IVES Technical Reviews
vine & wine

https://doi.org/10.20870/IVES-TR.2023.7868

No, *Brettanomyces bruxellensis* is not responsible for all woes!

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In wines, rice cake and sausage skin aromas are generally associated with the alteration of mousy off-flavours and constitute an increasingly prevalent deviation in some wines. In the present study microorganisms present in 25 altered wines were isolated. The spoilage yeast *Brettanomyces bruxellensis*, and lactic acid bacteria of the species *Oenococcus oeni* and *Lentilactobacillus hilgardii* were identified, and their ability to produce the *N*-heterocycles responsible for the mousy off-aroma was deterimined. *B. bruxellensis* was found in a minority of the wines altered by the mousiness.

Introduction

The defect of mousy off-flavours is characterised by aromas ranging from « basmati rice » and « sausage skin » to « mice urine », and is only perceptible through retro-nasal olfaction due to the low volatility of the compounds responsible for the alteration of wine pH [1]. These compounds can be produced by lactic acid bacteria, such as *Lentilactobacillus hilgardii* and *Oenococcus oeni*, as well as the yeast *Brettanomyces bruxellensis*, already known for producing the phenolic character of wines[2 3]. In general, this yeast is often held responsible for the mousiness defect, but is its incidence as significant as assumed? Very few studies focus on the microbial diversity of wines affected by mousiness. This study therefore aimed to identify the microorganisms present in 25 altered wines and investigate their capacity to induce the alteration in a model environment

Results

A total of 353 microbial isolates were obtained from the 25 wines exhibiting mousy off-aroma defects. These various microorganisms were identified using MALDI-TOF MS. 101 isolates revealed the presence of four species of yeast: *B. bruxellensis*, *Pichia manshurica*, *Priceomyces carsonii, and Saccharomyces cerevisiae*. Regarding the 252 lactic acid bacteria isolates, three species were identified: *O. oeni*, *L. hilgardii, and Pediococcus parvulus*. The frequencies of the identified species are shown in Figure 1.

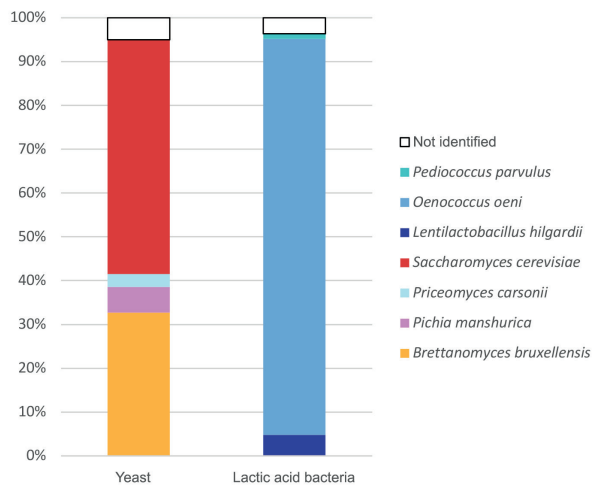

FIGURE 1. Microbial species identified among the isolates from the 25 altered[4].

The mousy off-flavor production capability of these various isolated microorganisms was evaluated in *N*HAM model medium (medium containing all known precursors to date to produce *N*-heterocycles). This capability was monitored by measuring the three *N*-heterocycles compounds recognised as being responsible for the mousy off-aromas, namely acetylpyrroline (APY), ethyltetrahydropyridine (ETHP) and acetyltetrahydropyridine (ATHP)[5]. Only 53 isolates produced the alteration: 13 from *B. bruxellensis*, 3 from *L. hilgardii*, and 37 from *O. oeni*, representing 100% of the tested isolates of these species. The study was then extended to 22 strains of *B. bruxellensis*, 20 strains of *O. oeni* and 10 strains of *L. hilgardii* from various collections (provided by CRBO (Bordeaux, France); GMGM laboratory (Strasbourg, France); AWRI (Adelaide, Australia), and DSM (Braunschweig, Germany)). The objective was to determine the variability in the production levels of the three *N*-heterocyclic compounds.

The results are presented in Figure 2. All tested strains produced at least two of these compounds. *B. bruxellensis* strains produced ATHP and ETHP, while lactic acid bacteria tended to predominantly produce APY and ATHP. Differences in production levels for the three compounds were observed among strains within the same species, but no correlation could be drawn between the genetic typing of the tested strains and their ability to produce the mousy off-flavor molecules under standardised conditions (*N*HAM medium).

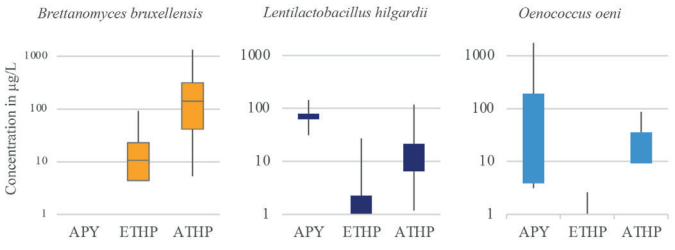

FIGURE 2. Alteration compounds in µg/L produced in a model medium by strains from collections[4].

These three producing species were detected in some of the studied wines but not in others. Their prevalence can be seen in in Figure 3. *O. oeni* is present in 84% of wines, while *B. bruxellensis* and *L. hilgardii* are found in only 20% and 12% of the wines respectively. For two of these wines, no microorganism belonging to the three species could be isolated. One of the two wines curiously contained

The translation of this article into English was offered to you by Moët Hennessy.

no microorganisms at all. The lack of information obtained on these wines does not exclude the possibility of filtration prior to sampling. However, the second wine contained other microorganisms, such as *Saccharomyces cerevisiae*. The possibility that the alteration was produced other than by these three species remains a working hypothesis.

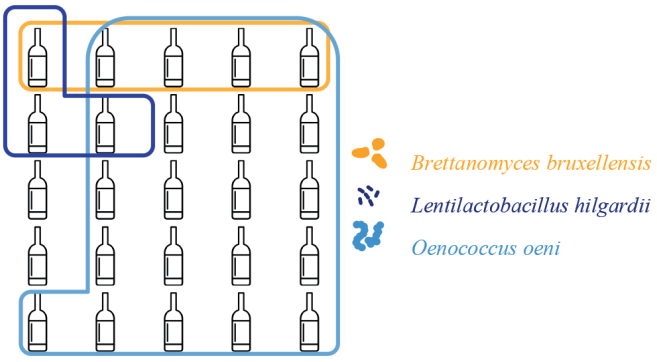

FIGURE 3. Prevalence of isolated species from 25 altered wines[4].

In another study conducted at Institut des Sciences de la Vigne et du Vin (ISVV, Villenave d'Ornon, France)[6], the three compounds were analysed in 62 defective wines following the development of a quantifying method for *N*-heterocycles responsible for alteration in wines. Each analysed wine contained at least one of the three compounds. In addition to *N*-heterocycles, 4-ethylphenol and 4-ethylguaiacol - responsible for the phenolic character produced by *B. bruxellensis* - were also measured. Among the 62 altered wines, only 8 of them showed significant amounts of volatile phenols (above the quantification limits), accounting for 12.9% of the wine. The absence of phenolic compounds in most of the wines affected by mousiness suggests that there is no correlation between both alterations.

Conclusion

The objective of this study was to determine the diversity of microorganisms in wines altered by mousy off-flavours and assess their production capability in a model medium. Most microorganisms isolated from the defective wines belonged to the species *O. oeni*, *B. bruxellensis* and *S. cerevisiae*. Of the four isolated yeast species, only *B. bruxellensis* demonstrated the ability to induce the alteration in the model medium. Similarly, only *O. oeni* and *L. hilgardii* exhibited this capacity among the three isolated lactic acid bacteria species. In most wines (16 out of 25), the only microbial species that produced the defect was *O. oeni*. On the other hand, *B. bruxellensis*, often associated with the alteration, was found in less than 20% of defective wines. ■

Acknowledgements: *This research was funded by the Franco-German Graduate College CDFA-03-18, the Nouvelle-Aquitaine Region and the State of Hesse, as part of the international alliance of projects Bordeaux-Adelaide-Geisenheim (BAG).*

1 Tempère, S., Chatelet, B., de Revel, G., Dufoir, M., Denat, M., Ramonet, P.-Y., Marchand, S., Sadoudi, M., Richard, N., Lucas, P., Miot-Sertier, C., Claisse, O., Riquier, L., Perello, M.-C., & Ballestra, P. (2019). Comparison between standardized sensory methods used to evaluate the mousy off-flavor in red wine. *OENO One, 53*(2). https://doi.org/10.20870/oeno-one.2019.53.2.2350

2 Chatonnet, P., Dubourdieu, D., Boidron, J., & Pons, M. (1992). The origin of ethylphenols in wines. *Journal of the Science of Food and Agriculture, 60*(2), 165–178. https://doi.org/10.1002/jsfa.2740600205

3 Peynaud, E., & Domercq, S. (1956). *Brettanyomyces* isolated from grapes and wine. Archiv Fur Mikrobiologie, 24(3), 266–280. https://doi-org.docelec.u-bordeaux.fr/10.1007/BF00419012

4 Moulis, P., Miot-Sertier, C., Cordazzo, L., Claisse, O., Franc, C., Riquier, L., Albertin, W., Marchand, S., De Revel, G., Rauhut, D., & Ballestra, P. (2023). Which microorganisms contribute to mousy off-flavour in our wines? OENO One, *57*(2), 177–187. https://doi.org/10.20870/oeno-one.2023.57.2.7481

5 Snowdon, E. M., Bowyer, M. C., Grbin, P., & Bowyer, P. K. (2006). Mousy off-flavor: A review. *Journal of Agricultural and Food Chemistry, 54*(18), 6465–6474. https://doi.org/10.1021/jf0528613

6 Kiyomichi, D., Franc, C., Moulis, P., Riquier, L., Ballestra, P., Marchand, S., Tempère, S., & de Revel, G. (2023). Investigation into mousy off-flavor in wine using gas chromatography-mass spectrometry with stir bar sorptive extraction. *Food Chemistry, 411*, 135454. https://doi.org/10.1016/j.foodchem.2023.135454