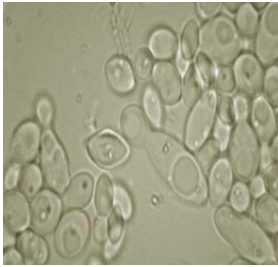


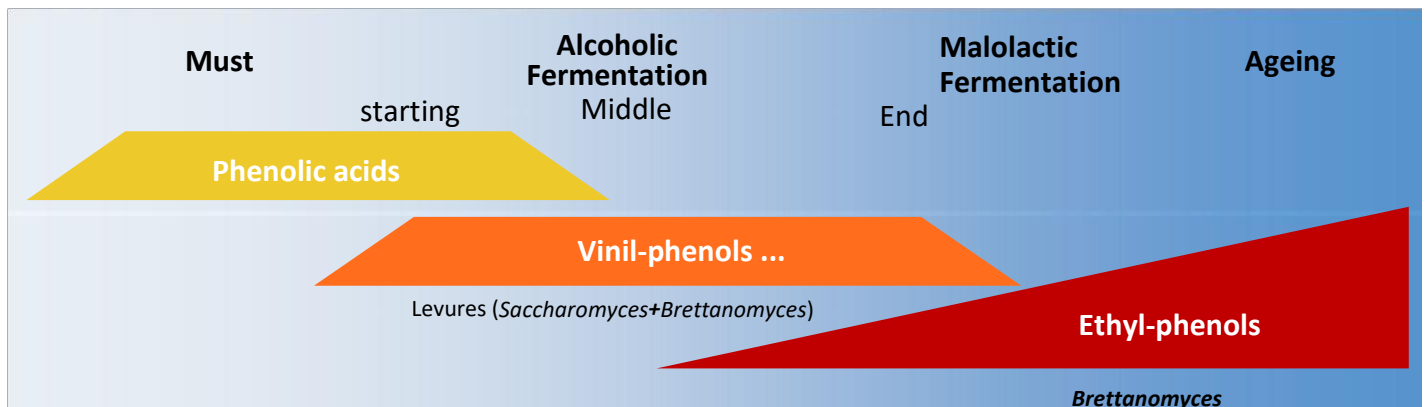
Detection of *Brettanomyces*

Only regular monitoring allows you to have a complete view of a population evolution



Brettanomyces are yeasts that alter wine, beer, cider, initially brought by fruit. They can therefore be present from fruit to bottling.

Perfectly adapted to fermentation conditions, they can survive and multiply in a deficient, acidic, alcoholic, or sulfur-rich environment. They are responsible for the production of volatile phenols that will give a bad taste and modify the aromas of wine or cider. These organoleptic deviations come from the transformation of phenolic acids into vinyl-phenol, vinyl-gaiacol and vinyl-catechol, then into ethylphenol, ethylgäicol and ethylcatechol.

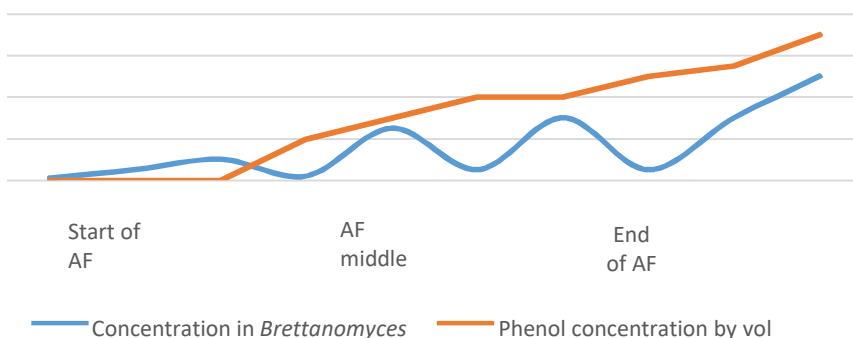


There are never volatile phenols without *Brettanomyces*, but there may be *Brettanomyces* without volatile phenols detectable at tasting.

Ideally, *Brettanomyces* should be detected as soon as possible.

To avoid their development and synthesis of molecules that will have to be eliminated later.

Example of evolution of volatile phenols compared to *Brettanomyces*



Although the *Brettanomyces* population evolves at all stages of fermentation, **volatile phenols** accumulate and **must be removed**.

In this case, late microbiological control can lead to an absence of contamination yeasts while **volatile phenols are already present**.

Detection of *Brettanomyces*

Brettia Test is a specific detection method of *Brettanomyces yeasts* by immunofluorescence.

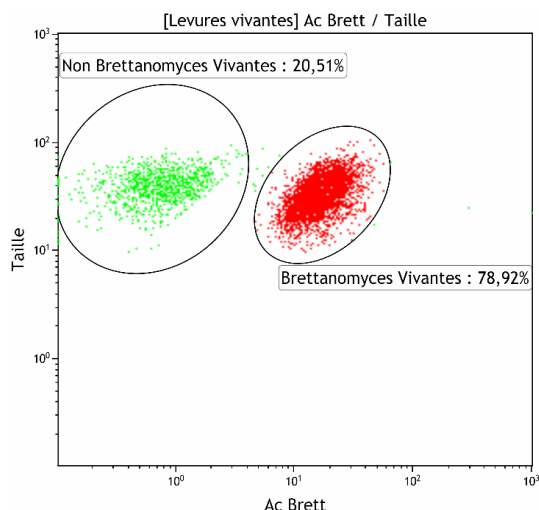
To date, there have been no real factual methods of rapid and specific detection that answer the important questions: the **presence, viability and activity of *Brettanomyces***. We have developed a specific method for the detection of these yeasts by immuno-cytometry.

Brettia Test uses antibodies, produced from *Brettanomyces* coming from different geographical origins, developed by Amarok Biotechnologies and coupled with a viability marker.

In this way, it is possible to *simultaneously* detect *Brettanomyces* and non-*Brettanomyces* and determine whether they are alive or dead and active or not.

Brettia Test provides analysis of live and dead yeasts in the sample in 2 hours.

With cytometry, it is also possible to discriminate between viable yeasts and viable non-culturable yeasts, all present in the live yeast population.



Advantages of the Brettia Test over current techniques.

Microbiological cultures

Historical technique using specific culture media. The average time to obtain a confirmed result is 5 to 7 days. Non-culturable viable yeasts are not counted.

- **Brettia test** characterizes all viable non-cultivable yeasts for less than 1 hour.

Microscopy

Visualization of microorganisms thanks to the appearance of cells. This method depends on the operator and only a small number of cell types can be identified.

- **Brettia test with cytometry can enumerate several thousand cells in seconds, and then identify small populations.**
- **Brettia test** brings the specificity of detection.

PCR

Genetic identification methods are the most specific. However, they need labs organized into forward-looking workflows: extraction is not done in the same place as amplification. They hardly distinguish between the living and the dead in standard protocols. It is also the most expensive technique.

- **Brettia test is a specific method at an affordable price, to identify viable and viable non-cultivable *Brettanomyces*. As you don't kill cells you can have all physiological states of these cells**

Cytometry with viability dye alone

Analytical methods that use only physiological dyes make it possible to follow the evolution of a production but without bringing specificity to what is observed.

- **Brettia test gives viability information in addition to specific identification.**