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Characterization of *Saccharomyces bayanus* CN1 for Fermenting Partially Dehydrated Grapes Grown in Cool Climate Winemaking Regions

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Abstract: This project aims to characterize and define an autochthonous yeast, *Saccharomyces bayanus* CN1, for wine production from partially dehydrated grapes. The yeast was identified via PCR and Basic Local Alignment Search Tool (BLAST) analysis as *Saccharomyces bayanus*, and then subsequently used in fermentations using partially dehydrated or control grapes. Wine grapes were dried to 28.0°Brix from the control grapes at a regular harvest of 23.0°Brix. Both the partially dehydrated and control grapes were then vinified with each of two yeast strains, *S. bayanus* CN1 and *S. cerevisiae* EC1118, which is a common yeast used for making wine from partially dehydrated grapes. Chemical analysis gas chromatography-flame ionization detector (GC-FID) and enzymatic) of wines at each starting sugar level showed that CN1 produced comparable ethanol levels to EC1118, while producing higher levels of glycerol, but lower levels of oxidative compounds (acetic acid, ethyl acetate, and acetaldehyde) compared to EC1118. Yeast choice impacted the wine hue; the degree of red pigment coloration and total red pigment concentration differed between yeasts. A sensory triangle test (n = 40) showed that wines made from different starting sugar concentrations and yeast strains both differed significantly. This newly identified *S. bayanus* strain appears to be well-suited for this style of wine production from partially dehydrated grapes by reducing the oxidative compounds in the wine, with potential commercial application for cool climate wine regions.

Keywords: winemaking; partially dehydrated grapes; appassimento; yeast; *Saccharomyces bayanus*; sensory; Ontario; climate change adaptation

1. Introduction

In an increasingly competitive international marketplace, important strategic considerations include a focus on the reliable production of high-value wines, and on styles that help differentiate and brand a wine region. This creates particular opportunities for the emerging wine regions of the New World, to adapt the traditions of the Old World while developing technological advancements in viticulture and oenology to assist in the expression of regionality [1]. In the recent past, winemakers in Ontario, Canada have highlighted their unique regional identity with products such as sparkling Icewine (e.g., Inniskillin Wines). Moving beyond that, there is room for additional signature products that can help define this region. Developing such wine styles and their corresponding production

technologies can support the sustainability of established appellations, as well as the development of nascent grape-growing regions.

The Ontario industry is economically important [2], and its success is intrinsically linked to its unique climate, which allows the growth of a range of premium *vinifera* grape varieties [3]. However, it can be challenging to achieve optimal grape ripeness in the shorter growing season that is associated with Ontario's cool climate [4]. Further, weather volatility is an additional threat to grape-growing in this region, with the most salient risks associated with temperature extremes, rainfall variability, and winter and frost damage [5]. Therefore, it is prudent to adopt innovative strategies in order to mitigate the risks associated with a changing climate and stabilize quality from vintage to vintage.

Postharvest grape-drying (appassimento) followed by vinification is a technique that is traditionally employed in Northern Italy for Amarone wine production [6]. This method consists of ripening grapes off-vine to produce withered or partially dehydrated fruit. The drying process increases the concentration of total soluble solids, phenolic compounds, and odorants in the grapes [7,8]. The wines produced from these grapes have a higher concentration of ethanol, volatile aroma compounds, and anthocyanins [9,10]. In Ontario, Canada, wines made from partially dehydrated grapes are regulated by the Vintners Quality Alliance (VQA) under the term Vin de Curé [11].

Despite these benefits, wines made from partially dehydrated grapes can have increased levels of undesirable oxidation compounds in the wine, most notably acetic acid, ethyl acetate, and acetaldehyde [10,12,13]. At elevated concentrations, these compounds can negatively affect the organoleptic quality of the wine [14], and in the case of acetic acid, exceed legal limits enforced by the VQA [11]. The development of these compounds is directly related to the high starting sugar concentration in the must that creates an environment of high osmotic stress for yeast.

Glycerol, the major compatible solute in *S. cerevisiae*, accumulates intracellularly as a survival response to hyperosmotic stress [15]. The accumulation of glycerol maintains cell volume and turgor pressure while limiting the efflux of intracellular water [15,16]. Glycerol formation is accompanied by an increase in NAD^+ production [17]. Under these conditions, the shift in redox balance ($NADH:NAD^+$ ratio) caused by the increased formation of glycerol is corrected via acetic acid production, which reduces NAD^+ to NADH [17–20]. Monitoring the development of glycerol and acetic acid during fermentation can therefore provide insights into the yeast's management of redox balance and hyperosmotic stress.

It has been suggested that autochthonous starter cultures have benefits for regional wines, including sparkling wines, in that they may be well-adapted to specific environmental conditions, and prospectively enhance the desired flavor and aroma profiles, which can impact the quality of regional wines [21–26]. We previously conducted a spontaneous fermentation of local Riesling Icewine must from Ontario, Canada and identified that *Candida dattilla* along with *Kloeckera apiculata* and *Cryptococcus laurentii* dominated the fermentation, and were still present at the end (day 30), whereas *S. cerevisiae* was not found [27]. In a later study, this *Candida dattilla* strain, which was initially identified using API Biomedical kits, was further identified as a *Saccharomyces* species by DNA sequencing of the 5.8S-ITS region. It was likely *S. bayanus* or *S. pastorianus*, but the identification could not be finalized past the genus (unpublished). Since *S. bayanus* is reported as producing lower acetic acid levels during wine fermentation [28], the strain isolated from Icewine grapes in Ontario was further tested on its own in the osmotically stressful Icewine fermentation condition. A pure starter culture of this yeast was built up and inoculated into filter-sterilized 41.6°Brix Riesling Icewine juice, where it produced 7.7% v/v ethanol compared to 10.8% v/v from the control *S. cerevisiae* K1-V1116. However, the isolated yeast produced 1.3-fold lower acetic acid/sugar consumed compared to K1-V1116 [29]. Although this yeast did attain the minimum alcohol required for Icewine of 7%, commercial Icewines in Canada have been found to range between 8.4–12.6% v/v ethanol and for Riesling Icewines, between 9.1–12.2% v/v [30]. The combined value of autochthonous yeast for the expression of regionality and the positive preliminary results in Icewine led us to characterize this yeast strain during the fermentation of must from partially dehydrated grapes, which provides a less

stressful sugar environment than Icewine juice, but still has potentially problematic oxidative quality concerns from this wine style [10,12,13].

In this study, a local yeast isolated from the skin of Riesling Icewine grapes [27] is tested in the fermentation of partially dehydrated grapes. Grapes were dried to 28.0°Brix and vinified with one of two yeast strains, *S. cerevisiae* EC1118, the commonly used yeast for this wine style, and the yeast of interest, CN1. Grapes picked at 23.0°Brix (a sugar level typical for red table wine production) were also fermented with the two yeast strains as a control.

The main objectives of our study are to (i) identify this locally-isolated yeast, (ii) determine its fitness for making wine from partially dehydrated grapes, and (iii) more fully understand the impact of high sugar fermentation on red wine composition, color, and sensory quality. The results from this study should assist in optimizing winemaking from partially dehydrated grapes in cool climate wine areas such as Ontario, Canada, as well as inform international wine regions that are seeking regional differentiation or further innovation of their wine styles.

2. Materials and Methods

2.1. Yeast Strains

Two yeast strains were selected to carry out the fermentations. The commercial *S. cerevisiae* strain EC1118, was purchased from Lallemand (Montreal, QC, Canada). The local strain was isolated from Riesling Icewine grapes from the Niagara Region in Ontario, at the Cool Climate Oenology and Viticulture Institute (CCOVI). Four genomic areas were analyzed to identify this yeast: the internal transcribed spacer regions (ITS1 and ITS2), including the *5.8S* gene of the ribosomal DNA (GenBank accession number: MH317189); the D1/D2 domain of a large subunit of the *26S rRNA* gene region (GenBank accession number: MH318011); the mitochondrial *β-tubulin* gene (GenBank accession number: MH339593); and the mitochondrial cytochrome oxidase II gene (*COX*II) (GenBank accession number: MH339594). The ITS1-*5.8S rRNA*-ITS2 gene region was amplified via PCR with the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG) and ITS4 (5′-TCCTCCGCTTATTGATATGC). The D1/D2 domain was amplified with the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5′-GGTCCGJGTTTCAAGACGG). The *β-tubulin* gene was amplified with the primer pair βtub3 (5′-TGGGCYAAGGGTYAYTAYAC) and βtub4r (5′-GCCTCAGTRAAYTCCATYTCRTCCAT), and the *COX*II gene was amplified with the primers COII5 (5′-GGTATTTTAGAATTACATGA) and COII3 (5′-ATTTATTGTTCRTTTAATCA). DNA sequencing analysis (Robarts Research Institute, London, ON, Canada) was performed on all four amplified genes, and the results were compared with all of the available sequence databases of DNA using the Basic Local Alignment Search Tool (BLAST).

2.2. Grape Harvest, Desiccation and Processing

Vitis vinifera Cabernet franc grapes were hand-harvested at Mazza Vineyards in Niagara-on the-Lake, Ontario, Canada, at approximately 23.0°Brix. First, 209 kg of grapes were picked and placed in perforated drying containers in a single layer. Grapes were divided into two parcels and delivered to two locations. One of the parcels was delivered to CCOVI (Brock University, St Catharines, ON, Canada) and processed on the following day after temperature stabilization overnight at room temperature. The other parcel was delivered to Cave Spring Cellars Barn (4424 Cave Spring Road, Beamsville, ON, Canada), which is dedicated to drying grapes for producing commercial Vin de Curé wines [11]. The drying containers were stacked 14 layers high, with adequate air space between each container to receive natural ventilation in the barn. Fifteen randomly selected clusters were collected weekly. The samples were hand-crushed in a plastic bag and strained through a metal strainer to collect must. Must samples were analyzed for soluble solids, pH, and titratable acidity. Once the target sugar concentration was reached (28.0°Brix), the partially dehydrated grapes were delivered to CCOVI for processing after temperature stabilization overnight. Grapes were crushed and destemmed (model Gamma 50, Mori-TEM; Florence, Italy) into 30-L steel fermentation vessels with

tight-fitting lids. Must was blanketed with CO_2, lids were secured, and vessels were stored at 22 °C prior to yeast inoculation. Must volume was estimated by multiplying weight by 0.75 for control must, and 0.60 for partially dehydrated grape must to account for desiccation effects. Then, 500 mg L^{-1} of diammonium phosphate (DAP; Laffort, Bordeaux, France) was added to the must and mixed by punch down. A further 250 mg L^{-1} of DAP was added on the third day of fermentation to reduce yeast stress.

2.3. Winemaking

Four sets of triplicate fermentations were carried out: (i) 23.0°Brix must fermented with *S. cerevisiae* EC1118, (ii) 23.0°Brix must fermented with *S. bayanus* CN1, (iii) 28.0°Brix must fermented with EC1118, and (iv) 28.0°Brix must fermented with CN1. Fermentations were conducted using the same microvinification protocols. *S. cerevisiae* EC1118 was rehydrated according to manufacturer's directions and plated out on yeast extract peptone dextrose plates (YPD, 1% yeast extract, 2% peptone, 2% dextrose, 2% agar). CN1 yeast was prepared from a frozen glycerol stock, and also plated out on YPD plates. Both yeasts were grown to appropriate colony size prior to preparing a starter culture in sterile-filtered grape juice. The starter cultures were built up in sterile-filtered Cabernet franc must, and then followed a step-wise acclimatization procedure as outlined in Kontkanen et al. [20]. The yeast strains were inoculated from YPD plates into 750 mL of 10°Brix sterile-filtered must with the addition of 2 g L^{-1} DAP and grown aerobically at 25 °C with shaking at 0.605× *g* until cell concentration reached 2×10^8 cells mL^{-1}, as determined by haemocytometry. Then, 750 mL of sterile-filtered 23.0°Brix control must was added to each build-up culture and held for 1 h at 25 °C with swirling every half hour. The 1.5 L of control cultures for both EC1118 and CN1 were added to 28.5 L of 23°Brix control must to reach an inoculum of 5.0×10^6 cells mL^{-1} in 30-L stainless steel fermentation vessels. The 28.0°Brix treatment required one more acclimatization step for both yeast, and 750 mL of sterile-filtered 28.0°Brix dehydrated grape must was added to each starter culture and held for 2 h at 25 °C with swirling every half hour, after which the 2.25-L culture was inoculated into 27.75 L of 28.0°Brix dehydrated grape must to reach an inoculum of 5.0×10^6 cells mL^{-1} in the 30-L fermentations.

After inoculation, the fermentations were gently mixed by punch down and moved to a temperature-controlled chamber at 22 °C. Fermentations were monitored once daily by recording soluble solids (hydrometer, °Brix) and temperature (thermometer, °C). The caps were punched down twice daily with 20 plunges per vessel using a separate punch-down tool for each yeast trial; this number was gradually reduced to four plunges near the end of the fermentation. As the cap started to fall, fermentations were blanketed with CO_2 to protect them from oxidation. Fermentations were considered complete once the yeast stopped consuming sugar (<5 g L^{-1}) and/or the sugar concentration stayed the same for three consecutive days, as confirmed by a wine scan analysis conducted by WineScan™ FT120 (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 glass carboys. Then, 50 mg L^{-1} of sulfur dioxide (as potassium metabisulfite) was added to each treatment, which were left to settle at room temperature. Wines were then racked and moved to a −2 °C chamber for cold stabilization. Wines were subsequently filtered through 0.45-µm filter pads, bottled in 750-mL glass wine bottles, with a manual bottler (Criveller Group; Niagara Falls, ON, Canada), closed with natural cork with an automated corker (model ETSILON-R, Bertolaso; San Vito, Italy), and stored in the CCOVI wine cellar (17.5 °C, 74.5% RH).

2.4. 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, NY, USA) for grape and must samples, and using a degree Brix hydrometer for fermentation time course samples. pH was determined using a pH meter (SympHony, VWR, SB70P, Mississauga, ON, Canada), and titratable acidity was determined by titration with 0.1 mol L^{-1} of NaOH to an endpoint

of pH 8.2 [31]. Glucose, fructose, glycerol, acetaldehyde, ethanol in must, amino acid nitrogen, ammonia nitrogen, acetic acid, lactic acid, and malic acid were determined with Megazyme Kits (K-FRUGL, K-GCROL, K-ACHD, K-ETOH, K-PANOPA, K-AMIAR, K-ACET, K-LATE, K-LMALL; Megazyme International Ireland, Limited, Bray Company, Wicklow, Ireland). Ethyl acetate and ethanol in wine were determined by gas chromatography (GC) using a Hewlett-Packard 6890 series gas chromatograph (Agilent Technologies Incorporated, Santa Clara, CA, USA) equipped with a flame ionization detector (FID), split/split-less injector, and Chemstation software (version E.02.00.493). Separations were carried out with a DB®-WAX (30 m, 0.25 mm, 0.25 µm) GC column (122-7032 model; Agilent Technologies, Santa Clara, CA, USA) with helium as the carrier gas at a flow rate of 1.5 mL min^{-1}.

2.5. Color Evaluation

Measures of color density, hue, degree of red pigment coloration, and total red pigments were conducted based on the methods of Iland et al. [32] by UV-Vis spectrophotometer (Cary 60, Agilent Technologies, Santa Clara, CA, USA).

2.6. Sensory Evaluation

A preliminary bench tasting (n = 4) of the wines established that the winemaking replicates within each treatment were similar enough to blend into representative treatments for difference testing. Therefore, four treatments were presented to the panelists (EC1118, 23.0°Brix; CN1, 23.0°Brix; EC1118, 28.0°Brix; CN1, 28.0°Brix). A balanced and randomized triangle test design composed of six sets of triads was used to compare all of the treatments to each other. Each participant (n = 40) tasted a total of 18 samples over the course of two sessions. The first session consisted of three sets of three wines, separated by forced three-minute breaks between each set to minimize fatigue and carry-over effects. Consumption of water and unsalted crackers was encouraged. The samples were coded with a three-digit randomly assigned code, and the participants were asked to evaluate them in the order presented. Participants were instructed to assess aroma by sniffing and flavor by tasting and expectorating the samples, and determine differences based on these observations. Their answers were recorded using the Compusense Five™ computer program (Compusense Inc., Guelph, ON, Canada). The same format was used for the second session, which was completed after a one-hour break. The evaluations took place in individual booths in the sensory evaluation lab at CCOVI, which was equipped with red lighting to mask possible color differences. Data was analyzed by comparing the number of correct responses to a critical value table for triangle tests [33].

2.7. Statistical Analysis

Analysis of variance (ANOVA) with mean separation by Fisher's Protected Least Significant Difference (LSD) test ($p < 0.05$) was conducted on chemical and color parameters using the XLSTAT statistical software package (Addinsoft, Version 7.1; New York, NY, USA).

2.8. Statement of Ethics

All of the subjects gave their informed consent for inclusion before they participated in the study. The protocol for the study was approved by Brock University's Research Ethics Board (file number 14-021-INGLIS).

3. Results

3.1. Yeast Strain Identification

The sequencing results of the ITS1-*5.8S rRNA*-ITS2 gene region and the D1/D2 domain gene region were only able to identify the isolate at the genus level as a *Saccharomyces* strain. Therefore, the mitochondrial genes *β-tubulin* [34] and *COX*II [35] were selected as biomarkers for

further identification. The amplified sequences of *β-tubulin* showed a 99% similarity in sequence identity with a query coverage of 100% to three *S. bayanus* strains (Table 1). The results from the *COX*II mitochondrial gene reported an identical level of similarity to CBS 380^T and CBS 395^T (Table 1), which are widely accepted type strains (taxonomic standards) of *S. bayanus* and *S. uvarum*, respectively [36,37]. Based on the Genbank sequence comparisons, we have identified this yeast as *S. bayanus*. Recent research reports the nearly identical similarity of the complete mitochondrial genome between these two potential species [38], further raising the question of whether *S. bayanus* and *S. uvarum* should be classified into two separate species (*S. bayanus*, *S. uvarum*) or two varieties under the species *S. bayanus* (*S. bayanus* var. *bayanus*, *S. bayanus* var. *uvarum*) [36].

Table 1. Homology of CN1 mitochondrial genes with GenBank sequences.

Gene Region	NCBI Database Strain for Sequence Comparison	GenBank Accession Number	Base Pairs *	Alignment Results		
				Max Score	Query Coverage	Sequence Identity
β-tubulin	*S. bayanus* Strain BCRC 21818	FJ238317.1	849/852	1555	100%	99%
	S. bayanus Strain BCRC 21964	FJ238319.1	848/852	1550	100%	99%
	S. bayanus Strain BCRC 21816	FJ238316.1	847/852	1546	100%	99%
	S. eubayanus Strain N/A	XM 018364800.1	815/852	1367	100%	96%
	S. pastorianus Strain BCRC 21420	FJ238324.1	813/852	1356	100%	95%
*COX*II	*S. bayanus* Strain CBS380^T	KX657743.1	632/635	1157	99%	99%
	S. uvarum Strain CBS395^T	KX657742.1	632/635	1157	99%	99%
	S. bayanus Strain CBS380	AP014933.1	632/635	1157	99%	99%
	S. bayanus x *S. uvarum* Strain CECT1991	JN676774.1	585/585	1081	91%	100%
	S. eubayanus Strain CRUB1975	KF530344.1	608/620	1079	97%	98%

* Number of base pairs in common between amplified sample and NCBI database sequence/total base pairs aligned.

3.2. Fermentation Kinetics and Metabolites

The must parameters for all treatments are listed in Table 2. The *S. bayanus* CN1 yeast consumed sugars at a higher rate than the control yeast EC1118 at the beginning of both fermentation treatments, but left 15.8 g L^{-1} unfermented sugar (mainly fructose) in the 28°Brix treatment wine (Figure 1, Table 3). Despite CN1 leaving residual sugar in the high brix ferments, CN1 produced a comparable level of ethanol to EC1118, and significantly less oxidative compounds (acetaldehyde, acetic acid, ethyl acetate) for both the control and wines made from the dehydrated grapes (Table 3). Regardless of the winemaking treatment, wines fermented with CN1 contained higher levels of glycerol, titratable acidity, and malic acid in comparison to wines fermented with EC1118, but lower lactic acid in the 23°Brix fermentation (Table 3).

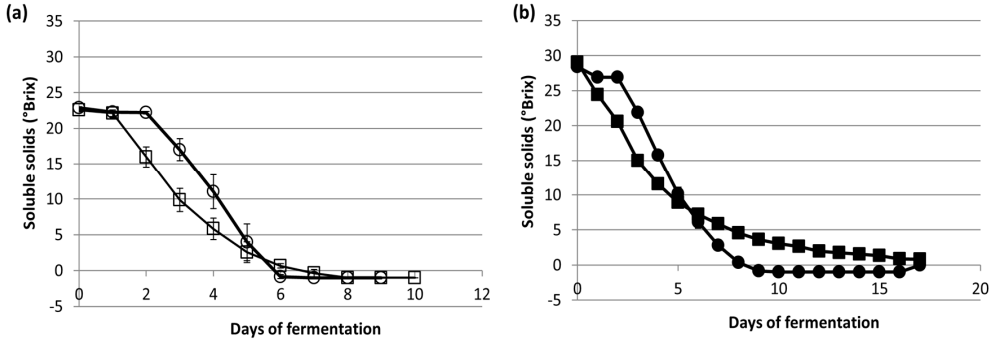

Figure 1. Soluble solid levels during fermentation. (**a**) The 23°Brix control must was inoculated with EC1118 (○) and CN1 (□); (**b**) the 28°Brix partially dehydrated grape must was inoculated with EC1118 (●) and CN1 (■). Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment).

Table 2. Chemical composition of Cabernet franc control must (23°Brix) and must from partially dehydrated grapes (28°Brix). Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Lowercase letters within the same parameter indicate differences between treatments (Fisher's Protected Least Significant Difference $(LSD)_{0.05}$).

Parameter	23°Brix EC1118	23°Brix CN1	28°Brix EC1118	28°Brix CN1
Reducing sugar (g L^{-1})	218 ± 8 ^{b}	198 ± 12 ^{a}	300 ± 3 ^{c}	301 ± 3 ^{c}
Glucose (g L^{-1})	108 ± 4 ^{b}	98 ± 6 ^{a}	145 ± 2 ^{c}	145 ± 1 ^{c}
Fructose (g L^{-1})	111 ± 5 ^{b}	100 ± 6 ^{a}	155 ± 2 ^{c}	156 ± 2 ^{c}
pH	3.39 ± 0.05 ^{a}	3.35 ± 0.01 ^{a}	3.34 ± 0.03 ^{a}	3.33 ± 0.03 ^{a}
Titratable acidity (g L^{-1} tartaric acid)	5.8 ± 0.2 ^{b}	6.1 ± 0.1 ^{c}	4.8 ± 0.0 ^{a}	4.9 ± 0.0 ^{a}
Ammonia nitrogen (mg N L^{-1})	17 ± 9 ^{b}	12 ± 2 ^{a,b}	8 ± 1 ^{a}	8 ± 2 ^{a,b}
Primary amino nitrogen (mg N L^{-1})	62 ± 13 ^{b}	47 ± 2 ^{a}	61 ± 3 ^{b}	63 ± 5 ^{b}
Ethanol (% *v*/*v*)	0.009 ± 0.004 ^{a}	0.005 ± 0.001 ^{a}	0.030 ± 0.006 ^{b}	0.031 ± 0.006 ^{b}
Glycerol (g L^{-1})	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.3 ± 0.1 ^{b}	0.3 ± 0.0 ^{b}
Malic acid (g L^{-1})	2.2 ± 0.3 ^{a}	2.1 ± 0.1 ^{a}	2.1 ± 0.1 ^{a}	2.0 ± 0.1 ^{a}
Lactic acid (g L^{-1})	0.04 ± 0.00 ^{a}	0.04 ± 0.11 ^{a}	0.05 ± 0.00 ^{b}	0.06 ± 0.00 ^{b}
Acetaldehyde (mg L^{-1})	<18 ^{a}	<18 ^{a}	<18 ^{a}	<18 ^{a}
Acetic acid (g L^{-1})	0.01 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.01 ± 0.00 ^{b}	0.01 ± 0.00 ^{b}
Ethyl acetate (mg L^{-1})	n/d †	n/d †	n/d †	n/d †

† n/d indicates the measurement is not detectable.

Table 3. Chemical composition of Cabernet franc control wine (23°Brix) and wine made from partially dehydrated grapes (28°Brix). Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Lowercase letters within the same parameter indicate differences between treatments (Fisher's Protected $LSD_{0.05}$).

Parameter	23°Brix EC1118	23°Brix CN1	28°Brix EC1118	28°Brix CN1
Reducing sugar (g L^{-1})	<0.07 ^{a}	0.2 ± 0.0 ^{a}	<0.07 ^{a}	15.8 ± 6.7 ^{b}
Glucose (g L^{-1})	<0.07 ^{a}	<0.07 ^{a}	<0.07 ^{a}	1.1 ± 0.7 ^{b}
Fructose (g L^{-1})	<0.07 ^{a}	0.1 ± 0.0 ^{a}	<0.07 ^{a}	14.7 ± 6.0 ^{b}
pH	3.78 ± 0.09 ^{b}	3.54 ± 0.04 ^{a}	3.74 ± 0.00 ^{b}	3.59 ± 0.05 ^{a}
Titratable acidity (g L^{-1} tartaric acid)	6.4 ± 0.3 ^{a}	9.4 ± 0.3 ^{c}	6.8 ± 0.2 ^{a}	8.1 ± 0.3 ^{b}
Ammonia nitrogen (mg N L^{-1})	<6 ^{a}	<6 ^{a}	<6 ^{a}	<6 ^{a}
Primary amino nitrogen (mg N L^{-1})	28 ± 3 ^{a}	24 ± 3 ^{a}	40 ± 2 ^{b}	36 ± 4 ^{b}
Ethanol (% *v*/*v*)	13.0 ± 0.3 ^{a}	12.6 ± 0.4 ^{a}	15.3 ± 0.7 ^{b}	14.7 ± 0.2 ^{b}

Table 3. *Cont.*

Parameter	23°Brix EC1118	23°Brix CN1	28°Brix EC1118	28°Brix CN1
Glycerol (g L^{-1})	8.5 ± 0.4 ^{a}	11.1 ± 0.6 ^{b}	11.2 ± 0.1 ^{b}	13.6 ± 0.2 ^{c}
Malic acid (g L^{-1})	1.6 ± 0.4 ^{a}	4.2 ± 0.2 ^{c}	1.9 ± 0.1 ^{a}	2.5 ± 0.1 ^{b}
Lactic acid (g L^{-1})	0.45 ± 0.42 ^{b}	0.04 ± 0.01 ^{a}	<0.03 ^{a}	<0.03 ^{a}
Acetaldehyde (mg L^{-1})	56 ± 7 ^{b}	38 ± 5 ^{a}	88 ± 7 ^{d}	70 ± 9 ^{c}
Acetic acid (g L^{-1})	0.30 ± 0.02 ^{c}	0.06 ± 0.01 ^{a}	0.36 ± 0.02 ^{d}	0.20 ± 0.02 ^{b}
Ethyl acetate (mg L^{-1})	36 ± 3 ^{b}	21 ± 3 ^{a}	37 ± 13 ^{b}	33 ± 2 ^{a}

3.3. Color and Sensory Evaluation

There were no significant differences between the wines in color density, which describes the intensity of wine color (Figure 2a). The hue, which is a measure of the shade of wine color, was lower in the 23°Brix CN1 wine (Figure 2b). The total red pigments in CN1 wines were lower than that in EC1118 wines for both winemaking treatments (Figure 2c). However, the degree of red pigment coloration was higher in CN1 wines than in EC1118 wines, suggesting a higher percentage of red-colored pigments in wines fermented by CN1 despite the lower concentrations of total red pigments (Figure 2c,d). Sensory evaluation also indicated perceptible differences between all of the wines with different yeast and starting sugar treatment (Table 4).

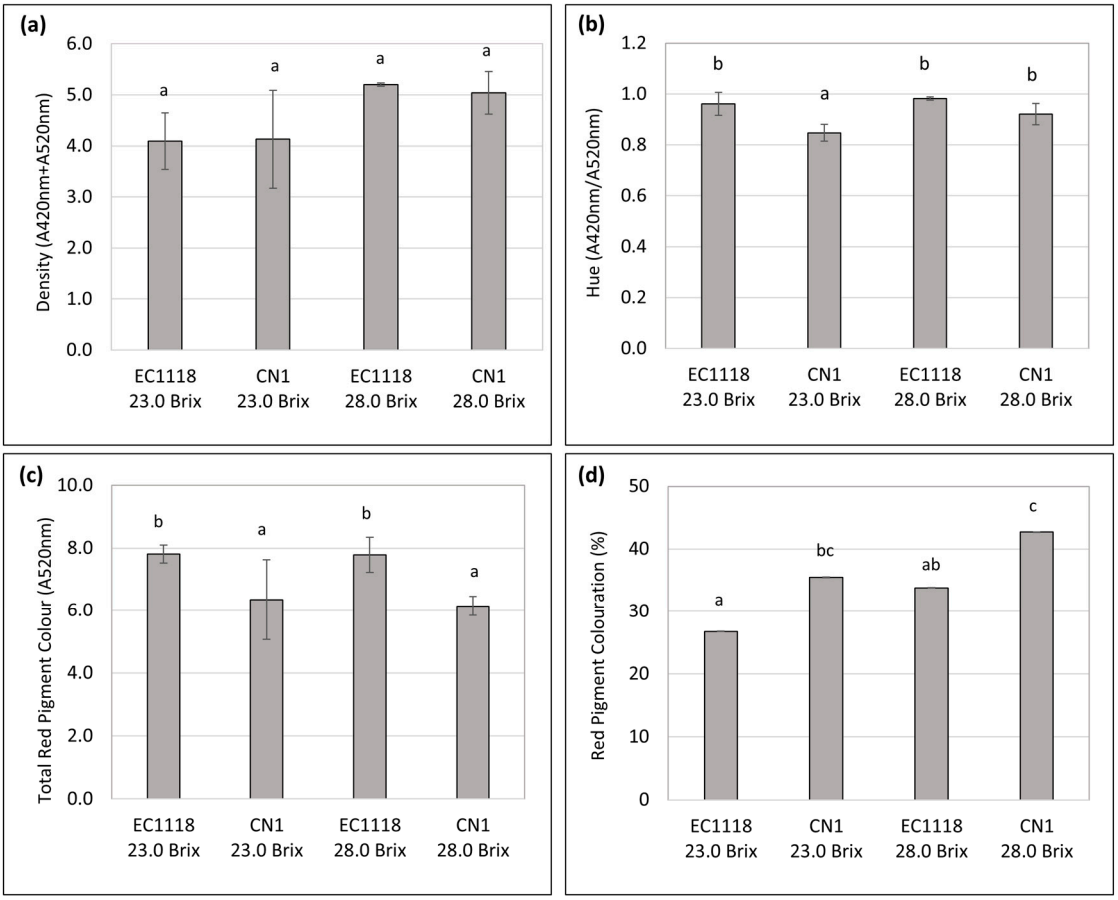

Figure 2. (**a**) Wine color density (**b**) wine hue, (**c**) total red pigment color (anthocyanins, oligomers, and polymers) and (**d**) degree of red pigment coloration (%) of control wines (23°Brix) and wines made from partially dehydrated grapes (28°Brix) vinified with either EC1118 or CN1. Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Lowercase letters indicate differences between treatments (Fisher's Protected $LSD_{0.05}$).

Table 4. Triangle test results to determine sensory differences between wines (n = 40). Significance was assessed by comparing the proportion of correct responses to critical values [33].

Paired Treatments	Correct	Incorrect	Total	Significance
23°Brix EC1118 vs. 23°Brix CN1	25	15	40	$p = 0.001$
28°Brix EC1118 vs. 28°Brix CN1	34	6	40	$p = 0.001$
23°Brix EC1118 vs. 28°Brix EC1118	25	15	40	$p = 0.001$
23°Brix EC1118 vs. 28°Brix CN1	32	8	40	$p = 0.001$
23°Brix CN1 vs. 28°Brix EC1118	26	14	40	$p = 0.001$
23°Brix CN1 vs. 28°Brix CN1	37	3	40	$p = 0.001$

4. Discussion

The main aim of this study is to investigate a low acetic acid-producing yeast, the newly identified yeast *S. bayanus* CN1, within the context of wine production from partially dehydrated grapes, which is a process that involves a high sugar fermentation and is often associated with undesirable oxidation compounds. The results presented in this study are based on chemical and preliminary sensorial analysis that demonstrate lower oxidation compounds produced by CN1 and perceptive differences from EC1118, which is the commonly used yeast for this winemaking style.

In an analysis of Amarone vinified with *S. cerevisiae* EC1118, the authors report concentrations of 0.56 ± 0.02 g L^{-1} acetic acid, 57.20 ± 2.12 mg L^{-1} ethyl acetate, 18.47% ethanol, and 6.41 ± 1.00 gL^{-1} residual sugar [39]. Their study reported a starting sugar concentration of 30°Brix, which is higher than the present study, contributing to the different but proportional results. An analysis of commercial Amarone wines over four vintages (1998–2001) reported similar acetic acid levels of 0.52–0.62 g L^{-1}, ethanol levels of 15.15–15.88%, and residual sugar levels of 0.29–0.8%, equating to 2.9–8 g L^{-1} [6]. The wines in this current study that were made from partially dehydrated grapes had a starting sugar concentration of 28.0°Brix, resulting in an ethanol range of 14.7–15.3%, which is proportional to the starting sugar concentration of the Amarone wines outlined in the literature. Similarly, the high starting sugar wines fermented in this study with EC1118 had an acetic acid concentration of 0.36 g/L^{-1}; this is lower than the Amarone values reported in the literature, which is likely due to the lower starting sugar concentration, while CN1 produced even lower levels of acetic acid at 0.20 g/L^{-1}. This result suggests the potential commercial application of the CN1 yeast to winemaking using partially dehydrated grapes to assist in mitigating the quality challenges associated with undesirable oxidation compounds in the final wine [10,13]. This wine style in Ontario in commercial production targets starting sugar concentrations of the dried fruit between 27–28°Brix. Although CN1 did not ferment the 28°Brix must to complete dryness, Amarone wines are also found with residual sugar [6,39]. Additionally, Alessandria et al. [40] found that autochthonous yeast yielded incomplete sugar transformation, but the authors suggest that this result should not be considered negative for this type of wine, as residual sugar is typical for some wines made from partially dehydrated grapes, offering an opportunity for stylistic considerations for the winemaker [39–42]. Further, studies are currently underway to evaluate the sugar range over which CN1 does ferment to dryness.

Despite the lower production of acetic acid by CN1 in the wines, this yeast produced higher concentrations of glycerol in comparison to EC1118. It has been well-established that glycerol is produced as an intracellular osmolyte in *S. cerevisiae* under hyperosmotic stress during wine fermentations accompanied by acetic acid production. The link between these two metabolites in *S. cerevisiae* under hyperosmotic stress is based on a redox balance of the NAD^+/NADH system. The formation of glycerol generates NAD^+ [15,17,43]. Acetic acid production from acetaldehyde reduces NAD^+ to NADH through the activity of a NAD^+-dependent aldehyde dehydrogenase, and corrects the redox shift [17,18,44]. We recently reported a 24-fold higher NAD^+/total NAD(H) ratio in *S. cerevisiae* on fermentation day 2 during fermentation of 39°Brix juice compared to 20°Brix juice, which was correlated with higher glycerol production followed by acetic acid production [17]. In this current study, higher acetic acid production under osmotic stress was also noted in both yeast

strains at the 28°Brix treatment compared to the 23°Brix treatment. However, *S. bayanus* CN1 produced more glycerol, but less acetic acid, in comparison to *S. cerevisiae* EC1118 at this higher brix condition. *S. bayanus* CN1 has a different response to osmotic stress than *S. cerevisiae*. Acetic acid may still be produced by *S. bayanus* as a response to glycerol production, but it may be further metabolized within the yeast as opposed to being released from the cell into the wine. Alternatively, a different metabolite may be used to reduce NAD^+ to NADH for redox balance, resulting in the lower acetic acid in the wine. Additional studies investigating the NAD(H) ratios in CN1 and yeast metabolites will provide insight on the mechanism and regulation of acetic acid production in high sugar fermentations in this yeast.

Wine color provides a quick reference of potential quality for consumers. The consumer can gather information about the wine's age, condition, body, and possible defects simply by looking at the wine as it leaves the bottle [45]. The basis for red wine color is anthocyanin content, and major secondary factors that are known to affect color density are pH and sulfur dioxide (SO_2) content. Interestingly, despite the low pigment content present in the wines vinified by *S. bayanus* CN1, at both sugar levels, they displayed a higher percentage of red-colored pigments than wines produced by EC1118. The CN1 control wine also showed a lower wine color hue compared to the other treatments. This could be caused by the lower pH in wines vinified by *S. bayanus*. It is accepted in the literature that the structure and color of anthocyanins are affected by pH, as acidification enhances the color intensity of red wine via the formation of the flavylium cation [46]. In addition to their direct role on color, anthocyanins can also contribute to the taste and chemical characteristics of wine because of their interactions with other molecules [47,48]. Therefore, they could have influenced the sensorially perceptible differences in the wines that were detected in this study. This is in agreement with the existing literature that found perceptible sensorial differences between the Amarone wines fermented with commercial *S. cerevisiae* yeast and those fermented with the inclusion of autochthonous yeast and non-*S. cerevisiae* yeast [39,49]. It is also important to note that there are differences in the wines in other categories; namely, orthonasal and/or retronasal sensory differences, as well as discrepancies in ethanol or residual sugar concentrations that could contribute to discriminating among the wines. The desirable higher percentage of red-colored pigments associated with CN1 and the established sensory differences amongst the treatments raise further questions about the organoleptic implications of using this yeast for wine production from partially dehydrated grapes. The differences are yet to be fully characterized; approaches such as quantitative sensory profiling and consumer preference testing would be useful in this regard.

5. Conclusions

This study lays the groundwork for further investigation of the potential of *S. bayanus* CN1 yeast for winemaking from partially dehydrated grapes in Ontario and other geographic regions that experience cool or marginal climates for grape growing. Although vinifying grapes for Vin de Curé poses risks for winemakers of increased oxidative compounds, the reward is in a high-value product that also adds diversity to the portfolio of a winery as well as its region. The findings on the isolate CN1 reported in this study are positive with respect to the legislated limits on oxidative compounds and desired red color hue, and have established sensory differences from the accepted commercial standard EC1118. Further to that, we recommend an additional sensory evaluation of wine made from *S. bayanus* CN1 in order to more fully understand its market potential. Additionally, understanding the difference between glycerol and acetic acid production of CN1 in comparison to *S. cerevisiae* EC1118 might contribute to the management of high acetic acid frequently associated with high sugar fermentations.

Author Contributions: D.L.I. and G.P. conceived and designed the experiments; L.D. and F.D.P. conducted the fermentations; L.D. performed the chemical analysis; J.K. conducted the color and sensory analysis; F.Y. prepared the yeast culture for fermentations; A.B., F.Y. and J.K. performed yeast identification; C.N. isolated the local *S. bayanus* strain. J.K., F.Y., G.P. and D.L.I. contributed to the writing of the manuscript.

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