Evaluation of the impact of a fungal-origin chitosan preparation on *Brettanomyces* in the context of wine aging

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Introduction

Brettanomyces bruxellensis development in wines is a continuous threat for wine quality. This undesirable yeast is able to develop during aging under difficult conditions, and to produce negative aromatic compounds such as volatile phenols related to sensory descriptors as animal-like, horse, barnyard, band aid and medicinal. Chitosan of fungal origin has been introduced as a new potential tool to control *Brettanomyces* in winemaking [1]. Recent studies showed the impact of chitosan application (racked off after 10 days) on contaminated wines, leading to the elimination of *Brettanomyces* cells [2]. Due to the necessity to control wine microbiological stability during aging in barrels, our research focuses on a new, long term application of an enological chitosan preparation, to prevent wine from *Brettanomyces* along the aging period (up to 9 months) at both experimental winery and at winery-scale.

Material and methods

The fungal origin chitosan preparation used (No Brett Inside™) is a powder with particles of average size of 50 µm, extracted from *Aspergillus niger*, produced by KitoZyme (Herstal, Belgium).
4 g/hL were added in the treated wines.
Several experimentations were run during 2 years in Tuscany, Italy.

At pilot-scale:

- 2 wines (Sangiovese and Merlot, 2011) after MLF;
- 3 different treatments (25L stainless-steel tanks) :
  - control (untreated);
  - chitosan 4 g/hL, no rack-off, no batonnage;
  - chitosan 4 g/hL, no rack-off, batonnage once a week .
- After the treatments, each tank was inoculated with pre-adapted *Brettanomyces* cells at 10^3 UFC/mL, and followed up to 6 months.

At winery-scale:

- 2 wines (Sangiovese and Merlot, 2011);
- Each grape must was separated into 2 lots inoculated wit the same ADY strain: one was co-inoculated with selected lactic acid bacteria and the other went through spontaneous MLF.
- The wine of each lot, at the end of MLF, was split into 3 barrels (225L) for 9 months aging, with different chitosan treatments:
  - control (untreated);
  - chitosan 4 g/hL, no rack-off, no batonnage;
  - chitosan 4 g/hL, no rack-off, batonnage once a week.

The same trial was repeated in a 2012 Sangiovese; after MLF achieved by co-inoculated lactic acid bacteria the wine was split into a larger number of barrels (4) per treatment condition.

Quantification of *Brettanomyces* cells was performed at the trials set-up and every month during the aging period, by plating on selective medium. Quantification of volatile phenols was performed at the trials set-up and at the end of aging by HPLC.

Impact of the treatments at pilot-scale

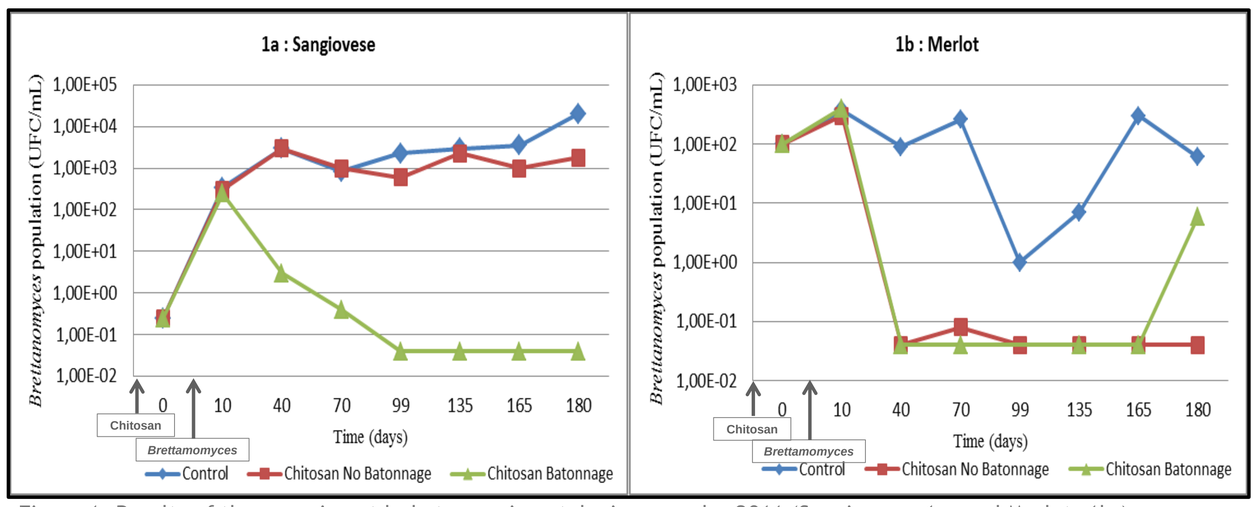

Figure 1: Results of the experiment led at experimental winery-scale, 2011 (Sangiovese -1a- and Merlot -1b-)

In this experiment, where *Brettanomyces* was inoculated on wines after the chitosan treatment, we observed a clear impact of chitosan on the evolution of *Brettanomyces* population along the 6 months. The best control of *Brettanomyces* contamination was achieved with batonnage on the Sangiovese wine, and with or without batonnage on the Merlot wine (Figure1). It is important to mention that wines had been previously desulphited and no SO_2 was added, which may explain the regrowth of *Brettanomyces* on the long-term.

Impact of the treatments at winery-scale

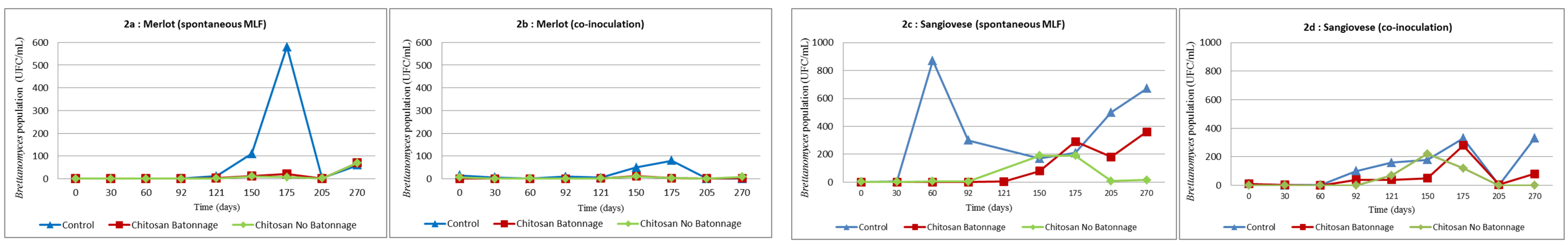

Figure 2: Results of the experiment led at winery-scale, 2011: Merlot (spontaneous MLF -2a- and co-inoculation -2b-); Sangiovese (spontaneous MLF -2c- and co-inoculation -2d-)

On 2011 Merlot wines (Figures 2a & 2b), *Brettanomyces* wild population was always below 100 UFC/mL, except for the control from spontaneous MLF. Chitosan addition, with or without batonnage, clearly helped to prevent *Brettanomyces* growth up to 6 months.

On 2011 Sangiovese wines (Figures 2c & 2d), where *Brettanomyces* populations were slightly higher, it is interesting to notice the positive impact of bacteria co-inoculation on *Brettanomyces* contamination, as previously described [3]. Furthermore, *Brettanomyces* development occurred earlier in untreated wines, confirming chitosan impact. Again, no SO_2 addition was done during the 6 first months. The 9 months of chitosan contact , did not show any effect on the chemical and sensory properties of the wine.

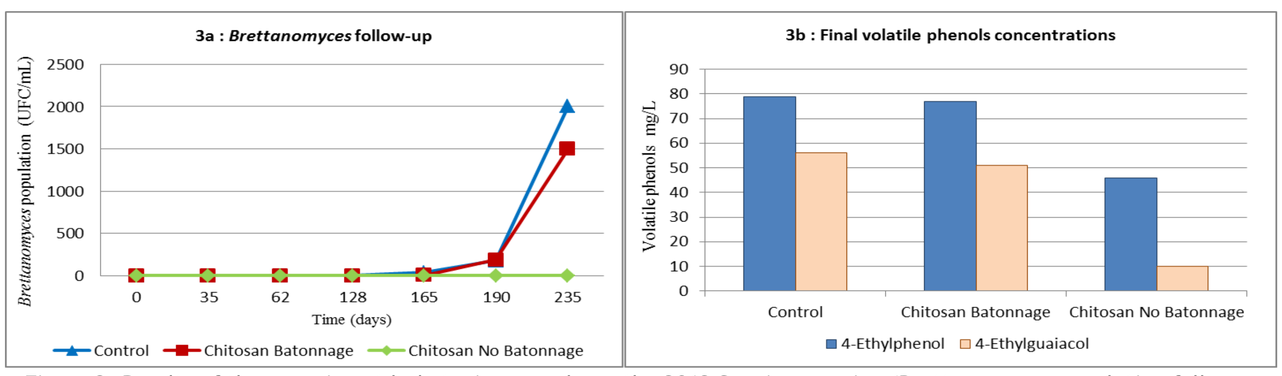

Figure 3: Results of the experiment led at winery-scale on the 2012 Sangiovese wine (Brettanomyces population follow-up -3a- and final volatile phenols concentrations -3b-)

Observations made on 2011 regarding the impact of chitosan on the long-term application to protect wines against *Brettanomyces*, were confirmed on a 2012 Sangiovese wine., The addition of chitosan without batonnage during aging led to lower *Brettanomyces* contamination and volatile phenols concentration.

Acknowledgements

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References

1. Gomez-Rivas et al. Selective antimicrobial action of chitosan against spoilage yeasts in mixed culture fermentations. J. Ind. Microbiol. Biotechnol. 31 (2004):16-22
2. Blateyron-Pic et al. Le chitosane d'origine fongique : un nouvel outil de choix pour lutter contre Brettanomyces dans les vins. Revue des œnologues. N°143 (2012), 27-28
3. Gerbaux et al. Influence of Inoculation with Malolactic Bacteria on Volatile Phenols in Wines. Am. J. Enol. Viticult. 60 (2009): 233-235.

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