

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 contents. 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 those chitosan-based formulations are 100% pure from *Aspergillus niger* and are complying with OIV strict specifications to which the EU regulation refers and have been successfully used by winemakers for the past 10 years. This Under Investigation will show their mode of action and the efficiency of these pure forms (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 the ones obtained from the mycelium of *Aspergillus niger*. If chitosan is from animal (crustaceans) or other fungal source, 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, the polymerization degree, the granulometry for example.

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 is consisting of pure chitosan associated with specific chitin-glucan acting in synergy. Chitosan plays the antimicrobial role and the chitin-glucan part helps to maximize the physical effect (elimination via flocculation with the injured cells and their sedimentation).

As an example, in Figure 1 shows an optical microscopy observation 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 constitutes a permanent threat to the quality of wines due to the production of volatile phenols (aromas of barnyard, band-aid, medicinal) which denature the sensory purity of the wine. These alteration yeasts 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 decided to compare the pure chitosan (**No Brett Inside™**) or chitosan combined with chitin-glucan (**Bactiless™**) to one of this blended products.

No Brett Inside™, with 100% *Aspergillus niger* pure chitosan, was studied 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 is NBI at a much lower dosage than the "so-called activated chitosans". It is as quick and efficient as a much higher dosage of the blended product, already 5 days after addition.

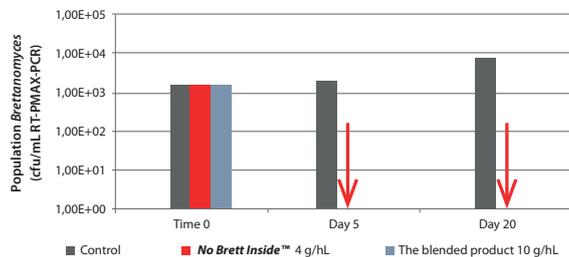


Figure 2. *Brettanomyces* death measured by Real-Time PMAX-PCR for wine without an antimicrobial agent, wine with NBI at 4 g/hL and a wine with the blended product 10 g/hL. (Collaboration with Excell Iberica 2017).

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 application, or has been used to delay or avoid malolactic fermentation.

In order to complete and increase our experience, our R&D team compared the use of **Bactiless™** to the blended product in terms of anti-bacterial action. In a trial conducted (Figure 3) in white wines

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

Similar results are obtained 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 numbers of lactic acid bacteria, however **Bactiless™** required less concentration (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.

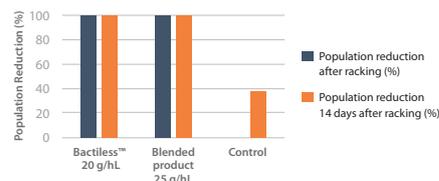


Figure 3. Decrease in population of lactic acid bacteria in white wine with **Bactiless™** compared to a blended product to prevent the onset of malolactic fermentation.

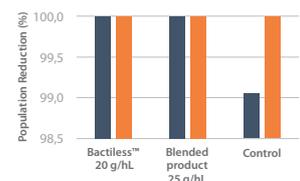


Figure 4. Decrease in population of *O. oeni* after malolactic fermentation with **Bactiless™** and a blended product.

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, and 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 affirm that **No Brett Inside™** and **Bactiless™** pure preparations don't impact negatively the wine sensory quality. On the contrary they contribute its higher preservation by reducing the microbial spoilage risk.