# Vintage 2008 – A heat wave and stuck fermentations

### Introduction

The number of queries related to stuck fermentations received each year by the AWRI has remained fairly constant for the past few years. However, this year, the number of queries received about this topic doubled. It is evident that the majority of the 'stuck ferment' queries were most likely related to a heat wave that affected much of southern Australia in the first half of March 2008.

Winemakers reported high Baumé (Bé) levels in harvested fruit, some approaching 30°Bé, that were the result of severe heat stress. Fermenting a must through to dryness with such a high sugar concentration would be challenging for any yeast due to the extremely high potential alcohol produced. Some winemakers reported having primary fermentations that had become stuck but which were undergoing malolactic fermentation (MLF) and contained high concentrations of volatile acidity (VA).

Stuck fermentation is a complex winemaking problem and considerable research has identified many causes. Therefore, it can be a very difficult task to identify the cause of a stuck fermentation in a particular wine (Bisson and Butzke 2000, Henschke 1997). However, discussions with many winemakers who experienced fermentation difficulties during the 2008 vintage emphasised a number of factors that might have increased the chances of fermentation problems this year and these are discussed in this article.

### High temperature and microbial growth

Daytime temperatures were not only very hot during the heat wave, but persisted for over two weeks. For example, Adelaide had 15 consecutive days above 35°C and 13 consecutive days above 37.8°C. The nights were also hot, and records for the hottest March nights were set in both Adelaide, where the temperature was 30.2° overnight on 13/14 March, and Melbourne, where the temperature was 26.9°C overnight on 17/18 March (Australian Government Bureau of Meteorology 2008). Furthermore, no rain was reported during this period and, in some areas, irrigation was limited due to drought conditions.

Winemakers reported that fruit sugar concentration continued to increase rapidly during the first week of the heat wave, however, during the second week the grapes dehydrated quickly and shrivelled. The presence of damaged berries and any berries split due to dehydration, or leakage of sugar from the berries due to loss of turgidity, can be expected to result in the berries carrying a higher than usual microbial load. Mechanical harvesting leads to further damage of the fruit. The fruit would be especially susceptible to damage given the lack of

turgidity of the dehydrated berries. This, in turn, stimulates further growth of indigenous microorganisms during transport to the winery, unless preventative action is taken.

Grape berry nutrients are necessary for the proper growth and metabolism of the inoculated fermentative yeast (Bell and Henschke 2005). The uncontrolled growth of microorganisms associated with the grapes and the harvesting equipment can lead to serious losses of grape berry nutrients. For example, non-*Saccharomyces* yeasts such as *Kl. apiculata*, *C. stellata*, and *C. pulcherrima* (typically present on grapes) are far more demanding of vitamins than *S. cerevisiae* (Bataillon et al. 1996, Fleet and Heard 1993).

Given the high day-time and night-time temperatures during the heat wave, the grape berry temperature at harvest would have been higher than usual. The higher temperature of the berries before and during harvest, and also during transport to the winery, would have more quickly activated the microorganisms associated with the grapes. After a lag phase, these microorganisms commence an accumulation of nutrients, including nitrogen compounds and vitamins.

The binding of sulfur dioxide (bisulfite ion) to glucose is significant in juices (Boulton et al. 1996), so the higher than usual sugar concentrations would have reduced the effectiveness of sulfur dioxide ($SO_2$) added to grape bins. Therefore, in cases where control measures were not taken, such as regular cleaning of harvester bins and increased use of $SO_2$ in grape bins, the development of large populations of indigenous microorganisms could have depleted nutrients. This would have consequently made completion of fermentation of high sugar musts very difficult for the inoculated yeast. Monk (1994) indicated that low growth rate or low cell yeast yield resulting from vitamin deficiencies can lead to sluggish or stuck fermentations. In addition, high populations of well established native yeasts can compromise the ability of the inoculated yeast to dominate the microflora (Petering et al. 1993), leading to further depletion of essential nutrients.

#### High fruit temperature and capacity of winery cooling systems

Winemakers reported fruit arriving at the crusher with temperatures in the 30°C to 35°C range and higher. On one particular day, fruit arrived at one winery when the ambient temperature was greater than 40°C. Such high temperatures put increased loads on refrigeration systems and some wineries' cooling systems did not have the capacity to cope with these increased loads. In addition, the rapid ripening of fruit compressed the intake period which further exacerbated this problem. An inability to chill musts efficiently and quickly would have allowed further growth of the indigenous yeasts and bacteria in the prevailing warm conditions. These conditions lead to further loss of essential nutrients and

increased the risk of stuck fermentations. Added to this, dehydrated and shrivelled fruit with a low juice volume make processing more difficult and can lead to blockages of must lines and heat exchangers. Such delays exacerbate the high temperature problem, and the increased $SO_2$-binding problem due to the high levels of sugar, by allowing further growth of unwanted microorganisms.

### High Bé fruit leads to high alcohol wine

The very high sugar concentrations encountered in musts after the heat wave would have made completion of fermentation very difficult, due to the production of larger than usual concentrations of ethanol. Ethanol is the most important inhibitor of growth of *Saccharomyces cerevisiae* (Henschke 1997). Kunkee (1991) showed that ethanol inhibits the transport into the cell of many nutrients and substrates such as glucose, ammonium and amino acids and suggested that ethanol increases the toxicity of other compounds, such as the medium chain length fatty acids. These medium chain length fatty acids can inhibit yeast growth at a concentration of approximately 3 mg/L in 10% ethanol (Larue and Lafon-Lafourcade 1989, Casey and Ingledew 1986). The fact that ethanol increases the toxicity of other compounds explains why some compounds might not be problematic early in fermentation, but might interfere with fermentation in the latter stages (Henschke 1997).

### Fermentation temperature

Given the high ambient temperatures during the heat wave and the problems encountered in cooling musts sufficiently, it is likely that high temperatures contributed to fermentation problems in some cases. A high sugar concentration in the must combined with excessive temperature can limit the amount of ethanol produced. Yeast are particularly heat sensitive in their growth phase and when the temperature is greater than approximately 30°C they become stressed; their viability is affected; they will produce more volatile acidity during fermentation; and a stuck fermentation is more likely (Ribéreau-Gayon et al. 2000). Increased temperature of fermentation also increases yeast consumption of nitrogen (Ribéreau-Gayon et al. 2000). In the case of the 'heat wave musts', the availability of yeast assimilable nitrogen (YAN) might have been limited if microbial growth had occurred (as discussed above), or if the concentration of YAN was low due to the prevailing drought conditions.

Over-heating yeast in the cap of a red fermenter (>35°C) might also be a problem. Although this is usually believed to be rare (Henschke 1997), the high ambient temperatures during the heat wave this year, combined with the compressed fruit intake due to rapid ripening reducing proper temperature control, might have made this possibility more likely.

**Yeast assimilable nitrogen (YAN) concentration**

Hook (2008) reported that juice from many vineyards contained low concentrations of YAN, which is the usable nitrogen fraction of the total nitrogen present. Both yeast cell growth and fermentation rate are related to initial YAN concentration and a direct relationship exists between fermentation rate and the amount of nitrogen utilised by yeast (Bell and Henschke 2005). Therefore, when all other factors are non-limiting, fermentation duration is a function of the initial YAN concentration. If the initial YAN is low, the risk of a slow or stuck fermentation is increased.

Analysis of YAN data obtained for several hundred juice samples (tank samples after crushing) from two regions in South Australia, over the last two vintages, does in fact show that the average YAN concentration for the 2008 vintage juices was lower than that for the 2007 vintage juices for both regions (Figure 1). However, analysis of the data also show that there is a greater variation in the results for the 2008 samples than the 2007 samples (Figure 2). The results indicated that whilst some juices contained sufficient concentrations

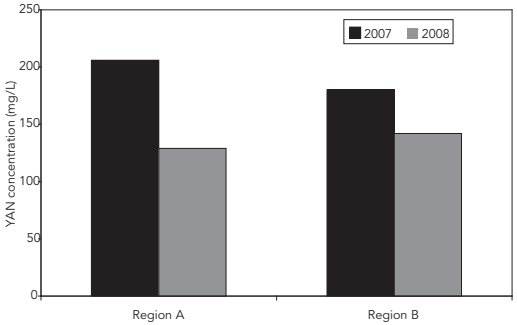


**Figure 1.** The average YAN concentration in juices from two regions in South Australia for the 2007 (dark shading) vintage and the 2008 vintage (light shading). For region A, n = 855 in 2007 and 1272 in 2008; for Region B, n = 414 in 2007 and 788 in 2008

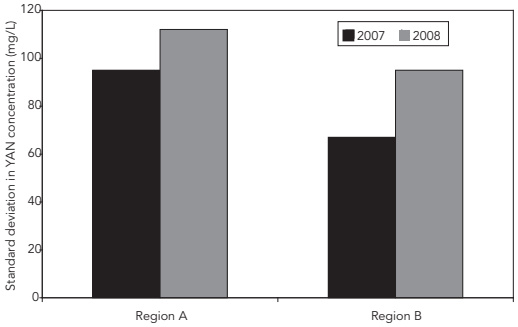


**Figure 2.** The standard deviation in the average YAN concentration in juices from two regions in South Australia for the 2007 (dark shading) vintage and the 2008 vintage (light shading). For region A, n = 855 in 2007 and 1272 in 2008; for Region B, n = 414 in 2007 and 788 in 2008

of YAN, others had relatively low YAN concentrations, which might have contributed to slow or stuck fermentations.

Various studies have been conducted to estimate the minimum concentration of nitrogen required to reduce the risk of slow or stuck fermentations in white wines. Due to variations in factors such as yeast strain, propagation and fermentation conditions, the estimates fall in a wide range (approximately 70–300 mg N/L). Nevertheless, as a rough guide, a figure of 140 mg N/L can be considered as a threshold; below this level the risk of a slow or stuck fermentation in a clarified white must of moderate sugar concentration becomes higher. However, yeast YAN demand increases with higher sugar concentration and higher fermentation temperatures (Jiranek et al. 1995). No studies have been published that estimate the minimum concentration of YAN required for red wine ferments. However, it is expected that the risk threshold concentration of nitrogen needed for red wine ferments would be lower than for clarified white musts, due to the presence of a higher amount of grape solids (Bell and Henschke 2005). Nevertheless, the risk threshold can be expected to increase with higher sugar concentration and higher fermentation temperatures.

Given that the average YAN concentrations for the two regions represented in Figure 1 were lower for the 2008 vintage than the 2007 vintage, raises the possibility that a lower than usual YAN concentration might have been a contributing factor to some of the stuck fermentations observed during 2008.

### Yeast – bacteria interaction and high VA

As mentioned previously, some winemakers reported that they had stuck fermentations containing high concentrations of VA and that many of these ferments had either finished, or were undergoing MLF. Some of these stuck ferments were tested at the AWRI and informal sensory analysis revealed that a number of them were also affected by mousy off-flavour. The results of analysis, conducted at the AWRI, of a number of red stuck fermentations showed they had high pH values; contained high concentrations of acetic acid; low concentrations of malic acid; and contained various microorganisms. The results of analysis of one red stuck ferment are presented in Table 1 and are fairly typical of many of the 'high VA' ferments tested.

As indicated above, once the structure of the berry is damaged, for example split due to dehydration or damaged due to mechanical harvesting, the native yeast and bacteria (including aerobic and anaerobic) can multiply. Rankine (1989) reported that growth and formation of volatile acidity by acetic acid bacteria is twice as fast at 23°C as at 18°C, and four times as fast at 28°C. Therefore, hot conditions can dramatically increase the rate of

formation of acetic acid by any acetic acid bacteria present on the grapes and could explain why one winemaker reported a VA concentration of 0.8 g/L in one batch of juice tested.

Growth of lactic acid bacteria (LAB) such as the lactobacilli and pediococci is encouraged at higher (>3.5) pH and unless controlled by acid additions and use of $SO_2$, substantial populations of these microorganisms might develop, especially under warm (30°C to 35°C) conditions (Fugelsang and Edwards 2007). LAB such as *Lactobacillus* sp. can produce acetic acid when growing on grape sugars. Strains such as *Pediococcus* sp. can also produce acetic acid when growing on pentose sugars (Sponholz 1993). In addition, when ethanol or acetaldehyde are present from fermentative yeast growth, the presence of grape sugars stimulates mousy compound formation by LAB (Costello and Henschke 2002), which would explain the mousy characters observed in the 'high VA' wines investigated at the AWRI.

Apart from spoilage, acetic acid and associated products of LAB metabolism represent potent inhibitors to fermentatively growing *Saccharomyces*, delaying the onset of fermentation and potentially causing fermentation to become stuck. In addition, there is the potential for more acetic acid to be produced by LAB at pH >3.5, both during growth on grape sugars and during MLF (Fugelsang and Edwards 2007).

California winemakers have also reported instances of stuck red wine fermentations attributed to pre-fermentation or early fermentative-phase growth of native lactobacilli. In these cases, the acetic acid levels generally ranged from 0.8 – 1.5 g/L, but sometimes were as high as 2 or 3 g/L, and in most cases the problem stemmed from fermentation of high pH (>3.5) musts without pre-fermentation addition of $SO_2$ (Fugelsang and Edwards 2007). Whilst there was potential for LAB activity to have contributed to the stuck fermentations observed in the 'high VA' wines, it is possible that other additional inhibitory mechanisms were involved, such as those previously discussed.

**Table 1.** Results of analysis of a typical 'high VA' 2008 red ferment

| Analysis | Result |
|---|---|
| Alcohol | 12.0 % v/v |
| Acetic acid | 1.94 g/L |
| Glucose + Fructose | 47.0 g/L |
| Malic acid | <0.05 g/L |
| pH | 3.81 |
| Microbiological | Yeast: *Saccharomyces* sp., non-*Saccharomyces* sp.<br>Bacteria: *Lactobacillus* sp., *Acetobacter* sp. |
| Sensory | Volatile, mousy off-flavour |

### Inhibitory substances – residual agrochemicals

Investigations conducted at the AWRI in conjunction with wineries have shown that high concentrations of copper in musts, for example, can slow the rate of fermentation. Tromp and de Klerk (1988) have also observed that the use of copper-containing fungicides can lead to sufficient copper residues in musts to cause lagging fermentation.

Whilst the presence of a residual agrochemical might not normally cause a fermentation to become stuck, its presence might have a larger inhibitory effect on fermentation when combined with a number of other inhibitory factors.

### Glucose to fructose ratio

Every year the AWRI receives queries regarding glucose to fructose ratio and residual fructose levels in relation to incomplete fermentations. It seems that *Saccharomyces* are slightly more 'glucophilic' than 'fructophilic': in fermentation mixtures of glucose and fructose, glucose will be consumed more quickly than fructose. This means that by the time the end of fermentation approaches, the last few grams of residual sugar will be fructose. However, under 'normal' circumstances, when nutrients are non-limiting and there are no inhibitory factors affecting fermentation, the yeast will utilise the fructose. As far as the AWRI is aware, it has not been conclusively demonstrated that a higher than usual glucose to fructose ratio in the juice is a cause of stuck fermentations. Several strains promoted to consume fructose at a more equitable rate to glucose are commercially available but their ability to avert stuck fermentations is still to be confirmed.

### Summary

There appear to have been a number of factors that might have caused or contributed to the stuck fermentations experienced by wineries in southern Australia following the heat wave that occurred during the first half of March this year. Winemakers should be aware of these factors and take steps, where possible, to minimise their effects, although some of these factors can occur individually without consequence. However, it is considered that high must sugar concentration was the most important factor in stuck or sluggish fermentations due to the higher ethanol produced.

The persistent drought conditions are believed to have contributed to a low YAN content, which is known to reduce yeast growth and fermentation power. Furthermore, the conditions during the 2008 heat wave were likely to have given rise to larger populations of indigenous microflora which would have removed nutrients from the juice at a greater rate than usual. Under such conditions, it is recommended:

- that the concentration of $SO_2$ added to grape bins be increased appropriately, perhaps double the amount that might usually be added, not only to reduce microbial activity, but to also reduce oxidative processes which occur more quickly at higher temperatures.
- it is essential to sanitise bins between loads. It is also recommended that bins be covered during transport to reduce further heating by exposure to direct sunlight. Winery equipment, such as receival bins, crushers, presses, must pumps and lines should also be regularly cleaned and sanitised since the surfaces of the equipment will more quickly become populated with best adapted yeasts and bacteria.

Given the high pH values observed in fruit harvested during the heat wave:

- tartaric acid additions should be made as soon as must tanks are mixed and the acidity parameters are known.
- lowering the pH will improve the efficacy of $SO_2$ by increasing its antimicrobial and antioxidant properties and inhibit the growth of unwanted microorganisms (including LAB such as *Lactobacillus* and *Pediococcus* spp.).
- monitor the pH during fermentation and adjust if necessary, in order to maintain a value in the range of 3.4–3.5. This will avoid an excessive increase in pH with the deposition of potassium bitartrate and help maintain microbiological stability, in combination with $SO_2$, after the MLF. The rapid growth of lactobacilli in the early stage of fermentation, which is an important factor in the cause of stuck fermentation with high residual sugar, can be controlled by low pH.
- lysozyme addition to must or ferments to control bacteria development has also been suggested.

Finally, winemakers should be aware that if their 2008 red wines contain higher than usual concentrations of residual sugar there is an increased risk of microbial growth in the future, especially growth of *Dekkera/Brettanomcyces* yeast.

### Additional resources

Choice of a yeast strain with known high tolerance to alcohol; optimal yeast preparation; and fermentation management strategies are very important to avoid stuck fermentations. These issues and strategies for managing sub-optimal fermentation are discussed in a presentation (entitled *Management of wine fermentation*) by Dr Paul Henschke, which is available for viewing by Australian winemakers and grapegrowers at the AWRI website (http://awri.streamcast.com.au/).

A paper entitled *Winemaking implications of drought* (prepared by Con Simos, Sally-Jean Bell, Peter Leske and Peter Godden) is also available at the AWRI website (http://www.awri.com.au/information_services/current/pdfs/Drought_response.pdf). This paper outlines some of the strategies available to winemakers to minimise the negative effects of hot weather on wine quality.

A method for restarting stuck fermentations (entitled *Prevention and management of stuck alcoholic fermentations*) is also available at the AWRI website (http://www.awri.com.au/practical_solutions/technical_notes/notes/TN05.pdf) and many winemakers have had success restarting stuck fermentations using this method. Similar methods are also promoted by yeast suppliers. Please note that the aeration steps and maintenance of adequate nutrient levels as outlined in the method are essential as the method is unlikely to succeed if these are neglected.

**Acknowledgement**

The author acknowledges the winemakers who provided information regarding stuck fermentations this year and who contributed juice and must compositional data and samples for analysis. Dr Paul Henschke is also thanked for many useful discussions on the subject and for critically reviewing this text.

**References**

Australian Government Bureau of Meteorology (2008) SPECIAL CLIMATE STATEMENT 15 An exceptional and prolonged heat wave in Southern Australia, Issued 20th March 2008 – updated 3rd April 2008, National Climate Centre. http://www.bom.gov.au/climate/current/statements/scs15b.pdf

Bataillon, M., Rico, A., Sablayrolles, J.M., Salmon, J.M., Barre, P. (1996) Early thiamin assimilation by yeasts under enological conditions: impact on alcoholic fermentation kinetics. J. Ferment. Bioeng. 82: 145–150.

Bell, S.J., Henschke, P.A. (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11: 242–295.

Bisson, L.F., Butzke, C.E. (2000) Diagnosis and rectification of stuck and sluggish fermentations. Am. J. Enol. Vitic. 51: 168–177.

Boulton, R.B., Singleton, V.L., Bisson, L.F., Kunkee, R.E. (1996) Principles and practices of winemaking. New York: Chapman & Hall: 460–461.

Casey, G.P., Ingledew, W.M. (1986) Ethanol tolerance in yeasts. CRC Crit. Rev. Microbiol. 13: 29–280.

Costello, P.J., Henschke, P.A. (2002) Mousy off-flavour of wine: precursors and biosynthesis of the causative N-heterocycles 2-ethyltetrahydropyridine, 2-acetyltetrahydropyridine, and 2-acetyl-1-pyrroline by *Lactobacillus hilgardii* DSM 20176. J. Agric. Food Chem. 50: 7079–7087.

Fleet, G.H., Heard, G.M. (1993) Yeasts – Growth during fermentation. Fleet, G.H. (ed.) In: Wine microbiology and biotechnology. Chur, Switzerland: Harwood Academic Publishers: 27–54.

Fugelsang, K.C., Edwards, C.G. (2007) Wine microbiology. Practical applications and procedures. Second edition. New York: Springer.

Henschke, P.A. (1997) Stuck fermentation: causes, prevention and cure. Allen, M., Leske, P., Baldwin, G. (eds) Proceedings advances in juice clarification and yeast inoculation, 15 August 1996, Melborne, Vic. Adelaide, SA: Australian Society of Viticulture and Oenology: 30–38, 41.

Hook, J. (2008) Heatwave effects on South Australian vineyards – observations in 2008. Aust. N.Z. Grapegrower Winemaker. June: 25–26.

Jiranek, V., Langridge, P., Henschke, P.A. (1995) Amino acid and ammonium utilization by *Saccharomyces cerevisiae* wine yeasts from chemically defined medium. Am. J. Enol. Vitic. 46: 75–83.

Kunkee, R.E. (1991) Relationship between nitrogen content of must and sluggish fermentation. Proceedings of the International Symposium on Nitrogen in Grapes and Wines, Seattle, USA. Davis, CA: American Society for Enology and Viticulture: 148–155.

Larue, F., Lafon-Lafourcade, S. (1989) Survival factors in wine fermentation. Van Uden, N. (ed.) In: Alcohol tolerance in yeasts and bacteria. Boca Raton, FL: CRC Press, Inc.: 193–215.

Monk, P. (1994) Nutrient requirements of yeast. Prac. Winery Vine. July/August: 24–27, 60.

Petering, J.E. Langridge, P., Henschke, P.A. (1993) Use of a marked wine yeast to determine efficiency of sulfur dioxide at low must temperature. Stockley, C.S., Johnstone, R.S., Leske, P.A., Lee, T.H. (eds) Proceedings of the eighth Australian wine industry technical conference; 25–29 October 1992, Melbourne Vic. Adelaide, SA.: Australian Wine Industry Technical Conference Inc.: 211.

Rankine, B.C. (1989) Making good wine. Melbourne: Macmillan: 282.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (2000) Handbook of enology volume 1: the microbiology of wine and vinifications. Chichester, England: John Wiley & Sons Ltd: 95, 307.

Sponholz, W.R. (1993) Wine spoilage by microorganisms. Fleet, G.H. (ed.) In: Wine microbiology and biotechnology. Chur, Switzerland: Harwood Academic Publishers: 395–420.

Tromp, A., de Klerk, C.A. (1988) Effect of copperoxychloride on the fermentation of must and wine quality. S.A. J. Enol. Vitic. 9: 31–36.

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