# Micro agglomeration and bioavailability of sterols – the revolutionary wine yeast protector

## Faster yeast rehydration

By David Ferreira*

The impact on the fermentation process and final wine quality are now greater due to climate change, with higher sugar/ethanol contents, higher pH's, off-flavours caused by yeast stress, sluggish or stuck fermentations and higher microbial contaminants. It is now crucial to select wine yeast in dry form (ADY) in the optimal physiological state to have healthy and active populations capable of completing alcoholic fermentation.

Over the last 20 years, scientific information on yeast nutrition management in winemaking has significantly increased. The role of key nutrients, such as nitrogen sources, oxygen and micronutrients like vitamins and minerals, have been the subject of research and publications, showing their impact on fermentative activity and wine sensory profile.

In parallel, specific research has focused on how sterols influence yeast's physiological state and metabolism and how it was directly correlated with a secure end of fermentation. It has been shown that using specific yeast autolysates rich in sterols during the rehydration step of the ADY increases the fermentation activity and vitality during fermentation, particularly in the last part, when alcohol levels become toxic for the yeasts. This is related to improving yeast cell stress resistance correlated with better membrane integrity.

ADY rehydrated with protectors (Go-Ferm Protect™/ Go-Ferm Protect Evolution™) maintains their viability and vitality until the end of fermentation, decreasing the risk of stuck or sluggish fermentations, avoiding undesirable aroma production, and resulting in an increase of the aroma compound synthesis by the yeast with the protectors. The incorporation of the sterols in the ADY rehydrated with protector leads to an increase in their membrane lipid composition and healthier cell membrane. The protector effect is notable at different temperatures, high potential alcohol, lack of oxygen or low turbidity in white and rosés.

### 1. Why are sterols so important? – new evidence

Sterols, especially ergosterol originating from yeast, are critical for yeast viability and vitality. They help with better assimilation of total YAN, leading to healthier cell membranes that are more efficient for the uptake of amino acids and assimilable nitrogen. This leads to higher yeast performance, efficiency and aromatic contribution, even under difficult conditions. For example, Figure 1 shows that with higher ergosterol concentration, the assimilation of amino acid is higher by the yeast cell.

In addition, sterols also help obtain higher and healthier yeast populations. Even when YAN is not limiting, yeast multiplying capacity is affected by other micronutrients, such as sterols, which are more frequently unbalanced or scarce in grape musts or with excessive clarification practices. The sterols help yeast produce viable and healthy generations during budding, leading to a higher final population concentration. For example, by increasing from 1 mg/L to 4 mg/L of ergosterol, the population (biomass) doubles (Figure 2).

Finally, sterols help with yeast performance. When YAN is more efficiently assimilated, it leads to a better sugar conversion ratio and, consequently, to regular and complete fermentation. Healthier yeast populations with increased viability perform better and more efficiently until the end of fermentation, as shown in Figures 3A and 3B.

## 2. Optimised sterol integration during rehydration

The above results confirming the importance of sterols, especially ergosterol, on viability, vitality and fermentation led to the development of an optimal rehydration protector for active dry yeast. The development of this new protector, Go-Ferm Sterol Flash™, was undertaken with two objectives in mind: efficiency and rapidity.

### Sterols... lots of sterols

Based on a thorough screening of Lallemand Oenology's yeast portfolio, our R&D team selected a natural specific and unique autolysate with the highest sterol content. Go-Ferm Sterol Flash™ was developed with this autolysate resulting in a protector with the highest possible sterol content. Furthermore, the sterol content is immediately bioavailable

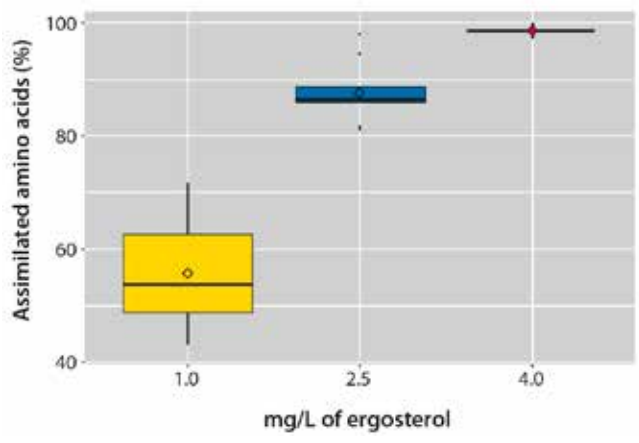



**FIGURE 1.** *Combined results of the percentage of assimilated amino acids by 10 different wine yeast strains at 80% of fermentation in a synthetic juice (200 g/L G+F, 400 mg/L YAN, 24°C) with 1 mg/L (yellow), 2.5 mg/L (blue) and 4 mg/L (red) of ergosterol.*

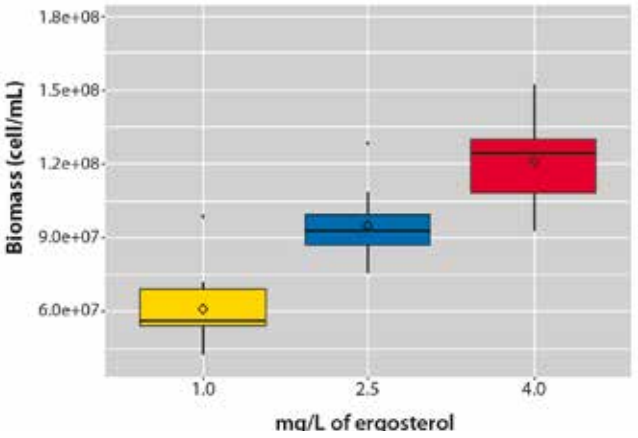



**FIGURE 2.** *Combined results of the biomass generated by 10 different wine yeast strains at 80% of fermentation in a synthetic juice (200 g/L G+F, 400 mg/L YAN, 24°C) with 1 mg/L (yellow), 2.5 mg/L (blue) and 4 mg/L (red) of ergosterol.*

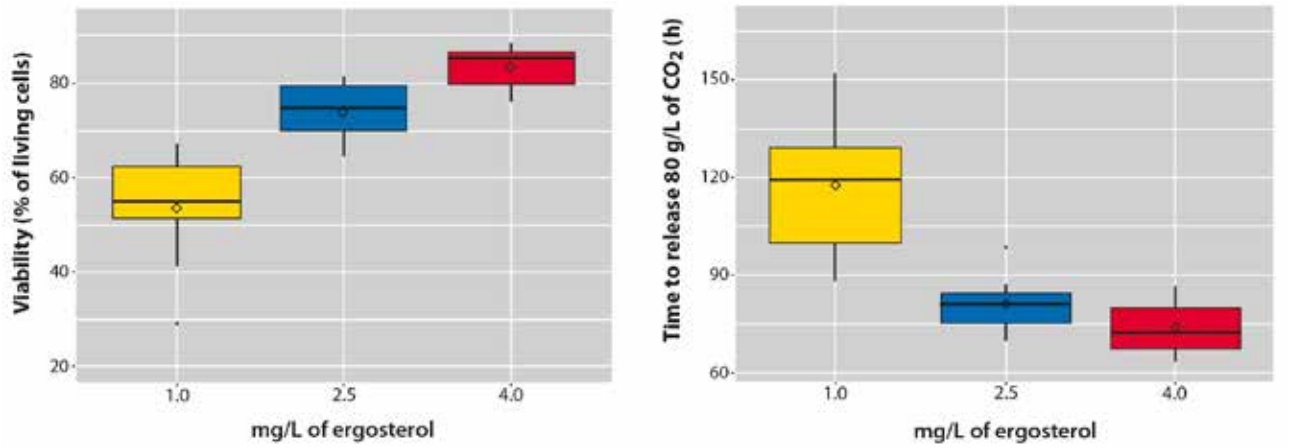



**FIGURE 3A AND 3B.** *A. Viability. B. Fermentation efficiency generated by ten different wine yeast strains at 80% of fermentation in a synthetic juice (200 g/L G+F, 400 mg/L YAN, 24°C) with 1 mg/L (yellow), 2.5 mg/L (blue) and 4 mg/L (red) of ergosterol.*

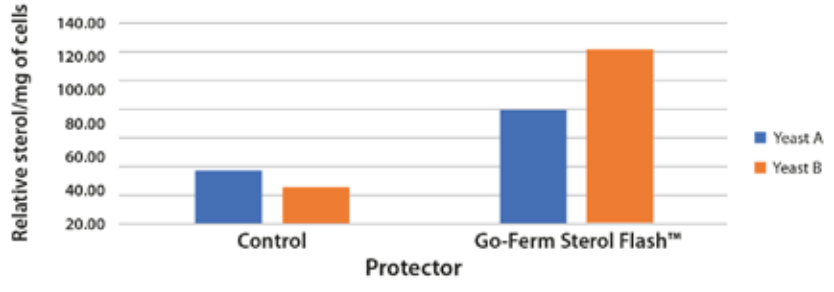



**FIGURE 4.** *Relative sterol content per mg of viable cells after a rehydration process of 20 minutes in water at 15°C without Go-Ferm Sterol Flash™ ("Control") and with Go-Ferm Sterol Flash™, using the wine yeast strains Yeast A and Yeast B.*

for the yeast so that it can be quickly integrated as soon as it is in contact with Go-Ferm Sterol Flash™ (Figure 4) during the rehydration phase. This is due to its specific micro-agglomerated form.

**Particle size matters**

To improve product manipulation, different physical forms and processes were studied (lyophilisation, compaction, tablets, etcetera). A micro agglomeration form was developed, and the resulting product, Go-Ferm Sterol Flash™, with the following characteristics:

- Improved manipulation – straightforward and simple to use and instant dispersion.
- Safe and compatible with the winemaking process.
- Maintains the sterols activity and impact.
- Very short rehydration time at room temperature.

As the name suggests, micro agglomeration agglomerates several product particles together. In Go-Ferm Sterol Flash™, no ligands or additives are used to achieve this state. The physical agglomeration occurs based on the (brief) contact with hot air that makes water evaporate from the inactivated yeast cream droplets. As water evaporates, the viscosity of each droplet progressively increases, leading to an optimal physical state where the collisions with other particles also being dried will efficiently make them stick together. At this point, strong bonds between particles are created, and as water evaporates, the particles become agglomerated and free-flowing.

Each particle is now bigger and heavier (4 - 5x), just enough so that it is no longer powdery when poured or manipulated (Figure 5).

The dispersion is also greatly improved as the bigger particle creates larger spaces between each particle so that more water can penetrate the interspace (Figure 6). A greater surface area is exposed to water, making it easier to dissolve.

The larger particle size improves the manipulation to the point where dispersion is easy and quick and carried out at room temperature.

**Better internalisation of sterols and better viability and vitality**

Using room temperature water for rehydration is challenging for ADY. However, with Go-Ferm Sterol Flash™, the high level and bioavailable sterols, the yeast can be rehydrated in cool water without losing vitality and viability. Go-Ferm Sterol Flash™ has a positive impact throughout the fermentation process, observed from the rehydration step to the end of fermentation. For example, at mid-fermentation, yeasts rehydrated with Go-Ferm Sterol Flash™ had better viability (Figure 7), as shown by the number of viable cells and the gain between the two types of rehydration (+64% for yeast A and +94% for yeast B). Wine yeast cells rebuilding their membranes now have higher chances of surviving, because of the optimal sterol integration.

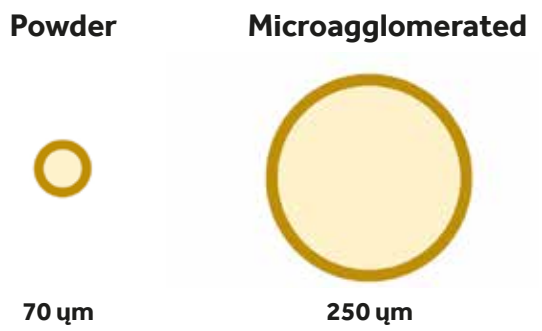



**FIGURE 5.** *Illustration of the particle size increase responsible for eliminating the powdery behaviour.*

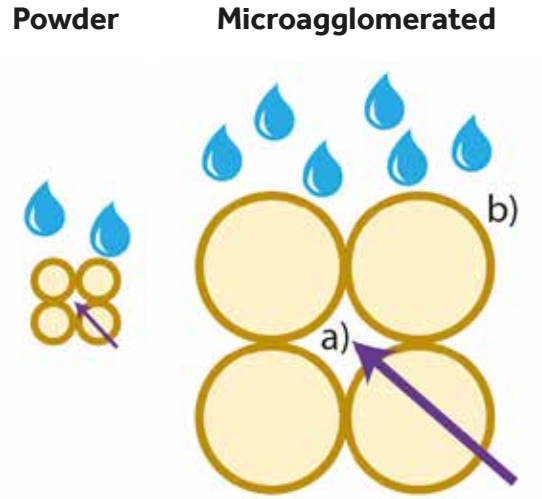



**FIGURE 6.** *Illustration of the improved dispersion mechanics, a) larger particles create larger spaces in between them; b) an increased surface area (up to 16x) greatly improves the dispersion potential of each particle.*

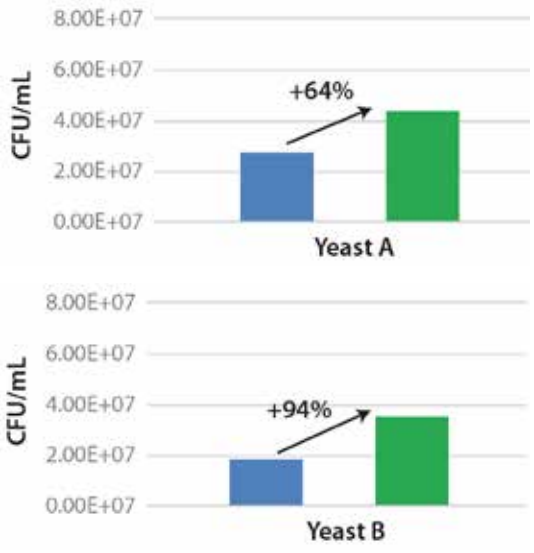



**FIGURE 7.** *Viability of two yeast strains, Yeast A and Yeast B, after the classic rehydration process or with Go-Ferm Sterol Flash™ as measured at mid-fermentation.*

### 3. 15 minutes, 15°C

Micro agglomeration facilitates a better bioavailability of sterols compared to all other types of protectors. Without micro agglomeration, obtaining such a high sterol content coupled with high dispersion would be impossible. With the high sterol content, the rehydration temperature can be as low as 15°C, the rehydration time is 15 minutes, and there is no need for acclimatisation to must temperature. The extra sterols and their fast dispersion render them highly bioavailable for the wine yeast, providing instant and extra protection for the cell (Figure 8). This preserves yeast cell integrity and overall vitality. This method of rehydration is only possible with Go-Ferm Sterol Flash™.

In summary, the protocol of rehydration is greatly simplified without compromising performance and a considerable amount of time, energy and labour is saved during yeast inoculation:

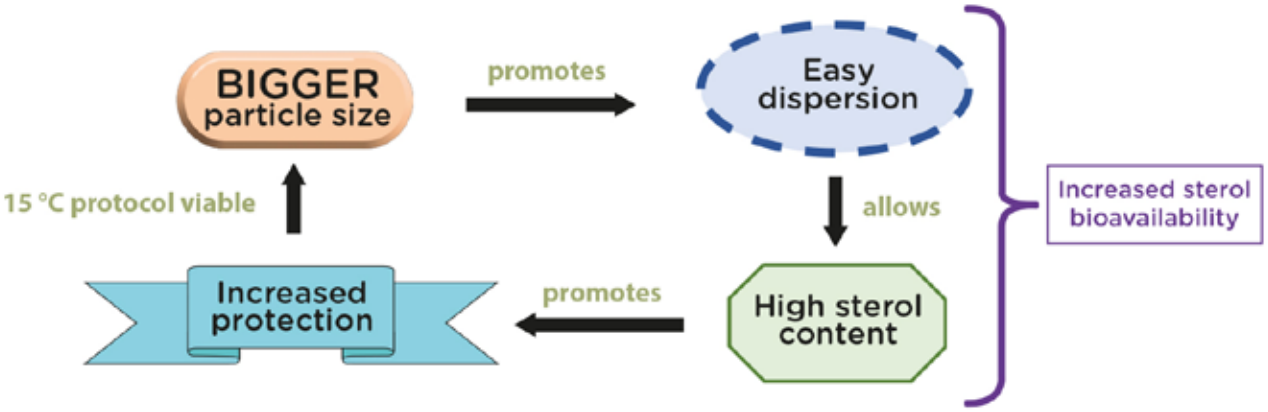



**FIGURE 8.** *Micro agglomeration and high sterol bioavailability.*

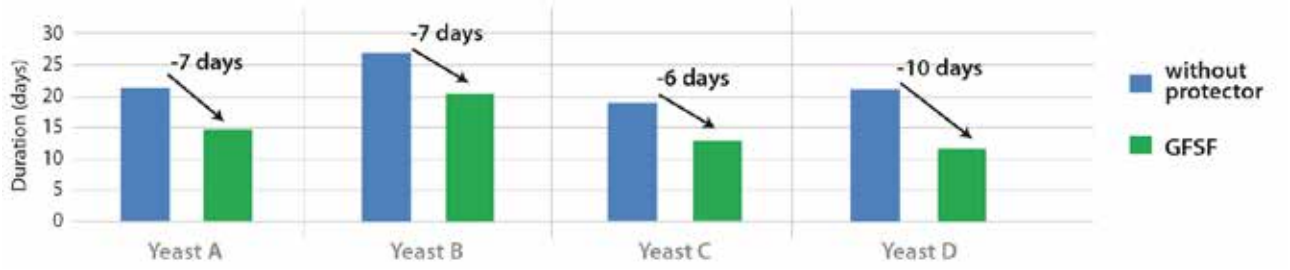



**FIGURE 9.** *Alcoholic fermentation time comparing Go-Ferm Sterol Flash™ and no protector with four different wine yeast in synthetic juice 250N.*

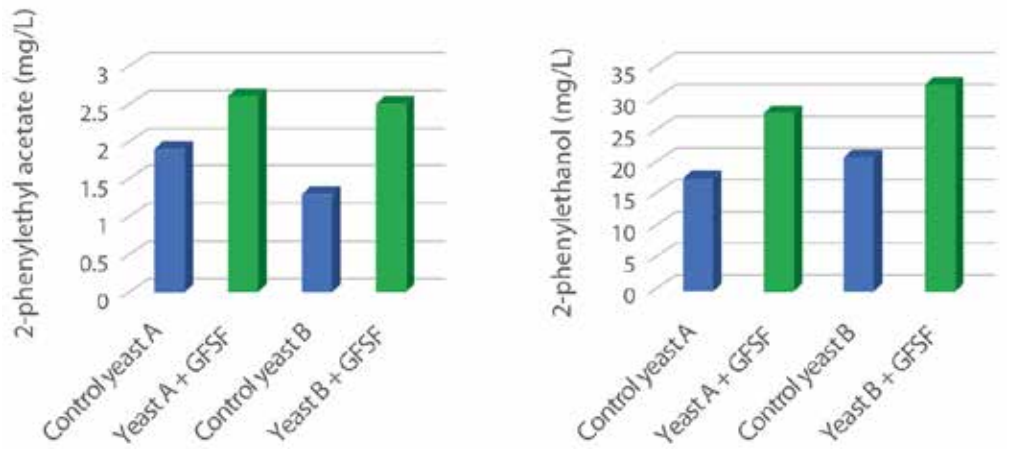



**FIGURE 10.** *Production of 2-phenyl ethyl acetate and 2-phenyl ethanol by two distinct oenological yeasts rehydrated with and without Go-Ferm Sterol Flash™.*

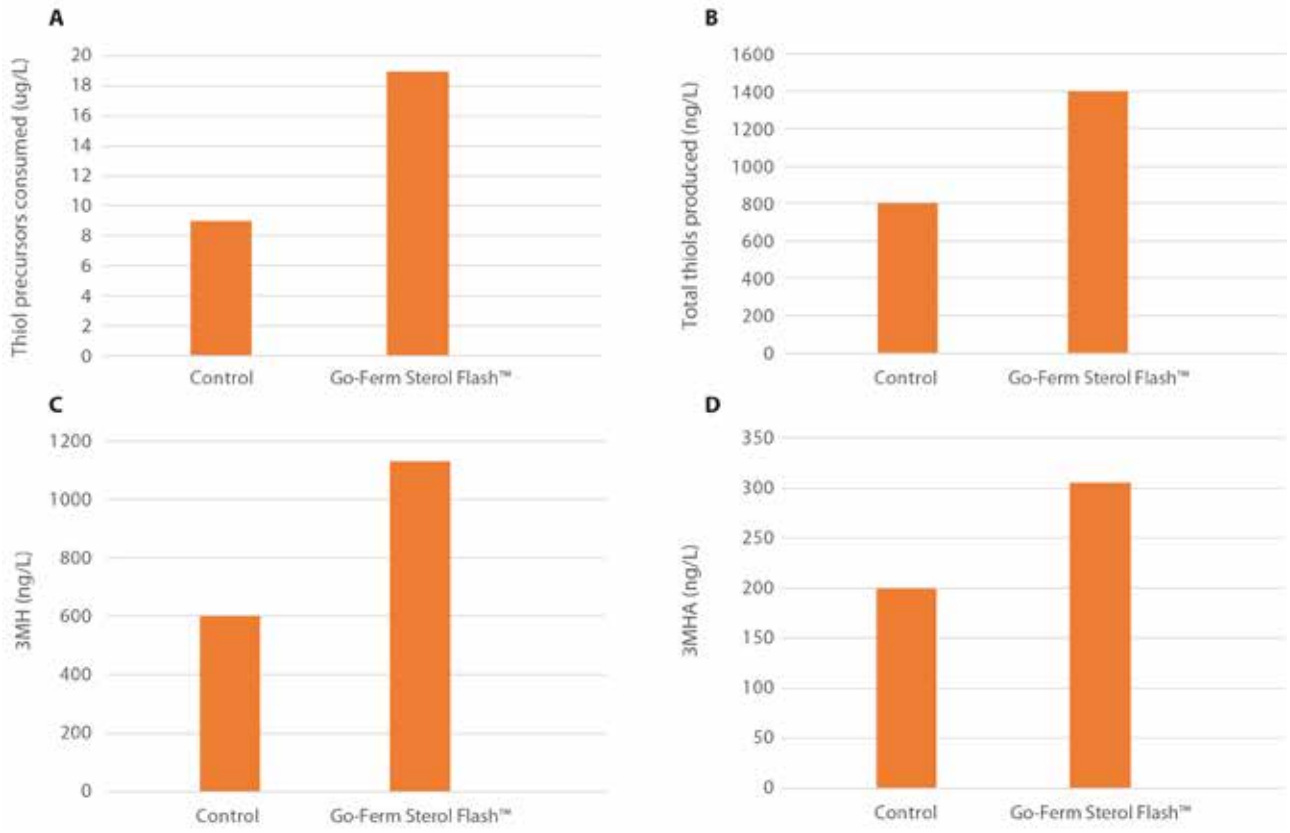
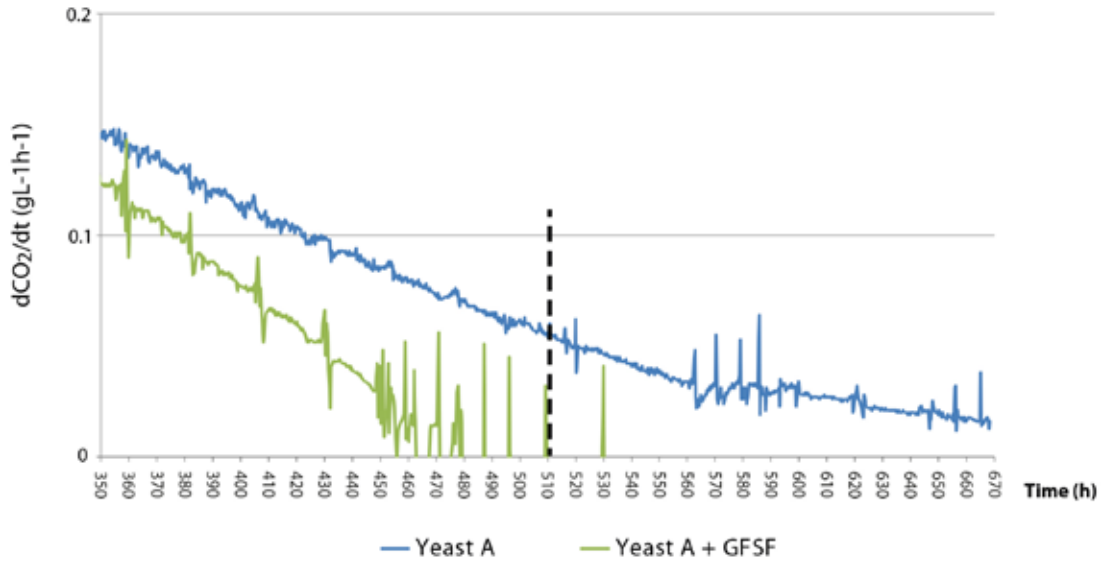



**FIGURE 11.** *A. Total thiol precursors converted. B. Total thiol produced. C. Total 3MH produced. D. Total 3MHA produced by yeast A rehydrated with Go-Ferm Sterol Flash™ versus control (no protector) in a Colombard (2020, France, 199 g/L sugars, YAN: 400 mg/L, NTU 30).*




| Yeast A | Yeast A + GFSF |
|---|---|
| Residual sugar = 7.2 g/L | Residual sugar = 0.3 g/L |

**FIGURE 12.** *Fermentation of rosé Syrah (INRAE France, 2020) with yeast A rehydrated without a protector or with Go-Ferm™ Sterol Flash under challenging conditions.*

- Tap water rehydration temperature (15°C).
- Quick and fast dispersion.
- Shorter yeast rehydrating time.
- Already acclimatised to must temperature.

### 4. Lab and winery trials

Go-Ferm Sterol Flash™ was tested under different conditions in many varieties, including red, white and rosé wines and controlled crash tests (where yeasts are tested in the most extreme conditions found in wine), with more than 20 different wine yeast strains for different applications and more than 50 trials made at all scales: from the laboratory to winery.

In Figure 9, for alcoholic fermentation time, Go-Ferm Sterol Flash™ was compared to no protector, with four different wine yeast rehydrated. The length of the alcoholic fermentation was faster with Go-Ferm Sterol Flash™ compared to without a protector. Since the protocol to use Go-Ferm Sterol Flash™ is faster and simpler, time gain is even more significant.

The addition of Go-Ferm Sterol Flash™ resulted in an increased production of different aroma compounds, such as acetate esters, ethyl esters and higher alcohols. Figure 10 shows two volatile aroma compounds, phenyl ethanol and 2-phenyl ethyl acetate, with increased concentration when the ADY is rehydrated with Go-Ferm Sterol Flash™. It also has an impact on thiol production, as shown in Figures 11A, B, C and D. The yeast A rehydrated with Go-Ferm Sterol Flash™ had a higher conversion of thiol precursors (Figure 11A), leading to the higher production of thiol compounds (total, 3MH and 3MHA). The dual action of higher sterols and their better integration results in healthier yeast cells with an optimised metabolism and hence aromatic expression in the wine.

Finally, in very difficult conditions, as those shown in Figure 12 depicting a rosé (Syrah) with high alcohol potential (14%), low YAN (66 mg/L) and low turbidity (30 NTU), the yeast rehydrated with Go-Ferm Sterol Flash™ and inoculated in a 14°C must, completed the fermentation. In contrast, the non-protected yeast resulted in a stuck fermentation.

### Conclusion

The innovation of Go-Ferm Sterol Flash™ relies on the unique synergy of its properties capable of simultaneously achieving the following objectives: improvement of yeast health to assure fermentation and aroma biosynthesis, while proposing a user-friendly product that simplifies its manipulation and application, to save time, labour and energy.

**Go-Ferm Sterol Flash™ brings to the winemaker:**

A. Easier manipulation = time and energy saved
   - Zero powdery levels.
   - Instant dispersion.
   - Rehydration media for yeast at tap water temperature (15°C).
   - No yeast acclimatisation is needed.

B. Increased protection level
   - Optimised sterol bioavailability.
   - Helps overcome challenging winemaking conditions.
   - Improvement of fermentation efficiency and positive sensory wine properties directly related to yeast metabolism and performance. ■

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