Grape juice contamination by Botrytis. Impact on the characteristics and foaming properties of yeast macromolecules released in the wine during alcoholic fermentation

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Introduction

Several studies have demonstrated the contribution of (glycol)proteins and polysaccharides (PS) in sparkling wine foam properties (Maujean et al., 1990; Brissonnet and Maujean, 1991; Abdallah et al., 2010; Coelho et al., 2011; Martínez Lapuente et al., 2013). Correlations between foam properties of grape juices, base wines, and sparkling wines with PS content and composition have been shown (Girbau Sola et al., 2002). More precisely some studies have identified yeast mannoproteins (MPs) released during alcoholic fermentation and autolysis as molecules involved in improving foam properties (Abdallah et al., 2010; Coelho et al., 2011). Also, Martínez Lapuente et al. (2015) suggested that MPs were not involved in foamability but were good foam stabilizers. Moreover, we know that *Botrytis* can degrade grape berry proteins (Marchal et al., 2006; Cilindre et al., 2007). Nevertheless, we know very little concerning the impact of *Botrytis cinerea* enzymatic activities onto 1) the characteristics of the macromolecules released by *Saccharomyces* in the juice/wine during the alcoholic fermentation and 2) their contribution to wine foamability. To answer this question, we have produced Wine from Synthetic Must (noted WSM) by fermenting synthetic "healthy" and "*Botrytis* contaminated" musts with 3 oenological yeast strains.

Botrytis cinerea culture in a synthetic must

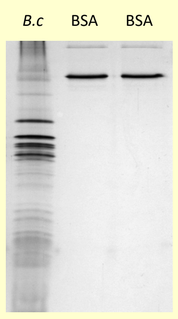
* Fungus isolated from Champagne's vineyard (*B.c 630*, INRA Versailles)
* Culture on a synthetic must to avoid grape berry macromolecules and phenolic compounds.
* Inoculation at 1,67.10^4 spores/mL in a Manteau medium.
* Agitation at 150 rpm – Culture during 22 days at 18°C.

Proteic composition of the *Botrytis* culture (22 days)

Studied by SDS-PAGE + Silver nitrate staining
Surnageant concentrated 14,7 x
Calibration : 30ng BSA/well

Total released proteins : **1.075 mg/L** (densitometric integration)

Detection of at least 21 bands/proteins released by the fungus during the culture
Presence of enzymes : Protease(s)? Mannosidase(s)? Glucanase(s)?



Proteolytic activity of *Botrytis* culture

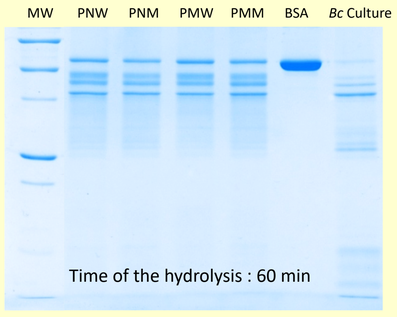
Grape juices, wines and *Botrytis* culture proteolytic activities were compared using the **BSA** as a substrate

Pinot noir must (**PNM**) and Pinot meunier must (**PMM**) were produced from 100% botrytized grape berries

Pinot noir wine (**PNW**) and Pinot meunier wine (**PMW**) were produced from the corresponding grape juices

All samples were diluted 1/10

Comparison of grape juices, wines and *Botrytis* culture proteolytic activities



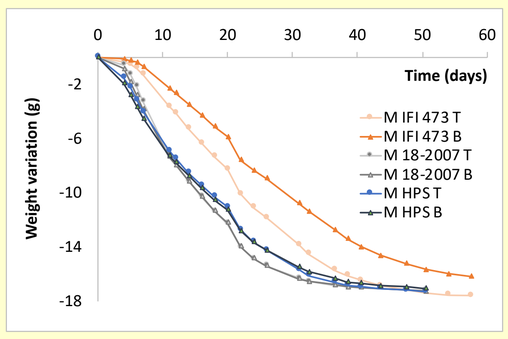
The proteolytic activity of *Botrytis* culture on the BSA corresponds to a grape juice with a 5% *Botrytis* contamination (estimation by densitometric integration of the native BSA band).

The « botrytized » synthetic must was then « contaminated » with 5% (v/v) of the *Botrytis* culture. This must not be considered as a fungus contamination, but rather like an enzymatic contamination,

Alcoholic fermentation (AF) of the Synthetic must and WSM preparation

* Each experiment in triplicate
* Oenological yeast strains **: IOC 18-2007** (IOC), **HPS** (Lallemand) , **IFI 473**
* Yeast inoculation: 2.10^6 cells/mL in 250mL Erlens
* Enzymatic « contamination » : 5% (v/v) with *Botrytis* culture
* AF at 18°C in a dark room followed by weighing the Erlens
* End of AF controlled by measuring reducing sugars + alcohol content (%v/v)
* Centrifugation of the WSM 15min at 17000g + filtration on 0,45µm membrane
* + 80mg/L SO_2 + ullage in cristal flasks + storage at 18°C

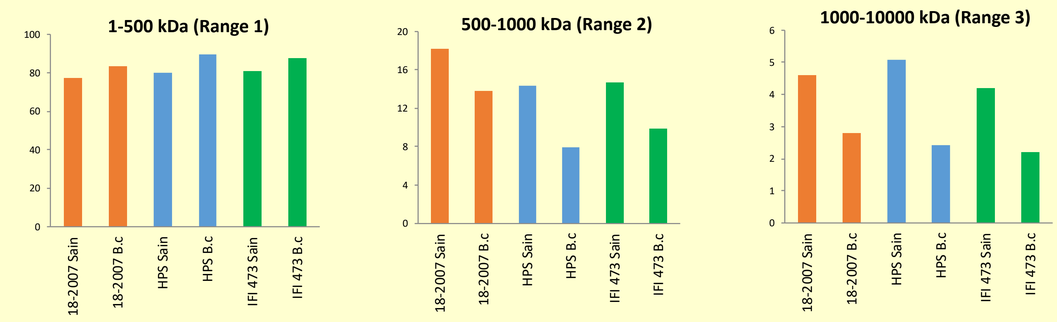
Kinetics of the AF of healthy and contaminated synthetic grape juices



- Very slow AF for all of the samples
- No impact of *Botrytis* culture on the AF kinetics for 18-2007 and HPS strains
- Incomplete AF for IFI 473 + *Botrytis* after 60 days

WSM Polysaccarides molecular weight distribution

In the WSM produced by 3 oenological strains, one can observe strong differences between the wines produced with the « healthy » and the « botrytized » synthetic juices whatever the range studied by size exclusion chromatography. Polysaccharides ranging from 1 to 500 kDa are present in higher quantities in the botrytized WSM. At the opposite, the molecules with MW higher than 500 kDa have contents much higher in the sound WSM. This proves that *Botrytis* hydrolytic enzymes (potentially mannosidases and glucanases) are capable to partially degrade the MPs released during the alcoholic fermentation. During wine storage in the cellar, the enzymes still present in the WSM could most probably continue their hydrolytic activites leading to stronger differences between healthy and sound WSM.



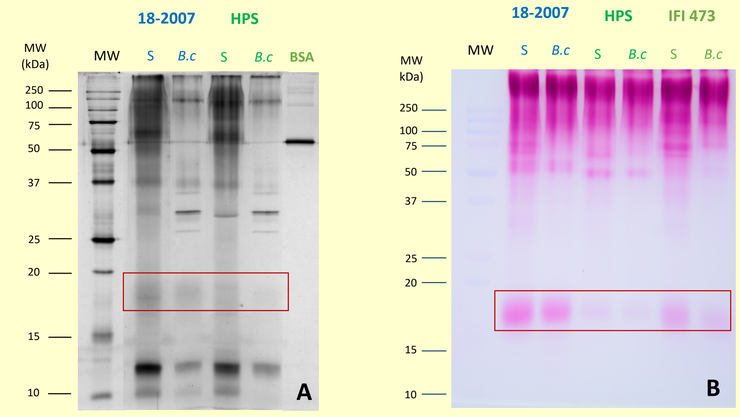
WSM proteic composition

As for the PS composition of the WSM, the SDS-PAGE analysis show strong differences between the sound wine and the wine produced from a synthetic grape juice containing enzymatic activities from a *Botrytis* culture. When compared to the sound WSM, the total protein content decreased by 53 to 63% for the *B.c*-18-2007 and *B.c*-HPS WSM respectively. For the 17kDa protein, the hydrolysis even reached 72 and 54%.

WSM protein analysis by SDS-PAGE

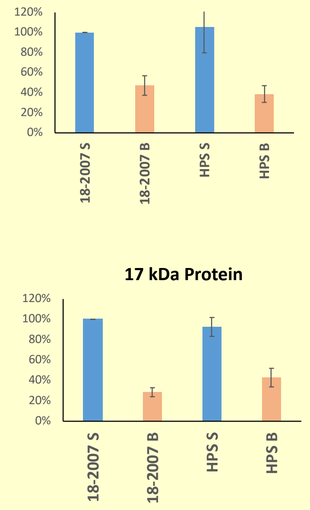
$AgNO_3$ (A) *and* PAS (B) stainings - Analysis 2 months after the end of the AF

S : Sound, *B.c : Botrytis cinerea,* MW : molecular weight markers



Protein content in the WSM

estimated by densitometric integration of the SDS-PAGE/$AgNO_3$ staining analysis



WSM preparation by UF : Dialysis 100x and Concentration 11x

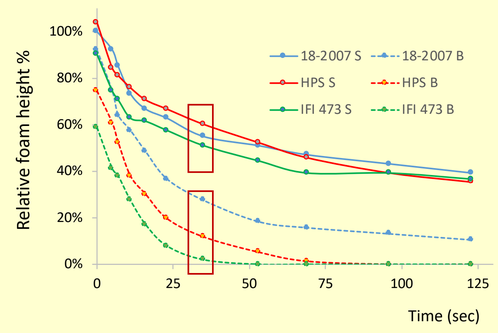
Volumes loaded : WSM : 20µL BSA : 16µL = 30 ng

Foaming properties

The evolution of the foam height following a foaming expansion test in 30mL tubes shows that the 3 healthy WSM exhibit a strong and stable foamability. Whatever the time, after 10 sec, the ANOVA indicates a significant difference between the « healthy » and the contaminated WSM. The PS and protein compositions of these 2 WSM groups explain these foaming behaviors, due to the hyrdolytic fungal enzymes present in the botrytized medium. The ANOVA shows no significant differences between the healthy WSM.

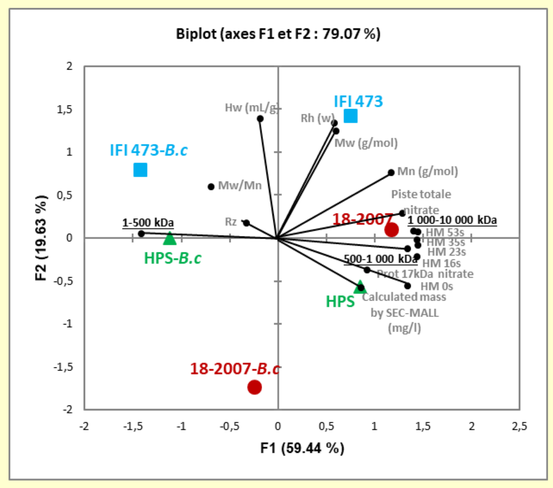
WSM foaming properties

WSM 18-2007 foam at t_0 = 100%



Conclusions

Botrytis enzymatic activities are partially capable to degrade the macromolecules released by *Saccharomyces* during the AF, *i.e* the polysaccharides, the glycoproteins and the proteins not glycosylated. This leads to a **strong decrease of the wine foaming** properties as shown in the ACP Biplot with an anticorrelation between 1) botrytized WSM + Low MW-PS) and 2) healthy WSM + foam HM + proteins + High MW yeast-PS. The 18-2007 stain seems to better resist to the fungus enzymes. Nevertheless, the yeast macromolecule degradation probably still continues during the wine storage. This aspect is currently studied.



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