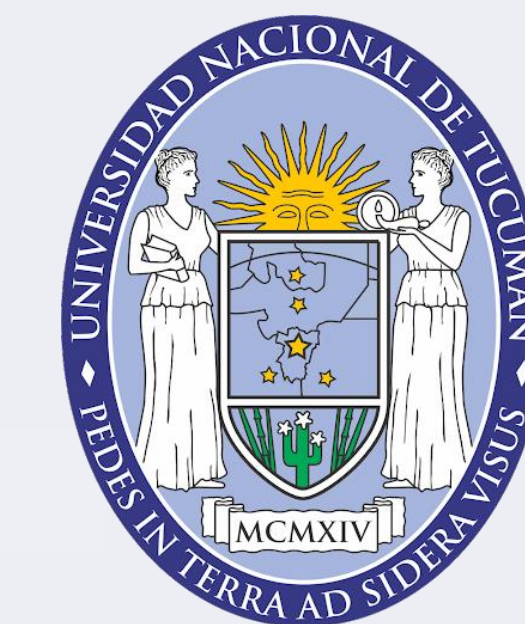




Saccharomyces cerevisiae

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INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin with harmful effects on human and animal health. OTA is the most commonly reported mycotoxin in wine and its presence also significantly affects the metabolism of yeasts. *Aspergillus carbonarius* and *A. niger* are the main OTA-producing fungal in grapes wine. During winemaking or storage, sulfur dioxide (SO₂), applied as sodium or potassium metabisulfite, is the most used product for the inhibition of undesirable microorganisms. However, the constant use of chemical antifungals can cause undesirable effects in the microbial processes. In addition, some fungicides used in sub-lethal doses reduce the fungal growth but increase the OTA production. The search of alternatives that allow reduction or even elimination of SO₂ is a challenge to the winemaking industry. In this sense, plant extracts and their metabolites have become popular on the mycotoxigenic fungi control. In previous assays we demonstrated that an antifungal plant constituent identified as 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (lapachol) combined with sodium metabisulfite completely suppressed the OTA accumulation at concentrations of 9.8 and 156.3 µg/mL, respectively. Lapachol synergized the antifungal activity of sodium metabisulfite and its use could reduce the doses of the commercial preservative in the prevention of fungal growth and OTA accumulation in grape juice and wine. Therefore, is important to evaluate the effect of this novel combination on the viability and fermentative activity of commercial yeasts used in wine fermentation.

OBJECTIVE

To evaluate the fermentation process carry out by the commercial yeast *Saccharomyces cerevisiae* EC-1118 under vinification conditions in presence of the mixture of lapachol and sodium metabisulfite

MATERIALS AND METHODS

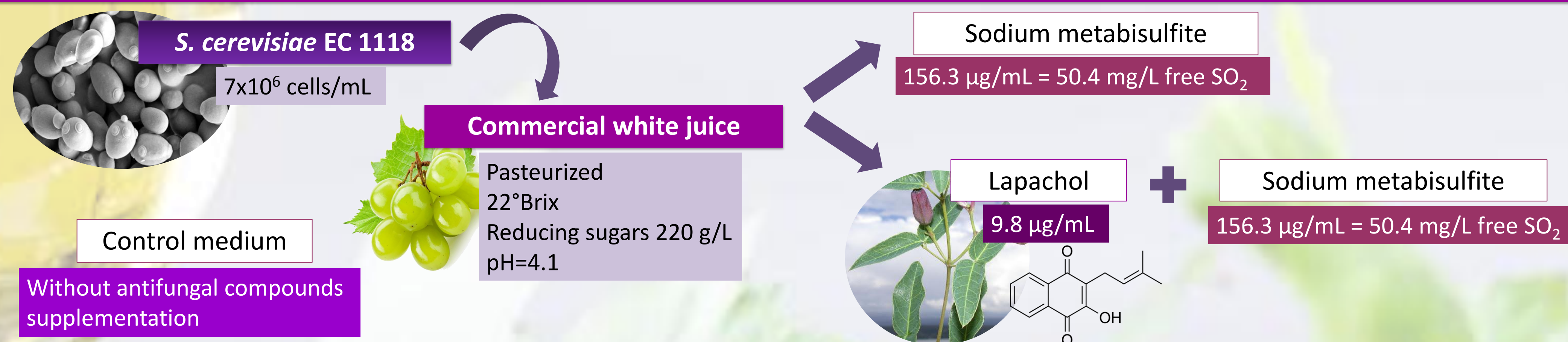
Microorganism and growth condition

A commercial *Saccharomyces cerevisiae* yeast strain (Lalvin EC-1118) was used to perform alcoholic fermentation. The yeast strain was rehydrated and routinely grown in YPD (extract peptone dextrose) medium (20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract and pH=5.0) at 28 °C.

Antifungal compounds

Lapachol used as natural antifungal compound was obtained from a dichloromethane extract of *Macfadyena cynanchoides* stems according to the protocol previously described by Apud et al. (2019). Sodium metabisulfite used as commercial chemical preservative was purchased from Merck Company.

Alcoholic Fermentation



The flasks were corked with an air trap with sulfuric acid and incubated at 22°C. Fermentations were monitored through releasing of CO₂ by determining the system weight loss every 24 h

During the process yeast viability was determined. The parameters ethanol (immersion refractometer), pH, total soluble solids (°Brix using a calibrated hand refractometer), residual reducing sugars (Fehling method) and free SO₂ (Ripper titration) were evaluated in the final product

RESULTS

Table 1 . Antifungal and antiochratoxigenic activity of lapachol + sodium metabisulfite on *A. carbonarius* and *A. niger*. (Apud et al. 2019)

Assay compound	Concentration (µg/mL)	<i>Aspergillus carbonarius</i>		<i>Aspergillus niger</i>	
		Biomass (mg)	ng OTA/mg biomass	Biomass (mg)	ng OTA/mg biomass
Growth control	0	1.00±0.01	100.74±6.8	1.00±0.01	62.71±2.8
Lapachol + Sodium metabisulfite	9.8 + 156.3	0.51±0.01	ND	0.85±0.01	ND
	19.5 + 312.5	0.35±0.03	ND	0.48±0.01	ND
	39.1 + 625	0.21±0.02	ND	0.22±0.03	ND

Values are expressed as mean ± standard deviation. ND: not determined (OTA was not detected)

Table 2. Evaluated physico-chemical parameters at the end of alcoholic fermentation

Assay parameter	Control	Sodium metabisulfite	Lapachol + Sodium metabisulfite
Yeast viability (cells/mL)	8.9 x 10 ⁷	9.4 x 10 ⁷	1.0 x 10 ⁸
Ethanol (%)	12.24	12.32	12.20
pH	3.88	3.84	3.75
°Brix	5.20	5.20	5.20
Residual reducing sugars (g/L)	3.58	3.86	4.00
Free SO ₂	-	32	32

Alcoholic fermentation finished at 14 days in control medium without additives. The presence of antifungal compounds provokes an extension of fermentation until for 21 days. The presence of combination of lapachol with sodium metabisulfite didn't affect the yeast development with respect to sodium metabisulfite alone, achieving a complete fermentation of grape juice at the same time. The parameters evaluated at the end of alcoholic fermentation showed similar values.

CONCLUSIONS

Lapachol did not show inhibitory effect on the viability of *S. cerevisiae* and allowed a normal course of the alcoholic fermentation with physico-chemical parameters values like the trial contained sodium metabisulfite.

The combination of sodium metabisulfite with lapachol could be used as strategy to reduce the doses of the commercial preservative in the prevention of fungal growth and OTA accumulation as antifungal treatment at post-harvest stage.

REFERENCES

Apud GR, Aredes-Fernández PA, Kritsanida M, Grougnet R, Sampietro DA. 2019. Antifungal activity of Bignoniaceae plants on *Aspergillus carbonarius* and *Aspergillus niger*. Nat Prod Res 1–4.