

ORIGINAL ARTICLE

Concentration effect of Riesling Icewine juice on yeast performance and wine acidityG.M. Pigeau^{1,2}, E. Bozza³, K. Kaiser⁴ and D.L. Inglis^{1,2,5}

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Abstract**Aims:** The objective of this study was to determine the effect of increasing juice soluble solids above 40°Brix on wine yeast's ability to grow and ferment the juice, with particular focus on acetic acid production, titratable acidity (TA) changes and the maximum amount of sugar consumed by the yeast.**Methods and Results:** Riesling Icewine juices at 40, 42, 44 and 46°Brix were inoculated with K1-V1116 at 0.5 g l⁻¹ and fermented at 17°C until sugar consumption ceased. Increasing soluble solids showed strong negative linear correlations with yeast growth, sugar consumption and ethanol production ($r = -0.999$, -0.997 and -0.984 , $P < 0.001$, respectively). Acetic acid, glycerol and TA production normalized to sugar consumed showed strong positive correlations to the initial juice concentration ($r = 0.992$, 0.963 and 0.937 , $P < 0.001$, respectively) but no correlation was found for ethanol production. The acetic acid produced as a function of sugar consumed was positively correlated to the glycerol produced ($r = 0.970$, $P < 0.001$). The final TA of the wines ranged between 11.8 and 13.7 g l⁻¹ tartaric acid, increasing by 2.3–3 g l⁻¹ over the starting juice. The increase in TA was positively correlated to the increase in acetic acid produced after normalizing the data to the amount of sugar consumed ($r = 0.975$, $P < 0.001$). The acid equivalents resulting from the increase in acetic acid accounted for 80–100% of the TA increase when converted to units of tartaric acid. In the final Icewines, acetic acid represented 19–20% of wine TA.**Conclusions:** Increasing Icewine juice concentration from 40 to 46°Brix increases the proportion of yeast sugar metabolism towards glycerol and acetic acid production to cope with the increased osmotic stress by decreasing yeast growth, sugar consumption rate, the total amount of sugar consumed and the total amount of ethanol produced. The high proportional contribution of acetic acid to titratable acidity in Riesling Icewine may affect acidity perception.**Significance and Impact of the Study:** We have determined that 10% v/v ethanol would not be achievable with initial juice concentrations above 42° Brix and that Riesling Icewine juice above 52.5°Brix would be theoretically unfermentable. The high proportional contribution of acetic acid to TA may be an important factor in the organoleptic balance of these Icewines.

Introduction

Icewine is a sweet dessert wine of critical importance to the Canadian wine industry. It is the flagship wine that initiated international trade for Canadian wines and represents a large proportion of the industry's revenue (Pickering 2006).

To produce an authentic Icewine according to the Vintners Quality Alliance (1999), the entire harvesting and pressing process must be carried out below -8°C . The berries are harvested and pressed in this frozen state to release the very concentrated juice, which is required to be a minimum of 35°Brix prior to fermentation (Vintners Quality Alliance 1999). In practice, individual Icewine juices have been reported to range from 32 to 46°Brix (Inglis *et al.* 2006). Icewine juice is concentrated in all soluble solids, including glucose and fructose, as well as acids such as tartaric and malic and nitrogenous compounds.

Fermenting Icewine presents many challenges for wine yeast. The high levels of solutes in Icewine juice place wine yeast under increased osmotic stress, leading to altered metabolism, growth and fermentation difficulties, and sometimes organoleptic faults in the wine (Kontkanen *et al.* 2004; Pigeau and Inglis 2005). Fermentations may take months to complete and may have difficulty attaining desired ethanol levels (Kontkanen *et al.* 2004). Hyperosmotic stress placed on yeast invokes their high osmolarity glycerol (HOG) response. Studies of laboratory yeast growth under salt-induced osmotic stress have shown that glycerol is produced in a HOG dependent manner (while oxidizing NADH to NAD^+) to serve as an internal osmolyte to balance the osmotic pressure placed on the cell (Blomberg and Adler 1989; Nevoigt and Stahl 1997; Blomberg 2000). This has also been shown for wine yeast exposed to hyperosmotic stress from high concentrations of fermentable sugars, such as those found in Icewine juice (Erasmus *et al.* 2003; Pigeau and Inglis 2005).

A problem associated with overproduction of glycerol during fermentation is the diversion of sugar metabolism away from ethanol and towards acetic acid production. Commercial yeast strains that naturally produce higher levels of glycerol also show a decrease in the concentration of ethanol in the finished wines (Scanes *et al.* 1998; Malacrino *et al.* 2005). Overproduction of glycerol by engineered wine yeasts has been shown to decrease ethanol and increase acetic acid generated during conditions simulating table wine fermentation (Remize *et al.* 1999).

We have previously shown that glycerol concentration is elevated and biomass accumulation is decreased during Icewine fermentation (Pigeau and Inglis 2005). Under these conditions, the intracellular NAD^+ generated from glycerol production would need to be reduced to NADH

to maintain redox balance. As a result of the lack of a transhydrogenase in yeast to convert reducing equivalents between the NAD^+/NADH system and the $\text{NADP}^+/\text{NADPH}$ system, yeast must rely on metabolite formation to maintain the intracellular redox balance for the coenzyme systems (van Dijken and Scheffers 1986). Acetic acid production has been suggested as a mechanism to balance the NAD^+ produced from glycerol formation during the hyperosmotic stress response (Miralles and Serrano 1995; Navarro-Avino *et al.* 1999). Elevated acetic acid concentrations have been found in Icewine and correlated to an increased expression of an NAD^+ dependent aldehyde dehydrogenase (Pigeau and Inglis 2005). Increased levels of both titratable acidity (TA) and volatile acidity (VA) have been found in high-sugar fermentations of musts derived from dried grapes (Caridi *et al.* 1999). A high concentration of acetic acid in Icewine is a concern because of regulatory issues regarding its upper allowable limit in Icewine of 2.1 g l^{-1} (Vintners Quality Alliance, 1999) and the association of acetic acid with wine spoilage.

The purpose of this work was to determine the upper concentration limit of Riesling Icewine juice that can still be fermented into wine. The effect of increasing Icewine juice concentration above 40°Brix was investigated to determine the impact on wine yeast growth, fermentative ability, yeast metabolism and ethanol, glycerol, acetic acid and TA production.

Materials and methods

Yeast strain

The commercial yeast strain used for wine fermentations was *Saccharomyces cerevisiae* K1-V1116, kindly provided by Lallemand Inc. (Montreal, QC, Canada).

Icewine juice for fermentation trials

Riesling Icewine juice was kindly provided by Coyote's Run Estate Winery (St David's, ON, Canada) and Niagara Vintage Harvesters Ltd (Virgil, ON, Canada). One millilitre of Cinn-free pectinase (Scott Laboratories, Inc. Petaluma, CA, USA) was added to the juice and after 24 h at 7°C , the clarified juice was racked. The juice was then diluted with sterile water from 46°Brix to 44, 42 and 40°Brix, aliquoted into sterile, 1 l bottles and stored at -40°C until fermentation.

Chemical composition of Icewine juice

The compositional profile of the Icewine juice was determined on the thawed juice prior to fermentation. The juice was thawed at 40°C and thoroughly mixed prior to

analysis. Soluble solids of all Icewine juice samples were determined with an ABBE bench top refractometer (American Optical, Buffalo, NY, USA). Juice acidity was determined by measuring pH with a Corning pH meter (model 455, Corning Inc., Corning, NY, USA) and TA (recorded in units of g l^{-1} tartaric acid) by titration against 0.067 N NaOH to a pH 8.2 endpoint (Zoecklein *et al.* 1995). Yeast assimilable amino acid nitrogen levels were determined in duplicate from each sample following the NOPA assay (Dukes and Butzke 1998). Glycerol, acetic acid, ammonia and glucose + fructose were determined with enzymatic test kits from Megazyme International Ireland Inc. (Bray, Co. Wicklow, Ireland).

Yeast inoculation procedure

Wine yeast were rehydrated and inoculated into Icewine juice using a yeast acclimatization procedure previously described (Kontkanen *et al.* 2004). Five grams of K1-V1116 wine yeast were rehydrated with 50 ml of sterile, 40°C Milli-RO water for 15 min, swirling gently every 5 min. Fifty millilitre of twofold diluted Icewine juice was then added to the rehydrated yeast. This starter culture was held at 25°C for 1 h, swirling gently every 30 min. Fifty millilitre of Icewine juice was then added to the starter culture and was held at 25°C for 2 h, swirling gently every 30 min. About 7.5 ml of starter culture was then used to inoculate 500 ml of each of the four different juice concentrations, resulting in a yeast inoculum of 0.5 g dry weight per litre.

Fermentation monitoring

Fermentations were carried out at 17°C in duplicate and continued until the yeast stopped consuming sugar, signalled by no further change in sugar concentration for 3 days via Lane-Eynon titrations (Zoecklein *et al.* 1995). Sampling of the fermentations occurred after stirring for 5 min to ensure a homogeneous mixture. Yeast cell concentrations were measured in the samples using a microscope and a haemocytometer counting chamber as

outlined by Zoecklein *et al.* (1995). The remaining fermentation samples were centrifuged at 13 648 g (Sorvall MC 12, Sorvall Inc., Newton, CT, USA) to remove fermenting yeast cells and metabolite concentrations were then measured in the supernatant. Ethanol was determined in duplicate by gas chromatography using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Corporation, Palo Alto, CA, USA) with a 30 m × 0.32 mm, 5% phenyl methyl silicone column as previously described (Pigeau and Inglis 2005). Samples were diluted tenfold and contained 1% 1-propanol as an internal standard.

Statistical analyses

An analysis of variance (ANOVA) was used to evaluate the effect of initial Brix on the various fermentation parameters (XLSTAT version 7.1 Addinsoft, New York, NY, USA). Differences between parameters at various Brix levels were determined using Fisher's least significant difference (LSD) test ($P < 0.05$).

Results

Initial Icewine juice

The chemical compositions of the initial Riesling juices are indicated in Table 1. Diluting the initial 46°Brix juice provided four juices that differed not only in sugar concentration, but also in the concentration of all other juice components including acids and nitrogenous compounds. Because of the buffering capacity of the juice, the pH was unaffected over the dilution range tested. Diluting the most concentrated Icewine juice (46°Brix) with water to achieve the 40–44°Brix juices is comparable to achieving these Brix values during Icewine grape processing as the final concentration of the juice is a function of the pressing temperature and the amount of water frozen out of the juice (Wuerdig *et al.* 1975). As the ambient temperature becomes colder, more water remains frozen in the grapes resulting in juice that is more concentrated in all

Table 1 Riesling Icewine juice composition prior to fermentation (mean value ± SD)

	40°Brix	42°Brix	44°Brix	46°Brix
Titrateable acidity (g l^{-1})	9.42 ± 0.14 b	9.73 ± 0.04 b	10.23 ± 0.03 ab	10.75 ± 0.35 a
Acetic acid (g l^{-1})	0.11 ± 0.02 c	0.15 ± 0.01 b	0.16 ± 0.01 b	0.21 ± 0.01 a
Glycerol (g l^{-1})	4.85 ± 0.05 b	5.40 ± 0.04 ab	5.80 ± 0.02 a	5.90 ± 0.27 a
pH	3.15 a	3.15 a	3.14 a	3.15 a
Ammonia nitrogen (mg l^{-1})	120 ± 4 b	122 ± 3 b	135 ± 0 a	138 ± 5 a
Amino acid nitrogen (mg l^{-1})	484 ± 4 d	522 ± 1 c	529 ± 1 b	568 ± 3 a
Glucose + fructose (g l^{-1})*	447 ± 1 d	467 ± 1 c	487 ± 5 b	508 ± 2 a

Lowercase alphabets indicate statistical difference between treatments for a given parameter using Fisher's Protected LSD_{0.05}.

*Initial glucose + fructose values were determined by enzymatic test kits (Megazyme Int).

soluble solids. The fermentations were not limited by nitrogen availability, as even in the most diluted condition at 40°Brix, there was a total yeast assimilable nitrogen level of 604 mg N l⁻¹. We have previously reported that K1-V1116 inoculated into 40°Brix Icewine juice only consumed 112 mg N l⁻¹ during the fermentation (Pigeau and Inglis 2005).

Icewine fermentation kinetics

Figure 1(a) illustrates the performance of K1-V1116 during the fermentation treatments. Yeast in the 46°Brix fermentation were greatly delayed in sugar consumption, requiring 10 days to begin utilizing the available sugar and only did so between 288 and 432 h. Yeast fermenting the diluted juices began to consume sugar almost immediately and continued for 18 days, after which, no further sugar was consumed over the next 21 days. The rates of sugar consumption, during the time when yeast were

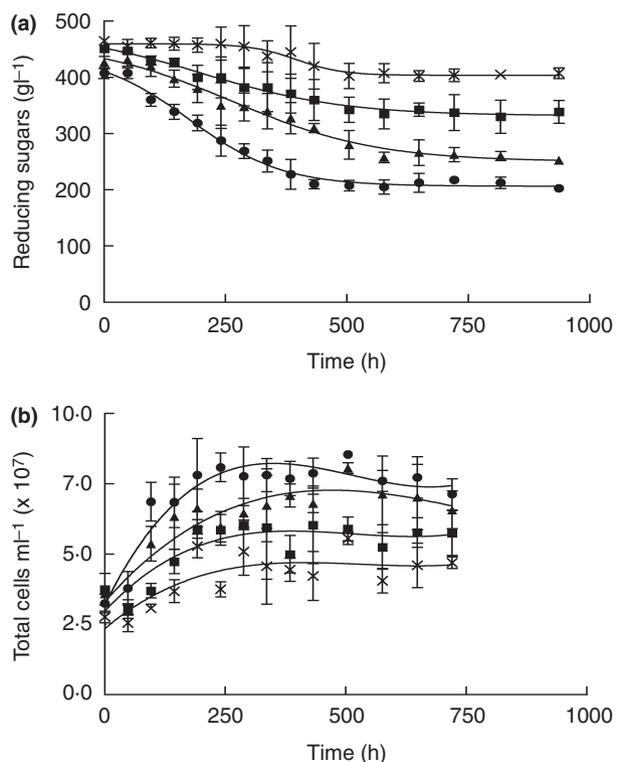


Figure 1 Icewine fermentation kinetics. Fermentations of Riesling Icewine juice at 40 (●), 42 (▲), 44 (■) and 46 (×), Brix were analysed every second day for (a) reducing sugar content by the Lane-Eynon method and (b) total cell production. Yeast cells consumed sugar at rates of 0.48, 0.30, 0.22 and 0.20 g l⁻¹ h⁻¹ and attained peak cell levels of 8.45, 7.40, 6.00 and 4.85 × 10⁷ cells per millilitre for the 40, 42, 44 and 46°Brix fermentations, respectively. Fermentations were performed in duplicate and samples from each trial were also tested in duplicate. The mean values (±SD) are shown.

actively taking up sugar, were negatively correlated with increasing juice concentration ($r = -0.999$, $P = 0.009$) and the level of soluble solids in the initial juice negatively correlated with the peak number of yeast cells in the fermentations ($r = -0.999$, $P = 0.0014$) (Fig. S1). Peak cell concentrations were lower and growth rates were slower as juice concentration increased (Fig. 1b).

Metabolite production

Residual sugar and yeast metabolites in the finished Icewines are shown in Table 2. Increasing the initial concentration of the Icewine juice had a negative impact on yeast sugar consumption, resulting in higher residual sugar and lower ethanol in the final wines as the initial juice concentration increased. In all the Icewines, the acetic acid, once converted to tartaric acid equivalents, represented between 19% and 20% of the final TA (Table 2). As yeast fermenting the four fermentation conditions consumed different amounts of sugar (Table 3) and yeast metabolites are by-products of sugar consumption, the metabolites produced were normalized to the grams of sugar consumed and then plotted as a function of initial juice Brix in order to compare the four conditions. Figure 2 shows that (i) acetic acid, (ii) glycerol and (iii) TA all increase as a function of the initial concentration of the Icewine juice. Interestingly, the amount of ethanol produced per gram of sugar consumed was not dependent on the initial juice concentration (Fig. 2d). After normalizing for the amount of sugar consumed, it was found that the increase in acetic acid had a strong linear correlation ($r = 0.970$) to the amount of glycerol produced during the fermentations (Fig. 3a) and that the increase in TA was strongly correlated ($r = 0.982$) to the increase in acetic acid (Fig. 3b).

Predictive extrapolation

The amount of sugar consumed and ethanol produced during the fermentations both exhibited strong negative correlations to initial Brix (Fig. 4). By extrapolating these relationships, a theoretical upper limit of the juice concentration for this juice that could no longer be fermented by yeast was determined to occur at 52.5°Brix.

Discussion

We have illustrated that the fermentation difficulties encountered during Icewine production are a function of the osmotic stress placed on the fermenting yeast by the concentrated solutes in the juice. It has been reported that yeast cells can overcome the severe sugar stress exerted by 35°Brix juice produced by partially dried grapes to

Table 2 Chemical composition of the finished riesling Icewines (mean value \pm SD)

	40°Brix	42°Brix	44°Brix	46°Brix
Residual glucose + fructose (g l ⁻¹)*	235.1 \pm 2.2 d	286.8 \pm 8.1 c	345.1 \pm 0.9 b	396.9 \pm 6.4 a
Titrateable acidity (g l ⁻¹)	11.8 \pm 0.1 b	12.4 \pm 0.5 b	13.2 \pm 0.4 a	13.1 \pm 0.3 a
Acetic acid (g l ⁻¹)	1.79 \pm 0.10 b	1.99 \pm 0.07 ab	2.09 \pm 0.06 a	2.11 \pm 0.08 a
% of TA represented by acetic acid†	19%	20%	20%	20%
Glycerol (g l ⁻¹)	15.95 \pm 0.76 ab	15.49 \pm 0.81 ab	16.50 \pm 0.77 a	14.97 \pm 0.13 b
Ethanol (g l ⁻¹)	94.0 \pm 0.9 a (11.9% v/v)	79.1 \pm 1.8 b (10.0% v/v)	60.4 \pm 1.6 c (7.7% v/v)	49.2 \pm 7.1 d (6.3% v/v)

Lowercase alphabets indicate statistical difference between treatments for a given parameter using Fisher's Protected LSD_{0.05}.

*Residual glucose + fructose values were determined by enzymatic test kits (Megazyme Int).

†Converted to units of tartaric acid for determination of % TA represented by acetic acid.

Table 3 Metabolites generated by yeast during fermentation of riesling Icewines (mean value \pm SD)

	40°Brix	42°Brix	44°Brix	46°Brix
Glucose + fructose consumed (g l ⁻¹)	211.5 \pm 1.6 a	180.0 \pm 4.3 b	142.0 \pm 2.8 c	110.9 \pm 4.2 d
TA produced (g l ⁻¹)	2.4 \pm 0.1 a	2.6 \pm 0.5 a	3.0 \pm 0.4 a	2.3 \pm 0.3 a
Acetic acid produced (g l ⁻¹)	1.67 \pm 0.96 b	1.88 \pm 0.96 ab	1.91 \pm 0.05 a	1.88 \pm 0.11 a
% of TA increase because of acetic acid produced*	87%	90%	80%	100%
Glycerol produced (g l ⁻¹)	11.09 \pm 0.76 a	10.10 \pm 0.80 ab	10.7 \pm 0.77 ab	9.03 \pm 0.08 b

Lowercase alphabets indicate statistical difference between treatments for a given parameter using Fisher's Protected LSD_{0.05}.

*Converted to units of tartaric acid for determination of % TA represented by acetic acid.

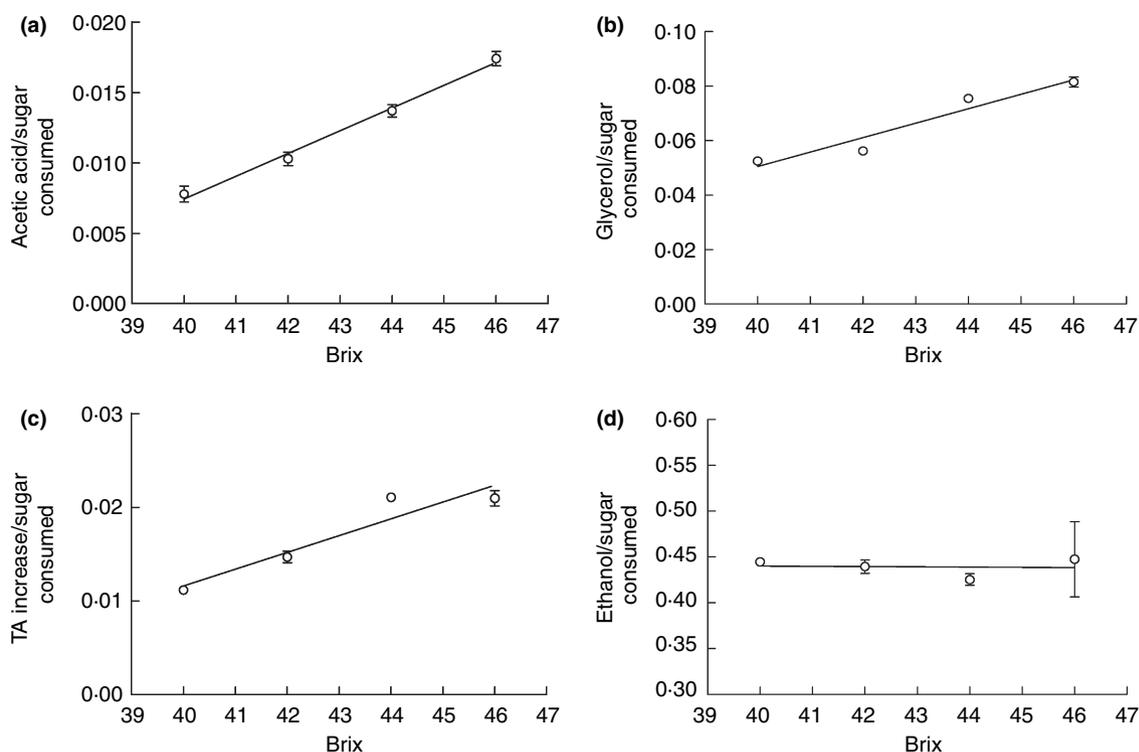


Figure 2 Metabolite production. Final metabolite levels (g l⁻¹) were measured in the Icewines and normalized to the amount of sugar consumed (g l⁻¹) in each. Fermentations were performed in duplicate and samples from each trial were also tested in duplicate. The mean values (\pm SD) are shown. Correlations between initial Brix and (a) acetic acid/consumed sugar ($r = 0.992$, $P < 0.001$), (b) glycerol/consumed sugar ($r = 0.963$, $P < 0.001$), (c) titrateable acidity increase/consumed sugar ($r = 0.937$, $P < 0.001$) were found. No correlation between (d) ethanol/consumed sugar ($r = 0.030$, $P = 0.9431$) was found.

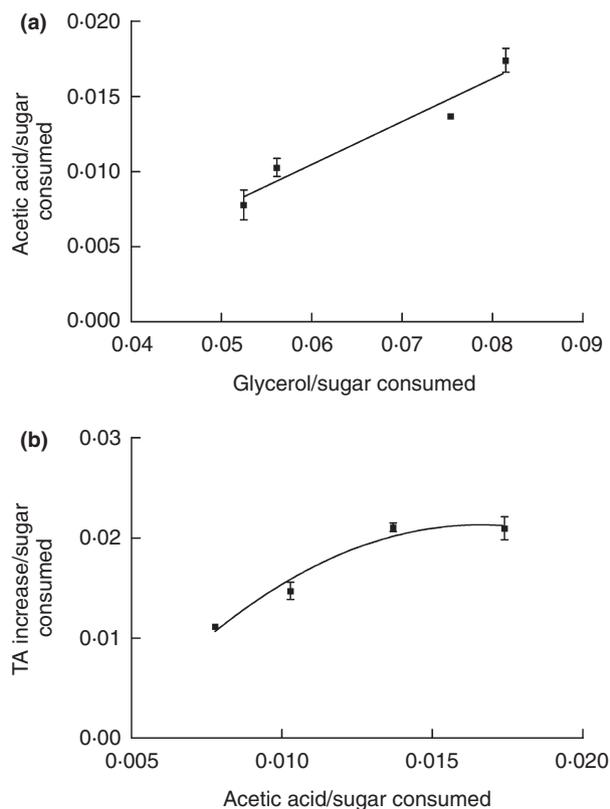


Figure 3 Relationship between metabolites. (a) The amount of acetic acid produced was found to positively correlate ($r = 0.970$, $P < 0.001$) with the amount of glycerol produced. (b) A positive correlation ($r = 0.975$, $P = 0.0012$) was found between the increase in titratable acidity and acetic acid produced. Metabolites reported were normalized to the amount of sugar consumed in each fermentation condition.

begin metabolic activity to produce high levels of alcohol (18–20%) (Malacrino *et al.* 2005). However, these studies have not addressed the upper limit of fermentable sugar concentration and have not examined musts over 40°Brix that are further concentrated in acids and other juice solutes, as in the case of Icewine juice. In agreement with (Caridi *et al.* 1999), we have found that yeast sugar consumption and ethanol concentration produced decrease with increasing juice concentration. It appears from our study that yeast are diverting metabolic resources away from growth and towards combating osmotic stress as yeast growth is negatively correlated and glycerol and acetic acid production are positively correlated with the Icewine juice concentration. The prolonged lag phase seen in the 46°Brix fermentation is noteworthy as the three other fermentations began to consume sugar within 48 h. This level of soluble solids may be approaching the upper limit of what *S. cerevisiae* K1-V1116 can actively ferment, which was theoretically determined to be 52.5°Brix under

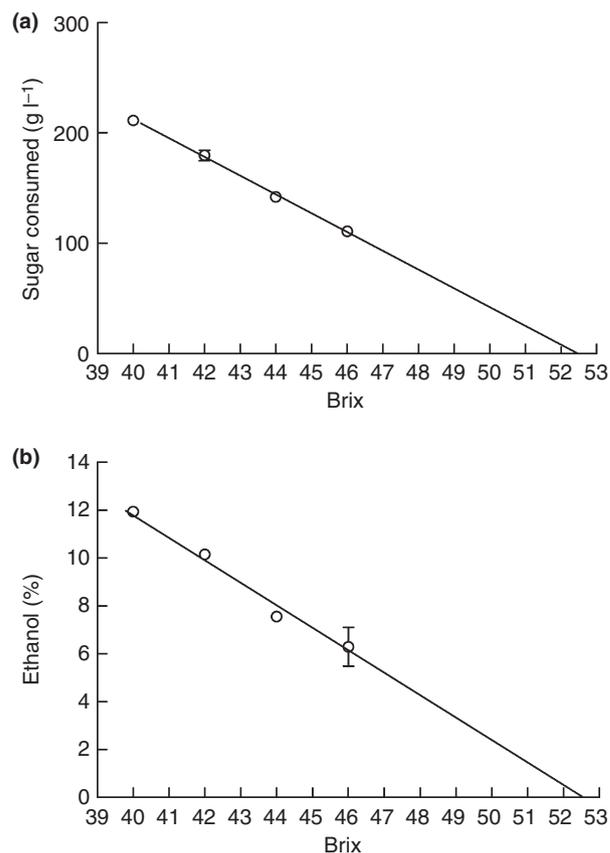


Figure 4 Extrapolative relationships. The (a) sugar consumed and (b) Ethanol produced by yeast during Icewine fermentations was plotted as a function of initial juice concentration. The strong negative correlations can be extrapolated to an Icewine juice concentration of approx. 52.5°Brix where no sugar would be consumed ($r = -0.997$, $P < 0.001$) and no ethanol would be produced ($r = -0.984$, $P < 0.001$).

our fermentation conditions. As the rate of sugar consumption and peak cell concentration are inversely proportional to the initial Brix of the juices, these observations reflect an adaptation phase of the yeast where the cells divert metabolic resources away from growth and towards coping with the increasing osmotic stress from the concentrated juices. Based on the fermentation conditions examined in this work, it appears that the osmotic stress exerted by Riesling Icewine juice above 42°Brix is prohibitive to producing Icewines with a minimum of 10% v/v ethanol. These results are of importance to the commercial production of Icewines as we have determined a theoretical upper limit of Icewine juice concentration above which may not allow the target ethanol of 10% v/v to be attained, the average ethanol found to be present in Canadian Riesling Icewines (Nurgel *et al.* 2004).

In order to survive in an osmotically stressful environment, yeast produce glycerol as a compatible solute

(Blomberg and Adler 1989; Nevoigt and Stahl 1997; Blomberg 2000). We see an increase in glycerol per amount of sugar consumed as initial Brix increases. We have previously shown increasing glycerol production in a wine yeast strain of *S. cerevisiae* fermenting Icewine requires upregulation of NADH-dependent glycerol 3-phosphate dehydrogenase (GPD1) producing intracellular NAD⁺ (Pigeau and Inglis 2005). Redox balance for this cofactor system may be achieved by the action of NAD⁺-dependent aldehyde dehydrogenases, producing acetic acid (Pigeau and Inglis 2005). In support of this relationship between glycerol and acetic acid production during Icewine fermentation, acetic acid production per gram of sugar consumed was shown to positively correlate with glycerol production per gram of sugar consumed over the Icewine juice concentration range of 40–46°Brix.

We have quantified the increase in TA that occurs during Icewine fermentation and shown that it correlates to the increased production of acetic acid. As acetic acid accounts for approx. 20% of the TA in the wines, clearly the acid profile in Riesling Icewines differs from that found in Riesling table wine (Frayne 1986) with a significant proportion in Icewine because of acetic acid. Icewine requires a high level of TA in the final wine to balance the high sweetness of the wines. A survey of 14 Riesling Icewines from Canada report an average TA of 11.8 g l⁻¹ tartaric acid and residual sugar of 219.2 g l⁻¹ comprised of glucose and fructose (Nurgel *et al.* 2004). The acetic acid produced during Icewine fermentation may assist in the sugar to acid balance in the wines by contributing to TA. However, as acetic acid is a much weaker acid with a higher pKa in comparison to tartaric and malic acid, the two main acids present in Riesling table wines, additional acidulation may still be required to reach the optimal acid to sugar balance in Icewines. In Canada, the Vintner's Quality Alliance (VQA) regulates Icewine production and has legislated an upper allowable limit of VA at 2.1 g l⁻¹ acetic acid although the odour detection threshold of acetic acid in Icewine has been reported as 3.185 g l⁻¹ (Cliff and Pickering 2006). The acetic acid present in Icewine is not an indicator of microbial spoilage, but rather is a natural by-product of wine yeast fermentation under hyperosmotic stress that may be of benefit in the overall sweetness to sourness balance of the wine as opposed to having a negative impact on overall wine quality. However, concerns regarding high acetic acid concentrations in Icewines remain merited as the potential contribution of metabolically produced acetic acid to elevating ethyl acetate in Icewine has not yet been investigated. As the odour detection threshold of ethyl acetate in Icewine has been reported as 0.198 g l⁻¹ (Cliff and Pickering 2006) and the average ethyl acetate concentration in Icewine has been reported as 0.24 ± 0.07 g l⁻¹ (Nurgel *et al.* 2004), Cliff

and Pickering (2006) suggest separate acceptance limits for ethyl acetate and acetic acid be established for Icewines.

Conclusions

Increasing Icewine juice concentration from 40 to 46°Brix decreases yeast growth, sugar consumption rate, the total amount of sugar consumed and the total amount of ethanol produced. Increasing Icewine juice concentration also raises the proportion of yeast sugar metabolism diverted away from growth and towards glycerol and acetic acid production to cope with the increased osmotic stress of the concentrated juice. Under the fermentation conditions examined, we have theoretically determined that Riesling Icewine juice above 52.5°Brix would not be fermentable and 10% v/v ethanol would not be achievable with initial juice concentrations above 42°Brix. The increase in acetic acid production during Icewine fermentation is mainly responsible for the increase in TA in the wines and may assist in the sugar to acid balance in the wine. The high proportional contribution of acetic acid to TA in Icewines may affect acidity perception as acetic acid is a weaker acid than tartaric and malic acids.

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References

- Blomberg, A. (2000) Metabolic surprises in *Saccharomyces cerevisiae* during adaptation to saline conditions: questions, some answers and a model. *FEMS Microbiol Lett* **182**, 1–8.
- Blomberg, A. and Adler, L. (1989) Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD⁺) in acquired osmotolerance of *Saccharomyces cerevisiae*. *J Bacteriol* **171**, 1087–1092.
- Caridi, A., Crucitti, P. and Ramondino, D. (1999) Winemaking of musts at high osmotic strength by thermotolerant yeasts. *Biotech Lett* **21**, 617–620.
- Cliff, M.A. and Pickering, G.J. (2006) Determination of odour detection thresholds for acetic acid and ethyl acetate in Icewine. *J Wine Res* **17**, 45–52.
- van Dijken, J. and Scheffers, W. (1986) Redox balances in the metabolism of sugars by yeasts. *FEMS Microbiol Rev* **32**, 199–224.

- Dukes, B.C. and Butzke, C.E. (1998) Rapid determination of primary amino acids in grape juice using *O*-pathaldehyde/*N*-acetyl-L-cysteine spectrophotometric assay. *Am J Enol Vitic* **49**, 125–134.
- Erasmus, D.J., van der Merwe, G.K. and van Vuuren, H.J.J. (2003) Genome-wide expression analyses: metabolic adaptation of *Saccharomyces cerevisiae* to high sugar stress. *FEMS Yeast Res* **3**, 375–399.
- Frayne, R.F. (1986) Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. *Am J Enol Vitic* **37**, 281–287.
- Inglis, D.L., Pigeau, G., Quai, J., Pistor, M. and Kaiser, K. (2006) Chemical composition of Vidal Icewine juice and nitrogen usage during fermentation. In *Sixth International Cool Climate Symposium for Viticulture and Oenology – Wine Growing for the Future*. ed. Creasy, G.L., Lincoln University, Christchurch, New Zealand, pp. 48. 79WT.
- Kontkanen, D., Inglis, D., Pickering, G. and Reynolds, A. (2004) Effect of yeast inoculation rate, acclimatization, and nutrient addition on Icewine fermentation. *Am J Enol Vitic* **55**, 363–370.
- Malacrino, P., Tosi, E., Caramia, G., Prisco, R. and Zapparoli, G. (2005) The vinification of partially dried grapes: a comparative fermentation study of *Saccharomyces cerevisiae* strains under high sugar stress. *Lett Appl Microbiol* **40**, 466–472.
- Miralles, V. and Serrano, R. (1995) A genomic locus in *Saccharomyces cerevisiae* with four genes up-regulated by osmotic stress. *Mol Microbiol* **17**, 653–662.
- Navarro-Avino, J.P., Prasad, R., Miralles, V.J., Benito, R.M. and Serrano, R. (1999) A proposal for nomenclature of aldehyde dehydrogenases in *Saccharomyces cerevisiae* and characterization of the stress-inducible *ALD2* and *ALD3* genes. *Yeast* **15**, 829–842.
- Nevoigt, E. and Stahl, U. (1997) Osmoregulation and glycerol metabolism in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **21**, 231–241.
- Nurgel, C., Pickering, G. and Inglis, D. (2004) Sensory and chemical characteristics of Canadian Icewines. *J Sci Food Agric* **84**, 1675–1684.
- Pickering, G. (2006) Icewine. The frozen truth. In *Sixth International Cool Climate Symposium for Viticulture and Oenology – Wine Growing for the Future*. ed. Creasy, G.L., Lincoln University, Christchurch, New Zealand, pp. 84–99.
- Pigeau, G. and Inglis, D. (2005) Upregulation of *ALD3* and *GPD1* in *Saccharomyces cerevisiae* during Icewine fermentation. *J Appl Microbiol* **99**, 112–125.
- Remize, F., Roustan, J.L., Sablayrolles, J.M., Barre, P. and Dequin, S. (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.
- Scanes, K.T., Hohmann, S. and Prior, B.A. (1998) Glycerol production by the yeast *Saccharomyces cerevisiae* and its relevance to wine: a review. *S Afr J Enol Vitic* **19**, 17–24.
- Wuerdig, G., Schlotter, H.A. and Th. Mueller (1975) Versuche zur Eisweinbereitung, Weinwirtsch. (*Neustadt/Wstr.*) **111**, 982–984.
- Zoecklein, B.W., Fugelsang, K.C. and Gump, B.H. (1995) *Wine Analysis and Production*. New York: Chapman and Hall.

Supplementary material

The following supplementary material is available for this article online:

Figure S1 The rate of sugar consumption and peak cells per millilitre is negatively associated with initial Brix. The rates of sugar consumption were determined through linear regression during the time frames when yeast were actively consuming sugar and were plotted as a function of initial Brix. Yeast sugar consumption rate was negatively correlated with initial Brix ($r = -0.999$, $P = 0.0009$). Peak cell levels were extrapolated from the curve maxima in Fig. 1b and were negatively correlated to initial Brix ($r = -0.999$, $P = 0.0014$).

This material is available as part of the online article from <http://www.blackwell-synergy.com>.