TECH POINT

MICROBIOLOGICAL STABILITY

CHALLENGES AND SOLUTIONS TO ADDRESS RISING pH LEVELS

Even though it is mostly made up of *Saccharomyces cerevisiae*, the must's microbial flora nevertheless includes **a wide variety of other species** (and even genera) of fungi and bacteria, **some of which are detrimental to the quality** of the finished product. As a result, it is essential to master microbiological balance and to control undesirable flora.

Rising pH levels in must are a consequence of global warming and **translate into a decrease in acidity**; this favors the development of these undesirable microorganisms. Traditionally used for its antiseptic and antimicrobial action, SO_2 is controversial due to its allergenicity. Also, it is **not always sufficient to cleanse the medium when pH is high** – non-*Saccharomyces* yeasts are much less sensitive to it and certain strains of *Brettanomyces bruxellensis*, for example, are resistant to it.

So it is necessary to find alternatives.

MASTERING THE **MICROBIAL DIVERSITY OF MUSTS**

At OENOFRANCE® our goal is **microbiological stabilization** done **in a more selective manner**, to develop alternative tools that can be used systematically in the cellar.

New formulations based on chitosan originating from fungi were developed by adapting to oenology a sequencing and analysis technique used in ecology and medicine.

This method, known as **targeted metagenomics, aims to study with precision the impact** of this molecule **on fungal and bacterial populations**, and provides information on the composition of the species that are present, how abundant they are and their diversity.

These tests have shown that **chitosan leads to significantly lower fungal diversity** (Figure 1), including that of non-*Saccharomyces* yeast populations.

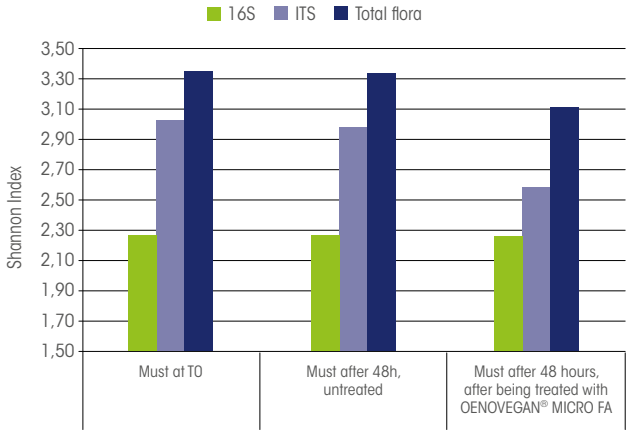

Figure 1. Evaluation of the microbial diversity of must before and after treatment with OENOVEGAN® MICRO FA. Measuring the Shannon index. This indicator reflects actual microbiological diversity because it takes into account alpha diversity (number of species present) as well as how abundant they are. High abundance of one species compared to other species significantly lowers the Shannon index. 16S: bacterial ribosomal RNA. ITS: region of the yeast's ribosomal DNA.

Table 1. Classification scheme of the Shannon diversity index

Relative values	Scheme of the Shannon diversity index
Very high	3.50 and above
High	3.00 - 3.49
Moderate	2.50 - 2.99
Low	2.0 - 2.49
Very low	1.99 and above

OENOFRANCE

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oenofrance.com

OENOVEGAN® MICRO FA

A 100% natural biocontrol tool with a **broad spectrum of action** used to **slow down the growth and reduce the population of spoilage microorganisms**, including *Brettanomyces*.

Microbiological stability

Helps to reduce doses of SO_2

100% natural

A synergistic association between

Activated chitosan + **Yeast hulls**

TO CONTROL MICROBIAL DIVERSITY IN MUST

EFFECTS OF OENOVEGAN® MICRO FA

ACTIVATED CHITOSAN

- LIMITS THE GROWTH OF MICROORGANISMS
- ENSURES SAFE FERMENTATION KINETICS
- ALTERNATIVE TO SO_2

YEAST HULLS

- DETOXIFIES THE MUST (UNDESIRABLE MOLECULES)
- MAKES IT POSSIBLE TO GET A CLEARER AROMATIC PROFILE

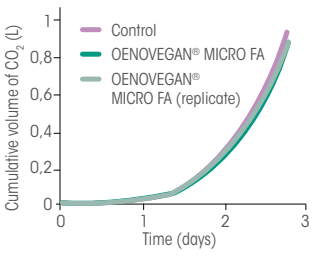
Chitosan is a polymer derived from chitin contained in the cell wall of microorganisms such as *Aspergillus niger*.
Positively charged in an acidic environment (ph<5.5), its molecule reacts by electrostatic attraction with negatively charged compounds contained in the walls of microorganisms, leading to membrane dysfunction and cell death.

OENOLOGICAL PROPERTIES

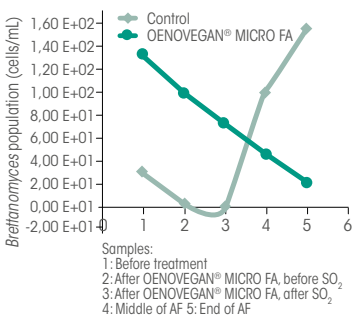
- Used on grapes and musts before alcoholic fermentation
- Reduces microbial diversity and allows native populations to be managed
- Helps to start AF
- Replaces or reduces the use of SO_2

The use of chitosan at this dosage does not affect the kinetics of alcoholic fermentation, thanks to the particular metabolism of *Saccharomyces cerevisiae*.

IMPACT OF CHITOSAN ON THE FERMENTATION KINETICS OF *SACCHAROMYCES CEREVISIAE*. TRACKING CO_2 RELEASE.



BRETTANOMYCES POPULATION GROWTH DURING VINIFICATION (MERLOT, BORDEAUX). ANALYSIS BY QPCR.



APPLYING TO MUST

Application timing: to grapes and/or must before AF.
Dosage: 15 to 20 g/hL based on microbiological risk.
Preparation: to be done in water.

THE DIFFERENCE BETWEEN BIOCONTROL AND BIOPROTECTION

Unlike bioprotection, which consists of colonizing a medium in the pre-fermentation phase with microorganisms selected to limit the development of other species, ***biocontrol aims to slow down and reduce the overall microbial diversity of the must. This ensures lasting protection against contamination and facilitates the development of yeasts of interest*** *(limiting competition) because Saccharomyces cerevisiae is a species that is not very sensitive to chitosan, the active compound used in this solution.*

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