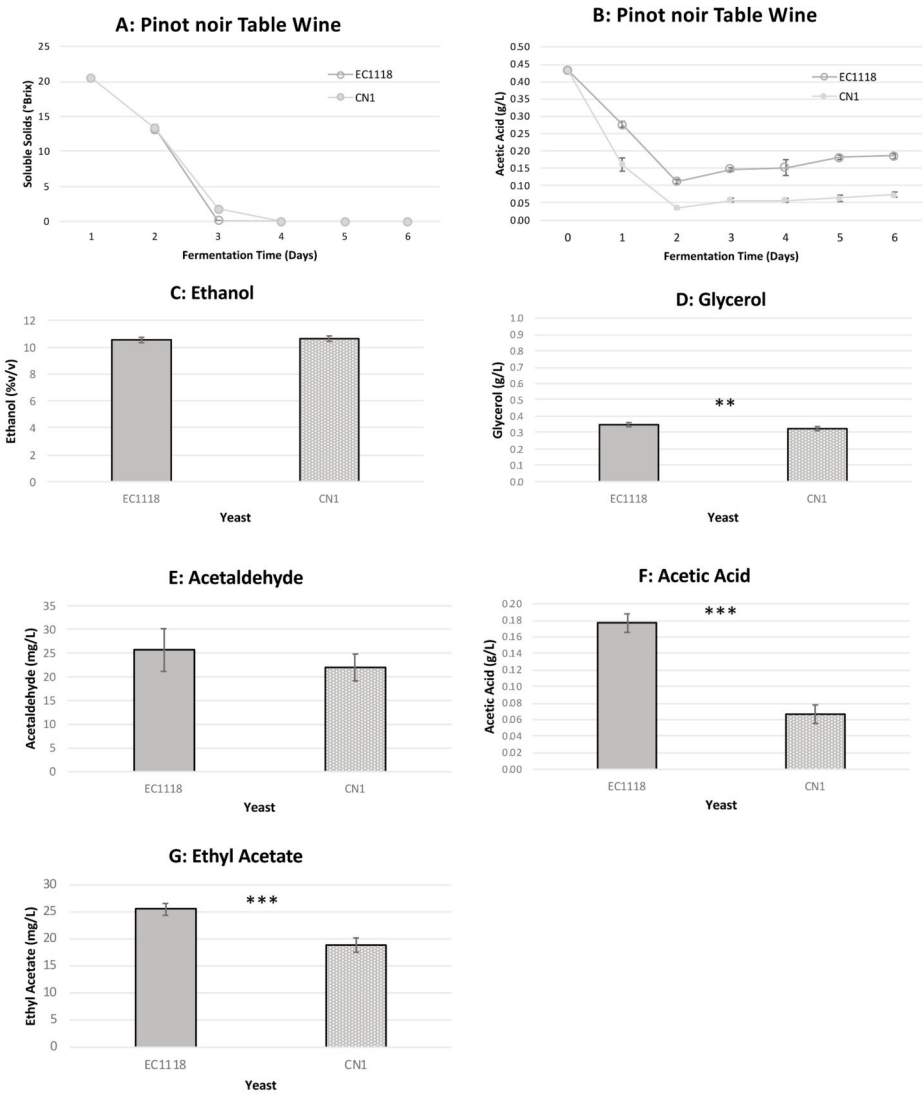


FIGURE 4. (A) Sugar consumption and (B) acetic acid consumption during fermentation of Pinot noir table wine with a high starting concentration of acetic acid. The yeast metabolites (C) ethanol, (D) glycerol, (E) acetaldehyde, (F) acetic acid and (G) ethyl acetate at the end of fermentation.
Each data point represents the mean measu-rement from triplicate fermentations, with the samples from each replicate measured in duplicate (± standard deviation). Asterisks (*p<0.05, **p<0.01, ***p<0.001) indicate significant differences between yeast strains (Student's *t*-Test).

(Kelly *et al*., 2018). The present study also showed a unique characteristic of acetic acid metabolism by this yeast isolate that further differentiates this yeast from commercial *S. cerevisiae* wine yeast such as EC1118. Not only does the *S. uvarum* isolate produce lower levels of acetic acid, but by day two of the fermentations the yeast appears to consume acetic acid at a rate faster than producing acetic acid, resulting in a drop in the acetic acid concentration in the wine. This characteristic of the yeast isolate may have application beyond an altered yeast osmotic stress response in high sugar juices (Pigeau and Inglis, 2005). If this yeast is able to consume acetic acid during fermentation, the *S. uvarum* isolate may have application in wines made from grapes with elevated starting concentrations of acetic acid, such as grapes infected with sour rot or suffering from the start of breakdown at harvest (Barata *et al*., 2011). Grapes delivered to wineries in Ontario, Canada have an allowable upper limit of VA for wineries of 0.2–0.24 g/L acetic acid, above which the grapes can be rejected by the winery based on quality standards set by individual wineries (McFadden-Smith, 2010). After observing the acetic acid metabolism during fermentation of the partially dried grapes,

the *S. uvarum* yeast isolate was tested in a juice that contained high VA due to sour rotted fruit. As the yeast isolate was able to consume acetic acid throughout the fermentation and reduce the acetic acid concentration seven-fold as well as signficiantly reduce ethyl acetate concentrations in the wine, it could prove useful for table wine production from juice with VA, especially in vintages with high pressures from sour rot. This application would be contingent on other juice quality factors in the high VA juice not being compromised.

The findings in this current study agree with existing literature that the composition of wines produced with various strains of *S. uvarum* yeasts have lower acetic acid levels and higher glycerol levels when compared to wines made with *S. cerevisiae* (Eglinton *et al*., 2000). However, the metabolism of acetic acid during fermentation, as the osmotic stress from sugars increases, indicates a differing mechanism for dealing with hyperosmotic stress in the *S. uvarum* isolate. The response of *S. uvarum* CN1 in this present study – producing higher glycerol yet lower acetic acid along with the further metabolism of acetic acid during fermentation – differs from the hyperosmotic stress response of *S. cerevisiae* wine yeast during high sugar fermentations (Pigeau and Inglis, 2005; Yang *et al*., 2017; Heit *et al*., 2018). In *S. cerevisiae* wine yeast under hyperosmotic stress from sugars, glycerol and acetic acid production are linked to the redox balance of the NAD+/NADH cofactor system and acetaldehyde stress (Pigeau and Inglis, 2007; Yang *et al*., 2017). Our results indicate that the *S. uvarum* isolate produces acetic acid in response to glycerol production but further metabolizes the acetic acid. The acetic acid is not converted into ethyl acetate so the *S. uvarum* acetylCoA synthetase required for ethyl acetate production may be down regulated, as reported in *S. cerevisiae* wine yeast under these conditions (Heit *et al*., 2018). It is unknown what the acetic acid is metabolized into at the present time but clearly the yeast are releasing it into the wine and then retaking it up for further metabolism. The mechanism for these differing responses between *S. cerevisiae* and *S. uvarum* is an area of future study.

Acetic acid is an important component and marker for influencing the final quality of wine, and at elevated levels it is associated with spoilage, can reduce varietal character, and can have a highly undesirable organoleptic effect (Lambrechts and Pretorius, 2000; Nurgel *et al*., 2004; Jackson, 2008). In Canada, the legal limit of acetic acid in wines is regulated based on the starting Brix measurement: at 27–28°Brix, the allowable limit is 1.5 g/L, at 28–32 °Brix the limit is 1.8 g/L, and over 32 °Brix the limit is 2.1 g/L (VQA, 2019). Amarone wines made from post-harvest dried grapes have a range of acetic acid and ethyl acetate reported in the starting juices and finished wines, which can also be influenced by microbial contamination of the fruit (Fedrizzi *et al*., 2011; Zapparoli *et al*., 2018). The *S. uvarum* isolate may prove useful in fermenting microbially contaminated partially dehydrated grapes with high starting VA due to both its low production of acetic acid during fermentation and its ability to consume acetic acid during fermentation.

Ethyl acetate, although often considered an oxidation fault that contributes to the sensory perception of VA at elevated concentrations (Jackson, 2008), is a volatile constituent that has been reported as providing a positive contribution to wines made from partially dehydrated grapes and may not contribute to the perception of spoilage (Moio and Piombino, 2013). As the *S. uvarum* isolate CN1 produces very low levels of ethyl acetate, any beneficial attributes of ethyl acetate in appassimento wines would be reduced if fermented with this isolate.

To control the soundness of the Cabernet franc grapes included in this study, fruit showing signs of fungal infection were discarded and the slow dry time resulted in relatively low starting concentrations of acetic acid, but variations in vintage can result in varied grape quality. Therefore, using the appropriate yeast can assist with meeting requirements for legal allowable limits of oxidation compounds in wines produced from partially dehydrated grapes. The *S. uvarum* isolate CN1 yeast represents an opportunity to regionally tailor this wine style and offer consumers a signature style that is suited to the climate and varietals available to industry. This *S. uvarum* isolate may also have application in wine production from grapes infected with sour rot containing high volatile acidity due to its ability to consume acetic acid during fermentation. Perhaps CN1 could be co-inoculated with another strain of *Saccharomyces* yeast or included in a succession of strains for fermentation to degrade acetic acid. This

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hypothesis remains to be tested and may be included in future research.

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