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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 (glyco)proteins and polysaccharides (PS) to 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 (IOC 18-2007 (IOC), HPS (Lallemand), IFI 473). The model juice was also fermented with EC1118, a wine yeast strain (Lallemand) and EKD-13 that is an EC1118 recombinant derivative, deleted for both copies of KNR4/SMI1 as described by Gonzalez-Ramos et al. (2008).

Botrytis cinerea culture in a synthetic must

- * Fungus isolated from Champagne's vineyard (B.c 630, INRA Versailles, France)
- * Culture on a synthetic must to avoid grape berry macromolecules and phenolic compounds.
- * Inoculation at 1,67.10⁴ spores/mL in a Manteau medium.
- * Agitation at 150 rpm Culture during 22 days at 18°C.

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

Studied by SDS-PAGE + Silver nitrate staining after Surnageant concentrated 14,7 x. Calibration with BSA. Total released proteins: 1.075 mg/L (densitometric integration)

Proteolytic activity of *Botrytis* culture

Grape juices and *Botrytis* culture proteolytic activities were compared using the **BSA** as a substrate Pinot noir must and Pinot meunier must were obtained from 100% botrytized bunches. 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).

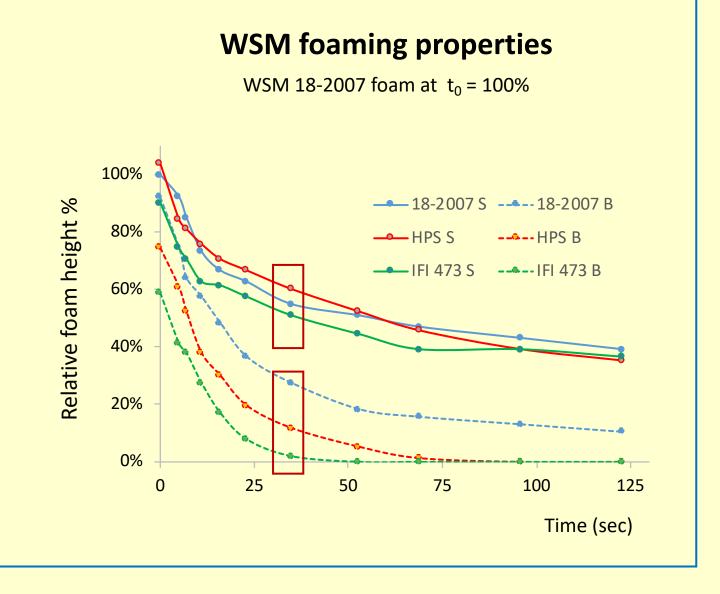
So, the « botrytized » synthetic must was « contaminated » with 5% (v/v) of the Botrytis culture **supernatant**. This must not be considered as a fungus contamination, but rather like an enzymatic contamination.

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

- * Each experiment was performed in triplicate
- * Oenological yeast strains: IOC 18-2007 (IOC), HPS (Lallemand), IFI 473
- * Native/Genetically modified yeast strains : EC 118, EKD 13
- * Yeast inoculation: 2.10⁶ cells/mL in 250mL shake flasks
- * Enzymatic « contamination » : 5% (v/v) with *Botrytis* culture
- * AF at 18°C in a dark room followed by weighing the shake flasks
- * End of AF controlled by measuring reducing sugars + alcohol content (%v/v)
- * Centrifugation of the WSM 15min at 17000g + filtration on 0,45µm membrane
- * Addition of 80mg/L SO₂ + ullage in crystal flasks + storage at 18°C

Foaming properties – Enological strains

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 behaviours, due to the hydrolytic fungal enzymes present in the botrytized medium. The ANOVA shows no significant differences between the healthy WSM.



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WSM Polysaccarides molecular weight distribution

In the WSM produced by 4 oenological strains and a recombinant strain, strong differences between the wines produced with the « healthy » and the « botrytized » synthetic juices can be observed whatever the size range, as analysed size by exclusion chromatography (SEC-MALLS).

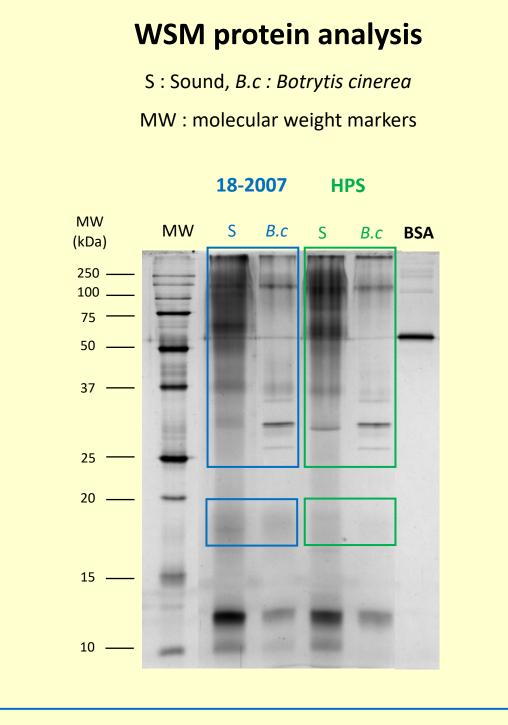
Polysaccharides ranging from 1 to 500 kDa are present in higher quantities in the botrytized WSM. At the opposite, the molecules with MW higher than 500 kDa have contents much higher in the healthy WSM. This indicates that *Botrytis* hydrolytic enzymes (potentially mannosidases and glucanases) are capable 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 activities leading to stronger differences between botrytized and healthy WSM.

		1.10 ³ - 100.10 ³ g/mol	100.10 ³ - 250.10 ³ g/mol	250.10 ³ - 500.10 ³ g/mol	500.10 ³ - 1.10 ⁶ g/mol	1.10 ⁶ - 10.10 ⁶ g/mol
IOC 18 sain	MOYENNE	13,2	27,4	36,6	18,2	4,6
	Ecart-type	9,2	8,6	0,6	1,8	1,4
IOC 18 Botrytisé	MOYENNE	24,2	22,5	36,6	13,8	2,8
	Ecart-type	5,8	0,6	1,5	2,5	1,3
	p-test Student	0,133	0,379	0,989	<u>0,067</u>	0,191
HPS sain	MOYENNE	7,3	37,0	38,2	14,4	5,1
	Ecart-type	4,8	6,1	4,8	4,1	1,2
HPS Botrytisé	MOYENNE	18,9	31,0	36,5	11,0	2,6
	Ecart-type	6,2	5,3	0,9	0,9	0,4
	p-test Student	<u>0,042</u>	0,266	0,568	0,239	<u>0,033</u>
Ifi 473 sain	MOYENNE	13,2	26,5	40,9	14,7	4,2
	Ecart-type	6,3	4,2	1,0	0,9	0,9
Ifi 473 Botrytisé	MOYENNE	29,4	20,7	37,5	9,9	2,2
	Ecart-type	8,9	0,6	6 <i>,</i> 7	1,1	0,6
	p-test Student	<u>0,061</u>	0,073	0,436	<u>0,004</u>	0,034
18-07 Sain	Moyenne	29,7	20,0	32,0	11,5	6,4
	écart type	2,0	1,7	1,4	0,2	0,2
19-07 Rotryticó	Moyenne	42,6	24,6	26,1	5,7	0,9
18-07 Botrytisé	écart type	5,1	2,1	3,7	0,4	0,2
	p-test Student	<u>0,014</u>	0,081	0,135	<u>0,000</u>	< 0,0001
EC 118 Sain	Moyenne	28,1	30,7	31,3	14,6	4,7
	Ecart-type	2,5	10,3	3,0	3,2	0,7
EC 118 Botrytisé	Moyenne	43,3	22,3	27,2	6,7	0,7
	Ecart-type	2,8	3,5	3,9	1,9	0,4
	p-test Student	<u>0,009</u>	0,254	0,227	<u>0,022</u>	<u>0,007</u>
EKD 13 Sain	Moyenne	19,4	24,9	41,2	13,8	0,7
	Ecart-type	1,8	1,9	1,6	1,7	0,2
EKD 13 Botrytisé	Moyenne	23,7	27,9	39,9	8,1	0,4
	Ecart-type	1,4	2,3	2,0	1,6	0,1
	p-test Student	<u>0,010</u>	0,079	0,332	0,002	0,052

WSM protein composition by SDS-PAGE (AF with enological yeast strains)

As for the PS composition of the WSM, the SDS-PAGE analysis (AgNO₃ staining) showed strong differences between the sound/healthy wine (S) and the wine produced from a synthetic grape juice containing enzymatic activities from a *Botrytis* culture (*B.c*). 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% respectively.

These results alone can largely explain the differences in foaming properties between the "wines" originating from healthy and botrytized model grape juices.

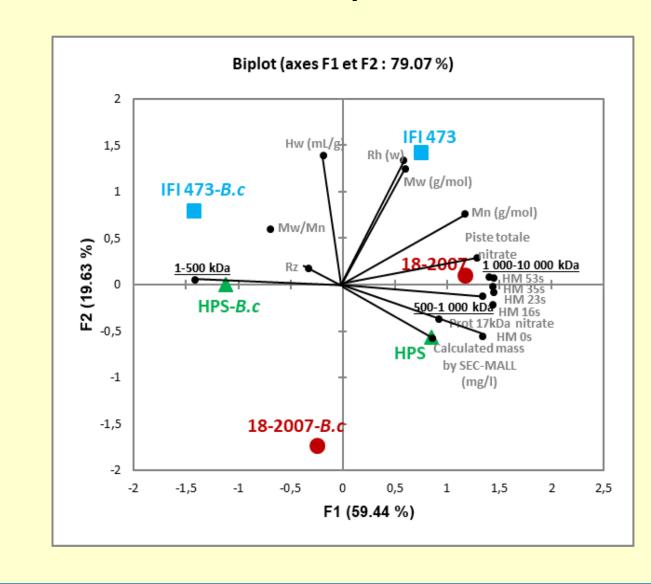


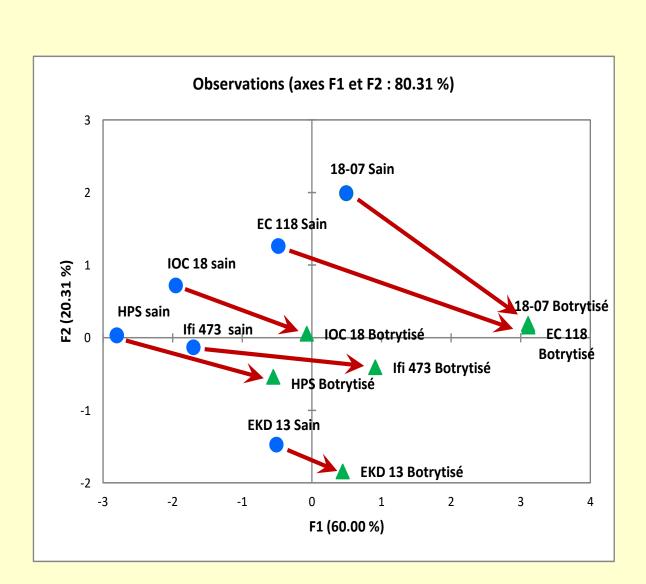
Conclusions

Botrytis enzymatic activities are capable to degrade the macromolecules released by enological strains of Saccharomyces during the AF, i.e the polysaccharides, the glycoproteins and the proteins not glycosylated.

This leads to a strong decrease of the macromolecules MWs as well as a strong decrease of the wine foaming properties as shown in the PCA Biplot with a negative correlation 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, degradation of yeast macromolecules probably continues to progress during the wine storage step. This aspect is currently studied.

PCA with all parameters





PCA with polysaccharides ranges

