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Bioprotection using a selected strain of *Metschnikowia pulcherrima* as an alternative to SO_2 for preventing the browning of white grape must

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Enzymatic browning of grape must still represent one of the major problems in oenology today[1], and is especially serious when the grapes are affected by grey rot[2]. Sulphur dioxide is the most common tool used in wineries to protect grape must against enzymatic browning. This additive is a potent inhibitor of tyrosinase and laccase[3] and also has antimicrobial properties[4]. However, the wine industry is currently trying to reduce and even eliminate sulphur dioxide due to its negative effects on the environment and human health. Hence, to prevent browning, some alternatives to sulphur dioxide additives have been proposed over recent years, such as the use of inert gases, oenological tannins, ascorbic acid, reduced glutathione, inactivated dry yeasts rich in glutathione and bioprotection using some Non-*Saccharomyces* yeasts[3,5,6]. Different microorganisms have been proposed for Bioprotection, but according to recent literature, *Metschnikowia pulcherrima* seems to be the most promising microorganism for wine bioprotection against enzymatic browning due to its high capacity for consuming oxygen[3,7]. The aim of this paper is therefore to study the bioprotective effect of a selected strain of *Metschnikowia pulcherrima* (MP1) against enzymatic browning caused by polyphenol oxidases.

Experimental design

Muscat of Alexandria grapes were harvested, pressed and diluted 5 times with a model grape must synthetic buffer at pH = 3.50; this made it possible to monitor it with higher precision, and also to standardise the pH of the different measurements. The diluted grape must was immediately supplemented or not with 20 mg/L of SO_2, 2 UA/mL of laccase activity and 250 mg/L of the selected *M. pulcherrima* MP1 (Level2 Initia™, Lallemand Inc, Montreal, Canada). Immediately, the samples were saturated with O_2 and its concentration was noninvasively monitored overtime by luminescence (Nomasense TM O2 Trace Oxygen Analyzer by Nomacorc S.A., Thimister Clermont, Belgium)[7]. Once oxygen consumption had attained asymptotic behaviour the samples were used for colour analysis and for HPLC analysis of hydroxycinnamic acids[7].

Statistics

All data are expressed as the arithmetic average ± standard deviation of three replicates. One-factor analysis of variance (ANOVA F test) was conducted using the SPSS 15.0 software (SPSS Inc., Chicago, IL). Differences were considered significant when the *p* value was less than 0.05.

Results and Discussion

Figure 1 shows the oxygen consumption rates of the diluted grape must supplemented or not with sulphur dioxide, *Metschnikowia pulcherrima* strain MP1 or with the combination of the two agents, in the absence or presence of 2 U/mL of laccase.

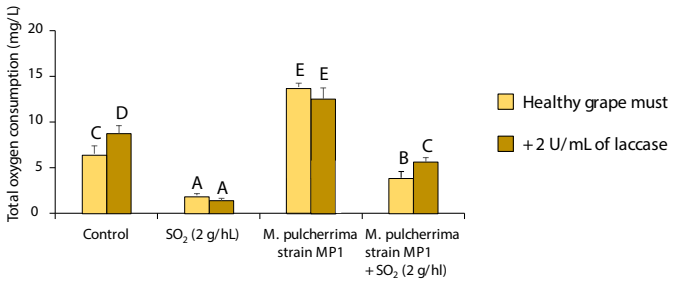


FIGURE 1. Influence of inoculation of *M. pulcherrima* strain MP1 on Total Oxygen Consumption.

As expected, in the absence of SO_2, the grape must actively consumed O_2, and total oxygen consumption (TOC) was significantly higher when the medium was supplemented with laccase. By contrast, TOC decreased drastically with the addition of sulphur dioxide, regardless of the presence of laccase. Inoculation with the selected *M. pulcherrima* MP1 strain significantly increased TOC in the absence of SO_2, confirming the strong oxygen consumption capabilities of this non-*Saccharomyces* yeast strain. However, in the presence of SO_2, the impact of *M. pulcherrima* MP1 strain on TOC was less pronounced, likely due to the inhibitory effect of SO_2 on its growth.

Figure 2 shows the absorbance at 420 nm (A420nm) and the CIEL*a*b* blue–yellow component (b*) of the samples supplemented or not with sulphur dioxide, *Metschnikowia pulcherrima* strain MP1 or the combination of the two, in the absence and presence of laccase as indicators of browning intensity.

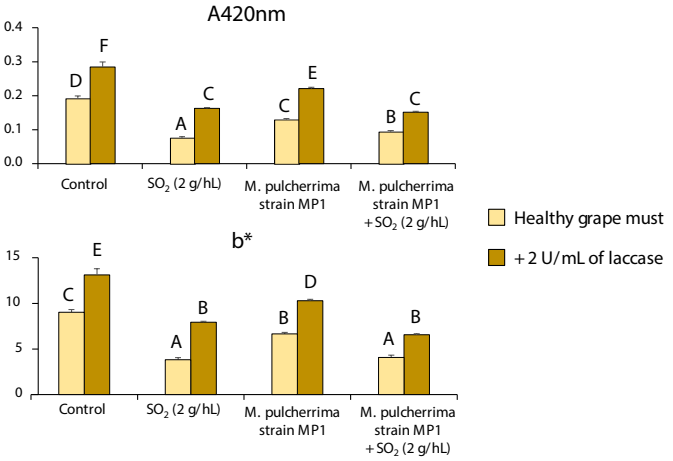


FIGURE 2. Influence of inoculation of *M. pulcherrima* strain MP1 on the intensity of the yellow colour (A420nm and b*) of the grape must.

The supplementation with sulphur dioxide exerted a clear protective effect against browning since the values of A420 and b* were significantly lower than in the control sample. This confirms the described protective effect of this additive for avoiding enzymatic browning[3,4]. The supplementation with *Metschnikowia pulcherrima*

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strain MP1, alone or combined with sulphur dioxide, also significantly decreased A420 and b* with respect to the control, confirming that this non-*Saccharomyces* yeast really protects grape must against enzymatic browning even in the absence of sulphur dioxide. However, the combination of *M. pulcherrima* with sulphur dioxide did not enhance the performance compared to sulphur dioxide alone, likely because sulphur dioxide inhibits *M. pulcherrima*, preventing it from exerting its protective effect.

In the presence of laccase all the samples showed significantly higher values of A420 and b* than their corresponding controls without laccase. This confirms the high oxidative capacity of this polyphenol oxidase. It also indicates that it is more difficult to completely protect against browning in grapes affected by grey rot, even when using sulphur dioxide. The protective effect of *M. pulcherrima* on wine colour was also observed in final hydroxycinnamic acids concentration that was higher than in the control (data not shown).

The different intensities of the colour of the four samples after the experimental process can be clearly seen in the photographs in Figure 3.

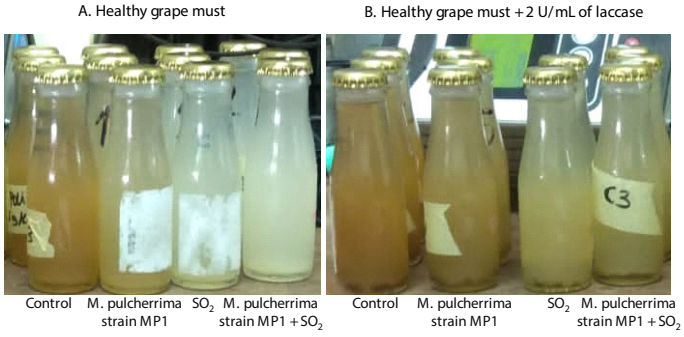


FIGURE 3. Influence of inoculation of *M. pulcherrima* strain MP1 on the intensity of the yellow colour of the grape must.

In conditions of healthy grape must, the control sample without anything added to it has undergone a clear browning process. By contrast, the samples supplemented with sulphur dioxide, alone or combined with *Metschnikowia pulcherrima* strain MP1, are very light in colour, which confirms that this additive protects perfectly against browning. The sample supplemented only with *Metschnikowia pulcherrima* strain MP1 is slightly browner in colour than the samples protected with sulphur dioxide, but it is much clearer than the control without any additive. However, in the presence of laccase the protective effectiveness of *Metschnikowia pulcherrima* strain MP1, and even that of sulphur dioxide, were not enough to prevent browning. This confirms that supplementation with *Metschnikowia pulcherrima* strain MP1 exerts a protective effect against browning in conditions of healthy grape must.

Conclusions

In this work, we analysed how supplementation with sulphur dioxide and/or *Metschnikowia pulcherrima* strain MP1 influence oxygen consumption kinetics, hydroxycinnamic acids concentrations, and browning intensity in two situations. The first situation was grape must from healthy grapes, and the second was grape must supplemented with the enzyme laccase to reproduce what occurs when the grapes are infected with *Botrytis cinerea*. The results show that sulphur dioxide drastically reduces the oxygen consumption rate and prevents grape must from browning even in the presence of laccase, although less effectively than in the absence of laccase. By contrast, the supplementation with *Metschnikowia pulcherrima* strain MP1 drastically increases the oxygen consumption rate, which reduces the oxygen available for polyphenol oxidases; in the absence of laccase, this results in a decrease in browning. However, in the presence of laccase, supplementation with *Metschnikowia pulcherrima* strain MP1 was not effective enough to prevent browning. It can therefore be concluded that *Metschnikowia pulcherrima* strain MP1 can be considered as an adequate alternative tool to sulphur dioxide for preventing browning in white grape must in conditions of healthy grapes. ■

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