PLANT ORIGIN α-PINENE AS AN ALTERNATIVE TO SULFUR DIOXIDE IN RED WINE

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Abstract

The present study aimed at finding an alternative to the widely used sulfur dioxide (SO_2) to control the wine spoilage yeast, *Brettanomyces bruxellensis.* The growth inhibitory effect of α-pinene was evaluated and compared with widely used SO_2, oenological tannins and chitosan. The primary screening was carried out through disc diffusion assay followed by the quantitative test in 96 well microtiter plates to evaluate the test compounds' minimum inhibitory concentration (MIC) against the growth of yeast. The compounds showing antimicrobial activity in primary screening were further assessed in real wine conditions for their capability of inhibiting the growth of *B. bruxellensis* and 4-ethylphenol production. Apart from the tannins and chitosan, the α-pinene showed an inhibitory effect towards *B. bruxellensis* growth. A concentration as low as, 0.625 g L^{-1} of -pinene in wine, could reduce the spoilage yeast population from 5.22 to <1.0 log CFU mL^{-1} and, ceased the 4-ethylphenol production. In conclusion, have found that the efficacy of α-pinene was better than the oenological tannins and chitosan.

Introduction

One of the most feared microbial spoilages in ageing red wines is volatile phenols production, associated with the development of the yeast *Brettanomyces bruxellensis*. Sulfur dioxide (SO_2) is widely used in winemaking to prevent or eliminate unwanted microbes. However, the use of SO_2 in the process of winemaking involves hygienic and technological risks. There is a growing tendency to cut down on its utilization in winemaking, since an excessive intake of it may cause toxicity for consumers, as well as originating negative reactions among specific individuals who may be affected by its presence, with the result of representing a primary cause of intolerance to wine. The European Union has classified SO_2 as a priority food allergen (EU Regulation No. 1169/2011, Annex II). For this, and as strains tolerant to SO_2 exist, alternate antiseptic molecules or methods are looked for by winemakers. The objective of this study was to evaluate the antimicrobial efficacy of different natural products against *B. bruxellensis*: different chitosan-based compounds, oenological tannins and terpene extracted from the pine tree, α-pinene. In order to select the best compound that could inhibit the growth of *B. bruxellensis*, our first approach was to conduct a disc diffusion assay. And thereafter, the Minimum Inhibitory Concentration (MIC) determination by the visual observation of the turbidity in the microtiter plate followed by counting the colony forming units (CFU) using surface agar plating onto GYP media. Finally, the effect of these compounds was tested in the laboratory, mimicking the winery condition in which spontaneous *B. bruxellensis* populations may attain significant densities, as in the case of wine ageing in oak barrels.

Tables and graphs

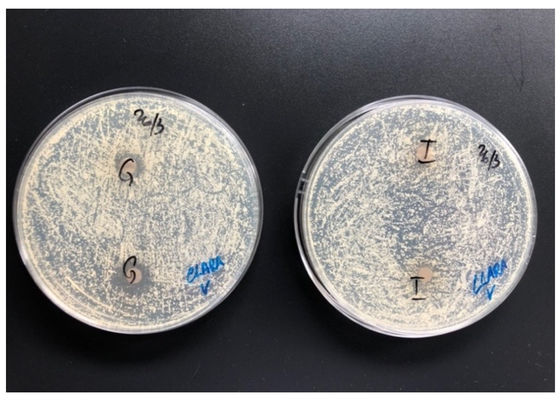

Figure 1. Disk diffusion assay. G, Tan'active T-80; I, Quitosano.

Table 1. The minimum inhibitory concentration of the tested compounds (log10 CFU mL^{-1}, nd-undetected, uc-uncountable)

SO_2		α-pinene		BrettOut F		BrettOut T		CleanBrett		Quitosano	
mg/mL	CFU/mL	mL/L	CFU/mL	mg/L	CFU/mL	mg/L	CFU/mL	mg/L	CFU/mL	mg/L	CFU/mL
25.00	nd	0.625	nd	0.13	8.13	7.50	7.40	37.50	7.86	62.50	9.00
12.50	nd	0.312	nd	0.06	uc	3.75	uc	18.75	uc	31.25	uc
6.25	nd	0.156	nd	0.03	uc	1.88	uc	9.38	uc	15.63	uc
3.13	nd	0.781	nd	0.02	uc	0.94	uc	4.69	uc	7.81	uc
1.56	nd	0.391	nd	0.01	uc	0.47	uc	2.34	uc	3.91	uc
0.78	nd	0.195	6.98	0.00	uc	0.23	uc	1.17	uc	1.95	uc
0.39	nd	0.098	uc	0.00	uc	0.12	uc	0.59	uc	0.98	uc
0.20	5.00	0.049	uc	0.00	uc	0.06	uc	0.29	uc	0.49	uc

Tan'active CE		Tan'active QS-SOL		Tan'active T-80		Tan'active QBC		Amora grape opera		Control	
mg/L	CFU/mL	mg/L	CFU/mL	mL/L	CFU/mL	mg/L	CFU/mL	mg/L	CFU/mL	mg/L	CFU/mL
3.13	8.11	3.13	8.06	3.13	8.12	3.13	8.18	3.13	7.32	0	9.00
1.56	uc	1.56	uc	1.56	uc	1.56	uc	1.56	uc	0	uc
0.78	uc	0.78	uc	0.78	uc	0.78	uc	0.78	uc	0	uc
0.39	uc	0.39	uc	0.39	uc	0.39	uc	0.39	uc	0	uc
0.20	uc	0.20	uc	0.20	uc	0.20	uc	0.20	uc	0	uc
0.10	uc	0.10	uc	0.10	uc	0.10	uc	0.10	uc	0	uc
0.05	uc	0.05	uc	0.05	uc	0.05	uc	0.05	uc	0	uc
0.02	uc	0.02	uc	0.02	uc	0.02	uc	0.02	uc	0	uc

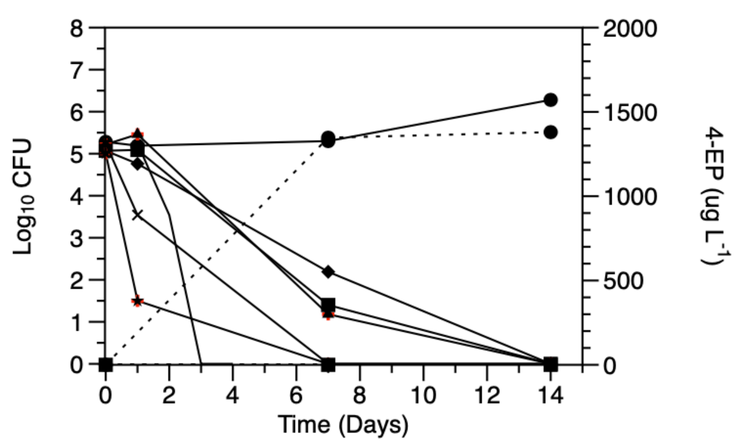

Figure 2. Effect of commercial and commercial compounds (T-80 ▲, BrettOut F ■, BrettOut T◆ , Sulphur dioxide ★, α-pinene ✕ control ●; solid line-CFU mL^{-1}, dottled line- 4-EP) against *B. bruxellensis.*

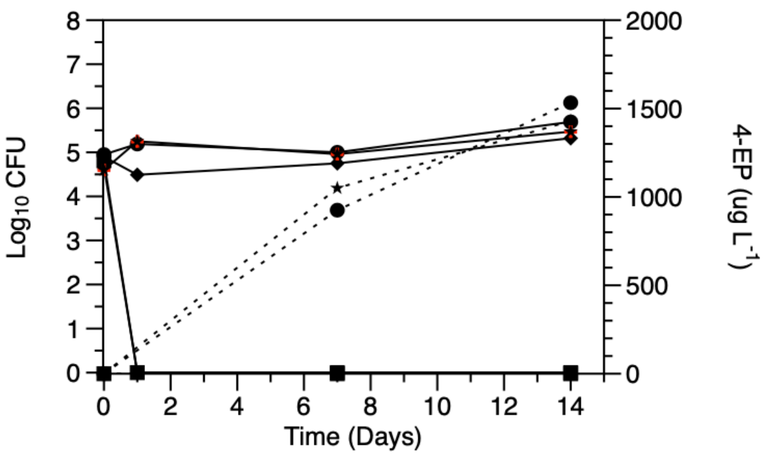

Figure 3. α-pinene effect (1.25 ml L^{-1} ▲, 0.625 ml L^{-1} ■, 0.312 ml L^{-1} ◆ , 0.156 ml L^{-1} ★, control ●; solid line-CFU mL^{-1}, dottled line- 4-EP) on *B.bruxellensis* cells growth and 4-ethylphenol production in wine.

Results

- Among ten test compounds, a commercial tannin preparation (Tan'Active T-80) named in the legend "G" (Figure 1), showed a zone of inhibition around the diffusion disc. The rest of the compounds did not show any zone of inhibition (except SO_2 which served as control). However, all the test compounds were claimed to inhibit the *B. bruxellensis* by the industry producers.

- Among the commercial preparations of oenological tannins, named by the manufacturer T-80, the MIC required to inhibit *B. bruxellensis* growth in wine was 500 mg mL-1, while the concentration advised by the producer was 10 g mg hL-1. The MIC for α-pinene was found to be 0.39 mL L^{-1} (Table 1). The rest of the test compounds did not show inhibition, as the CFU counts were uncountable on the surface plate (Table 1). The inhibitory effect on the yeast growth, decreased cells count from log 5.22 to <1 CFU mL with no 4-ethylphenol production within day 7 days of observation (Figure 2).

- In real wine conditions, the minimum concentration of α-pinene that could inhibit the growth of *B. bruxellensis* in wine was 0.625 ml L-1 (Figure 3). At this concentration, there was no production of 4-ethylphenol observed on days 7 and 14. The viability loss was achieved right after the addition of α-pinene. On the other hand, at concentrations of 0.156 ml L-1 and 0.312 ml L-1 cellular death was not observed and 4-ethylphenols increased up to 1430 ug L-1 in 15 days (Figure 3). These results, showing the lethal effect at low concentrations, if valid for other yeasts, could provide stronger evidence that α-pinene is an antimicrobial agent suitable for the control of yeast spoilage in wine.

- The α-pinene could be a suitable candidate as an alternative for SO_2 in winemaking since it is a natural compound and possesses adequate antioxidative and antibacterial abilities. This is the first time that α -pinene is shown to control *B. bruxellensis* population in wine during storage. It is of oenological significance that the lowest concentration used, efficiently prevented *B. bruxellensis* growth as well as 4-ethylphenol production in the red wine.

Conclusion

Alpha-pinene was found to be the best alternative to sulfur dioxide as it was capable of performing antimicrobial activity against *B. bruxellensis* during wine storage with a concentration of 0.625 mL L^{-1}. Moreover, α-pinene is a vegetal extract, thus entirely natural. This would help winemakers to contrast yeast spoilage without the use of chemicals, to obtain high-quality wines and, above all, to cater those wine consumers that are sensitive to the effects of sulphites.

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