

Chitosan

under investigation

Nº1

BIOLOGICAL SOLUTION TO CONTROL CONTAMINANTS IN WINE

Managing the risk associated with microbial spoilage is a key point throughout the life of a wine. The commonly used product to limit microbial contaminants associated with sensory deviation is SO₂ although the market is looking for wines with lower sulphite content. One way to control the spoilage microbes is by preventing their development through bioprotection (selected yeast, co-inoculation with selected bacteria (see UI Bacteria #1 and #4)), and our research has led to safe and effective biological alternatives against contaminants, especially *Brettanomyces* and lactic and acetic bacteria. They are fungal-origin chitin derivatives: pure chitosan (**No Brett Inside™**) and chitosan associated with chitin-glucan (**Bactiless™**). Both of these chitosan-based formulations are 100% pure from *Aspergillus niger* and compliant with OIV strict specifications and EU regulations and have been successfully used by winemakers for the past 10 years. This Under Investigation will explain their mode of action and the efficiency of these chitosan-based products (100%-natural, biodegradable, non-GMO and non-allergenic).

WHAT ARE CHITOSAN AND CHITIN-GLUCAN?

Aspergillus niger is a fungus from which chitin and chitin-glucan are extracted. Chitosan is derived from chitin via deacetylation. The only approved chitosan and chitin-glucan in the wine industry are those obtained from the mycelium of *Aspergillus niger*. Chitosan derived from animal (crustaceans) or other fungal sources, it is not approved for use in wine.

In collaboration with our partner Kitozyme since 2003, we screened and selected the **best raw-materials as pure compounds** based on the physico-chemical diversity of the chitosans and chitin-glucans. The diversity of the different chitosans relies on physico-chemical characteristics such as the deacetylation grade, degree of polymerization and the granulometry.

The antibacterial and antifungal properties of our chitosan-based formulas (**No Brett Inside™** or **NBI** and **Bactiless™**) have been extensively studied and their antimicrobial efficient action has been proven: strong

inhibition of *Brettanomyces* (especially for **NBI**) and acetic and lactic acid bacteria (especially for **Bactiless™**). The latter consists of pure chitosan associated acting in synergy with specific chitin-glucan. Chitosan plays the antimicrobial role and the chitin-glucan portion helps to maximize the physical effect (elimination via flocculation with the damaged cells and their sedimentation).

As an example, in Figure 1 shows an optical microscopy image of *Brettanomyces* cells treated with chitosan (NBI) from Taillandier et al, 2012, where we can see:

- Physical effect: aggregation due to charge interaction between **NBI** and the yeast cells
- Biological effect: die-off of *Brettanomyces* cells



Figure 1. Optical microscopy of *Brettanomyces* cells with **No Brett Inside™**

THE EFFICIENCY OF THE PURE FORMS OF CHITOSAN AND CHITIN-GLUCAN?

Brettanomyces is a permanent threat to the quality of wine due to the production of volatile phenols (barnyard, band-aid, medicinal aromas) which reduce the sensory quality of the wine. These contaminating yeast can develop in difficult environments at any time during the life of a wine, but particularly during the aging phase. SO₂ is the most commonly used method to control *Brettanomyces* but some strains of this contaminants are resistant to SO₂.

There are also other chitosan-based products available on the market to control contaminants that are blends with organic acids and/or yeast derivatives. Our R&D group have compared the efficacy of the pure chitosan (**No Brett Inside™**) or chitosan combined with chitin-glucan (**Bactiless™**) to one of this blended products (activated-chitosan).

No Brett Inside™, 100% *Aspergillus niger* pure chitosan, was evaluated against a blended product with the RT-PMAX-PCR method that provides a more accurate evaluation of live *Brettanomyces* cells (no risk of false positive counts). Figure 2 shows how efficient NBI is at a much lower dosage than the "so-called activated chitosans". It is as quick and efficient as the blended product used at the recommend dosage, evident at 5 days after addition.

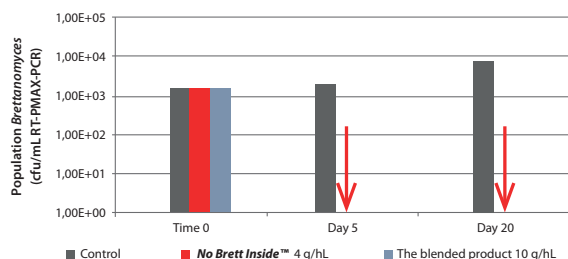


Figure 2. *Brettanomyces* death measured by Real-Time PMAX-PCR for wine without an antimicrobial agent, wine with NBI at 4 g/hL and a wine with the blended product 10 g/hL. (Collaboration with Excell Iberica 2017).

Also, **Bactiless™**, since its development in 2016, has been shown to reliably decrease the population of undesirable lactic and acetic acid bacteria in white and rosé wines, in sparkling base wine, Cognac or spirit production, or has been used to delay or avoid malolactic fermentation.

In order to extend our understanding of the anti-bacteria action of chitosan/chitin-glucan, our R&D team compared the use of **Bactiless™** to the blended product. A trial conducted (Figure 3) in white

wines where malolactic fermentation needed to be prevented, **Bactiless™** and the blended product prevented the onset of MLF. However, **Bactiless™** exhibited the same performance at a lower dosage, due to its highest purity and the blended preparation (same positive trends of results were observed in red wines).

Similar results we observed when microbial stability is required after malolactic fermentation as shown in Figure 4. Both **Bactiless™** and the blended product showed their ability to reduce the lactic acid bacteria population, however **Bactiless™** required a lower dose (20 g/hL) compared to the blended product (25 g/hL) as the quality of chitosan in **Bactiless™** is more efficient, even at a lower dose.

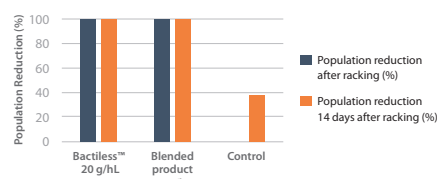


Figure 3. Decrease in population of lactic acid bacteria in white wine with **Bactiless™** compared to a blended product to prevent the onset of malolactic fermentation.

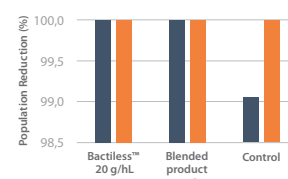


Figure 4. Decrease in population of *O. oeni* after malolactic fermentation with **Bactiless™** and a blended product.

IN SUMMARY

- The chitosan of **No Brett Inside™** and the chitosan and chitin-glucan of **Bactiless™** will prevent the growth of contaminants in a more efficient way versus blended formulas, as it is more concentrated and with a higher chitosan purity, a lower dosage is needed for a very good efficiency.
- **No Brett Inside™** and **Bactiless™** are uniquely formulated from 100% *Aspergillus niger* with the purest and certified form of chitosan, which is the only form approved by the OIV.
- **No Brett Inside™** and **Bactiless™** can be part of a strategy to reduce the use of SO₂ in wines.
- For more than 15 years of numerous trials, we can confirm that **No Brett Inside™** and **Bactiless™** pure preparations don't negatively impact the wine sensory quality. They contribute their high preservation by reducing the microbial spoilage risk.