The 15 key points for alcoholic fermentation (AF)

Or how to always complete your AF's successfully

*AF : Alcoholic Fermentation

GROUPE ICV
L'art & l'expertise du vin

The 15 key points for alcoholic fermentation (AF)

" YEAST ARE AUTONOMOUS LIVING BEINGS "

15 main factors directly influence their life, activity and survival during alcoholic fermentation.

These factors have been validated for selected yeast and in a cellar that practices good hygiene. Specific preventive actions to manage contaminants and unhealthy grapes are not addressed here.

" THE END RESULT OF THE AF DEPENDS ON MASTERY OF THESE 15 FACTORS "

Different wine yeast will have variable reactions to these factors.

The purpose of this document is to describe the critical points to be mastered in order to ensure the complete success of an AF. Operational indicators, i.e. parameters that can be measured in the cellar, and several solutions are proposed for each of these points.

THE 4 MAIN OBJECTIVES OF THE ALCOHOLIC FERMENTATION (AF)

- Ensure complete and rapid fermentation of the sugars
- Limit the production of volatile acidity (VA) during the first third of fermentation
- Avoid the production of sulphur compounds with unpleasant odors throughout the fermentation
- Achieve the desired aromas and taste.

Throughout the document unless otherwise stated, the terms "yeast" and "wine yeast" refer to yeasts of the species Saccharomyces cerevisiae.

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ON INOCULATION

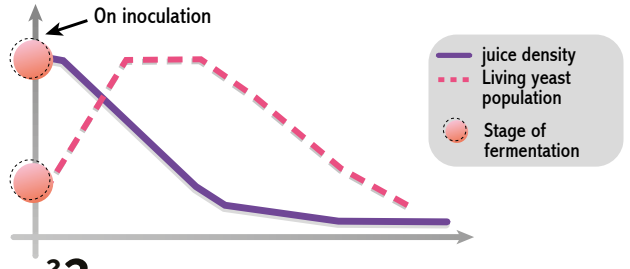
Osmotic and thermal shock in juice

Osmotic shock is due to the sugar concentration of the juice. It is the first major event in the life of a population of wine yeast. This shock impacts the physiology of the cells throughout their life, i.e. until the end of fermentation. In particular, the ability of the yeast biomass to consume all of the fermentable sugars depends indirectly on the intensity of the initial osmotic shock and the ability of the yeast to adapt to it.

Different yeast vary in their ability to withstand osmotic shock, especially in sugar-rich juices above 13% potential alcohol.

This is an important selection criterion for wine yeast to be used to ferment high sugar musts and juices.

The second shock concerns temperature. The difference between the temperature of the water used to rehydrate the yeast (normally 35°C/95°F) and the temperature of the juice impacts the ability of the yeast to take up a certain number of compounds such as nitrogen from the medium. This difference directly or indirectly limits the growth and activity of the biomass.



Question/Answer

To succeed when adding yeast, what should I prepare?

> *The important thing is to make sure that everything is available and working: clean container, hot water and thermometer, a mixing utensil and a stopwatch, to prepare the yeast under the best conditions.*

KEY POINT N° 1

Adaptation of the yeast to the high sugar content of the must: the initial osmotic shock

This shock can be evaluated directly from the natural potential alcohol level. The higher it is, the more energy the yeast has to expend to maintain the balance between the inside and the outside of the cell. The yeast has to synthesize more glycerol*[2].

At the same time, it produces more acetic acid. This production increases when the fatty acid* and sterol* resources are insufficient and when the yeast is subject to other stresses: temperature, SO_2, etc. Although there is no (or very little) alcohol in the medium, **irreparable damage to the yeast membrane* may occur at this stage and will affect future generations of yeast until the end of fermentation** and in particular when the yeast are of insufficient quality or when **good rehydration practices** have not been correctly applied.

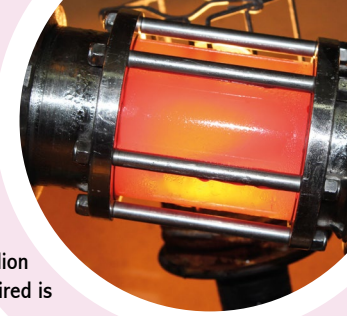
There is no real way to reduce the source of risk associated with this factor since, in general, ripeness depends on market and/or sensory objectives. It is essentially a factor that is present and that we can rebalance, from the point of view of the alcoholic fermentation and the yeast, by the choice of yeast inoculation rate (increasing it in proportion to the sugar concentration) and by managing the following points.

Question/Answer

But how do I choose my yeast?

> *The first point is to be sure of its capacities and to evaluate them in relation to the grapes that will be harvested and the planned vinification. How much sugar can it ferment? How much assimilable nitrogen does it need? What temperatures can it withstand without major risk? Then, you have to assess its impact on the profile of the final wine, and for that there is nothing better than to taste wines from comparative trials or do your own experiments with several tanks.*

For all terms followed by *, see the lexicon at the end of the document.



KEY POINT N° 2

Concentration of stress-resistance factors in the cells

Stress-resistance factors are important **for the proper functioning of the cell membrane: they are the unsaturated fatty acids* and sterols***. Their concentration can be evaluated indirectly by the dose of active dry yeast (ADY) that is added. During cell multiplication in grape juice, the yeast has a fermentative metabolism*. It is difficult for it to synthesize these stress-resistance factors. **The initial concentration in the cells will be diluted throughout the cell multiplication phase.** Conversely, during industrial production in a real fermenter, where the Crabtree effect* is avoided, the yeast respires. With this respiratory metabolism, it can more easily synthesize and accumulate these stress-resistance factors.

In AF tanks, multiplication takes place during the consumption of the first 50-60 grams of sugar, i.e. at approximately "5-6 drop in °Brix". A grape juice is completely colonized by a wine yeast population when there are approximately 100 million cells per millilitre, the moment when peak fermentation rate or V_{max} is reached (see "Did you know"). This maximum colonization level has relatively little to do with the initial population. This means that to reach 100 million per millilitre, the number of generations required is fewer when the initial population is high. There is, therefore, less dilution of the initial stock of resistance factors. Up to 30 g/hL, **the higher the dose of ADY, the richer the cells are in resistance factors during multiplication and they remain so when the maximum population is reached.**

Apart from the special case of inoculation without rehydration (see "Did you know?"), **it is essential to strictly follow the rehydration conditions given on the active dry yeast packet, in particular the water temperature (which should be measured) and the rehydration time (which should be monitored).**

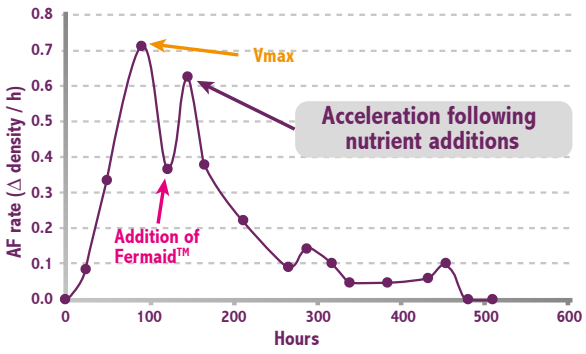
The concentration of stress resistance factors in the yeast can also be increased through two points, namely the management of cold settling (point 3) and the addition of stress resistance factors during rehydration (point 4).

Did you know?

AF fermentation rate

> *It can be **measured** in several ways. Researchers use the release of carbon dioxide (CO_2) per unit of time (t). The formula is then $V = dCO_2/dt$ where dCO_2 is the quantity of CO_2 released over time dt. In the cellar it can be monitored by regularly recording the density (D) and the time of day (t) when this measurement is carried out. In this case, $V = -dD/dt$ (with the minus sign so that it keeps a positive value) or more simply $V = (D_1 - D_2) / (t_2 - t_1)$ where D_1 is the density measured at time t_1 and D_2 the density measured at time t_2 (and $t_2 > t_1$). The curve then looks like this:*

Example of fermentation rate curve based on density



*The interest is to measure and visualize V_{max}, the maximum fermentation rate. It precedes the **most favorable moment** for the addition of assimilable nitrogen and oxygen to secure the fermentation kinetics. These additions re-accelerate the rate of the AF, hence the second peak in the curve above.*

KEY POINT N° 3

Cold settling of Whites and Rosés: removal of sterols and fatty acids*

With cold settling, the removal of particulate material and pectic flakes (light juice lees)* also removes the unsaturated fatty acids* that are of interest to the yeast. These lipids (fatty acids and phytosterols) encourage both the consumption of assimilable nitrogen by the yeast and the formation of membrane sterols that allow the yeast to survive during the last quarter of the fermentation. They are hydrophobic and therefore not in solution in the juice. This factor can be **indirectly** evaluated by the intensity of clarification (final NTU or concentration of suspended solids).

Clarification degree is often dictated by the style of wine required. Below 200 NTU (or for juices that are perfectly clear to the naked eye) it is advisable to offset the risks through other factors: a higher ADY inoculation rate, addition of protectors and complex nutrients*, reduced thermal shock.

The use of "neutral" additives to create turbidity, such as cellulose or yeast hulls, does not affect the fermentation kinetics but has mainly a sensory effect.

Question/Answer

I settle my juices so that they are very clear. Is this dangerous?

> *It can be if the potential alcohol is above 12.5 – 13%. But we can partially compensate for this risk by increasing the yeast inoculation rate and above all by preparing the yeast in a rehydration water suspension containing GoFerm Protect Evolution™.*

KEY POINT N° 4

Addition of fatty acids*, sterols*, amino acids* and micronutrients* on inoculation

The first operational indicator* is the ratio between the **addition of complex protectors* containing inactivated yeast such as GoFerm Protect Evolution**™ and the inoculation rate, at the time of rehydration. At this moment, the yeast has the ability to take up the sterols in these preparations with the micronutrients they contain. These protectors do not supply assimilable nitrogen directly but promote a better physiological and structural condition of the yeast wall and membrane.

Like all living things, yeasts need amino acids*, fatty acids*, micronutrients* (vitamins* and minerals) during the cell multiplication phase. In addition, they need sterols* to withstand the osmotic shock and the alcohol. In juice, some of these elements are present but not bioavailable when the yeast need them most.

The addition of organic or complex protectors that contain these different elements in a bioavailable form can **reduce the risk of nutritional stress on the yeast.** At the time of inoculation, an addition contributes to cell multiplication of the yeast under healthier conditions.

Each wine yeast has its own needs. Nutrient addition levels are determined by the choice of wine yeast and other factors, such as the sanitary state of the harvest (a poor sanitary state leads to lower levels of assimilable nitrogen), the potential alcohol (more sugar to ferment = greater need for assimilable nitrogen), the turbidity level, the thermal regime applied, temperature extremes during AF, any oxygenation that is carried out, etc. The second operational indicator at this phase (overlapping with the so-called "cell multiplication" phase dealt with in chapter B) is the addition (or not) of organic or complex nutrients such as **Fermaid O**™.

The purpose of these two steps (protector during rehydration and early nutrient addition) is to reach a sufficient population level for good **fermentation capacity** and an adequate **survival level** to successfully complete the alcoholic fermentation.

KEY POINT N° 5

Induced deficiencies

Beyond the initial concentration of vitamins, trace elements and assimilable nitrogen in the must in which the yeast biomass will grow, deficiencies can result from the growth of "indigenous" microorganisms (i.e. those naturally present in the tank ecosystem, whatever their species or their provenance). These induced deficiencies can lead to sensory deviations (e.g. sulfur off compounds), analytical deviations (e.g. volatile acidity) or to sluggish or even stuck fermentations.

Question/Answer

If my grape lacks nitrogen, should I compensate from the start?

> *It is better to divide the addition in 2, with the most important addition being the one made at around 1/3 through the alcohol fermentation (AF). And with a preference for organic nitrogen.*

Likewise, the pre-multiplication of a starter with visible fermentation activity, places a very high population of yeast in a very small volume of must, induces deficiencies with similar effects (see "Did you know?").

Did you know?

The bad idea of "pre-multiplication of yeast"

Rehydrating the yeast and then starting to ferment them "to let them acclimatize" under standard conditions is very risky. The amount of yeast calculated for the total volume of the tank multiplies and starts to ferment in just a few % of this total volume. It ***therefore quickly lacks vitamins, micro-nutrients and assimilable nitrogen.*** *The yeast multiplied under these* ***stress conditions*** *(very unfavorable biomass / nutrient ratio) generally result in difficult fermentations, higher volatile acidities and more frequent negative sulphur off compounds.*

Direct inoculation, at an **early stage**, as well as **the absence of sensitivity to the killer** (competitive) factor* (neutral or K2 phenotype) of the chosen yeast, are the ways to limit this risk.

The operational indicators* are the absence of pre-multiplication, the sensitivity or not of the yeast, and the time between starting to fill the fermentation tank and introducing the yeast.

N.B. : in case of sequential inoculation (in general a non - Saccharomyces followed by a Saccharomyces), the consumption of nutrients by the "initial" yeast must be taken into account and offset by appropriate additions to provide the best possible conditions for the second yeast.*

KEY POINT N° 6

Temperatures of Whites and Rosés: thermal shock in cold juices

This shock can be directly evaluated by the difference of temperature between the must and the water at the end of rehydration. The greater this difference, the more the yeast will be stressed. A temperature difference greater than 10°C/18°F will have physiological consequences throughout the fermentation.

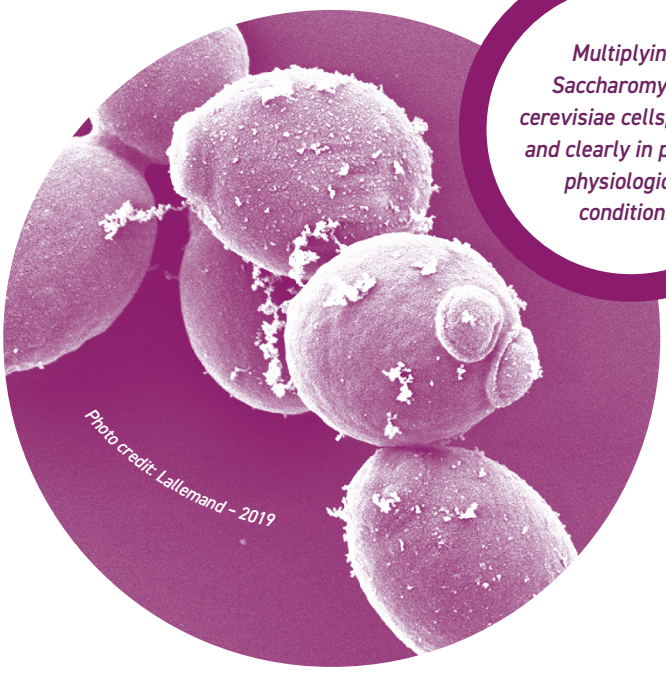
Did you know?

Good rehydration

> *This is **crucial** for the proper establishment of the yeast population. To succeed, the **right tools** are needed: a tub, hot water, thermometer and stopwatch. If only one of these is missing, failure is guaranteed. The ICV provides pictorial procedures that take several scenarios into account (with or without protector, with or without acclimatization). The "standard" case calls for **water at 35°C/95 °F** at a volume **ten times greater** than the mass of the yeast to be rehydrated. The yeasts are **gently mixed** in the water. **A gentle and thorough stirring is carried out after 10 to 15 minutes** and repeated 10 to 15 minutes later. After this second stirring, the tank is inoculated or the acclimatization steps are started.*

Inoculation without rehydration: good or bad idea?

> *3 years of trials were necessary to verify that **only a small number of yeasts** could withstand direct inoculation on grapes or in unfermented must. In all cases, the **inoculation rate must be increased** to offset the inevitable mortality and the practice should be **limited to fermentation conditions analyzed as favorable for all the other key points** of the AF (potential alcohol, nutrient availability, temperatures, etc.).*



Multiplying Saccharomyces cerevisiae cells, turgid and clearly in perfect physiological condition.

Photo credit: Lallemand - 2019

Different yeast have different sensitivity to this stress. When the winemaking technique imposes a thermal shock, it is advisable to choose a yeast that is more resistant to these conditions, or to offset the risk through other factors: higher ADY inoculation rate, gradual acclimatization to the juice temperature, addition of complex protectors.

Question/Answer

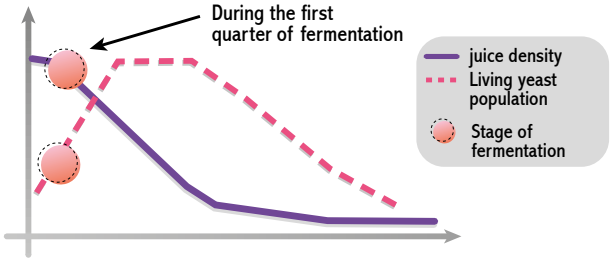
Do I have to wait until my tank is full before inoculating, sometimes the day after starting to fill it?

> *It's not a good idea. You should inoculate when the first red grapes arrive, or at the start of filling for white, rosé or thermo-vinification, with acclimatisation when necessary. This is the best way to establish your yeast, minimise contamination and avoid nutrient deficiencies.*

DURING THE FIRST QUARTER OF FERMENTATION

Multiplication of yeast cells

The multiplication phase corresponds to the consumption of the first 50-60 grams of sugar, i.e. up to approximately "5-6 drop in °Brix" (V_{max}* reached). After the initial osmotic shock, this is another key phase for the yeast. Most of its metabolism is directed towards the syntheses that allow the creation of daughter cells.



For these synthesis, yeast needs various sources of nutrients*: **all forms of assimilable nitrogen*** (in particular amino acids*) and lipids (polyunsaturated fatty acids*, sterols) (see point 4 above). **When there are imbalances, the cell membrane* may already be damaged and production of acetic acid may increase.** Nitrogen supplements and their addition times should be chosen according to the desired style of wine.

KEY POINT N° 7

Low temperature during the first 48 hours

Low temperatures during the cell growth phase (in general ≤ 15°C/59°F) limit the ability of yeasts to assimilate available nitrogen, especially in the form of ammonia. The overall capacity of the yeast to take up nitrogen from the medium decreases as the alcoholic fermentation progresses, an initial "deficiency" induced by low temperature during the first 48 hours may not be offset by increased nitrogen consumption later in the fermentation.

Some yeasts are more sensitive than others to these conditions.
The relevant indicator is the must temperature.

Question/Answer

With cold soaking before fermentation, is it necessary to inoculate when filling?

> Yes. Otherwise contaminating flora can become established that will consume the nitrogen and vitamins that the yeast will then lack. There are many biocontrol solutions both for Saccharomyces and for non-Saccharomyces.

KEY POINT N° 8

Oxygen addition At initial density minus 2.5 °Brix

During cell multiplication, yeast uses this oxygen to synthesise monounsaturated fatty acids* and ergosterol*. This puts the cell membrane* in better physiological condition and limits the risk of excessive volatile acidity* production. Some yeasts are more sensitive to this than others: for them, daily additions of O_2 (1 to 2 mg/L) during the multiplication phase systematically lead to lower levels of VA.

To avoid the risk of oxidation, **it is important to add this oxygen when the population is sufficiently large and the juice is completely saturated with CO_2.**

The addition of 3 to 5 mg/L of dissolved oxygen is effective. This can be done by pumping over the entire volume of the tank via an open tub, rack and return with aeration, or direct injection of oxygen using a device designed and validated for this purpose, such as a clicker or sparging system.

Question/Answer

I'm afraid of oxidising the aromas. Can I do without adding aeration?

> It is a major risk not to aerate, especially at 1/3 AF when any added oxygen is completely consumed by the yeasts in a few seconds. There are precise tools that allow you to aerate just what is needed for the yeast.

KEY POINT N° 9

Reds: yeast multiplication rate and effect on temperature control

When yeast multiplies rapidly, the kinetics of ethanol production are higher, a fast initial rate leads to an increase in temperature that is difficult to control, especially for large fermentation tanks. **This leads to deterioration in the physiological condition of the cell membrane***, even if the alcohol concentration in the juice is still modest. This early deterioration limits the cell's resistance at the end of fermentation.

In grape juice, the cell multiplication rate is highest at 28°C/82°F. To avoid excessive production of alcohol at the start of fermentation, it is recommended to **maintain the juice below 25°C/77°F up to "initial density minus 30 points"**, or to limit the drop in density to no more than 2.5 °Brix per day.

Such a thermal regime (T < 25°C/77°F) also makes it possible to perform longer extractions under low-alcohol conditions, to promote diffusion of tannins and hydrophilic polysaccharides, which can be the most interesting from the sensory point of view.

Question/Answer

I like to practice high temperature cap management in order to aid extraction. Are there any reasons to avoid this?

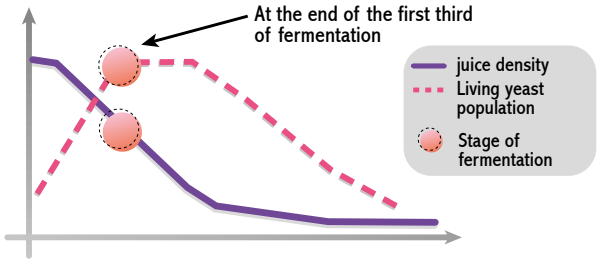
> *Yes, the hotter the grapes, the more the yeasts suffer and die, regardless of when they "get hot". The alternatives are quite simple: perform one or two early rack and return operations and increase the enzyme addition rate at filling, when this is authorised.*

AT THE END OF THE FIRST THIRD OF FERMENTATION

The stationary phase of the yeast population

At initial density minus 30 points, or more generally between density 1060 and 1040 (or after reaching V_{max}), the yeast population has completely colonized the medium. The active multiplication of cells stops. The yeast direct their metabolism* towards **resistance to difficult conditions in the medium**: a decreasing nutrient availability and an increasing ethanol concentration.

At this point, **the yeast cell can still store membrane resistance compounds**: sterols* and monounsaturated fatty acids*. It can also store ammonia* and amino acids* for synthesis of proteins involved in membrane transport systems.



KEY POINT N° 10

Reds: maximum temperature reached during fermentation

This is the only factor that has a direct lethal effect on the cells. This effect depends on the condition the yeast is in. The more the yeast has been stressed before, the lower the maximum temperature it can tolerate.

There are known differences between the resistance of different wine yeasts.

For most Mediterranean and Rhône grape varieties, **above 28°C/82°F there is a strong risk of very high mortality**, which will not allow survival of a population sufficient to complete the consumption of the sugars. For the reasons indicated above, it is recommended to **further reduce this temperature when the sugar concentration is greater than 22 °Brix.**

Question/Answer

I usually do fining during the AF. Does this disturb the yeasts?

> *Fining agents have no known effect on fermentation, whether it's bentonite or No Brett Inside™, for example, which have been studied in detail.*

KEY POINT N° 11

Addition of fatty acids, sterols and amino acids / Addition of ammonium phosphate*

AT THE END OF THE FIRST THIRD OF FERMENTATION

The operational indicator* is the addition of organic or complex nutrients* containing inactivated yeast or yeast autolysate, with their addition rates (see "Did you know?"). These nutrients provide all forms of **assimilable nitrogen***. The addition of nutrients* that contain these various components in a bioavailable form can reduce the risk of nutritional stress on the yeast.

Did you know?

Nitrogen requirements

*They depend on the **quantity of sugar to be fermented and the yeast chosen**, some having clearly greater needs than others. By subtracting what is already naturally present in the must, it is possible to **calculate the deficit** and thus define the strategy to make up for it through judicious additions. **Each tank must be checked** before the start of fermentation to determine the availability in the must, as the variability between vineyard plots and between vintages is very high in the Mediterranean and Rhône areas. Several strategies are possible depending on the nitrogen source used and the kinetic and sensory objectives. In general, or in the absence of measurement of the initial assimilable nitrogen, a first addition is recommended just after inoculation and a second at the end of the first third of fermentation.*
Strongly increasing the inoculation rate decreases the number of cell divisions accomplished. It is an alternative when assimilable nitrogen nutrients are not available, even if it does not always resolve this kind of situation.

Different wine yeast have different nutritional needs. The addition ratios depend on the wine yeast and **other measurable factors**: initial assimilable nitrogen, possible addition of nutrients at inoculation, potential alcohol, thermal shocks, maximum temperature, oxygenation.

The other operational indicator* is the addition of ammonium sulphate or phosphate* with its addition rate. In some grapes and juices, excessive addition can cause nutritional imbalance, deviations in the metabolism* of sulphur and the production of sulphur off compounds*. Ammonia is very quickly absorbed by the yeast and can, in reds in particular, lead to a significant increase in temperature with the consequences mentioned in point 10.

Diammonium phosphate is not the most effective nitrogen nutrient. Very simple in chemical form, it requires the yeast to expend energy to be able to take it up as a source of nitrogen in place of other more complex molecules that it needs (amino acids then peptides then proteins), for the regeneration of membrane transport systems.

KEY POINT N° 12

Oxygen addition

AT INITIAL DENSITY MINUS 30 POINTS

At the end of population growth, the yeast uses this oxygen to synthesise monounsaturated fatty acids* and sterols*. This allows it to maintain the **cell membrane* in good physiological condition.**

The addition of 5 to 8 mg/L of dissolved oxygen is effective. This can be done by pumping over twice the volume of the tank via an open tub, rack and return with aeration (with a Venturi on both rack and return), or direct injection of oxygen using a device designed for this purpose, such as a clicker or sparging system, either continuously (in one go, over a maximum 36 to 48 hours) or over several smaller additions.

This O_2 addition is synergistic with the addition of assimilable nitrogen: it is advisable to manage points 11 and 12 together.

Question/Answer

Can copper residues have an effect on fermentation?

> Our recent work at the ICV and with Lallemand shows that they don't. You have to add very high doses on inoculation (> 10 mg/L) before you will see a small negative effect on drawing out the lag phase of some yeasts.

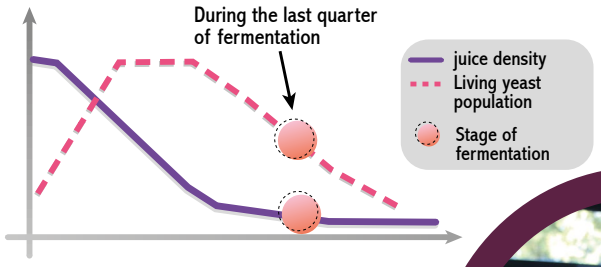
If I co-inoculate the yeast and bacteria, will the AF be more difficult?

> No. Lactic acid bacteria (Oenococcus oeni, Lactiplantibacillus plantarum) cannot compete with Saccharomyces. If there is a problem, it means that one or other of the 15 key points needs to be re-evaluated.

DURING THE LAST QUARTER OF FERMENTATION

Survival of the yeast cells

During the last third of the fermentation, most of the cells are physiologically old, in survival mode. This is the consequence of the previous steps. At this stage, few changes will have a direct effect on individual cells, apart from high temperature which would accelerate their death. **Their good survival essentially depends on good fermentation practices applied in the previous steps.** The practical actions at this stage are aimed only at keeping the entire population of cells in suspension and therefore in contact with the juice. **There are virtually no real possibilities for improving the yeast physiology.**



KEY POINT N° 13

Regularly keeping the yeast in suspension

At the end of fermentation, the rate of CO_2 production is low and there is therefore little natural movement in the juice. As a result, the yeast stay in suspension for a shorter time. **Yeast that settle at the bottom of the tank are no longer in contact with the juice, which still contains sugar. They die from the lack of an energy source and their inability to maintain their internal pH.** There are fewer active yeast, and the dead yeast in a reducing medium frequently release sulphur off compounds*. The more difficult the fermentation conditions (initial osmotic shock and high final ethanol content) and the taller the tanks, the more important it is to regularly keep the yeast in suspension.

The operational indicator is the number of times per day that all yeast are put back in suspension.

Different methods are effective in practice: rack and return, stirring in small containers, agitation using a CO_2 or N2 cane or by adding dry ice pellets, continuous internal pumping using a submersible pump. Closed-circuit pumping over is ineffective because preferential channels are created in the juice and limit stirring at the bottom of the tank. **Stirring also helps to manage the risks of sulphur off compounds** by homogenising the different parts of the tank. It also helps to release yeast mannoproteins* which contribute to the colloidal balance of the wine.

KEY POINT N° 14

Organic nitrogen addition

The yeast retains the ability to take up assimilable nitrogen, essentially in its organic form. In case of sluggish fermentation, small daily additions can help ensure more complete consumption of the sugars.

N.B. **O_2 additions**, in the event of sluggish or stuck fermentation, must however be **avoided**. They have no effect on the fermentation activity of the yeast (apart from the "mechanical" effect of re-suspension which can be achieved in other ways - see point 13) and they can, on the contrary, contribute to the development of contaminants such as acetic acid bacteria or Brettanomyces.

KEY POINT N° 15

Low temperature in the survival phase

Low temperatures at the end of fermentation (below 18°C/64°F) slow down the fermentative metabolism. This results in a lower rate of CO_2 production and therefore less natural movement in the juice.

The risks described in point 13 are increased. **Thermal shock during this phase can stress the cells, which increases the risk of sluggish or stuck fermentations.**

Question/Answer

My fermentation is slow to finish. What should I do?

> At this point, there aren't many solutions. Avoid extreme temperatures, keep the yeasts in suspension (without O_2!) and add small amounts of organic nitrogen such as RESKUE™ specific yeast derivative. After the fact, it is a good idea to take stock to identify what went wrong in the process by reviewing the 15 key points.

If, despite all these precautions, I have a stuck AF, what should I do?

> Follow our guidelines on restarting a stuck fermentation using RESKUE™ and UVAFERM 43 RESTART™ wine yeast.

The table below is an example of a risk assessment grid

CELLAR WORK INDICATORS	HIGH RISK LEVELS (Non-Compliant)	LOW RISK LEVELS (Compliant)	Values for tank No.	Compliance (C, L or NC)
1. Potential alcohol	> 225 g/L of G+F (~21°Brix)	≤ 200 g/L of G+F (~18.5°Brix)		
2. ADY inoculation rate	<10 g/hL of ADY (~0.8lb/1000 US gal)	≥ 30 g/hL of ADY (~2.4lb/1000 US gal)		
3. Induced deficiency	Sensitive yeast or inoculation delay of more than 2h or subculturing/ pre-multiplication	Direct inoculation with neutral or killer yeast		
4. Whites and rosés: turbidity	<50 NTU	150 NTU with 2 to 3% pectic flakes		
5. Addition of protectors	No addition	1.25 times the yeast inoculation rate		
6. Thermal shock	Yeast-juice difference > 10°C/18°F	Acclimatisation in stages, with steps of 10°C/18°F maximum		
7. Temperature during the first 48 hours	< 15°C/59°F	> 18°C/64°F		
8. Addition of oxygen at initial density minus 2.5°Brix	No addition	3 to 5 mg/L of dissolved O_2 and 1 to 2 mg/L/day until 1/3 AF		
9. Reds: temperature during the peak of fermentation	> 28°C/82 °F under the cap	< 25°C/77 °F under the cap		
10. Reds: maximum temperature	> 28°C/82 °F under the cap	< 23°C to 28°C/73°F to 82°F under the cap (depending on initial G+F)		
11. Addition of nutrients at 1/3 AF	No addition when needed or not evaluated	Complex or organic nitrogen depending on the precise deficiency		
12. Addition of oxygen at 1/3 AF	No effective addition	5 to 8 mg/L dissolved O_2		
13. Regularly keeping the yeasts in suspension	No stirring	Daily stirring		
14. Organic nitrogen addition below 5 °Brix	No addition	Small daily additions		
15. Temperature below 5 °Brix	< 16°C/61°F	Between 18 and 20°C./64°F and 68°F		

The examples of "Non-Compliant" and "Compliant" levels take into account the low nutritional levels of high Brix juices in assimilable nitrogen.

Guidelines for interpretation of the overall risk:

1. If the potential alcohol (point 1) or the maximum temperature in red fermentation (point 10) or the temperature during the first 48 hours (point 7) are classed as "Non-Compliant", it is essential that all other points are "Compliant". It will also be necessary to take all possible measures to reach a "Compliant" temperature

2. If one of these indicators is classed as "Limit", it is very risky to have more than 2 "Non-Compliant" key points, even if all the other points are "Compliant".

3. If there is no "Non-Compliant" key point, it is essential that the majority of key points are "Compliant".

Good Fermentation Practices for high sugar juices

Good Fermentation Practices (GFP) are the operational choices that make it possible to **achieve the 4 main fermentation objectives** (see page 1) **with good control of the risks.** For a cuvée, to develop a strategy that optimizes GFP, we start by **listing the key points in the chronological order** of their action on the yeast. We can then determine risk thresholds for each key point **with operational indicators that can be measured in real time in a simple and precise way.**

To fine-tune the organization of work, for each key point we can set a **Compliant** level **(C)**, a **Limit** level **(L)** and a **Non-Compliant** level **(NC)**. This makes it possible to assess the risks during preparation for the harvest and to undertake **priority actions: bringing "Non-Compliant" points into compliance.** In the event of a problem, it is a tool for exhaustive analysis of possible causes.

Potential alcohol has a special place: it is usually set by the style required for the product, and when it is high it increases all other risks.

LEXICON

• Alcoholic fermentation
Anaerobic metabolic pathway by which yeasts produce their energy from the sugars in the juice. With this metabolism, it is difficult for the yeast to produce certain important constituents: monounsaturated fatty acids and sterols.

• Amino acids
The most effective source of assimilable nitrogen for the yeast. Most high brix juices have relatively low amino acid concentrations.

• Ammonium phosphate
Source of assimilable nitrogen for the yeast. Very simple in its chemical form, ammonium phosphate is rapidly absorbed by the yeast. This does not make it the most effective source of nitrogen. On the contrary. To use this source of nitrogen in protein synthesis, yeast expend more energy than during amino acid uptake.

• Assimilable nitrogen
Nitrogen in forms that the yeast is able to use. These are mainly amino acids and ammonium. High Brix juices naturally and frequently have very low levels of assimilable nitrogen. These deficiencies aggravate fermentation risks and they are the cause of imbalances between nitrogen and sulphur metabolisms.

• Crabtree effect
Tendency for the yeast to "waste" sugars. While the respiratory metabolic pathway offers a better energy yield, Saccharomyces cerevisiae, even when the growth medium is permanently saturated with O_2, prefers alcoholic fermentation over the respiration when the sugar concentration in the growth medium exceeds a certain value. This value is between 0.1 and 0.2 g/L.

• Glycerol
Compound produced by the yeast to balance the difference in osmotic pressure between the inside and outside of the cell. The sweeter the juice, the more glycerol the yeast produces. Its production at high levels carries a risk of increased volatile acidity production. On tasting, glycerol produces a transient sensation of sweetness on the mid-palate. It contributes much less than polysaccharides to volume and length on the palate.

• Killer (competitive) factor
At must pH and in the absence of significant levels of ethanol, only Killer factor K2 is active. Yeast known as "Killer K2" (or simply "killer") produce a toxin that eliminates sensitive yeast. "Neutral" yeast do not produce toxins and are not sensitive to them.

• Mannoproteins
Polysaccharide macromolecules of the yeast cell wall. These polysaccharides are released into the medium by the yeast during fermentation and during ageing on lees. Mannoproteins actively contribute to the colloidal balance of the wine: stabilisation of colour, structure and aromas. Different yeast have different capacities to release them (the quantity and quality of the polysaccharides).

• Membrane
Organelle that allows exchanges between the interior of the yeast cell and the juice. The chemical composition, physiological condition and age of the membrane are key points for yeast activity and survival. When this organelle is in good condition, it allows the yeast to withstand osmotic shock in the juice, then to be fully active and finally to withstand alcohol. If the membrane undergoes significant damage when the cell is young (first quarter of fermentation) it will be in a critical condition in the aged phase at the end of fermentation.

• Metabolism
Set of biochemical reactions taking place inside a yeast cell that ensure its survival and reproduction.

• Micronutrients
Present in micro quantities, elements essential to the life of the yeast. These are mainly mineral salts (also identified as trace elements when they are at concentrations <1 ppm) and vitamins. These elements are present in sufficient quantities in juices under normal conditions. However, their bioavailability to the yeast is not always guaranteed.

• Monounsaturated fatty acids
Constituents of the cell membrane. Allow good fluidity and good resistance to stress. The main stresses are osmotic shock on inoculation, high temperatures and high alcohol concentration at the end of fermentation.

• Nutrients
All the elements that the yeast cell must draw from the medium to ensure its vital functions: energy production and syntheses. Mediterranean and Rhône juices have low levels of some nutrients, assimilable nitrogen in particular.

• Operational indicator
A parameter that can be measured in real time, in a simple and precise way, in the cellar. It makes it possible to evaluate the level and compliance of the various key points for fermentation. For example, the operational indicator for osmotic shock is the natural potential alcohol content.

• Pectic flakes (light juice lees)
Particles created by the flocculation of hydrolyzed pectins, certain proteins and other hydrophobic molecules. This flocculation occurs during the first stages of the static cold settling of white and rosé juices. Pectic flakes are rich in (phyto)sterols and polyunsaturated fatty acids: for the yeast, they are stress-resistance factors. In the case of static cold settling without fining and with pectolytic enzymes, the pectic flakes settle last and are positioned above the heavy solids. The pectic flakes can be recovered differentially and then reincorporated into the clear juice before inoculation. This rebalances the content of the clear juice in stress-resistance factors for the yeast.

• Protectors

Group of compounds not directly involved in the construction of the basic building blocks of the yeast cell but having an effect on its ability to divide, maintain activity or survive. This includes sterols, vitamins, micro-nutrients (see these terms), which are naturally present and can be supplemented, usually by the addition of inactivated yeast and/or yeast autolysate. Providing little or no assimilable nitrogen, they do not, strictly speaking, have a nutrient role.

• Sterols

Compounds in the lipid family. Constituents of the cell membrane of the yeast (ergosterol) and the grape (phytosterols). Sterols contribute to the proper functioning of the yeast membrane and, in particular, its resistance to various stresses. Highly clarified juices are low in sterols, which are also less assimilable by yeast than ergosterol. In red winemaking, at the start of fermentation and in the absence of alcohol, the phytosterols from the bloom of the grape are not soluble. In juice, yeast can only synthesize sterols in the presence of a small amount of dissolved oxygen. During industrial production of active dry yeasts, yeast cells synthesize sterols more readily and can accumulate them. During alcoholic fermentation, this initial stock is diluted with the production of daughter cells (and hence with each subsequent generation).

• Vitamins

Substances necessary for the proper functioning of the metabolism, without being sources of energy. Only small quantities are required. Juices contain sufficient vitamins under normal conditions. When there are deficiencies, the yeast metabolism is highly disrupted. Deficiencies are most often due to contamination during the pre-fermentation phases.

• Volatile acidity

In the absence of a bacterial problem, the volatile acidity consists of the acetic acid produced by the yeast. This production takes place during the first third of fermentation when the yeast produces glycerol to balance the osmotic pressure between the inside and outside of the cell.

• Volatile sulphur off compounds

Various compounds produced by the yeast from different sources in the juice: SO_2, vineyard spray residues, certain amino acids. These compounds have very unpleasant odors: rotten egg, garlic, burnt matches, rubber, etc. Different yeast have a varying tendency to produce them. When the yeast is stressed and does not have sufficient and balanced nitrogen nutrition or has to cope with other deficiencies (vitamins, trace elements or sterols, for example) it produces more of these compounds. This is a major risk to be managed throughout the fermentation of high Brix juices.

• Yeast

Autonomous single-celled fungus. Like all living things, yeast cells rely on the medium they are in to draw their nutrients and release their waste. The medium is the juice. The main nutrients are glucose and fructose, amino acids and ammonia, fatty acids, vitamins, minerals. The main compounds released are ethanol, glycerol, mannoproteins, acetic acid and several hundred more or less aromatic compounds. The balance changes with the genetic potential of the yeast, the age of the cells, the composition of the juice and the conditions imposed by the winemaker: sulphite addition, temperature, oxygenation, etc…