

STUCK FERMENTS: CAUSES AND SOLUTIONS

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An analysis by LAFFORT of the possible causes for stuck fermentations and more importantly, ways to treat them, has led to a strategic approach to the effective management of stuck fermentations, coupled with the innovative use of products purpose-designed for problem solving. Even in a very difficult year for stuck fermentations, such as 2008 in South Australia, the stuck fermentations experienced were corrected using the LAFFORT protocol.

INTRODUCTION

2008 was a shocking year in Australian winemaking for stuck fermentations, particularly in South Australia, and given the recent spate of excessively hot weather in SA and Victoria we can presumably expect some similar fermentation difficulties for the '09 vintage. Many of these were induced by the much-discussed heat wave experienced in South Australia in March 2008, however not all can be attributed solely to cooked fermentations. In subsequent discussions with several winemakers, from both big and small production facilities and in various regions it has become apparent that yeast nutrition, or rather lack thereof, was a major to stuck fermentation problems. It is therefore prudent that we seek a greater understanding of stuck fermentations prior to the 2009 harvest.

CAUSES OF STUCK FERMENTATIONS

A stuck fermentation can be defined as any fermentation "where the rate of fermentation of sugar slows dramatically, or where the end of fermentation is protracted" (Boulton *et al.*, 1999a). There are several known causes for stuck fermentations:

- Nutrient deficiency
- Excessive must/juice sugar
- Excessive alcohol
- Excessive heat (or cold)
- Toxins

Any or all of these problems can lead to wine attenuation and the production of off-avours. Typically, off-avours are sulphide derivatives, and are generated due to the metabolism by yeast of sulphur-containing proteins in the absence of sufficient nitrogen. Stuck fermentations represent an enormous cost to the Australian wine industry, both in monetary and quality terms (figure 1).

Per 100 hL	Yeast only	Yeast and nutrients	Curative (1 attempt)	Curative (2 attempts)
Item / Approximate cost	\$	\$	\$	\$
Yeast 300 ppm	170	170	170	170
Dynastart 300 ppm		125	125	250
Bioactiv 400 ppm			85	170
Thiazote 200 ppm			10	20
Actiflore Bayanus 500 ppm			235	470
Labour (1 cellar hand, 48 h)			160	320
Energy (warming)			20	40
Quality loss 20 c/L per restart			2000	4000
Total	170	295	2805	5440
Wine quality	Average	Highest	Low	Lower...

Fig. 1. A comparison of the cost and influence on wine quality of different fermentation strategies based on the use of yeast with or without rehydration nutrients against the cost of restarting stuck fermentations.

Problem: Nutrient deficiency

In order to function effectively, *Saccharomyces* species require:

- Nitrogen (from ammonium ions or amino acids)
- Phosphate
- Vitamins
- Minerals

Many winemakers assume that the must will contain sufficient levels of these nutrients, however given that Australia has some of the oldest and most nutrient-depleted soils in the world, and that many regions have been in drought for several years, that assumption could be dangerous. Poor soil nutrition availability affects plant health, hence Australian winemakers potentially inherently suffer poor must (and therefore yeast) nutrition. For an excellent discussion of the parameters affecting soil nutrient status and uptake see Dry and Coombe, 2005.

Whilst some nutrients are used stoichiometrically, others such as vitamins are not (Boulton *et al.*, 1999a), and can be used through several metabolic cycles. Thus, a deficiency of vitamins or enzymatic co-factors may not be immediately evident, and so may generate a problem part-way through a fermentation. This situation is particularly likely where "mother cultures" have been used to inoculate a must, given that these yeast cells have already completed many growth phases. Any infection of the grapes with rot will also deplete the must nutrient pool (Boulton *et al.*, 1999a), as fungal growth consumes similar metabolites to yeast.

Solution: Direct yeasting with rehydration nutrients

The use of direct yeasting rather than a mother culture will vastly reduce the potential for micronutrient deficiency. Additionally, optimum yeast metabolic activity is achieved when using a rehydration nutrient. For several years now LAFFORT has demonstrated that the use of DYNASTART®, containing no inorganic nitrogen, increases yeast metabolic function and improves the aromatic expression (enzymatic function) of yeast (van der Westhuizen, 2006; Bowyer *et al.*, 2008a; Bowyer *et al.*, 2008b; Swiegers *et al.*, 2008;). Furthermore, it is provided essentially only to the chosen yeast strain since it is added to the rehydration water prior to the dry yeast, and not to the general microbial pool in the must. Anecdotally, we have also seen a major reduction in the requirement for inorganic nitrogen (diammonium phosphate) supplementation in fermentations where DYNASTART® has been used, which was particularly beneficial for DYNASTART® users last vintage when DAP was in critically short supply. It is also noteworthy that users of DYNASTART® suffered minimal fermentation difficulties in the 2008 vintage.



Problem: Excessive must/juice sugar

High concentrations of sugar are known to inhibit microbial growth through the extreme osmotic stress induced, which is why sugar is able to function as a preservative in jams. Must sugar, therefore, exerts significant osmotic stress on the yeast during rehydration, leading to increased cell death and poor initial fermentation progress. This is particularly relevant in Australia given that the sugar content of Australian wine grapes is typically higher than many other winemaking countries.

Solution: Attention to rehydration protocol

In 2007 LAFFORT sponsored two honours projects at the University of Adelaide to optimise the yeast rehydration process. The full results of these studies will be published at a later date, but in terms of high sugar juice the most pertinent result for this discussion is that at higher sugar concentrations, higher yeast inoculation rates led to more complete fermentations (data not shown).

Most yeast manufacturers recommend an addition of sugar or grape juice to the rehydration medium prior to the addition of the dry yeast, to provide some fuel for the yeast and also to reduce the osmotic stress differential experienced when the rehydrated yeast are added to the must.

Problem: Excessive alcohol

Clearly this problem is related to high must sugar levels. Some winemakers are under the assumption that some yeast strains produce more alcohol than others, and whilst this is true to a very small extent due to minor metabolic differences between strains, it does not normally lead to significant deviations in alcohol levels in the final wine. Perceived higher alcohol production in high sugar musts is an artefact: given that a certain amount of sugar is consumed for yeast growth (without alcohol production), and that this amount of sugar is roughly constant for a given inoculation rate, the remaining sugar available for conversion to ethanol appears to be proportionately higher in a higher sugar must. Thus, a higher amount of alcohol is produced in a higher sugar must, since proportionately less sugar is consumed in the growth phase (figure 2).



Fig. 2. A comparison of low (a) and high (b) sugar musts in terms of sugar consumed for growth (pink) and ethanol production (green). In the low sugar must, a smaller proportion of overall sugar is converted into ethanol, whilst in the high sugar must a proportionately greater amount of sugar is converted into ethanol. This leads to the misunderstanding surrounding excessive alcohol production of some yeast strains in high sugar musts.

Increasing ethanol concentrations leads progressively to:

- Inhibited amino acid and sugar uptake
- Increased membrane leaching of micronutrients
- Loss of co-factors for enzymatic function (Boulton *et al.*, 1999b).

Solution: Yeast strain choice and membrane fortification

Given that wine style is driven by the consumer, and that currently in Australian domestic and export markets the consumer is demanding riper-style wines, it is not practical to simply "pick the fruit earlier". The solution to the problem lies rather in the choice of a suitable yeast strain for a given fermentation and in the membrane fortification of that strain.

Yeast strains vary in their susceptibility to ethanol toxicity, which is why they ferment to different levels of alcohol. For example, ZYMAFLORE VL1® ferments to a maximum recommended ethanol concentration of 14 %, whilst ACTIFLORE® BO213 can ferment to 18 % alcohol. It was partially for this reason that LAFFORT embarked several years ago on developing hybridised yeast strains (the ZYMAFLORE® X-series yeasts), where particular traits can be selected for during the development process. In this way, high ethanol tolerance became an integral part of the developmental phase of the X-series yeast strains. It is important, therefore, to choose the right yeast strain for a given fermentation based on the must physiochemical status, notably the alcohol potential.

Since yeast membrane function is increasingly impaired as ethanol concentration rises, fortification of the membrane integrity is mandatory for high sugar musts. This is primarily achieved through the incorporation of sterols and other survival factors (e.g. fatty acids) into the yeast membrane during rehydration. Although yeast are capable of producing sterols themselves, this only occurs in an aerobic medium (Boulton *et al.*, 1999b), hence mother cultures are far more likely to provide sterol-deficient yeast. Along with the nutritive elements mentioned previously, DYNASTART® also provides these essential membrane fortification components, selectively to the inoculum yeast strain as opposed to the general microbial pool in the must.

Problem: Excessive heat (or cold)

Excessive cold or heat is detrimental to yeast function. In the case of excessive cooling, typically yeast activity is dramatically slowed or arrested, without total destruction of the cells. In the case of excessive heating, however, typically most cells are destroyed. Many stuck fermentations experienced in South Australia in 2008 related to overheating of fermentations, a combined result of excessive and extended high ambient temperatures and insufficient cooling capacity. Another instance where overheating can manifest itself is during rehydration, where the metabolic activity of the rehydrated yeast increases the temperature of the inoculum. This can occur at a rapid rate given the high concentration of yeast and the small volume of the inoculum, hence extra caution is required at the rehydration stage. Figure 3 indicates the importance of temperature management during rehydration, through an examination of yeast cell structure using an electron microscope of yeast cells rehydrated at different temperatures.

Solution: Choosing thermally tolerant yeast strains and attending the rehydration phase

Certain yeast strains tolerate high fermentation temperatures better than others. As an example, the LAFFORT Médoc isolate ZYMAFLORE F10® was recently used to develop the hybrid strain FX10® (Gourraud *et al.*, 2009). At each stage of back-crossing, the best of 10 progeny was selected according to thermal and ethanol tolerance characteristics. In this manner the new strain, FX10®, was developed to possess, after 4 back-crosses, 93 % genetic equivalence with the parent F10 strain, but with enhanced thermal and ethanol tolerance.

As noted, the rehydration phase of the yeast is critical for fermentation security, not only in terms of adequate nutrition and minimising osmotic stress, but also in terms of rehydration temperature. The second study at the University of Adelaide in 2007 identified that the optimum temperature of rehydration was found to be around 40 °C, with significant cell loss at higher temperatures as evidenced by electron microscopy (figure 3). It is critical that the inoculum temperature does not exceed this level, and this can easily happen if the inoculum is left unattended for any length of time during preparation. Successive timely additions of cool grape juice are typically made periodically during yeast rehydration in order to provide a gradual acclimatisation of the yeast to the temperature and sugar concentration of the juice to be inoculated. This inoculum has the potential, if not monitored carefully, to heat up rapidly, and so to vastly reduce cell viability and thus endanger the fermentation.

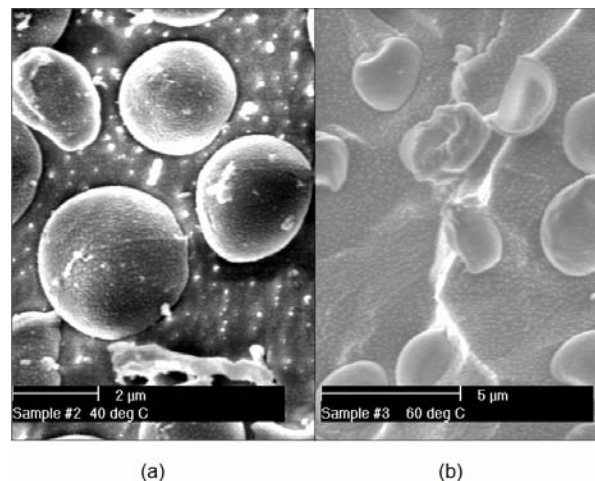


Fig. 3. A comparison of yeast cell integrity after rehydration at (a) 40°C and (b) 60°C as viewed under a cryo-scanning electron microscope. Cells rehydrated at 40°C are plump and well-structured, whilst those rehydrated at 60°C display significant damage.

Problem: Toxins

Several toxins can find their way into wine, from residual pesticides (Ruediger *et al.*, 2004) to simply having too much SO₂ present in the fruit bins. Less known toxins (Boulton *et al.*, 1999c) include:

- Organic acids (present in all grapes)
- Acetic acid (an organic acid produced by yeast when stressed)
- Fluoride (present in many pesticides and town water)
- Medium-length fatty acids (produced by yeast when stressed); and
- Killer factors, which are small peptides toxic toward certain yeast strains.

Many wineries suffered fermentation difficulties in 2008 arising from elevated volatile acidity (VA) levels. Several reports were made in the 2008 vintage of fruit received with elevated VA's. This in turn made fermentation difficult. We would not suggest attempting to restart a stuck fermentation if the VA level is above 0.8 g/L (as acetic acid), due to acetic acid's known inhibitory effects on yeast. It is also worth remembering that a difficult primary fermentation typically leads to a problematic malolactic fermentation, due to the expression of toxic fatty acids by yeast when stressed, as depicted in figure 4.

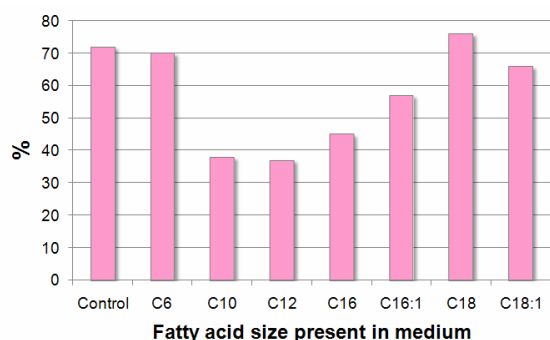


Fig. 4. An indication of the inhibitory effect of fatty acids on malolactic fermentation. C10 and C12 fatty acids are shown to be especially inhibitory, as indicated by a decreased malolactic fermentation rate over 12 days where those acids are present in a red wine (pH 3.4; 12 % alc.) inoculated with 107 cfu/mL of lactic acid bacteria.

Solution: Reduce yeast stress and detoxify the medium

Yeast stress is minimised when metabolic activity is optimised, hence DYNASTART® should be used in any restart culture. A detoxification agent is also required to treat any stuck fermentation, since yeast under stress release stress indicators such as acetic acid and medium-chain fatty acids. These compounds must be removed before any restart attempt is made. BIOACTIV® is a product developed by LAFFORT to fulfil three roles in restarting stuck fermentations: detoxification, yeast nutrition and yeast physical support. The genuine yeast hulls in BIOACTIV® provide the first two functions, since fatty acids are membrane-soluble and yeast hulls are a good nutrition source for yeast. Yeast support is provided in the form of cellulose, which keeps the restart culture suspended and improves fermentation kinetics: physical proximity between the yeast and the sugars is mandatory for fermentation. Indeed, many winemakers use BIOACTIV® as a rapid response tool for stuck fermentations in the lagging phase, by keeping some on-hand during vintage and adding it to a fermentation at the first sign of retardation of the fermentation rate. In many instances this quick action is sufficient to allow fermentation completion.

SUMMARY

While there are few guarantees in the world of winemaking, there are steps we can take to minimise our risks and maximise wine quality, aided by some understanding of cause and effect. LAFFORT has developed specific tools to help winemakers in this regard, and a protocol for restarting stuck fermentations that has proved to be particularly effective, which can be provided on request.

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