TECHNICAL BOOKLET

MANAGING *BRETTS* RISK

Brettanomyces bruxellensis **is a non-*saccharomyces* yeast found on the grapes' skins and also within the winery. A large diversity of strains are known. It can also be found in containers (tanks made from different materials, barrels, hoses), as well as on winery equipment. These so-called spoilage yeasts produce volatile phenols that have an irreversibly detrimental effect on the wine's quality, even at low population levels: decreased fruitiness, odours of "gouache", leather or "stable".**

This practical booklet aims to give information and simple, effective tools for the preventive and curative management of *Brettanomyces*.

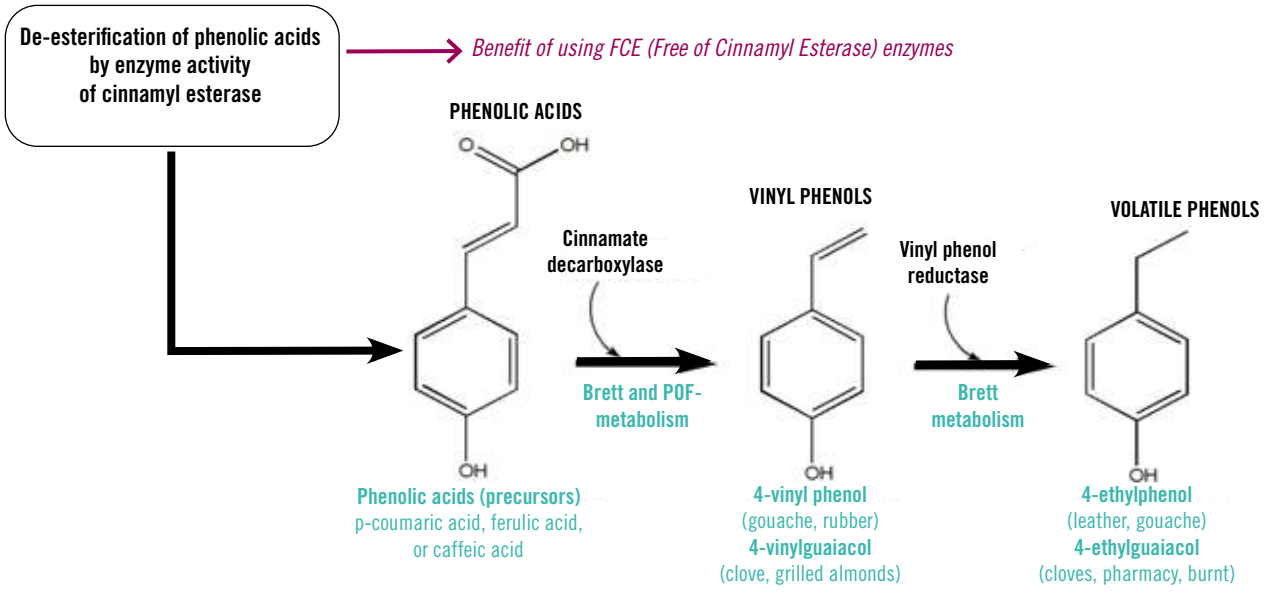
Brettanomyces, a yeast that is particularly well adapted to wine:

Despite ever increasing knowledge of *Brettanomyces* yeasts, they remain **one of the main causes of organoleptic spoilages in wine**. This is, in part, due to winemakers' desire to decrease enological additives, especially sulfites. Today, there is no authorised method that effectively removes ethyl phenols from wines. It is therefore essential to learn more about *Brettanomyces*, in order to better control their presence and development.

- It is resistant to enological pH and temperatures
- It is tolerant to high alcohol contents
- Some strains are very resistant to SO_2 (triploid strains)
- It has low nutritional requirements (low needs in assimilable nitrogen, vitamins, etc. less so than *S. cerevisiae*)
- It is comfortable with or without oxygen
- It is resistant to phenolic compounds

MECHANISMS OF ETHYL PHENOL SYNTHESIS BY *BRETTANOMYCES* AND THEIR SENSORY IMPACT

Wine is more impacted by *Brettanomyces* if it contains a large amount of **phenolic acids, the precursors to volatile phenols**. Several factors determine the amount that is present, and their synthesis into volatile phenols: concentration of phenolic acids in the grapes, vinification conditions, the yeast strain used (ex POF-), as well as the use of certain enzymes that have cinnamoyl esterase activity. These are the different formation pathways of volatile phenols:



STRATEGIES TO COMBAT *BRETTANOMYCES*

Until now, strategies against spoilage microorganisms such as *Brettanomyces* have been essentially preventive:

- **Biocontrol**: use of non-saccharomyces strains during pre-fermentation operations
- Limiting contaminations through **rigorous hygiene**
- Controlling the wine's ecological niche through **good fermentation management** (use of adapted yeast and co-inoculation, with selected malolactic bacteria)
- Avoiding the production of *Brettanomyces*' substrates through te use of **enzymes without cinnamyl esterase activity, and with selected yeasts.**

However, during the maturation phase there are few tools that are totally effective against Brettanomyces which do not have an impact on the wine. This is why Lamothe-Abiet developed two innovative and effective enological solutions: **Killbrett®** and **Killbact®**, for **preventive** or **curative** use.

1. EFFECTIVELY ELIMINATE BRETT USING CHITOSAN

Killbrett® makes use of chitosan, an innovative biotechnological tool recognised for microbiological stabilisation of wines. Killbrett® is a simple and effective solution for winemakers to control spoilage microorganisms.

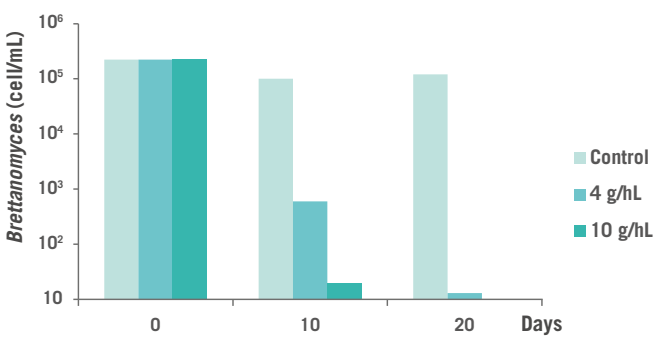
From a dosage of 4 g/hL, the use of chitosan can significantly decrease populations of undesirable microorganisms.

TRIAL RESULTS: EFFECT OF KILLBRETT® ON *BRETTANOMYCES* POPULATIONS

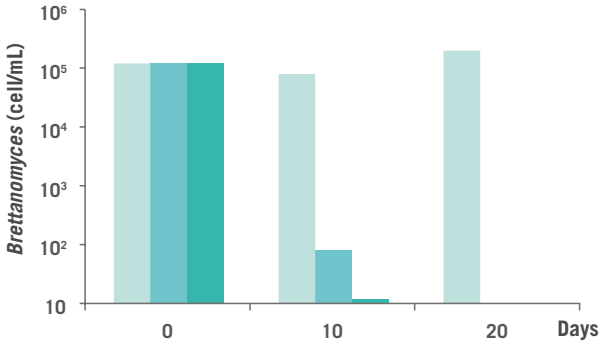
Several trials of Killbrett® on wines naturally contaminated by high populations of *Brettanomyces* have shown:

- A significant decrease in their population in less than 10 days, starting from 4 g/hL
- Even faster action at 10 g/hL (maximum authorised dosage)

Bordeaux, Cabernet-Sauvignon 2018
initial population of *Brettanomyces* : 2,2.10^5 cell/mL
(free SO_2 = 25 mg/L; T = 15°C; pH = 3,81)



Loire Valley, Cabernet-Franc 2018
initial population of *Brettanomyces* : 1,2.10^5 cell/mL
(free SO_2 = 21 mg/L; T = 16°C; pH = 3,69)

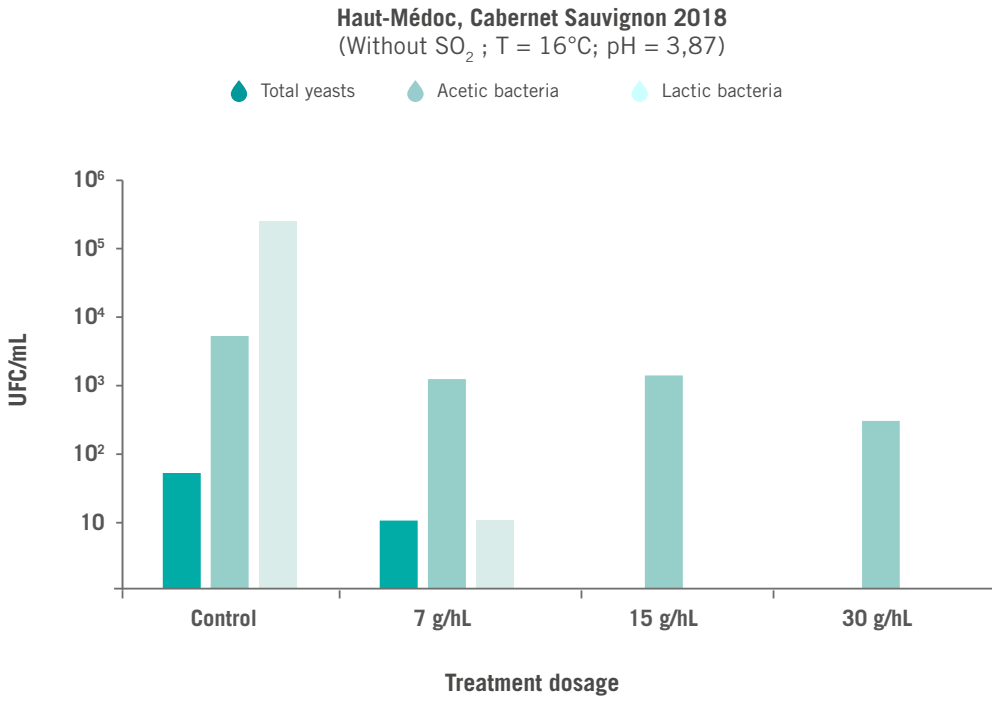


2. A BROADER SPECTRUM FOR MICROBIAL MANAGEMENT DURING MATURATION

Killbact® is a specifically formulated tool derived from chitosan and lysozyme for the **reduction and control of microorganism populations** such as undesirable yeasts, malolactic bacteria and acetic bacteria.

The chitosan and lysozyme work in synergy to give a good tool for avoiding the negative impacts linked to spoilage microorganisms present in wine (reduction of risks of volatile acidity or ethyl phenols).

TRIAL RESULTS: THE IMPACT OF DIFFERENT DOSAGES OF KILLBACT® ON TOTAL YEAST, MALOLACTIC BACTERIA AND ACETIC BACTERIA POPULATIONS



USAGE IN CASE OF *BRETTANOMYCES* CONTAMINATION

- Use a dose of Killbrett® between 4 and 10g/hL (Maximum Authorised dosage = 10 g/hL) or between 7 and 20 g/hL of Killbact® according to microbial load and winemaking conditions.
- Rehydrate in 10 times its weight in water and add evenly to the wine.
- Rack after ten days, avoiding aeration.
- Analytical control: *Brett* count or quantitative PCR and analysis of ethyl phenols after racking.
- Can be added with a glucanase enzyme (Vinotaste Pro) for faster and/or easier clarification (must be racked after ten days)

LAMOTHE-ABIET OVERVIEW:
POINTS OF VIGILANCE TO PREVENT *BRETTANOMYCES* THROUGHOUT THE PROCESS

MICROBIOLOGICAL RISK

RISK MANAGEMENT

Harvest

High population of microbial flora (moulds, non-*saccharomyces* yeasts like *Brett*, bacteria…) on grapes and additional contamination possible from equipment. Risk of multiplication during maceration.

Reception maceration

- Sort the grapes
- Bioprotection (Use of Excellence® B-Nature)
- Use of SO_2 according to grape quality
- Strict hygiene of equipment
- Use of Killbrett® or Killbact® in preventive dosages

NB: thermovinfication can reduce yeast population, including *Brettanomyces*

- Stuck or sluggish AF due to *Brettanomyces* development
- Possibly persits after AF

AF

- Quick inoculation of yeast and adapted nutrition (Gamme Excellence®)
- Control the end of AF and avoid residual sugars
- Racking at end of AF
- Killbrett® or Killbact® after AF (if MLF not desired)

NB : If there is *Brettanomyces* present and/or problems of stuck or sluggish fermentation: add Killbrett®, then re-inoculate using selected AF restart yeast

Before MLF: microbiological vacuum, risk of *Brettanoymces* development before bacteria multiplication

MLF

- MLF with selected malolactic bacteria strains (faster)
- Adapted and sufficient sulfite addition as soon as MLF over
- Use of Killbrett® or Killbact® (treatment then racking after 15 days)
- Racking
- Keep winery temperature <14°C

- Outside contamination through blending or poorly cleaned equipment

Maturation

- Adjust active SO_2
- Killbact®: 7 to 15g/hl for global microbial management and 5 to 15g/hL of Killbrett® is *Brettanomyces* present
- Clean the wine: fining, racking, clarification
- Analyse wines before blending, regular monitoring

Bottling

- Be sure of absence of *Brettanomyces* and low populations of spoilage microorganisms
- Avoid cross contamination
- Keep temperature low <12°C

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