

MAXAFERM®

Addition to must of a specific bio regulator
for improved control of alcoholic fermentation.
Effect on fermentation speed and wine characteristics.

Sluggish or stuck and stopped fermentation is difficult to predict, and is a major problem for wine-makers.

There is a greater probability of the problem occurring in a must that lacks assimilable nitrogen or has a high sugar concentration, and also with particular varieties such as Chardonnay.

Some vineyard plots have also been identified as frequently giving rise to such problems although the root cause of fermentation problems is still not known.

Nevertheless, fermentation difficulties arise from a progressive reduction in yeast viability, which in turn is caused by the combined effect of several factors: loss of permeability in the cytoplasmic membrane of the yeast through insufficient synthesis of sterols, the adsorption of toxic fatty acids, an increase in the concentration of ethanol, a reduction in the quantity of assimilable nitrogen and a high concentration of CO₂.

This wide variety of causes has two effects. Firstly, it is not possible to predict fermentation difficulties by prior analysis of the must with any degree of certainty. And secondly, adding ammonium salt is not a panacea but only treats the relatively simple problem of shortage of assimilable nitrogen.

*The purpose of this study is to define a specific fermentation activator to be added to the fermentation must in order to prevent (or treat) the majority of cases of stuck fermentation. The defined formula incorporates ammonium salts, thiamin and thermally inactivated yeast. The strain used, *Saccharomyces cerevisiae*, was selected for its high level of ergosterols and zymosterols.*

► The main causes of fermentation problems

There are many causes of sluggish or stuck fermentation (Table 1).

They can be classified into three categories:

- Those due to the initial composition of the must: deficiencies in assimilable nitrogen and such vitamins as thiamin (Bataillon et al. 1996), a high concentration of fermentable sugar and a low pH value;
- Insufficient control of the vinification procedures during the clarification and yeasting phases (erroneous choice of strain, poor rehydration and must acclimatization), oxygenation and temperature control (Ribereau-Gayon et al. 1998; Sablayrolles, 1998);
- Production by the yeast of inhibitors such as ethanol, carbon gas and saturated fatty acids C8 and C10 (Lafon-Lafourcade et al. 1984).

Fermentation problems are rarely due to one single factor in isolation but are usually the result of a combination of several factors that lead to a significant loss of yeast viability.

However, it has been shown that some causes occur more frequently than others (Sablayrolles et al. 1996). Nitrogen availability is essential for launching the initial exponential development of the yeast, and also for the continued renewal of the "pool" of enzyme proteins and trans-membrane transporters.

An increased ethanol content in the fermentation must has an effect on the permeability of the cytoplasmic yeast membrane and thus on the activity of the transmembrane sugar transporters (Salmon, 1989). Thus, residual sugars, dominated by fructose, cannot be metabolized by the yeast and therefore cannot be fermented. The yeast must adapt the composition of its plasmic membrane by continued synthesis of sterols and unsaturated fatty acids.

This synthesis results from the presence of a minimal concentration of dissolved oxygen.



▶ Designing a specific fermentation bio-regulator

Composition

The purpose of the present study is to design a complex fermentation bio-regulator in order to solve the majority of problem fermentation cases encountered by wine-makers.

The composition of this activator (Maxaferm®), and the significance of each of its components, is shown in Table 2.

Composition optimization results mainly from the yeast strain *S. cerevisiae* chosen for formulating the product.

Hence, we carefully examined all the yeast strains produced by DSM in order to establish the degree of the retention of ergosterols, zymosterols, trehalose and glutathion in each of them.

The Research Department of DSM has succeeded in identifying the yeast strains with the highest sterol content and is employing the most effective for the final formulation of a fermentation activator. During the optimization phase, the effectiveness of the product was tested to assess the reduction in fermentation time of a Chardonnay must which, under normal conditions, suffered from sluggish fermentation.

The Maxaferm® formula enables sluggish and stuck fermentation to be avoided and treated.

The presence of inactivate yeast enables CO₂ to be evacuated and toxic yeast metabolites to be adsorbed (short-chain saturated fatty acids) and is also a source of sterols, phospho-lipids, amino acids and other micro-nutriments for fermentation yeast.

Maxaferm® also includes thiamin and ammonium salts that encourage growth and the constant renewal of metabolizing enzymes, thus improving the viability of the yeast.

▶ Model vinification trials on sluggish fermentation prevention

The effect of Maxaferm® on fermentation speed was tested during micro-vinification of Chardonnay musts, which show signs of sluggish fermentation under standard conditions.

The fermentation temperature was set at 24°C.

The speed of fermentation was monitored by measuring the production of carbon gas (Sablayrolles et al. 1987).

The musts used had all led to sluggish fermentation of periods in excess of 300 hours, regardless of the yeast strain added. All different varieties of active dried yeast were added at a dose of 20 g per hectoliter.

▶ Significance of the addition conditions and impact on the total fermentation period

We compared the efficacy of the fermentation activator with the time and the condition of the addition to the must.

When the fermentation activator was added at the time of putting into tanks at a dose of 300 mg per liter, the main effect observed was a reduction in the latency phase and a rapid start-up of the exponential growth phase of the yeast (Figure 1).

This early addition of the activator did not, however, have any discernible effect on the total fermentation period, the percentage viability of the yeast or the residual sugar content after 200 hours of fermentation.

Yet, the addition at mid-fermentation had the immediate effect of reactivating alcoholic fermentation which was then completed in less than 200 hours.

A split dose of 2 x 150 mg per liter, added at the beginning and at mid-fermentation, also had a very positive effect on fermentation speed (not shown).

▶ The effect of various types of fermentation speed activators and yeast viability

The effect of the addition of Maxaferm® was compared with that of di-ammonium phosphate dosed at 20 g per hectoliter, either alone or combined with a moderate oxygenation (7 mg per liter).

These trials showed that the most effective treatment for preventing sluggish fermentation is the simultaneous addition of 300 mg per liter de Maxaferm® and oxygen at mid-fermentation (Figure 2).

In this trial, fermentation was completed at 150 hours while the control must still contained over 10 g of residual sugar after 300 hours.

These results on the total fermentation period were in perfect correlation with the yeast viability values at the end of fermentation (Figure 3).

The other modes tested (di-ammonium phosphate and oxygen, added alone or in combination) gave intermediate results on the two criteria assessed. In samples treated with Maxaferm® alone or in combination with oxygen, viable yeast represented over 50 % of total cells at the end of fermentation, while viability at the end of the process with other treatments tested was about 40-45 %.

▶ Vinification trials and the effect on wine characteristics

Effect on the total fermentation period

Models vinification trials on the 1999 harvest were carried out jointly with the "Centre Technique Interprofessionnel de la Vigne et du Vin" (ITV-France, Tours).

The must used was obtained from White Sauvignon, sulfated at 5 g per hectoliter, and clarified with the addition of pectolytic enzymes (Rapidase® CB) at 2 g per hectoliter.

The initial concentration of reductive sugars was almost 200 g per liter.

After yeasting (with Collection Cépage Sauvignon LW07) at 20 g per hectoliter, the alcoholic fermentation of the control must was complete after 17 days at a temperature of between 19 and 20 °C. It can be seen that the control must used for the tests does not show any specific problem leading to stuck fermentation.

The addition of Maxaferm® at 30 g per hectoliter, after a reduction of 0.035 in density (fourth day of fermentation) led to a reduction of 13 days in the total period of fermentation.

The use of this activator thus constitutes an efficient means of reducing the fermentation period and optimizing vat management in wineries.

Effect on the characteristics of the wine obtained

Various samples of White Sauvignon fermented without (control) and with the addition of 30 g per hectoliter of Maxaferm® were submitted to oenological analysis (Table 3) and to a sensorial evaluation.

Comparing the analytical profiles of the wine (Table 3) shows that the use of a fermentation activator has a positive effect on several significant parameters such as:

- a lower final concentration of reductive sugar;
- a reduction of volatile acid by 0.17 g per liter, or a reduction of 30 % by comparison with the control wine;
- an increase in glycerol retention of 10 %.

Other parameters, such as the volumetric alcohol content, total acidity and absorption at 420 nm, were unchanged.

Both wines were then subjected to sensorial evaluation by a panel from ITV-France, experts in wine from the Loire Valley area.

The panel judged the sensorial parameters such as nose intensity and quality, taste, visual appearance, balance, bitterness and acidity and their findings were subjected to statistical analysis. Both wines were assessed as being identical for each parameter evaluated.

The addition of Maxaferm® during fermentation did not lead to sensorial modification of the wine.

Vinification trials: dose effect and total fermentation period

Model vinification trials on the most recent harvests were carried out jointly with CIVAM in Corsica. The must used was obtained by direct pressing of White Vermentino, sulfated at 4 g per hectoliter, and clarified by static cold settling after the addition of pectolytic enzyme (Rapidase CB®) at 2 g per hectoliter.

Initial turbidity was adjusted to 100 NTU before yeasting at 20 g per hectoliter with Equinox B1 strain. The harvest used for this study was from a plot identified as giving sluggish fermentation musts. The fermentation must thus obtained (TAP of 13.5 % vol.) lasted for 31 days at a temperature of 18-19°C.

The addition of Maxaferm® was effected on the second day of fermentation and a dose dependent effect was observed.

The addition of 10 g per hectoliter enabled the total period to be reduced to 21 days.

A dose of 30 g per hectoliter had a quite amazing effect as fermentation was completed in 13 days, a reduction of almost 60 % by comparison with the control.

Analytical and sensorial characteristics (evaluated by a panel from CIVAM Corsica) of the wine obtained showed no significant difference.

This further trial confirmed the value of the fermentation bio-regulator as a tool for vat management in wineries, without compromising the quality of the wine.

Conclusion

The fermentation activator formulated by the Research Department of DSM and tested in the present study is seen to be very effective in reducing alcoholic fermentation time and in avoiding the main causes of fermentation problems.

The addition of Maxaferm® at mid-fermentation significantly reduces the frequency and degree of fermentation problems (sluggish and stuck fermentation).

Moreover, certain analytical parameters can be improved without causing the least sensorial change in the wine.

This product can also be used for treating stuck fermentation in parallel with the inoculation of strains such as Fermichamp® (strain 67J INRA Narbonne).

Table 1:
Principle causes of fermentation problems.

COMPOSITION OF THE MUST		
Factor	Consequence	Comments
Nitrogen shortage.	Exponential growth of the yeast and slow fermentation speed.	The must content depends on: <ul style="list-style-type: none"> ■ the soil and method of cultivating the vineyard (grassed, ...), ■ the variety ■ the ripeness of the grape.
High concentration of reductive sugar.	Initial death of the yeast and high final alcohol content.	Choosing a yeast strain that is resistant to osmotic pressure and high alcohol content enables this problem to be overcome.
Thiamin shortage.	Yeast reproductive capacity limited.	Thiamin must be added very early, before fermentation, to encourage yeast development

VINIFICATION PROCESS		
Factor	Consequence	Comments
Excessive SO ₂ .	Yeast inhibition. High concentration of ethanol.	Toxicity increases with a low pH and a excessive clarification
Must deprived of certain nutritional elements.		Fine solid particles: <ul style="list-style-type: none"> ■ contain lipids (unsaturated fatty acids); ■ allow nucleation of CO₂ and then its elimination. Must turbidity should be maintained at 80 to 200 NTU following the state of health of the harvest and the yeast strain used.
Oxygen shortage.	High alcohol toxicity leading to the loss of yeast viability.	The effect of oxygen shortage is aggravated by clarification and by excessive measures for protecting against oxidation. By adding oxygen at mid-fermentation, the risk of oxidation is almost negligible.
Extreme temperatures.	A temperature of over 32 °C fosters yeast mortality. A temperature below 15 °C slows activity.	Tolerance of temperature extremes varies greatly between yeast strains Heat increases the effect of alcohol on the yeast membrane. Avoid any thermal shock greater than 10 °C at the time of inoculation (loss of viability of the initial yeast population).

AUTO-TOXIC METABOLITES OF YEAST		
Factor	Consequence	Comments
Ethanol.	Ethanol, in synergy with other inhibitors, leads, to yeast death.	The alcohol generation capacity of the selected yeast is greater than that of natural "wild" yeast.
CO ₂ excess.	Yeast death..	Some yeast strains tolerate a high level of CO ₂ .
C8, C10 Fatty acids	Toxic for the fermentation yeast.	The cell wall of the yeast adsorbs these inhibitors.

Table 2:
Composition of the DSM refined fermentation activator.

Component	Comments
Inactivated yeast.	Elimination of carbonic gas. Adsorption of toxic fatty acids. Source of sterols, glutathion, amino acids and trace elements.
Ammonium sulfate, Di-ammonium hydrogenophosphate.	Encourages yeast reproduction and long term viability.
Thiamin.	Encourages exponential yeast growth.

Table 3:
Comparative analysis of Sauvignon white wine fermented without (control) and with the addition of 30 g per hectoliter of Maxaferm® after fourth day of fermentation.
Data: ITV-France (Tours).

Parameter	Control	Wine obtained using Maxaferm®
Reductive sugar (g per liter)	1,3	0,9
Volatile acidity (g per litre-H ₂ SO ₄)	0,46	0,29
Ethanol content (% vol.)	12,3	12,4
pH	3,08	3,03
Total acidity (g per litre-H ₂ SO ₄)	5,25	5,5
Glycerol (g per liter)	5,9	6,5
Potassium (g per liter)	0,52	0,54
Absorbance at 420 nm per 1 cm	0,035	0,034

Figure 1:
Speed of alcoholic fermentation in a Chardonnay must with an history of sluggish fermentation. Control (blue curve), after addition of 300 mg per liter of Maxaferm® at the beginning (green curve) and at mid-fermentation (red curve).
Data: UMR Sciences for Oenology (INRA-Montpellier, France).

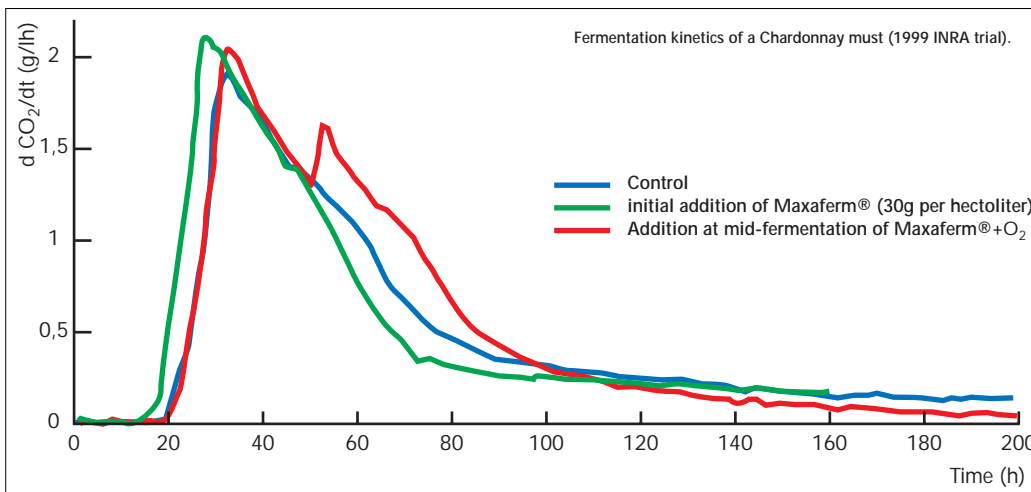


Figure 2 :

Completion of fermentation obtained by various treatments of a Chardonnay must. Addition of di-ammonium hydrogenophosphate (200 mg per liter) and Maxaferm® (300 mg per liter), alone or combined with oxygen (7 mg per liter) Additions carried out when must density had reached 1040.

Data: UMR Sciences for Oenology (INRA-Montpellier, France).

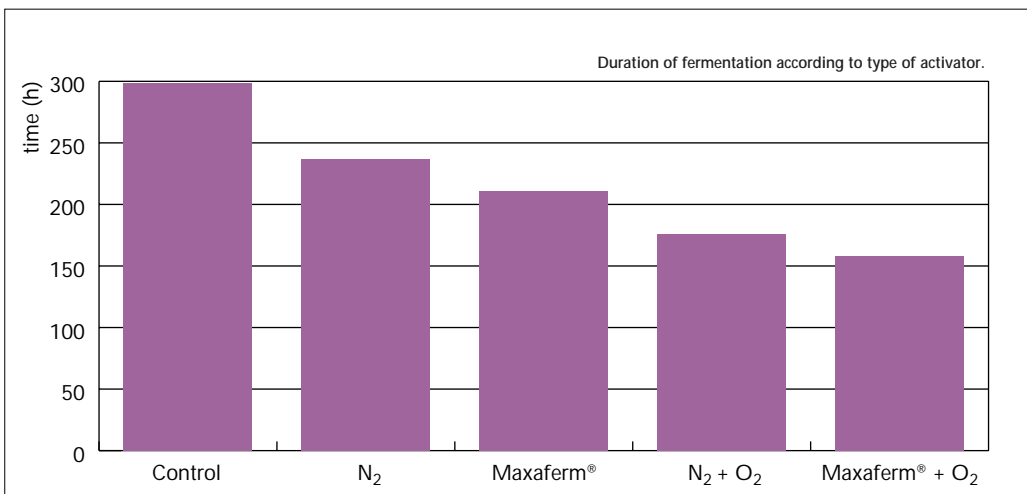
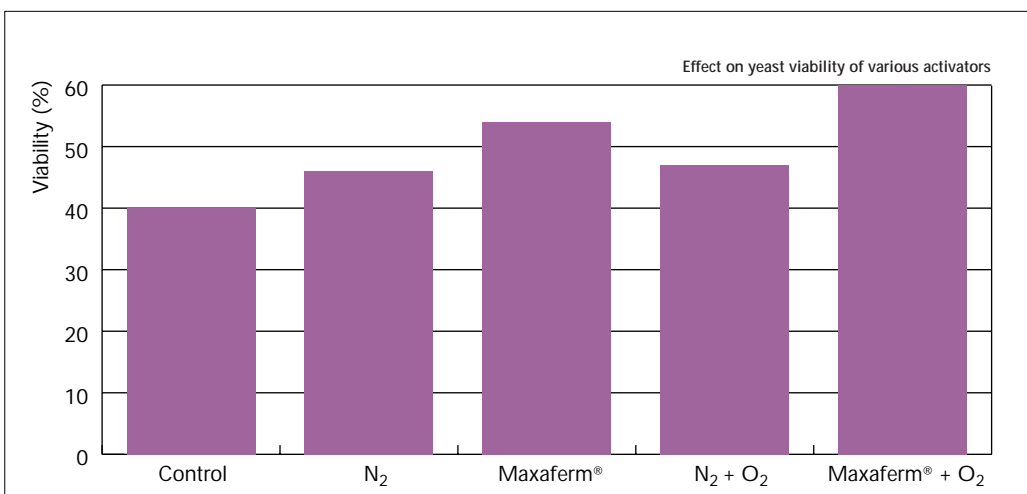


Figure 3 :

Viability of yeast cells (in %) at the end of alcoholic fermentation of a Chardonnay must submitted to various treatments. The addition of di-ammonium hydrogenophosphate (200 mg per liter) and Maxaferm® (300 mg per liter), alone or combined with oxygen (7 mg per liter), when must density had reached 1040.

Data: UMR Sciences for Oenology (INRA-Montpellier, France).



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