A Case Study: Yeast performance during the 1996 vintage at Rosemount Estate

IAN LONG
Rosemount Estate

Introduction
During the 1996 vintage at Rosemount Estate, the yeast performance can best be described in two words: ‘stuck fermentations.’ Prior to 1996, a small number of stuck and slow fermentation problems had been dealt with but, due to their low incidence, were not considered to be of real concern. With some effort, these fermentations always proceeded to completion even though at slow rates. In contrast, the fermentation problems encountered during vintage 1996 required considerable effort to resolve.

Fermentations that would normally complete in ten to twelve days remained incomplete after three to four weeks. Fermentation temperatures were raised to unacceptably high levels with little effect on fermentation rate. Bottling dates for several 1996 wines were fast approaching and half of the wines in the winery had failed to complete fermentation.

Rosemount Estate was left with three main questions to resolve:

• Why did these fermentations stick?
• How can they be prevented from sticking?
• What is the best way of restarting the stuck fermentations?

Through extensive research, trial and error, Rosemount Estate was able to answer these questions and overcome most of the problems. This experience at Rosemount Estate is the basis of this presentation.

Why did the fermentations stick?
In order to determine why the fermentations were sticking, the following vintage operational areas were investigated:

• juice processing and clarification
• fermentation monitoring
• temperature control
• yeast strain selection
• yeast rehydration and inoculation

Juice processing and clarification
At Rosemount Estate, ‘premium’ white juices are cold settled and racked prior to fermentation, while ‘commercial’ white juices are clarified by centrifugation immediately following pressing. At best, juices prior to fermentation are cellar bright while it is most common for Chardonnay juices to contain some light solids after racking. Juice filtration is only carried out during wet vintages where there is a high incidence of Botrytis infection in the vineyard.

After considering these factors, it was Rosemount Estate’s conclusion that the juice processing procedures were not significant in influencing the incidence of stuck fermentations.

White juice composition would typically be a soluble solids level of between 11.0 to 13.5 Baume, titratable acidity levels from 6 to 8 g/L, pH levels in the range 3.00 to 3.30 and Free SO₂ levels between zero to a maximum of 15 ppm.

Fermentation monitoring
Immediately following inoculation, yeast counts and fermentation Baumes were measured every two hours. Once cell numbers exceed 4 × 10⁷ cells/mL and a one Baume decrease was recorded, the fermentations were cooled from 18°C (inoculation temperature) to between 12–14°C. Baumes were then checked every 12 hours. Sensory analysis of the fermentations were also carried out every 12 hours to check for sulphide development and where necessary correct with the addition of diammonium phosphate.

Temperature control
Fermentation temperatures were monitored every two hours. All fermentation tanks have dimple plate type brine cooling, controlled by a basic computer program, with external heat exchangers being used where necessary to supplement the in-place cooling. Target temperatures for all fermenters are updated every twelve hours in order to maintain consistent fermentation rates. Although Rosemount Estate does not believe its temperature control system is perfect, fermentation temperature fluctuations during the 1996 vintage were initially not considered responsible for causing the high incidence of stuck fermentations. In previous years, almost all stuck fermentation problems were linked to overcooling of fermentations.

Following the establishment of the yeast population, the decrease in fermentation temperature to 12–14°C is achieved by cooling through a heat exchanger. When stuck fermentations became more prevalent, in an attempt to overcome the problem, fermentation temperatures were raised to 18–20°C. These temperatures, unacceptably high for quality wine production, had no significant effect on either accelerating sluggish fermentations or in decreasing the incidence of stuck fermentations.

Yeast strain selection
Prior to the 1996 vintage, Rosemount Estate had predominately used EC1118 type yeast strains. However, concerned for the high level of nitrogen supplementation, it was decided to consider alternative yeast strains for the 1996 vintage. The yeast strain 796 was chosen on the basis of its lower nitrogen requirement. The experience with yeast strain 796 suggested that it was most suited for fermentation of low Baume juices, and Fermentation problems encountered during vintage 1996 were initially not considered responsible for causing the high incidence of stuck fermentations. In previous years, almost all stuck fermentation problems were linked to overcooling of fermentations.

Following the establishment of the yeast population, the decrease in fermentation temperature to 12–14°C is achieved by cooling through a heat exchanger. When stuck fermentations became more prevalent, in an attempt to overcome the problem, fermentation temperatures were raised to 18–20°C. These temperatures, unacceptably high for quality wine production, had no significant effect on either accelerating sluggish fermentations or in decreasing the incidence of stuck fermentations.

Yeast strain selection
Prior to the 1996 vintage, Rosemount Estate had predominately used EC1118 type yeast strains. However, concerned for the high level of nitrogen supplementation, it was decided to consider alternative yeast strains for the 1996 vintage. The yeast strain 796 was chosen on the basis of its lower nitrogen requirement. The 1996 experience with yeast strain 796 suggested that it was most suited for fermentation of low Baume juices, for example Rosemount Estate Semillon. Yeast strain 796 appeared less suited for use in high baume juices or for grape varieties where fermentation difficulties might be expected (e.g. Chardonnay). In these applications, yeast strain 796 showed inability to complete fermentation. This brought about the replacement of yeast strain 796 with more suitable strains immediate improvements were recorded, with fermentations proceeding to dryness at normal fermentation temperatures.

Yeast rehydration and inoculation
For the start of the 1996 vintage, the yeast rehydration tech-
nique used by Rosemount Estate was unchanged from previous years and was based on the recommendations provided by the yeast manufacturers. The procedure was as follows:

- For every 1 kilogram of yeast, prepare a mixture of 5 litres water and 5 litres of the juice to be inoculated. This mixture to be gently heated to 40°C.
- Slowly sprinkle the yeast into the juice/water mixture while stirring to mix.
- Leave until the yeast suspension has expanded to twice its original volume.
- Add the yeast to the tank and pump over to mix.

Athough pleased with the yeast rehydration procedure, Rosemount Estate explored ways of improving the technique so as to give yeast the best possible chance of success. After many hours of research, plus numerous telephone calls to The Australian Wine Research Institute and many of the major Australian wine companies, Rosemount Estate concluded that its rehydration technique could be improved by introducing a number of minor improvements. Following many discussions with various wine companies, it was apparent that others shared the view of Rosemount Estate that the preparation of dried yeast through propagation, as opposed to direct inoculation, was a more efficient and effective means of utilising dried yeast. Please refer to Appendix I for a detailed description of the yeast preparation and propagation procedures.

How can the fermentations be prevented from sticking?
The search for an improved yeast rehydration technique initially yielded more questions than solutions. In studying the instructions given on six different dried yeast packets, six different rehydration techniques were identified. The variations included:

- Temperature of rehydration water
- Time of rehydration
- Inclusion/exclusion of juice in the rehydration medium
- Stirring/not stirring yeast during rehydration
- Adjustment of temperature of yeast mixture before adding to fermentation tank

Further discussions with staff at The Australian Wine Research Institute revealed that all of the above factors are important to the successful rehydration of yeast. Based on these discussions, Rosemount Estate formulated its own rehydration technique. Refer to Appendix II for a detailed description of the yeast rehydration procedure. The major points of this technique are:

- Temperature— the optimum appears to be 37°C.
- Juice addition— initial rehydration should be carried out in a low baume mixture (3°Be) of approximately four parts water to one part juice.
- Timing of rehydration— timing is critical during the initial rehydration stage. Once the yeast cells are fully rehydrated they will quickly utilize the available sugar in the rehydration mixture. Yeast viability will decrease rapidly if the yeast is allowed to exhaust the available sugar.
- Aclimatization of the yeast to the juice temperature and baume— this is done by the gradual addition of juice to the yeast suspension.

- Stirring during rehydration— during the initial rehydration phase the cell wall is relatively porous. Research indicates that stirring during this period can lead to loss of vital components from within the yeast cell, resulting in reduced cell viability.
- Interference by other chemicals— during the initial rehydration phase the yeast cells are particularly vulnerable to the effects of chemicals such as SO₂ and chlorine. Water used for rehydration should be free from chlorine. The sugar source used in the rehydration phase should be free from SO₂ and other chemicals such as pesticide residues. In trials using cane sugar instead of grape juice, significant increases in the activity of the yeast during rehydration was noted, indicating that even very low levels of SO₂ will have a detrimental effect on the yeast.

The overall aim of the rehydration procedure is to maximise the number of viable yeast cells that are acclimatized to the environment to which they are to be introduced. By rehydrating in a medium that is free from chemical contamination and by avoiding temperature shock in the initial rehydration stage, this aim should be successfully achieved.

The new procedure is not significantly different from the previous Rosemount Estate procedure and remains consistent with the general recommendations of yeast manufacturers. However the new procedure is much more detailed and contains a number of additional steps. Further research will be carried out next vintage to simplify the procedure whilst still meeting all requirements.

Rosemount Estate felt confident that through a better understanding of the characteristics of the various yeast strains, through improvements to the rehydration technique and greater use of yeast cultures that the problems of stuck fermentations would be overcome. This was reflected by the way in which the fermentations in the latter part of the 1996 vintage proceeded. However Rosemount Estate still had one other major problem to address— what to do with the ‘stockpile’ of stuck fermentations?

What is the best way of restarting the stuck fermentations?
Various techniques were employed in an effort to restart stuck fermentations including:

- Warming the stuck ferment to elevated temperatures (e.g. 20°C)
- Constant mixing of the fermentation tank
- Additions of diammonium phosphate
- Direct reseeding with rehydrated yeast

In general, these techniques were not successful. Further searching of the literature revealed a number of methods for the preparation of rescue cultures for stuck fermentations. Success with these techniques was variable.

In studying the rescue culture techniques, it was apparent that they all shared a similar approach— that is to prepare a culture of yeast and then to slowly feed into it portions of the stuck fermentation. As the sugar is consumed, more of the stuck fermentation is introduced. The theory for this approach is that the yeast cells are gradually acclimatized to the harsh environment of the stuck fermentation (high alcohol, high acid, possible killer factor etc).

Rosemount Estate found that in practice the stuck fermentation was being fed in fairly slowly and in quite a number of steps, the result being that by the time all of the stuck...
ferment had been introduced, the yeast viability had fallen to such a low level that fermentation had virtually stopped once again. Furthermore, most rescue techniques indicate that one should wait until the sugar level of the rescue culture has decreased to low levels before more of the stuck fermentation is introduced. This leads to two problems, firstly the sugar level of the rescue culture, rather than being on a constant decline, is actually fluctuating up (by the addition of the stuck fermentation) and down as the fermentation continues in the period between additions. Secondly, there is a high risk of the culture fermenting to dryness (with subsequent rapid loss of yeast viability) before all of the stuck fermentation has been added.

Guided by the available literature, Rosemount Estate began to develop its own yeast rehydration technique. In principle Rosemount Estate wanted to achieve high viable yeast cell numbers, acclimatized to the environment to which they were to be introduced so as to achieve rapid and complete fermentation. To achieve these objectives, the developed technique involves five steps:

Step one—rehydration of yeast as per method described in Appendix Two.

Step two—introduction of the rehydrated yeast into a volume of grape juice.

Step three (first stage of acclimatization)—after a significant Baume drop the volume of the culture is doubled with the stuck ferment.

Step four (second stage of acclimatization)—after a further Baume drop, double the volume again with the addition of stuck fermentation.

Step five—after a further Baume drop, the rescue culture is added back to the main batch of the stuck fermentation.

Throughout steps two to four, the yeast culture should be continuously aerated and the culture supplemented with nutrients. Prior to the preparation of the rescue culture, the stuck fermentation is warmed and supplemented with nutrients. Timing is critical throughout this procedure as steps one to five will all take place within one to two hours. Volume increases at the correct times along with aeration and nitrogen supplementation are critical to achieving high cell viability. Cell numbers in the culture, at the end of step five and prior to the addition back to the main fermentation, should be approximately $1 \times 10^6$ cells/mL.

Throughout this process, there are no increases in the sugar concentration of the culture. It is always falling and never approaches dryness to minimize viability losses. The additions of stuck fermentation to the culture help acclimate the yeast to high alcohol levels. However due to the short time span of the buildup phases the number of viable cells introduced into the stuck fermentation is very high.

By the adoption of this method, all stuck fermentations at Rosemount Estate were successfully completed. A detailed description of the procedure is given in Appendix III.

**Conclusion**

From the experiences of vintage 1996 the use of inappropriate yeast strains led to a situation where there was serious threat to wine quality and production efficiency. Only through significant amounts of research was this situation overcome. It should be recognized that perfect conditions do not exist in the cellar environment. Suppliers of dry wine yeast should strive to develop a better understanding of the performance of their products in a normal cellar environment and hence be better placed to make recommendations on their suitability for various applications.

**Acknowledgments**

Rosemount Estate gratefully acknowledges the assistance of many wine industry personnel and the staff of The Australian Wine Research Institute, in particular Dr Paul Henschke.

### Appendix I

1. **Propagation of yeast culture**

   *Utilising propagation tank which should have agitator and facility for air sparging*

   **Rate of inoculation**—1 kg yeast per 2000 litres in propagator

   **1. Juice preparation**
   - **1.1 Juice specifications**
     - Temperature 20°C
     - Baume—maximum 10° (adjust with water if necessary)
     - Addition of 2000 ppm diammonium phosphate
     - Addition of 10 ppm cerevit (or similar nutrient); mix.
     - Minimal Free SO$_2$ and low Total SO$_2$; No botrytis
   - **1.2 Method**
     - Transfer required amount of juice to propagation tank. Add DAP and nutrient and begin agitation and air sparging.

   **2. Rehydration**
   - **2.1 Rate**
     - For every 1 kg yeast require 8 litres water and 2 litres juice.
   - **2.2 Method**
     - Adjust warm water/juice mixture temperature to 37°C
     - Slowly sprinkle yeast onto surface of water without stirring
     - Leave undisturbed for exactly 15 minutes.

   **3. Aclimatization**
   - **3.1 Rate**
     - For every 10 litres rehydrated yeast mixture require 20 litres juice ex-propagator
   - **3.2 Method**
     - To each 10 litres yeast mixture slowly add 5 litres juice.
     - Wait 5 minutes.
     - Slowly add remaining 15 litres juice. Wait 5 minutes.

   **4. Add yeast mixture to propagator**

   **5. agitation and aeration should be continuous**

   **6. Maintain temperature at 25–28°C**

   **7. Culture is ready to use when cell numbers exceed $3 \times 10^6$ cells/mL**

**Notes**

- **i**. Air requirement: 35 litres/min per 100 litre of culture (7 litres/min if oxygen is used)
- **ii**. If a yeast count of $3 \times 10^6$ cells/mL cannot be achieved this indicates insufficient supply of oxygen
- **iii**. If the culture is not for immediate use the temperature should be reduced to 10°C to maintain sugar level after maximum cell numbers are achieved.
APPENDIX II
Direct inoculation with dry yeast
Rate 1kg yeast per 4,000 litres juice

1. Juice preparation
   warm tank to 17°C
   add 200 mg/L DAP

2. Rehydration
   2.1 Rate
   For every 1 kg yeast require 8 litres water and 2 litres juice
   2.2 Method
   Warm water/juice mixture to 37°C

3. Acclimatization
   3.1 Rate
   For every 10 litre yeast mixture require 20 litres juice
   3.2 Method
   To each 10 litres yeast mixture slowly add 5 litres juice.
   Wait 5 minutes
   Slowly add yeast suspension to the remaining 15 litres juice. Wait 5 minutes

4. Addition of the yeast preparation to the tank. Mix well.

APPENDIX III
Rescue culture for stuck fermentation
Rate 1 kg yeast per 2000 litre stuck fermentation

1. Stuck ferment preparation
   Warm the stuck fermentation to 20°C

2. Juice preparation
   2.1 Addition rates
   For every 1 kg yeast require 80 litres juice, 160 g DAP, 1 g cerevit.
   2.2 Method
   Transfer juice to propagator tank, warm to 30°C and add DAP and cerevit.
   Begin agitation and aeration of the juice

3. Rehydration
   3.1 Rate
   For every 1 kg yeast require 8 litres water and 2 litres juice
   3.2 Method
   Warm water/juice mixture to 30°C
   Slowly sprinkle yeast onto surface of water without stirring. Leave undisturbed for 15 minutes

4. Acclimatization
   4.1 Rate
   For every 10 litres yeast mixture require 20 litres juice
   ex propagator

5. Addition yeast mixture to the propagator

6. Further additions
   • When sugar level falls to 3°Be, double the volume in the propagator by adding the stuck fermentation.
   • Add 50 g DAP per 1 kg of yeast.
   • Check Baume after volume increase.
   • When sugar level falls to half of that at end of step 6 double the volume in the propagator using stuck ferment.
   • Add 50 g DAP per 1 kg of yeast. Check Baume after building up

7. Final inoculation step
   When sugar level falls to half of that at end of step 6, add culture back to the main volume of stuck fermentation.

Notes
i) agitation and aeration of the culture should be maintained until end of step 7.
ii) agitation of the main fermentation should be maintained until fermentation is complete.