

Overcoming stuck and sluggish fermentations

BY Gordon Specht, Lallemend

Stuck or sluggish fermentations have plagued many if not all winemakers at some point in their career. In one survey conducted in France (Association for the Development of Wine Biotechnology, 1996), more than 60% of responding winemakers admitted to having experienced this problem.

Stuck and sluggish fermentations ranks as the fourth most important enological concern to winemakers, according to an American Vineyard Foundation survey (See *PWV*, May/June 2003, p. 5). However, considering that surveyed winemakers' first two concerns are actually related to viticulture and cork-taint, fermentation difficulties can be seen as ranked second in importance only to the influences of winemaking on wine composition and flavor.

Yeast does the fermenting in the winemaking process. If fermentation slows down or stops, some change in fermentation conditions clearly has curtailed the yeast's cellular activity. What causes stuck and sluggish fermentations? The answers are complex and usually interrelated.

Technical information was contributed to this text by: Clayton Cone, alcoholic fermentation and facility hygiene consultant; Dominique Delteil, Institut Cooperatif du Vin scientific director; Laurent Dulau, Vinidea Srl owner and oenology consultant; Anne Julien, Lallemend fermented beverage R&D director; Lanfranco Parronetto, alcoholic fermentation consultant in Italy; Gianni Trioli, Vinidea Srl owner and oenology consultant; Paula Vagnoli, Lallemend Italia R&D director.

Nutritional imbalance

The principal nutrient deficiency impeding fermentation is lack of usable nitrogen. During growth, yeast cells must consume nitrogen to reproduce nuclear protein and cell protein.¹ They also need nitrogen to produce enzymes. Various sources of nitrogen are available in the must, but not all are accessible to the growing yeast cells.

While the importance of nitrogen is known, it has not been given adequate attention in the wine industry. Many winemakers fail to add a sufficient amount of usable nitrogen; indeed, nitrogen is often added only when problems arise. By that time it is usually too late.

The best form of usable nitrogen seems to be a combination of diammonium phosphate (DAP) and autolysed yeast extract. A good balance of yeast-accessible vitamins and minerals, such as thiamine, niacin, folic acid, and calcium pantothenate, are also necessary for good yeast growth and fermentation.^{2,3,4}

Aerobic survival factors

Lipids are an essential component of the yeast cell membrane. They are necessary for the budding and growth of yeast cells in the early stage of fermentation and to protect the yeast cell from alcohol toxicity in later stages.⁵ Once the yeast enters the anaerobic growth phase, each budding cycle depletes the amount of lipids by half. If insufficient lipids are available, the cell's semi-permeable membrane does not function properly. It cannot maintain the osmotic balance of nutrients (sugar) inside the yeast cell and by-products (alcohol) outside, so the growth cycle is restricted.

Adding oxygen somewhere between one-third and midway through fermentation helps the yeast produce its

own lipids.⁶ Another way to prevent the depletion of lipids is to add them just after the yeast inoculation in the form of yeast hulls. The cell wall of yeast hulls contains not only lipids but significant amounts of polysaccharides, including chitin.

Chitin increases the surface area in the must, which helps keep the yeast cells from settling to the bottom of a fermentor, where they may become weak and stressed. Improving the yeast's ability to stay in suspension is especially important if you are fermenting highly clarified juices or musts from concentrates or after bentonite settling or heavily ameliorated or very cool fermentations.

Temperature management

Temperature plays an important role during yeast multiplication early in the fermentation process. When yeast multiply rapidly, they produce ethanol faster than they can excrete it. As a result, alcohol accumulates within the cell and negatively alters the physiological state of the cellular membrane. This early excess of intracellular alcohol will limit the cell's resistance to alcohol toxicity toward the end of fermentation, contributing to stuck or sluggish fermentation.

From a yeast's viewpoint, and depending on the initial sugar concentration of the must, the optimal fermentation temperature begins under 25°C (77°F) and takes more than three days to increase to a maximum of 28°C (82°F) for a 22.5° Brix juice or must.

The more yeast is stressed early in fermentation, the lower the peak fermentation temperature it can tolerate.⁷ Different thresholds of resistance have also been found among different enological yeast strains.

Natural wine yeast strain selection

To avoid stuck and sluggish fermentations, take several criteria into account when selecting yeast strains:

- **Lag phase and multiplication** — A yeast strain's ability to compete with wild microflora and dominate a fermentation depends on its lag phase (The initial phase where the yeast builds up its cell food reserves after rehydration and adding it to the fermentor before the cells start to divide. This usually takes about six to 12 hours

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on average in a typical wine fermentation.) length and its rate of multiplication. The lag phase should be short since the longer the lag phase, the longer the contaminating organisms have an opportunity to flourish and cause off-taints.

This depends on the fermentation conditions but ideally you want don't want the exponential growth phase to occur so quickly that the alcohol accumulates in the cell, causing problems later on, but you also don't want it to be too slow since it might not dominate the fermentation. About every four to eight hours would be a good average time period for every doubling. Usually about one-third of the sugars are used up by the end of the yeast's exponential growth phase.

- Suitability for the selective conditions of the must — Yeast strains have widely varying capacities to withstand difficult fermentation conditions, particularly high alcohol (14%) content, low pH values and extreme temperatures, which can vary from 12°C (50°F) to 35°C (95°F).

- Fermentation performances in must with nitrogen and oxygen deficiencies — These also vary with different strains.

- Resistance to the competitive-factor proteins. There are three major categories of yeasts:

- * "Competitive-positive," which dominate competitive-sensitive yeasts,

- * "Competitive-sensitive," which are dominated by competitive-positive yeasts and do not produce competitive-factor proteins,

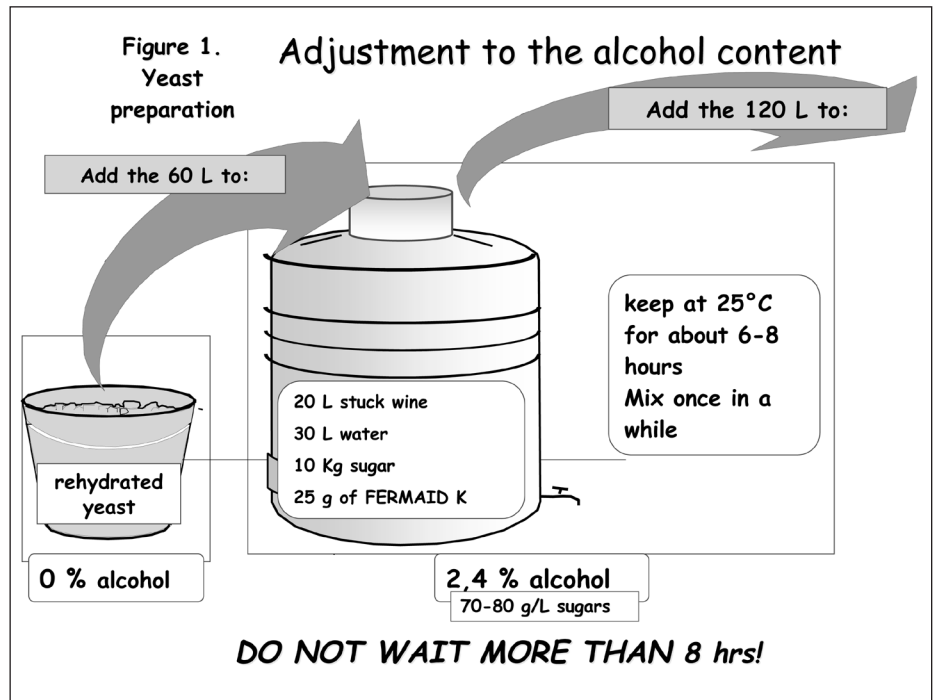


Figure 1. Activation and adaptation of the yeast starter.

* "Competitive-neutral," which are not affected by and do not produce the proteins.

The K2 competitive-factor protein is most commonly found among the competitive-positive yeast strains that produce the competitive-factor protein.⁹ This K2 competitive-factor protein is also the only one showing any significant activity at the pH conditions found in wines. The use of

strains with resistance to the K2 protein is therefore strongly recommended, especially when it can be assumed that the must contains a large K2 yeast population.

Yeast rehydration

When using active-dried wine yeast, proper rehydration is critical in order to avoid problem fermentations. The drying stage removes the living cell's extra-cellu-

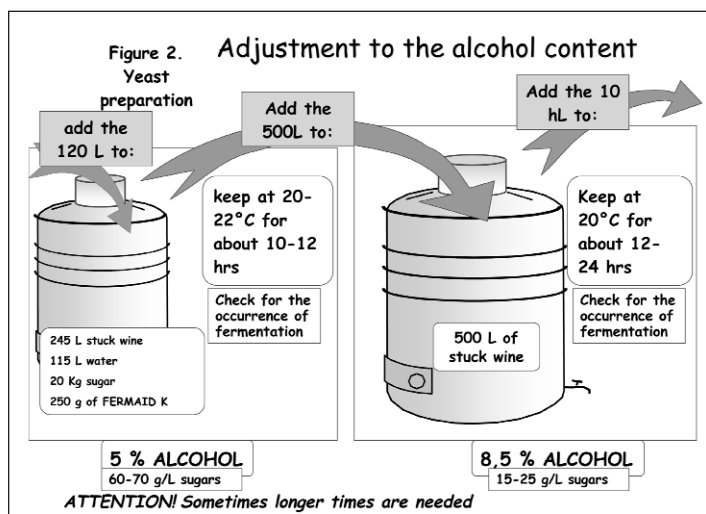


Figure 2. Expansion of the adapted yeast starter.

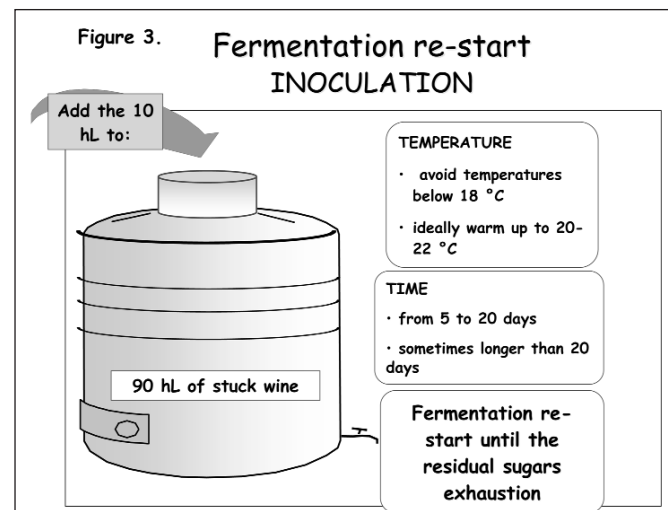
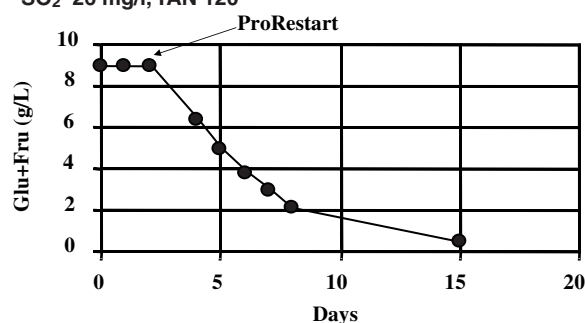


Figure 3. Inoculation of the adapted starter culture.

ProRestart® at 14.2% EtOH



Evolution of residual sugars, Syrah 2001. pH 3.62, volatile acidity 0.39 g/l, total acidity 5.6 g/l, free SO₂ 10 mg/l, total SO₂ 26 mg/l, YAN 120



ProRestart® at pH 3.02



Evolution of residual sugars, Pinot 2001. Alcohol 11.9%, volatile acidity 0.33 g/l, total acidity 7.5 g/l, free SO₂ 13 mg/l, total SO₂ 45 mg/l, pH 3.02, FAN 120

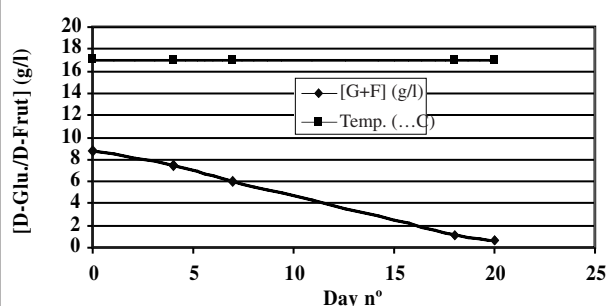


Figure 4 & 5. Restarting a stuck wine under high alcohol and low pH using encapsulated yeast.

lar water, most of the water within the cell, and also the water bound to the cell's organelles. In the dryer, the yeast cells shrink and desiccate, and essentially go into a deep sleep.

To be functional again, the dried yeast cells must reabsorb all this lost water. When the dried yeast comes in contact with water (or any other liquid), the cells literally act like dried sponges, sucking up the needed water in seconds.

Yeast cells will not disperse in must effectively if not properly rehydrated, and they can also lose a large amount of cytoplasm (the guts of the cell),¹⁰ reducing the efficiency of oxygen and nutrient transfer to the cells. This impedes growth and activity, leading to sluggish or stuck fermentations. Proper rehydration can ensure healthy yeast cells and good fermentation characteristics.

It is best to rehydrate in water rather than in must, even though must contains sugars that may help shorten the lag phase, because must may also contain SO₂ or residual fungicides that could be lethal during the rehydration stage. Once rehydrated, the yeast cells can resist SO₂ and low levels of fungicides, but not during liquid uptake.

Avoid cold-shocking the yeast by adjusting the yeast suspension temperature. Do this by slowly (over 30 to 60 seconds) mixing an equal amount of juice to be fermented with the rehydrated yeast suspension. The temperature difference between the rehydration medium and the must to be inoculated should never be greater than 10°C (18°F).

Finally, be sure to inoculate with a high enough initial yeast population (2 to 6 million CFU per ml for most wine fermentation depending on the yeast strain and sugar content of the must). This is especially important because of the trend to harvest grapes at higher sugar maturities. This helps overcome an over-dilution of the initial yeast cell inoculation making it possible to reach the maximum yeast cell population necessary for a healthy fermentation.⁷

Natural inhibitors

Natural inhibitors, such as medium chain fatty acids¹¹ and acetic acid, also contribute to stuck and sluggish fermentations.¹² High levels of acetic acid may be produced by contaminating organisms or selected wine yeasts, especially under a deficiency of aerobic factors.¹³ Osmotic shock due to high starting sugar concentrations will also cause the yeast to lose much of its activity.⁷

Residual pesticides

Pesticide residues have also been implicated as a cause of fermentation problems. Metholachlor and Propanil have been shown to be the main compounds that inhibit yeasts.¹⁴ Some pesticides, such as dichlofluanide, can increase the length of the lag phase, thus delaying the start of fermentation.¹⁵

Pesticide residues can either act directly (as fungicides do) or indirectly (as when the yeast has to detoxify the fermentation medium before it can start fermenting). In years of late-season

drought, high concentrations of pesticide residues may remain on the grapes, resulting in higher rates of stuck and sluggish fermentations.

Stuck and sluggish fermentation remedies

There are a number of ways to restart a stuck fermentation. Two methods are proposed below. The first method follows recommendations proposed by Lanfranco Parronetto and Paola Vagnoli of Lallemand, Italy, based on their experiences with troubleshooting stuck fermentations over many years. The second method involves the use of encapsulated yeast technology.

Many stuck fermentations are difficult to trace back to their root causes. When this is the case, the Parronetto-Vagnoli procedure for restarting the fermentation is as follows:

If you can rule out inhibitors or residual pesticides as the cause which is very difficult and expensive to analyse for, you can skip the addition of adsorbants during a stuck wine preparation. Stuck ferments caused by an obvious microbial contamination and/or a high level of inhibitors will require more emphasis on their removal during the stuck wine preparation.

A. Stuck wine preparation

1. Take the necessary precautions to avoid growth of spoilage bacteria by adding SO₂ (avoid adding too much which will inhibit the rescue yeast). Gently rack the wine off the yeast lees to help eliminate potential inhibitory sub-

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stances attached to the lees, which could leach back into the wine. Filter the wine if more practical.

2. Adsorb the inhibitory substances with 25 g/hL yeast hulls and 125 g/hL cellulose. Avoid adding yeast hulls in excess of 25 g/hL as too much can impart a yeasty character to the wine. For cellulose to be effective, it should be added at five times the yeast hulls' addition rate.

3. Gently stir the yeast hulls and cellulose into the stuck wine to ensure good dispersal and contact, then allow them to settle for about 48 hours.

4. After allowing the adsorbents to work for 48 hours, rack (or filter if more practical). Add 25 g/hL Fermaid K and 50 g/hL of cellulose. The stuck wine is now ready to be inoculated with the rescue yeast that may be prepared as you are finishing the racking off or filtering of the wine.

B. Rescue yeast preparation

The following protocol is based on restarting 100 hL of stuck wine at 12% alcohol with 15g/hL residual sugar. If you had 1,000 hL or 10hL of stuck wine, nothing would be different except for the scaling up or down depending on the volume.

Select a strain that is both alcohol-tolerant and a vigorous fermenter, such as Uvaferm 43, Lalvin DV-10, Lalvin ICV K1(V-1116), or Enoferm L2226. Avoid using any yeast strain that has already been used in the fermentation. Generally it is sufficient to add 50 g/hL. However, if you lack good temperature control in the cellar, use 100 g/hL.

Suspend 5 kg of Go-Ferm yeast-starter nutrient into 60L of clean water at 43°C (110°F). Once the suspended Go-Ferm solution drops to 40°C (104°F), add 5 kg of the selected yeast to this suspension.

Add the yeast slowly to the water while gently stirring to avoid clumping. Allow the rehydration suspension to sit for at least 15 and no more than 30 minutes before addition to initial wine/water mixture.

Add the 60 liters of rehydrated and suspended yeast solution to a mixture of 20L of stuck wine, 30L of water, 10kg of sugar, and 25g of Fermaid K to help adapt the yeast to increasing levels of alcohol (see Figure I).

Maintain at 25°C (77°F) for six to eight hours with occasional stirring. DO NOT ALLOW this step to progress beyond eight hours as all the reducing sugars will

be used by the yeast. They will have difficulty recovering from the lack of food.

After six to eight hours, expand the above 120 L mixture to a 500 L mixture of (see Figure II):

245 L stuck wine

115 L water

20 kg sugar

250 g Fermaid K

Maintain this mixture at 20°C to 22°C (68°F to 72°F) and monitor for fermentation activity. It should take about 10 to 12 hours to ferment 50% of the reducing sugars, but it may take longer if the conditions are more difficult. Once 50% of the reducing sugars have been fermented, it is necessary to add this 500 L (5hL) mixture to 5 hL of the stuck wine for the next expansion. DO NOT WAIT to make the next expansion, and do not allow the fermentation to progress too far beyond 50% sugar depletion. If you wait and all the reducing sugar is used up, there will be a rapid loss of yeast activity.

Maintain this 10hL mixture at 20°C (68°F) and monitor the fermentation activity until 50% of the reducing sugars are fermented, which may take about 12 to 24 hours. Again, it is very important to make the next expansion before the sugar level drops too far.

C. Inoculation of the stuck wine

At this stage, the 10hL alcohol-adapted yeast starter can be directly inoculated into the remaining 90hL of stuck wine (Figure III). Make sure when using this approach to keep the wine temperature between 20°C and 22°C (68°F to 72°F) and to avoid letting it drop below 18°C (65°F). The remaining fermentable sugars should ferment out in as quickly as five days, but may take longer than 20 days.

Under more difficult restart conditions, consider adding the stuck wine to the 10 hL alcohol-adapted yeast starter in small amounts. This may be necessary when the previous expansion stages progress too slowly, or when the wine has a high alcohol (over 14%) and/or free SO₂ level, or is held at a low temperature.

If incremental additions of the stuck wine to the rescue yeast preparation are necessary, do them in three phases:

Phase I — Add 10hL of the stuck wine and wait 48 to 72 hours for the fermentation to appear active before going on to the next phase.

Phase II — Add 30hL of stuck wine and wait another 48 to 72 hours for the fermentation to appear active once again before going on to the final phase.

Note: In both Phase I and II, it is critical to avoid letting the yeast use up all fermentable sugars before continuing on to the next phase.

Phase III — Add the remaining 50hL and allow the fermentation to complete.

Using encapsulated yeast technology

The second method for restarting stuck fermentations has been used successfully for many years in secondary in-bottle fermentation. This relatively new use of encapsulated yeast technology offers both greater convenience and improved wine quality compared to traditional methods of restarting stuck fermentations. The method reduces wine handling and keeps spoilage to a minimum.

Background

The technique of encapsulating yeast cells in alginate gel, a natural polysaccharide extracted from seaweed, allows the food and byproducts to move throughout the gel without releasing the *Saccharomyces* yeast cells into must. The encapsulated beads are made of two different layers: the inner layer containing the yeast cells and the outer layer made of sterile alginate. The beads are partially dehydrated in a fluidized bed column and stored at 4°C until ready for use.

Pro-Restart is a new product developed and patented by Proenol and Lallemant for completing stuck or sluggish fermentations quickly and easily. It uses selected rescue yeasts that have been acclimatized to a harsh wine environment during the encapsulation process. This treatment allows easy direct inoculation into a stuck or sluggish fermentation.

Yeast handling and inoculation

Dried Pro-Restart encapsulated yeast beads are rehydrated by removing them from the recommended 4°C storage and allowing them to reach room temperature to avoid thermal shock. Before rehydration, the beads are placed into nylon mesh bags and distributed throughout the nylon bag to ensure good contact with the wine. Use 75g/hL of stuck wine.

Add the beads to a volume of water five times their weight that also contains 10 grams of sugar (grape or other) per liter.

Leave them in this rehydration solution for four to five hours before inoculation.

At inoculation, the temperature difference between the encapsulated yeast beads and the wine must be less than 10°C. If several bags are added to the same tank, they must be placed at different heights for better distribution, so the fermentation gets re-activated throughout the entire body of wine. Hang a weight (ballast) beneath the bags to prevent them from floating.

Shake the bags several times each day to release the CO₂ accumulated around the beads. Stir or pump the wine over once or twice per day without aeration. Leave the beads in the wine until the desired residual sugar endpoint is reached.

Pro-Restart will not work miracles restarting stuck fermentations in difficult wine conditions. However, the beads are a useful alternative to traditional restart methods, especially where impact on final wine quality is a concern. Their convenience and their lowering of the quality loss risks associated with using a traditional restart protocol. Figures IV and V show data under low alcohol and low pH conditions. In North America in 2003, one stuck red wine with over 16% potential alcohol was successfully restarted. (personal comm. from Vinquiry).

Summary

The best way to deal with stuck and sluggish fermentations is through prevention, but no matter what precautions you

take, problematic fermentations may occur.

Whatever method you decide to adopt for resolving your stuck fermentations, begin your approach by finding out the basic numbers in order to adjust these parameters if necessary — especially the pH, alcohol, residual sugars, volatile acidity, total and free SO₂ — of the stuck wine. It also helps to have a good microscope or access to a competent microbiology lab in order to see the microbial soup. That will help you determine what actions to take if needed as the best approach for restarting the fermentation. When in doubt, contact your fermentation products supplier or micro-lab for assistance. ■

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