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Study on the distribution of *Brettanomyces bruxellensis* contamination on the inner surface of a new barrel

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The Vicard cooperage has recently developed and patented an exclusive process for etching the inner surface of barrel staves (Scarstave™) with the aim of improving the effects of barrel toasting (*photo 1*). In an extension to this innovation, a specific study was commissioned at the LEC in order to gain an insight into the behaviour of the microbiological flora in wine towards this new surface in contact with the liquid. Among the microbiological incidents feared, the development of *Brettanomyces bruxellensis* when wine is matured in barrels causes not only well-known spoilage of the wine but also adverse consequences for the wooden container (1). Indeed the ability of these yeasts to colonize the inner surface of barrels and the contamination problems encountered when reusing these barrels later are recurring issues for coopers and their clients. This is why the purpose of this study is to observe the distribution of *Brettanomyces bruxellensis* populations on the inner surface of a barrel that has contained wine artificially offering optimum conditions for the development of this yeast.

Photo 1: Interior of a barrel treated with the Scarstave™ process.

Materials and methods

For this experiment the cooper specially built a new 55-litre barrel with the following characteristics:

- Wood type: Fine-grain French oak;
- Steam bending;
- Toast: Medium +, heads not toasted;
- The barrel body alternated between etched and non-etched staves.

The strains of *Brettanomyces bruxellensis* and the seeding protocol were supplied by Dr Jean-François Gilis, Microbiology and Biotechnology R & D Manager at Vivelys, who also carried out the microbiological analyses.

The wine used was a blend of cabernet and merlot at the end of the malolactic fermentation stage and had not undergone any stabilization treatment (active S02 < 0.3 mg/L). In order to optimize the development of *Brettanomyces bruxellensis*, the wine was implanted with sugars (glucose - fructose) and para-coumaric acid, then treated with Chloramphenicol and Actidione in order to limit competition from other microorganisms (2). After the wine was transferred the full barrel was kept at 20 °C, with the bung at the top and not hermetically sealed, conserving a substantial vapour phase.

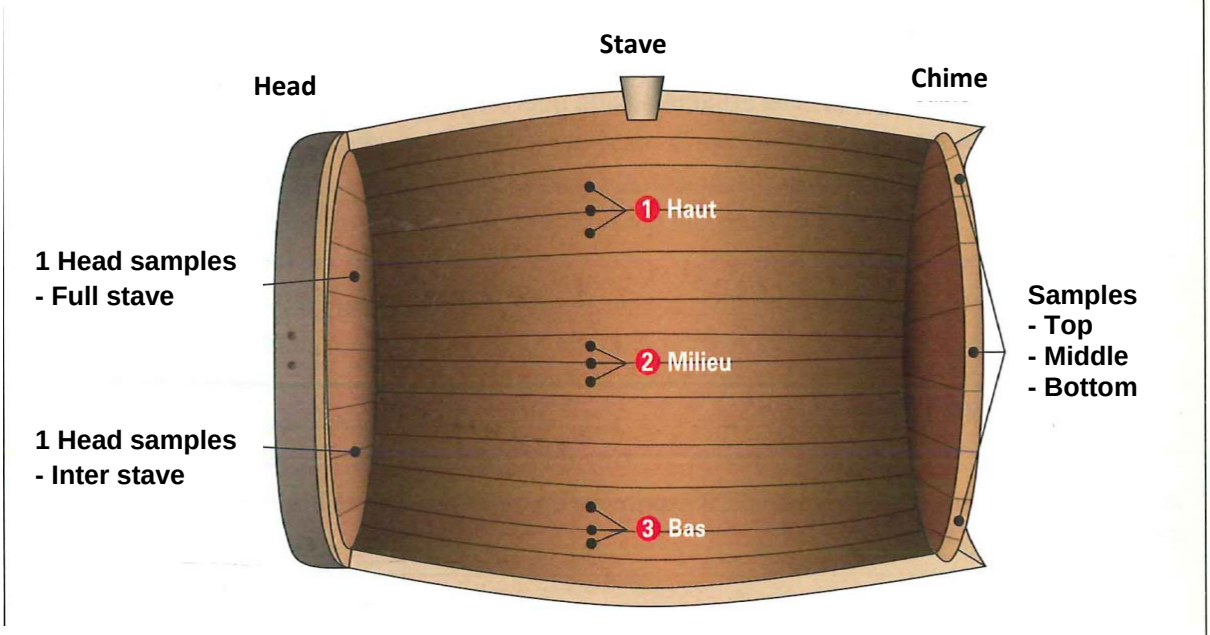
Samples were taken at regular intervals in order to monitor progress in the development of microbiological activity over the course of the 3-month trial (olfaction and dosage of the 4-Ethyphenols & 4-Ethylgaiacol by SPME-CPG-SM[(1)]). At the moment when the experiment was stopped, the concentrations in 4-ethylphenols and in 4-ethylgaiacol had stabilized to respectively 2200 µg/L and 160 µg/L.

Once drained and with the lees removed (quick rinse with demineralized water), the staves were numbered and the barrel was dismantled.

The zones sampled for analysis were distributed as follows (figure 1):

- 3 sampling heights: top, middle and bottom;
- Inner parts of the barrel: chime (junction between body and head), body (etched and non-etched staves, and inter-stave zone), head (full stave and inter-stave zone).

Figure 1: Diagram explaining the sampling zones inside the barrel.



3 Series of samples

1 Top
- Full stave, non-etched
- Inter-stave
- Full stave, non-etched

2 Middle
- Full stave, etched
- Inter-stave
- Full stave, etched

3 Bottom
- Full stave, non-etched
- Inter-stave
- Full stave, etched

The sampling tools were disinfected with ethanol between each sampling operation. The wood chips obtained were placed in sterile bottles and sent immediately to the partner laboratory for microbiological analyses. The microbiological analyses of the wood samples were performed by QPCR (scorpion probes) after maceration in a sterile grape juice to put the microorganisms back into suspension and reactivate them. The results obtained are expressed in cells per gram of wood.

(1) Solid Phase Micro Extraction™ and Gas Chromatography – Mass Spectrometry.

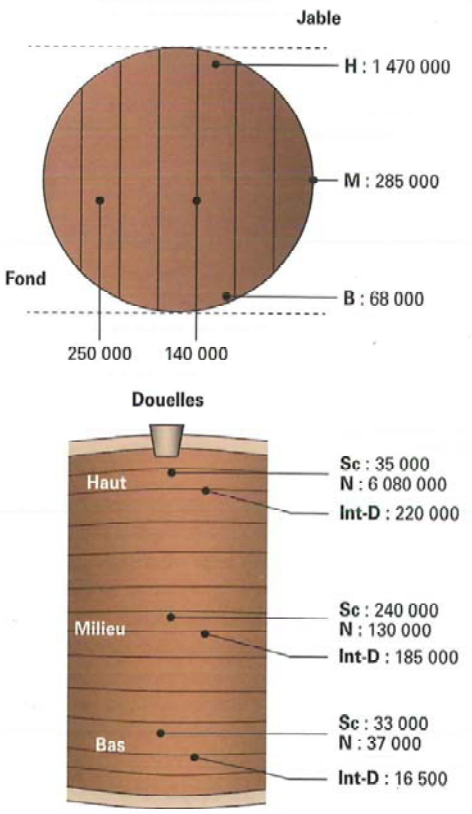
Results and discussion

The main results obtained are summarized in figures 2 and 3 *(p.4&5).*
Details of the *Brettanomyces bruxellensis* populations in the various inner parts of the barrel according to sampling height are given in figure 4 *(p.5).*
The *Brettanomyces bruxellensis* populations measured decrease rapidly between the upper and lower parts of the barrel: this is in line with the observations of Peynaud and Domercq (1954), who showed that *Brettanomyces bruxellensis* are veil yeasts likely to accumulate on the surface of wines during maturing (3).
The unusual distribution of populations on the etched staves could not be explained, although these results were confirmed by further samples.

Conclusions

The process of etching the surface of staves does not appear to be a supplementary risk factor in the event of *Brettanomyces bruxellensis* development when wine matures in barrels.
As the upper part of the barrel is likely to contain larger populations of *Brettanomyces bruxellensis*, this zone must be monitored more closely during the cleaning and decontamination of used barrels.

Figure 2: Results of the QPCR analyses expressed in cells per gram of wood.



Sc: Etched staves	**N:** Non-etched staves	**Int-D:** Inter-stave

Figure 3: Distribution of average populations of *Brettanomyces bruxellensis* expressed in cells/g of wood.

It can be observed that the chime is a part which favours the accumulation of *Brettanomyces bruxellensis*, while there is no significant difference between the populations measured on the other pieces of wood.

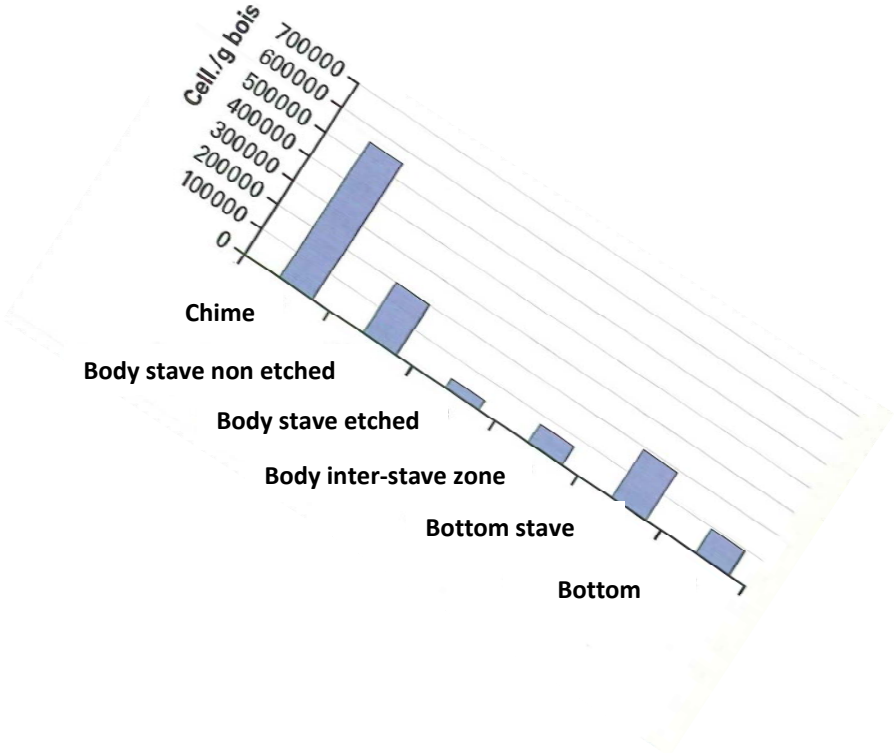


Figure 4: Details of the distribution of *Brettanomyces bruxellensis* populations expressed in cells/g of wood.