Modulation of metal content and antioxidant activity in wine by native non-*Saccharomyces* yeasts

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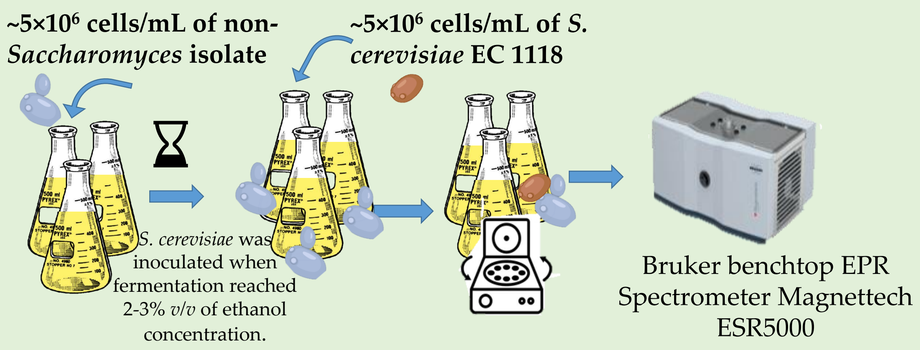
Introduction

The utilisation of non-*Saccharomyces* (NS) yeasts in the winemaking procedure recently became a trend in the wine industry due to their contribution to the wine aroma. Still, there is a lack of information about how using NS yeasts affects the wine's antioxidative properties and concentrations of metals present in grape juice. In the present study, 1,1-diphenyl-2-picrilhydrazyl (DPPH) assay and electron paramagnetic resonance spectroscopy (EPR) were used to explore the antioxidant activity and concentrations of iron (Fe^{3+}) and manganese (Mn^{2+}) in experimental white wines[1]. The sequential fermentation trials were set up with seven indigenous NS yeast on Maraština grape sterile must to provide insight into the NS yeast mechanism.

Materials and methods

- Indigenous yeasts: ***Metschnikowia pulcherrima* K-6 (*Mp*), *Metschnikowia chrysoperlae* K-11 (*Mc*), *Metschnikowia sinensis/shanxiensis* P-7 (*Ms*), *Lachancea thermotolerans* P-25 (*Lt*), *Pichia kluyveri* Z-3 (*Pk*), *Hanseniaspora uvarum* Z-7 (*Hu*)** and ***Hanseniaspora guilliermondii* N-29 (*Hg*)** (**Institute for Adriatic Crops collection**) and commercial *Saccharomyces cerevisiae* EC 1118 (*Sc*) (Lallemand)
- Control: monoculture fermentation with *Sc*
- **Experimental design:** sequential fermentation of sterile white Maraština (*V. Vinifera* L) grape juice with non-*Saccharomyces* isolate-*S. cerevisiae* in three replications



- **Instrumental analysis and conditions**

Antioxidant activity was measured by Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Germany) with the following parameter set: microwave frequency 9.5 GHz, modulation amplitude 0.20000 mT, magnetic field modulation frequency 100 kHz and magnetic field range from 331.00 mT to 343.00 mT. The DPPH assay was used to determine free radical scavenging capacity. 20 µL aliquots of the tested samples were mixed with 980 µL of a 0.1 mM ethanolic solution of the DPPH radical. The blank sample consisted of 20 µL of 96% ethanol instead of the sample. The calibration curve of ascorbic acid was used to calculate the concentration of antioxidants in samples. Concentrations of the ascorbic acids were: 0.3, 0.1, 0.06, 0.04, 0.03, and 0.01 mg/mL. 20 mL of ascorbic acid solution was mixed with 980 µL of a 0.10 mM ethanolic solution of the DPPH radical.

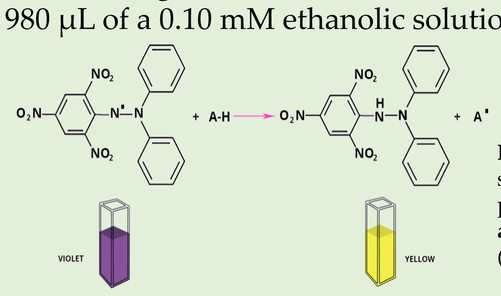


Figure 1. DPPH reacts with antioxidative species and transforms from a paramagnetic species (purple, **EPR active**) into a diamagnetic species (yellow, **EPR inactive**)

Proportion of Fe and Mn were measured by Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Germany) with parameter set: microwave frequency 9.5 GHz, modulation amplitude 0.5 mT, magnetic field modulation frequency 100 kHz and magnetic field range from 100.00 mT to 500.00 mT.

Results

The antioxidative acitivity was expressed by percent inhibition of DPPH and ascorbic acid equivalents (Figure 2). Percent inhibition of DPPH (DPPH%) was calculated according to the formula $DPPH\% = 100*(A_{blank} - A_{sample})/A_{blank}$, where A_{blank} is the amplitude of the blank DPPH solution and A_{sample} denotes the amplitude of the sample spectra

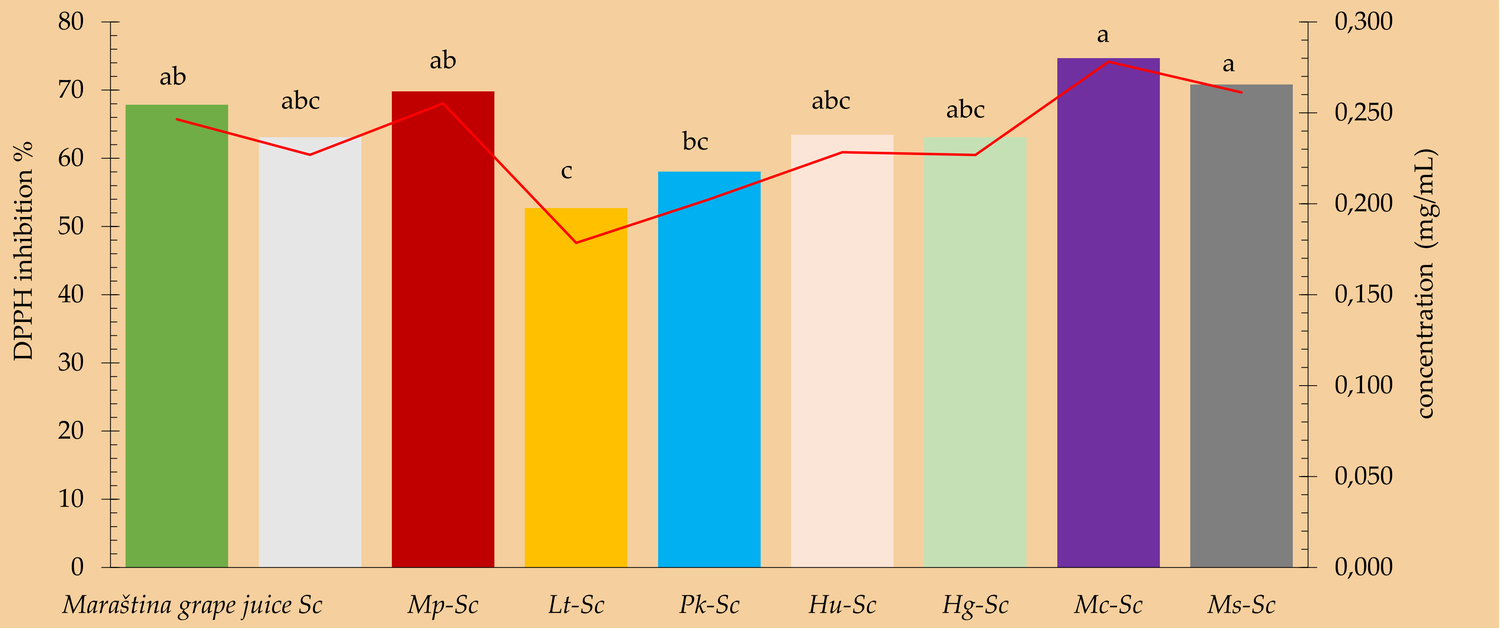


Figure 2. Antioxidative activity (%) and ascorbic acid equivalents (mg/mL) of Maraština grape juice and different wines. Different letters in the column indicate a significant difference ($p < 0.05$).

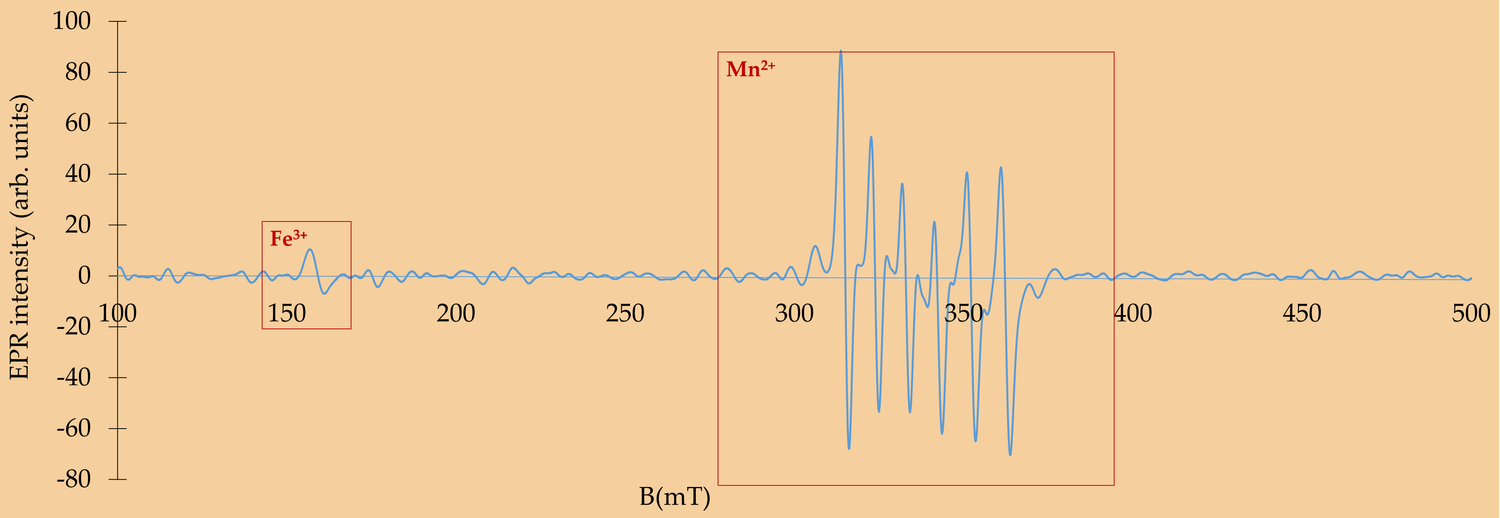


Figure 3. EPR spectra of the Maraština grape juice.

Fe^{3+} and Mn^{2+} were detected in Maraštine grape juice, while copper (Cu^{2+}) is under Mn^{2+} or hindered so it can be concluded that copper is undetected.

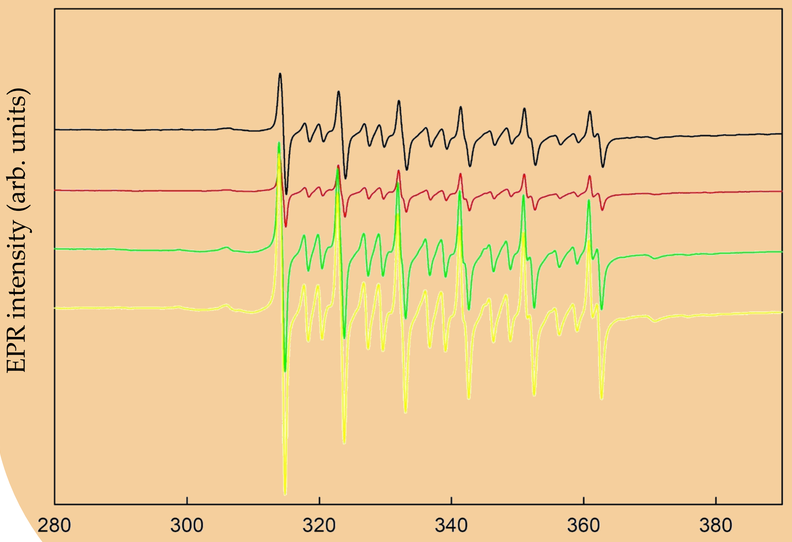


Figure 4. Mn^{2+} EPR spectra for grape juice (black line) and wines produced with different yeasts in sequential fermentations: *Hu-Sc* (red line), *Hg-Sc* (green line) and *Mc-Sc* (yellow line). B-magnetic flux density.

Higher intensity means higher Mn^{2+} concentration and the difference in EPR spectral shape responds to the change in microstructure around the manganese ion.

Conclusions

- All yeast from the *Metschnikowia* genera (*M. pulcherrima, M. chrysoperlae, M. sinensis/shanxiensis)* increased the antioxidant activity of wines, while *L. thermotolerans* significantly reduced it compared to the initial grape juice.
- The application of indigenous *H.uvarum, H. guilliermondii,* and *M. sinensis/shanxiensis* yeasts reduced the concentration of Mn^{2+} in wine compared to the Maraština grape juice, especially in the case of *M. sinensis/shanxiensis.*

Acknowledgements

All Authors gratefully acknowledge the COST Action 19116 PLANTMETALS for the given opportunity to present this work and the Croatian Science Foundation for financing Research project IP-2020- 02-1872.

References

[1]Fuhrman, B., Volkova, N., Suraski, A., Aviram, M. (2001). White wine with red wine-like properties: increased extraction of grape skin polyphenols improves the antioxidant capacity of the derived white wine. *J. Agric. Food Chem*. 49(7), 3164-8.