Impact of Yeast Strain on the Production of Acetic Acid, Glycerol, and the Sensory Attributes of Icewine

Daniel J. Erasmus,[1] Margaret Cliff,[2] and Hennie J.J. van Vuuren[3]*

Abstract: High concentrations of volatile acids, consisting mainly of acetic acid, are often found in icewine. Wine yeasts produce acetic acid as a by-product of the hyperosmotic stress response caused by high sugar concentrations (>35 Brix) in grape must. Volatile acid levels in icewine often exceed legal limits. We compared seven commercially available wine yeast strains (ST, N96, Vin13, Vin7, EC1118, 71B, V1116) for icewine production. Yeast strains were evaluated for acetic acid and glycerol formation, fermentation rates, and sensory characteristics. Fermentations were conducted using both synthetic grape must and Riesling icewine grape must obtained from a commercial winery. Fermentations were conducted until approximately 11% (v/v) ethanol was produced. The yeast strains fermented Riesling icewine must at different rates and fermentations were completed in 17 to 49 days. Acetic acid and glycerol formation were significantly different ($p < 0.05$) and linked to the yeast strain. Sensory analysis of the icewines produced with the different yeast strains showed significant differences for overall quality, perceived sulfur-like aroma, and color ($p < 0.05$). ST, N96, and EC1118 were identified as the most suitable yeast strains for the production of icewine.

Key words: icewine, volatile acidity, wine yeast, osmotic stress

Icewine, or *eiswein*, was first produced in 1794 in Germany, where grapes were left on vines until they froze. Freezing of berries concentrates both the sugars and flavor compounds in the grapes, leading to wines with intense aroma and flavor notes and high residual sugar concentrations (Cliff et al. 2002). Icewines are today produced in Germany, Austria, and Canada, predominantly from Riesling grapes. In Canada, the Vintners Quality Alliance (VQA) regulates the production of icewine. Grapes for icewine production must be frozen naturally, on the vine, and can only be harvested at temperatures equal to or below -8°C. The natural freezing process results in grape must with exceptionally high sugar concentrations that can be as high as 50 Brix. During harvesting no single pressing may produce grape must lower than 32 Brix, with the final average in the fermentation tank not below 35 Brix (BC Wine Institute 2000).

The production of icewine is often problematic, with issues of protracted and stuck alcoholic fermentations and high volatile acidity (VA). International and Canadian laws regulate the maximum level of VA allowed in icewine, which, according to Canadian federal regulations, is 1.3 g/L. However, Canadian icewines often contain greater than 1.3 g/L VA (mainly acetic acid), resulting in financial losses to wineries. Microbiological agents associated with VA can be divided into three groups: spoilage bacteria (Drysdale and Fleet 1988), non-*Saccharomyces* yeasts (Fleet and Heard 1993), and wine yeasts (*Saccharomyces* sp.) used as starter cultures for wine production. Although the former two groups are more frequently the cause of high VA in table wines, wine yeast seems to be a major contributor of VA in high Brix musts (Erasmus et al. 2003, Shimazu and Watanabe 1981).

During growth in icewine grape musts, *S. cerevisiae* is exposed to extreme conditions of osmotic stress. The water activity (A_w) of 40% (w/v) sugar grape juice is ~0.939 compared to A_w = 0.981 for 22% (w/v) sugar grape juice (Erasmus et al. 2003). High-density DNA microarrays showed that the transcription of 589 genes in wine yeast was affected more than 2-fold in grape juice containing 40% sugar when compared to 22% sugar (Erasmus et al. 2003). This sugar-induced osmotic stress up-regulated the structural genes involved in the synthesis of acetic acid from acetaldehyde and of glycerol from dihydroxyacetone phosphate. The osmoregulatory response in *S. cerevisae* has been well-characterized (see Hohmann 2002 for a review). Yeast cells adapt to osmotic stress environments by producing glycerol as a compatible solute. Glycerol prevents the efflux of water from the cell into the environment, thereby preventing dehydration of the yeast. The key enzyme in the pathway for glycerol formation is a NADH-dependent glycerol-3-phosphate dehydrogenase that converts dihydroxyacetone phosphate to glycerol-3-phosphate with the concomitant oxidation of NADH to NAD^+. The shift in redox balance (NADH:NAD^+ ratio) caused by increased formation of glycerol is corrected by using acetic acid as a redox sink to convert NAD^+ back to NADH (Michnick et al. 1997, Remize et al. 1999). Wine yeasts produce acetic acid

[1]Ph.D. student, [3]Professor and Eagles Chair, Wine Research Center, Faculty of Agricultural Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4 Canada; [2]Research scientist, Pacific Agriculture and Agri-food Research Center, Summerland, BC V0H 1Z0 Canada.

*Corresponding author [Tel: 604-822-0418; fax: 604-822-5143; email: hjjvv@interchange.ubc.ca]

Acknowledgments: We thank the scientists from PARC who acted as tasters and for use of their sensory analysis facilities and the Wine Research group for a critical discussion of the manuscript. This research was supported by a grant to HJJVV from the BC Wine Institute, AAFC, and NSERC (AAFPJ 240132-00).

Manuscript submitted March 2004; revised August 2004

by the oxidation of acetaldehyde to acetate by $NAD(P)^+$-dependent (acet)aldehyde dehydrogenases (Remize et al. 2000). Several studies have linked the production of acetic acid to increased glycerol production (Eglinton et al. 2002, Remize et al. 1999, Valadi et al. 1998). The glycerol concentration in table wines ranges from 3 to 12 g/L, but in general ~7 g/L is present (Mattick and Rice 1970, Rankine and Bridson 1971).

Wine yeast strains vary greatly in their ability to form acetic acid (Delfini and Cervelli 1991). The overproduction of glycerol during wine fermentations for its positive sensory attributes leads to an increase in acetic acid concentration (Remize et al. 1999). However, the degree of acetic acid formation was yeast-strain dependent. Although Remize and coworkers used the same strategy for all the yeast strains, acetic acid and glycerol production varied greatly among the strains. It is therefore conceivable that yeast strains experiencing the same osmotic pressure will respond by producing different concentrations of glycerol and acetic acid. In the case of icewine, the choice of yeast strain might, therefore, determine if a wine will be accepted or rejected because of legal requirements. To our knowledge, yeast strains have not yet been evaluated for icewine production.

The aim of this study was to compare seven different wine yeast strains for (1) acetic acid and glycerol formation, (2) fermentation rates in synthetic and icewine grape must, and (3) impact on sensory characteristics of icewine.

Materials and Methods

Yeast strains and media. Seven commercially available yeast strains were used in this study: Vin13, N96, and Vin7 (Anchor Yeast, Industria, South Africa); EC1118, V1116, and 71B (Lallemand Inc., Montreal, Canada); and Zymaflore ST (J. Laffort & Cie, Bordeaux, France). Recommended rehydration methods are the following: for Vin13, N96, and Vin7: a 7 Brix must for 30 min at 37 to 40°C; for EC1118, V1116, and 71B: sterile tap water for 30 min at 40°C; and for Zymaflore ST: a sucrose solution with water (100 g/L) at 38 to 40°C for 30 min.

Synthetic must. The synthetic grape must used in this study contained equimolar amounts of glucose and fructose at final concentrations of 20, 40, 45, or 50% (w/v), with 4.5 g/L L-malic acid, 0.3 g/L citric acid, 4.5 g/L tartaric acid, 2 g/L ammonium sulfate, 1.7 g/L yeast nitrogen base (without ammonium sulfate or amino acids), 1 mL/L Tween 80, and 5 mg/L oleic acid (Husnik 2001). The pH of the synthetic must was adjusted to 3.2 with 0.5 N KOH and filter-sterilized (0.22 µm). Riesling icewine juice of 40 Brix was obtained from a commercial vineyard in Ontario (Table 1). Icewine juice was stored in 1-L batches at -30°C until use.

Determination of nitrogen content of Riesling icewine must. An enzymatic kit (Ammoniak/Ammonia) was used to determine the ammonium concentration in the must according to the manufacturer's instructions (Roche Molecular Biochemicals, Laval, Canada). Free α-amino nitrogen was measured with a spectrophotometer using *o*-phthaldialdehyde and *N*-acetyl-L-cysteine (Dukes and Butzke 1998).

Experimental winemaking. All musts were inoculated with active dry yeast (ADY) to a final concentration of 6 x 10^6 cells/mL and fermented at 20°C. Fermentation progress was followed by weight loss. Since icewine contains ~11% (v/v) ethanol, fermentations were stopped when concentrations reached ~11% ± 0.5% ethanol. Fifty milliliter samples for analyses of acetic acid, glycerol, and ethanol were centrifuged at 5000 rpm for 5 min at 4°C (Sorvall RC5C plus, rotor model SLA300; Newton, CT), filter-sterilized (0.22 µm), and stored at -30°C until analyzed.

Two types of media, synthetic grape must and Riesling icewine grape must, were used to study the impact of yeast strain on the production of acetic acid, glycerol, and fermentation rate. The synthetic grape must containing either 20% or 40% (w/v) sugar was divided into 200-mL batches and placed in 250-mL Kimax bottles fitted with fermentation locks. ADY were rehydrated in 20% sugar synthetic grape must diluted 1:2 with deionized water and incubated at 40°C for 30 min. Fermentations were stirred slowly for 1 min on a magnetic stirrer before samples were taken to monitor cell growth by spectrophotometry using absorbance (λ = 600 nm). Fermentations were conducted in triplicate.

Icewine fermentations were conducted using 500-mL batches of Riesling icewine grape must in 500-mL Kimax bottles fitted with fermentation locks. Six yeast strains (Vin13, N96, Vin7, EC1118, V1116, and 17B) were rehydrated in two ways: in sterile tap water and in icewine must diluted to 7 Brix. The Zymaflore ST strain was rehydrated in a 10% (w/v) sucrose solution or in the diluted 7 Brix icewine must. Rehydrated cultures were incubated for 30 min at 40°C before the icewine grape must was inoculated. Fermentations were performed twice in duplicate.

Icewine for sensory analysis was produced as described above, except that fermentations took place in 2-L flasks. Fermentations were performed in duplicate at 20°C and the rate was monitored by weight loss. Fermentations were stopped by transferring the flasks to 4°C, with the headspace of the fermentation vessel filled with N_2-gas. As soon as yeast cells settled to the bottom of the flask, 150 mg/L SO_2 was added. Prior to bottling, a further 50 mg/L SO_2 was added, followed by filtration through a 0.45-µm filter into

Table 1 Composition of Riesling icewine must used for evaluating yeast strains.

Soluble solids (Brix)	40
pH	3.2
Titratable acidity (g/L)	10.9
Ammonia (mg N/L)	145
Free amino nitrogen (mg N/L)	370
Yeast assimilable nitrogen (mg N/L)	515

375-mL screw-top bottles. Wines were bottle-aged for five months. To minimize bottle-to-bottle variation, two bottles (375 mL) were combined to form a composite sample for sensory evaluation for each of the yeast strains.

Quantification of acetic acid, glycerol, and ethanol. Acetic acid, glycerol, and ethanol were quantified by injecting 10 µL of diluted icewine (12-fold) and synthetic must samples (9-fold for 40% and 6-fold for 20% sugar synthetic must) into an Agilent (Palo Alto, CA) 1100 series HPLC with a photo diode array set at λ = 210 nm and a refractive index detector maintained at 35°C. The HPLC was fitted with Supelcogel C610H (model 59320-U; Supelco, Bellefonte, PA) analytical cation exchange column and Supelguard C610H (model 59319; Supelco) column and operated at 30°C. A degassed 0.22-µm filtered 10 mM H_3PO_4 mobile phase was applied to the column at a flow rate of 0.5 mL/min. Run time of samples was 35 min. Compounds were quantified using external standards dissolved in 10 mM H_3PO_4. Analyses were performed at least in duplicate.

Analysis of color, viscosity, and titratable acidity. The color of icewines was determined by CIELAB tristimulus values, L (lightness), a (red-green), and b (yellow-blue), using a scanning spectrophotometer (model DU640B; Beckman, Fullerton, CA). The viscosity of 16-mL icewines samples was determined with a Brookfield viscometer (model DV-II; Brookfield Engineering Labs, Stoughton, MA) equipped with a LV spindle. The viscometer was set at 60 rpm at 25°C. Calibration of the viscometer was confirmed with a 4.3 mPa.s viscosity standard**.** Titratable acidity was determined according to A.O.A.C. method number 926.12 (Caputi 1996) and expressed as g/L tartaric acid.

Sensory methodology. Icewines were evaluated in duplicate according to a complete randomized design. Wines were coded with 3-digit random numbers and presented in random order to the judges. Different codes were assigned for each of the color, aroma, flavor, and quality assessments. Each assessment was conducted on a separate scorecard. Wine samples (20 mL) for visual (color) assessment were placed in plastic petri dishes and evaluated under natural light against a white background. Aroma, flavor, and quality were assessed using 25-mL icewine samples at room temperature in 210-mL INAO-ISO glasses covered with plastic petri dishes. All assessments were conducted in individual tasting booths.

Twelve judges, 11 from the Pacific Agriculture and Agri-Food Research Centre and one from the University of British Columbia Wine Research Centre, participated in the study. All judges were experienced in wine-quality and sensory evaluations.

Five of the 12 judges participated in an initial benching session to identify possible sensory attributes and screen the wines. During this session, two icewines (71B, Vin7) were determined to be excessively oxidized and were dropped from further evaluation and some were identified to have a high perceived sulfur-like aroma. The judges determined it would be advantageous to dissipate the sulfur-like aroma by swirling the glass in order to better evaluate underlying fruity character.

All 12 judges participated in the training session, during which they revised and refined the sensory attributes until a consensus was obtained (Table 2). Judges were also familiarized with the tasting/rinsing protocol and unstructured line scales. The judges were required to follow a strict tasting/rinsing protocol: swirl and sniff the glass for the aroma assessments, sip and swirl the wine in the mouth for flavor/quality evaluations, and rinse with sparkling and still water between assessments.

Evaluations took place on two successive mornings. Each tasting consisted of two sessions (08:30 to 09:00 and 10:30 to 11:30). During the first session, judges evaluated color and aroma attributes (sulfur-like aroma 1, fruity character 1) on scorecards one and two. Once completed, lids were removed and the glasses swirled three times (09:45, 10:00, and 10:15) to dissipate sulfur-like aromas. During the second session, judges evaluated aroma (sulfur-like aroma 2, fruity character 2), flavor (fruitiness, acidity, mouthfeel, aftertaste), and overall quality on scorecards three, four, and five, respectively. Judges were requested to take a 5-min break between the acidity and mouthfeel attributes. All assessments were conducted in individual tasting booths. Judges scored each attribute on a 10-cm unstructured line scale, anchored at 1 cm and 9 cm with low (short, thin) and high (long, thick), respectively. Data were quantified by measuring the distance of the judge's mark from the origin.

Statistical analyses. A two-factor analysis of variance (ANOVA) was used to evaluate the effect of yeast strain and rehydration method on acetic acid and glycerol production. Differences among yeast strains were determined using Fisher's least significant difference (LSD) test ($p < 0.05$). Two-factor ANOVA with replication (Excel; Microsoft, Redmond, WA) was used to evaluate the effect of judge, wine, and judgeXwine for each of the sensory attributes. Differences among wines were determined using Fisher's LSD test ($p < 0.05$). A paired *t*-test was used to evaluate the changes in perceived sulfur-like and fruity aromas from the first session to the second session. Principal component

Table 2 Definitions of sensory attributes evaluated in icewines.

Attribute	Definition
Sulfur-like aroma	Intensity of any sulfur-like aroma (H_2S, mercaptan, rubbery, garlic, onion, diesel), ranging from low to high
Fruity	Intensity of fruitlike aroma and taste (peach, apricot, honey, citrus, dried fruit, jam), notes ranging from low to high
Acidity	Intensity of sour taste, ranging from low to high
Mouthfeel (body)	Degree of mouthfeel coating or body, ranging from thin (low body) to thick (viscous)
Aftertaste	Duration of flavor sensations that remain in the mouth after expectoration, ranging from short to long
Overall quality	Composite response of all sensations (visual, aroma, taste, aftertaste), ranging from low to high

analyses (PCA) (Minitab) were performed using the correlation matrix on the mean sensory scores for the statistically significant attributes. Preliminary evaluation of judgeXwine interactions showed that judges scored consistently except for the sulfur-like aroma for the second session. However, that did not influence the significance of the F value, when examined using the techniques described by Cliff and Dever (1996). A Pearson-correlation matrix analysis was performed between physicochemical and sensory attributes.

Results

Fermentation of yeast strains in synthetic grape must. Yeast strains N96 and Vin7 were the fastest fermenters at 20% sugar followed by EC1118 and Vin13, V1116, 71B, and ST. The order of completion to produce ~11% ethanol (v/v) from 40% sugar was N96, Vin13, EC1118 and V1116, Vin7 and ST, and 71B (Table 3). As expected, the yeast strains also grew to lower cell densities and fermented slower in 40% synthetic must in comparison to 20% synthetic must.

Of all the yeast strains, ST grew to the highest cell densities in fermentations conducted with 20% sugar synthetic must (Table 3). ST was followed by (from high to low cell densities): EC1118, V1116, N96, Vin13, 71B, and Vin7. 71B and Vin7 grew to substantially lower cell densities than ST, N96, V1116, and Vin13 in 20% sugar synthetic must (Table 2). Analysis of cell densities in fermentations conducted using 40% sugar synthetic must revealed that N96 and ST grew to higher cell densities than V1116, Vin13, EC1118, 71B, and Vin7. Vin7 reached much lower cell densities, in both 20% and 40% sugar synthetic must), compared to the other strains (Table 3).

Effect of sugar concentration on production of acetic acid and glycerol in fermented synthetic must. All seven yeast strains produced more acetic acid and glycerol in synthetic must containing 40% sugar compared to that with only 20% sugar (Figure 1A and B). Depending on yeast strain, acetic acid increased ~3- to 6-fold: ST (3.7-fold), Vin13 (4.5-fold), N96 (5.6-fold), V1116 (2.7-fold), EC1118 (4.2-fold), 71B (3.7-fold), and Vin7 (3-fold). N96 produced the lowest amount of acetic acid at 20% sugar followed by ST, Vin13, EC1118, 71B, V1116, and Vin7. ST produced the lowest amount at 40% sugar, followed by Vin13, N96, EC1118, V1116, 71B, and Vin7.

Glycerol production by ST at 40% sugar was the lowest followed by (from low to high) N96, EC1118, Vin13, V1116, 71B, and Vin7 (Figure 1B). At 20% sugar, glycerol formation by N96 was the lowest, followed by V1116, Vin13, ST, EC1118, Vin7, and 71B.

Regardless of yeast strain used, acetic acid and glycerol concentrations were directly related to sugar concentration; the higher the sugar concentration, the more acetic acid and glycerol were produced. This relationship was linear since the correlation coefficients for all three yeast strains were >0.950 for both acetic acid and glycerol (Figure 1C and D).

Yeast-strain specificity of fermentation rate and production of acetic acid and glycerol. Riesling icewine must fermentations were completed (~11.0% v/v ethanol produced) after 17 days by N96, followed by EC1118, V1116, Vin13, and ST. Strains Vin7 and 71B were not able to ferment the desired amount of sugars to produce ~11% ethanol, even after 49 days (Figure 2). Formation of acetic acid ($F = 75.43$, $p < 0.0001$) and glycerol ($F = 57.84$, $p < 0.0001$) by the different yeast strains showed significant variation (Figure 3), with ST producing the least and Vin7 the most acetic acid. Icewines fermented with Vin13, N96, EC1118, and V1116 did not differ significantly with respect to the concentration of acetic acid produced. ST produced significantly less glycerol and Vin7 produced significantly more glycerol than the other yeast strains. Vin13, EC1118, and N96 did not produce significantly different concentrations of glycerol. V1116 and 71B did not differ significantly in glycerol production.

Physicochemical and sensory analysis. Icewines produced by yeast strains N96 and EC1118 had the highest mean scores for overall quality, followed by ST, V1116, and Vin13 (Table 4). Icewine produced by Vin13 was significantly lower in quality than icewines produced by N96 and EC1118 ($F = 3.28$, $p < 0.05$), while icewines produced by Vin13, ST, and V1116 did not differ significantly. Icewines produced with N96, EC1118, ST, and V1116 were the highest in overall quality and not significantly different from one another. Overall quality assessment had a significant correlation with spectrophotometric color measurements: lightness ($r = 0.873$, $p < 0.1$), red-green ($r = -0.817$, $p < 0.1$), and yellow-blue ($r = -0.903$, $p < 0.05$). Color assessments by the judges revealed that icewine from Vin13 was significantly ($F = 31.8$, $p < 0.0001$) more intense in yellow color than the other icewines (Table 5). EC1118 produced icewine that was significantly darker in color than icewines produced with V1116 and ST, but not N96. Icewines from ST and V1116 were significantly lighter in color than those pro-

Table 3 Fermentation time, maximum cell densities and ethanol produced by seven wine yeast strains in 20% and 40% sugar synthetic must. Values are the means of three fermentations ± standard deviation.

Strain	Fermentation time (hr)		Maximum cell density (OD_{600nm})		Ethanol % (v/v)	
	20%	40%	20%	40%	20%	40%
ST	336	432	7.5 ± 0.1	3.8 ± 0.2	10.9 ± 0.04	11.0 ± 0.06
N96	192	288	6.5 ± 0.1	3.9 ± 0.04	10.5 ± 0.15	10.9 ± 0.22
Vin13	216	404	6.4 ± 0.4	3.2 ± 0.1	10.8 ± 0.12	11.5 ± 0.12
EC1118	216	404	6.7 ± 0.1	3.4 ± 0.1	10.7 ± 0.22	10.5 ± 0.31
V1116	264	404	6.6 ± 0.2	3.7 ± 0.3	10.9 ± 0.07	11.5 ± 0.42
71B	336	504	5.4 ± 0.2	3.0 ± 0.1	11.0 ± 0.22	10.8 ± 0.12
Vin7	192	432	5.2 ± 0.1	2.7 ± 0.1	10.6 ± 0.32	10.5 ± 0.16

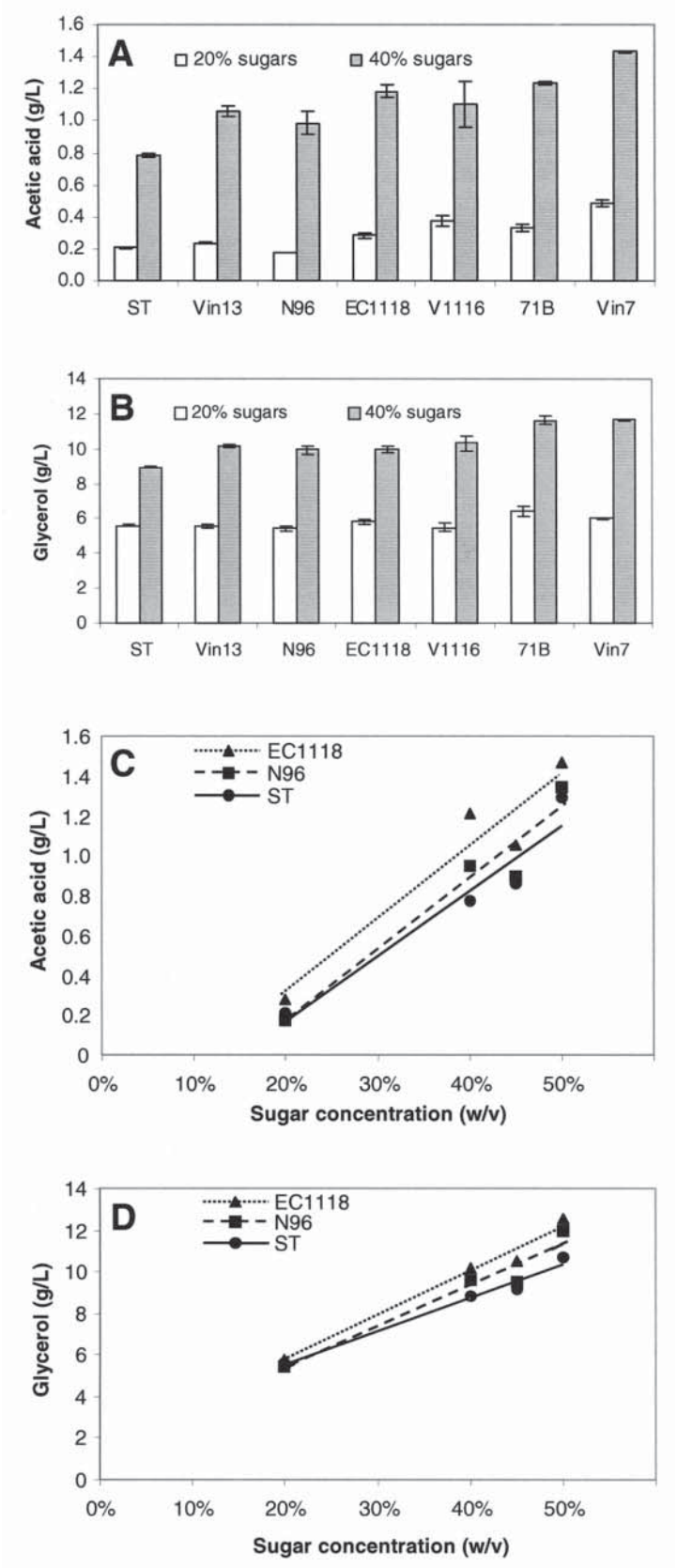

Figure 1 Formation of acetic acid (**A**) and glycerol (**B**) by seven different wine yeast strains in synthetic must containing either 20 or 40% (w/v) sugar. Results are mean values ± standard deviation from three fermentations. A linear relationship was found for (**C**) acetic acid (ST r = 0.966, N96 r = 0.970, EC1118 r = 0.959) and (**D**) glycerol (ST r = 0.990, N96 r = 0.972, EC1118 r = 0.989) with increasing sugar concentration.

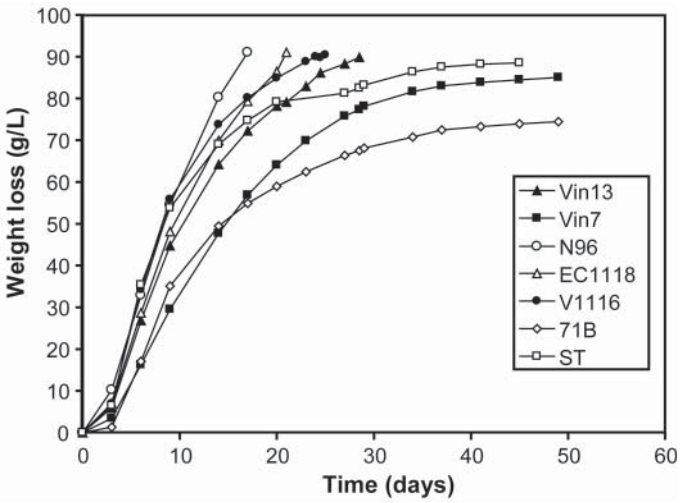

Figure 2 Fermentation rates of seven yeast strains in Riesling icewine must. Results are mean values from six different fermentations. Difference between replicates was <10%.

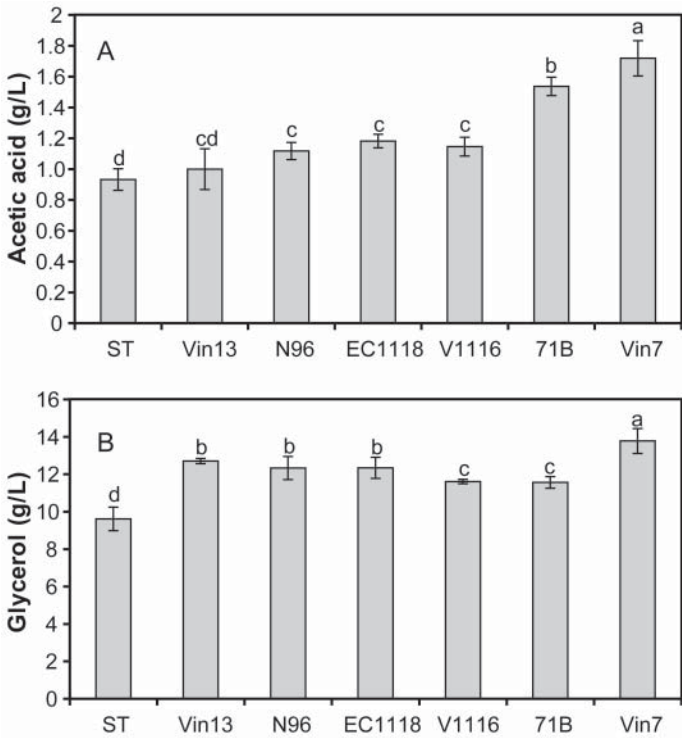

Figure 3 Formation of (**A**) acetic acid (LSD = 0.097) and (**B**) glycerol (LSD = 0.575) by seven different yeast strains in icewine. Results are mean values ± standard deviation from six fermentations.

duced by Vin13 and EC1118, but not N96. The physicochemical and sensory analysis of color showed high correlations, indicating that the evaluations were tracking the same underlying phenomenon. Lightness had an inverse correlation (r = -0.960, $p < 0.05$), whereas red-green (r = 0.957, $p < 0.05$) and yellow-blue (r = 0.838, $p < 0.1$) were positively correlated with color assessments by the judges. Furthermore, a statistically significant correlation was

Table 4 Analysis of sensory attributes of icewines used in sensory analysis. Values are the means of 12 determinations.

	ST	Vin13	N96	EC1118	V1116
Overall quality	49.8ab[a]	38.5b	52.2a	52.0a	46.3ab
Color	27.1c	57.8a	35.3bc	41.7b	31.6c
Sulfur-like aroma 1	50.8a	39.5ab	45.5abA[b]	33.5b	50.6aA
Sulfur-like aroma 2	47.3a	41.3ab	33.3bB	34.2b	34.8bB
Fruity aroma 1	40.7a	43.7a	43.9a	42.9a	42.5a
Fruity aroma 2	40.5a	42.4a	46.9a	48.1a	40.8a
Fruity flavor	40.9a	41.0a	37.9a	39.5a	39.6a
Acidity	59.4a	55.2a	62.2a	58.1a	56.1a
Mouthfeel/body	56.1a	49.2a	49.6a	48.8a	46.8a
Aftertaste	55.8a	47.9a	55.1a	49.6a	55.4a

[a]Lowercase letters indicate statistical difference between yeast strains for a sensory attribute using Fisher's LSD ($p < 0.05$).

[b]Uppercase letters indicate statistical difference between session 1 and session 2 for a particular aroma attribute.

Table 5 Analysis of physicochemical properties of icewines used in sensory analysis. Values are the means of three determinations ± standard deviation.

	ST	Vin13	N96	EC1118	V1116
TA (g/L)	10.0	10.1	10.2	10.2	9.83
pH	3.641	3.609	3.639	3.632	3.593
Viscosity (mPa.s)	3.28 ± 0.01	3.43 ± 0.01	3.43 ± 0.04	3.21 ± 0.00	3.40 ± 0.01
Color assessment					
L (lightness)	96.16 ± 0.005	94.99 ± 0.006	95.98 ± 0.021	95.87 ± 0.005	95.94 ± 0.020
a (red-green)	-2.10 ± 0.010	-1.63 ± 0.006	-2.12 ± 0.200	-1.93 ± 0.017	-2.06 ± 0.004
b (yellow-blue)	18.86 ± 0.021	21.17 ± 0.064	18.10 ± 0.202	19.35 ± 0.045	19.27 ± 0.103

found between red-green and yellow-blue ($r = 0.955$, $p < 0.05$).

Aroma differences among the icewines produced by the yeast strains were limited to sulfur-like aroma. Icewine produced with EC1118 had significantly ($F = 5.151$, $p < 0.01$) lower mean scores than ST and V1116 for sulfur-like aroma during the first session (sulfur-like aroma 1, Table 4). There were no significant differences between the icewines with respect to fruity aroma, acidity, mouthfeel, aftertaste, and fruity flavor. The swirling of the glasses decreased sulfur-like aroma significantly only for icewines produced with N96 ($t = 2.64$, $p < 0.05$) and V1116 ($t = 2.84$, $p < 0.001$). Icewines fermented with EC1118, V1116, and N96 were judged to have significantly ($F = 5.058$, $p < 0.01$) lower perceived sulfur-like aroma notes than wine made with ST during the second session (sulfur-like aroma 2, Table 4). Sulfur-like aroma from the second session had an inverse correlation with acetic acid concentration in the icewine ($r = -0.925$, $p < 0.05$). Acetic acid had a significant correlation ($r = 0.868$, $p < 0.1$) with ethanol.

Discussion

The choice of yeast strain can contribute to the consistent production of high-quality wines with unique styles and characteristics. Therefore, the identification of wine yeast strain(s) that produce low concentrations of acetic acid, conduct fermentations efficiently, and produce icewine with ideal sensory characteristics is crucial for the production of high-quality icewines.

Fermentation rate and growth is yeast-strain specific. The seven yeast strains differed with respect to the time required to produce at least 11% ethanol from 20% and 40% sugar. Vin7, ST, and 71B were the slowest fermenters at 40% sugar. The other yeast strains all finished in approximately the same order at both 20% and 40% sugar. Moreover, 71B and Vin7 produced the highest concentrations of acetic acid. Slow fermentation rates and high production of acetic acid indicate that these two strains are unsuitable for icewine fermentations.

N96 grew to high cell densities and fermented the fastest at both 20% and 40% sugar in synthetic must. Vin7 and 71B both grew to lower cell densities and fermented slower. ST, however, grew to high cell densities but fermented slower than the other yeasts in synthetic must at both sugar concentrations. Although ST is a slow fermenter, this yeast produced the least acetic acid. N96 was the fastest fermenter and produced relatively little acetic acid. Vin13, EC1118, and V1116 fermented at an acceptable rate and produced moderate concentrations of acetic acid.

Osmotically stressful environments and yeast-strain differences. Data obtained with 40% sugar synthetic must and 40 Brix icewine must showed similar trends. N96 was the fastest fermenter, and ST, although a slow fermenter, produced the least acetic acid. Vin7 and 71B were both the slowest fermenters and produced the highest concentrations of acetic acid in both the synthetic must and Riesling icewine must. Vin13, EC1118, and V1116 followed a similar pattern in the icewine fermentations as in the 40% sugar synthetic must of being moderate fermenters and acetate producers. However, unlike in the synthetic must, 71B was not able to produce 11% ethanol.

Vin7 and 71B both produced the highest concentrations of acetic acid and glycerol. This might indicate that these two strains require more glycerol to adapt to high osmolarity or that they have a decreased ability to retain glycerol as a compatible solute in the cytoplasm, hence the higher

concentrations of glycerol in the media. In contrast, ST produced the least glycerol but was a slow fermenter. That may be due to the insufficient formation of glycerol to act as compatible solute, which in turn may be the cause of the observed low metabolic activity (slow fermentation) and acetic acid formation by ST cells. N96, EC1118, Vin13, and V1116 produced more glycerol than ST but less than 71B and Vin7. N96, EC1118, Vin13, and V1116 fermented much faster than ST, 71B, and Vin7. Therefore it seems that N96, EC1118, V1116, and Vin13 are more successful at adapting to high-stress environments than ST, 71B, and Vin7. The two *Saccharomyces bayanus* strains (N96 and EC1118) were the fastest fermenters in icewine grape must.

The use of synthetic grape must to evaluate yeast strains for icewine production provided reliable analytical data, but does not allow for the evaluation of these strains for their sensory characteristics during icewine production. Furthermore, icewine grape must used in commercial wineries is not filter sterilized and contains other organisms that may influence acetic acid levels. Analysis of icewine produced with nonsterile icewine grape must revealed significant differences ($p < 0.05$) for acetic acid levels when using different wine yeast strains (Figure 3A). This indicates that even if nonsterile icewine must is used, wine yeast is still the major factor that contributes to high volatile acidity in high Brix musts.

Sensory analysis of icewines produced with different yeast strains. Principal component analysis (PCA) of sensory attributes that were significantly different ($p < 0.05$) revealed 99.3% of the total variation (Figure 4). PC I explained 55.1% of the variation in the data set; it was weighted in the positive and negative directions with overall quality and color, respectively. PC II explains an additional 34.9%, which was primarily due to the presence or absence of sulfur-like aroma, and PC III 9.3% (data not shown). Icewines produced with N96 and EC1118 were located in the lower right quadrant, which is associated with high-quality icewine. Icewines produced with ST and V1116 were characterized by higher perceived sulfur-like aroma. Vin13 located to the left of the PCA plot produced icewine with a darker yellow color. The statistically significant positive correlation between overall quality and spectrophotometric analysis indicates that high-quality icewine was associated with a light yellow color. N96, ST, and V1116 produced icewine with a lighter yellow color than Vin13 and EC1118. The high correlation between a (red-green) and b (yellow-blue) indicates that as yellow increases, red color also increases. This is consistent with a study that found both yellow and brown color increase simultaneously for British Columbia, Ontario, and German icewines (Cliff et al. 2002). The redness factor can be perceived as a brownish color in "white" wine. Brown color is usually associated with oxidized wines. It is not clear how the choice of yeast strain contributed to this aspect, since great care was taken by the authors to treat all wines consistently. Longer fermentations may lead to oxidized/brownish wines. However, yeast strains such as ST that required longer fermentations produced lighter colored icewine than Vin13 and EC1118.

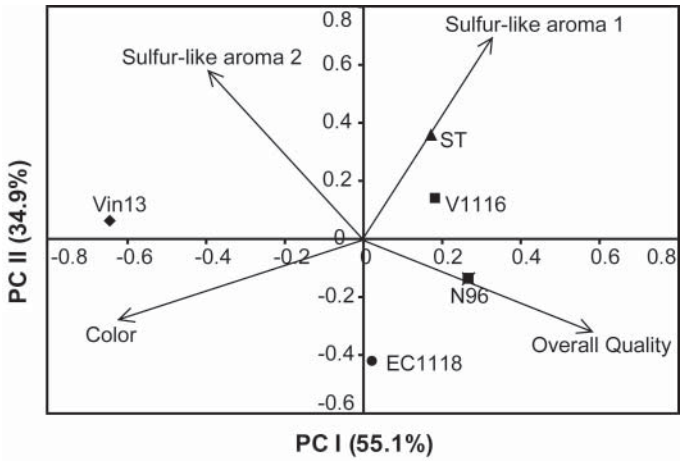

Figure 4 Principal component analysis of statistically significant ($p < 0.05$) sensory attributes of icewines produced with five different wine yeast strains.

The near 180° angle for overall quality and sulfur-like aroma of the second session indicate an inverse correlation (Figure 4), suggesting that sulfur-like aroma detracts from overall quality. The near 90° angle between overall quality and sulfur-like aroma of the first session indicates that these two vectors had no correlation. High levels of hydrogen sulfide often cause sulfur-like aroma in wine. The production of this compound is associated with sluggish or stuck fermentations, primarily because of a lack of nitrogen in the must. Levels of 140 mg N/L seem sufficient to prevent stuck or sluggish fermentations (Agenbach 1977). Bely et al. (2003) suggest that 190 mg N/L is an optimal concentration to limit acetic acid formation in high Brix musts. The addition of nitrogen to fermentations has also been shown to reduce hydrogen sulfide formation (Vos et al. 1979). The Riesling icewine must in this study had sufficient nitrogen (515 mg N/L) to prevent stuck or sluggish fermentations. ST is a slow fermenter and produced icewine with high perceived sulfur-like aroma, indicating that it might have a greater demand for nitrogen than N96, EC1118, and Vin13. Interestingly, a negative correlation was found for sulfur-like aroma from the second session and acetic acid. These two flaws do not seem to occur simultaneously in the icewine. The positive correlation between acetic acid and ethanol formation indicates that as more sugar is consumed, the yeast could potentially form more acetic acid. Winemakers can possibly prevent acetic acid formation by stopping icewine fermentations as soon as sufficient ethanol is produced. Sulfur dioxide additions (50 and 100 mg/L) had no significant effect on acetic acid formation by N96, EC1118, and ST (data not shown).

Glycerol is responsible for viscosity and mouthfeel in dry table wine. Glycerol only increases perceived viscosity at high levels (>25 g/L) in table wines (Noble and Bursick 1984). Since the level of glycerol in icewine during this

study was well below 25 g/L (a maximum of 13.8 g/L produced by Vin7), it is doubtful if it had an effect on viscosity or mouthfeel. Since icewines have residual sugar concentrations in excess of 150 g/L (Cliff et al. 2002), it is doubtful if glycerol can influence this property of icewine, even if the concentrations exceeded 25 g/L. The residual sugar content most likely has a greater effect on viscosity than glycerol. Because of the high residual sugar concentration of icewine, it is also doubtful that glycerol would contribute to sweetness.

Conclusions

This study illustrates the effect of wine yeast strain on the production of high volatile acidity in icewine and emphasized the importance of the choice of yeast strain. Two of the seven yeast strains produced acetic acid at levels above the legal limit of 1.3 g/L under the conditions used. However, it would be prudent to select strains that produce as little as possible acetic acid in icewine, since fermentation conditions/practices and grape must composition vary among wineries and among vintages. ST, N96, and EC1118 were identified as the three strains most suitable for icewine production. ST produced the least acetic acid, but was a slow fermenter and had high perceived sulfur-like aroma. N96 and EC1118 produced higher quality icewines, had lower levels of perceived sulfur-like aroma, and had faster fermentation rates. N96 and EC1118 produced significantly more acetic acid than ST, but were still below the legal limit of 1.3 g/L. Future studies on ST, N96, and EC1118 to refine fermentation conditions would be valuable to produce high-quality icewines.

Literature Cited

Agenbach, W.A. 1977. A study of must nitrogen content in relation to incomplete fermentations, yeast production and fermentation activity. *In* Proceedings of the South African Society for Enology and Viticulture, pp. 66-87. Cape Town.

Bely, M., A. Rinaldi, and D. Dubourdieu. 2003. Influence of assimilable nitrogen on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. J. Biosci. Bioeng. 96:507-512.

BC Wine Institute. 2000. Wine Standards and Regulations, p. 29. British Columbia Wine Institute, Kelowna.

Caputi, A. 1996. Wines. *In* Official Methods of Analysis of AOAC International. Vol. 2. 16th ed. P. Cunniff (Ed.), p. 8. AOAC International, Gaithersburg, MD.

Cliff, M.A., and M.A. Dever. 1996. Sensory and compositional profiles of British Columbia Chardonnay and Pinot noir wines. Food Res. Int. 29:317-323.

Cliff, M.A., D. Yuksel, B. Girard, and M.A. King. 2002. Characterization of Canadian ice wines by sensory and compositional analyses. Am. J. Enol. Vitic. 53:46-53.

Delfini, C., and F. Cervetti. 1991. Metabolic and technological factors affecting acetic acid production by yeasts during alcoholic fermentation. Vitic. Enol. Sci. 46:142-150.

Drysdale, G.S., and G.H. Fleet. 1988. Acetic acid bacteria in winemaking: A review. Am. J. Enol. Vitic. 39:143-154.

Dukes, B.C., and C.E. Butzke. 1998. Rapid determination of primary amino acids in grape juice using an *o*-phthaldialdehyde/N-acetyl-L-cysteine spectrophotometric assay. Am. J. Enol. Vitic. 49:125-134.

Eglinton, J.M., A.J. Heinrich, A.P. Pollnitz, P. Langridge, P.A. Henschke, and M. de Barros Lopes. 2002. Decreasing acetic acid accumulation by a glycerol overproducing strain of *Saccharomyces cerevisiae* by deleting the *ALD6* aldehyde dehydrogenase gene. Yeast 19:295-301.

Erasmus, D.J., G.K. van der Merwe, and H.J.J. van Vuuren. 2003. Genome-wide expression analyses: Metabolic adaptation of *Saccharomyces cerevisiae* to high sugar stress. FEMS Yeast Res. 3:375-399.

Fleet, G.H., and G.M. Heard. 1993. Yeasts: Growth during fermentation. *In* Wine Microbiology and Biotechnology. G. H. Fleet (Ed.), pp. 27-54. Harwood Academic, Chur, Switzerland.

Hohmann, S. 2002. Osmotic stress signaling and osmoadaptation in yeasts. Microbiol. Mol. Biol. Rev. 66:300-372.

Husnik, J.I. 2001. Genetic construction of malolactic wine yeasts. M.S. thesis, University of Guelph, ON, Canada.

Mattick, L.R., and A.C. Rice. 1970. Quantitative determination of lactic acid and glycerol in wines by gas chromatography. Am. J. Enol. Vitic. 21:205-212.

Michnick, S., J.L. Roustan, F. Remize, P. Barre, and S. Dequin. 1997. Modulation of glycerol and ethanol yields during alcoholic fermentation in *Saccharomyces cerevisiae* strains overexpressed or disrupted for *GPD1* encoding glycerol 3-phosphate dehydrogenase. Yeast 13:783-793.

Noble, A.C., and G.F. Bursick. 1984. The contribution of glycerol to perceived viscosity and sweetness in white wine. Am. J. Enol. Vitic. 35:110-112.

Rankine, B.C., and D.A. Bridson. 1971. Glycerol in Australian wines and factors influencing its formation. Am. J. Enol. Vitic. 22:6-12.

Remize, F., E. Andrieu, and S. Dequin. 2000. Engineering of the pyruvate dehydrogenase bypass in *Saccharomyces cerevisiae*: Role of the cytosolic Mg(2+) and mitochondrial K(+) acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic fermentation. Appl. Environ. Microbiol. 66:3151-3159.

Remize, F., J.L. Roustan, J.M. Sablayrolles, P. Barre, and S. Dequin. 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. Appl. Environ. Microbiol. 65:143-149.

Shimazu, Y., and M. Watanabe. 1981. Effects of yeast strains and environmental conditions on the formation of organic acids in must fermentation. J. Ferm. Tech. 59:27-32.

Valadi, H., C. Larsson, and L. Gustafsson. 1998. Improved ethanol production by glycerol-3-phosphate dehydrogenase mutants of *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 50:434-439.

Vos, P.J.A., W. Zeeman, and H. Heymann. 1979. The effect on wine quality of di-ammonium phosphate additions to musts. *In* Proceedings of the South African Society for Enology and Viticulture, pp. 87-104. Cape Town.