Fungal chitosan is an efficient alternative to sulphites in specific winemaking situations

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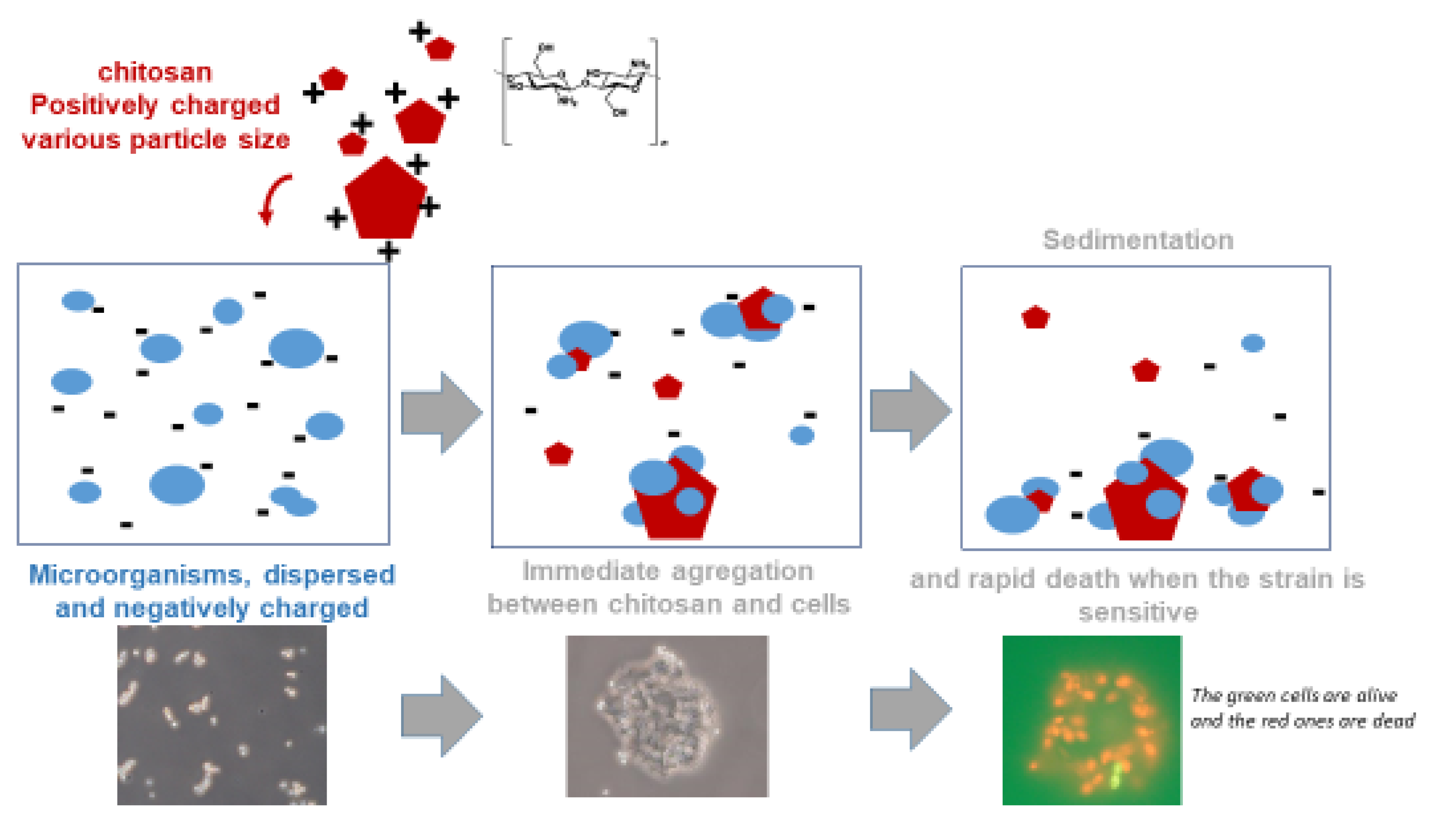
The most common method to prevent or eliminate microbes in wine is sulfur dioxide (SO_2) addition. However, as risk of acute allergy exists, the European Union has classified SO_2 as one of the 14 priority food allergens (EU Regulation N°1169/2011, Annex II). Winemakers thus need alternate and efficient antiseptic methods to reduce total SO_2 content in wines. The resolutions of the 7th general assembly of the International Organization of Vine and Wine (OIV/OENO 338A/2009) and the European Union (EC 53/2011) authorized the addition of fungal chitosan to reduce spoilage microorganism populations especially *Brettanomyces bruxellensis*.



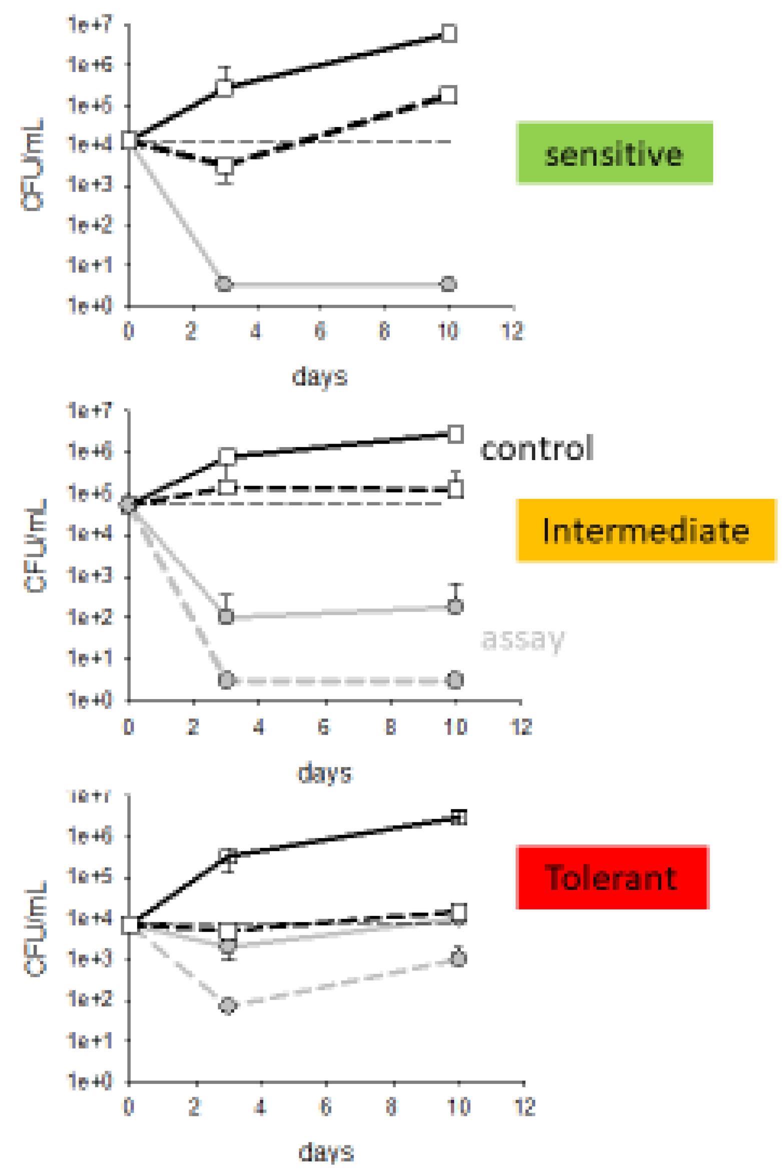


Figure 1. Chitosan mode of action (A) and distinct behaviors observed among wine microbes (B). *In the figures describing the profiles (B), chitosan is added at t=0; the gray lines represent the populations in the assays, the black ones those in the untreated control; the dotted lines represents the population in the lees (sedimented living cells) and the continuous lines the population in the wine above (unsedimented living cells).*

Chitosan is a partially acetylated polysaccharide of glucosamine. It is positively charged at wine pH, which allows it to interact with the negatively charged microorganisms and particles present in the wine. The immediately formed aggregates then sediment into the lees, where most of the sensitive individuals rapidly die and living populations decrease under the detection threshold (Figure 1). However, a high variability prevails within most species and sensitive, intermediate and tolerant strains can be observed. Furthermore, wine components can also interact with chitosan and prevent interaction with microbes. Chitosan thus displays different efficiencies depending on the wine biological and chemical parameters or on the winemaking stage when the treatment is performed.

In order to state on the **relevance of chitosan treatment**, we have carried out tests with more than 200 strains in 27 species in the presence of 2 distinct chitosans called F1 and F4 and displaying distinct molecular weights (Figure 2). Most species are affected, at least transiently, by chitosan treatment.

- ✓ Among bacteria, the acetic ones are highly tolerant, and *Oenococcus oeni* appeared highly sensitive, while the behavior of other lactic acid bacteria was highly variable depending on the strain.
- ✓ Among wine yeasts, *Saccharomyces* species were quite tolerant while *Brettanomyces* and other non-*Saccharomyces* species displayed variable behavior with however a high proportion of sensitive and intermediate strains.
- ✓ The study also shows that structural differences among fungal chitosans impact their efficiency. F1 (30kDa MW) which is the form most often found for wine applications is more efficient than F4 (400kDa).

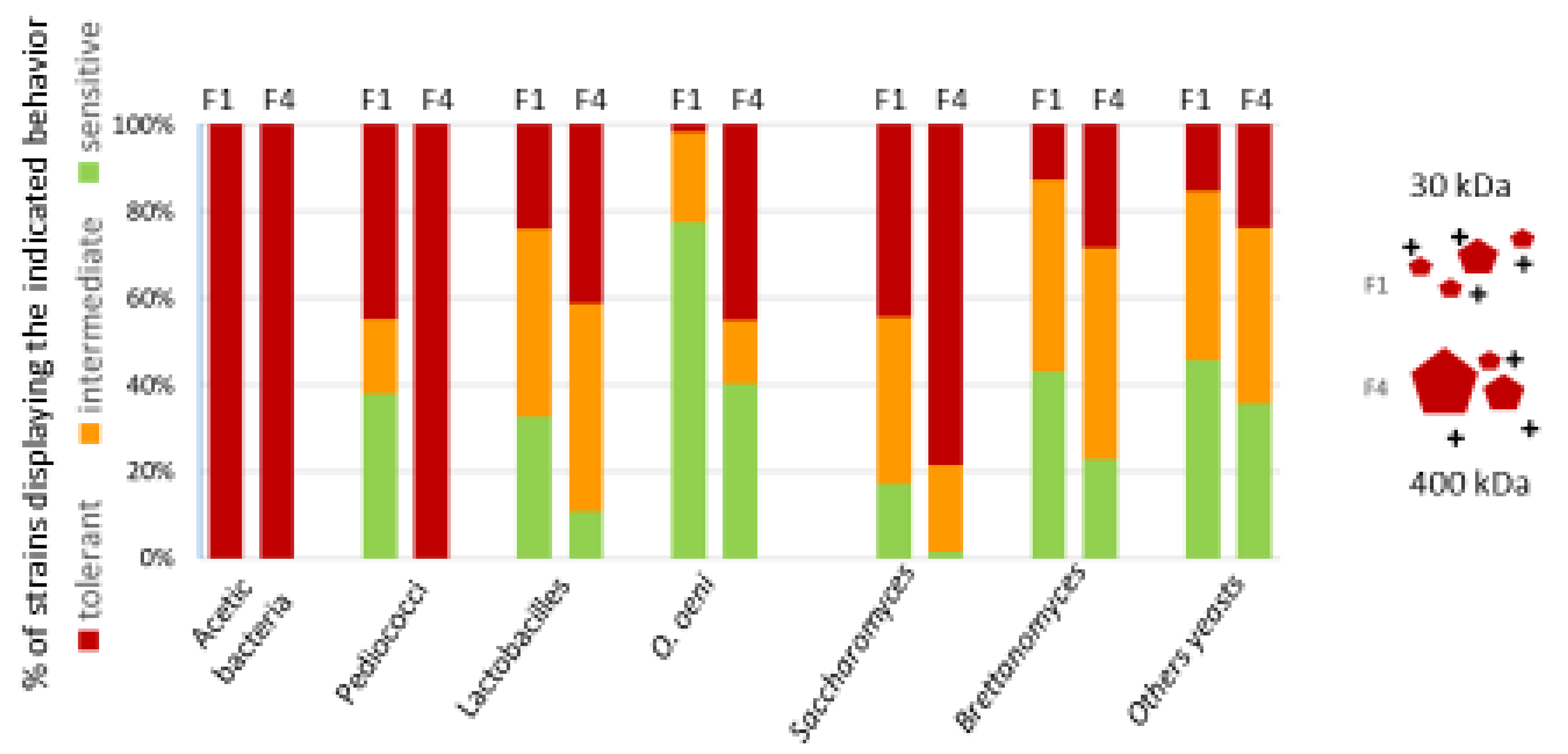


Figure 2. Proportion of sensitive, intermediate and tolerant strains in various groups of wine microbes.

Table 1. Sum up of winemaking situations explored

Winemaking stage	Results
Before alcoholic fermentation (AF); prefermentary stage	No effect on *Hanseniaspora uvarum* (no protection). No effect on subsequent fermentations if inoculation
Elimination of *B. bruxellensis* at the end of AF	Efficient on sensitive and intermediate strains if racking is performed; random spontaneous MLF
Elimination of *B. bruxellensis* after FML	Can be efficient and longlasting if sensitive strains present; variable result with intermediate strains. Often efficient on sulphite tolerant strains. Racking is necessary after treatment
Elimination of acetic bacteria during aging	No effect
Preventive treatment against *B. bruxellensis* during aging	Efficient and longlasting on sensitive strains. No effect on tolerant ones.

The relevance of chitosan treatment was then explored in different winemaking situations in several white and red wines (Table 1). Though chitosan does not solve all the microbial spoilage issues, this study reveals that chitosan can be an interesting alternative to sulphites in certain situations. Furthermore, when the antiseptic effect is high, it seems durable and wines are protected for microbial spoilage over long periods.

The organoleptic consequences of the treatment (dose 10g/hL) were evaluated on four red and two white wines, through triangle tests (ISO 4120:2004) and a panel gathering half expert and half naïve tasters. No significant difference could be observed, and when the difference was perceived by some expert, the non treated wine was not always the preferred one, i.e. chitosan treatment was not perceived as a fault.

Conclusion

Chitosan treatment can be very efficient, long-lasting and without danger for wine quality in certain specific winemaking situations. In other ones, it is completely useless and other antiseptic methods should be preferred.

More results and method description in Miot-Sertier et al., https://doi.org/10.1016/j.ijfoodmicro.2022.109907