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Assessment of the impact of chitosan treatment on microorganisms in enology

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As part of the move to reduce SO_2 in wines, the empirical use of chitosan to eliminate the spoilage yeast *Brettanomyces bruxellensis* is becoming a frequent alternative. Chitosan treatment has an effect on a number of microbial species, at least temporarily, but there is wide variability in response within strains of each species. In addition, the effectiveness of chitosan also depends on the physical and chemical parameters of the wine. However, when chitosan treatment is effective, it appears to be long-lasting.

Chitosan in enology

The Chitowine project, funded by the French National Research Agency from 2018 to the end of 2022, aimed to improve understanding of the mechanism of action of chitosan through a detailed assessment of its effectiveness in various enological applications. In particular, this research was based on the scientific knowledge acquired concerning the major microbial groups found in enology.

Chitosan has been used since 2009 (OIV/Oeno, 338A/2008 and European regulation EU/53/2011 Annex I), including in Organic Agriculture (Regulation EU 1584/2018), to treat wines contaminated by the spoilage yeast *Brettanomyces bruxellensis*. This polysaccharide derived from chitin is positively charged at wine pH, enabling it to interact with negatively charged particles in wine, notably microorganisms. However, research into its effectiveness against *B. bruxellensis* and other enological microorganisms has shown mixed results. In addition, while it was recommended for use in aging until recently, its early application is now being promoted by suppliers, without any real scientific justification, particularly for use in the pre-fermentation phase.

What is the impact of chitosan treatment on microorganisms in enology?

A large-scale study of 206 strains of 27 yeast and bacteria species from the enological ecosystem was carried out in wine, under standardized conditions[1]. For some species, such as *Saccharomyces cerevisiae*, *B. bruxellensis* and *Oenococcus oeni*, strains were selected according to how representative they were of the genetic groups[2 3 4]. These strains were adapted and then allowed to grow in a wine fermented in the laboratory. Each was treated with 4 or 10 g/hL chitosan and racked after 3- or 10-days' contact time. A control, simply racked under the same conditions, was used to determine the survival of the microbial strains under the test conditions but in the absence of treatment.

The response of *Brettanomyces bruxellensis* to chitosan addition falls into three main categories

This study demonstrated the existence of three types of *B. bruxellensis* response to chitosan treatment (Figure 1):

- **SENSITIVE** strains, which from the 3rd day of contact show very low levels of viable and cultivable populations (or below the method's detection threshold) both in the lees and in the racked wine fraction.
- **INTERMEDIATE** strains, for which few viable and cultivable cells are detected in the racked wine fraction, while significant populations of microorganisms are present in the lees.
- **TOLERANT** strains, temporarily affected by the treatment, but with significant viable and cultivable populations in the racked wine fraction and lees at ten days.

Chitosan is thus generally effective against *B. bruxellensis*[2]. In evidence of this, under laboratory conditions in standardized wines, 41 % of *B. bruxellensis* strains are "sensitive" to chitosan, while 38 % show an "intermediate" response.

Finally, various trials have shown that, after racking, chitosan treatment eliminates nearly 80 % of the strains among approximately fifty tested in red wine. Nevertheless, some strains remain unaffected by treatment, viable and capable of growing and producing volatile phenols in a treated wine.

For the same wine and a given strain, the dose used – 4 or 10 g/hL (as recommended by the OIV) – has little impact on the strain's behavior, only on the magnitude of the result.

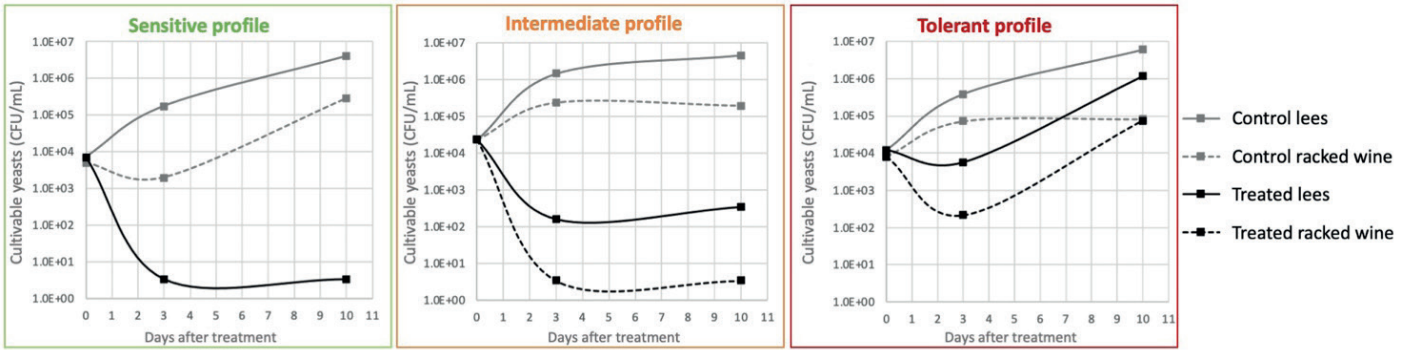

FIGURE 1. Trend curves for the responses of different B. bruxellensis microbial strains observed following chitosan treatment at 10 g/hL in the same red wine. The solid lines correspond to the lees of the treated wine (black) or control wine (gray). The dotted lines correspond to the racked wine fraction, treated (black) or the control (gray).

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Is there a link between the genetic group of *B. bruxellensis* yeasts and their response to chitosan?

The link between SO_2 sensitivity and the genetic group of *B. bruxellensis* was demonstrated in 2018[5]. The CHITOWINE project thus investigated the existence of a potential link between genetic group and chitosan response in approximately fifty strains[6]. The susceptibility of *B. bruxellensis* strains to chitosan treatment was established for isolates from each of the 6 major genetic groups.
At the doses of chitosan recommended in enology (4 to 10 g/hL), the study failed to establish a clear and robust link between genetic group and strain susceptibility (Figure 2). However, a large proportion of strains resistant to SO_2 (AWRI group 1499) are sensitive to chitosan.

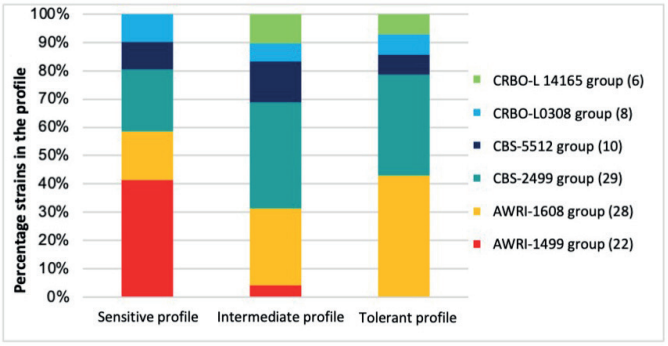

FIGURE 2. Distribution of strains of *B. bruxellensis* by genetic group (number of trials in brackets) according to their susceptibility to chitosan treatment at 10 g/hL in the same red wine.

What about other microorganisms found in enology?

The study was widened to include 27 enological yeast and bacteria species. They were also found to adopt the three main behavior patterns in response to chitosan, in varying proportions depending on the species or group of species (Figure 3).
Treatment of red wine with 10 g/hL chitosan has an effect on most of the microorganisms studied, at least temporarily. For example, among the *S. cerevisiae* yeasts tested, more than half are effectively eliminated by chitosan. The effects are variable for non-*Saccharomyces* yeast species found in the pre-fermentation phase. In the laboratory, most strains of *Hanseniaspora uvarum* are unaffected by early chitosan treatment. For yeasts of interest such as *T. delbrueckii* or *M. pulcherrima*, results are highly disparate and clearly depend on the strain in question.
Treatment of red wine has very little effect on acetic acid bacteria, during either vinification or aging: chitosan had no effect on the viability of any of the strains tested. Lastly, the effect on lactic acid bacteria is variable, with a mosaic of responses, except in the case of *O. oeni* which was strongly affected by chitosan in more than 90 % of the trials.

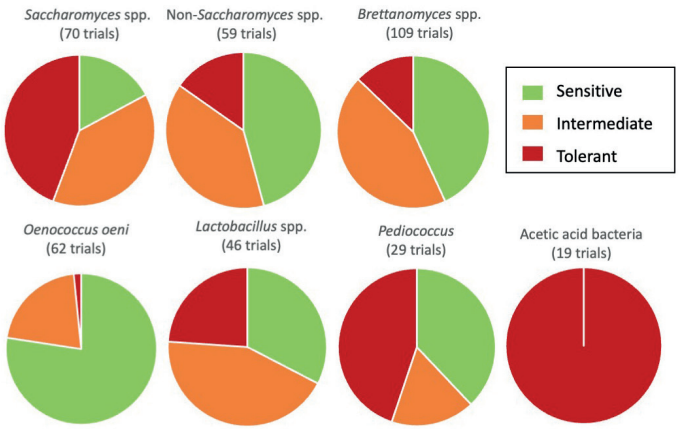

FIGURE 3. Distribution by sensitive, intermediate or tolerant profile for microbial strains tested within a species or group of microorganisms after treatment with 10 g/hL chitosan in the same red wine.

Does the effectiveness of chitosan vary according to the stage of vinification?

The nature of the wine alters the availability of chitosan and the capacity of microorganisms to overcome its effects.
Early application of chitosan at the vat-filling stage does not replace the use of sulfite to control the predominance of undesirable yeasts such as *H. uvarum*, confirming laboratory trials (Figure 3). However, early application does not appear to disrupt progress of the alcoholic fermentation when the must is inoculated with a selected strain of *S. cerevisiae*. On the other hand, an early application, although effective, leads to an unpredictable progress of the malolactic fermentation. This is partly explained by the sensitivity of *O. oeni* strains to chitosan. Nevertheless, a sustainable elimination of *B. bruxellensis* is possible, provided that an effective racking is performed (data currently being published). Lastly, the treatment is not an effective way to control acetic acid bacteria.

Conclusion

The response of enological microorganisms to chitosan treatment of wine falls into three categories: sensitive, intermediate and tolerant. Effective racking after treatment results in the long-lasting elimination of the spoilage yeast *B. bruxellensis* for 80 % of the strains tested in this study. It is reassuring to note that most of the strains tested in this study belonging to genetic group AWRI 1499 (known for its sulfite tolerance[5]) are sensitive to chitosan. In addition, early treatment does not seem to influence the smooth progress of the alcoholic fermentation, but is of little or no interest for the control of non-*Saccharomyces* yeasts or acetic acid bacteria. However, when applied too early, chitosan makes progress of the malolactic fermentation unpredictable and is thus not recommended. ■

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Sources : *Sourced from the research articles: "Brettanomyces bruxellensis displays variable susceptibility to chitosan treatment in wine." (Front. Microbio, 2021) and "Assessment of chitosan antimicrobial effect on wine microbes" (Int. Journal of Food Microbiol., 2022)*

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