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Grape juice contamination by *Botrytis*. Impact on the formation of yeasts and macromolecules released in the wine during alcoholic fermentation

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

**Botrytis cinerea culture in a synthetic must**
- Fungus isolated from Champagne's vineyard (8 ± 630, INRA Versailles)
- Culture on a synthetic must to avoid grape berry macromolecules and phenolic compounds.
- Inoculation at 1.67.10^3 spores/mL in a Manteau medium.
- Agitation at 150 rpm — Culture during 22 days at 18°C.

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

**Protein content in the WSM**

**Alcoholic fermentation (AF) of the Synthetic must and WSM preparation**
- Each experiment in triplicate
- Oenological yeast strains : IOC 18-2007 (IOC), HPS (lalettement) ; IIF 473
- Yeast inoculation: 2.10^2 Cell/mL in 250mL Erlen.
- Enzymatic « contamination » : 5% (v/v) with Botrytis culture
- AF at 18°C in a dark room followed by weighing the Erlen
- End of AF controlled by measuring reducing sugars + alcohol content (°W/v)
- Centrifugation of the WSM 15min at 17000g + filtration on 0.45μm membrane
- * + 80mg/L SO2 + absence in crustal flask + storage at 18°C

**Kinetics of the AF of healthy and contaminated synthetic grape juices**
- Very slow AF for all the samples
- No impact of Botrytis culture on the AF kinetics for 18-2007 and HPS strains
- Incomplete AF for IIF 473 + Botrytis after 60 days

**WSM Polysaccharides 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 wine whatever the range studied by size exclusion chromatography. Polysaccharides ranging from 1 to 500 kDa are present in higher quantities in the contaminated 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 MRP released during the alcoholic fermentation. During wine storage in the cellar, the enzymes still present in the WSM could most probably continue their hydrolytic activities leading to stronger differences between healthy and sound WSM.

**WSM proteose 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 Bc-1 18-2007 and Bc-HPS WSM respectively. For the 17Daa protein, the hydrolysis even reached 72 and 54%.

**Foaming properties**
These properties represent the foam height following a foaming expansion test in 30mL tubes shows that the 3 healthy WSM exhibit a strong and stable foamy ability. 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 hydrolytic fungal enzymes present in the botrytized medium. The ANOVA shows no significant differences between the healthy WSM.

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

**References**