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Survival of wine microorganisms in the bottle during storage

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• During barrel aging, wine cannot be sterilized, and each enological operation is a possible source of contamination. Therefore, by the end of aging, the microbial population is often 1,000 to 10,000 cfu/ml, including *Acetobacter, Saccharomyces,* and *Oenococcus*, as well as *Pediococcus* and *Brettanomyces*.

• The goal of this study was threefold: 1) to determine the microbial populations at the end of aging (newly bottled, unfiltered wines); 2) to compare the impact on microbial populations of the most common prebottling filtration methods; and 3) to inventory the microbial populations in very old vintages (Sounds like fun to me!).

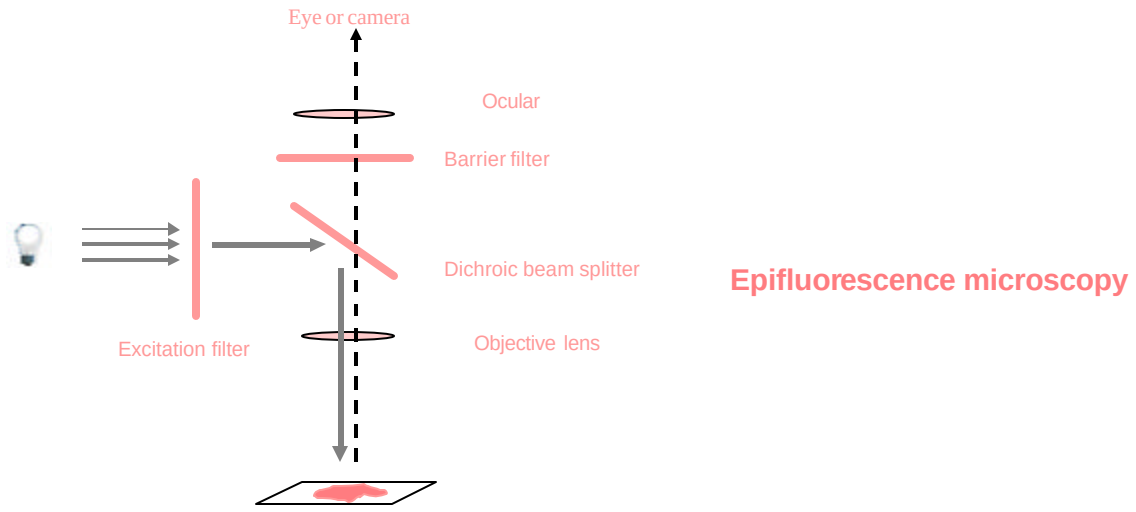
• For objective 1) above, the authors used the **2003 vintage** from 3 estates that had bottled their wines without filtration (Saint-Emilion, Pessac-Léognan, and Pauillac). For objective 2), they compared the filtration methods of 2 of the estates: a K700 (7.0 µm) pad followed by a K300 (4.0 µm) (Saint-Emilion); and the following pad sequence: K900 (10.0 µm), K300 (4.0 µm), K100 (1.0 µm), and EK (0.3 µm) (Pauillac). (As we know, the larger the pad number, the larger the pore). For objective 3), they used 10 older Bordeaux vintages (we are talking **1909 to 1981**!), all of which had corks in good condition, had been sealed with wax, and had been bottled without filtration.

• To identify the microorganisms, the authors used 3 methods: 1) an *epifluorescence microscope*, to determine viable microflora (*epi*fluorescence, by the way, is simply fluorescence in which the source of light comes from above, instead of below); 2) *plate cultures* using an array of selective media that allowed them to distinguish among: total yeast, non-*Saccharomyces* yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB); and 3) *DNA molecular tools*, such as PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and RFLP (restriction fragment length polymorphism). Three bottle replications were analyzed for each assay, and the authors noted how much the microbial populations varied from one bottle to another (generally by as much as10%).

• **Microorganisms in newly-bottled wines.** At bottling, the authors could detect 3 species: *S. cerevisiae, H. uvarum,* and *B. bruxellensis*. After 6 months in bottle, the only species that could be detected was *B. bruxellensis.* After 10 months in bottle, volatile phenol concentrations (which the authors also measured to help them complement their microorganism detection techniques) increased significantly in all wines. These dynamics varied depending on the winery. For example, Pauillac and Saint-Emilion tended to have higher AAB, and Pessac-Léognan had the highest volatile phenols –meaning higher Brett- after 10 months.

• **Microorganisms in filtered vs. non-filtered wines.** 1) The least stringent of the filtrations (K700 followed by to K300) significantly reduced total yeast populations, non-*Saccharomyces* populations, and volatile phenol concentrations. The most stringent (second sequence above, ending in an EK, or sterilizing pad), eliminated total yeast in every vintage tested and prevented the increase of volatile phenols. Filtration method did not have an effect on LAB populations.

2) Interestingly, the impact of filtration on yeast populations varied according to vintage. In 1994, K300 filtration was sufficient to eliminate all yeasts, but in 1995 there was only partial reduction with this type of filter. Similarly, K100 was sufficient in 1994 to eliminate LAB populations (*O. oeni* was the only species found this year), but an EK pad was needed to ensure elimination of LAB populations in the remaining years, when *P. parvulus* was also detected.



• **Microorganisms in older wines.** Most of the older bottled wines contained high *total yeast* populations. For example, a Pauillac wine bottled in 1926 contained about 2,000 cfu/ml viable and culturable non-*Saccharomyces* yeast in 2006 -when the study was done-; and a Pessac-Léognan wine bottled in 1949 contained 4 x10^6 cfu/ml. (that's 97 years remaining alive in a bottle of wine!) Only 40% of the older bottles contained LAB. Finally, AAB were not found in any of the older wines.

Some food for thought from the authors:

_ whereas *Saccharomyces* and acetic acid bacteria were mostly not detected in the newer bottled wines at 6 months and at 1 year (for these latter oxygen is essential to survive), non-*Saccharomyces* yeast and lactic acid bacteria were able to survive in the bottle for a very long time. Survival of LAB populations could be explained by the fact that malolactic fermentation was not encouraged in old Bordeaux vintages.

_ of the non-*Saccharomyces* yeasts, the main species found was *B. bruxellensis.* This is likely attributed to its exceptional survival ability under minimal nutrient conditions.

_ EK filtration was required to remove all bacteria, but K100 filtration was sufficient to remove all yeast. On the one hand, the smaller the filter pore size, the more effectively microbes are removed. But, according to these authors, very fine filters can impact wine aromatics and color, and therefore, it is crucial to find the right compromise. Their recommendation is that when the bacterial population is lower than the yeast population, K100 works well; and when the reverse is the case –particularly when *Pediococcus* is present- an EK filtration is recommended. How do we know which is larger, the yeast or the bacterial populations? This can be known with the help of a pre-bottling microbial analysis.

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